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Factors affecting outcomes for young women with breast cancer

by

Thomas Christopher Maishman

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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FACTORS AFFECTING OUTCOMES FOR YOUNG WOMEN WITH BREAST CANCER

by Thomas Christopher Maishman

Breast cancer is the most common cancer in young women (aged 40 years or younger), with over 2,000 new cases each year in the United Kingdom. Younger women have been found to develop more aggressive tumours coupled with lower survival and higher local-recurrence rates compared to older women. The exact reasons for this remain unclear, and evidence that age is an independent factor for poor prognosis still remains limited. There is further need for more in-depth research into this area to help inform both patients and the clinical teams treating these patients.

The aim of this thesis was to study factors affecting outcomes for young women with breast cancer to provide additional data for clinicians and their patients, to weigh up the optimum approach to reduce the risk of death in newly diagnosed young breast cancer patients.

This thesis includes research comprising a collection of published works in young women with invasive breast cancer, using data from a large prospective cohort study. The findings have demonstrated that the oestrogen receptor status of the tumour, together with the ethnicity and body mass index of patients, were found to be significant independent prognostic factors affecting survival in this young age group, whilst reported family history of breast cancer, surgical type and BRCA mutation status were not found to be significant prognostic indicators.

There is a need for caution when extrapolating data from older patient cohorts in order to determine the most appropriate treatment management options for younger women. Future research should be carried out in order to investigate new treatment approaches for this age group, and should take into account these prognostic factors to provide clinicians with sufficient information to decide the optimum treatment approach to reduce the rate of death for young women with invasive breast cancer.

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Academic Thesis: Declaration Of Authorship

I, Thomas Christopher Maishman, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Factors affecting outcomes for young women with breast cancer.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

   i. Copson E, Eccles B, Maishman T, et al. Prospective observational study of breast cancer treatment outcomes for UK women aged 18-40 years at diagnosis: the POSH study. *J Natl Cancer Inst* 2013;105(13):978-88.
   ii. Copson E, Maishman T, Gerty S, et al. Ethnicity and outcome of young breast cancer patients in the United Kingdom: the POSH study. *Br J Cancer* 2014;110(1):230-41.
   iii. Copson ER, Cutress RI, Maishman T, et al. Obesity and the outcome of young breast cancer patients in the UK: the POSH study. *Ann Oncol* 2015;26(1):101-12.
   iv. Eccles BK, Copson ER, Cutress RI, Maishman T, et al. Family history and outcome of young patients with breast cancer in the UK (POSH study). *Br J Surg* 2015;102(8):924-35.
   v. Copson E, Maishman T, Tapper W, et al. Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. *Lancet Oncol* 2018; 19(2):169-180.
   vi. Maishman T, Cutress RI, Hernandez A, et al. Local Recurrence and Breast Oncological Surgery in Young Women With Breast Cancer: The POSH Observational Cohort Study. *Ann Surg* 2017; 266(1):165-172.

vii. Maishman T, Copson E, Stanton L, et al. An evaluation of the prognostic model PREDICT using the POSH cohort of women aged 40 years at breast cancer diagnosis. *Br J Cancer* 2015;112(6):983-91.

Signed: .....

Date: .....

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I would like to thank all of my co-authors on the seven papers presented here, with special thanks to Ellen Copson and Ramsey Cutress; my clinical and surgical gurus. Lorraine Durcan, queen of data, Paul Pharoah, expert on PREDICT (and left-truncation), and Professor Doug Altman, expert on anything at all statistical.

I would like to thank the thousands of women who participated in the POSH study. These young women with breast cancer are a unique group of people, often diagnosed with very aggressive and high grade tumours and, without whom, this large prospective study would not have been possible for this vitally important research.

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Chapter 1: Introduction

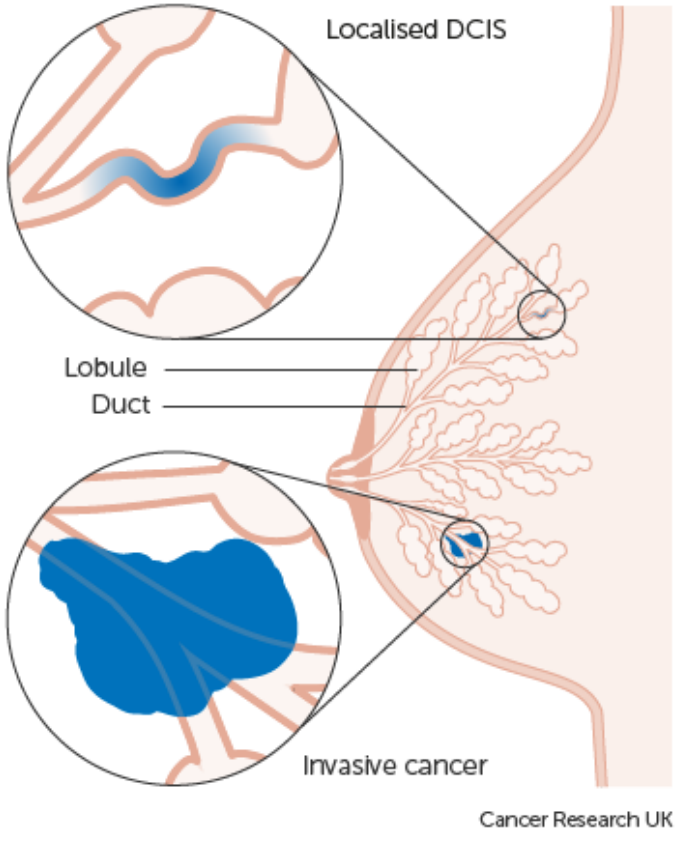
1.1 Breast cancer

Breast cancer is the most common cancer in the United Kingdom (UK), accounting for 15% of all newly diagnosed cancers in the UK, with approximately 55,000 new cases diagnosed each year[1]. Ninety-nine percent of breast cancer occurs in women, with 1 in 8 UK women developing breast cancer in their lifetime. In terms of mortality, breast cancer in UK women is the second most common cause of cancer death, with approximately 11,000 deaths each year in the UK[1].

1.1.1 Types of breast cancer

Invasive breast cancer is the most common type of breast cancer. Also known as 'no special type' and previously described as 'ductal carcinoma', invasive breast cancer develops in the cells that line the ducts of the breast (**Figure 1**), and grows through the lining of the ducts into the surrounding breast tissue[2].

Figure 1 Types of breast cancer[2]



The term 'no special type' refers to the cancer cells, which usually show no distinctive or 'special' features when looked at through a microscope.

Other types of breast cancer include: inflammatory breast cancer, where the cancer cells block the lymph channels in the breast, resulting in a breast which may appear red and swollen; medullary breast cancer, where cancer cells tend to be bigger, which often occurs in young women who have inherited a faulty BRCA1 gene; and lobular carcinoma in situ (LCIS), where some cells in the breast lobules have started to become abnormal[3].

1.1.2 Clinical and pathological features of breast cancer

In addition to the type of breast cancer, other characteristics or features are often used to describe different breast cancers. These include:

- The 'stage' of the tumour i.e. the size of the tumour and whether it has spread to other parts of the body/organs – the larger the tumour and greater extent to which the tumour has spread, the higher the stage. The tumour stage is broken down into a TNM staging system[4,5]:
    - Tumour (T) stage – the extent or size of the tumour i.e. how large the tumour is and whether it has grown into nearby areas. The higher the T stage, the larger and/or more widely spread the tumour (see **Table 3** in **Appendix A1** for a more detailed breakdown of the T staging categories);
    - Node (N) stage – whether any cancer cells have spread and are found in the lymph nodes (N1), or not (N0);
    - Metastasis (M) stage – whether the cancer has spread to distant sites (different parts of the body) e.g. distant organs such as the lungs or liver (M1), or not (M0).
- The histological 'grade' of the tumour i.e. how abnormal the tumour cells appear under a microscope – the more undifferentiated the tumour cells appear, the higher the grade (see **Table 4** in **Appendix A2** for a more detailed breakdown of the grade categories)[4];
- The oestrogen receptor (ER) status of the tumour – the ER is a hormone receptor (protein) found in the breast cells. The ER status of the tumour determines whether the cancer has receptors for oestrogen (ER positive [ER+]), or not (ER negative [ER-])[5,6];
- The progesterone receptor (PR) status of the tumour – like ER, the PR is another hormone receptor (protein) found in the breast cells. The PR status of the tumour determines whether the cancer has receptors for progesterone (PR positive [PR+]), or not (PR negative [PR-])[6];
- The human epidermal growth factor receptor 2 (HER2) status of the tumour – HER2 is one of a number of drivers of breast cancer growth. A tumour which is HER2 positive (HER2+) is where the HER2 gene is amplified (i.e. there are too many copies of the gene) which in turn encourages the breast cells to grow and divide in an uncontrolled manner[6];
- The triple negative (TNT) status of the tumour – this term is used to denote tumours that do not have any of the three tumour receptors: ER, PR and HER2. This is categorised as TNT vs. non-TNT.

1.1.3 Treatment of invasive breast cancer

Treatment of breast cancer varies depending on the breast cancer type, tumour stage, grade, and receptor status. For invasive breast cancer, a combination of chemotherapy, surgery, radiotherapy and/or hormone therapy may be used[2].

Chemotherapy given before surgery (known as neo-adjuvant chemotherapy) may be given to decrease the size of the tumour to reduce the extent of surgery, whilst chemotherapy given after surgery (known as adjuvant chemotherapy) is usually given when there is a risk that some of the cancer cells have spread to other parts of the body[7].

Different types of breast surgery may be used depending on a combination of factors, such as the location and size of the tumour, and the decision of the patient. The two main types of surgery used are:

- breast conserving surgery (BCS), such as a wide local excision or lumpectomy – used to remove the area of the cancer in the breast whilst leaving as much of the healthy breast tissue remaining as possible[7];
- mastectomy – used to remove the entire breast including the breast tissue and tissues that cover the chest muscles[7].

Radiotherapy is usually given after surgery (adjuvant radiotherapy), but patients may also have additional radiotherapy targeted at the tumour bed following BCS (known as a radiotherapy boost) or further radiotherapy to the chest wall following a mastectomy (known as chest wall radiotherapy).

Hormone therapy, also known as hormone treatment or endocrine therapy, is a treatment which blocks the effects of oestrogen and progesterone on breast cancer cells or by blocking the body's ability to produce hormones. It is usually only effective for patients with ER+ tumours, and similar to chemotherapy, it can be given before surgery (neo-adjuvant hormone therapy) or after surgery (adjuvant hormone therapy)[6,7].

1.1.4 Invasive breast cancer in young women (aged ≤40 years)

Approximately 4% of UK invasive breast cancer cases occur in young women (aged ≤40 years), however it remains the most frequent malignancy in this age group, with over 2,000 new cases diagnosed each year in the UK[1]. Breast cancer screening in the UK is usually offered to women aged between 50 and 70 via three-yearly mamograms[8]. In some areas of England, screening is also made available to women from 47 to 73 as part of an ongoing trial[8,9]. However, in general for women

aged under 50, the risk of breast cancer is considered low and mammograms more difficult to read in younger women due to their breast tissue being of higher density. Yearly magnetic resonance imaging (MRI) scans are currently offered to women aged between 30 to 40 who have an increased risk of breast cancer or have a genetic BRCA1 or BRCA2 mutation, and also in women aged from 20 who have a genetic TP53 mutation[8]. Nevertheless, the majority of breast cancers in young women are diagnosed symptomatically.

Young age is associated with poorer survival in women with invasive breast cancer, and a higher risk of experiencing a local relapse (where the cancer re-appears in the same area of the body), or a distant relapse (where the cancer develops in a different area of the body) compared to older patients[10-24]. Younger women with breast cancer also present with an increased incidence of adverse biological features in tumours, such as high grade, ER+, HER2+, and/or N1 stage tumours[17,19,20,22-30]. Across all age groups, evidence suggests that the overall incidence of invasive breast cancer is lower in Black women compared to White/Caucasian women, however in younger women the risk of developing breast cancer is higher in Black women compared to White/Caucasian women[31-35], and evidence also suggests that Black women have a poorer prognosis compared to non-Black patients[35-41]. Although obesity does not appear to increase the risk of developing breast cancer in pre-menopausal women[42,43], it is associated with poorer survival, particularly in pre-menopausal women[44-46].

In terms of treatment decisions of invasive breast cancer in young women, compared to older patients, evidence suggests that younger patients might not benefit as much from hormonal therapy following adjuvant chemotherapy[47-49]. Studies of pre- and post-menopausal women with breast cancer also demonstrate that chemotherapy alone might be insufficient for younger patients, indicating a move towards investigating more tailored or age-based treatments[49-51]. Outcomes according to surgical type (BCS vs. mastectomy) were found to be equivalent across all age groups, including in patients aged <40 years, however this evidence was based on very few young women[14,52,53]. Nevertheless, the use of adjuvant radiotherapy following BCS was shown to have the largest benefit in women under 40 years[54], and a Randomised Controlled Trial (RCT) of pre-menopausal women undergoing mastectomy showed that chest wall radiotherapy significantly improved outcomes[55].

In terms of family history and the genetic make-up of patients, studies show conflicting information with regards to patients with a positive reported family history of breast cancer, with an improved[56-58], impaired[59,60] and unchanged[61-64] survival advantage all found. Research into cancer risk genes has found that the BRCA1 and BRCA2 genes are two examples of genes which increase the risk of

developing breast cancer if the genes become altered[65]. Genetic testing for the BRCA gene provides three possible results:

- a positive result – where a pathogenic BRCA mutation is found;
- a negative result – where either no mutation is detected or a variant of no clinical significance (a polymorphism) is found;
- a variant of unknown significance (VUS) – where part of the gene appears different to the way in which it should appear normally. However, the risk of causing cancer has not yet been determined by researchers[66].

Young patients are more likely to carry a BRCA1 or BRCA2 gene, and evidence of a possible survival advantage for BRCA gene-mutation carriers has also been reported[16,67-69]. However, BRCA genetic testing has only recently become more routine practice, emphasising the need for further research into whether a positive reported family history of breast cancer as well as BRCA status affects outcome in young women.

The exact reasons for these differences remain unclear. Young women have more dense breasts, with a higher ratio of glandular tissue to fat, whereas after the menopause the breasts are less dense as the glandular tissue is gradually replaced by fat[9], however evidence that age is an independent factor for poor prognosis remains limited[19,22,70]. A number of currently available prognostic tools have been developed to encapsulate some these factors in order to more accurately assess the survival of young women, and in turn help to determine the long-term benefit of treatments; these include the Adjuvant! Online[71] and PREDICT[70]. However, the number of young patients that were included in both the development and validation of these tools was limited[72], and there remained a further need for more in-depth research into this area to help inform both patients and the clinical teams treating these patients.

1.2 Aim of thesis

To study factors affecting outcomes for young women with invasive breast cancer to provide additional data for clinicians, and their patients, to weigh up the optimum approach to reducing the risk of death in newly diagnosed young breast cancer patients.

This is a highly important research area; whilst there is a large body of evidence on factors affecting outcomes for breast cancer patients, only a very small number of these patients included in the research were aged 40 years or younger at their date of diagnosis. It was therefore of key interest to determine whether the effects observed in the general breast cancer population also reflect those in young women with invasive breast cancer in particular.

1.3 Primary research questions

The specific primary research questions for each of the published papers (referred to in this thesis as '**Paper 1**' through to '**Paper 7**') are as follows:

- **Paper 1** – To describe the clinical presenting characteristics, including the pathology and treatment, of young woman with invasive breast cancer, and to compare survival outcome according to ER status;
- **Paper 2** – To describe the pathology and treatment of young women with invasive breast cancer according to their ethnic origin, and to compare survival outcome according to ethnic origin;
- **Paper 3** – To explore the associations of obesity (using body mass index [BMI]) with tumour pathology, treatment, and survival outcome in young women with invasive breast cancer;
- **Paper 4** – To compare tumour pathology and survival outcome in young women with invasive breast cancer according to reported family history of breast cancer;
- **Paper 5** – To investigate the effect of a germline BRCA1 or BRCA2 status on survival outcome in young breast cancer patients;
- **Paper 6** - To assess clinical and surgical factors affecting local recurrence and survival outcome in young breast cancer patients;
- **Paper 7** – To establish how well the prognostic tool PREDICT performs in estimating survival outcome in a large cohort of young women with invasive breast cancer.

1.4 Structure of thesis

This thesis combines the work of seven key papers with the purpose of providing insight into the many putative factors found to affect young women and their prognosis following invasive breast cancer diagnosis. Each of the seven papers included in this thesis focuses on specific factors and a specific research area, and this work brings these together with a cohesive message about identified risk factors and treatment options, and their impact on various outcomes in this patient group. Each of these studies have made a significant contribution to learning in the field, utilising data from a large prospective observational study, thereby providing a high level of evidence from which clinical practice and patient outcomes can be improved. For each paper, I was the lead statistician and conducted all analyses; full details of my contributions for are outlined in the relevant chapters.

Chapter 2 of this thesis provides a detailed description of the study population used for the analyses in the papers, together with a breakdown of the data selection criteria used. In order to help guide through complexities of both the nature of the data and the appropriate methodology required to

answer the objectives of each paper, this chapter also provides detailed descriptions of the study endpoints, along with a comprehensive breakdown of the various statistical methods and analyses undertaken.

Chapters 3 to 9 outline the seven published papers, with each of the papers including any supplementary material presented as published in each journal, along with a detailed breakdown of my author contributions for each paper. Specifically, **Chapters 3 to 7** (**Papers 1** to **5**) look at possible effects of clinical, pathological, patient and genetic characteristic factors on survival. **Chapter 8** (**Paper 6**) describes the possible effect of surgical type on outcome, in terms of overall mortality as well the chance of experiencing a local or distant relapse. **Chapter 9** (**Paper 7**) provides an evaluation of the prognostic tool, PREDICT, using a large cohort of young women with invasive breast cancer, to describe how well the existing prognostic tool works in the young breast cancer population.

Finally, **Chapter 10** provides a summary of the findings from the seven papers, together with a critical appraisal of the published work, establishing the contribution of the current findings to this important area of research, as well as suggestions for future research.

Chapter 2: Study Population and Methods

2.1 Study Population

The study population used for the analyses in **Papers 1** through **7** in this thesis is the Prospective Study of Outcomes in Sporadic and Hereditary Breast Cancer (POSH), which received approval from the South West Multicentre Research Ethics Committee (MREC 00/6/69). POSH is a multicentre prospective observational cohort study of young women diagnosed with invasive breast cancer from the UK, designed to assess whether the prognosis of patients with breast cancer is influenced by inherited genotype and clinical and pathological factors. Approximately 3000 women were recruited between 2000 and 2008 from 127 hospitals across the UK, and were considered eligible if diagnosed with invasive breast cancer between 1 January 2000 and 31 January 2008 at an age of 40 years or younger. An additional subgroup of approximately 40 women aged 41-50 years diagnosed with invasive breast cancer in the study period were recruited if they had a known BRCA1 or BRCA2 gene mutation[73]. However, these women were excluded from all analyses described in this thesis (as outlined in **Table 1** below).

Recruited patients were treated according to local protocols and written informed consent was obtained for all patients. Patient medical records were used to collect clinical data including information on personal characteristics, tumour pathology, disease stage, diagnosis and treatment, risk reducing surgery, new primary tumours, recurrence and survival. Patients completed questionnaires on their family history of breast and ovarian cancer. Annual follow up was used to capture information on recurrence and survival until death or loss to follow up[73].

The POSH cohort is a unique and valuable resource as it represents one of the largest prospective studies of young breast cancer patients to date. The design of the study also had numerous advantages over retrospective studies, including, but not limited to: minimising ascertainment bias, in which the results are systematically distorted by knowledge of what treatment each patient is receiving; and enabling for more accurate and standardised data collection to be carried out[73]. For example, a key limitation of retrospective studies is the amount and nature of incomplete information, such as tumour pathology information and good quality DNA, required to clearly define the genetic status of patients.

2.2 Data and selection criteria

For each of the seven papers included in this thesis, data were obtained from the POSH study population. However, as each paper posed a unique question and the analyses were performed at

different times, various selection criteria were imposed according to a pre-specified statistical analysis plan (see **Appendix A3**) and the number of patients available for the analysis population slightly varied.

Table 1 below provides a breakdown of the key selection criteria used in the analyses of each paper, together with the number of patients included in the analysis populations, and the amount of follow-up information available at the time of analyses:

Table 1 Selection criteria and analysis population breakdown by paper

Paper	Key inclusion criteria of analysis population	Key exclusion criteria of analysis population	Number of patients included in the analysis population	Date of last follow-up data download
1	Patients aged 40 years or younger at diagnosis	• Gene carriers aged 41-50 years • Missing primary tumour data information • Evidence of invasive cancer unavailable for pathology	n=2956	11 April 2012
2	Patients aged 40 years or younger at diagnosis	• Gene carriers aged 41-50 years • Missing primary tumour data information • Evidence of invasive cancer unavailable for pathology	n=2956	11 April 2012
3	Patients aged 40 years or younger at diagnosis	• Gene carriers aged 41-50 years • Missing primary tumour data information • Evidence of invasive cancer unavailable for pathology • Missing BMI information	n=2843	22 October 2013
4	Patients aged 40 years or younger at diagnosis	• Gene carriers aged 41-50 years • Missing primary tumour data information • Evidence of invasive cancer unavailable for pathology	n=2956	22 October 2013
5	Patients aged 40 years or younger at diagnosis without a TP53 gene mutation	• Gene carriers aged 41-50 years • No follow-up information available • Metastatic (M1 stage) at diagnosis • Not genetically tested • TP53 gene carriers	n=2733	26 July 2016
6	Patients aged 40 years or younger at diagnosis	• Gene carriers aged 41-50 years • Missing primary tumour data information • Evidence of invasive cancer unavailable for pathology • Metastatic (M1 stage) at diagnosis	n=2882	26 June 2015
7	Patients aged 40 years or younger at diagnosis	• Gene carriers aged 41-50 years • Missing primary tumour data information • Evidence of invasive cancer unavailable for pathology • Metastatic (M1 stage) at diagnosis Key prognostic information required for the PREDICT tool unavailable (including lack of follow-up information available)	n=2827	22 October 2013

2.3 Study Endpoints

Due to the unique nature of the research question of each paper, a number of study endpoints were used to assess and describe the invasive breast cancer over time. A description of each endpoint is provided below, together with a list of which papers included each endpoint in the analyses.

2.3.1 Overall Survival (OS)

Defined as time from the date of invasive breast cancer diagnosis to the date of death from any cause. Patients who had not died or were lost to follow-up at the time of analyses were censored at their date of last follow-up. **Incorporated in all papers.**

2.3.2 Breast Cancer Specific Survival (BCSS)

Defined as time from the date of invasive breast cancer diagnosis to the date of death from breast cancer only. Patients who died from non-breast cancer deaths were censored at the date of death. Patients who had not died or were lost to follow-up at the time of analyses were censored at their date of last follow-up. **Incorporated in Paper 7 only.**

2.3.3 Distant Disease Free Survival (DDFS)

Defined as time from the date of invasive breast cancer diagnosis to the date of distant relapse or death from any cause (whichever event occurred first), where distant relapse was defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, central nervous system and bone metastases. Patients who had not died, had not experienced a distant relapse, or were lost to follow-up at the time of analyses were censored at their date of last follow-up. **Incorporated in Paper 5 only.**

2.3.4 Distant Disease Free Interval (DDFI)

Defined as time from the date of invasive breast cancer diagnosis to the date of distant relapse or death from breast cancer (whichever event occurred first), where distant relapse was defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, central nervous system and bone metastases. Patients who died from non-breast cancer deaths were censored at the date of death. Patients who had not died, had not experienced a distant relapse, or were lost to follow-up at the time of analyses were censored at their date of last follow-up. **Incorporated in Papers 1, 2, 3, 4 & 6.** For **Paper 2**, the term 'Distant Relapse-Free Survival' (DRFS) was used to describe DDFI i.e. with the same definition as DDFI. However, for the purposes of this thesis, the term 'DDFI' has been used to ensure consistency throughout.

2.3.5 Post Distant Relapse Survival (PDRS)

Defined as time from the date of distant relapse to death from any cause, where distant relapse was defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, central nervous system and bone metastases. Patients who had not experienced a distant relapse were excluded from the analyses. Patients who had not died or were lost to follow-up at the time of analyses were censored at their date of last follow-up. **Incorporated in Paper 5 only.**

2.3.6 Local-Recurrence Interval (LRI)

Defined as time from the date of invasive breast cancer diagnosis to the date of local relapse, where local relapse was defined as either an ipsilateral recurrence or ipsilateral new primary (whichever event occurred first following BCS), or chest-wall recurrence following mastectomy. The local-recurrence event was counted as an event if, where applicable, the date of any 'non-event' (death from breast cancer, distant relapse, ipsilateral local axillary recurrence, ipsilateral regional nodes recurrence, and/or contralateral recurrence) was more than three months after the date of the local-recurrence event. If, however, the date of the 'non-event' was within three months of the local-recurrence event then the patient was censored at the date of the 'non-event' (as the 'non-event' would then be considered the overarching event superseding the local-recurrence event). Deaths from other causes after local recurrence did not affect the event. Patients who had not experienced a local-recurrence event, had not experienced a 'non-event', or were lost to follow-up at the time of analysis were censored at their date of last follow-up. **Incorporated in Paper 6 only.**

2.4 Statistical Methods

All analyses were conducted according to pre-specified statistical analysis plans (SAPs) (see **Appendix A3**), and carried out using STATA version 11.2 or later[74-77] (see **Table 2** in **Section 2.5** for further details).

Due to the nature of the data and complexities associated with the research questions of the papers, comprehensive descriptions of the various statistical methods used are provided below.

2.4.1 Summary statistics

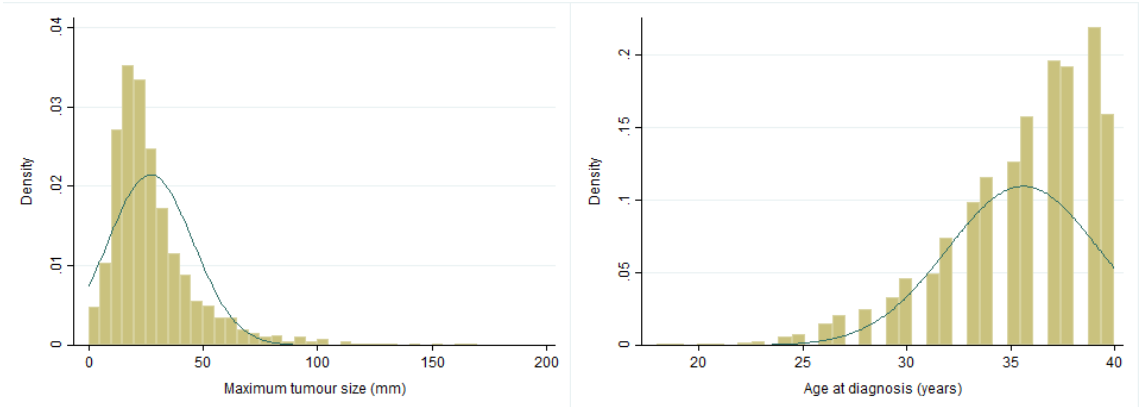
Summary statistics were computed in the majority of the papers. For example, when comparing a binary/categorical variable across two or more groups to ascertain whether the distribution of the binary/categorical variable differs significantly across groups, the number and proportion of patients in each category can be calculated for each group and then formally compared using a Pearson Chi-squared (χ^2) test[78,79]. Using a 5% level of significance, a p-value of less than 0.05

indicates that the distribution of the binary/categorical variable differs significantly across groups, whilst a p-value of 0.05 or more indicates that the distribution is not significantly different across groups. This method is not restricted to a maximum number of categories being compared, nor by a maximum number of groups being compared. However, the test must only be used to compare binary/categorical variables (e.g. BRCA status, ethnicity, grade, etc.), as opposed to continuous variables (e.g. tumour size, age at diagnosis, height, etc.). In addition, the test must not be used when the expected number in any of the categories is less than five; a particular issue when analysing small samples[79], but not for these analyses.

When comparing a continuous variable across two groups, the median of the variable can be calculated for each group and formally compared using a Mann-Whitney U test[80], or when comparing across more than two groups, the extended Kruskal-Wallis test[81]. Similar to the Pearson χ^2 test in interpretation, a p-value of less than 0.05 indicates that the distribution of the continuous variable differs significantly across groups, whilst a p-value of 0.05 or more indicates that the distribution is not significantly different across groups. A key advantage of both the Mann-Whitney U and Kruskal-Wallis tests is that they are both non-parametric tests i.e. they do not assume that the continuous data being analysed follows the Normal distribution, for which the use of a mean and subsequent parametric test such as a Student t-test, etc. would be appropriate to use. This is a particularly important factor when analysing data such as maximum invasive tumour size or age at diagnosis from the POSH cohort, which are highly skewed and do not follow a 'bell-shaped' curve observed in a Normally distributed variable i.e. they do not follow a Normal distribution (see **Figure 2a** and **2b** below). In these cases, the use of a mean and subsequent parametric test would be inappropriate.

Figure 2 Histogram of a) maximum invasive tumour size, and b) age at diagnosis[†]

a) **b)**



[†] Data taken from the POSH cohort

For the analyses implemented in this thesis, the number and proportion of patients by clinical, pathological and treatment information categories (binary/categorical variables only) were calculated and formally compared using Pearson χ^2 tests for each of the comparator groups listed below, with tests carried out on patients with complete data. The median, range, inter-quartile range (IQR) of clinical, pathological, and treatment information (continuous variables only) were then calculated and formally compared using Mann-Whitney U tests (or Kruskal-Wallis tests where specified) for each of the comparator groups listed below, with tests carried out on patients with complete data.

- ER- vs. ER+ patients in **Paper 1**;
- Individual ethnic groups (White/Caucasian vs. Black patients, White/Caucasian vs. Asian patients, and Asian vs. Black patients) in **Paper 2**;
- Individual BMI categories (Underweight/Healthy vs. Overweight patients, Underweight/Healthy vs. Obese patients, and Overweight vs. Obese patients) in **Paper 3**[‡];
- Patients with a negative reported family history (FH-) vs. patients with a positive reported family history (FH+), and patients with at least one first degree relative (FDR) vs. patients with at least one second degree relative (SDR) in **Paper 4**;
- Patients without a BRCA1 and/or 2 gene mutation (BRCA-) vs. patients with a BRCA1 and/or 2 mutation (BRCA+), patients without a BRCA1 gene mutation (BRCA1-) vs. patients with a BRCA1 mutation (BRCA1+), and patients without a BRCA2 gene mutation (BRCA2-) vs. patients with a BRCA2 mutation (BRCA2+) in **Paper 5**;
- Patients undergoing a mastectomy vs. patients undergoing BCS in **Paper 6**.

2.4.2 Kaplan-Meier estimates

A common method used to describe survival of patients over time, or more generally, the proportion of patients event-free over time, is calculating Kaplan-Meier estimates[82]. These estimates are obtained by computing the probabilities of an event occurring at a certain time-point and multiplying these successive probabilities by earlier computed probabilities[83]. At each time-point, the number of patients known to be event-free and at risk (referred to simply as the 'number at risk') is re-calculated, and is used as the denominator to calculate the probabilities. These estimates can also be graphically displayed by plotting them over time in a Kaplan-Meier plot.

[‡] A closed testing approach was used in **Paper 3** whereby binary/categorical and continuous variables were first compared across BMI categories overall (Underweight/Healthy vs. Overweight vs. Obese patients) using Pearson χ^2 tests and Kruskal-Wallis tests. If a significant difference was found when comparing BMI categories overall, further tests comparing individual BMI categories were then carried out using Pearson χ^2 tests and Mann-Whitney U tests.

For example, when assessing Overall Survival (OS) in the POSH cohort, time is measured from the date of diagnosis to death from any cause and the proportion of patients still alive over a set period of time (e.g. at five or ten years) is of key interest in this case. The main benefit of using Kaplan-Meier estimates over alternative methods is that it is still able to utilise censored data; in which the event-free time for a patient cannot be accurately established[84]. For OS in the POSH cohort, if a patient is known to be still alive at their last follow-up visit, the Kaplan-Meier estimates are able to use the time interval up to this date but then censor the patient so that the they are not included in the number at risk in future time points.

For **Papers 1** through **5**, Kaplan-Meier estimates and corresponding plots were used to describe all study endpoints overall and by comparator groups. For **Paper 6**, only OS and DDFI were described using Kaplan-Meier estimates.

2.4.3 Nelson-Aalen estimates for cause-specific hazards

Kaplan-Meier estimates are not always appropriate for describing all types of survival or time-to-event data[85]. In some instances, competing risks can be problematic and should be considered with due care. A competing risk is an event that can either prevent or modify the chances of the event of interest from occurring[86]. For example, for LRI in the POSH cohort, the event of interest was a local recurrence. However, if a patient died before a local recurrence it would have been impossible to establish whether a local recurrence would have occurred if the patient had not died. Similarly, if a patient experienced a distant recurrence before a local recurrence, the distant recurrence event then superseded an otherwise possible local recurrence event.

In the presence of completing risks, calculating the cause-specific Nelson-Aalen estimates and producing a corresponding cause-specific Nelson-Aalen cumulative hazard plot provides an alternative method to describe time-to-event data[85,87,88]. The cause-specific Nelson-Aalen estimates describe the risk of experiencing an event over a set period of time by using the number of events and number at risk at set time-points to estimate the cumulative hazard rate function from the time-to-event data, censoring individuals failing from competing causes. For example, for OS in the POSH cohort, the hazard function is the probability that a person who has previously survived to a set time-point, dies in the next time period, and a Nelson-Aalen cumulative hazard plot would describe the risk of patients dying from any cause over time. Similarly, for LRI in the POSH cohort, the cause-specific Nelson-Aalen cumulative hazard plot describes the risk of experiencing a local recurrence event over time.

For **Paper 6**, cause-specific Nelson-Aalen estimates and corresponding plots were used to describe LRI overall and by comparator groups.

2.4.4 Cox proportional hazards regression models

A frequently used method to assess the effect of a variable (or several variables) on the time to the event of interest is to produce a Cox proportional hazards regression model, or Cox regression model[89].

To understand how the Cox regression model works, using a covariate (a variable fitted in a model) such as BRCA status, fit this in the model to assess the OS of BRCA+ vs. BRCA- patients. The Cox regression model compares the hazard rate over time of BRCA+ patients and compares this to the hazard rate of BRCA- patients using a 'hazard ratio' (HR), a measure of the relative risk of the event (death in this instance) occurring in a set time period. Using the BRCA- patients as a reference category:

- a HR of less than 1 would indicate that BRCA+ patients have a lower risk of dying compared to BRCA- patients;
- a HR of greater than 1 would indicate that BRCA+ patients have a higher risk of dying compared to BRCA- patients;
- a HR of 1 would indicate that BRCA+ patients have the same risk of dying compared to BRCA- patients.

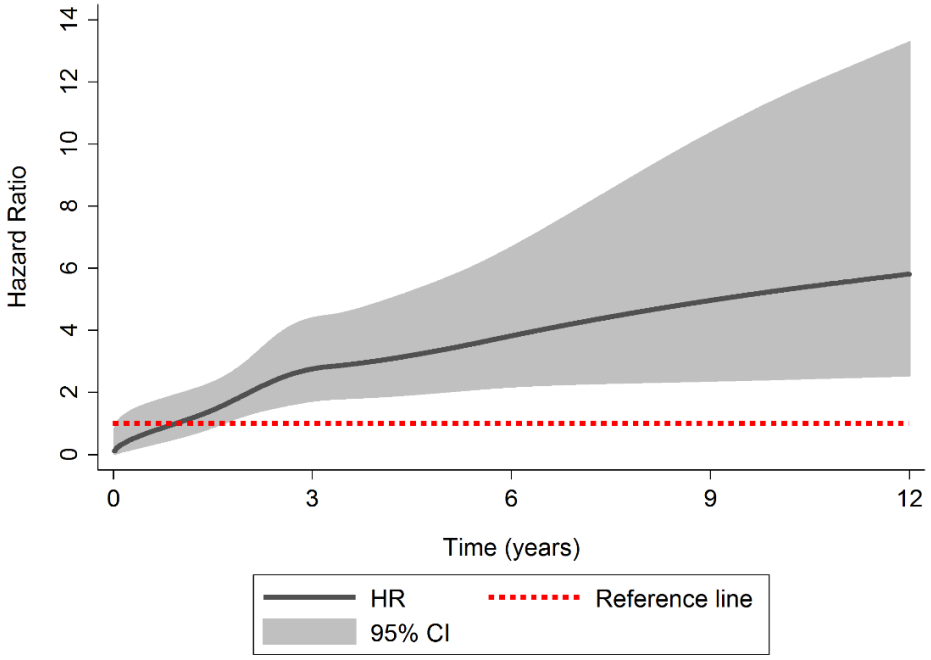
An associated 95% Confidence Interval (CI) and p-value can be calculated to help determine the accuracy of the estimated HR. For example, a HR (with 95% CI, and p-value) of 2 (1.5 to 2.5, $p<0.05$) would indicate a statistically significantly greater risk of dying i.e. there is a 95% chance that the true HR in the population would fall within the range of 1.5 to 2.5, and so the HR result calculated is considered to be statistically significantly greater. Similarly, a HR (95% CI, p-value) of 2 (0.5 to 3.5, $p\geq0.05$) would indicate a greater, but not statistically significantly greater, risk of dying i.e. there is a 95% chance that the true HR lies between 0.5 to 3.5, and so the HR could in fact be less than 1, and therefore not considered to be statistically significantly greater.

Various adjustments to the Cox regression model can be made to account for other factors (called covariates when incorporated into a regression model). For example, for OS in the POSH cohort, if the comparator of interest was BRCA status but an important factor to take into account was age at diagnosis i.e. if it was expected that the effect of BRCA status would vary by age, both an 'unadjusted' and 'adjusted' model would be fitted. An unadjusted model is a type of univariable analysis (UVA), whilst an adjusted model is a type of multivariable analysis (MVA). The unadjusted model in this case would simply fit BRCA in the model, whilst the adjusted model would fit both BRCA and age at diagnosis in the model in order to make an important adjustment for the potential confounding factor. There is no limit to the number of covariates allowed to be included in MVA

but the more factors added, the smaller the number in each possible group, and the larger the inaccuracy of any estimates produced. In some cases, the covariate(s) should be removed if the numbers are too small. However, a delicate balance is required as it is important that any possible confounding factors be pre-specified and included (where possible) in any MVA, as was the case in all MVA for the papers included in this thesis.

A key assumption of the Cox regression model is that the hazard rates of the comparator covariate categories are proportional over time. For example, for OS in the POSH cohort, if a HR of 2 was obtained from a Cox regression model for BRCA+ vs. BRCA- patients, this would indicate that at any time-point, the risk of death for BRCA+ was twice that of BRCA- patients. However, in a number of cases, this assumption is not met i.e. the effect of the factor varies over time (illustrated in **Figure 3**, below). In this instance, a Cox regression model would not be appropriate to use, and an alternative method would be required.

Figure 3 Hazard ratio plotted over time - example of non-proportional hazards[‡]



A simple solution to non-proportional hazards is to fit a Cox regression model stratified (split) by the factor, otherwise known as a stratified Cox regression model. However, whilst this method is statistically appropriate, a key drawback of this type of regression model is that a HR would not be produced for the factor being stratified in the model. Thus, for cases where the factor is not of primary interest, a stratified Cox regression model presents a straightforward solution to the

[‡] Excerpt taken from **Figure 2B** from **Paper 6**, with reference line at 1.

problem of 'time-varying effects'. Whilst for cases where the factor is of primary interest, an alternative modelling technique is required (see **Section 2.4.5** below).

Papers 2 through **6** all incorporated Cox regression models for the UVA, and **Papers 2**, **3** and **6** used stratified Cox regression models for the MVA, including a selection of sensitivity MVA incorporated in **Paper 6** (to adjust for the amount of missing data – see **Section 2.4.6** for further details).

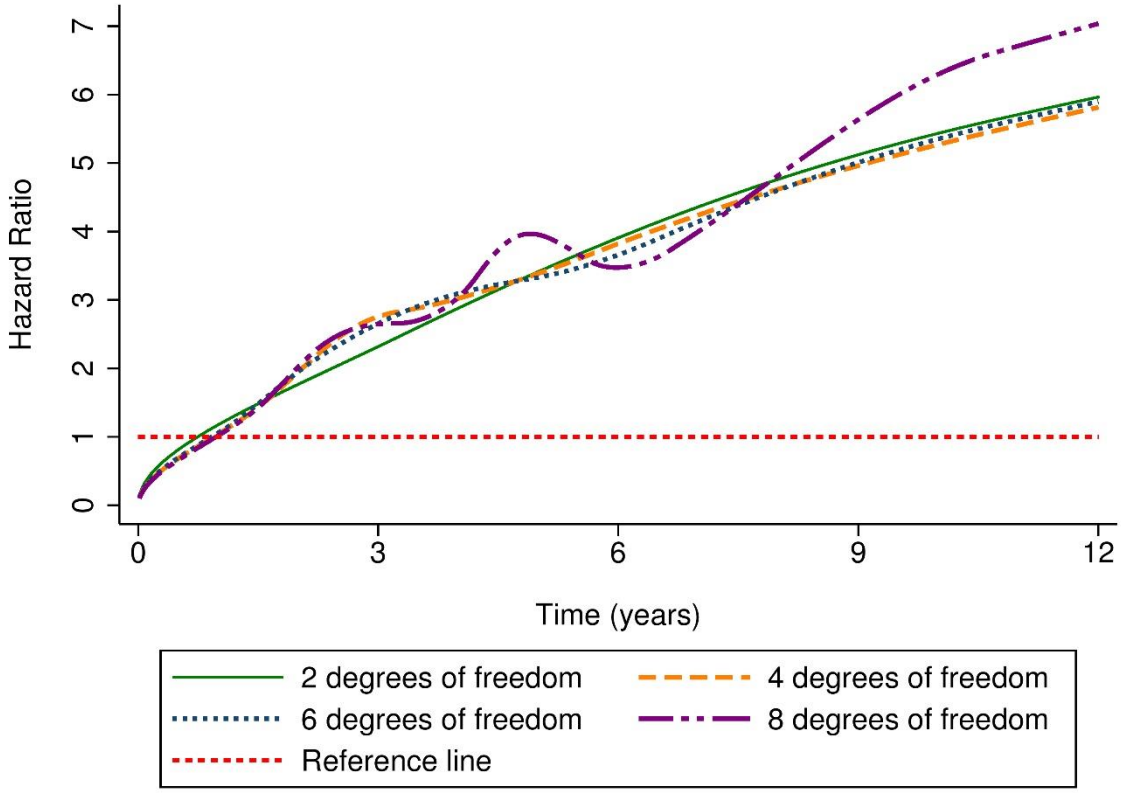
2.4.5 Flexible parametric survival models

In the case of time-varying effects, and where it is of interest to provide the HR of the time-varying factor, a flexible parametric survival model (FPSM) can be fitted[90,91]. This model uses 'restricted cubic spline functions' to model the baseline cumulative hazard, and can be used to model both proportional hazards as well as complex time-dependent effects. Restricted cubic splines are piecewise polynomial functions which ensure a smooth overall curve by forcing the fitted function to be linear at set positions, called 'knots'[76]; thus enabling a 'regression line' to provide appropriate estimates. The number of knots can be directly specified in the creation of the model by denoting the number of 'degrees of freedom' required (further details described below).

Key benefits of the FPSM are the ability to provide both HR estimates (with 95% CIs) at set time-points, and to produce clear graphical displays of the hazard rates and hazard ratios over time (see **Figure 3**, above).

In order to fit a FPSM, the model first requires some calibration, including specification of the degrees of freedom for the baseline hazard function as well as for any time-dependent effects. For example, specifying two degrees of freedom for the baseline hazard function will provide one internal knot at the 50th centile of the baseline distribution function, specifying three degrees of freedom would provide two internal knots at the 33rd and 67th centile, specifying four degrees of freedom would provide three internal knots at the 25th, 50th and 75th centile, etc. The same applies to the degrees of freedom for the time-dependent effects. However, a delicate balance is required when specifying the degrees of freedom; the higher the number, the more fitted the model becomes, but if the model is fitted too well it becomes 'over-fitted' i.e. too specified and the estimates become less transferrable (see the '8 degrees of freedom' line in **Figure 4**, overleaf).

Figure 4 Hazard ratio plotted over time - example of varying degrees of freedom[§]



A method used to establish the optimum degrees of freedom is the Akaike Information Criterion (AIC)[92]. The AIC is an index calculated using the likelihood of the fitted model; the lower the AIC, the better the model fit. In the example shown in **Figure 4** above, the lowest AIC was found when using four degrees of freedom in the FPSM. A further check to establish the suitability of the fitted model is by overlaying the hazard rates produced from the FPSM onto the 'standard' smoothed hazard rates, which are calculated as a weighted kernel-density estimate using the estimated hazard contributions[93]. Provided that the two curves do not deviate dramatically from one another, the suitably of the FPSM can be verified.

Papers 1, **5** and **6** all incorporated FPSMs for the UVA, and **Papers 1**, **4**, **5** and **6** used FPSMs for the MVA, including a selection of sensitivity MVA incorporated in **Paper 6** (to adjust for the amount of missing data – see **Section 2.4.6** for further details).

All FPSMs were created using the 'stpm2' command in STATA[91].

[§] Modelled using data taken from the POSH cohort.

2.4.6 Multiple-imputation

In clinical research, missing data are a common and often unavoidable issue that can be problematic and undermine the validity of results, as well as reducing the power and precision of estimates produced[94]. An example of the amount of missing data observed in the POSH cohort and its impact on MVA was demonstrated in **Paper 6**, when a MVA Cox regression model was fitted to establish the effect of surgical type on OS. The model was adjusted for age at diagnosis, tumour invasive size, tumour (overall) size, focality, N stage, grade, ER status, HER2 status, adjuvant radiotherapy, and hormonal therapy. In addition, patients undergoing neo-adjuvant chemotherapy were excluded from this analysis leaving a possible 2,429 eligible patients to be analysed in the model. However, 806 (33%) patients had at least one missing value in one or more of the fitted covariates which left just 1623 (67%) patients who could be analysed in the 'complete-case' analysis (where the analysis is not adjusted for missing data).

Multiple-imputation is an efficient and robust method that can be used to handle missing data in MVA[94] e.g. for the example outlined above, multiple-imputation would enable all 2,429 eligible patients to be included in the analysis. The multiple-imputation process for MVA in survival analyses can be described as follows[94]:

- First, creating several different plausible imputed datasets, with missing values replaced with values predicted based on the observed data i.e. by incorporating all of the covariates included in the MVA, in addition to the censored indicator (whether a patient experiences an event or not), and the cumulative baseline hazard (which can be created using the Nelson-Aalen estimate of the cumulative hazard function)[94,95];
- Second, fitting the appropriate statistical models (e.g. Cox regression model, FPSM, etc.) to each of the imputed datasets, which produces a series of different estimates;
- Finally, combining the different estimates to provide an overall estimate (result) by averaging them as recommended by Rubin's rules[96], which takes into account the variability of the different estimates from each of the imputed datasets.

The number of imputed datasets to be created (number of imputations to run) can be chosen using a simple rule of thumb which states that the number of imputations should be at least equal to the percentage of incomplete cases[97] i.e. for the example above where 33% of patients have incomplete data, the number of imputations would be rounded up to 40.

Key assumptions are often required when carrying out multiple-imputation, namely that the data are assumed to be missing is random. To explain this in more detail, reasons for missingness are placed into three key categories[98]:

- MCAR (Missing Completely At Random) – The missing data does not depend on the unobserved or observed data i.e. the data are missing at random and for no particular reason;
- MAR (Missing At Random) – The missing data does not depend on the unobserved data but may depend on the observed data. For example, if modelling blood-pressure as a function of gender, and some values of blood-pressure are missing because males are less likely to disclose their blood-pressure, this would be considered MAR. MCAR could be considered as a particular case of MAR;
- MNAR (Missing Not At Random) – Missing data depends on the unobserved data. For example, if patients are prematurely withdrawn from the study due to high blood-pressure, the missing blood-pressure measurements would not be MAR. The measurements of patients with high blood-pressure would be missing and the reason for patient withdrawal would depend on the missing blood-pressure values.

In terms of multiple-imputation for MVA in survival analyses, the missing data must be MAR (or MCAR), and the censoring must also be non-informative i.e. the time to censoring distribution and time to event distribution are independent (MAR)[99].

In the development of the SAP of each paper, the amount of missing data and reasons for missingness were assessed for all variables. All missing data were assumed to be either MAR or MCAR, and censoring was assumed to be non-informative.

Paper 4 incorporated multiple-imputation for the MVA using the 'ice' command in STATA[100-102] in conjunction with FPSMs, whilst **Paper 6** used the 'ice' command to carry out a selection of sensitivity MVA in conjunction with both FPSMs and stratified Cox regression models.

In **Paper 5**, the updated 'mi' command in STATA was used for analyses[98]. In addition, 'conditional multiple-imputation', where only selected missing data are imputed, was implemented using the 'mi' command; carried out as a sensitivity analysis in **Paper 5** in order to establish whether missing HER2 data affected any of the MVA estimates. Missing HER2 data were investigated in this manner due to the quantity of missing data as well as the way in which HER2 data were collected in the POSH cohort. HER2 testing was only widely introduced in UK hospitals after 2006; prior to this, testing was more likely to have been carried out in patients who had progressed. Therefore, patients for whom HER2 status was already known were more likely to have had a worse prognosis. Hence, comparing patients selected on the basis of HER2 testing with patients who may or may not have been HER2 tested would be biased as the patients who have been HER2 tested could appear worse by comparison. Conditional multiple-imputation was therefore incorporated using the following approach (illustrative example shown in **Figure 5** below):

i) First, starting with the initial data (see **Figure 5i** - with patients diagnosed prior to 2006 shown in green);

ii) Second, removing any existing HER2 values recorded for patients diagnosed prior to 2006 (see **Figure 5ii** - with removed values shown in green);

iii) Third, running the multiple-imputation model on all patients to create the imputed datasets (see **Figure 5iii** – showing *one* of the example imputed datasets, with values imputed shown in green);

iv) Finally, where available replacing any of the newly imputed HER2 values for patients diagnosed prior to 2006 with the actual values recorded (see **Figure 5iv** – showing *one* of the example imputed datasets, with previously recorded values replaced shown in green).

Figure 5 Illustrative example of conditional multiple-imputation on HER2 data

i) *Initial data*

Patient number	Year of diagnosis	HER2 value
1001	2004	Missing
1002	2005	Negative
1003	2005	Positive
1004	2006	Positive
1005	2007	Missing
1006	2007	Positive
…etc…		

→ ii) *Removal*

Patient number	Year of diagnosis	HER2 value
1001	2004	Missing
1002	2005	Missing
1003	2005	Missing
1004	2006	Positive
1005	2007	Missing
1006	2007	Positive
…etc…		

→ iii) *Imputation*

Patient number	Year of diagnosis	HER2 value
1001	2004	Positive
1002	2005	Negative
1003	2005	Negative
1004	2006	Positive
1005	2007	Negative
1006	2007	Positive
…etc…		

→ iv) *Replacement*

Patient number	Year of diagnosis	HER2 value
1001	2004	Positive
1002	2005	Negative
1003	2005	Positive
1004	2006	Positive
1005	2007	Negative
1006	2007	Positive
…etc…		

2.4.7 Left-truncation

Left-truncation[99], which could lead to possible survival bias in the case of the POSH cohort study, occurs when there is a large time difference between the date of diagnosis and the date of recruitment into the study; patients who died before reaching the date of recruitment might otherwise have joined the study if the date of recruitment was equal to the date of diagnosis. As a result, the study sample could reflect a group of patients with a better prognosis compared to the population.

To determine the extent of this possible bias in the POSH study, a sensitivity analysis was carried out for **Paper 5**, whereby an adjustment in the MVA of the primary comparison comparing OS in BRCA+ patients with BRCA- patients was made, with an additional adjustment of time from date of diagnosis to date of blood draw (date of recruitment to the study). This was carried out in Stata using the 'enter' and 'origin' options in the 'stset' survival set up command, with date of diagnosis specified as the 'origin' date, and date of blood draw specified as the 'enter' date accordingly.

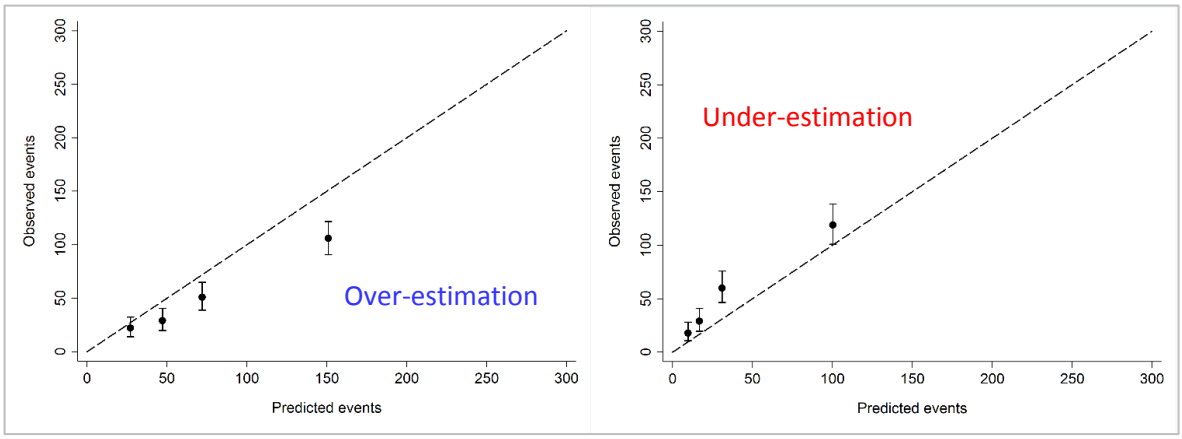
2.4.8 Model Calibration

Validation of a prognostic tool or model using a validation dataset can be assessed in a number of ways. A commonly used approach to evaluate a survival prognostic model is through 'model calibration'; a method which compares the predicted mortality estimates from the model with the observed mortality at set timepoints[70,103,104]. The predicted and observed deaths are then compared using goodness-of-fit Pearson χ^2 tests[70,103-105]. The calibration can be performed in this manner on: (i) the complete dataset; (ii) within strata of the prognostic and other specified variables; and (iii) within specified quantiles of the predicted mortality e.g. quartiles, quintiles, deciles, etc. of the predicted mortality. This enables an evaluation of how well the tool performs: (i) overall; (ii) within each of the specified variable categories; and (iii) over a specified distribution of the predicted mortality, respectively.

Model calibration can also be graphically illustrated by way of 'calibration plots'; created by plotting the observed outcomes (with 95% CIs) against the predicted outcomes, and adding a diagonal reference line to enable a visual assessment of the predicted estimates produced from the model. Values falling below the reference line represent an overestimation in the predicted outcomes, whilst values above the line represent an underestimation (see example in **Figure 6** below).

Figure 6 Example of model calibration plots[**]

a) **b)**



For **Paper 7**, model calibration was performed on all patients at five, eight and ten years overall, within categories for a number prognostic variables, and within quartiles of the predicted risk. Calibration plots by quartiles of the predicted risk were then produced for the five, eight and ten year comparisons, split by ER and/or HER2 status.

** Excerpt taken from a) **Figure 1B** and b) **Figure 1D** from **Paper 7**.

2.4.9 Model Discrimination (ROC analyses)

Model discrimination is another frequently used method to evaluate survival prognostic models, which can be assessed by calculating the 'area under the receiver-operator-characteristic [ROC] curve' (AUC). For prognostic survival models, the ROC curve is a plot of sensitivity against 1-specificity, where sensitivity represents the proportion of patients who were accurately predicted to have died, and specificity represents the proportion of patients who were accurately predicted to have survived. The AUC is a measure of how well the model identifies patients with a worse survival. Specifically, the AUC is the probability that the predicted mortality of a randomly selected patient who died is higher than that of a randomly selected patient who survived[106]. The higher the AUC, the more accurate the model is at identifying patients with a worse survival.

For **Paper 7**, ROC curves and corresponding AUCs were produced, split by ER and HER2 status at five, eight and ten years.

2.5 Summary

Table 2 below, provides a summary of the analysis population and methods used in each paper. The table includes the key comparator groups evaluated in the paper, the study endpoints assessed, the statistical methods implemented, the number of patients included in the primary analysis population, UVA and MVA, and the version of STATA software used for the analyses.

Table 2 Summary of analysis population, endpoints and methods used by paper

Paper	Key comparator groups	Study endpoints	Statistical methods implemented *(including sensitivity analyses)*	Number of patients included in: a) analysis population; b) UVA†; c) MVA‡	Version of STATA used
1	ER- vs. ER+	OS, DDFI	• Summary statistics (Pearson χ^2 tests and Mann-Whitney U tests) • Kaplan-Meier estimates • UVA & MVA - FPSMs (Complete-case)	a) n=2956 b) n=2944 c) n=2701	v11.2[74]
2	White/Caucasian vs. Black vs. Asian	OS, DDFI¥	• Summary statistics (Pearson χ^2 tests and Mann-Whitney U tests) • Kaplan-Meier estimates • UVA - Cox Regression • MVA - Stratified Cox Regression *for DDFI only* (Complete-case)	a) n=2956 b) n=2895 c) n=2581	v11.2[74]

Paper	**Key comparator groups**	**Study endpoints**	**Statistical methods implemented** ***(including sensitivity analyses)***	**Number of patients included in:** **a) analysis population;** **b) UVA†;** **c) MVA‡**	**Version of STATA used**
3	Underweight/healthy vs. Overweight vs. Obese	OS, DDFI	• Summary statistics (Pearson χ^2 tests, Kruskal-Wallis tests and Mann-Whitney U tests) • Kaplan-Meier estimates • UVA - Cox Regression • MVA - Stratified Cox Regression (Complete-case)	a) n=2843 b) n=2843 c) n=2325	v11.2[74]
4	FH- vs. FH+, and FDR vs. SDR	DDFI	• Summary statistics (Pearson χ^2 tests and Mann-Whitney U tests) • Kaplan-Meier estimates • UVA - Cox Regression • MVA - FPSMs (Multiple-imputation)	a) n=2956 b) n=2827 c) n=2932††	v11.2[74]
5	BRCA+ vs. BRCA+, and BRCA1+ vs. BRCA2+	OS, DDFS, PDRS	• Summary statistics (Pearson χ^2 tests and Mann-Whitney U tests) • Kaplan-Meier estimates • UVA – Cox regression **and** FPSMs • MVA - FPSMs (Multiple-imputation) • *Sensitivity MVA - FPSMs (Conditional Multiple-imputation)*	a) n=2733 (558¥¥) b) n=2733 (558¥¥) c) n=2733†† (558¥¥)	v14.2[77]
6	Mastectomy vs. BCS	LRI, OS, DDFI	• Summary statistics (Pearson χ^2 tests and Mann-Whitney U tests) • Kaplan-Meier estimates • Nelson-Aalen estimates • UVA – FPSMs **and** Cox regression • MVA - FPSMs (Complete-case) **and** stratified Cox Regression (Complete-case) • *Sensitivity MVA - FPSMs (Multiple-imputation), **s**tratified Cox Regression (Multiple-imputation), and Competing Risk Regression (Complete-Case)*	a) n=2882 b) n=2859 c) n=1623‡‡	v13.1[76]

Paper	**Key comparator groups**	**Study endpoints**	**Statistical methods implemented** ***(including sensitivity analyses)***	**Number of patients included in: a) analysis population; b) UVA†; c) MVA‡**	**Version of STATA used**
7	N/A (Numerous prognostic factors)	OS, BCSS	• Model calibration • Model discrimination	a) n=2827 b) n=2827 c) N/A	v12.1[75]
BCS=Breast Conserving Surgery; BCSS=Breast Cancer-Specific Survival; DDFI=Distant Disease-Free Interval; DDFS=Distant Disease-Free Survival; ER=Oestrogen Receptor; FDR=First Degree Relative; FH=Family History; FPSM=Flexible Parametric Survival Model; LRI=Local-Recurrence Interval; MVA=Multivariable Analyses; OS=Overall Survival; PDRS=Post Distant-Relapse Survival; SDR=Second Degree Relative; UVA=Univariable Analyses.					
† Patients excluded from univariable analyses either if follow-up information was unavailable or if patients were not categorised within any of the main comparator groups.					
‡ Patients excluded from the univariable analysis either if follow-up information was unavailable, if patients were not categorised within any of the main comparator groups, or if patients had missing information for any of the adjusted covariates included in the multivariable analysis.					
¥ Termed DRFS (Distant Relapse-Free Survival) in the publication.					
†† Multiple-imputation implemented in the multivariable analysis so patients excluded only if follow-up information was unavailable.					
‡‡ Excludes patients undergoing neo-adjuvant chemotherapy					
¥¥ Analysis subgroup population of patients with triple-negative breast cancer					

Chapter 3: Paper 1 - Prospective observational study of breast cancer treatment outcomes for UK women aged 18–40 years at diagnosis: the POSH study

Authors	Copson E, Eccles B, **Maishman T**, Gerty S, Stanton L, Cutress RI, Altman DG, Durcan L, Simmonds P, Lawrence G, Jones L, Bliss J, Eccles D; POSH Study Steering Group.
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Date Received	12 November 2012
Date Accepted	26 April 2013
Date Published	30 May 2013
Citations to date	78

3.1 Contribution

As the lead statistician and scientific lead at the Southampton Clinical Trial Unit (SCTU):

- Researched all statistical methods to be implemented in the analyses – including research into Flexible Parametric Survival Models (FPSMs) to use for time-varying covariates;
- Developed and authored the Statistical Analysis Plan (SAP) – including the organisation and participation of meetings to develop the SAP, and the creation of all draft and final versions of the SAP;
- Responsible for central data monitoring, data cleaning and data interpretation – liaising with the study team to identify extensive data queries with the POSH data, and for the resolution of all data queries raised with data management;
- Developed and conducted all statistical programming and analyses – including the creation of all database and analysis programming required for the analyses;
- Designed, developed and produced all manuscript figures and tables – including the development of the time-varying hazard plots by ER status, the transcription of all results, the creation of all draft and final versions of the manuscript figures and tables;
- Co-authored manuscript – listed as 3rd named author, drafted the statistical methods section of the manuscript, extensively involved in the interpretation of results including the interpretation of the FPSM results, reviewed the entire manuscript including sense checks and result checking, involved in the resolution of reviewer comments and responses.

Professor Diana Eccles, chief investigator of the POSH study, chair of the POSH Study Steering Group, and senior last author of **Paper 1**, confirms the accuracy of contribution outlined above of Thomas Christopher Maishman.

Signed: .....

Date: .....

ARTICLE

Prospective Observational Study of Breast Cancer Treatment Outcomes for UK Women Aged 18–40 Years at Diagnosis: The POSH Study

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Background Breast cancer at a young age is associated with poor prognosis. The Prospective Study of Outcomes in Sporadic and Hereditary Breast Cancer (POSH) was designed to investigate factors affecting prognosis in this patient group.

Methods Between 2000 and 2008, 2956 patients aged 40 years or younger were recruited to a UK multicenter prospective observational cohort study (POSH). Details of tumor pathology, disease stage, treatment received, and outcome were recorded. Overall survival (OS) and distant disease-free interval (DDFI) were assessed using Kaplan-Meier curves. All statistical tests were two-sided.

Results Median age of patients was 36 years. Median tumor diameter was 22 mm, and 50% of patients had positive lymph nodes; 59% of tumors were grade 3, 33.7% were estrogen receptor (ER) negative, and 24% were human epidermal growth factor receptor 2 (HER2) positive. Five-year OS was higher for patients with ER-positive than ER-negative tumors (85.0%, 95% confidence interval [CI] = 83.2% to 86.7% vs 75.7%, 95% CI = 72.8% to 78.4%; $P < .001$), but by eight years, survival was almost equal. The eight-year OS of patients with ER-positive tumors was similar to that of patients with ER-negative tumors in both HER2-positive and HER2-negative subgroups. The flexible parametric survival model for OS shows that the risk of death increases steadily over time for patients with ER-positive tumors in contrast to patients with ER-negative tumors, where risk of death peaked at two years.

Conclusions These results confirm the increased frequency of ER-negative tumors and early relapse in young patients and also demonstrate the equally poor longer-term outlook of young patients who have ER-positive tumors with HER2-negative or -positive disease.

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Breast cancer remains the most common cancer in females in the United Kingdom (1). Approximately 4% of cases occur in women younger than 40 years of age (1). Young age at diagnosis is associated with an increased risk of recurrence and inferior survival compared to older patients (2–12). The exact reasons for this remain unclear.

Numerous publications describe an increased incidence of adverse biological features in tumors from young breast cancer patients. These include high grade (3,5,9,13–15), vascular invasion (5,14,15), lymph node involvement (3,7,9,13,16), absence of hormone receptors (3–5,7,9,14,15), and increased frequency of human epidermal growth factor receptor 2 (HER2) overexpression (15–18). It is controversial whether these adverse features fully explain the poor outcome of young breast cancer patients. The St Gallen 1998 consensus identified diagnosis at age 35 years or younger as a very poor prognostic factor and recommended use of adjuvant chemotherapy regardless of other tumor features, although no evidence was given to support this threshold (19). Evidence that age is an independent marker of poor prognosis remains limited (5,9,20–22).

An underlying genetic predisposition to breast cancer is characterized by young age of disease onset, yet even at a very young age of diagnosis most individuals do not have an identifiable mutation in a known breast cancer predisposition gene (23,24). The primary aim of the Prospective Study of Outcomes in Sporadic and Hereditary Breast Cancer (POSH) is to determine whether the prognosis of patients with breast cancer is altered by inherited genetic factors. In this initial publication, we use Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines to report presenting characteristics, pathology, treatment, and survival of this large cohort of patients with young-onset breast cancer (25).

Patients and Methods

POSH is a multicenter prospective observational cohort study of young women diagnosed with breast cancer in the United Kingdom between 2000 and 2008 (http://www.southampton.ac.uk/medicine/research/posh.page). The detailed study protocol was published in 2007 (26). This study received approval from the South West Multi-center Research Ethics Committee (MREC 00/6/69).

Patients

Female patients were recruited from 127 UK hospitals. Patients were eligible if diagnosed with invasive breast cancer between January 1, 2000 and January 31, 2008 at an age of 40 years or younger. Potential recruits were identified within 12 months of initial diagnosis. All patients received treatment according to local protocols. Written informed consent was obtained (26).

Women aged 41–50 years were recruited if they had a known *BRCA1/2* gene mutation and were diagnosed with invasive breast cancer, but were excluded from these analyses.

Study Variables and Data Sources

Details of personal characteristics, tumor pathology, disease stage, and treatment received were collected from medical records. Pathology and imaging data were verified with copies of original reports from sites. For patients treated with neoadjuvant chemotherapy, initial tumor diameter was derived from radiological reports. Family history and personal risk factors were collected using a questionnaire completed by participants at recruitment (26).

Detailed clinical follow-up data, including date and site of disease recurrence, were obtained from medical records at 6 and 12 months and at yearly intervals after diagnosis until death or loss to follow-up. Patients were flagged in the National Health Service Medical Research Information Service to facilitate automatic notification of date and cause of death. This article presents analyses conducted on follow-up data received until April 11, 2012.

To rule out any systematic ascertainment bias, cohort characteristics were compared with data from the West Midlands Cancer Intelligence Unit (WMCIU), the lead national registry for breast cancer. Data on all known invasive breast cancers diagnosed within England for the same age range and time period were provided.

Biological Analyses

Estrogen receptor (ER), progesterone receptor (PR), and HER2 receptor status of primary tumors were determined from diagnostic pathology test reports. Tissue microarray (TMA) data from 1336 cases were used to corroborate and supplement missing clinical data on receptor status. Genetic testing results have been recorded for trial participants who underwent formal genetic assessment and diagnostic *BRCA1/2* screening at a regional clinical genetics center. Additional research testing is in progress.

Statistical Analysis

Details of the target sample size (n = 3000) are reported in the protocol (26). The statistical analysis was conducted according to a prespecified plan (available on request) (25). Analyses were performed with Stata software, version 11.2 on records with complete data (levels of missingness were reported). Summary statistics were used to describe the cohort, and key data were compared with information from the WMCIU. All reported *P* values are two-sided. Overall survival (OS) and distant disease-free interval (DDFI) were assessed using Kaplan-Meier curves. These were defined as time from date of invasive breast cancer diagnosis to death from any cause (OS), and to distant relapse or death from breast cancer (DDFI). Patients who had not experienced an event at the time of analysis were censored at their date of last follow-up. The effect of ER status on survival varies over time (27). Therefore, to assess the effect of ER status, a flexible parametric survival model was fitted to OS and DDFI using the Stata stpm2 command with ER as a time-dependent covariate (28). In each case, we explored varying degrees of freedom for the baseline hazard rate and time-dependent effect using the Akaike information criterion and overlaying the flexible parametric model hazard curves onto the smoothed hazard rates. The best model fit for OS (DDFI) was found by setting the degrees of freedom to three (four) and two (two) for the baseline hazard rate and time-dependent effect, respectively. The model was unadjusted for any other factors. The resulting time-varying hazard ratio and hazard and survival rates were plotted over time by ER status.

Results

The POSH study recruited 3095 patients across England (n = 2695), Scotland, Wales, and Northern Ireland. After excluding 139 trial participants (Figure 1), 2956 patients were included in this analysis. Recruitment peaked in 2005 (Supplementary Figure 1, available online). A total of 11 594 female patients aged 18–40 years were registered with invasive breast cancer in England during 2000–2007 (WMCIU data, Supplementary Table 1, available online). POSH participants recruited from England thus represent 23% of the available population during the recruitment period.

Patient Characteristics

Table 1 demonstrates patient demographics and breast cancer risk factors. Median age at diagnosis of breast cancer was 36 years (range = 18 to 40 years).

Presentation and Diagnostics

Symptomatic presentation accounted for 98% (2900) of the cohort. Thirty women presented with screening-detected malignancies while on surveillance programs due to a previously identified *BRCA1/2* mutation in the patient (n = 3) or family (n = 6) or a strong family history of breast cancer (n = 21). A mammogram was performed in 2687 patients, (90.9%) and ultrasound in 2636 patients (89.2%). Two hundred twenty patients (7.4%) underwent magnetic resonance imaging of the breasts. No imaging modality data were available in 82 patients (2.8%).

Tumor Pathology

Median tumor diameter was 22 mm, and 50% of patients had positive lymph nodes; 59% of tumors were grade 3; 33.7% were ER negative, and 24% were HER2 positive. Despite similar pathological T stage, the difference in nodal status between patients with ER-positive tumors and those with ER-negative tumors was

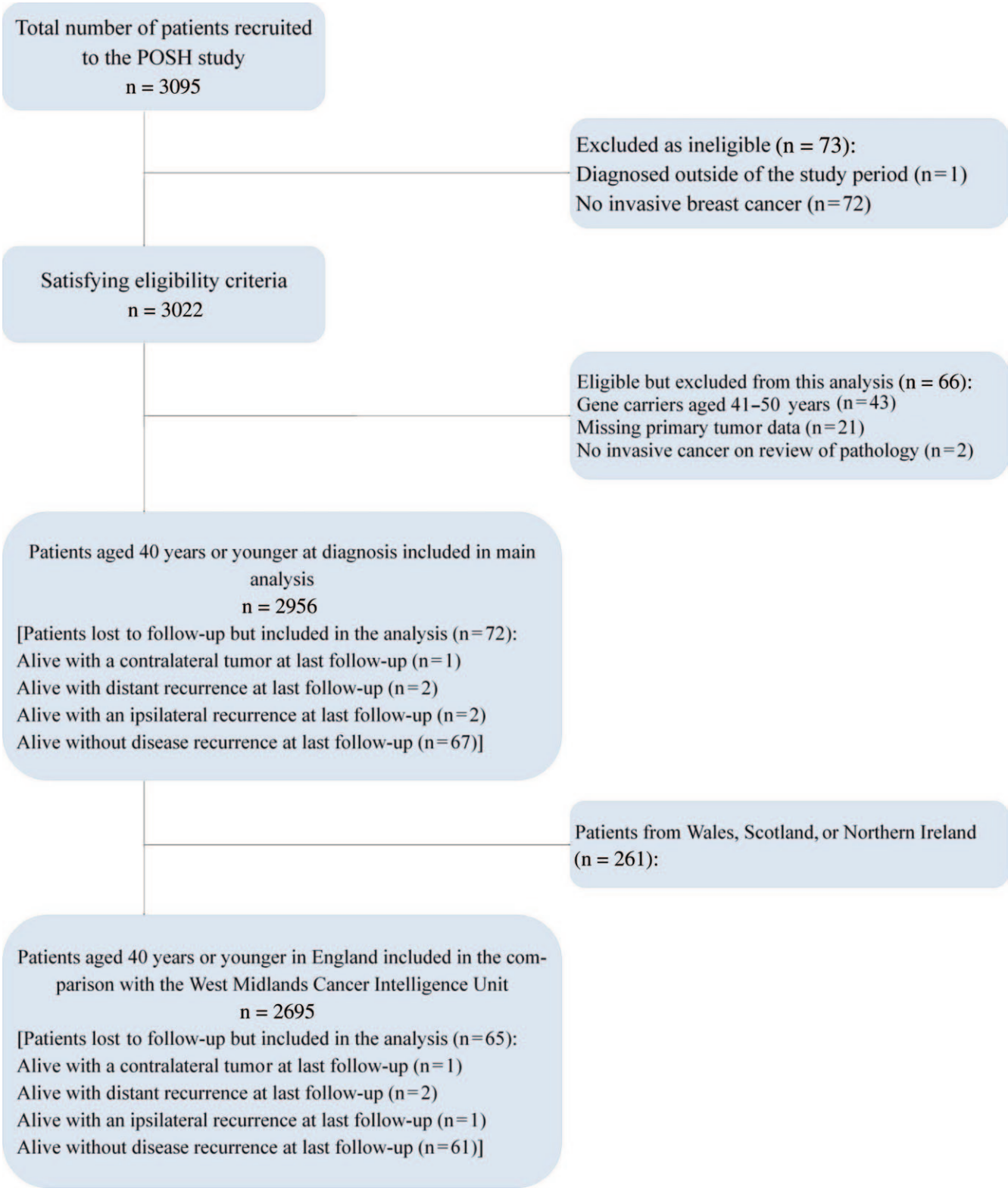

Figure 1. Flow diagram for the Prospective Study of Outcomes in Sporadic and Hereditary Breast Cancer (POSH).

statistically significant (Table 2). For larger tumors, downstaging between clinical and pathological T stage was demonstrated, reflecting the more frequent use of neoadjuvant chemotherapy (Supplementary Table 2, available online). Tumors were reported as ER positive in 65.9% and ER negative in 33.7% of cases. HER2 overexpression was recorded in 24.3% (717) of patients overall. However, on randomly selected study-specific TMAs, 243 of 1336 patients (18.2%) had HER2 overexpression. Five hundred eighty-eight (19.9%) patients had ER-, HER2-, and (if available) PR-negative tumors based on clinical report data, and 148

Table 1. Patient characteristics and risk factors*

Characteristic/risk factor	Median (range, IQR) or No. of patients (%)
Age at diagnosis, y	36 (18 to 40, 33 to 38), N = 2956 (100%)
18–25	46 (1.6%)
26–30	270 (9.1%)
31–35	900 (30.5%)
36–40	1740 (58.9%)
Duration of follow-up, mo	60 (1 to 136, 45 to 75), N = 2956 (100%)
Presentation	
Symptomatic	2900 (98.1%)
Screen detected	30 (1.0%)
Other	12 (0.4%)
Missing/unknown	14 (0.5%)
Age at menarche, y	13 (8 to 18, 12 to 14), N = 2956 (100%)
Body mass index, kg/m^2	24.6 (14.7 to 59.5, 22.1 to 28.4), n = 2842 (96.1)
Missing/unknown	114 (3.9%)
Age at first birth, y	27 (13 to 40, 23 to 30), n = 2080 (70.4%)
Missing/unknown	876 (29.6%)
No. with children	2097 (70.9%)
No. of children, median (range, IQR)	2 (1 to 8, 1 to 2)
No. without children	834 (28.2%)
Missing/unknown	25 (0.9%)
Use of contraceptive pill	
Ever	2598 (87.9%)
Never	358 (12.1%)
Smoker	
Ever	1455 (49.2%)
Never	1408 (47.6%)
Missing/unknown	93 (3.2%)
Menopausal status	
Premenopausal	2885 (97.6%)
Perimenopausal	5 (0.2%)
Postmenopausal	7 (0.2%)
Missing/unknown	59 (2.0%)
No. of patients with first- or second-degree relatives with breast cancer	
First degree	418 (14.1%)
Second degree	554 (18.7%)
No. of relatives with breast cancer	
0	1874 (63.4%)
1	702 (23.8%)
2	199 (6.7%)
>2	75 (2.5%)
Missing/unknown	106 (3.6%)

* IQR = interquartile range.

(14.1%) based on study TMA results. Comparing the cohort with available English national data demonstrates that study patients are representative (Supplementary Table 1, available online).

Treatment

Most patients (98.6% [2915]) had surgical treatment, and 27 had only surgery with no other modality of treatment (Table 3). Four hundred sixty (15.6%) patients received neoadjuvant chemotherapy. The majority of these (329) had T1/2 tumors, 57 had T3 tumors, and 68 patients had T4 (including inflammatory cancer). Adjuvant chemotherapy was given to 72.8% (2152) of patients, and 1.8% (54) patients received palliative chemotherapy. Thirty-six different adjuvant regimens were reported; the most common was 5-fluorouracil/epirubicin/cyclophosphamide in 1020 patients. The frequency of the three most common regimens varied over time (Supplementary Figure 1, available online). Use of trastuzumab was reported in 47.6% (363) of HER2-positive/ borderline patients. In patients with ER-positive tumors, tamoxifen use was recorded in 88.6% (1726) and an aromatase inhibitor in 2.8% (55).

BRCA1/BRCA2 Testing

By December 31, 2012, 26% (n = 763) patients have had genetic testing. *BRCA*-tested patients were not representative of the whole cohort, as they were either tested following referral to genetics services or were selected for research testing because of prespecified characteristics (n = 436), such as tumor pathology. Further testing of the cohort is ongoing as part of the study (29). A pathogenic mutation has been found in 218 patients (*BRCA1* in 136, *BRCA2* in 78, *TP53* in four).

Follow-up and Survival

At the time of analysis, length of follow-up ranged from one month to 11 years (median = 5 years). Only 72 patients (2.4%) had been lost to follow-up. There have been 613 deaths (20.7%) and cause of death is breast cancer in 578 patients (94.3% of deaths).

There were two treatment-related deaths, five breast cancer deaths, and six noncancer deaths, with missing data in 24 patients. A total of 13 non–breast cancer malignancies have been reported (Supplementary Table 4, available online).

Six hundred thirteen women (21%) died, of whom 578 (94%) died from breast cancer. Seven hundred twelve women (24%) developed a distant recurrence, of whom 149 are still alive. Kaplan-Meier survival curves are plotted in Figure 2, A–D. Median survival from date of first distant relapse to death was longer in patients with ER-positive tumors than those with ER-negative tumors (23.4 vs 10.8 months). Isolated local relapse events were few (89 ipislateral, 63 contralateral) and will be explored in a subsequent article.

Table 2. Tumor characteristics

Characteristic	ER negative (n = 997) (33.7%) No. of patients	ER positive* (n = 1947) (65.9%) No. of patients	Total† (N = 2956) (100%) No. of patients	*P*‡
Histological grade				
1	6 (0.6%)	155 (8.0%)	163 (5.5%)	<.001
2	100 (10.0%)	871 (44.7%)	972 (32.9%)	
3	864 (86.7%)	871 (44.7%)	1742 (58.9%)	
Missing/unknown	27 (2.7%)	50 (2.6%)	79 (2.7%)	
Histological type				
Ductal	909 (91.2%)	1637 (84.1%)	2556 (86.5)	<.001
Lobular	7 (0.7%)	127 (6.5%)	134 (4.5%)	
Ductal and lobular	8 (0.8%)	70 (3.6%)	78 (2.6%)	
Medullary	28 (2.8%)	3 (0.2%)	31 (1.1%)	
Metaplastic	10 (1.0%)	1 (0.1%)	11 (0.4%)	
Mixed	6 (0.6%)	19 (1.0%)	26 (0.9%)	
Other	7 (0.7%)	56 (2.9%)	64 (2.2%)	
Unclassified adenocarcinoma	9 (0.9%)	8 (0.4%)	17 (0.6%)	
Not graded§	0 (0%)	2 (0.1%)	2 (0.1%)	
Missing/unknown	13 (1.3%)	24 (1.2%)	37 (1.3%)	
Distribution of cancer				
Multifocal	176 (17.7%)	620 (31.8%)	797 (27.0%)	<.001
Localized	709 (71.1%)	1156 (59.4%)	1873 (63.4%)	
Missing/unknown	112 (11.2%)	171 (8.8%)	286 (9.7%)	
PR status‖				
Negative	813 (81.5%)	219 (11.3%)	1033 (35.0%)	<.001
Positive	80 (8.0%)	1261(64.8%)	1342 (45.4%)	
Missing/unknown	104 (10.4%)	467 (24.0%)	581 (19.7%)	
HER2 status‖				
Negative	631 (63.3%)	1205 (61.9%)	1839 (62.2%)	.431
Positive	256 (25.7%)	461 (23.7%)	717 (24.3%)	
Borderline	12 (1.2%)	33 (1.7%)	45 (1.5%)	
Missing/unknown	98 (9.8%)	248 (12.7%)	355 (12.0%)	
M stage				
M0	969 (97.2%)	1880 (96.6%)	2860 (96.8%)	.350
M1	21 (2.1%)	52 (2.7%)	74 (2.5%)	
Missing/unknown	7 (0.7%)	15 (0.8%)	22 (0.7%)	
Pathological T stage (all patients)				
T0	39 (3.9%)	33 (1.7%)	73 (2.5%)	.005
T1	448 (44.9%)	959 (49.3%)	1411 (47.7%)	
T2	397 (39.8%)	765 (39.3%)	1167 (39.5%)	
T3	65 (6.5%)	123 (6.3%)	189 (6.4%)	
T4	3 (0.3%)	3 (0.2%)	6 (0.2%)	
Tis	10 (1.0%)	11 (0.6%)	21 (0.7%)	
Tx	27 (2.7%)	49 (2.5%)	77 (2.6%)	
Missing/unknown	8 (0.8%)	4 (0.2%)	12 (0.4%)	
Pathological T stage (excluding neoadjuvant patients¶)				
T0	2 (0.3%)	4 (0.2%)	6 (0.2%)	.703
T1	398 (48.8%)	869 (51.9%)	1270 (50.9%)	
T2	352 (43.2%)	689 (41.2%)	1044 (41.8%)	
T3	47 (5.8%)	89 (5.3%)	137 (5.5%)	
T4	0 (0%)	2 (0.1%)	2 (0.1%)	
Tis	0 (0%)	2 (0.1%)	2 (0.1%)	
Tx	10 (1.2%)	18 (1.1%)	29 (1.2%)	
Missing/unknown	6 (0.7%)	0 (0%)	6 (0.2%)	

(Table continues)

Table 2 (Continued).

Characteristic	ER negative (n = 997) (33.7%) No. of patients	ER positive* (n = 1947) (65.9%) No. of patients	Total† (N = 2956) (100%) No. of patients	P‡
N stage (excluding neoadjuvant patients¶)				
N0	456 (56.0%)	753(45.0%)	1213 (48.6%)	<.001
N1	348 (42.7%)	903 (54.0%)	1252 (50.2%)	
1–3	220 (63.2%)	597 (66.1%)	817 (65.3%)	
4–9	78 (22.4%)	200 (22.2%)	279 (22.3%)	
≥10	49 (14.1%)	106 (11.7%)	155 (12.4%)	
Missing/unknown	1 (0.3%)	0 (0)	1 (0.1%)	
Missing/unknown	11 (1.4%)	17 (1.0%)	31 (1.2%)	
	Median (range, IQR) or No. of patients	**Median (range, IQR) or No. of patients**	**Median (range, IQR) or No. of patients**	**P#**
Maximum diameter invasive tumor, mm	22 (1 to 199,15 to 31)	22 (0 to 170,15 to 35)	22 (0 to 199,15 to 33)	.156
(all patients)	912 (91.5%)	1840 (94.5%)	2763 (93.5%)	
Missing/unknown	85 (8.5%)	107 (5.5%)	193 (6.5%)	
Maximum diameter invasive¶ tumor, mm	22 (1.5 to 199, 15 to 30)	22 (1 to 150, 16 to 33)	22 (1 to 199, 15 to 32)	.206
(exc neoadjuvant)	796 (79.8%)	1643 (84.4%)	2446 (82.8%)	
Missing/unknown	19 (2.3%)	30 (1.8%)	50 (2.0%)	
Maximum tumor diameter**, mm	26 (0.6 to 199, 18 to 37)	27 (0 to 190, 19 to 42)	27 (0 to 199,18 to 40)	.005
(all patients)	928 (93.1%)	1856 (95.3%)	2795 (94.6%)	
Missing/unknown	69 (6.9%)	91 (4.7%)	161 (5.5%)	
Maximum tumor diameter**¶, mm	26 (3 to 199, 18 to 35),	27 (1 to 190, 19 to 41)	26 (1 to 199, 19 to 40)	.006
(exc neoadjuvant patients)	801 (80.3%)	1653 (84.9%)	2461 (83.3%)	
Missing/unknown	14 (1.7%)	20 (1.2%)	35 (1.4%)	
No. of axillary lymph nodes recovered	13 (0 to 46, 8 to 18)	12 (0 to 53, 7 to 17)	12 (0 to 53, 8 to 17)	.022
(all patients)	981 (98.4%)	1920 (98.6%)	2910 (98.4%)	
Missing/unknown	16 (1.6%)	27 (1.4%)	46 (1.6%)	
No. of axillary lymph nodes recovered	13 (0 to 46, 8 to 18)	12 (0 to 53, 7 to 17)	12 (0 to 53, 7 to 17)	.088
(exc neoadjuvant patients)¶	807 (99.0%)	1660 (99.2%)	2472 (99.0%)	
Missing/unknown	8 (1.0%)	13 (0.8%)	24 (1.0%)	
No. of positive axillary lymph nodes	3 (1 to 42, 1 to 6)	2 (1 to 50, 1 to 5)	2 (1 to 50, 1 to 5)	.212
(all patients)	426 (42.7%)	1067 (54.8%)	1495 (50.6%)	
Missing/unknown	22 (2.2%)	34 (1.8%)	59 (2.0%)	
No. of positive axillary lymph nodes (exc	2 (1 to 42, 1 to 5)	2 (1 to 50, 1 to 5)	2 (1 to 50, 1 to 5)	.708
neoadjuvant patients)¶	349 (35.0%)	910 (46.7%)	1260 (42.6%)	
Missing/unknown	13 (1.3%)	18 (0.9%)	34 (1.2%)	

* ER positive defined as hormone receptor level equivalent to Allred score of ≥3. ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PR = progesterone receptor.

† Total column includes data from the whole cohort, ie, ER positive, ER negative, and ER status unknown (12 patients).

‡ *P* values obtained from the Pearson χ^2 test between ER status and each categorical variable (excluding missing/unknown data). All statistical tests were two-sided.

§ Not graded as pathology from axillary node, no primary detected.

‖ Includes data from tissue microarray as well as primary POSH data.

¶ Total number of patients excluding neoadjuvant (n = 2496).

P values obtained from the Mann-Whitney test between ER status and each continuous variable (excluding missing/unknown data). All statistical tests were two-sided.

** Maximum tumor diameter includes ductal carcinoma in situ.

The estimated five-year OS for the entire POSH cohort was 81.9%, and DDFI was 76.6%. Patients with ER-positive tumors had an estimated five-year OS of 85.0% compared with 75.7% for those with ER-negative tumors ($P < .001$ at five years). DDFI at five years was 78.5% for patients with ER-positive tumors and 72.7% for patients with ER-negative tumors ($P < .001$ at five years). At eight years, OS for the whole cohort was 67.6% and there was no difference in survival (67.5% vs 67.7%, $P = 0931$ at eight years) or DDFI (68.3% vs 68.1%, $P = .965$ at eight years) between patients with ER-positive and ER-negative tumors. The flexible parametric survival model for OS (Figure 2, E and F; Supplementary Table 3 and Supplementary Figure 3, available online) shows the hazard ratio and hazard and survival rates over time by ER status. It graphically illustrates that the risk of death prior to five years is greater for patients with ER-negative tumors and after five years is greater for patients with ER-positive tumors. The result is that by eight years the survival curves converge. The OS model survival and hazard rate estimates closely match the Kaplan-Meier estimates and smoothed hazard rate, estimates respectively, indicating a good model fit (Figure 2D compared to Figure 2F; Supplementary

Table 3. Treatment details

Characteristic	ER negative (n = 997) (33.7%)	ER positive (n = 1947) (65.9%)	Total* (N = 2956) (100%)
Definitive breast surgery, no.			
Breast conserving surgery	521 (52.2%)	879 (45.1%)	1497 (50.6%)
Mastectomy	458 (45.9%)	1037 (53.3%)	1409 (47.7%)
Nodal surgery only	3 (0.3%)	6 (0.3%)	9 (0.3%)
No surgery	13 (1.3%)	25 (1.3%)	39 (1.3%)
Missing/unknown	2 (0.2%)	0 (0%)	2 (0.1%)
Chemotherapy timing, no.			
Adjuvant†	772 (77.4%)	1378 (70.8%)	2152 (72.8%)
Neoadjuvant	182 (18.2%)	274 (14.1%)	460 (15.6%)
Palliative	17 (1.4%)	36 (1.8%)	54 (1.8)
Not applicable	26 (2.6%)	259 (13.3%)	290 (9.8%)
Missing/unknown	0 (0%)	0 (0%)	0 (0%)
Chemotherapy regimen, no.			
Anthracycline based	690 (69.2%)	1245 (63.9%)	1938 (65.6%)
Anthracycline and taxane	264 (26.5%)	416 (21.4%)	684 (23.1%)
Taxane based	13 (1.3%)	7 (0.4%)	20 (0.7%)
Other‡	4 (0.4%)	20 (1.0%)	24 (0.8%)
None	26 (2.6%)	259 (13.3)	290 (9.8%)
Missing/unknown	0 (0%)	0 (0%)	0 (0%)
Adjuvant trastuzumab, no.			
Yes	129 (12.9%)	234 (12.0%)	363 (12.3%)
Other treatment period/no/ missing/unknown§	868 (87.1%)	1713 (88.0%)	2593 (87.7%)
Adjuvant radiotherapy, no.			
Yes	816 (81.9%)	1536 (78.9%)	2358 (79.8%)
BCS + adjuvant RT	490 (60.1%)	844 (55.0%)	1339 (56.8%)
Mastectomy + adjuvant RT	321 (39.3%)	685 (44.6%)	1007 (42.7%)
Nodal surgery only	2 (0.3%)	3 (0.2%)	6 (0.3%)
No surgery	3 (0.4%)	4 (0.3%)	6 (0.3%)
No/missing/unknown	167 (16.8%)	367 (18.8%)	598 (20.2%)
Adjuvant hormone treatment, no.			
Yes	98 (9.8%)	1790 (91.9%)	1823 (61.7%)
No/missing/unknown	899 (90.2%)	157 (8.1%)	1133 (38.3%)
Ovarian suppression (in any treatment period), no.			
Medical (LHRH agonist)	122 (12.2%)	659 (33.8%)	784 (26.5%)
Irradiation	0 (0%)	11 (0.6%)	11 (0.4%)
Oophorectomy	73 (7.3%)	325 (16.7%)	398 (13.5%)

* Total column includes data the whole cohort, ie, ER positive, ER negative, and ER status unknown (12 patients). BCS = breast conserving surgery; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; LHRH = Luteinising Hormone Releasing Hormone; PR = progesterone receptor; RT = radiotherapy.

† Excluding any treatment for M1 disease.

‡ For example, cyclophosphamide, methotrexate, and 5-fluorouracil or any regimen not containing an anthracycline or taxane.

§ Due to the data collection methods and emerging knowledge of HER2 and guidance through the study, this is likely to be inaccurate.

Table 3, available online). The change in hazard ratios over time by ER status is still apparent even after adjustment for tumor size, grade, and nodal status in a multivariable flexible parametric model (Supplementary Figure 3, available online).

Although HER2-positive compared to HER2-negative tumors showed lower OS and DDFI at all time points in patients with ER-positive tumors, the difference was only statistically significant for DDFI at five years (five-year DDFI: 71.4% vs 78.3%, $P = .00823$; eight-year DDFI: 60.3% vs 66.3%, $P = .227$; five-year OS: 81.4% vs 84.1%, $P = .206$; eight-year OS: 57.6% vs 65.4%, $P = .159$) (Figure 3).

For patients with ER-negative tumors, again, HER2-positive compared to HER2-negative tumors were associated with worse DDFI and OS at all time points. The difference in DDFI was statistically significant at five and eight years (five-year DDFI: 62.2% vs 73.9%, $P = .00141$; eight-year DDFI: 53.4% vs 70.7%, $P = .00438$) and OS was statistically significantly lower at 8 years (five-year OS: 70.2% vs 75.2%, $P = .154$; eight-year OS: 58.4% vs 68.3%, $P = .0476$) (Figure 3).

Discussion

We present the first outcome analysis of a large prospective cohort study of young-onset breast cancer patients receiving modern breast cancer treatment. As anticipated, the major cause of death in this young trial cohort was breast cancer. The estimated five-year OS of our cohort (82%) is almost identical to 2005–2009 relative survival national statistics in 15- to 39-year-olds with breast cancer (83.5%). This confirms that the POSH cohort is representative of the wider population and that the survival of patients younger

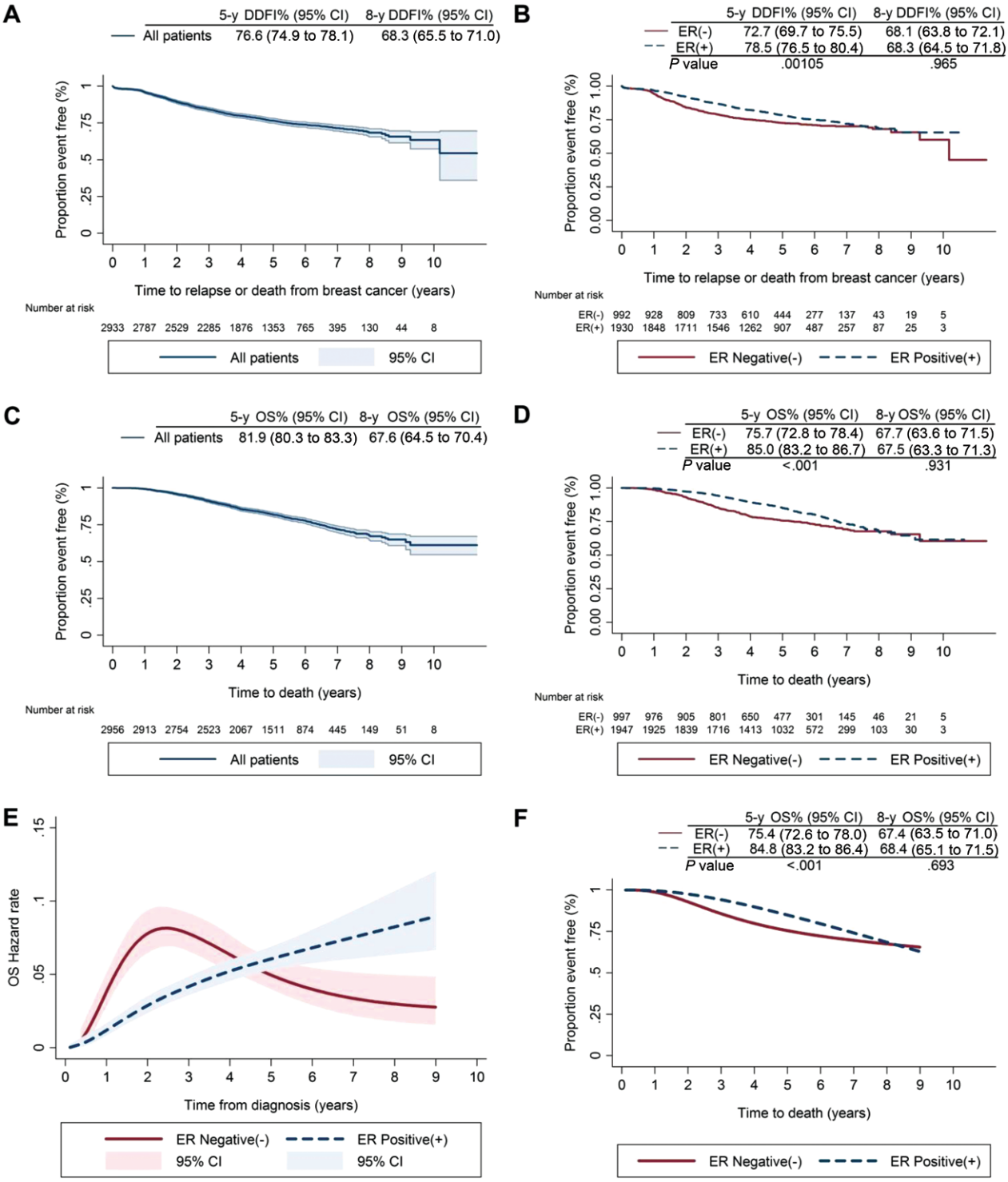

Figure 2. Kaplan-Meier distant relapse-free survival estimates for all patients **(A)** and for patients with estrogen receptor (ER)–negative and -positive tumors **(B)**. Kaplan-Meier overall survival (OS) estimates for all patients **(C)** and patients with ER-negative and -positive tumors **(D)**. Time-varying hazard OS estimates for patients with ER-negative and -positive tumors, showing the time-varying hazard rates by ER status **(E)** and survival rates by ER status **(F)**. All statistical tests were two-sided. CI = confidence interval; DDFI = distant disease-free interval.

than 40 years of age at diagnosis is worse than that of patients aged 40–69 years (five-year relative survival = 89.1% to 90.4%) (1).

Our prospective data clearly demonstrate the influence of ER status over time on recurrence risk and OS in young patients. The estimated five-year OS of patients with ER-positive tumors was 9% higher than patients with ER-negative tumors; however, by eight years the survival of young breast cancer patients with ER-positive tumors was no better than that of patients with ER-negative tumors. Whereas our data indicated falling ER-negative hazard rates and rising ER-positive hazard ratios after

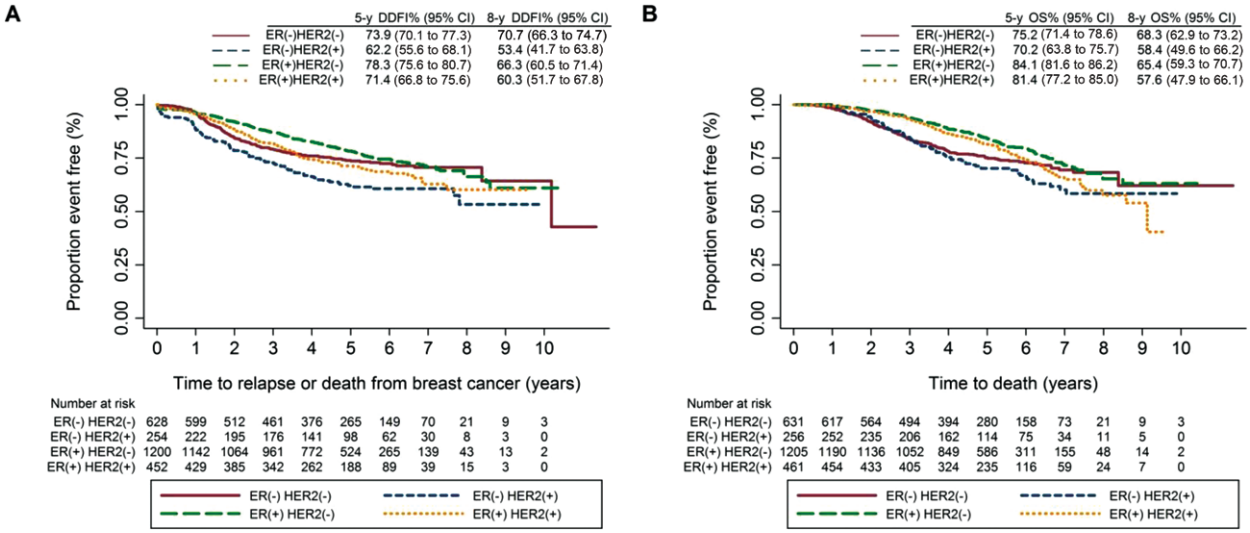

Figure 3. Kaplan-Meier distant relapse-free survival **(A)** and overall survival (OS) **(B)** estimates, by estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status. All statistical tests were two-sided. CI = confidence interval; DDFI = distant disease-free interval.

five years, a previous analysis of Surveillance Epidemiology and End Results (SEER) data showed falling ER-negative hazard rates and constant ER-positive rates crossing at seven years; however, this latter analysis was in non-age-selected patients (30). A more recent report using SEER data observed that patients younger than 40 years of age with ER-positive tumors had an increased hazard of breast cancer–specific mortality compared with that for patients with ER-negative tumors at 5–10 years after diagnosis (31). The increase in breast cancer–specific mortality hazard was notably less marked in older patients with ER-positive tumors. It should, however, be noted that our 10-year follow-up data are currently limited.

Notably, 10.2% of POSH patients with ER-positive tumors relapse between five and eight years (Figure 2B). Adjuvant hormone therapy is generally prescribed for a five-year period. Our results raise the question of duration of hormonal therapy in some premenopausal women. Although the National Surgical Adjuvant Breast and Bowel Project B-14 extension indicated that continuation of adjuvant tamoxifen beyond five years did not confer additional benefits, this trial was limited to node-negative patients and only 31% of patients were younger than 49 years of age (32). Data from the MA.17 clinical trial suggests that extended hormone therapy with letrozole may be beneficial in patients who are premenopausal at diagnosis but became amenorrheic during adjuvant treatment (33). This is more likely to occur in women aged 41–50 years than in those aged 40 years and younger, so further investigation is clearly required to confirm the optimum length of hormone treatment in the youngest age groups. The recently published Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) randomized trial, which included 1270 women aged 45 years or younger at diagnosis with ER-positive tumors, indicates that continuation of tamoxifen for 10 years rather than stopping at five years reduces breast cancer recurrence and mortality in both pre- and postmenopausal women (34).

Ovarian suppression (medical, irradiation, and/or oophorectomy) was documented in 703 of the POSH patients with ER-positive tumors. Although chemotherapy-induced amenorrhea has been associated with improved prognosis, the use of ovarian suppression in addition to chemotherapy and tamoxifen remains controversial (35). Our data reflect the timing of this trial prior to guidance from the National Institute for Clinical Excellence in the UK, recommending use of ovarian suppression plus chemotherapy and tamoxifen in a clinical trial setting only (36).

The vast majority of POSH patients (88%) with early breast cancer received chemotherapy in addition to local treatments, suggesting general compliance with the 1998 St Gallen recommendations (19). Most patients received anthracycline-based chemotherapy. Inevitably, standard systemic treatment regimens changed during the recruitment period of this study, including the incorporation of taxanes into adjuvant regimens. Kroman et al. reported that a diagnosis of breast cancer at a young age (particularly younger than 35 years) was a poor prognostic factor, but that the age effect was only clinically significant in patients who did not receive systemic cytotoxic therapy (37). However, in Kroman's series a much smaller proportion of stage 1–3 patients (65%) received chemotherapy than in POSH. Similarly, Fredholm et al. found that the excess risk in young women was most evident in women with early tumors but also reported low frequency of chemotherapy in these patients (9). A recent meta-analysis confirms that absolute benefits from systemic therapy are higher in patients younger than 45 years of age but that the proportional reduction in risk of relapse is largely independent of age (38). A more detailed exploration of the age effect is beyond the scope of this descriptive publication but will be addressed in future analyses.

Numerous previous publications have reported larger tumors in younger patients with increased nodal involvement. Our findings are consistent with these reports; there was a lower frequency of T1 tumors in our cohort than reported recently for unselected UK patients (47.7% vs 58.2%) and a larger frequency of positive lymph nodes (50.2% vs 38.4%) (39). This may explain why the POSH cohort mastectomy rate (51%) is higher than that reported for non-age-selected symptomatic and screening-detected UK patients (43% in 2007) (39). The upper age criterion for this trial is

below the minimum age for the UK breast screening program and this trial excluded previous history of malignancy; therefore, our data do not include any examples of routine screening-detected or radiation-induced breast cancer. However, 30 patients were undergoing early screening because of a family history of breast cancer or known *BRCA1/2* mutation.

Biological characteristics of tumors were consistent with other published series of women aged younger than 35 or 40 years with a high proportion of grade 3 (59%) and ER-negative (34%) tumors (3,5,9,14–16). Patients with an ER-negative tumor were twice as likely to have a grade 3 tumor than those with ER-positive tumors; but ER-positive tumors had a higher frequency of nodal involvement (54.0% vs 42.7% of ER-negative tumors). Although the reported frequency of HER2 overexpression was 24%, HER2 status was not routinely tested in the United Kingdom prior to 2006. Retrospective testing of primary tumors at subsequent presentation of metastatic disease would be likely to inflate the proportion of positive results among those tested. For the 1336 tumors tested on TMAs, the proportion of HER2-positive tumors was 18.2%. This is within the range reported elsewhere for all breast tumors. Overall, 19.9% of our patients were negative for HER2 overexpression, ER, and (where available) PR. Other series have described triple-negative tumors in 23%–25% of patients aged 40 years and younger (16,40).

As anticipated, a positive HER2 status is associated with a lower five-year and eight-year OS in both patients with ER-positive and those with ER-negative tumors; however, this difference is only statistically significant for patients with ER-negative tumors at eight years. Our data indicate that the eight-year OS of ER-positive/HER2-positive patients is no better than that of ER-negative/HER2-positive patients and is inferior that of to ER-positive and ER-negative patients with HER2-negative tumors. However, use of adjuvant trastuzumab was recorded for less than 50% of our cohort, which may be explained by the fact that 53.3% of the POSH cohort was diagnosed before 2005 when adjuvant trastuzumab came into routine use in the United Kingdom. It is therefore likely that these figures are not entirely representative of the modern oncological management of HER2-positive breast cancer. Most of these patients who did not receive adjuvant trastuzumab received it for metastatic disease.

POSH is a cohort study and we have therefore not directly compared our data with older women. However, we have reported according to the STROBE guidelines to ensure complete transparency in relation to our findings and future analyses. Although national registry data are incomplete, the data presented in this study appear to be comparable with national data over the same time period so are likely to be representative. One limitation of the data presented here is that ER, PR, and HER2 results were obtained from local pathology reports with variations in scoring systems. PR testing was not routinely performed at many sites during recruitment. A slightly lower proportion of our patients (2.5%) had distant metastases at presentation than in retrospective series of women younger than 35 years (3.2%) (9) or 40 years of age (2.9%–7·0%) (9–16). This may represent bias against recruitment of this group to an observational study.

As one of the few prospective studies on medium-term outcome in this age group, POSH already provides a unique data set. Further analysis from the POSH study data will provide important insights into long-term outcomes for early-onset breast cancer and the influences of genetic variation on tumor pathology and response to treatment.

We have described the presenting characteristics, pathology, and treatment of 2956 women diagnosed with breast cancer aged 40 years or younger in the United Kingdom. Despite modern oncological treatments, this group of women has a poor prognosis. Our data confirm the high frequency of ER-negative tumors in young breast cancer patients and the association of this phenotype with high tumor grade and risk of early disease recurrence. However the equally poor medium-term outcome of ER-positive tumors in this patient group, in both HER2-positive and HER2-negative subgroups, highlights the need for new treatment approaches in all younger women, including extended adjuvant hormonal therapy and possibly age-selected trials.

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Note

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SUPPLEMENTARY MATERIAL

Supplementary Document 1

POSH Collaborators

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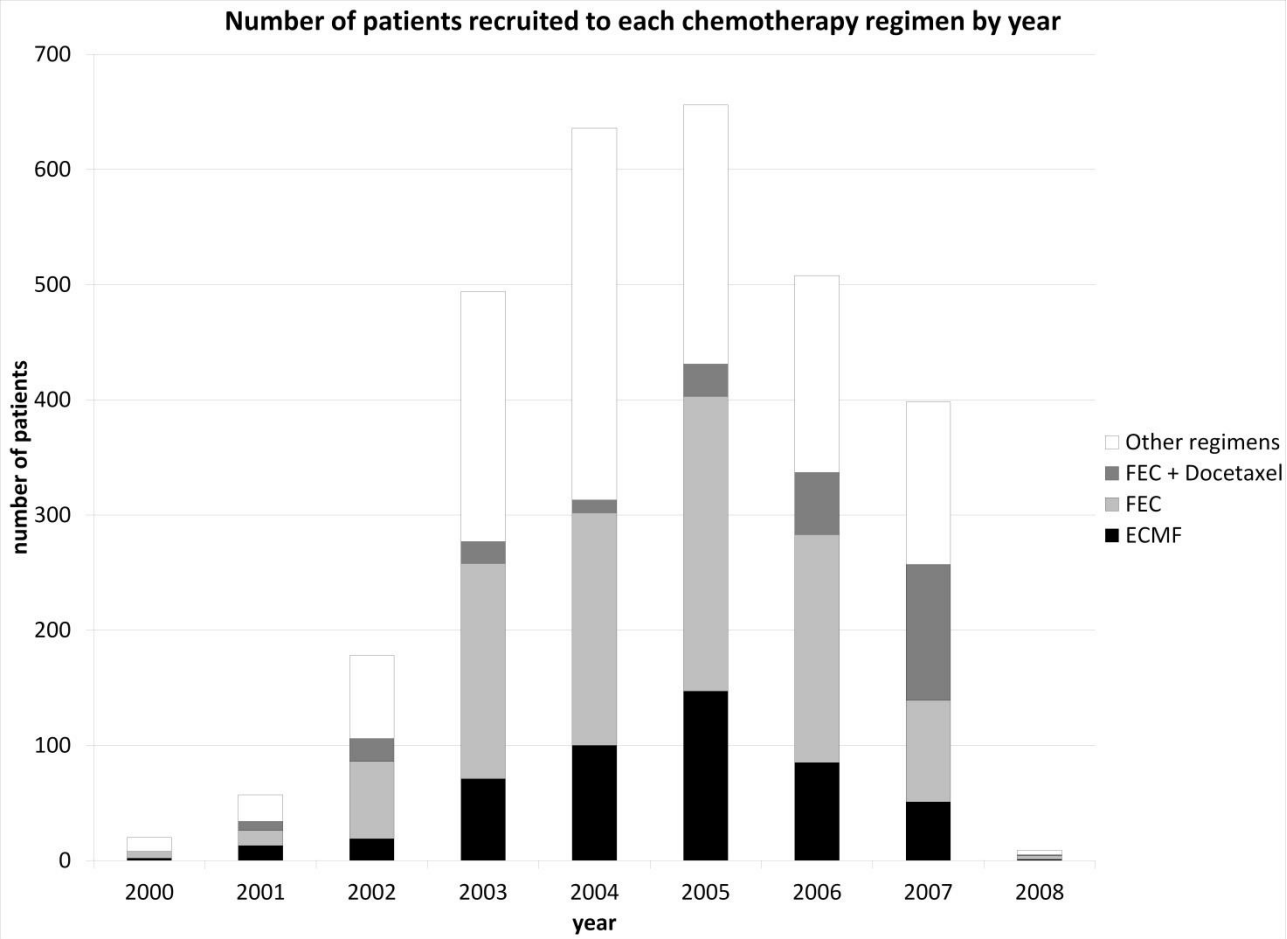
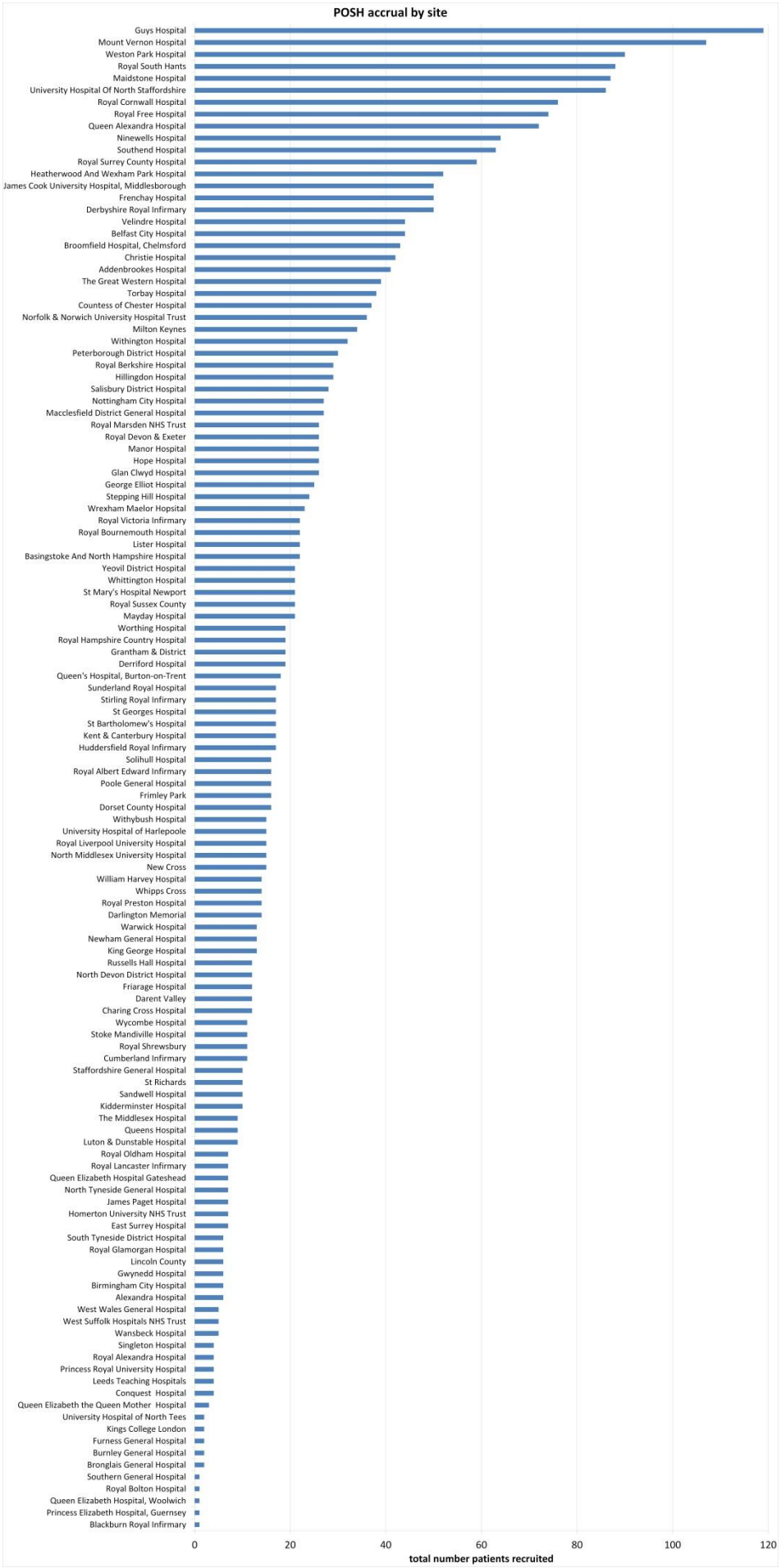
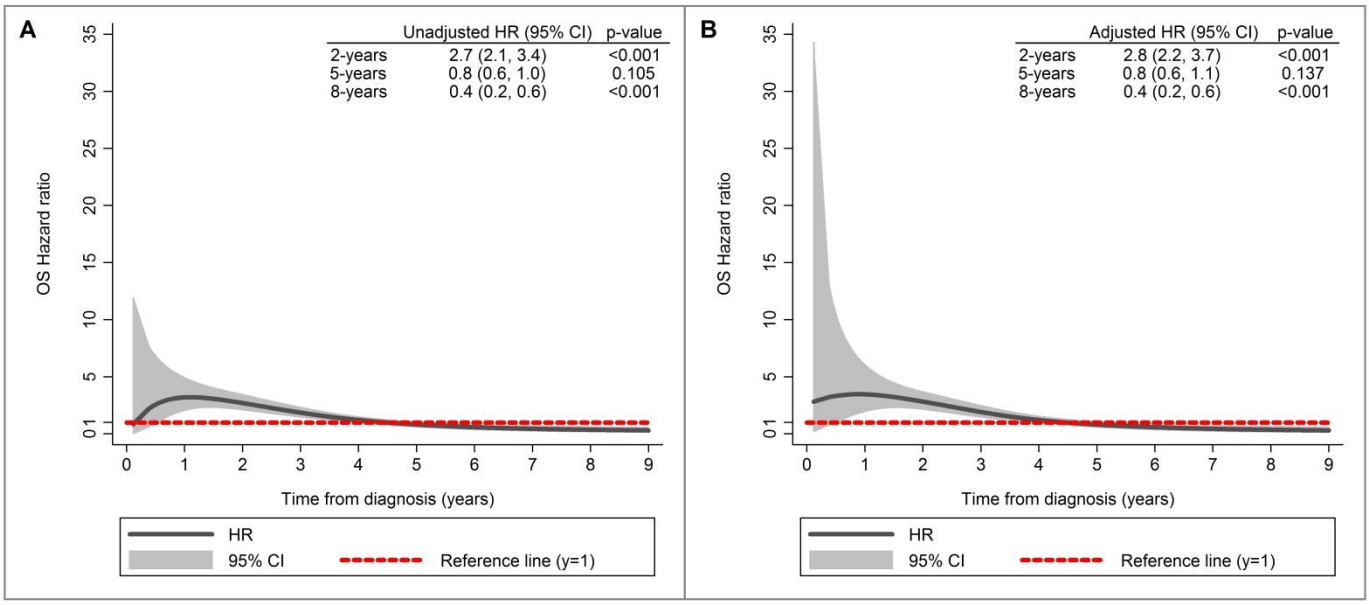
Supplementary Figure 1. Number of patients recruited to POSH study by year



Supplementary Figure 2. Number of patients recruited by site



Supplementary Figure 3. Time-varying OS hazard ratios for ER status which have been (A) unadjusted (B) adjusted for tumour size, grade and nodal status in the multivariate flexible parametric model.



Supplementary Table 1. Comparison with National data from WMCIU (England only)

Characteristic	**POSH cohort (only patients diagnosed in England) (n=2695)**	**West Midlands Cancer Intelligence Unit (n=11594)**	**Proportion of POSH cohort patients included in the West Midlands Cancer Intelligence Unit data set (%)**
	Number of patients (%)†	**Number of patients (%)†**	
Age at diagnosis			
18 to 25	45 (1.7%)	205 (1.8%)	22.0%
26 to 30	248 (9.2%)	1075 (9.3%)	23.0%
31 to 35	838 (31.1%)	3298 (28.4%)	25.4%
36 to 40	1564 (58.0%)	7016 (60.5%)	22.3%
Year of diagnosis			
2000	20 (0.7%)	1397 (12.0%)	1.4%
2001	44 (1.6%)	1346 (11.6%)	3.3%
2002	165 (6.1%)	1450 (12.5%)	11.4%
2003	445 (16.5%)	1463 (12.6%)	30.4%
2004	570 (21.2%)	1457 (12.6%)	39.1%
2005	606 (22.5%)	1572 (13.6%)	38.5%
2006	468 (17.4%)	1537(13.3%)	30.4%
2007	360 (13.4%)	1372 (11.8%)	26.2%
Histological grade†			
1	152 (5.8%)	840 (9.7%)	18.1%
2	876 (33.4%)	3246 (37.4%)	27.0%
3	1594 (60.8%)	4588 (52.9%)	34.8%
Not graded	1 (0.04%)	0 (0%)	0%
Missing/unknown	72 (2.7%)	2920 (25.2%)	2.5%
Maximum diameter invasive tumour, in mm†			
<15			
15 to 20	530 (21.0%)	1974 (23.5%)	26.9%
>20 to 35	593 (23.6%)	2218 (26.4%)	26.7%
>35 to 50	871 (34.6%)	2567 (30.5%)	39.3%
>50	298 (11.8%)	934 (11.1%)	31.9%
Missing/unknown	226 (9.0%)	715 (8.5%)	31.7%
	177 (6.6%)	3186 (27.5%)	5.6%
N stage†			
N0	1268 (48.1%)	2838 (41.1%)	44.7%
N1	1367 (51.9%)	4067 (58.9%)	48.2%
Missing/unknown	60 (2.2%)	4689 (40.4%)	1.3%
ER Status*			
Missing/unknown	12 (0.4%)	9440 (81.4%)	NA
PR Status*			
Missing/unknown	525 (19.5%)	10147 (87.5%)	NA
HER2 Status*			
Missing/unknown	329 (12.2%)	10412 (89.8%)	NA

*Details not included as large amount of missing data from West Midlands Cancer Intelligence unit, POSH data represented in Table 2.

†Percentage given is excluded missing data so direct comparisons of percentages for representativeness can be performed.

Supplementary Table 2. Clinical T stage and Pathological T stage of primary breast cancer cross tabulated

Clinical T stage	Pathological T stage								
	T0	**T1**	**T2**	**T3**	**T4**	**Tis**	**Tx**	**Missing/ unknown**	**Total**
T0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
T1	14 (1.0%)	926 (66.1%)	389 (27.8%)	53 (3.8%)	0 (0%)	4 (0.3%)	12 (0.9%)	4 (0.3%)	1402 (100%)
T2	42 (4.1%)	264 (25.5%)	602 (58.2%)	76 (7.3%)	4 (0.4%)	12 (1.2%)	31 (3.0%)	4 (0.4%)	1035 (100%)
T3	8 (7.4%)	21 (19.4%)	41 (38.0%)	24 (22.2%)	0 (0%)	1 (0.9%)	11 (10.2%)	2 (1.9%)	108 (100%)
T4 (not T4d / inflammatory)	3 (12.5%)	6 (25.0%)	4 (16.7%)	3 (12.5%)	1 (4.2%)	0 (0%)	7 (29.2%)	0 (0%)	24 (100%)
T4d/inflammatory	6 (8.8%)	15 (22.1%)	19 (27.9%)	13 (19.1%)	1 (1.5%)	3 (4.4%)	11 (16.2%)	0 (0%)	68 (100%)
Missing/unknown	0 (0%)	179 (56.3%)	112 (35.2%)	20 (6.3%)	0 (0%)	1 (0.3%)	4 (1.3%)	2 (0.6%)	318 (100%)
Total	73 (2.5%)	1411 (47.7%)	1167 (39.5%)	189 (6.4%)	6 (0.2%)	21 (0.7%)	77 (2.6%)	12 (0.4%)	2956 (100%)

Supplementary Table 3. OS smoothed hazard rates and corresponding time-varying hazard rates and hazard ratios by ER status

Time from diagnosis	Smoothed hazard rate estimate per 1,000 person-years (95% CI)		Smoothed hazard rate difference between ER negative and positive patients (95% CI)	p-value	Time-varying hazard rate estimate per 1,000 person-years (95% CI)		Time-varying hazard rate difference between ER negative and positive patients (95% CI)	p-value	Time-varying HR (95% CI)	p-value
	ER Negative patients	ER Positive patients			ER Negative patients	ER Positive patients				
2-years	6.5 (5.4, 7.8)	2.4 (2.0, 2.9)	4.1 (2.9, 5.3)	<0.001	7.8 (6.5, 9.3)	2.9 (2.4, 3.5)	4.9 (3.5, 6.3)	<0.001	2.7 (2.1, 3.4)	<0.001
5-years	4.1 (3.4, 5.1)	5.1 (4.5, 5.7)	-0.9 (-2.0, 0.1)	0.0915	5.0 (4.0, 6.1)	6.0 (5.3, 6.9)	-1.1 (-2.4, 0.2)	0.0915	0.8 (0.6, 1.0)	0.105
8-years	2.5 (1.5, 4.1)	6.9 (5.3, 8.9)	-4.4 (-6.3, -2.4)	<0.001	3.0 (1.8, 4.9)	8.2 (6.4, 10.7)	-5.3 (-7.6, -2.9)	<0.001	0.4 (0.2, 0.6)	<0.001

Supplementary Table 4. Types of non-breast primary cancer

Types of Non-breast primary Cancer	No. of patients
Melanoma	2
Choroidal Melanoma	1
Cervical Squamous cell carcinoma	2
Thyroid Adenocarcinoma	1
Ovarian Adenocarcinoma	1
Non-Hodgkins Lymphoma	1
Basal Cell carcinoma	1
Acute Myeloid Leukaemia	2
Endometrial carcinoma	1
Borderline Endometroid tumour*	1
Anaplastic Oligodendroglioma*	1
Total	13

*Both cancers occurred in the same patient

Chapter 4: Paper 2 - Ethnicity and outcome of young breast cancer patients in the United Kingdom: the POSH study

Authors	Copson E, **Maishman T**, Gerty S, Eccles B, Stanton L, Cutress RI, Altman DG, Durcan L, Simmonds P, Jones L, Tapper W; POSH study steering group, Eccles D.
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4.1 Contribution

As the lead statistical methodologist and scientific lead at the SCTU:

- Responsible for study methodology and research of all statistical methods to be implemented in the analyses;
- Developed and authored the SAP – including the organisation and participation of meetings to develop the SAP, and the creation of all draft and final versions of the SAP;
- Responsible for central data monitoring, data cleaning and data interpretation – liaising with the study team to identify data queries, and for the resolution of all data queries raised with data management;
- Developed and conducted all statistical programming and analyses – including the creation of all database and analysis programming required for the analyses;
- Designed, developed and produced all manuscript figures and tables – including the creation of all draft and final versions of the manuscript figures and tables;
- Co-authored and reviewed the manuscript – listed as 2nd named author, drafted the statistical methods section of the manuscript, involved in the interpretation of results, reviewed the entire manuscript including sense checks and result checking, involved in the resolution of reviewer comments and responses.

Professor Diana Eccles, chief investigator of the POSH study, chair of the POSH Study Steering Group, and senior last author of **Paper 2**, confirms the accuracy of contribution outlined above of Thomas Christopher Maishman.

Signed: .....

Date: .....

FULL PAPER

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Keywords: breast cancer; prognosis; ethnicity

Ethnicity and outcome of young breast cancer patients in the United Kingdom: the POSH study

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Background: Black ethnic groups have a higher breast cancer mortality than Whites. American studies have identified variations in tumour biology and unequal health-care access as causative factors. We compared tumour pathology, treatment and outcomes in three ethnic groups in young breast cancer patients treated in the United Kingdom.

Methods: Women aged ⩽40 years at breast cancer diagnosis were recruited to the POSH national cohort study (MREC: 00/06/69). Personal characteristics, tumour pathology and treatment data were collected at diagnosis. Follow-up data were collected annually. Overall survival (OS) and distant relapse-free survival (DRFS) were assessed using Kaplan–Meier curves, and multivariate analyses were performed using Cox regression.

Results: Ethnicity data were available for 2915 patients including 2690 (91.0%) Whites, 118 (4.0%) Blacks and 87 (2.9%) Asians. Median tumour diameter at presentation was greater in Blacks than Whites (26.0 mm *vs* 22.0 mm, $P=0.0103$), and multifocal tumours were more frequent in both Blacks (43.4%) and Asians (37.0%) than Whites (28.9%). ER/PR/HER2-negative tumours were significantly more frequent in Blacks (26.1%) than Whites (18.6%, $P=0.043$). Use of chemotherapy was similarly high in all ethnic groups (89% B *vs* 88.6% W *vs* 89.7% A). A 5-year DRFS was significantly lower in Blacks than Asians (62.8% B *vs* 77.0% A, $P=0.0473$) or Whites (62.8 B% *vs* 77.0% W, $P=0.0053$) and a 5-year OS for Black patients, 71.1% (95% CI: 61.0–79.1%), was significantly lower than that of Whites (82.4%, 95% CI: 80.8–83.9%, W *vs* B: $P=0.0160$). In multivariate analysis, Black ethnicity had an effect on DRFS in oestrogen receptor (ER)-positive patients that is independent of body mass index, tumour size, grade or nodal status, HR: 1.60 (95% CI: 1.03–2.47, $P=0.035$).

Conclusion: Despite equal access to health care, young Black women in the United Kingdom have a significantly poorer outcome than White patients. Black ethnicity is an independent risk factor for reduced DRFS particularly in ER-positive patients.

Although the overall incidence of invasive breast cancer remains lower in Black women than White women, the risk of developing breast cancer is higher in Blacks than Whites in women aged 45 and under (Newman and Alfonso, 1997; Harding and Rosato, 1999; Ward *et al*, 2004, Chlebowski *et al*, 2005; Smigal *et al*, 2006; Jack *et al*, 2009). There is now substantial evidence that Black women with breast cancer have a poorer prognosis than non-Black patients (Joslyn and West, 2000; Jatoi *et al*, 2003; Carey *et al*, 2006;

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Grann *et al*, 2006; Albain *et al*, 2009). This disparity in outcome is widening with time (Menashe *et al*, 2009). Whether ethnicity is an independent prognostic factor remains controversial.

Many studies have attributed the inferior outcomes in Black women to an increased incidence of adverse biological features. Blacks have an increased incidence of larger and higher-grade tumours with more lymph node involvement than Whites (Elledge *et al*, 1994; Dignam, 2000; Joslyn and West, 2000; Carey *et al*, 2006; Bowen *et al*, 2008). Blacks are also more likely to have ER-negative tumours than Whites, and there are reports of an increased percentage of triple-negative (ER/PR/Her2-) and basal cell tumours in this ethnic group (Carey *et al*, 2006; Bauer *et al*, 2007). The mean age of diagnosis is lower in Blacks than Whites, and this may partially explain the increased incidence of aggressive biological features in some non-age-matched studies (El-Tamer and Wait, 1999; Newman and Alfonso, 2007).

Most studies have been confounded by other factors, with most published data derived from American populations where access to diagnostic health care and treatment is affected by economic status and may vary between different ethnic groups (Bickell *et al*, 2006). Lower uptake of screening and lower rates of chemotherapy use in Blacks compared with other ethnic groups have been reported, with an association between health insurance status and receipt of chemotherapy (Freedman and Yea, 2011). Variations in other social and cultural factors between ethnic groups may also promote differential outcomes (Gerend and Pai, 2008).

Published data on the effect of ethnicity on breast cancer in the United Kingdom are limited but demonstrate a similar effect of ethnicity on outcome as the American studies, (Wild *et al*, 2006; Bowen *et al*, 2008; Jack *et al*, 2009). In their retrospective study of 102 Black and 191 White British women, Bowen *et al* (2008) observed a higher frequency of grade 3 tumours, lymph node-positive disease, negative oestrogen receptor and progesterone receptor status and triple-negative tumours in Black women than White women, with significantly worse survival in Blacks than Whites for patients with small (<2.0) tumours only. The cancer registry based analysis of Jack *et al* (2009) also reported significantly worse overall survival in Black African women than White women after adjustment for age, stage and treatment (HR: 1.24). This variation was less marked when breast cancer specific mortality was examined (HR: 1.09).

The POSH study is a prospective observational study of patients aged less than 41 years with breast cancer, diagnosed and treated in the United Kingdom (Eccles *et al*, 2007). This cohort of almost 3000 patients diagnosed and treated within the 21st Century represents, to the best of our knowledge, the largest prospective study of young breast cancer patients to date. All patients were managed within the National Health Service (NHS), and therefore had equal access to diagnostic, surgical and oncology services. Screening for breast cancer is not offered to women below age 40 years in the United Kingdom, thus removing this potentially confounding factor. Here we report the pathology and treatment of these patients according to their ethnic origin and compare outcome in White, Black and Asian patients.

PATIENTS AND METHODS

POSH is a multicentre prospective observational cohort study of young women diagnosed with breast cancer in the United Kingdom between 2000 and 2008, (http://www.southampton.ac.uk/medicine/research/posh.page).

The detailed study protocol was published in 2007 (Eccles *et al*, 2007). This study received approval from the South West Multicentre Research Ethics Committee (MREC 00/6/69).

Patients. Female patients were recruited from 127 UK hospitals. Patients were eligible if diagnosed with invasive breast cancer between 01 January 2000 and 31 January 2008 at an age of 40 years or younger. Potential recruits were identified within 12 months of initial diagnosis. All patients received treatment according to local protocols. Written consent was obtained.

Study variables and data sources. Details of personal characteristics, tumour pathology, disease stage and treatment received were collected from medical records. Pathology and imaging data have been verified with copies of original reports from sites. For patients treated with neo-adjuvant chemotherapy, initial tumour diameter was derived from radiological reports. Family history and personal risk factors were collected using a questionnaire completed by participants at recruitment. Ethnicity was self-reported, and patients were subsequently categorised into ethnic and racial categories according to National Institute of Health reporting guidelines, (NIH policy on reporting race and ethnicity data: subjects in clinical research 8-2001 http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html). Patients were categorised as Black if they reported 'Black British', 'African', 'Black Caribbean', 'Caribbean' or 'West Indian' ethnicity and Asian if they reported, 'Asian', 'British Asian', 'Asian-Pakistani' or 'Indian subcontinent' ethnicity.

Detailed clinical follow-up data, including date and site of disease recurrence, were obtained from medical records at 6 months, 12 months and at yearly intervals post diagnosis until death or loss to follow-up. Patients were flagged in the NHS Medical Research Information Service to facilitate automatic notification of date and cause of death. This paper presents analyses conducted on follow-up data received until 11 April 2012.

Tumour receptor status data. ER, PR and HER2 receptor status of primary tumours was primarily determined from routine diagnostic pathology tests. Hormone receptor levels equivalent to an Allred score of $\geqslant 3$ were categorised as positive. Tissue microarray, (TMA) data from central pathology review at St. Bartholomew's Hospital, London has been performed on 1336 randomly selected tumour samples. TMA results for ER, PR and HER2 receptor status have been used to corroborate clinical data or supplement missing data points on receptor status for these 1336 patients. BRCA1/2 mutation testing is in progress.

Statistical analysis. Details of the target sample size (3000) are reported in the protocol (Eccles *et al*, 2007). The statistical analysis was conducted according to a pre-specified plan and as recommended by STROBE (STrengthening the Reporting of Observational Studies in Epidemiology) guidelines (von Elm *et al*, 2007). Analyses were performed in STATA v11.2 on records with complete data (levels of missingness were reported). Summary statistics were used to describe the cohort. Where appropriate, Pearson's chi-squared or Mann–Whitney tests were performed in order to identify whether there were any specific differences in the characteristics between ethnic categories.

Overall survival (OS) and distant relapse-free survival (DRFS) were assessed using Kaplan–Meier curves. These were defined as time from date of invasive breast cancer diagnosis to death from any cause (OS) and to distant relapse or death from breast cancer (DRFS). Patients who had not experienced an event at the time of analysis were censored at their date of last follow-up. Multivariate analyses using Cox regression were performed according to the pre-specified analysis plan to adjust for the effect of confirmed prognostic factors (tumour grade, total tumour diameter, nodal status, ER status and body mass index) on DRFS in the different ethnic groups. The proportionality assumption was assessed by inspecting the Nelson-Aalen plots and Schoenfeld residuals and was satisfied in each case.

RESULTS

The POSH study recruited 3095 patients across England (2695), Scotland, Wales and Northern Ireland. After excluding 139 trial participants (Figure 1), 2956 patients were included in this analysis.

Patient characteristics and presentation. Self-reported ethnicity was available for 2915 (98.6%) patients. Of these 2902 reported a single ethnic/ racial category; 2690 (92.7%) were classified as White/ Caucasian, 106 (3.7%) as Black, 86 (3.0%) as Asian; and 20 (0.7%) were from 'other' ethnic groups. Thirteen patients reported mixed ethnicity: eight Black/Caucasian, three Caribbean/White, one Caribbean/Irish and one Chinese/White. In view of the small number of mixed ethnicity patients, these patients were categorised as Black (12) or Asian (1) for the purpose of further analyses. Patients from 'other' ethnic groups ($n=20$) were excluded from further analyses.

Table 1 demonstrates patient demographics and breast cancer risk factors in White, Black and Asian ethnic groups. Median age at diagnosis of breast cancer was significantly lower in Asians than Whites (35 years A *vs* 36 years W, $P=0.001$) or Blacks (35 years A *vs* 36 years B, $P=0.0472$). Median body mass index was significantly higher in Black patients than Whites (26.9 kg m^{-2} B *vs* 24.6 kg m^{-2} W, $P<0.001$) and Asians (26.9 kg m^{-2} B *vs* 24.1 kg m^{-2} A, $P<0.001$). The proportion of patients with at least one child was 71.6% of Whites, 69.0% of Blacks and 79.1% of Asians, with no statistically significant differences between groups. The median number of children in patients who had at least one child was significantly higher in Blacks than Whites ($P=0.011$). Symptomatic presentation accounted for 98% (2900) of the trial cohort, and mode of presentation was similar in all ethnic categories.

Tumour pathology. Median total tumour diameter was significantly greater in Blacks than Whites (26.0 mm B *vs* 22.0 mm W, $P=0.0103$), and multifocal tumours were more frequent in Blacks (43.4%) than Whites (28.9%, $P=0.002$) Table 2. The median total tumour diameter for unifocal disease was signicantly smaller than the median total tumour diameter of patients with multifocal disease (<0.001). There was an increased frequency of grade 3 tumours in Blacks (68.1%) compared with Whites (60.4%), and a higher proportion of Blacks had positive nodal involvement than Whites (56.1% B *vs* 50.8% W) but these differences were not significant. Both Blacks and Asians had a higher frequency of ER-negative tumours than Whites (37.6% B, 42.5% A, 33.5% W), but this was not statistically significant. There were no significant differences in the frequency of HER 2 overexpressing tumours between ethnic groups. However, the frequency of ER/PR/HER2-negative tumours was significantly higher in Blacks (26.1%) than Whites (18.6%, $P=0.043$). Data for tumour grade, histological type, nodal status, presence of metastases and ER status were missing in 0–4.6% of cases only. Data for tumour distribution, PR status and HER2 status were missing more frequently, in up to 16.1%, 20.5% and 13.7% of cases, respectively.

Treatment. Most patients 98·6% (2915) had surgical treatment (Table 3). Rates of breast conserving surgery were lower in Blacks than Whites or Asians (39.8% B *vs* 48.1% W *vs* 48.3% A). Use of chemotherapy in early breast cancer patients was similarly high in all ethnic groups (89% B *vs* 88.6% W *vs* 89.7% A), but a higher proportion of Blacks received neo-adjuvant chemotherapy than Whites or Asians (23.7% B *vs* 14.8% W *vs* 20.7% A). The numbers of patients receiving anthracycline/taxane chemotherapy were similar in each ethnic group. Missing data for trastuzumab and hormonal therapy including ovarian suppression precluded the use of chi-squared tests to compare the proportions of ethnic categories receiving these treatments.

Follow-up and survival. At the time of analysis, length of follow-up ranged from 1 month to 11 years (median 5 years). Only 72 patients (2·4%) had been lost to follow-up. Isolated local relapse

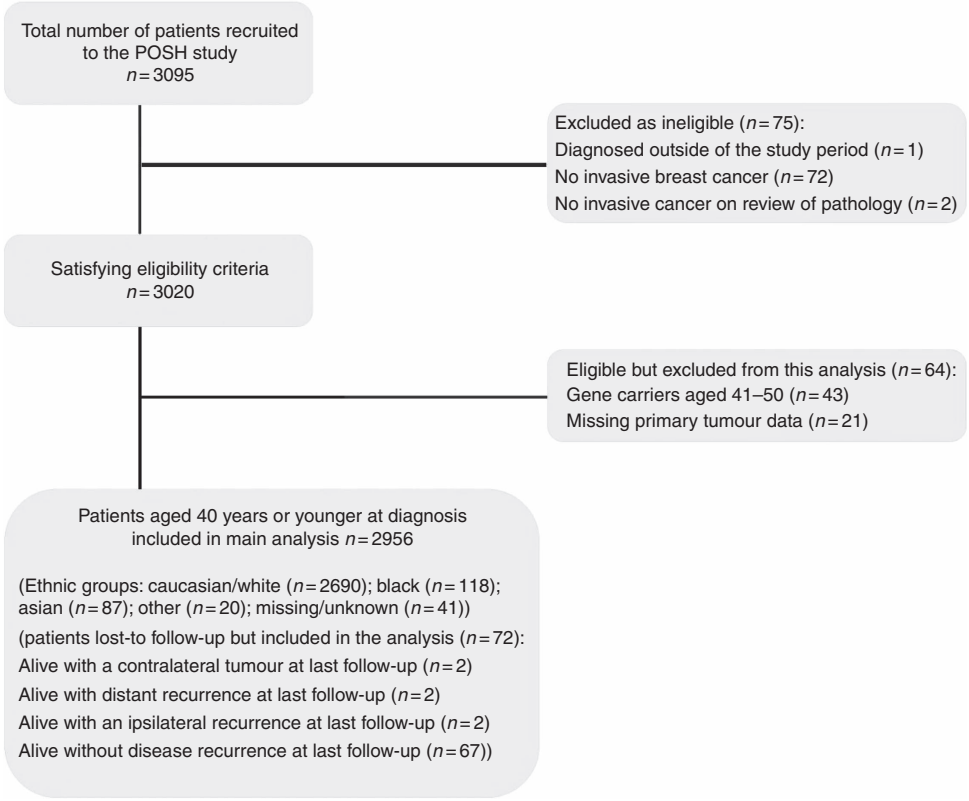

Figure 1. Flow Diagram for the Prospective Study of Outcomes in Sporadic and Hereditary Breast Cancer (POSH).

Table 1. Patients' characteristics and risk factors

Characteristic	All[a]: $n=2956$ (100.0%)	W: $n=2690$ (91.0%)	B: $n=118$ (4.0%)	A: $n=87$ (2.9%)	P-value[‡‡]
Median (range, IQR), number of patients					
Age at diagnosis, in years	36 (18–40, 33–38), 2956	36 (18–40, 34–38), 2690	36 (18–40, 33–38), 118	35 (23–40, 32–37), 87	W *vs* B: $P=0.463$ (NS) W *vs* A: $P<0.001$ B *vs* A: $P=0.0472$
Duration of follow-up, in months	60.5 (1.4–136.1, 45.4–75.4), 2956	60.8 (1.4–136.1, 46.5–76.3), 2690	47.8 (8.1–95.7, 34.1–70.8), 118	56.0 (8.0–102.0, 38.2–71.0), 87	—
Age at menarche, in years	13 (8–18, 12–14), 2956	13 (8–18, 12–14), 2690	12 (8–17, 12–14), 118	13 (9–18, 12–13), 87	W *vs* B: $P=0.560$ (NS) W *vs* A: $P=0.391$ (NS) B *vs* A: $P=0.921$ (NS)
Body mass index, in kg m^{-2} Missing/unknown	24.6 (14.7–59.5, 22.1–28.4), 2842 114 (3.9%)	24.6 (14.7–59.5, 22.1–28.4) , 2610 80 (3.0%)	26.9 (18.9–49.1, 23.5–31.2) , 113 5 (4.2%)	24.1 (17.2–39.0, 21.3–26.5), 79 8 (9.2%)	W *vs* B: $P<0.001$ W *vs* A: $P=0.0401$ B *vs* A: $P<0.001$
Age at first birth, in years Missing/unknown	27 (13–40, 23–30), 2080 876 (29.6%)	27 (13–40, 23–30), 1898 792 (29.4%)	26 (14–37, 20–30), 79 39 (33.1%)	25 (16–36, 22–29), 65 22 (25.3%)	W *vs* B: $P=0.200$ (NS) W *vs* A: $P=0.154$ (NS) B *vs* A: $P=0.965$ (NS)
Number with children	2097 (71.6%)	1912 (71.6%)	80 (69.0%)	68 (79.1%)	W *vs* B: $P=0.545$ (NS)
Number without children, *n*(%) Missing/unknown	834 (28.5%) 25 (0.9%)	760 (28.4%) 18 (0.7%)	36 (31.0%) 2 (1.7%)	18 (20.9%) 1 (1.2%)	W *vs* A: $P=0.128$ (NS) B *vs* A: $P=0.921$ (NS)
Number of children – median (range, IQR), n for patients with $\geqslant 1$ child	2 (1–8, 1–2), 2097	2 (1–8, 1–2), 1912	2 (1–4, 2–3), 80	2 (1–5, 2–3), 68	W *vs* B: $P=0.011$ W *vs* A: $P=0.087$ (NS) B *vs* A: $P=0.688$ (NS)
Number of patients (%)					
Presentation:					
Symptomatic Screen detected Other Missing/unknown	2900 (98.6%) 30 (1.0%) 12 (0.4%) 14 (0.5%)	2645 (98.6%) 26 (1.0%) 11 (0.4%) 8 (0.3%)	116 (98.3%) 1 (0.9%) 1 (0.9%) 0 (0%)	86 (100.0%) 0 (0%) 0 (0%) 1 (1.2%)	W *vs* B: $P=0.770$ (NS) W *vs* A: $P=0.548$ (NS) B *vs* A: $P=0.479$ (NS)
Age at diagnosis, in years					
18 to 25 26 to 30 31 to 35 36 to 40	46 (1.6%) 269 (9.1%) 900 (30.5%) 1741 (58.9%)	39 (1.5%) 240 (8.9%) 810 (30.1%) 1601 (59.5%)	2 (1.7%) 13 (11.0%) 35 (29.7%) 68 (57.6%)	4 (4.6%) 9 (10.3%) 37 (42.5%) 37 (42.5%)	W *vs* B: $P=0.880$ (NS) W *vs* A: $P=0.003$ B *vs* A: $P=0.109$ (NS) W *vs* B: $P<0.001$
Use of contraceptive pill					
Ever Never	2598 (87.9%) 358 (12.1%)	2421 (90.0%) 269 (10.0%)	80 (67.8%) 38 (32.2%)	49 (56.3%) 38 (43.7%)	W *vs* A: $P<0.001$ B *vs* A: $P=0.093$ (NS)
Smoker					
Ever Never Missing/unknown	1455 (50.8%) 1408 (49.2%) 93 (3.2%)	1368 (52.4%) 1243 (47.6%) 79 (2.9%)	47 (41.2%) 67 (58.8%) 4 (3.4%)	16 (18.8%) 69 (81.2%) 2 (2.3%)	W *vs* B: $P=0.020$ W *vs* A: $P<0.001$ B *vs* A: $P=0.001$
Menopausal status					
Premenopausal Perimenopausal Postmenopausal Missing/unknown	2885 (99.6%) 5 (0.2%) 7 (0.2%) 59 (2.0%)	2634 (99.6%) 4 (0.2%) 7 (0.3%) 45 (1.7%)	114 (100.0%) 0 (0%) 0 (0%) 4 (3.4%)	86 (98.9%) 1 (1.2%) 0 (0%) 0 (0%)	W *vs* B: $P=0.788$ (NS) W *vs* A: $P=0.090$ (NS) B *vs* A: $P=0.251$ (NS) W *vs* B: $P=0.126$ (NS)
No. of patients with first or second degree relatives with breast cancer					
First degree Second degree	418 (14.7%) 554 (19.4%)	382 (14.7%) 521 (20.0%)	18 (15.8%) 14 (12.3%)	5 (6.0%) 9 (10.8%)	W *vs* A: $P=0.003$ B *vs* A: $P=0.091$ (NS)
No. of relatives with breast cancer					
0 1 2 >2 Missing/unknown	1874 (65.8%) 702 (24.6%) 199 (7.0%) 75 (2.6%) 106 (3.6%)	1696 (65.2%) 651 (25.0%) 189 (7.3%) 67 (2.6%) 87 (3.2%)	82 (71.9%) 21 (18.4%) 5 (4.4%) 6 (5.3%) 4 (3.4%)	69 (83.1%) 12 (14.5%) 1 (1.2%) 1 (1.2%) 4 (4.6%)	W *vs* B: $P=0.076$ (NS) W *vs* A: $P=0.006$ B *vs* A: $P=0.168$ (NS)

Abbreviations: A = Asian; B = Black; IQR = Inter-Quartile Range; NS = not significant; W = White.

[a]Includes patients in an Other or Missing/unknown Ethnic group.

[††]*P*-values from Pearson's chi-squared test between Ethnic groups and each categorical variable (excluding Other Ethnic groups and missing/unknown data).[‡‡]*P*-values from Mann–Whitney test between Ethnic groups and each continuous variable (excluding Other Ethnic groups and missing/unknown data).

Table 2. Tumour characteristics

	All[a]: $n=2956$ (100.0%)	W: $n=2690$ (91.0%)	B: $n=118$ (4.0%)	A: $n=87$ (2.9%)	*P*-value[††]
Number of patients %)					
Histological grade					
1	163 (5.7%)	147 (5.6%)	1 (0.9%)	10 (11.8%)	W *vs* B: $P=0.055$ (NS)
2	972 (33.8%)	891 (34.0%)	35 (30.0%)	24 (28.2%)	W *vs* A: $P=0.045$
3	1742 (60.6%)	1586 (60.4%)	77 (68.1%)	51 (60.0%)	B *vs* A: $P=0.004$
Missing/unknown	79 (2.7%)	66 (2.5%)	5 (4.2%)	2 (2.3%)	
Histological type					
Ductal	2556 (87.6%)	2320 (87.3%)	101 (87.8%)	81 (94.2%)	W *vs* B: $P=0.882$ (NS)
Lobular	134 (4.6%)	126 (4.7%)	5 (4.4%)	1 (1.2%)	W *vs* A : $P=0.260$ (NS)
Ductal and Lobular	78 (2.7%)	74 (2.8%)	2 (1.7%)	1 (1.2%)	B *vs* A : $P=0.445$ (NS)
Other	149 (5.1%)λλ	137 (5.2%)	7 (6.1%)	3 (3.5%)	
Not graded[a]/missing/unknown	39 (1.3%)	33 (1.2%)	3 (2.5%)	1 (1.2%)	
Distribution of cancer					
Localised	1873 (70.2%)	1741 (71.1%)	56 (56.6%)	46 (63.0%)	W *vs* B: $P=0.002$
Multifocal	797 (29.9%)	707 (28.9%)	43 (43.4%)	27 (37.0%)	W *vs* A: $P=0.133$ (NS)
Missing/unknown	286 (9.7%)	242 (9.0%)	19 (16.1%)	14 (16.1%)	B *vs* A: $P=0.395$ (NS)
Pathological T stage (all patients)					
T0	73 (2.5%)	63 (2.4%)	4 (3.4%)	3 (3.5%)	W *vs* B: $P<0.001$
T1	1411 (47.9%)	1300 (48.5%)	40 (33.9%)	42 (48.3%)	W *vs* A: $P=0.370$ (NS)
T2	1167 (39.6%)	1064 (39.7%)	45 (38.1%)	36 (41.4%)	B *vs* A: $P=0.014$
T3	189 (6.4%)	167 (6.2%)	18 (15.3%)	2 (2.3%)	
T4	6 (0.2%)	5 (0.2%)	0 (0%)	1 (1.2%)	
Tis	21 (0.7%)	18 (0.7%)	1 (0.9%)	1 (1.2%)	
Tx	77 (2.6%)	62 (2.3%)	10 (8.5%)	2 (2.3%)	
Missing/unknown	12 (0.4%)	11 (0.4%)	0 (0%)	0 (0%)	
N stage					
N0	1417 (48.9%)	1302 (49.2%)	50 (43.9%)	40 (48.2%)	W *vs* B: $P=0.260$ (NS)
N1	1484 (51.2%)	1342 (50.8%)	64 (56.1%)	43 (51.8%)	W *vs* A: $P=0.850$ (NS)
Missing/unknown	55 (1.9%)	46 (1.7%)	4 (3.4%)	4 (4.6%)	B *vs* A: $P=0.547$ (NS)
M stage					
M0	2860 (97.5%)	2613 (97.6%)	111 (94.9%)	84 (96.6%)	W *vs* B: $P=0.069$ (NS)
M1	74 (2.5%)	65 (2.4%)	6 (5.1%)	3 (3.5%)	W *vs* A: $P=0.545$ (NS)
Missing/unknown	22 (0.7%)	12 (0.5%)	1 (0.9%)	0 (0%)	B *vs* A: $P=0.563$ (NS)
ER Status[b]					
Positive	1947 (66.1%)	1782 (66.5%)	73 (62.4%)	50 (57.5%)	W *vs* B: $P=0.358$ (NS)
Negative	997 (33.9%)	898 (33.5%)	44 (37.6%)	37 (42.5%)	W *vs* A: $P=0.080$ (NS)
Missing/unknown	12 (0.4%)	10 (0.4%)	1 (0.9%)	0 (0%)	B *vs* A: $P=0.477$ (NS)
PR Status[b]					
Positive	1342 (56.5%)	1215 (56.8%)	65 (58.0%)	40 (50%)	W *vs* B: $P=0.793$ (NS)
Negative	1033 (43.5%)	925 (43.2%)	47 (42.0%)	40 (50%)	W *vs* A: $P=0.230$ (NS)
Missing/unknown	581 (19.7%)	550 (20.5%)	6 (5.1%)	7 (8.1%)	B *vs* A: $P=0.270$ (NS)
HER2 Status[b]					
Positive	717 (28.1%)	657 (28.3%)	22 (20.2%)	22 (29.7%)	W *vs* B: $P=0.065$ (NS)
Negative	1839 (72.0%)	1664 (71.7%)	87 (79.8%)	52 (70.3%)	W *vs* A: $P=0.789$ (NS)
Missing/unknown	400 (13.5%)	369 (13.7%)	9 (7.6%)	13 (14.9%)	B *vs* A: $P=0.138$ (NS)
TNT Status[c]					
TNT	537 (19.0%)	478 (18.6%)	30 (26.1%)	19 (23.2%)	W *vs* B: $P=0.043$
Not TNT	2296 (81.0%)	2099 (81.5%)	85 (73.9%)	63 (76.8%)	W *vs* A: $P=0.291$ (NS)
Missing/unknown	123 (4.2%)	113 (4.2%)	3 (2.5%)	5 (5.8%)	B *vs* A: $P=0.641$ (NS)

Table 2. (Continued)

	All[a]: $n=2956$ (100.0%)	W: $n=2690$ (91.0%)	B: $n=118$ (4.0%)	A: $n=87$ (2.9%)	*P*-value[††]
Median (range, IQR), number of patients					
Maximum tumour diameter[d] in mm (all patients)					W *vs* B: $P=0.0103$
median (IQR, range), n Missing/unknown	22 (15–33, 0–199), 2763 193 (6.5%)	22 (15–33, 0–199), 2527 163 (6.1%)	26 (15–50, 1–110), 103 15 (12.7%)	26 (15–35, 0.15–98), 80 7 (8.1%)	W *vs* A: $P=0.762$ (NS) B *vs* A: $P=0.0940$ (NS)
No. of positive axillary lymph nodes recovered (all patients)					
median (IQR, range), *n*	2 (1–5, 1–50), 1495	2 (1–5, 1–50), 1352	3 (1–7, 1–19), 65	1 (1–4, 1–20), 43	W *vs* B: $P=0.496$ (NS)
No. of positive axillary lymph nodes recovered (all patients), *n*(%)					
1–3	952 (63.7%)	859 (63.5%)	41 (63.1%)	32 (74.4%)	W *vs* A: $P=0.0143$
4–9	357 (23.9%)	324 (24.0%)	14 (21.5%)	9 (20.9%)	B *vs* A: $P=0.0169$
10+	186 (12.4%)	169 (12.5%)	10 (15.4%)	2 (4.7%)	W *vs* B: $P=0.754$ (NS)
Total	1495 (100.0%)	1352 (100.0%)	65 (100.0%)	43 (100.0%)	W *vs* A: $P=0.220$ (NS)
Missing/unknown	59 (2.0%)	50 (1.9%)	4 (3.4%)	4 (4.6%)	B *vs* A: $P=0.204$ (NS)

Abbreviations: ER = oestrogen receptor; HER2 = human epidermal growth factor receptor 2; IQR = inter-quartile range; PR = progesterone receptor; TNT = triple negative.
[a]Includes patients in an other or missing/unknown ethnic group.
[b]Includes data from TMA as well as primary POSH data.
[c]Includes patients with an ER negative, HER2-negative and PR-negative status.
[d]Maximum tumour diameter includes ductal carcinoma *in situ*.
[††]*P*-values obtained from the Pearson's chi-squared test between ethnic groups and each categorical variable (excluding other ethnic groups and missing/unknown data).[‡‡]*P*-values obtained from the Mann–Whitney test between ethnic groups and each continuous variable (excluding Other Ethnic groups missing/unknown data).

events were rare with ipsilateral relapses occurring in 3.0% of Whites, 3.4% of Blacks and 1.2% of Asians, and contralateral tumours occurring in 2.1% of Whites, 0.0% of Blacks and 3.5% of Asians. Kaplan–Meier (KM) survival curves are plotted in Figures 2 and 3. The estimated 5-year OS for the entire POSH cohort was 81.9% (95% CI: 80.3–83.3%) and DRFS 76.6 (74.9–78.1%, table 4). The 5-year OS for Black patients, 71.1% (95% CI: 61.0–79.1%), was significantly lower than that of Whites (82.4%, 95% CI 80.8–83.9%, W *vs* B: $P=0.0160$). The 5-year OS for Asian patients was between that of Whites and blacks and not significantly different from either of these ethnic groups (78.7%, 95% CI 66.7–86.7%). A 5-year DRFS was significantly lower in Blacks 62.8% (95% CI: 52.1–71.8%) than both Whites (77.0%, 95% CI: 75.3–78.6%) and Asians (77.0%, 95% CI: 65.1–85.3%; W *vs* B: $P=0.0053$; B *vs* A: $P=0.0473$). There was no significant difference in a 5-year DRFS between Whites and Asians (W *vs* A: $P=0.991$).

Use of a multivariate model to adjust DRFS for total tumour diameter, grade, nodal status and patient body mass index (BMI) in all patients confirms that Black ethnicity is a significant independent marker of poor prognosis with a hazard ratio of 1.50 (95% CI: 1.06–2.13, $P=0.023$, Table 5) compared with Whites. Separate multivariate analyses of ER-negative and positive tumours indicate that the independent prognostic power of Black ethnicity is no longer significant in ER-negative tumours when adjustments are made for total tumour diameter, grade, nodal status and patient BMI (HR: 1.31 Blacks, 95% CI: 0.73–2.36, $P=0.369$), although the direction of effect is still the same. However, in ER-positive patients, Black ethnicity remains an independent marker of poor prognosis (HR:1.60, 95% CI: 1.03–2.47, $P=0.035$).

DISCUSSION

The primary aim of the POSH prospective, multicentre study is to determine whether the prognosis of patients with breast cancer is altered by inherited genetic factors. The presenting characteristics, pathology, treatment and survival of this large cohort of early-onset breast cancer patients diagnosed and treated in the first decade of this century have recently been published (Copson *et al*, 2013); genetic analysis of the study cohort is in progress. The POSH study cohort provides a unique opportunity to compare the outcomes of different ethnic groups in an age group that is not eligible for breast screening and in a population that receives entirely public funded health care, thus eliminating these potentially confounding socio-economic factors. We present here the clinical course of Blacks, Asians and Whites recruited to this study.

Our data confirm conclusions from retrospective studies that Blacks have a tendency towards more biologically aggressive tumours with significantly larger tumours and increased incidence of triple-negative tumours, and trends towards increased frequency of grade 3 and node-positive tumours (Elledge *et al*, 1994; Dignam, 2000; Joslyn and West, 2000; Carey *et al*, 2006; Bowen *et al*, 2008). Our finding of a higher incidence of multifocal tumours in Blacks than Whites is in agreement with other data (Litton *et al*, 2007). The incidence of multifocal disease in all ethnic groups of this cohort was higher than in some comparable series; this is likely to reflect the fact that multi-focality was defined pathologically following surgery in this study, rather than radiologically before surgery. The presence of multifocal disease was not incorporated into our multivariate analysis as both the definition of multi-focality and the independent prognostic effect of this feature over and above total tumour diameter remain controversial (Coombs and Boyages, 2005).

All of our cohort were diagnosed and treated within the UK NHS according to local protocols and therefore had equal access to standard therapies. There was no difference in receipt of chemotherapy for early breast cancer between ethnic groups, unlike the data from Bickell *et al* (2006) who reported use of appropriate chemotherapy in only 67% of Blacks compared with 78% of Whites. The increased use of neo-adjuvant rather than adjuvant chemotherapy in Blacks in our cohort is likely to be due

Table 3. Treatment details

	All[a]: $n=2956$ (100.0%)	W: $n=2690$ (91.0%)	B: $n=118$ (4.0%)	A: $n=87$ (2.9%)	*P*-value[††]
Number of patients (%)					
Definitive surgery					
Breast conserving surgery	1409 (47.7%)	1294 (48.1%)	47 (39.8%)	42 (48.3%)	W *vs* B: $P<0.001$
Mastectomy	1497 (50.7%)	1355 (50.4%)	64 (54.2%)	44 (50.6%)	W *vs* A: $P=0.978$ (NS)
Nodal surgery only	9 (0.3%)	6 (0.2%)	2 (1.7%)	0 (0%)	B *vs* A: $P=0.255$ (NS)
No surgery	39 (1.3%)	33 (1.2%)	5 (4.2%)	1 (1.2%)	
Missing/unknown	2 (0.1%)	2 (0.1%)	0 (0%)	0 (0%)	
Chemotherapy timing					
Adjuvant[b]	2152 (72.8%)	1985 (73.8%)	77 (65.3%)	60 (69.0%)	W *vs* B: $P=0.007$
Neo-adjuvant	460 (15.6%)	397 (14.8%)	28 (23.7%)	18 (20.7%)	W *vs* A: $P=0.221$ (NS)
Palliative	54 (1.8%)	46 (1.7%)	5 (4.2%)	3 (3.5%)	B *vs* A: $P=0.942$ (NS)
Not applicable	290 (9.8%)	262 (9.7%)	8 (6.8%)	6 (6.9%)	
Chemotherapy regimen					
Anthracycline &/or taxane	2642 (89.4%)	2405 (89.4%)	110 (93.2%)	80 (92.0%)	W *vs* B: $P=0.239$ (NS)
Other[c]	24 (0.8%)	23 (0.9%)	0 (0%)	1 (1.2%)	W *vs* A: $P=0.653$ (NS)
None	290 (9.8%)	262 (9.7%)	8 (6.8%)	6 (6.9%)	B *vs* A: $P=0.505$ (NS)
Adjuvant trastuzumab					
Yes	363 (12.3%)	332 (12.3%)	9 (7.6%)	11 (12.6%)	—
Other treatment period/no/ missing/unknown	2593 (87.7%)	2358 (87.7%)	109 (92.4%)	76 (87.4%)	
Adjuvant radiotherapy					
Yes	2358 (79.8%)	2160 (80.3%)	87 (73.7%)	67 (77.0%)	—
No/missing/unknown	598 (20.2%)	530 (19.7%)	31 (26.3%)	20 (23.0%)	
ER-positive patients only	**All[a]: $n=1947$ (100.0%)**	**W: $n=1782$ (91.5%)**	**B: $n=73$ (3.8%)**	**A: $n=50$ (2.6%)**	
Adjuvant hormone treatment					
Yes	1725 (88.6%)	1591 (89.3%)	59 (80.8%)	42 (84.0%)	—
No/missing/unknown	222 (11.4%)	191 (10.7%)	14 (19.2%)	8 (16.0%)	
Ovarian suppression (in any treatment period) Medical (LHRH agonist)					
Yes	655 (33.6%)	605 (34.0%)	21 (28.8%)	11 (22.0%)	—
No/missing/unknown	1292 (66.4%)	1177 (66.1%)	52 (71.2%)	39 (78.0%)	
Irradiation					
Yes	11 (0.6%)	11 (0.6%)	0 (0%)	0 (0%)	—
No/missing/unknown	1936 (99.4%)	1771 (99.4%)	73 (100.0%)	50 (100.0%)	
Oophorectomy					
Yes	324 (16.6%)	307 (17.2%)	5 (6.9%)	5 (10.0%)	—
No/missing/unknown	1623 (83.4%)	1475 (82.8%)	68 (93.2%)	45 (90.0%)	

Abbreviations: A = Asian; B = Black; NS = not significant; W = White.
[a]Includes patients in an Other or Missing/unknown Ethnic group.
[b]Excluding any treatment for M1 disease.
[c]For example, CMF or anything not containing an anthracycline or taxane.
[††]*P*-values from Pearson's chi-squared test between ethnic groups and each categorical variable (excluding other ethnic groups and missing/unknown data).[‡‡]*P*-values from Mann–Whitney test between ethnic groups and each continuous variable (excluding Other Ethnic groups and missing/unknown data).

to the increased frequency of larger tumours in this ethnic group. Use of anthracyline/taxane combination chemotherapy was similar in each ethnic category, in contrast to Griggs *et al* (2007a) who reported increased use of non-standard chemotherapy in American Blacks . There are also reports that Blacks are more likely to receive reduced dose chemotherapy and to discontinue chemotherapy prematurely (Griggs *et al*, 2007b; Hershman *et al*, 2009). Such treatment modifications could reflect higher rates of co-morbidities in Blacks compared with Whites, although Blacks do not have increased rates of neutropenic complications despite lower baseline white blood counts (Tammemagi *et al*, 2005; Hershman *et al*, 2009). We currently have insufficient data to compare chemotherapy dose density in our cohort. However, increased mortality in Blacks compared with Whites enroled in SWOG chemotherapy

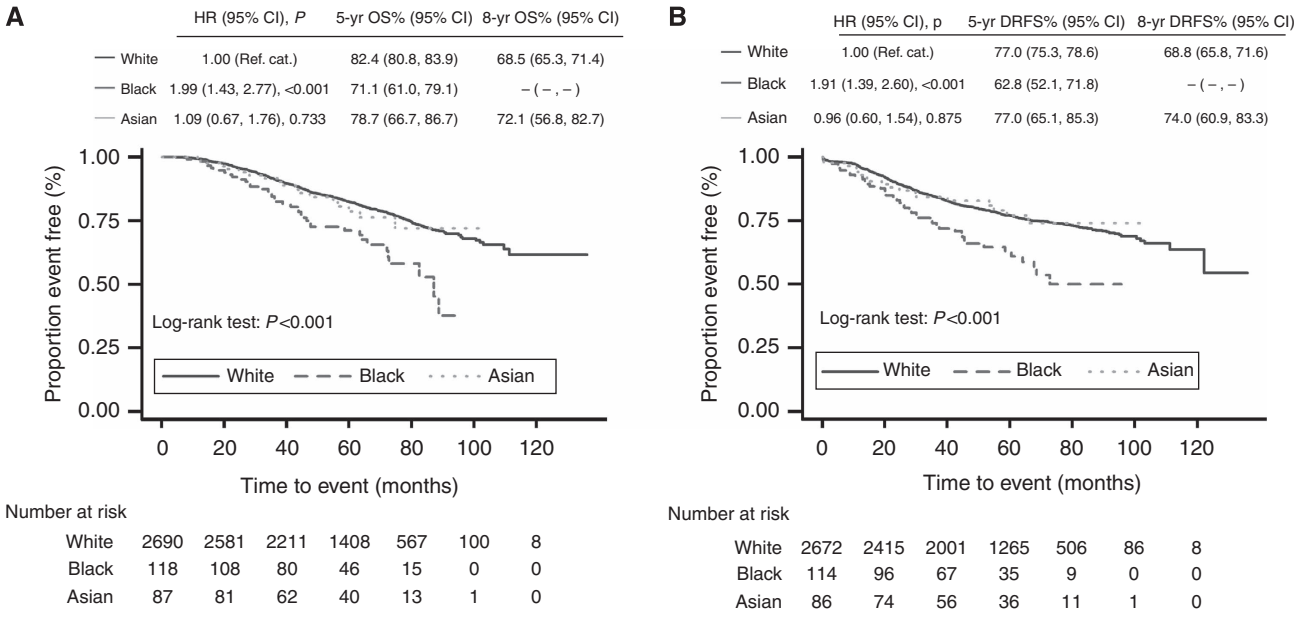

Figure 2. Kaplan–Meier (**A**) OS and (**B**) DRFS estimates for Caucasian/White, Black and Asian patients.

Table 4. OS and DRFS estimates at 5 and 8 years

	Kaplan–Meier estimates				Comparisons		
	All	**W**	**B**	**A**	**W *vs* B (W—B)**	**W *vs* A (W—A)**	**B *vs* A (B—A)**
Group of patients	**A 5-year OS% (95% CI), Number at risk,**				**A 5-year OS% difference (95% CI), *P*-value**		
All patients	81.9 (80.3, 83.3), 1511	82.4 (80.8, 83.9), 1408	71.1 (61.0, 79.1), 46	78.7 (66.7, 86.7), 40	11.3 (2.1, 20.5), $P=0.0160$	3.7 (−6.2, 13.7), $P=0.471$	−7.5 (−20.9, 5.9), $P=0.273$
	A 8-year OS% (95% CI), Number at risk,				**A 8-year OS% difference (95% CI), *P*-value**		
All patients	67.6 (64.5, 70.4), 149	68.5 (65.3, 71.4), 145	− (− , −), 0	72.1 (56.8, 82.7), 2	—	−3.6 (−16.8, 9.6), $P=0.607$	—
	A 5-year DRFS% (95% CI), Number at risk,				**A 5-year DRFS % difference (95% CI), *P*-value**		
All patients	76.5 (74.8, 78.1), 1353	77.0 (75.3, 78.6), 1265	62.8 (52.1, 71.8), 35	77.0 (65.1, 85.3), 36	14.2 (4.2, 24.2), $P=0.0053$	0.1 (−10.0, 10.2), $P=0.991$	−14.2 (−0.1, −28.2), $P=0.0473$
ER negative	72.5 (69.5, 75.3), 444	73.0 (69.8, 75.9), 411	58.0 (40.4, 72.0), 12	80.1 (62.6, 90.1), 17	15.0 (−1.3, 31.4), $P=0.0716$	−7.2 (−20.8, 6.5), $P=0.307$	−22.2 (−43.0, −1.3), $P=0.0368$
ER Positive	78.5 (76.5, 80.4), 907	79.1 (77.0, 81.0), 852	65.2 (51.0, 76.1), 23	74.5 (56.9, 85.8), 19	13.9 (1.1, 26.7), $P=0.0326$	4.5 (−9.9, 18.9), $P=0.551$	−9.4 (−28.4, 9.7), $P=0.340$
Triple Negative	73.3 (69.1, 77.0), 214	72.8 (68.4, 76.8), 191	65.8 (43.9, 80.8), 10	94.7 (68.1, 99.2), 10	7.1 (−12.0, 26.1), $P=0.478$	−21.9 (−32.8, −11.0), $P<0.001$	−29.0 (−50.1, −7.8), $P=0.0073$
	A 8-year DRFS% (95% CI), Number at risk,				**A 8-year DRFS % difference (95% CI), *P*-value**		
All patients	68.3 (65.5, 71.0), 130	68.8 (65.8, 71.6), 126	− (− , −), 0	74.0 (60.9, 83.3), 2	—	−5.2 (−16.7, 6.3), $P=0.382$	—
ER negative	68.1 (63.8, 72.1), 43	68.6 (64.1, 72.6), 42	− (− , −), 0	− (− , −), 0	—	—	—
ER positive	68.3 (64.5, 71.8), 87	68.8 (64.8, 72.4), 84	− (− , −), 0	74.5 (56.9, 85.8), 2	—	−5.8 (−20.5, 9.0), $P=0.454$	—
Triple negative	70.5 (65.5, 74.9), 15	69.9 (64.6, 74.5), 14	− (− , −), 0	− (− , −), 0	—	—	—

Abbreviations: W = White; A = Asian; B = Black; CI = confidence interval; DRFS = distant recurrence-free survival; OS = overall survival.

studies has been demonstrated despite similar relative dose intensity of adjuvant chemotherapy (Hershman *et al*, 2009).

As anticipated, the major cause of death in this cohort was breast cancer. Our data show clearly that both DRFS and OS were significantly lower in Blacks than Whites. This is in agreement with the findings of a number of previous USA and UK studies (Joslyn and West, 2000; Jatoi *et al*, 2003; Carey *et al*, 2006; Grann *et al*, 2006; Wild *et al*, 2006; Jack *et al*, 2009; Albain *et al*, 2009). Data on the association between Asian ethnicity and breast cancer prognosis are more limited, but our data are consistent with other studies showing no significant difference in the outcome of Whites and Asians (Wild *et al*, 2006).

Our multivariate analyses indicate that the inferior outcomes of Blacks are not fully explained by an increased frequency of adverse

Table 5. Multivariate Analyses—DRFS

		Unadjusted[a]			Adjusted[b]		
Group of patients	**Ethnic category**	**N[c]**	**HR (95% CI)**	***P*-value**	**N[d]**	**HR (95% CI)**	***P*-value**
All patients	W	2872	1 *(Reference cat.)*	—	2581	1 *(Reference cat.)*	—
	B		1.91 (1.39, 2.60)	<0.001		1.50 (1.06, 2.13)	0.023
	A		0.96 (0.60, 1.54)	0.875 (NS)		0.85 (0.48, 1.51)	0.578 (NS)
ER-negative patients only	W	974	1 *(Reference cat.)*	—	862	1 *(Reference cat.)*	—
	B		1.73 (1.04, 2.87)	0.034		1.31 (0.73, 2.36)	0.369 (NS)
	A		0.82 (0.40, 1.65)	0.575 (NS)		0.76 (0.31, 1.85)	0.546 (NS)
ER-positive patients only	W	1888	1 *(Reference cat.)*	—	1716	1 *(Reference cat.)*	—
	B		2.03 (1.36, 3.02)	<0.001		1.60 (1.03, 2.47)	0.035
	A		1.04 (0.55, 1.94)	0.910 (NS)		0.90 (0.42, 1.90)	0.776 (NS)
Triple negative patients only	W	524	1 *(Reference cat.)*	—	477	1 *(Reference cat.)*	—
	B		1.39 (0.71, 2.73)	0.340 (NS)		1.18 (0.52, 2.69)	0.693 (NS)
	A		0.19 (0.03, 1.33)	0.094 (NS)		0.28 (0.04, 2.03)	0.209 (NS)

Abbreviations: A = Asian; B = Black; CI = Confidence Interval; DRFS = Distant Recurrence-Free Survival; W = White.
[a]Univariate analyses: results obtained by fitting a Cox model with ethnic grouping as the only covariate.
[b]Multivariate analyses: results obtained by fitting a Cox model with ethnic grouping as a covariate and adjusting for body mass index, tumour grade, size and nodal status.
[c]Number of Caucasian/White, Black and Asian patients.
[d]Number of Caucasian/White, Black and Asian patients with complete data for body mass index, tumour grade, size and nodal status.

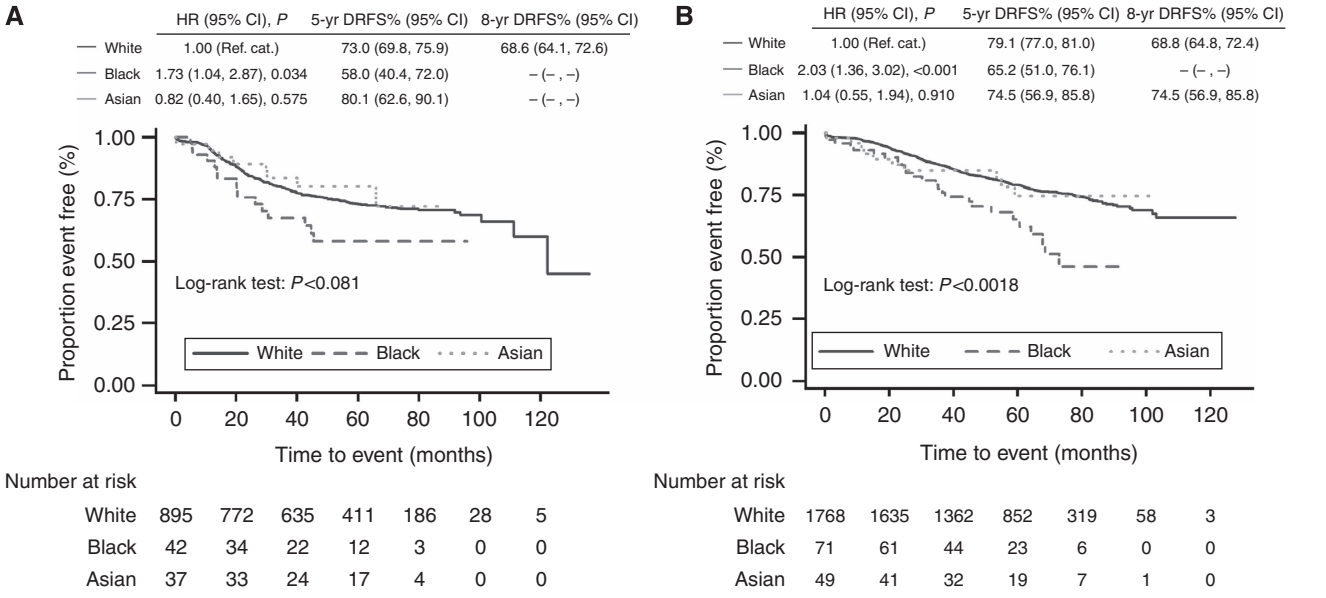

Figure 3. Kaplan–Meier DRFS estimates for Caucasian/White, Black and Asian (**A**) ER-negative patients and (**B**) ER-positive patients.

pathological features. In particular, our separate analyses of ER-positive, ER-negative and triple-negative patients confirm previous reports that the poor prognosis of Blacks is not fully explained by the increased incidence of triple-negative tumours (Albain *et al*, 2009), although we cannot entirely exclude other confounding biological factors. Our analysis is based on biological features obtainable from routine histopathological review; it is feasible that our results could be explained by differences in tumour gene expression profiles. An excess of luminal B tumours (a subtype of ER-positive breast cancer defined by increased proliferation, relative resistance to chemotherapy compared with other highly proliferative breast cancers, and poor outcome with endocrine therapy) in Blacks could, for example, explain our findings, (Perou *et al*, 2000). Alternatively, it is well established that breast cancers diagnosed during or within a year of pregnancy tend to be more aggressive than cancers in nulliparous women (reviewed by Azim *et al* (2012)), and it is possible that our results could be explained by a higher number of pregnancy associated tumours in Blacks than other ethnic groups. Our data set does not permit us to make direct assessments about the number of pregnancy-related cancers in our cohort, as pregnancy was assessed as a risk factor for breast cancer rather than a potential prognostic factor, and we therefore lack data on the date of second and subsequent pregnancies. However, the fact that Blacks who had already started their families by the time of their diagnosis were more likely to have a larger number of children than Whites suggests that Blacks would have spent more time being pregnant before their cancer diagnosis and could therefore have been at a higher risk of pregnancy-related breast cancer.

Although previous publications have reported persistence of ethnicity as an independent prognostic factor in both ER-negative and positive patients after adjustment for other pathological

factors, we found this in ER-positive patients only. Albain *et al* (2009) also found a greater hazard ratio in Black premenopausal ER-positive patients than ER-negative patients (1.74 *vs* 1.29), and Hershman *et al* (2009) commented that their finding of no interaction between race and tumour ER status should 'not be overinterpreted' given their small sample size and the long survival of their non-age selected patients . The small number of events in our ER-negative Black patients may affect our ability to demonstrate an independent effect of ethnicity in ER-negative patients. However, our finding that Black ethnicity is particularly an independent prognostic factor particularly in young ER-positive patients could suggest that either the use or effectiveness of hormonal therapy may vary significantly between ethnic groups. Bickell *et al* (2006) reported significantly lower use of adjuvant hormonal therapy in Blacks (71%) than Whites (80%) in women treated in America. Unexpectedly, we also found a lower percentage of ER-positive Black patients (80.8%) treated with hormonal therapy than White patients (89.3%); however, we cannot confirm that this is a statistically significant difference owing to missing data. It has been reported that compliance with tamoxifen is significantly lower in non-Whites than Whites (Partridge *et al*, 2003). Reduced compliance with hormonal therapy remains a possible explanation for our observations, although we did not collect compliance data. Our finding that more oophorectomies were performed in White than Asian or Black women could reflect a higher proportion of identified BRCA mutations in our Whites than Black or Asian patients as reported elsewhere (Ademuyiwa and Olopade, 2003).

Pharmocogenetics may also have a role in ethnic populations with different genetic structure. Some CYP2D6 variants associated with 'poor metabolism' of tamoxifen are more common in Blacks than other ethnic groups (Bradford, 2002; Gaedigk *et al*, 2002). However, it is currently controversial whether CYP2D6 genotype directly affects breast cancer survival in patients on adjuvant tamoxifen (Abraham *et al*, 2010). Albain *et al* (2009) reported inferior outcome in Blacks compared with Whites in other 'sex-specific' cancers as well as breast cancer . This suggests that other hormonal influences could interact with genetic factors. However, studies of the association between polymorphisms of the *CYP1A1* gene (involved in oestrogen metabolism) and risk of breast cancer in African-Americans have been inconclusive (Taioli *et al*, 1995; Bailey *et al*, 1998).

Many publications have examined the role of socio-economic factors in the presentation and outcome of malignancies. In a systematic review of mostly American studies, socio-economic position was been found to explain part of the variation in OS between ethnic groups but did not account for differences in breast cancer survival (McKenzie and Jeffreys, 2009). Other social issues may also cause disparities in breast cancer outcome in different ethnic groups. McKenzie and Jeffreys (2009) noted that, 'although there is a lack of major systemic genetic differences between ethnic groups, there are extensive differences in lifestyle'. Their systematic review however found little evidence to indicate that smoking or alcohol use could explain the inferior survival of Blacks compared with Whites, unlike BMI which did explain some of this variation (McKenzie and Jeffreys, 2009).

Health systems such as the UK NHS are designed to provide equal access to health care; however, this does not automatically equate to equal use of health care (Forbes *et al*, 2011). Recent immigration is frequently associated with linguistic and cultural challenges, which may act as barriers to accessing health care. It has also been reported that Blacks are less aware of symptoms of breast cancer than other groups and are less likely to self-check (Forbes *et al*, 2011). Therefore, the increased average tumour size may reflect a cultural tendency to delay presentation, as all our patients were below the minimum age for breast cancer screening. However, there was no significant difference between the rates of nodal involvement in the different ethnic groups in our cohort. Details of follow-up routines were not collected in this study; however, the independent effect of ethnicity persists when limiting analyses to hospitals treating both White and Black patients (data not shown). Clearly, our cohort contains a much smaller number of Black patients than in many of the American published series. However, the proportion of Black patients in the POSH cohort is very similar to the English population as a whole (2.9%). English cancer registry data for 2007 includes ethnicity data on only 80% of patients but indicate that 3.8% of breast cancers diagnosed in under 50 year olds were in Black women (National Cancer Intelligence Network, 2011), suggesting that our data are representative of the premenopausal English population. The average age of diagnosis is lower in Black women than Caucasians in the United Kingdom as elsewhere (Bowen *et al*, 2008). We cannot exclude selection bias; patients who agree to participate in clinical trials may not fully represent the general population. However, previous work indicates these patients may be more compliant with treatment than non-trial patients (Antman *et al*, 1985).

Categorisation of patients into broad ethnic categories on the basis of self-reported ethnicity is a simplification of a very complex picture. We have not attempted to differentiate between Blacks of African and Caribbean descent because of small patient numbers. However, previous data suggest that there is disparity in the outcome of these two groups (Wild *et al*, 2006). The complexities of categorising ethnicity and the controversies associated with analysing data from individuals with mixed ethnicity have been highlighted previously (Agyemang *et al*, 2005; Aspinall, 2011); however, here we have been transparent in our management of these data. Place of birth may provide additional information about genetic ancestry (Ingleby, 2008).

Other limitations of this study include the low number of ER-negative patients in the Black and Asian groups. The target sample size of the POSH study ($n = 3000$) was calculated to detect a 10% difference in event rates between BRCA mutation carriers and sporadic early-onset breast cancer patients. It is likely that this study was underpowered to demonstrate an independent effect of ethnicity in ER-negative patients. Incomplete data on HER2 status reflect the time course of recruitment to this study, with routine HER2 testing largely being introduced from 2005 onwards, while missing PR data are largely accounted for by the fact that some of the recruiting hospitals did not routinely assess PR status during the study period. In addition, inconsistencies in the reporting of trastuzumab use, hormonal therapy and ovarian suppression have resulted in missing data, which has precluded a formal analysis of the use of these treatments in different ethnic groups. We have also not attempted to classify the socio-economic status of patients, as the POSH study did not collect data on income or education.

However, the POSH cohort is the first prospective study of young breast cancer patients treated within the UK NHS and, unlike previous registry based retrospective series, includes extensive data on treatment as well as pathology. This analysis of the effect of ethnicity on breast cancer outcome is strengthened by our use of a pre-specified analysis plan and STROBE reporting guidelines. Ethnicity was also directly self-reported by study participants, rather than inferred from other information, and we have been explicit in our categorisation of ethnic groups. Our data also benefits from the fact that all patients received 'modern' oncological therapies, in contrast to some historical registry studies.

We have recently published a comprehensive description of the entire POSH cohort, which confirms the poor medium term outcome of both ER-positive and ER-negative patients aged 40 years or under at the time of diagnosis (Copson *et al*, 2013). The cause for the poor outcome of young breast cancer patients currently remains controversial; the POSH study ultimately aims to

determine whether this is in part due to underlying inherited genetic mutations.

This publication from the POSH study provides valuable confirmation that Black ethnicity is an independent marker of poor prognosis in this young age group. Further research is clearly required to establish whether the effect of Black ethnicity is indeed more marked in ER-positive than ER-negative disease, and if so whether this is related to the use or effectiveness of Tamoxifen. In addition, there is a need to clarify the contribution of socio-economic position, education and breast cancer awareness to outcome of early breast cancer in different ethnic groups in the United Kingdom. The fact that almost 50% of patients in all ethnic groups presented with tumours $\geqslant 2.0$ cm does of course raise questions about the need for screening in younger women. However, as robust evidence for screening mammography in this group does not exist, and there are demonstrable differences in breast awareness between different ethnic groups then improving education and understanding of breast cancer in these populations may be effective in reducing the differences observed in tumour size in this study (Forbes *et al,* 2011).

CONCLUSION

We present the first prospective study of young breast cancer patients in the United Kingdom to analyse outcome data according to ethnicity. Our results confirm that Black patients have an increased risk of breast cancer recurrence than Whites despite equal access to health care including adjuvant therapies. Black ethnicity is an independent indicator of poor prognosis in young women with invasive breast cancer, suggesting that current treatment approaches may be less effective in this population. Further studies are required to investigate this in more detail and to optimise the management of this patient group.

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CONFLICT OF INTEREST

EC has received honoraria from Roche and RIC has received honoraria from GSK and Pfizer. RE has received educational support from Vista Diagnostics, Tepnel (now GenProbe), Illumina and Janssen-Cilag. The remaining authors declare no conflict of interest.

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APPENDIX

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SUPPLEMENTARY MATERIAL

Supplementary Document 1

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Chapter 5: Paper 3 - Obesity and the outcome of young breast cancer patients in the UK: the POSH study

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5.1 Contribution

As the lead statistical methodologist and scientific lead at the SCTU:

- Responsible for study methodology and research of all statistical methods to be implemented in the analyses;
- Developed and authored the SAP – including the organisation and participation of meetings to develop the SAP, and the creation of all draft and final versions of the SAP;
- Responsible for central data monitoring, data cleaning and data interpretation – liaising with the study team to identify data queries, and for the resolution of all data queries raised with data management;
- Developed and conducted all statistical programming and analyses – including the creation of all database and analysis programming required for the analyses;
- Designed, developed and produced all manuscript figures and tables – including the creation of all draft and final versions of the manuscript figures and tables;
- Co-authored and reviewed the manuscript – listed as 3rd named author, drafted the statistical methods section of the manuscript, involved in the interpretation of results, reviewed the entire manuscript including sense checks and result checking, involved in the resolution of reviewer comments and responses.

Professor Diana Eccles, chief investigator of the POSH study, chair of the POSH Study Steering Group, and senior last author of **Paper 3**, confirms the accuracy of contribution outlined above of Thomas Christopher Maishman.

Signed: .....

Date: .....

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Obesity and the outcome of young breast cancer patients in the UK: the POSH study

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Background: Obese breast cancer patients have a poorer prognosis than non-obese patients. We examined data from a large prospective cohort study to explore the associations of obesity with tumour pathology, treatment and outcome in young British breast cancer patients receiving modern oncological treatments.

Patients and methods: A total of 2956 patients aged ≤40 at breast cancer diagnosis were recruited from 126 UK hospitals from 2001 to 2007. Height and weight were measured at registration. Tumour pathology and treatment details were collected. Follow-up data were collected at 6, 12 months, and annually.

Results: A total of 2843 eligible patients (96.2%) had a body mass index (BMI) recorded: 1526 (53.7%) were under/healthy-weight (U/H, BMI <25 kg/m^2), 784 (27.6%) were overweight (ov, BMI ≥25 to <30), and 533 (18.7%) were obese (ob, BMI ≥30). The median tumour size was significantly higher in obese and overweight patients than U/H patients (Ob 26 mm versus U/H 20 mm, $P < 0.001$; Ov 24 mm versus U/H 20 mm, $P < 0.001$). Obese and overweight patients had significantly more grade 3 tumours (63.9% versus 59.0%, $P = 0.048$; Ov 63.6% versus U/H 59.0% $P = 0.034$) and node-positive tumours (Ob 54.6% versus U/H 49.0%, $P = 0.027$; Ov 54.2% versus U/H 49%, $P = 0.019$) than U/H patients. Obese patients had more ER/PR/HER2-negative tumours than healthy-weight patients (25.0% versus 18.3%, $P = 0.001$). Eight-year overall survival (OS) and distant disease-free interval (DDFI) were significantly lower in obese patients than healthy-weight patients [OS: hazard ratio (HR) 1.65, $P < 0.001$; DDFI: HR 1.44, $P < 0.001$]. Multivariable analyses adjusting for tumour grade, size, nodal, and HER2 status indicated that obesity was a significant independent predictor of OS and DDFI in patients with ER-positive disease.

Conclusions: Young obese breast cancer patients present with adverse tumour characteristics. Despite adjustment for this, obesity still independently predicts DDFI and OS.

Key words: obesity, breast cancer, prognosis

introduction

Obesity is a significant risk factor for post-menopausal breast cancer [1]. Studies indicate that obesity does not increase the risk of developing pre-menopausal breast cancer [2, 3]. However, there is increasing evidence that a high body mass index (BMI) is associated with poorer outcomes in breast cancer patients of all ages [4, 5]. A recent meta-analysis of 82 studies (not including these data) reported that obese women had poorer overall survival (OS) [hazard ratio (HR) 1.41] than non-obese women, with a more marked effect in pre-menopausal (OS HR 1.75) than post-menopausal women (OS HR 1.34) [4].

The underlying reason for this association is not clear. Patients with a high BMI tend to present with larger tumours and some studies report more biologically adverse features including grade 3 tumours and nodal involvement in obese patients [6, 7]. Patients with a high BMI may also receive less effective treatment for early breast cancer. Ewertz et al. [7] reported that chemotherapy and endocrine therapy were less effective in patients with BMIs of ≥30 kg/m^2 and a recent review suggests that up to 40% of obese cancer patients may receive suboptimal chemotherapy doses [8].

At least 26% of British women and 35% of American women are currently obese. These figures are predicted to increase to

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43% and 52% by 2030 [9]. Furthermore, almost 50% of UK women aged 24–45 and 56% of American women aged 20–39 are overweight or obese [10, 11]. The POSH study is a prospective observational study of patients aged <41 years with breast cancer, diagnosed and treated in the UK [12]. This cohort of almost 3000 patients diagnosed between 2001 and 2008 represents, to the best of our knowledge, the largest prospective study of young breast cancer patients to date. Patients' height and weight were measured at registration. In this *post hoc* analysis, we describe the associations of BMI with tumour pathology, treatment, and outcome in these patients.

patients and methods

POSH is a multicentre prospective observational cohort study of young women diagnosed with breast cancer in the UK between 2000 and 2008 (http://www.southampton.ac.uk/medicine/research/posh.page).

The detailed study protocol was published in 2007 [12]. This study received approval from the South West Multi-centre Research Ethics Committee (MREC 00/6/69).

patients

Female patients were recruited from 127 UK hospitals. All patients were diagnosed with invasive breast cancer between 2000 and 2008 at an age of 40 years or younger, as previously described [12, 13].

study variables and data sources

Details of personal characteristics, tumour pathology, disease stage, and treatment received were collected from medical records. Pathology and imaging data were verified with copies of original reports from sites. For patients treated with neoadjuvant chemotherapy, initial tumour diameter was derived from radiological reports. Family history and personal risk factors were collected using a questionnaire completed by participants at recruitment. BMI was calculated from height and weight measured at recruitment. Patients were stratified into World Health Organisation (WHO) defined BMI categories under or healthy weight (BMI<25 kg/m^2), overweight (25≤ BMI <30 kg/m^2), and obese (BMI ≥30 kg/m^2) [14].

Detailed clinical follow-up data, including date and site of disease recurrence, were obtained from medical records at 6, 12 months, and at yearly intervals post-diagnosis until death or loss to follow-up. Patients were flagged in the NHS Medical Research Information Service to facilitate automatic notification of date and cause of death. This paper presents analyses conducted on follow-up data received until 22 October 2013.

tumour receptor status data

ER, PR, and HER2 receptor status of primary tumours were primarily determined from routine diagnostic pathology tests. Hormone receptor levels equivalent to an Allred score of ≥3 were categorized as positive.

statistical analysis

Details of the target sample size (3000) are reported in the protocol [12]. The statistical analysis was conducted according to a pre-specified plan and as recommended by STROBE (STrengthening the Reporting of Observational Studies in Epidemiology) guidelines [15]. Analyses were carried out in STATA v11·2 on records with complete data (levels of missingness were reported). Summary statistics were used to describe the cohort. Where appropriate, the Pearson χ^2, Kruskal–Wallis, or Mann–Whitney tests were carried out to identify any specific differences in the characteristic variables between BMI categories.

OS and distant disease-free interval (DDFI) were assessed using the Kaplan–Meier curves. These were defined as time from date of invasive breast cancer diagnosis to death from any cause (OS), and to distant relapse or death from breast cancer (DDFI). Patients who had not experienced an event at the time of analysis were censored at their date of last follow-up. As the effect of ER status on survival varies over time, the multi-variable analyses (MVA) models were stratified by ER status [13, 16]. MVA were carried out using the Cox regression to adjust for age at diagnosis and tumour-related factors known to affect prognosis (grade, total tumour diameter, nodal status, and HER2 status) according to our original statistical analysis plan. Following our publication of data demonstrating that race has an independent effect on the outcome of patients in the POSH cohort, we carried out additional MVA including race as an adjustment factor [17].

results

The POSH study recruited 3095 patients across England (2695), Scotland, Wales, and Northern Ireland. After excluding 252 trial participants (supplementary Figure S1, available at *Annals of Oncology* online), 2843 patients were included in this analysis.

One thousand five hundred and twenty-six patients (53.7%) were under or healthy weight (U/H) including 36 patients with a BMI of ≤18.5 kg/m^2, 784 (27.6%) were overweight, and 533 (18.7%) were obese. The median age was significantly lower in U/H patients than overweight (U/H 36 versus Ov 37, $P = 0.0063$) and obese patients (U/H 36 versus Ob 37, $P = 0.0322$). The median follow-up was 5.87 years for the entire cohort but was significantly longer in U/H patients than overweight (U/H 5.98 versus Ov 5.79, $P = 0.0023$) and obese patients (U/H 5.98 versus Ob 5.62, $P = 0.0014$). Table 1 demonstrates patient demographics and breast cancer risk factors in the different BMI categories.

pathology

The median tumour size was significantly higher in obese and overweight patients than U/H patients Ob 26 mm versus U/H 20 mm, $P < 0.001$. Ov 24 mm versus U/H 20 mm, $P < 0.001$). Obese patients and overweight patients had significantly more grade 3 tumours (Ob 63.9% versus U/H 59.0%, $P = 0.048$; Ov 63.6% versus U/H 59.0% $P = 0.034$) and node-positive tumours (Ob 54.6% versus U/H 49.0%, $P = 0.027$; Ov 54.2% versus U/H 49%, $P = 0.019$) than U/H patients. ER-negative tumours were significantly more frequent in obese patients than U/H patients (40.1% versus 31.7%, $P < 0.0001$), whereas the proportion of HER-positive tumours was similar across patient groups (P = NS). ER/PR/HER2 triple negative tumours were more frequent in obese than U/H (25.0% versus 18.3%, $P = 0.001$) or overweight patients (25% versus 19.4%, $P = 0.020$) (Table 2).

treatment

Most patients had surgical treatment (Table 1). The distribution across definitive surgery types is significantly different between obese and U/H patients ($P = 0.021$) with rates of breast conserving surgery higher in obese patients (49.3%) compared with U/H patients (46.3%). A higher proportion of obese patients received neoadjuvant chemotherapy (22.1%) than overweight (15.4%) or U/H patients (12.9%). More obese and overweight patients received anthracyline/taxane chemotherapy than U/H patients.

Table 1. Patients' characteristics, risk factors, and treatment details

Characteristic	All[a] [n = 2843 (100%)]	Underweight/healthy (U/H), BMI<25, n = 1526 (53.7%)	Overweight (Ov), 25≤BMI<30, n = 784 (27.6%)	Obese, BMI≥30, n = 533 (18.8%)	P-value[‡‡] (Kruskal–Wallis and Mann–Whitney tests)
	Median (range, IQR), number of patients				
Age at diagnosis (years)	36 (18–40, 33–38), 2843	36 (18–40, 33–38), 1526	37 (18–40, 34–39), 784	37 (24–40, 34–39), 533	Kwallis test: $P = 0.0091$ Mann–Whitney: U/H versus Ov: $P = 0.0063$ U/H versus Obese: $P = 0.0322$ Ov versus Obese: $P = 0.851$ (NS)
Duration of follow-up (months)	5.87 (0.12–13.23, 4.44–7.46) , 2843	5.98 (0.12–13.23, 4.63–7.69), 1526	5.79 (0.69–11.65, 4.13–7.22), 784	5.62 (0.38–11.97, 4.14–7.23), 533	Kwallis test: $P = 0.0005$ Mann–Whitney: U/H versus Ov: $P = 0.0023$ U/H versus Obese: $P = 0.0014$ Ov versus Obese: $P = 0.637$ (NS)
	(Risk factor characteristics)				
Age at menarche (years)	13 (8–18, 12–14), 2843	13 (9–18, 12–14), 1526	13 (8–18, 12–13), 784	13 (8–18, 11–13), 533	Kwallis test: $P < 0.001$ Mann–Whitney: U/H versus Ov: $P < 0.001$ U/H versus Obese: $P < 0.001$ Ov versus Obese: $P < 0.001$
Age at first birth (years)	27 (13–40, 23–30), 2018	27 (14–40, 23–30), 1043	27 (13–39, 23–30), 584	25 (15–38, 21–29), 391	Kwallis test: $P < 0.001$ Mann–Whitney: U/H versus Ov: $P = 0.552$ (NS) U/H versus Obese: $P < 0.001$ Ov versus Obese: $P < 0.001$
Missing/unknown	825 (29.0%)	483 (31.7%)	200 (25.5%)	142 (26.6%)	
Number with children	2040 (72.1%)	1059 (69.7%)	589 (75.6%)	392 (74.0%)	All BMI categories: $P = 0.007$
Number without children	788 (27.9%)	460 (30.3%)	190 (24.4%)	138 (26.0%)	U/H versus Ov: $P = 0.003$
Missing/unknown	15 (0.5%)	7 (0.5%)	5 (0.6%)	3 (0.6%)	U/H versus Obese: $P = 0.064$ Ov versus Obese: $P = 0.500$ (NS)
	Number of patients (%)				P-value[††] (Pearson χ^2 test)
Presentation:					All BMI categories: $P = 0.572$ (NS)
Symptomatic	2797 (98.6%)	1498 (98.4%)	775 (99.1%)	524 (98.5%)	
Screen detected	28 (1.0%)	17 (1.1%)	6 (0.8%)	5 (0.9%)	
Other	12 (0.4%)	8 (0.5%)	1 (0.1%)	3 (0.6%)	
Missing/unknown	6 (0.2%)	3 (0.2%)	2 (0.3%)	1 (0.2%)	
Race:					All BMI categories: $P = 0.005$
Caucasian/White	2610 (92.5%)	1406 (93.1%)	718 (91.9%)	486 (91.7%)	Underweight/Healthy versus
Black	113 (4.0%)	46 (3.1%)	32 (4.1%)	35 (6.6%)	Overweight: $P = 0.621$ (NS)
Asian	79 (2.8%)	47 (3.1%)	25 (3.2%)	7 (1.3%)	Underweight/Healthy versus
Other	19 (0.7%)	11 (0.7%)	6 (0.8%)	2 (0.4%)	Obese: $P < 0.001$
Missing/unknown	22 (0.8%)	16 (1.1%)	3 (0.4%)	3 (0.6%)	Overweight versus Obese: $P = 0.026$

Continued

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Table 1. *Continued*

Characteristic	All[a] [*n* = 2843 (100%)]	Underweight/healthy (U/H), BMI<25, *n* = 1526 (53.7%)	Overweight (Ov), 25≤BMI<30, *n* = 784 (27.6%)	Obese, BMI≥30, *n* = 533 (18.8%)	*P*-value[‡‡] (Kruskal–Wallis and Mann–Whitney tests)
Use of contraceptive pill					All BMI categories: *P* = 0.986 (NS)
Ever	2512 (88.4%)	1348 (88.3%)	692 (88.3%)	472 (88.6%)	
Never	331 (11.6%)	178 (11.7%)	92 (11.7%)	61 (11.4%)	
Smoker					All BMI categories: *P* = 0.915 (NS)
Ever	1409 (51.0%)	758 (51.2%)	391 (51.3%)	260 (50.2%)	
Never	1353 (49.0%)	724 (48.9%)	371 (48.7%)	258 (49.8%)	
Missing/unknown	81 (2.9%)	44 (2.9%)	22 (2.8%)	15 (2.8%)	
Menopausal status					All BMI categories: *P* = 0.269 (NS)
Premenopausal	2784 (99.6%)	1490 (99.4%)	775 (99.9%)	519 (99.6%)	
Perimenopausal	5 (0.2%)	5 (0.3%)	0	0	
Postmenopausal	7 (0.3%)	4 (0.3%)	1 (0.1%)	2 (0.4%)	
Missing/unknown	47 (1.7%)	25 (1.8%)	8 (1.0%)	12 (2.3%)	
No. of patients with 1st or 2nd degree relatives with breast cancer					All BMI categories: *P* = 0.429 (NS)
1st degree					
2nd degree	399 (14.5%)	231 (15.5%)	104 (13.8%)	64 (12.4%)	
Missing/unknown	539 (19.6%)	297 (20.0%)	138 (18.3%)	104 (20.2%)	
	86 (3.0%)	39 (2.6%)	29 (3.7%)	18 (3.4%)	
	(Treatment details)				
Definitive surgery					All BMI categories: *P* = 0.024
Breast conserving surgery	1345 (47.3%)	706 (46.3%)	376 (48.0%)	263 (49.3%)	U/H versus Ov: *P* = 0.679 (NS)
Mastectomy	1452 (51.1%)	798 (52.3%)	401 (51.2%)	253 (47.5%)	U/H versus Obese: *P* = 0.021
Nodal surgery only	36 (1.3%)	17 (1.1%)	6 (0.8%)	13 (2.4%)	Ov versus Obese: *P* = 0.015
No surgery	9 (0.3%)	4 (0.3%)	1 (0.1%)	4 (0.8%)	
Missing/unknown	1 (0.04%)	1 (0.07%)	0	0	
Chemotherapy timing					All BMI categories: *P* < 0.0001
Adjuvant[b]	2117 (74.5%)	1147 (75.2%)	596 (76.0%)	374 (70.2%)	U/H versus Ov: *P* = 0.019
Neo-adjuvant	436 (15.3%)	197 (12.9%)	121 (15.4%)	118 (22.1%)	U/H versus Obese: *P* < 0.001
Palliative	49 (1.7%)	24 (1.6%)	14 (1.8%)	11 (2.1%)	Ov versus Obese: *P* = 0.018
Not applicable	241 (8.5%)	158 (10.4%)	53 (6.8%)	30 (5.6%)	
Chemotherapy regimen					All BMI categories: *P* = 0.001
Anthracycline and/or taxane	2579 (90.7%)	1355 (88.9%)	728 (92.9%)	496 (93.1%)	U/H versus Ov: *P* = 0.007
Other[c]	23 (0.8%)	13 (0.9%)	3 (0.4%)	7 (1.3%)	U/H versus Obese: *P* = 0.003
None	241 (8.5%)	158 (10.4%)	53 (6.8%)	30 (5.6%)	Ov versus Obese: *P* = 0.119 (NS)
Adjuvant trastuzumab					—
Yes	347 (12.2%)	191 (12.5%)	89 (11.4%)	67 (12.6%)	
Other treatment period/no/missing/unknown	2496 (87.8%)	1335 (87.5%)	695 (88.7%)	466 (87.4%)	
Adjuvant radiotherapy					—
Yes	2276 (80.1%)	1213 (79.5%)	629 (80.2%)	434 (81.4%)	
No/missing/unknown	567 (19.9%)	313 (20.5%)	155 (19.8%)	99 (18.6%)	

ER-positive patients only	All	U/H	Overweight	Ob	
	n = 1867 (100%)	BMI<25	25≤BMI<30	BMI≥30	
		n = 1040 (55.7%)	*n* = 509 (27.3%)	*n* = 318 (17.0%)	
	Number of patients (%)				
Adjuvant hormone treatment					—
Yes	1664 (89.1%)	934 (89.8%)	453 (89.0%)	277 (87.1%)	
No/missing/unknown	203 (10.9%)	106 (10.2%)	56 (11.0%)	41 (12.9%)	
Ovarian suppression (in any treatment period)					
Medical (LHRH agonist)					
Yes	414 (22.2%)	239 (23.0%)	1 03 (20.2%)	1 72 (22.6%)	—
No/missing/unknown	1453 (77.8%)	801 (77.0%)	406 (79.8%)	246 (77.4%)	
Irradiation					
Yes	10 (0.5%)	6 (0.6%)	3 (0.6%)	1 (0.3%)	—
No/missing/unknown	1857 (99.5%)	1034 (99.4%)	506 (99.4%)	317 (99.7%)	
Oophorectomy					
Yes	321 (17.2%)	170 (16.4%)	87 (17.1%)	64 (20.1%)	
No/missing/unknown	1546 (82.8%)	870 (83.7%)	422 (82.9%)	254 (79.9%)	—

[††]*P*-values obtained from the Pearson χ^2 test between BMI categories and each categorical variable (excluding missing/unknown data).
[‡‡]*P*-values obtained from the Kruskal–Wallis and/or Mann–Whitney test between BMI categories and each continuous variable (excluding missing/unknown data).
[a]Excludes 113 patients with missing BMI information.
[b]Excluding any treatment for M1 disease.
[c]For example, CMF or anything not containing an anthracycline or taxane.

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Table 2. Tumour characteristics

Characteristic	All[a†] [*n* = 2843 (100%)]	Underweight/healthy, BMI<25, *n* = 1526 (53.7%)	Overweight, 25≤BMI<30, *n* = 784 (27.6%)	Obese, BMI≥30, *n* = 533 (18.8%)	*P*-value[‡‡] (Kruskal–Wallis and Mann–Whitney tests)
	Median (range, IQR), number of patients				
Maximum tumour diameter[b] in mm (all patients), median (IQR, range), *n*	22 (0–199, 15–34), 2666	20 (0–170, 14–30), 1446	24 (0–199, 17–34), 741	26 (0.5–130, 19–40), 479	Kwallis test: $P < 0.001$
Missing/unknown	177 (6.2%)	80 (5.2%)	43 (5.5%)	54 (10.1%)	Mann–Whitney:
					U/H versus Ov: $P < 0.001$
					U/H versus Obse: $P < 0.001$
					Ov versus Obese: $P < 0.001$
	Number of patients (%)				*P*-value[††] (Pearson χ^2 test)
Histological type					All BMI categories: $P = 0.008$
Ductal	2464 (87.8%)	1312 (87.4%)	694 (89.0%)	458 (87.2%)	U/H versus Ov: $P = 0.039$
Lobular	125 (4.5%)	63 (4.2%)	34 (4.4%)	28 (5.3%)	U/H versus Obese: $P = 0.012$
Ductal and lobular	76 (2.7%)	53 (3.5%)	13 (1.7%)	10 (1.9%)	Ov versus Obese: $P = 0.350$ (NS)
Medullary	31 (1.1%)	18 (1.2%)	9 (1.2%)	4 (0.8%)	
Metaplastic	11 (0.4%)	4 (0.3%)	1 (0.1%)	6 (1.1%)	
Mixed	24 (0.9%)	6 (0.4%)	11 (1.4%)	7 (1.3%)	
Other	58 (2.1%)	36 (2.4%)	14 (1.8%)	8 (1.5%)	
Unclassified adenocarcinoma	17 (0.6%)	9 (0.6%)	4 (0.5%)	4 (0.8%)	
Not graded[e]/missing/unknown	37 (1.3%)	25 (1.6%)	4 (0.5%)	8 (1.5%)	
Histological grade					All BMI categories: $P = 0.038$
1	140 (5.1%)	83 (5.6%)	34 (4.5%)	23 (4.4%)	For Grade 3 versus Grade 1+ 2
2	937 (33.8%)	529 (35.5%)	244 (32.0%)	164 (31.7%)	U/H versus Ov: $P = 0.034$
3	1695 (61.2%)	879 (59.0%)	485 (63.6%)	331 (63.9%)	U/H versus Obese: $P = 0.048$
Not graded/missing/unknown	71 (2.5%)	35 (2.3%)	21 (2.7%)	15 (2.8%)	Ov versus Obese: $P = 0.903$ (NS)
Distribution of cancer					All BMI categories: $P = 0.352$ (NS)
Localized	1808 (70.1%)	957 (69.4%)	503 (69.6%)	348 (72.8%)	
Multifocal	772 (29.9%)	422 (30.6%)	220 (30.4%)	130 (27.2%)	
Missing/unknown	263 (9.3%)	147 (9.6%)	61 (7.8%)	55 (10.3%)	
N stage					All BMI categories: $P = 0.018$
N0	1356 (48.5%)	766 (51.0%)	354 (45.8%)	236 (45.4%)	U/H versus Ov: $P = 0.019$
N1	1439 (51.5%)	736 (49.0%)	419 (54.2%)	284 (54.6%)	U/H versus Obese: $P = 0.027$
Missing/ unknown	48 (1.7%)	24 (1.6%)	11 (1.4%)	13 (2.4%)	Ov versus Obese: $P = 0.884$ (NS)

M stage					All BMI categories: $P = 0.547$ (NS)
M0	2758 (97.6%)	1482 (97.8%)	760 (97.4%)	516 (97.0%)	
M1	69 (2.4%)	33 (2.2%)	20 (2.6%)	16 (3.0%)	
Missing/unknown	16 (0.6%)	11 (0.7%)	4 (0.5%)	1 (0.2%)	
ER status[c]					All BMI categories: $P = 0.002$
Positive	1867 (65.8%)	1040 (68.3%)	509 (65.1%)	318 (59.9%)	U/H versus Ov: $P = 0.122$ (NS)
Negative	969 (34.2%)	483 (31.7%)	273 (34.9%)	213 (40.1%)	U/H versus Obese: $P < 0.001$
Missing/unknown	7 (0.3%)	3 (0.2%)	2 (0.3%)	2 (0.4%)	Ov versus Obese: $P = 0.055$ (NS)
PR status[c]					All BMI categories: $P = 0.001$
Positive	1279 (56.0%)	720 (58.9%)	355 (55.7%)	204 (48.0%)	U/H versus Ov: $P = 0.193$ (NS)
Negative	1006 (44.0%)	503 (41.1%)	282 (44.3%)	221 (52.0%)	U/H versus Obese: $P < 0.001$
Missing/unknown	558 (19.6%)	303 (19.9%)	147 (18.8%)	108 (20.3%)	Ov versus Obese: $P = 0.013$
HER2 status[c]					All BMI categories: $P = 0.843$
Positive	690 (27.5%)	381 (28.2%)	180 (26.4%)	129 (27.3%)	
Negative	1773 (70.7%)	944 (69.9%)	492 (72.0%)	337 (71.3%)	
Borderline	44 (1.8%)	26 (1.9%)	11 (1.6%)	7 (1.5%)	
Missing/unknown	336 (11.8%)	175 (11.5%)	101 (12.9%)	60 (11.3%)	
ER/HER2/PR status[d]					All BMI categories: $P = 0.005$
Triple negative	536 (19.9%)	267 (18.3%)	144 (19.4%)	125 (25.0%)	U/H versus Ov: $P = 0.529$ (NS)
Not triple negative	2162 (80.1%)	1190 (81.7%)	597 (80.6%)	375 (75.0%)	U/H versus Obese: $P = 0.001$
Missing/unknown	145 (5.1%)	69 (4.5%)	43 (5.5%)	33 (6.2%)	Ov versus Obese: $P = 0.020$

[††] P-values obtained from the Pearson χ^2 test between BMI categories and each categorical variable (excluding missing/unknown data).

[‡‡] P-values obtained from the Kruskal–Wallis and/or Mann–Whitney test between BMI categories and each continuous variable (excluding missing/unknown data).

[a]Excludes 113 patients with missing BMI information.

[b]Maximum tumour diameter includes DCIS (ductal carcinoma *in situ*).

[c]Includes data from TMA as well as primary POSH data.

[d] (TNT) includes patients with an ER-negative, HER2-negative, and PR-negative status.

[e]Not graded as pathology from axillary node, no primary detected.

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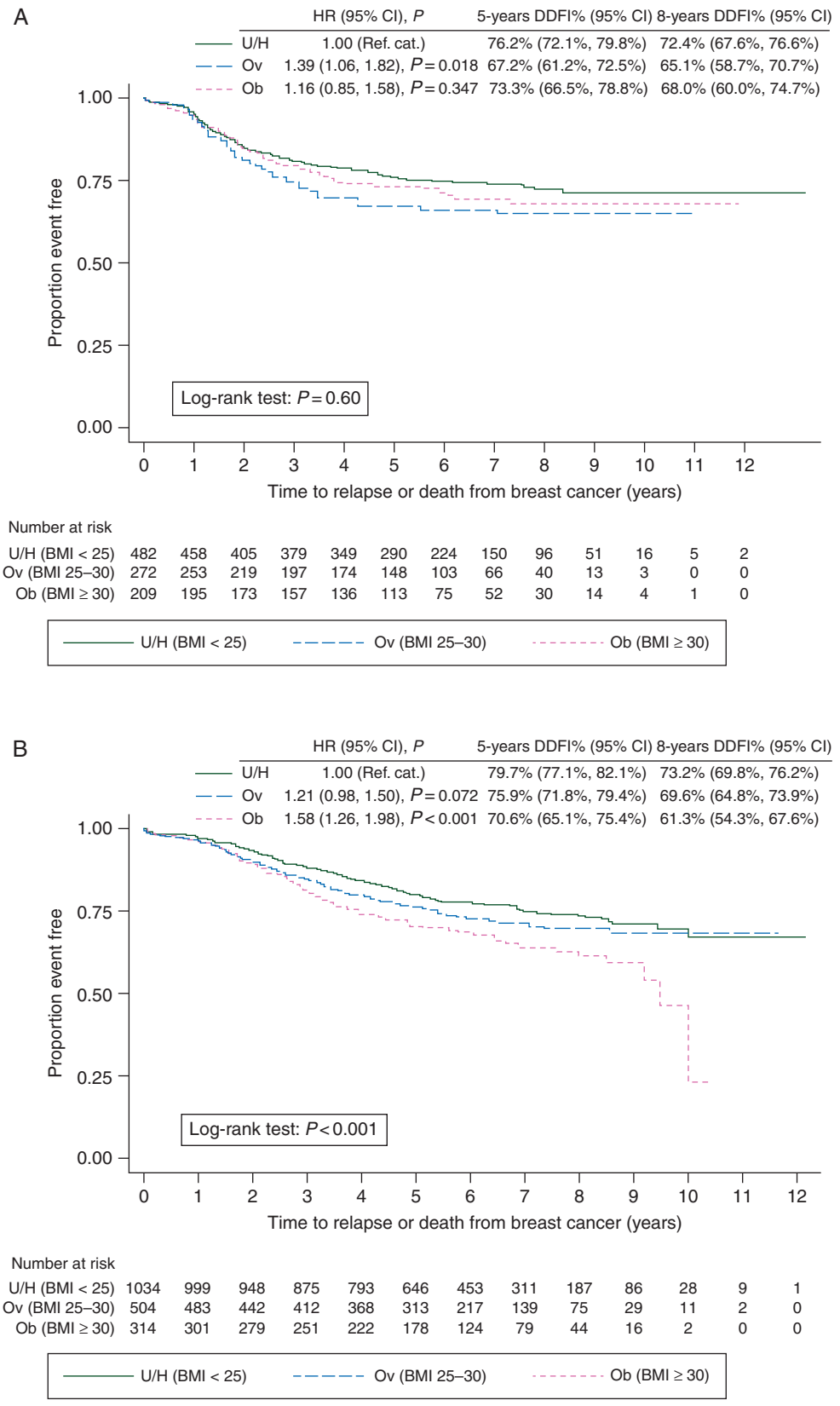

Figure 1. Kaplan–Meier distant disease-free survival estimates for under/healthy weight patients, overweight, and obese patients with (A) ER-negative tumours and (B) ER-positive tumours.

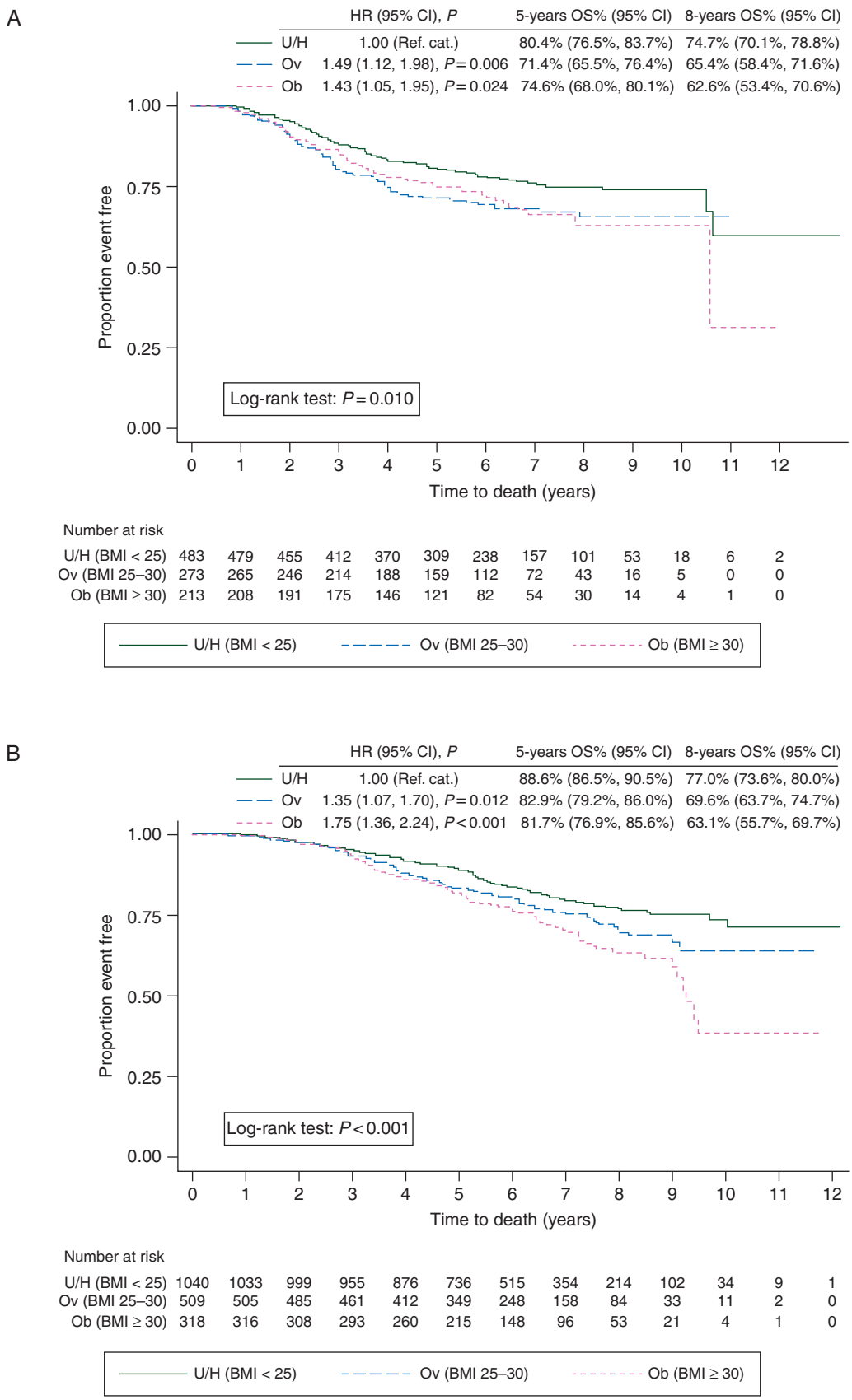

Figure 2. Kaplan–Meier overall survival estimates for under/healthy weight patients, overweight, and obese patients with (A) ER-negative tumours and (B) ER-positive tumours.

Table 3. Multivariable analyses: DDFI and OS

Group of patients	BMI Cat.	DDFI-unadjusted[a]			DDFI-adjusted[b]			DDFI-adjusted[c]		
		n[d]	HR (95% CI)	*P*-value	*n*[e]	HR (95% CI)	*P*-value	*n*[f]	HR (95% CI)	*P*-value
All	U/H	2821	1 *(Ref. cat.)*	—	2315	1 *(Ref. cat.)*	—	2284	1 *(Ref. cat.)*	—
	Ov		1.29 (1.09, 1.52)	0.003		1.22 (1.01, 1.45)	0.034		1.21 (1.01, 1.45)	0.042
	Ob		1.44 (1.20, 1.72)	<0.001		1.25 (1.02, 1.53)	0.033		1.22 (1.00, 1.51)	0.052 (NS)
ER−	U/H	963	1 *(Ref. cat.)*	—	789	1 *(Ref. cat.)*	—	778	1 *(Ref. cat.)*	—
	Ov		1.39 (1.06, 1.82)	0.018		1.20 (0.89, 1.61)	0.231 (NS)		1.21 (0.90, 1.63)	0.211 (NS)
	Ob		1.16 (0.85, 1.58)	0.347 (NS)		1.02 (0.72, 1.44)	0.910 (NS)		1.00 (0.70, 1.41)	0.988 (NS)
ER+	U/H	1852	1 *(Ref. cat.)*	—	1526	1 *(Ref. cat.)*	—	1506	1 *(Ref. cat.)*	—
	Ov		1.21 (0.98, 1.50)	0.072 (NS)		1.20 (0.95, 1.51)	0.118 (NS)		1.19 (0.94, 1.49)	0.146 (NS)
	Ob		1.58 (1.26, 1.98)	<0.001		1.39 (1.08, 1.79)	0.010		1.37 (1.06, 1.76)	0.017
Group of patients	BMI Cat.	OS-unadjusted[a]			OS-adjusted[b]			OS-adjusted[c]		
		n[d]	HR (95% CI)	*P*-value	*n*[e]	HR (95% CI)	*P*-value	*n*[f]	HR (95% CI)	*P*-value
All	U/H	2843	1 *(Ref. cat.)*	—	2325	1 *(Ref. cat.)*	—	2294	1 *(Ref. cat.)*	—
	Ov		1.41 (1.18, 1.68)	<0.001		1.30 (1.07, 1.57)	0.009		1.29 (1.06, 1.57)	0.010
	Ob		1.65 (1.36, 2.01)	<0.001		1.36 (1.10, 1.69)	0.005		1.35 (1.08, 1.68)	0.007
ER−	U/H	969	1 *(Ref. cat.)*	—	792	1 *(Ref. cat.)*	—	781	1 *(Ref. cat.)*	—
	Ov		1.49 (1.12, 1.98)	0.006		1.28 (0.94, 1.74)	0.119 (NS)		1.30 (0.95, 1.77)	0.100 (NS)
	Ob		1.43 (1.05, 1.95)	0.024		1.21 (0.85, 1.71)	0.301 (NS)		1.16 (0.81, 1.66)	0.411 (NS)
ER+	U/H	1867	1 *(Ref. cat.)*	—	1533	1 *(Ref. cat.)*	—	1513	1 *(Ref. cat.)*	—
	Ov		1.35 (1.07, 1.70)	0.012		1.28 (0.99, 1.64)	0.056 (NS)		1.28 (0.99, 1.65)	0.057 (NS)
	Ob		1.75 (1.36, 2.24)	<0.001		1.47 (1.11, 1.94)	0.006		1.47 (1.12, 1.95)	0.006

[a]Univariate analyses: results obtained by fitting a Cox model with BMI grouping as the only covariate.

[b]Multivariable analyses: results obtained by fitting a Cox model with BMI grouping as a covariate while also adjusting for, tumour grade, size, nodal status, HER2 status, age at diagnosis, and stratified by ER status.

[c]Multivariable analyses: results obtained by fitting a Cox model with BMI grouping as a covariate while also adjusting for, tumour grade, size, nodal status, HER2 status, race, age at diagnosis, and stratified by ER status.

[d]Number of patients with BMI information.

[e]Number of patients with complete data for BMI, tumour grade, size, ER, HER2, age at diagnosis, and nodal status.

[f]Number of patients with complete data for BMI, tumour grade, size, ER, HER2, ethnicity, age at diagnosis, and nodal status.

follow-up and survival

At the time of analysis, length of follow-up ranged from 1 month to 13 years (median 5.87 years). Only 87 patients (3.1%) had been lost to follow-up. Isolated local relapse events were few and will be explored in detail in a subsequent article [13].

DDFI at 5- and 8-years were significantly lower in obese (HR 1.44, $P < 0.001$) and overweight patients (HR 1.29, $P = 0.003$) than in U/H patients (5-year DDFI: U/H 78.6%, Ov 72.9%, Ob 71.3%; 8-year DDFI U/H 73.0%, Ov 68.1%, Ob 63.5%) (Figure 1). OS rates at 5- and 8-years were also significantly lower in obese (HR 1.65, $P < 0.001$) and overweight patients (HR 1.41, $P < 0.001$) (5-year OS: U/H 86.0%, Ov 78.9%, Ob 78.8%; 8-year OS: U/H 76.3%, Ov 68.2%, Ob 62.7%) (Figure 2). MVA with adjustment for age at diagnosis, tumour grade, size, nodal status, and HER2 status (Table 3) indicated that obesity was a significant independent predictor of both OS (HR 1.36, $P = 0.005$) and DDFI (HR 1.25, $P = 0.033$). On separate MVA of ER-positive and ER-negative patients, obesity was an independent prognostic factor in ER-positive patients (OS HR 1.47, $P = 0.006$ and DDFI HR 1.39, $P = 0.010$) but not ER-negative patients. Being overweight was also a significant independent predictor of inferior OS (HR 1.29, $P = 0.010$), and DDFI (HR 1.21, $P = 0.042$) but for the whole cohort only; significance was lost in this group on separate MVA of ER-positive and ER-negative patients.

discussion

The POSH study is the largest prospective study of obesity and breast cancer outcome in pre-menopausal women to date [13]. In keeping with the general population of UK 24–45 year olds, 46.3% of our cohort was/patients were overweight or obese at recruitment [10]. The POSH cohort is representative of the general UK breast cancer population for this age group in terms of other characteristics [13].

This study confirms previous studies and meta-analyses results showing that obesity is associated with poorer OS in comparison with U/H patients, with an unadjusted HR for OS of 1.65. The POSH data represent a young patient group, who are less likely to have significant co-morbidities than older patients. As described previously, 94% of deaths in this cohort were due to breast cancer [13]. Our data also support other studies which have found inferior DDFI associated with obesity [4, 5] suggesting that the adverse effect of obesity in this young patient group is due to breast cancer recurrence rather than obesity- associated non-breast cancer mortality.

Our data confirm previous reports of more advanced disease in obese patients [7]. All patients in our study were below the minimum age for UK national breast screening, over 98% presented with symptomatic tumours. The increasing mean tumour diameter and increased nodal involvement in patients with a higher BMI may be entirely due to body habitus but could also represent delays in self-referral or referrals from primary to secondary care.

Our data also confirm previous reports that obese patients have more biologically adverse tumours, with increased proportions of grade 3, ER-negative and triple negative tumours. The reason for this is unclear, but obesity is a subclinical inflammatory state and activated macrophages in adipose tissue producing pro-inflammatory mediators could potentially affect the tumour micro-environment [18]. Obesity is also associated with raised levels of adipocytokines including leptin, and insulin/insulin-like growth factor which have direct mitogenic/anti-apoptotic activity [19].

Our MVA results support other studies showing obesity to be an independent poor prognostic factor reducing both DDFI? and OS in unselected breast cancer patients adjusted for pathological features [6, 7, 20]. This effect could be due to direct biological effects of obesity on the tumour but obesity may influence the delivery of effective breast cancer treatments.

Ewertz et al. [7] suggested reduced use of chemotherapy in non-age selected obese patients as a potential explanation for this outcome. However, we found greater overall usage of anthracycline/ taxane therapy in the obese patient group. Most cytotoxics are dosed according to body surface area, a formula that was not designed for use at extremes of weight, and optimum dosing of chemotherapy in obese individuals is unresolved. A recent review indicated that up to 40% of obese cancer patients receive capped chemotherapy doses [8].

We do not have complete data on chemotherapy dose intensity for the entire study cohort but analysis of chemotherapy prescription records for the 77 POSH participants treated with adjuvant chemotherapy at the Southampton Oncology Centre indicates that obese patients were significantly more likely to receive a dose delay than healthy weight patients (33.3% versus 5.9%, $P = 0.0068$, data not shown). Gouerant et al. [21] also reported reduced dose intensity of docetaxel among obese patients.

We found that obesity is an independent prognostic factor in ER-positive but not ER-negative young breast cancer patients. Smaller numbers in our ER-negative group reduces power, but we found no dose–response relationship for ER-negative patients. while there is one for the ER positive patients. Although previously published series reported no evidence that breast cancer outcome differs by hormone receptor, our finding is supported by the recently presented data from 80 trials showing a significant association between obesity and prognosis in pre-menopausal and perimenopausal women with ER-positive disease only [5, 22]. More specifically, Sparano et al. [23] have shown an association between obesity and inferior outcome in ER-positive, HER 2-negative disease only.

An analysis of the ABCSG-12 trial reported that BMI significantly influenced the efficacy of anastrozole plus goserelin in premenopausal patients but did not influence the prognosis of patients treated with tamoxifen plus goserelin [24]. In the POSH cohort, 88.6% of ER-positive patients received tamoxifen, but the use of ovarian suppression was limited (17.2% medical suppression, 22.2% oophorectomy; not mutually exclusive). Our data could suggest that tamoxifen without ovarian suppression may be less effective in high BMI patients. Our study did not collect data on adherence, so we cannot exclude the possibility of an association between obesity and reduced tamoxifen adherence in young women

This study is strengthened by its prospective nature and use of BMIs calculated from objective measurements of height/weight at registration. Many previous reports have relied on

self-reported height/weight and some analyses have been compromised by BMI data being available only for patients who received chemotherapy [25]. Data from interventional clinical trials may be biased by selection of healthier obese patients than the general population. We have also consistently used standard WHO definitions of BMI categories [14]; some publications have used alternative definitions and this may have influenced meta-analyses.

Higher proportions of overweight and obese black or American African groups compared with Caucasian women may lead to confounding [26]. Race is an independent prognostic factor for recurrence and OS in this patient cohort [17], but the addition of race as an adjustment factor in our MVA (Table 3) did not alter our finding that obesity is an independent prognostic factor in ER-positive patients (OS HR 1.47, $P = 0.006$).

In conclusion, our data provide important confirmatory evidence that obesity at diagnosis is associated with poor outcome in young British breast cancer patients and is an independent prognostic marker in ER-positive patients. Further research is needed to optimize treatments for this patient group.

acknowledgements

We acknowledge the POSH collaborators and all the patients who participated in this study. Participating principal investigators are listed in supplementary material S1, available at *Annals of Oncology* online and on the study website: http://www.southampton.ac.uk/medicine/research/posh.page. We also thank our colleagues in the NIHR Southampton Biomedical Research Centre-Nutrition for their helpful discussions. RE acknowledges support from the NIHR to the Biomedical Research Centre at The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, (http://www.ncrn.org.uk/Portfolio/index.htm).

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disclosures

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SUPPLEMENTARY MATERIAL

Supplementary Document 1

POSH Collaborators

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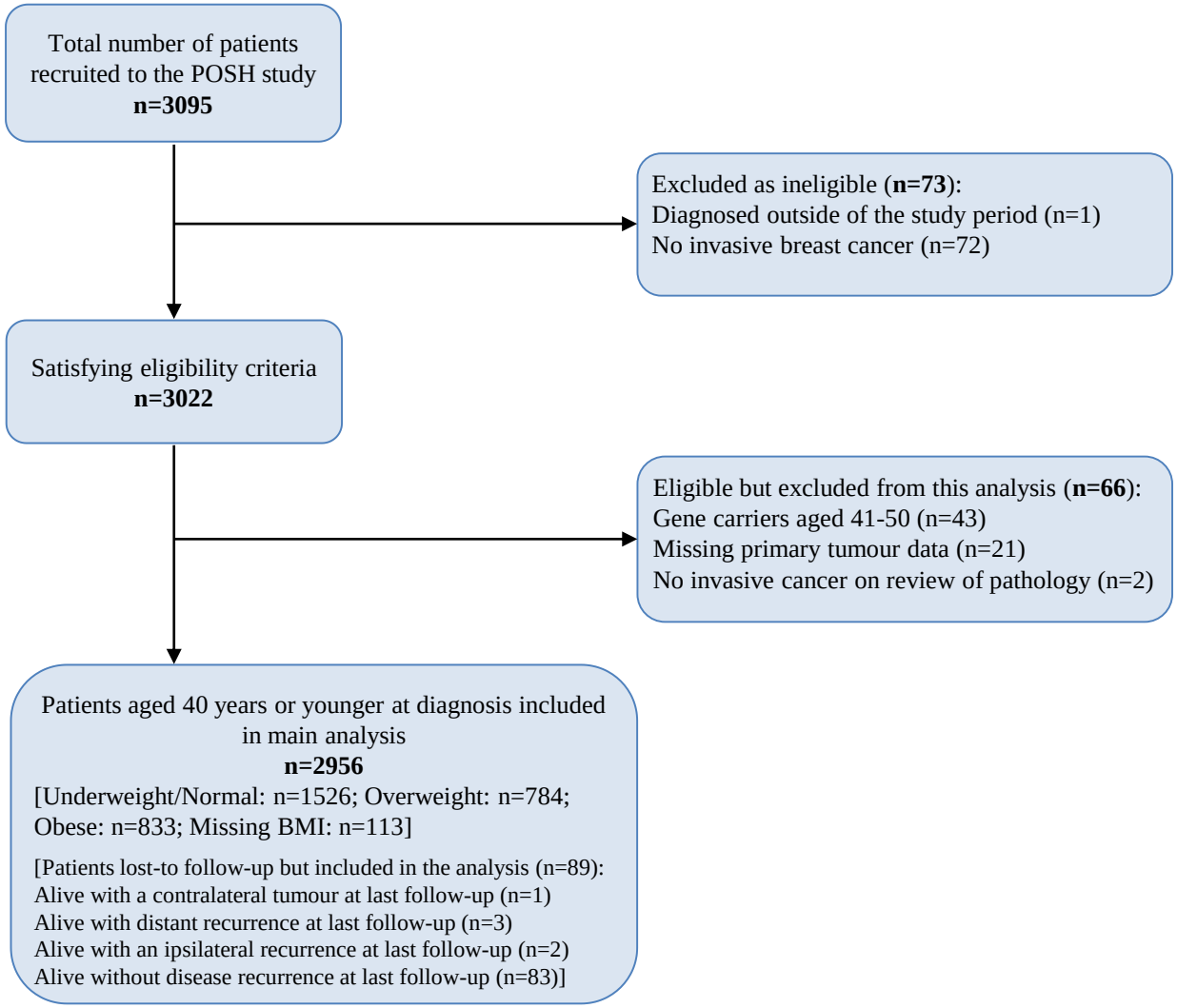
Total number of patients recruited to the POSH study
n=3095
Excluded as ineligible (n=73):
Diagnosed outside of the study period (n=1)
No invasive breast cancer (n=72)
Satisfying eligibility criteria
n=3022
Eligible but excluded from this analysis (n=66):
Gene carriers aged 41-50 (n=43)
Missing primary tumour data (n=21)
No invasive cancer on review of pathology (n=2)
Patients aged 40 years or younger at diagnosis included in main analysis
n=2956
[Underweight/Normal: n=1526; Overweight: n=784; Obese: n=833; Missing BMI: n=113]
[Patients lost-to follow-up but included in the analysis (n=89):
Alive with a contralateral tumour at last follow-up (n=1)
Alive with distant recurrence at last follow-up (n=3)
Alive with an ipsilateral recurrence at last follow-up (n=2)
Alive without disease recurrence at last follow-up (n=83)]

Chapter 6: Paper 4 - Family history and outcome of young patients with breast cancer in the UK (POSH study)

Authors	Eccles BK, Copson ER, Cutress RI, **Maishman T**, Altman DG, Simmonds P, Gerty SM, Durcan L, Stanton L, Eccles DM; POSH Study Steering Group.
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6.1 Contribution

As the lead statistical methodologist and scientific lead at the SCTU:

- Responsible for study methodology and research of all statistical methods to be implemented in the analyses, including the use of multiple imputation and FPSMs for time-varying covariates;
- Developed and authored the SAP – including the organisation and participation of meetings to develop the SAP, and the creation of all draft and final versions of the SAP;
- Responsible for central data monitoring, data cleaning and data interpretation – liaising with the study team to identify data queries, and for the resolution of all data queries raised with data management;
- Developed and conducted all statistical programming and analyses – including the creation of all database and analysis programming required for the analyses;
- Designed, developed and produced all manuscript figures and tables – including the creation of all draft and final versions of the manuscript figures and tables;
- Co-authored and reviewed the manuscript – listed as 4th named author, drafted the statistical methods section of the manuscript, extensively involved in the interpretation of results including the interpretation of the results of the FPSM using multiple imputation, reviewed the entire manuscript including sense checks and result checking, involved in the resolution of reviewer comments and responses.

Professor Diana Eccles, chief investigator of the POSH study, chair of the POSH Study Steering Group, and senior last author of **Paper 4**, confirms the accuracy of contribution outlined above of Thomas Christopher Maishman.

Signed: .....

Date: .....

Original article

Family history and outcome of young patients with breast cancer in the UK (POSH study)

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Background: Young patients presenting to surgical clinics with breast cancer are usually aware of their family history and frequently believe that a positive family history may adversely affect their prognosis. Tumour pathology and outcomes were compared in young British patients with breast cancer with and without a family history of breast cancer.

Methods: Prospective Outcomes in Sporadic *versus* Hereditary breast cancer (POSH) is a large prospective cohort study of women aged less than 41 years with breast cancer diagnosed and treated in the UK using modern oncological management. Personal characteristics, tumour pathology, treatment and family history of breast/ovarian cancer were recorded. Follow-up data were collected annually.

Results: Family history data were available for 2850 patients. No family history was reported by 65·9 per cent, and 34·1 per cent reported breast/ovarian cancer in at least one first- or second-degree relative. Patients with a family history were more likely to have grade 3 tumours (63·3 *versus* 58·9 per cent) and less likely to have human epidermal growth factor receptor 2-positive tumours (24·7 *versus* 28·8 per cent) than those with no family history. In multivariable analyses, there were no significant differences in distant disease-free intervals for patients with *versus* those without a family history, either for the whole cohort (hazard ratio (HR) 0·89, 95 per cent c.i. 0·76 to 1·03; $P = 0·120$) or when stratified by oestrogen receptor (ER) status (ER-negative: HR 0·80, 0·62 to 1·04, $P = 0·101$; ER-positive: HR 0·95, 0·78 to 1·15, $P = 0·589$).

Conclusion: Young British patients presenting to breast surgical clinics with a positive family history can be reassured that this is not a significant independent risk factor for breast cancer outcome.

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Introduction

Breast cancer in young women (aged 15–39 years) is relatively uncommon but associated with lower survival rates than in 40–70-year-old women[1]. Approximately 25 per cent of breast cancers in developed countries are thought to be related to hereditary factors and these usually present at a younger age than sporadic breast tumours[2]. At the time of diagnosis in breast surgical clinics, patients will usually be aware of their family history, and often wish to understand the significance of this for their treatment and prospects of cure. Genetic testing will be performed only to identify highly penetrant single genes, *BRCA1* and *BRCA2* currently being the most relevant, and is usually carried out at a later stage following initiation of treatments. Studies with selection based on family history (rather than mutation analysis) since the 1990s have shown inconsistent results, with improved[3,4], impaired[5,6] and unchanged[7–12] survival. Conflicting evidence may be partly due to different study populations or inconsistent definitions of a positive family history in the literature, which depends on both the number and closeness of affected relatives and variable use of genetic testing. A recent analysis[13] based on population samples of over 10 000 patients with colorectal cancer showed that patients with familial colorectal cancer have a significantly better survival than those with sporadic disease.

Younger women with breast cancer have an increased incidence of adverse biological features, such as oestrogen receptor (ER) negativity and high grade[14,15]. These adverse prognostic features are also the typical phenotype

of cancers in *BRCA1* mutation carriers[16–19], but it is unknown whether carrying a *BRCA1* or *BRCA2* mutation confers an additional effect on prognosis independent of known biological factors. However, breast cancer genetics is complex, and the majority (approximately 80 per cent) of familial breast cancers even in the young are not related to *BRCA1* or *BRCA2*[20–28]. It is still unclear whether *BRCA*-associated breast cancers have a different prognosis to sporadic cancers[29–31].

Thus, even though *BRCA* testing is now more frequently undertaken, especially in young patients, the larger picture of whether a positive family history of breast cancer independently affects prognosis is important to understand, and remains controversial with no clear consensus.

Prospective Outcomes in Sporadic *versus* Hereditary breast cancer (POSH)[14,32] is a large prospective cohort study of patients aged less than 41 years with breast cancer diagnosed and treated in the UK using modern oncological management. This analysis compared the pathology and outcome of the POSH cohort according to family history to determine whether the presence, or degree, of family history is an independent prognostic factor in young patients with breast cancer.

Methods

POSH is a multicentre prospective observational cohort study of young women diagnosed with breast cancer at an age of 40 years or younger in the UK, between 1 January 2000 and 31 January 2008, from 127 UK hospitals[14]. Potential recruits were consented within 12 months of initial diagnosis. All patients received treatment according to local protocols. Written consent was obtained. The detailed study protocol was published in 2007[32]. This study received approval from the South West Multi-centre Research Ethics Committee (MREC 00/6/69).

Study variables and data sources

Details of personal characteristics, tumour pathology, disease stage and treatment received were collected from medical records. Pathology and imaging data were verified with copies of original reports from sites. For patients treated with neoadjuvant chemotherapy, initial tumour diameter was derived from radiological reports. Maximum invasive tumour size was defined as size of invasive tumour by longest diameter excluding any ductal carcinoma *in situ* (DCIS), or in multifocal cancer as the sum of the longest diameters. Study participants were asked to complete a family history questionnaire at recruitment, comprising details of all first- and second-degree relatives, including current age or age at death, any cancer diagnosis, age at cancer diagnosis and type of treatment. Details of any other family member who had a history of malignancy were also requested. From this information a pedigree was drawn. Patients were categorized as having a positive family history (FH+) if they reported at least one affected first- or second-degree relative, where affected was defined as reported diagnosis of cancer of the breast and/or ovary. Participants were asked to complete a separate lifestyle questionnaire to provide details of other risk factors.

Detailed clinical follow-up data, including date and site of disease recurrence, were obtained from medical records at 6 months, 12 months and yearly intervals after diagnosis until death or loss to follow-up. Patients were flagged in the National Health Service Health and Social Care Information Centre to facilitate automatic notification of date and cause of death. This paper presents analyses conducted on follow-up data received until 22 October 2013.

Tumour receptor status

ER, progesterone receptor (PR) and human epidermal growth factor receptor (HER) 2 status of primary tumours were determined primarily from routine diagnostic pathology reports according to local laboratory methods. Where given, hormone receptor levels equivalent to an Allred score of at least 3 were categorized as positive. Tissue microarray (TMA) data from central pathology review at St Bartholomew's Hospital, London, was performed on the 1336 tumour samples that had been returned by participating hospitals and represented on TMAs at the time of analysis. TMA results for ER, PR and HER2 receptor status were used to corroborate clinical data or to supplement missing data points on receptor status.

Statistical analysis

Details of the target sample size (3000) are reported in the protocol[32]. The statistical analysis was conducted according to a prespecified plan, in line with published guidance[33]. Summary statistics used to describe the cohort and tumour characteristics were compared between family history categories for the whole cohort, and for ER-positive and ER-negative tumour groups, using χ^2 or Mann–Whitney *U* tests.

Distant disease-free interval (DDFI) was defined as time from date of invasive breast cancer diagnosis to distant relapse or death from breast cancer, and assessed using Kaplan–Meier curves. Patients who had not experienced an event at the time of analysis were censored at the date of last follow-up. As a result of the time-varying effects of

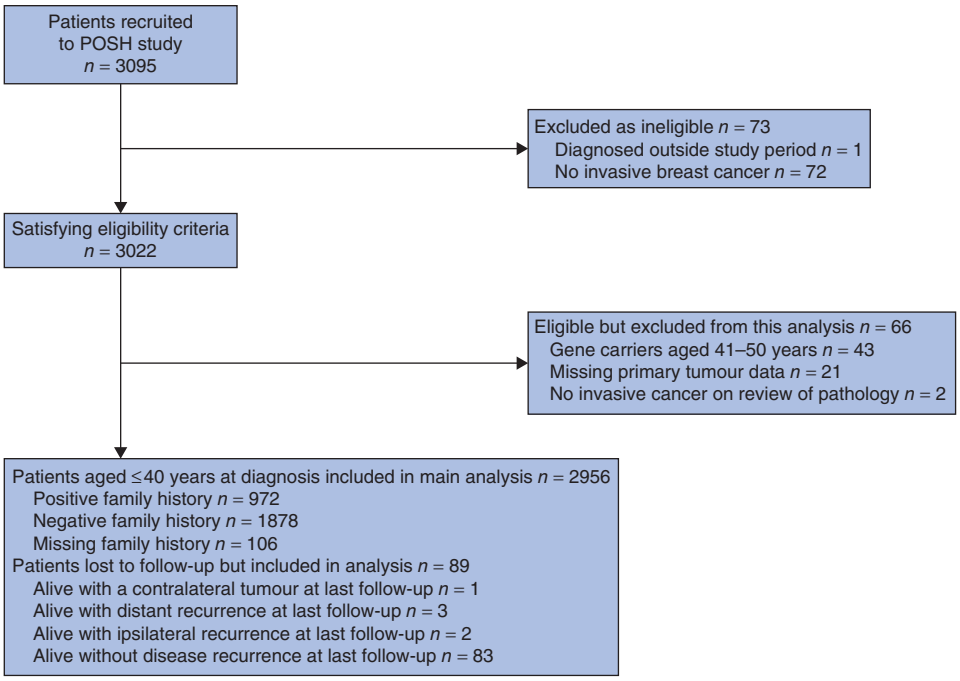

Fig. 1 Flow chart for Prospective Outcomes in Sporadic *versus* Hereditary breast cancer (POSH) study

ER status[34] and to adjust for potential confounders, multivariable analyses were based on the flexible parametric survival model[35]. This model included family history as a binary variable and all of the following co-variables measured at breast cancer diagnosis, regardless of statistical significance: age, tumour size (fitted as a log-transformed continuous co-variable as it has a skewed distribution), tumour grade, axillary node status, lymphovascular invasion, distribution of tumour, ER status, PR status and HER2 status.

All missing data were assumed to be either missing at random or missing completely at random, and censoring was assumed to be non-informative. Multiple imputed data sets were generated using the ice command and multivariable analyses of these carried out using the mim command in Stata® version 11.2 (StataCorp LP, College Station, Texas, USA). Sensitivity analyses were performed by fitting multivariable models, which excluded M1 disease and/or non-symptomatic patients (data not shown). An additional multivariable analysis was performed with patients stratified by ER status.

Results

Patient characteristics and family history

The POSH study recruited 3095 patients across England (2695), Scotland, Wales and Northern Ireland. A total of 139 trial participants were excluded (*Fig. 1*) and, of the 2956 remaining patients, family history data were available for 2850 (96·4 per cent). Some 1878 patients (65·9 per cent) reported no family history of breast/ovarian malignancy (FH− group) and 972 (34·1 per cent) reported breast/ovarian cancer in one or more first- or second-degree relative (FH+ group). Median age at diagnosis was 36 (range 18–40 years for FH− group, 21–40 years for FH+ group). There was no significant difference between family history groups. Surveillance-detected tumours were more frequent in the FH+ group (29 (3·0 per cent) *versus* 1 (0·1 per cent)) and in patients reporting an affected first-degree relative (25, 6·0 per cent) compared with those with an affected second-degree relative (4, 0·7 per cent) (*Table 1*).

Tumour pathology

The distribution of grade was significantly different between family history groups ($P = 0·040$); patients with a positive family history more likely to have a grade 3 tumour than those with a negative family history (63·3 *versus* 58·9 per cent). There were no significant differences in median tumour diameter, presence of lymphovascular invasion, incidence of node involvement or presence of metastases at diagnosis between the FH+ and FH− groups. The frequency of ER- and PR-positive tumours

Table 1 Patient and tumour characteristics by family history of breast cancer and by patients with a first- or second-degree relative

	No family history† ($n = 1878$)	Family history† ($n = 972$)	P¶	First-degree relative‡ ($n = 418$)	Second-degree relative‡ ($n = 554$)	P¶
Maximum invasive tumour size (mm)*	22 (0–199, 15–34)	22 (1–170, 15–32)	0·745#	20 (1–100, 15–30)	23 (1–170, 16–35)	0·004#
Unknown	117 (6·2)	60 (6·2)		25 (6·0)	35 (6·3)	
Maximum tumour size, including DCIS (mm)*	27 (0–199, 19–40)	26 (1–180, 18–40)	0·417#	25 (1–180, 17–39·5)	27 (1–170, 18–41)	0·098#
Unknown	100 (5·3)	47 (4·8)		20 (4·8)	27 (4·9)	
Presentation			< 0·001			< 0·001
Symptomatic	1870 (99·7)	931 (96·3)		384 (92·3)	547 (99·3)	
Surveillance	1 (0·1)	29 (3·0)		25 (6·0)	4 (0·7)	
Other	4 (0·2)	7 (0·7)		7 (1·7)	0 (0)	
Unknown	3 (0·2)	5 (0·5)		2 (0·5)	3 (0·5)	
Distribution of tumour			0·165			0·981
Localized	1211 (71·5)	615 (68·9)		266 (68·9)	349 (68·8)	
Multifocal	483 (28·5)	278 (31·1)		120 (31·1)	158 (31·2)	
Unknown	184 (9·8)	79 (8·1)		32 (7·7)	47 (8·5)	
Tumour grade			0·040			0·281
1	113 (6·2)	43 (4·5)		17 (4·2)	26 (4·8)	
2	637 (34·9)	306 (32·2)		121 (29·7)	185 (34·1)	
3	1076 (58·9)	602 (63·3)		270 (66·2)	332 (61·1)	
Unknown	52 (2·8)	21 (2·2)		10 (2·4)	11 (2·0)	
Pathological node status			0·774			0·117
N0	898 (48·7)	473 (49·3)		215 (52·2)	258 (47·1)	
N1–3	946 (51·3)	487 (50·7)		197 (47·8)	290 (52·9)	
Unknown	34 (1·8)	12 (1·2)		6 (1·4)	6 (1·1)	
Metastasis category			0·900			0·414
M0	1824 (97·5)	942 (97·6)		408 (98·1)	534 (97·3)	
M1	46 (2·5)	23 (2·4)		8 (1·9)	15 (2·7)	
Unknown	8 (0·4)	7 (0·7)		2 (0·5)	5 (0·9)	
ER status§			0·608			0·713
Negative	625 (33·4)	333 (34·4)		146 (35·0)	187 (33·9)	
Positive	1246 (66·6)	636 (65·6)		271 (65·0)	365 (66·1)	
Unknown	7 (0·4)	3 (0·3)		1 (0·2)	2 (0·4)	
PR status§			0·853			0·186
Negative	658 (43·6)	335 (43·2)		136 (40·5)	199 (45·2)	
Positive	852 (56·4)	441 (56·8)		200 (59·5)	241 (54·8)	
Unknown	368 (19·6)	196 (20·2)		82 (19·6)	114 (20·6)	
HER2 status§			0·031			0·196
Negative	1172 (71·2)	648 (75·3)		276 (77·5)	372 (73·7)	
Positive	474 (28·8)	213 (24·7)		80 (22·5)	133 (26·3)	
Unknown	232 (12·4)	111 (11·4)		62 (14·8)	49 (8·8)	
TNT status			0·579			0·590
TNT	343 (19·2)	183 (20·0)		82 (20·9)	101 (19·4)	
No TNT	1448 (80·9)	730 (80·0)		311 (79·1)	419 (80·6)	
Unknown	87 (4·6)	59 (6·1)		25 (6·0)	34 (6·1)	
Lymphovascular invasion			0·989			0·564
No	903 (52·0)	471 (52·0)		206 (53·1)	265 (51·2)	
Yes	833 (48·0)	435 (48·0)		182 (46·9)	253 (48·8)	
Unknown	142 (7·6)	66 (6·8)		30 (7·2)	36 (6·5)	

Values in parentheses are percentages unless indicated otherwise; *values are median (range, i.q.r.). †Some 106 patients (3·6 per cent) with unknown family history excluded from these analyses; ‡1878 (63·5 per cent) with no family history and 106 (3·6 per cent) with unknown family history excluded from these analyses. §Includes data from tissue microarrays as well as primary Prospective Outcomes in Sporadic *versus* Hereditary breast cancer (POSH) study. DCIS, ductal carcinoma *in situ*; ER, oestrogen receptor; PR, progesterone receptor; HER, human epidermal growth factor receptor; TNT, triple negative test (ER-. PR- and HER-negative). ¶χ^2 test, except #Mann–Whitney U test.

Table 2 Patient and tumour characteristics by family history of breast cancer and oestrogen receptor status

	ER-negative only†‡			ER-positive only†§		
	No family history ($n = 625$)	Family history ($n = 333$)	P¶	No family history ($n = 1246$)	Family history ($n = 636$)	P¶
Maximum invasive tumour size (mm)*	23 (1–199, 15–32)	20 (1–117, 15–30)	0·025#	22 (0–150, 15–35)	23 (1–170, 16–35)	0·269#
Unknown	58 (9·3)	18 (5·4)		59 (4·7)	41 (6·4)	
Maximum tumour size, including DCIS (mm)*	27 (0·6–199, 18–40)	25 (1–131, 17–34·5)	0·035#	27 (0–190, 19–41)	28 (1–180, 18–43)	0·609#
Unknown	49 (7·8)	13 (3·9)		51 (4·1)	33 (5·2)	
Presentation			< 0·001			< 0·001
Symptomatic	623 (99·8)	318 (96·1)		1240 (99·7)	610 (96·4)	
Surveillance	1 (0·2)	10 (3·0)		0 (0)	19 (3·0)	
Other	0 (0)	3 (0·9)		4 (0·3)	4 (0·6)	
Unknown	1 (0·2)	2 (0·6)		2 (0·2)	3 (0·5)	
Distribution of tumour			0·794			0·121
Localized	443 (80·5)	245 (79·8)		761 (66·9)	369 (63·2)	
Multifocal	107 (19·5)	62 (20·2)		376 (33·1)	215 (36·8)	
Unknown	75 (12·0)	26 (7·8)		109 (8·8)	52 (8·2)	
Tumour grade			0·175			0·089
1	6 (1·0)	0 (0)		106 (8·7)	42 (6·8)	
2	64 (10·6)	32 (9·8)		572 (47·0)	274 (44·1)	
3	534 (88·4)	296 (90·2)		538 (44·2)	305 (49·1)	
Unknown	21 (3·4)	5 (1·5)		30 (2·4)	15 (2·4)	
Pathological node status			0·156			0·521
N0	333 (54·4)	196 (59·2)		560 (45·7)	277 (44·1)	
N1–3	279 (45·6)	135 (40·8)		666 (54·3)	351 (55·9)	
Unknown	13 (2·1)	2 (0·6)		20 (1·6)	8 (1·3)	
Metastasis category			0·033			0·336
M0	605 (97·4)	330 (99·4)		1212 (97·6)	610 (96·8)	
M1	16 (2·6)	2 (0·6)		30 (2·4)	20 (3·2)	
Unknown	4 (0·6)	1 (0·3)		4 (0·3)	6 (0·9)	
PR status †			0·879			0·932
Negative	516 (91·0)	263 (90·7)		141 (15·0)	72 (14·8)	
Positive	51 (9·0)	27 (9·3)		800 (85·0)	414 (85·2)	
Unknown	58 (9·3)	43 (12·9)		305 (24·5)	150 (23·6)	
HER2 status †			0·105			0·126
Negative	395 (70·0)	225 (75·3)		774 (71·7)	423 (75·3)	
Positive	169 (30·0)	74 (24·8)		305 (28·3)	139 (24·7)	
Unknown	61 (9·8)	34 (10·2)		167 (13·4)	74 (11·6)	

Values in parentheses are percentages unless indicated otherwise; *values are median (range, i.q.r.). †Includes data from tissue microarrays as well as primary Prospective Outcomes in Sporadic *versus* Hereditary breast cancer (POSH) study. ‡Thirty-eight patients (3·8 per cent) with oestrogen receptor (ER)-negative disease and unknown family history, and §66 (3·4 per cent) with ER-positive disease and unknown family history were excluded from these analyses. DCIS, ductal carcinoma *in situ*; PR, progesterone receptor; HER, human epidermal growth factor receptor. ¶χ^2 test, except #Mann–Whitney U test.

did not vary significantly between family history groups. Patients with no family history were significantly more likely to have a HER2-positive tumour (28·8 *versus* 24·7 per cent; $P = 0·031$). There were no significant differences in any of the pathological features between patients with first- or second-degree affected relatives other than the median maximum invasive tumour size, which was smaller in patients with first-degree affected relatives (20 *versus* 23 mm; $P = 0·004$). After exclusion of the small number of surveillance-detected tumours, median invasive tumour size was still smaller in patients with affected first-degree relatives (20 *versus* 24 mm; $P = 0·016$). Median invasive tumour size in patients with no family history was 22 mm ($P = 0·030$ and $P = 0·169$ *versus* patients with affected first- and second-degree relatives respectively).

When patients with ER-positive and -negative tumours were analysed separately, there was no longer a significant difference in distribution of tumour grade and HER2 status for FH+ and FH− groups (*Table 2*). Among women with ER-negative tumours the distribution of M0 and M1 was significantly different, with more metastatic disease in the FH− group (2·6 *versus* 0·6 per cent; $P = 0·033$). In the

Table 3 Treatment details by family history of breast cancer and by patients with a first- or second-degree relative

	No family history* ($n = 1878$)	Family history* ($n = 972$)	*P*#	First-degree relative† ($n = 418$)	Second-degree relative† ($n = 554$)	*P*#
Definitive surgery			0·007			0·484
Breast-conserving surgery	932 (49·6)	434 (44·7)		181 (43·3)	253 (45·7)	
Mastectomy	910 (48·5)	529 (54·4)		232 (55·5)	297 (53·6)	
Node surgery only	8 (0·4)	1 (0·1)		0 (0)	1 (0·2)	
No surgery	28 (1·5)	8 (0·8)		5 (1·2)	3 (0·5)	
Chemotherapy timing			0·806			0·030
Adjuvant	1370 (72·9)	720 (74·1)		327 (78·2)	393 (70·9)	
Neoadjuvant	284 (15·1)	147 (15·1)		49 (11·7)	98 (17·7)	
Palliative	36 (1·9)	15 (1·5)		4 (1·0)	11 (2·0)	
Not applicable	188 (10·0)	90 (9·3)		38 (9·1)	52 (9·4)	
Chemotherapy regimen			0·397			0·975
Anthracycline and/or taxane	1677 (89·3)	871 (89·6)		375 (89·7)	496 (89·5)	
Other‡	13 (0·7)	11 (1·1)		5 (1·2)	6 (1·1)	
None	188 (10·0)	90 (9·3)		38 (9·1)	52 (9·4)	
Adjuvant trastuzumab			–			–
Yes	230 (12·2)	109 (11·2)		36 (8·6)	73 (13·2)	
No, other treatment period, unknown§	1648 (87·8)	863 (88·8)		382 (91·4)	481 (86·8)	
Adjuvant radiotherapy			–			–
Yes	1532 (81·6)	758 (78·0)		316 (75·6)	442 (79·8)	
No, unknown§	346 (18·4)	214 (22·0)		102 (24·4)	112 (20·2)	
Ovarian suppression (ER-positive disease only)¶	$n = 1246$	$n = 636$		$n = 271$	$n = 365$	
Hormone therapy			–			–
Tamoxifen or AI	1142 (91·7)	592 (93·1)		255 (94·1)	337 (92·3)	
No, unknown	104 (8·3)	44 (6·9)		16 (5·9)	28 (7·7)	
LHRH agonist			–			–
Yes	374 (30·0)	262 (41·2)		120 (44·3)	142 (38·9)	
No, unknown	872 (70·0)	374 (58·8)		151 (55·7)	223 (61·1)	
Irradiation			–			–
Yes	2 (0·2)	9 (1·4)		6 (2·2)	3 (0·8)	
No, unknown	1244 (99·8)	627 (98·6)		265 (97·8)	362 (99·2)	
Oophorectomy			–			–
Yes	170 (13·6)	148 (23·3)		72 (26·6)	76 (20·8)	
No, unknown	1076 (86·4)	488 (76·7)		199 (73·4)	289 (79·2)	

Values in parentheses are percentages. *Some 106 patients (3·6 per cent) with unknown family history excluded from these analyses; †1878 (63·5 per cent) with no family history and 106 (3·6 per cent) with unknown family history excluded from these analyses. ‡Cyclophosphamide, methotrexate and fluorouracil, or anything not containing an anthracycline or taxane. §Likely to be inaccurate owing to data collection methods. ¶In any treatment period. ER, oestrogen receptor; AI, aromatase inhibitor; LHRH, luteinizing hormone-releasing hormone. #χ^2 test.

ER-negative subgroup only there was a significant difference in tumour diameter (invasive, $P = 0·025$; including DCIS, $P = 0·035$) between family history groups, with a median invasive tumour diameter of 23 and 20 mm in the FH– and FH+ groups respectively.

Treatment

Detailed information on multimodal treatment according to family history group is shown in *Table 3*. The distribution of primary surgery was significantly different between FH– and FH+ groups, but not between those with first- and second-degree relatives. More women in the FH+ group had a mastectomy (54·4 *versus* 48·5 per cent). Chemotherapy timing and regimen were similar in both family history groups, the majority having anthracycline and/or taxane-based adjuvant chemotherapy. However, there was a significant difference in chemotherapy timing in women with first-degree relatives compared with those who had second-degree relatives with breast cancer; more women with affected first-degree relatives had adjuvant chemotherapy (78·2 *versus* 70·9 per cent) and fewer received neoadjuvant chemotherapy (11·7 *versus* 17·7 per cent). The majority (over 90 per cent) of women with ER-positive cancers received hormone therapy, with little difference between the family history groups.

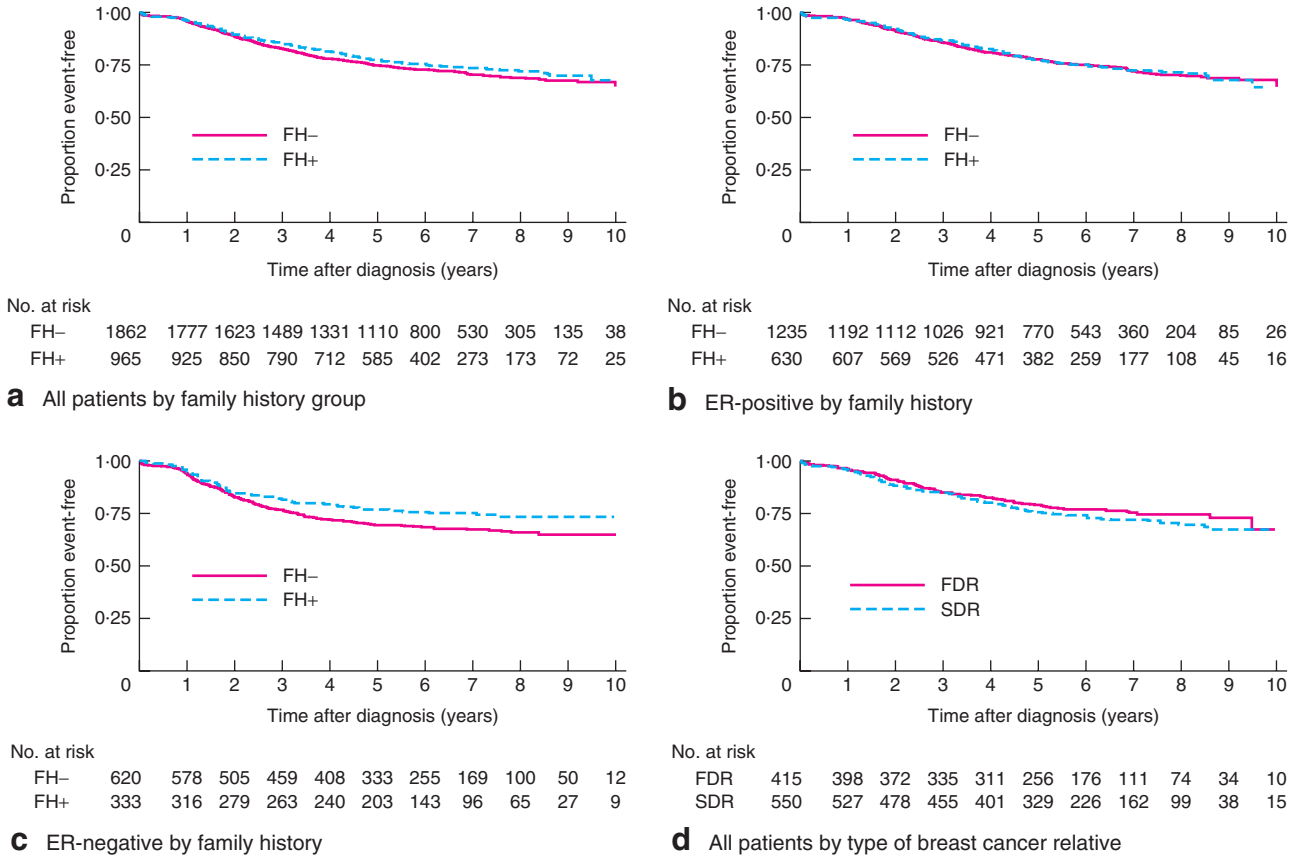

Fig. 2 Distant disease-free interval by univariable analysis for **a** all patients by family history (FH) group, **b** patients with oestrogen receptor (ER)-positive disease by family history group, **c** patients with ER-negative disease by family history group, and **d** all patients by type of breast cancer relative. FDR, first-degree relative; SDR, second-degree relative

Table 4 Multivariable analyses of impact of family history on distant disease-free interval by oestrogen receptor status

	Unadjusted*			Adjusted†		
	No. of patients	Hazard ratio	*P*	No. of patients	Hazard ratio	*P*
All patients	2827			2932		
No family history		1·00 (reference)			1·00 (reference)	
Family history		0·88 (0·75, 1·02)	0·100		0·89 (0·76, 1·03)	0·120
ER-negative	953			993		
No family history		1·00 (reference)			1·00 (reference)	
Family history		0·74 (0·57, 0·96)	0·021		0·80 (0·62, 1·04)	0·101
ER-positive	1865			1935		
No family history		1·00 (reference)			1·00 (reference)	
Family history		0·98 (0·81, 1·18)	0·813		0·95 (0·78, 1·15)	0·589

Values in parentheses are 95 per cent c.i. ER, oestrogen receptor. *Cox model with complete-case analysis; †flexible parametric survival model using multiple imputed data.

Follow-up and survival

Median follow-up was 5·9 years at the time of analysis. There was no difference in follow-up time between family history groups over the whole cohort or when split by ER status (*Table S1*, supporting information). Estimated 5-year DDFI rates were 74·9 per cent for the FH– group and 77·4 per cent for the FH+ group, and 8-year DDFI rates were 68·7 and 72·0 per cent respectively, but the difference was not significant (hazard ratio (HR) 0·88, 95 per cent c.i.

Table 5 Hazard ratios for distant disease-free interval for all patients in unadjusted and adjusted analyses

	Unadjusted*		Adjusted†	
	Hazard ratio	*P*	Hazard ratio	*P*
Non-time varying				
Age at diagnosis (years) (continuous)	0·97 (0·95, 0·99)	0·002	0·98 (0·96, 0·99)	0·009
Maximum invasive tumour diameter (continuous, log transformed)	1·96 (1·74, 2·21)	< 0·001	1·37 (1·19, 1·56)	< 0·001
Family history				
Negative	1·00 (reference)		1·00 (reference)	
Positive	0·88 (0·75, 1·02)	0·100	0·89 (0·76, 1·03)	0·120
HER2 status				
Negative	1·00 (reference)		1·00 (reference)	
Positive	1·38 (1·18, 1·61)	< 0·001	1·04 (0·89, 1·22)	0·627
PR status				
Negative	1·00 (reference)		1·00 (reference)	
Positive	0·68 (0·58, 0·79)	< 0·001	0·68 (0·52, 0·88)	0·005
Tumour grade				
1	1·00 (reference)		1·00 (reference)	
2	2·20 (1·36, 3·56)	0·001	1·37 (0·84, 2·23)	0·205
3	3·12 (1·95, 4·99)	< 0·001	1·71 (1·05, 2·78)	0·030
Node status				
N0	1·00 (reference)		1·00 (reference)	
N1	2·82 (2·40, 3·30)	< 0·001	1·09 (0·40, 2·99)	0·860
No. of positive lymph nodes				
0	1·00 (reference)		1·00 (reference)	
1–3	2·10 (1·75, 2·52)	< 0·001	1·56 (0·57, 4·27)	0·389
4–9	3·67 (2·98, 4·51)	< 0·001	2·29 (0·82, 6·40)	0·112
≥ 10	6·56 (5·22, 8·26)	< 0·001	3·58 (1·27, 10·04)	0·016
Lymphovascular invasion				
No	1·00 (reference)		1·00 (reference)	
Yes	2·56 (2·19, 3·00)	< 0·001	1·51 (1·27, 1·80)	< 0·001
Distribution				
Localized	1·00 (reference)		1·00 (reference)	
Multifocal	1·38 (1·18, 1·61)	< 0·001	1·09 (0·93, 1·28)	0·262
Time varying				
ER status at 2 years				
Negative	1·00 (reference)		1·00 (reference)	
Positive	0·71 (0·60. 0·83)	< 0·001	0·93 (0·74, 0·16)	0·522
ER status at 5 years				
Negative	1·00 (reference)		1·00 (reference)	
Positive	0·93 (0·74, 1·16)	0·522	2·06 (1·51, 2·81)	< 0·001
ER status at 8 years				
Negative	1·00 (reference)		1·00 (reference)	
Positive	2·06 (1·51, 2·81)	< 0·001	3·69 (2·11, 6·47)	< 0·001

Values in parentheses are 95 per cent c.i. HER, human epidermal growth factor receptor; PR, progesterone receptor; ER, oestrogen receptor. *Cox regression with complete-case analysis; †flexible parametric survival analyses using multiply imputed data.

0·75 to 1·02; $P = 0·100$) (*Fig. 2a*). When the analysis was repeated for women with ER-positive and -negative disease separately, the HR for DDFI remained non-significant for the ER-positive subgroup (HR 0·98, 0·81 to 1·18; $P = 0·813$) (*Fig. 2b*). However, in the ER-negative subgroup, patients with a positive family history had a superior outcome (HR 0·74, 0·57 to 0·96, $P = 0·021$), with a 5-year DDFI rate of 77·2 per cent compared with 69·7 per cent among those with no family history (*Fig. 2c*). There was no significant difference between DDFI in patients with an affected first-degree *versus* second-degree relative for the entire cohort (HR 1·17, 0·90 to 1·52; $P = 0·234$) (*Fig. 2d*) or when divided by ER status (ER-positive: HR 1·05, 0·77 to 1·45, $P = 0·761$; ER-negative: HR 1·44, 0·91 to 2·27, $P = 0·117$).

In multivariable analysis adjusting for receptor status, age at diagnosis, tumour grade, tumour size, node status, distribution of cancer and lymphovascular invasion, DDFI was similar between FH– and FH+ groups for all patients (HR 0·89; $P = 0·120$) and when categorized by ER status (ER-negative: HR 0·80, $P = 0·101$; ER-positive: HR 0·95, $P = 0·589$) (*Table 4*). Variables independently contributing

significantly to DDFI were age at diagnosis, tumour grade, size, node involvement, lymphovascular invasion and PR-positive disease. However, family history was not a contributing variable in a range of tested models (*Table 5*). To assess the possibility that the definition of FH+ (having either an affected first-degree relative or second-degree relative, or both) may have affected the result, a sensitivity analysis was conducted in which patients with an affected second-degree relative only were included in the FH− group. This analysis also showed no significant improvement in survival for patients with affected first-degree relatives compared with women with no family history (data not shown).

Discussion

The present data indicate that a positive family history of breast or ovarian cancer does not provide a significant independent contribution to the risk of distant disease recurrence in young patients with breast cancer after adjustment for known prognostic factors. Univariable analysis stratified by ER status showed that patients with ER-negative disease and a positive family history had a significantly better outcome than those with ER-negative tumours with no family history. Patients with a family history had higher-grade tumours and were more likely to have HER2- negative disease (at the 5 per cent significance level), reflecting the likely dominance of *BRCA1* mutations as the genetic cause in this subgroup, particularly among patients presenting with ER-negative disease[36]. However, background genetic aetiology is likely to be accounted for by a wide spectrum of genes and modes of inheritance, particularly in the FH+/ER-positive group. Among patients with ER-negative tumours, although patients in the FH+ group had superior outcome to those in the FH− group, once adjusted for known pathological risk factors the difference between the family history groups was no longer significant. More women with a positive family history underwent a mastectomy rather than breast-conserving surgery. Given that median tumour size was the same in both groups and there were not statistically more multifocal cancers in the FH+ group, the presence of a family history may have influenced the surgeon and/or woman to choose mastectomy. A recently published study[37] of patients eligible for breast-conserving surgery treated by a single surgeon found that family history was not independently associated with women's choice for mastectomy. In the present study the majority of patients were treated with modern anthracycline and/or taxane-based regimens, trastuzumab if the tumour was HER-2 positive and hormone therapy if ER-positive.

The major strengths of the POSH study and this analysis are its prospective nature and large sample size, with no selection of patients other than by young age, thus avoiding the potential inclusion bias of studies selecting for specific characteristics. There was minimal loss to follow-up and few missing data. The greatest amount of missing data was for PR and HER2 status, reflecting historical local pathology practices. The reasons for missing PR and HER2 data are the same for both FH+ and FH− groups, so this is unlikely to bias the analysis in any systematic way. Other than by age, there was no selection for high-risk individuals or breast cancer families, so the results are generalizable to the young British breast cancer population[14].

Potential limitations of this study include survival bias. Patients were enrolled up to 1 year after diagnosis. Very early deaths (within 1 year) might therefore be under-represented; however, the proportion of patients presenting with distant metastatic disease in the cohort was similar to the proportion recorded in national registry data[14]. Excluding patients presenting with M1 disease from the analysis did not change the results (data not shown). The median follow-up in this first analysis was 5·9 years. This is relatively short, particularly for patients with ER-positive disease, which tends to relapse later. A further limitation was that self-reporting of family history was not confirmed independently, but many studies have demonstrated that the reliability of self-reporting is fairly robust for breast cancer in close relatives[4,8,9,38].

The present study used family history defined at a single time point at enrolment into the study. Despite the young age of this cohort, nearly two-thirds did not have a positive family history. Family history is dynamic as patients seek more information about other family members' medical history and as new diagnoses arise. Retrospective or registry studies may be vulnerable to such changes, and may not accurately describe the situation facing a young patient in a surgical clinic at the time that they face a new diagnosis of breast cancer.

Most previous studies have found no clear evidence of a prognostic effect of family history, although the evidence is inconclusive, with a paucity of large prospective studies in women with young-onset disease. A prospective population-based study[9] of 905 women, which selected for a high percentage of young women (aged less than 35 years) at 'high genetic risk' from the Ontario Familial Breast Cancer Registry, found no overall significant association between family history and recurrence or overall survival. The largest investigation to date of the effect of family history on prognosis is a Swedish population registry-based study[12] of over 17 000 patients, which found a modest non-significant improved survival for patients with a family

history in the subgroup of women aged 50 years or less, with a HR of 0·82 (95 per cent c.i. 0·60 to 1·12). A further large population-based study[8] using pooled data from 4153 patients in three breast cancer family registries (California, Ontario, Melbourne) also found no mortality differences between patients with and without a family history. The two largest of these studies[8,12] did not report the use of breast cancer screening, and the largest did not include data about stage or tumour type[12].

The present study specifically recruited young women for whom the question of family history is more likely to be of direct relevance. Two studies[3,4] focusing on young women found an improvement in survival in patients with a positive family history. Malone and colleagues[3] analysed results from 1260 women in two retrospective population-based American studies of women with breast cancer aged under 46 years; they reported that women with an affected first-degree relative had a significant reduction in mortality (HR 0·7, 95 per cent c.i. 0·5 to 0·9) independent of other prognostic factors. Although the study focused on young women with a very long follow-up of 19 years, it was retrospective, enrolling women from 1983 to 1992 and selected for ethnicity using pooled data from two North American studies. In addition, about one-third of the patients did not receive chemotherapy. A much smaller study[4] comparing breast cancer survival in 95 patients with familial disease (proband aged less than 46 years) with survival in 329 matched controls reported a relative survival advantage (6·11, 95 per cent c.i. 2·81 to 13·28) in familial cases in multivariable analysis[4]. Differences in study population, definition of family history, treatment and small size of the second study may account for the differing results compared with the findings of the present study. To explore the stability of the definition of family history, the present authors conducted multivariable analysis with the FH+ group including only patients with at least one affected first-degree relative; second-degree relatives were included in the FH− group. This analysis also showed no significant improvement in survival for patients with affected first-degree relatives *versus* those with no family history.

Patients with a recognized positive family history are more likely to be known to genetics services and may be attending surveillance programmes. Few tumours in this study were detected as a result of surveillance imaging. In the UK, women do not start breast screening until 47 years of age, apart from those who fit into the highest-risk group defined by a very strong family history or a known *BRCA1/2* mutation. Unsurprisingly, the majority of patients with surveillance-detected tumours were in the FH+ group, but there was no difference in median tumour size between the FH+ and FH− groups. However, patients with an affected close relative (first-degree *versus* second-degree relative and first-degree relative *versus* no family history) had significantly smaller tumours, both in analyses of all patients and after excluding the small number of tumours detected by surveillance. This probably reflects an earlier self-presentation of these women to medical services. Earlier presentation may be offset by the high-grade tumours in the FH+ group, particularly the subgroup with affected first-degree relatives, negating any outcome difference.

In general, younger women have a greater fear of breast cancer recurrence than older women[23]. Furthermore, patients with a strong family history may have a high level of anxiety about recurrence and death from breast cancer after witnessing cancer within their family[39]. Patients who present with breast cancer in the context of a personal family history of the disease may seek reassurance from their surgeon that they are at no higher risk from recurrence or death from this breast cancer than similar patients with no family history. This study demonstrates that family history *per se* is not an independent prognostic feature for recurrence in young-onset breast cancer treated in the modern era.

Collaborators

Members of the POSH steering group are: D. Eccles, P. Simmonds (University of Southampton and University Hospital Southampton Foundation Trust, Southampton, UK); D. G. Altman (University of Oxford, Oxford, UK); P. Pharoah, R. Warren, F. Gilbert (University of Cambridge, Cambridge, UK); L. Jones (Barts Cancer Institute, Queen Mary University of London, London, UK); R. Eeles (Institute of Cancer Research, London, UK); D. G. R. Evans (University of Manchester, Manchester, UK); A. Hanby (University of Leeds, Leeds, UK); A. Thompson (MD Anderson Cancer Center, Houston, Texas, USA); S. Hodgson (Imperial College, London, UK); H. Hammad (Guy's and St Thomas' NHS Foundation Trust, London, UK); S. Lakhani (University of Queensland, Brisbane, Queensland, Australia).

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Age at diagnosis, duration of follow-up and presence of lymphovascular invasion by family history of breast cancer and oestrogen receptor status (Word document)

Table S1 Age at diagnosis, duration of follow-up and presence of lymphovascular invasion by family history of breast cancer and oestrogen receptor status

	ER-negative only†‡			ER-positive only†§		
	No family history (*n* = 625)	Family history (*n* = 333)	*P*¶	No family history (*n* = 1246)	Family history (*n* = 636)	*P*¶
Age at diagnosis (years)*	36 (19 to 40, 33–38)	36 (24–40, 33–38)	0.085	37 (18–40, 34–39)	37 (21–40, 34–39)	0.336
Unknown	0 (0)	0 (0)		0 (0)	0 (0)	
Duration of follow-up (years)*	5.54 (0.38–11.97, 3.58–7.35)	5.80 (0.59–13.23, 4.03–7.46)	0.165	5.98 (0.12–12.41, 4.75–7.56)	5.89 (0.69–11.76, 4.70–7.40	0.749
Unknown	0 (0)	0 (0)		0 (0)	0 (0)	
Lymphovascular invasion			0.567#			0.683#
No	310 (54.5)	174 (56.5)		589 (50.8)	297 (49.7)	
Yes	259 (45.5)	134 (43.5)		571 (49.2)	300 (50.3)	
Unknown	56 (9.0)	25 (7.5)		86 (6.9)	39 (6.1)	

Values in parentheses are percentages unless indicated otherwise; *values are median (range, i.q.r.). †Includes data from tissue microarrays as well as from primary Prospective Outcomes in Sporadic *versus* Hereditary breast cancer (POSH) study. ‡Thirty-eight patients (3.8 per cent) with oestrogen receptor (ER)-negative disease and unknown family history, and §66 (3.4 per cent) with ER-positive disease and unknown family history were excluded from these analyses. ¶Mann–Whitney *U* test, except #χ^2 test.

Chapter 7: Paper 5 - Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study

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7.1 Contribution

As the lead statistical methodologist, scientific lead at the SCTU and joint first author:

- Responsible for study methodology and research of all statistical methods to be implemented in the analyses, including the use of multiple imputation and FPSMs for time-varying covariates;
- Developed and authored the SAP – including the organisation and participation of meetings to develop the SAP, and the creation of all draft and final versions of the SAP;
- Responsible for central data monitoring, data cleaning and data interpretation – liaising with the study team to identify extensive data queries with the POSH data, and for the resolution of all data queries raised with data management;
- Developed and conducted all statistical programming and analyses – including the creation of all database and analysis programming required for the analyses;
- Designed, developed and produced all manuscript figures and tables – including the creation and development of the forest plots of UVA and MVA results, time-varying hazard plots by BRCA status, the transcription of all results, the creation of all draft and final versions of the manuscript figures and tables;

[6] Joint first authors

- Co-authored and reviewed the manuscript – listed as joint 1st named author, drafted the methods and results section of the manuscript, extensively involved in the interpretation of results including the interpretation of the results of the FPSM using multiple imputation, reviewed the entire manuscript including sense checks and result checking, extensively involved in the resolution of both reviewer and editor comments and responses;
- Jointly responsible for manuscript submission and administrative correspondence.

Professor Diana Eccles, chief investigator of the POSH study, chair of the POSH Study Steering Group, and senior last author of **Paper 5**, confirms the accuracy of contribution outlined above of Thomas Christopher Maishman.

Signed: .....

Date: .....

Germline *BRCA* mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study

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Summary

Background Retrospective studies provide conflicting interpretations of the effect of inherited genetic factors on the prognosis of patients with breast cancer. The primary aim of this study was to determine the effect of a germline *BRCA1* or *BRCA2* mutation on breast cancer outcomes in patients with young-onset breast cancer.

Methods We did a prospective cohort study of female patients recruited from 127 hospitals in the UK aged 40 years or younger at first diagnosis (by histological confirmation) of invasive breast cancer. Patients with a previous invasive malignancy (except non-melanomatous skin cancer) were excluded. Patients were identified within 12 months of initial diagnosis. *BRCA1* and *BRCA2* mutations were identified using blood DNA collected at recruitment. Clinicopathological data, and data regarding treatment and long-term outcomes, including date and site of disease recurrence, were collected from routine medical records at 6 months, 12 months, and then annually until death or loss to follow-up. The primary outcome was overall survival for all *BRCA1* or *BRCA2* mutation carriers (*BRCA*-positive) versus all non-carriers (*BRCA*-negative) at 2 years, 5 years, and 10 years after diagnosis. A prespecified subgroup analysis of overall survival was done in patients with triple-negative breast cancer. Recruitment was completed in 2008, and long-term follow-up is continuing.

Findings Between Jan 24, 2000, and Jan 24, 2008, we recruited 2733 women. Genotyping detected a pathogenic *BRCA* mutation in 338 (12%) patients (201 with *BRCA1*, 137 with *BRCA2*). After a median follow-up of 8·2 years (IQR 6·0–9·9), 651 (96%) of 678 deaths were due to breast cancer. There was no significant difference in overall survival between *BRCA*-positive and *BRCA*-negative patients in multivariable analyses at any timepoint (at 2 years: 97·0% [95% CI 94·5–98·4] *vs* 96·6% [95·8–97·3]; at 5 years: 83·8% [79·3–87·5] *vs* 85·0% [83·5–86·4]; at 10 years: 73·4% [67·4–78·5] *vs* 70·1% [67·7–72·3]; hazard ratio [HR] 0·96 [95% CI 0·76–1·22]; p=0·76). Of 558 patients with triple-negative breast cancer, *BRCA* mutation carriers had better overall survival than non-carriers at 2 years (95% [95% CI 89–97] *vs* 91% [88–94]; HR 0·59 [95% CI 0·35–0·99]; p=0·047) but not 5 years (81% [73–87] *vs* 74% [70–78]; HR 1·13 [0·70–1·84]; p=0·62) or 10 years (72% [62–80] *vs* 69% [63–74]; HR 2·12 [0·82–5·49]; p= 0·12).

Interpretation Patients with young-onset breast cancer who carry a *BRCA* mutation have similar survival as non-carriers. However, *BRCA* mutation carriers with triple-negative breast cancer might have a survival advantage during the first few years after diagnosis compared with non-carriers. Decisions about timing of additional surgery aimed at reducing future second primary-cancer risks should take into account patient prognosis associated with the first malignancy and patient preferences.

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Introduction

Although only 5% of breast cancers are diagnosed in women aged younger than 40 years, a high proportion of deaths from breast cancer occur in this age group, which includes a higher number of patients who carry a pathogenic *BRCA1* or *BRCA2* mutation compared with patients with onset of breast cancer at an older age.[1–3] Second primary breast cancers are more frequent in high-risk gene carriers, and this higher frequency drives early genetic testing to inform surgical decision making; however, whether a germline *BRCA1* or *BRCA2* mutation has independent prognostic implications after an initial cancer diagnosis is unclear.

BRCA1 loss of function mutations are associated with high-histological-grade, oestrogen-receptor-negative, progesterone-receptor-negative, and HER2-negative (triple negative) breast cancer with a basal-like gene expression profile.[4] *BRCA2*-associated breast tumours are usually high-grade, oestrogen-receptor positive, and HER2-negative.[5,6] *BRCA1* mutation carriers have been reported to have enhanced sensitivity to neoadjuvant chemotherapy with cytotoxic drugs.[7]

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For more about the **POSH study** see http://www.southampton.ac.uk/medicine/research/posh.page

For the **BOADICEA algorithm** see http://ccge.medschl.cam.ac.uk/boadicea/

See Online for appendix

Research in context

Evidence before this study

At the initiation of this cohort study (Dec 3, 1999), we searched the PubMed database using the search terms [*BRCA1* OR *BRCA2*] AND [breast cancer or breast neoplasm] AND [survival OR prognosis OR mortality] and identified a few published retrospective studies reporting prognosis in *BRCA* mutation carriers. On Dec 5, 2016, we did another PubMed search for studies of patients who carried a *BRCA1* or *BRCA2* mutation and their prognosis, using the following search terms: "(*BRCA*) AND (survival or prognosis or outcome or mortality) AND (breast neoplasms or breast neoplasm or breast cancer or breast tumour)". Our search was not limited by date or language. We also hand-searched references cited in review papers for additional papers. Previous studies and meta-analyses have reported inconsistent effects of *BRCA1* and *BRCA2* mutations on the outcomes of early breast cancer with better, worse, and similar outcomes for patients with a *BRCA1* or *BRCA2* mutation compared with patients with sporadic breast cancer. These conflicting results might be explained by methodological issues with ascertainment biases introduced by retrospective and selective identification of cases, incomplete genetic testing, small numbers, an absence of adjustment for clinical variables, including treatment, and short follow-up.

Added value of this study

POSH is, to our knowledge, the largest prospective cohort study to compare breast cancer outcomes of patients with a *BRCA1* or *BRCA2* mutation with patients with sporadic cancer. Our findings showed that patients with young-onset breast cancer who have a *BRCA* mutation have a similar overall survival to non-carriers. However, in patients with triple-negative breast cancer, *BRCA* mutation carriers might have a survival advantage compared with non-carriers during the first few years after diagnosis. Our study was strengthened by unbiased recruitment, universal and central genetic testing at the end of the study, and comprehensive pathological, clinical, and follow-up data.

Implications of all the available evidence

Decisions about timing of risk-reducing surgery should take into account primary tumour prognosis and patient preference.

Published studies and meta-analyses have reported better, worse, and similar outcomes for patients with a *BRCA1* or *BRCA2* mutation compared with patients with sporadic breast cancer.[8–14] A comprehensive meta-analysis of 66 studies of breast cancer survival in patients with a *BRCA1* or *BRCA2* mutation compared with non-carrier patients or the general breast cancer population, which assessed study quality as well as outcome data, concluded that "it is not yet possible to draw evidence based conclusions about the association between *BRCA1* [or] *BRCA2* mutation carriership and breast cancer prognosis".[12] We undertook the Prospective Outcomes in Sporadic versus Hereditary breast cancer (POSH) study, the primary aim of which was to determine the effect of inherited *BRCA1* or *BRCA2* mutations on outcomes in patients with young-onset breast cancer.[15,16]

Methods

Study design and participants

We did a prospective cohort study at 127 hospitals in the UK (appendix pp 1–2). We recruited young women (aged 18–40 years) diagnosed with primary breast cancer in the UK. Patients were eligible if they were diagnosed with invasive breast cancer aged 40 years or younger. Potential recruits were identified by local breast cancer clinicians, nurses, or research clinical trial practitioners within 12 months of initial diagnosis of invasive breast cancer and the date of diagnosis was defined as the first histological confirmation of invasive breast cancer. All histological subtypes, disease stages (I–IV), comorbidities, and performance statuses were permitted. Patients with a previous invasive malignancy (with the exception of non-melanomatous skin cancer) were excluded.

Written informed consent was obtained from all participants. Ethical approval was granted in 2000 (MREC 00/6/69) and the study was approved for recruitment as part of the UK National Cancer Research Network (NCRN) portfolio in 2002, subsequently the NIHR portfolio. The protocol was published in 2007.[15]

Procedures

All patients received treatment according to local protocols. Details of personal characteristics, tumour pathology, disease stage, and surgical and cytotoxic treatment data were collected from medical records at study entry. Family history was collected by questionnaire. The BOADICEA algorithm, without adjustment for pathological subtype, was used to estimate the probability that an individual might carry a *BRCA1* or *BRCA2* pathogenic variant.[17] Pathology and imaging data were verified with copies of the original reports from sites. For patients treated with neoadjuvant chemotherapy, the initial diameter of the tumour was derived from radiological reports.

The oestrogen-receptor, progesterone-receptor, and HER2-receptor status of the primary tumours was determined from reports of local routine pathology testing of diagnostic core biopsies or tumour resections for clinical use. Hormone-receptor concentrations equivalent to an Allred score of 3 or more were categorised as positive. Immunohistochemical staining of tissue microarrays in some cases enabled clinical source data for oestrogen-receptor, progesterone-receptor, and HER2-receptor statuses to be corroborated; tissue microarray scores were used to supplement missing datapoints for these receptors.[16]

DNA for genotyping was extracted from whole blood samples submitted at recruitment. A multiplex amplicon-based library preparation system, Fluidigm Access Array (Fluidigm UK, Cambridge, UK), targeted a panel of breast-cancer-susceptibility genes (including *BRCA1, BRCA2,* and *TP53*) for sequencing using an Illumina HiSeq2500 Next Generation Sequencing Platform (Illumina, Little Chesterford, UK; appendix pp 20–21). Targeted-sequence capture cannot reliably identify large exonic deletions or duplications, therefore multiplex ligation probe analysis was used for patients who met current UK guideline thresholds for clinical genetic testing.[17,18] Predicted protein truncating variants (frameshift, nonsense, and canonical-splice site and large rearrangements) plus other variants (mainly mis-sense) unequivocally defined as pathogenic on the basis of multiple lines of evidence and expert review were assigned to the *BRCA*-mutation carrier group (*BRCA*-positive). All pathogenic variants were confirmed by Sanger sequencing. All other patients, including those with *BRCA1* or *BRCA2* variants of uncertain significance or very low penetrance, were assigned to the same group as no mutation found (*BRCA*-negative) or excluded if they were found to carry a pathogenic variant of *TP53*. For the purposes of this analysis, mutations in other breast cancer genes were not curated.

The study protocol and patient information specified that patients would not be informed of the research genetic-testing results; however, patient information sheets gave information about seeking clinical genetic referral. Clinical referrals for genetic testing were made by the treating physician according to local protocols. Genetic test reports for the study patients generated by UK National Health Service (NHS) diagnostic laboratories were collected as part of the medical record.

Detailed clinical follow-up data, including date and site of disease recurrence, were obtained from medical records at 6 months, 12 months, and annually thereafter, until death or loss to follow-up. Patients were flagged in the NHS medical research information service for automatic notification of date and cause of death.

Outcomes

The primary outcome was overall survival, defined as the time from first diagnosis to death from any cause. The secondary outcomes were distant disease-free survival, defined as time from first diagnosis to first distant disease excluding local (in breast) recurrence.

Statistical analysis

The original study sample size of a minimum of 2000 patients was estimated based on a prevalence of *BRCA1* or *BRCA2* pathogenic mutations of 10%, and an absolute difference in event rate at 2 years between mutation carriers and non-carriers of 10% (20% in mutation carriers compared with 10% in sporadic cases).[15] We also considered a prevalence of *BRCA1* or *BRCA2* mutations of 5% and 15%, and larger sample sizes. Good recruitment and data returns enabled us to continue study recruitment beyond 2000 participants providing sufficient power for multivariable analyses.

We did the statistical analyses according to a prespecified plan (appendix pp 22–31).[19] The analysis population included all eligible patients recruited to the cohort who had available data for the primary tumour and genotyping, were aged 40 years or younger at the date of diagnosis, did not carry a *TP53* gene, and who did not present with metastatic disease at presentation (M1 stage). A prespecified subgroup of the analysis population was patients with triple-negative breast cancer (ie, oestrogen-receptor-negative, HER2-negative, and progesterone-receptor-negative or unknown). All analyses were done for both the overall analysis population and the triple-negative breast cancer subgroup population, unless specified otherwise. Key patient data were described by *BRCA* mutation status, and formal comparisons by *BRCA* mutation status were done using Mann-Whitney tests (for continuous variables) and Pearson χ^2 tests (for categorical variables) for patients with complete data. We used Kaplan-Meier plots to show survival data by *BRCA* status at 2, 5, and 10 years. The 2-year comparison was chosen because this timepoint was specified for the original sample size; the 5-year and 10-year comparisons were chosen because they are commonly used in such studies and are clinically relevant timepoints. Patients who did not have an event were censored at the date of their last follow-up. Hazard ratios (HRs) and 95% CIs for

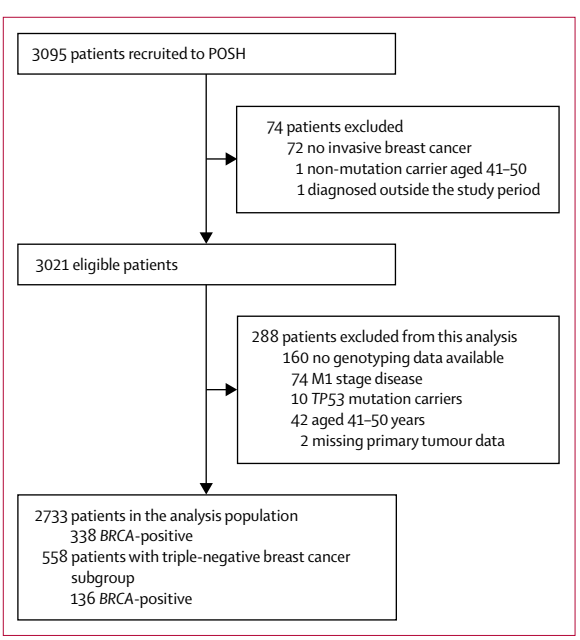

Figure 1: **Trial profile**
BRCA-positive=patient with *BRCA1* or *BRCA2* pathogenic mutation. Patients were categorised as *BRCA*-negative if no *BRCA* pathogenic mutation was found or they had a *BRCA1* or *BRCA2* variant of uncertain significance or very low penetrance.

univariable analyses and multivariable analyses (for the primary and secondary outcomes) were calculated using Cox proportional-hazards models, or flexible parametric survival models for those that involved time-varying hazards.[20] For each flexible parametric survival model, varying degrees of freedom for the baseline-hazard rate

	All patients (n=2733)	*BRCA1*-positive (n=201)	*BRCA2*-positive (n=137)	*BRCA*-positive (n=338)	*BRCA*-negative (n=2395)	p value*
Age at diagnosis (years)	36 (34–38, 18–40)	35 (32–38, 22–40)	37 (33–38, 21–40)	36 (32–38, 21–40)	37 (34–39, 18–40)	*BRCA*-positive *vs* *BRCA*-negative p<0·0001, *BRCA1*-positive *vs* *BRCA2*-positive p=0·014
BMI (kg/m²)						*BRCA*-positive *vs* *BRCA*-negative p=0·48, *BRCA1*-positive *vs* *BRCA2*-positive p=0·40
<25	1427/2632 (54%)	114/192 (59%)	70/133 (53%)	184/325 (57%)	1243/2307 (54%)	
≥25 to <30	714/2632 (27%)	47/192 (25%)	41/133 (31%)	88/325 (27%)	626/2307 (27%)	
≥30	491/2632 (19%)	31/192 (16%)	22/133 (17%)	53/325 (16%)	438/2307 (19%)	
Missing	101 (4%)	9 (5%)	4 (3%)	13 (4%)	88 (4%)	
Ethnicity						*BRCA*-positive *vs* *BRCA*-negative p=0·28, *BRCA1*-positive *vs* *BRCA2*-positive p=0·99
White	2494/2698 (92%)	178/196 (91%)	122/134 (91%)	300/330 (91%)	2194/2368 (93%)	
Black	103/2698 (4%)	10/196 (5%)	6/134 (5%)	16/330 (5%)	87/2368 (4%)	
Asian	80/2698 (3%)	5/196 (3%)	4/134 (3%)	9/330 (3%)	71/2368 (3%)	
Other	21/2698 (<1%)	3/196 (2%)	2/134 (2%)	5/330 (2%)	16/2368 (<1%)	
Missing	35 (1%)	5 (3%)	3 (2%)	8 (2%)	27 (1%)	
Histological grade						*BRCA*-positive *vs* *BRCA*-negative p<0·0001, *BRCA1*-positive *vs* *BRCA2*-positive p<0·0001
1	156/2658 (6%)	2/197 (1%)	0	2/326 (<1%)	154/2332 (7%)	
2	904/2658 (34%)	16/197 (8%)	40/129 (31%)	56/326 (17%)	848/2332 (36%)	
3	1598/2658 (60%)	179/197 (91%)	89/129 (69%)	268/326 (82%)	1330/2332 (57%)	
Missing or not graded	75 (3%)	4 (2%)	8 (6%)	12 (4%)	63 (3%)	
Oestrogen-receptor status						*BRCA*-positive *vs* *BRCA*-negative p<0·0001, *BRCA1*-positive *vs* *BRCA2*-positive p<0·0001
Negative	908/2719 (33%)	151/200 (76%)	21/136 (15%)	172/336 (51%)	736/2383 (31%)	
Positive	1811/2719 (67%)	49/200 (25%)	115/136 (85%)	164/336 (49%)	1647/2383 (69%)	
Missing	14 (<1%)	1 (<1%)	1 (<1%)	2 (<1%)	12 (<1%)	
HER2 status						*BRCA*-positive *vs* *BRCA*-negative p<0·0001, *BRCA1*-positive *vs* *BRCA2*-positive p=0·18
Negative	1763/2412 (73%)	164/176 (93%)	111/125 (89%)	275/301 (91%)	1488/2111 (71%)	
Positive	649/2412 (27%)	12/176 (7%)	14/125 (11%)	26/301 (9%)	623/2111 (30%)	
Missing	321 (12%)	25 (12%)	12 (9%)	37 (11%)	284 (12%)	
Progesterone-receptor status						*BRCA*-positive *vs* *BRCA*-negative p<0·0001, *BRCA1*-positive *vs* *BRCA2*-positive p<0·0001
Negative	951/2208 (43%)	144/171 (84%)	23/107 (22%)	167/278 (60%)	784/1930 (41%)	
Positive	1257/2208 (57%)	27/171 (16%)	84/107 (79%)	111/278 (40%)	1146/1930 (59%)	
Missing	525 (19%)	30 (15%)	30 (22%)	60 (18%)	465 (19%)	
†Triple-negative breast cancer status						*BRCA*-positive *vs* *BRCA*-negative p<0·0001, *BRCA1*-positive *vs* *BRCA2*-positive p<0·0001
No	2175/2733 (80%)	78/201 (39%)	124/137 (91%)	202/338 (60%)	1973/2395 (82%)	
Yes	558/2733 (20%)	123/201 (61%)	13/137 (10%)	136/338 (40%)	422/2395 (18%)	
Maximum invasive tumour size (mm)	22 (15–33, 0–170)	21 (15–30, 1–140)	25 (16–32, 1–92)	22 (15–31, 1–140)	22 (15–34, 0–170)	*BRCA*-positive *vs* *BRCA*-negative p=0·97, *BRCA1*-positive *vs* *BRCA2*-positive p=0·060
Missing	156 (6%)	10 (5%)	14 (10%)	24 (7%)	132 (6%)	

(Table 1 continues on next page)

and time-dependent effect were explored to obtain the best-model fit. All missing data were assumed to be either missing at random or missing completely at random, and censoring was assumed to be non-informative. Prespecified sensitivity analyses included the generation of corresponding complete-case multivariable analysis model results.

Post-hoc sensitivity analyses were done to explore the possible reasons for some of the results in the triple-negative breast cancer group. Additionally, to investigate the degree of potential bias from time of diagnosis to blood draw for genetic testing at registration, a multivariable analysis model adjusting for the time from diagnosis to blood draw was generated accordingly for the analysis population only. We considered if the longer survival of *BRCA* mutation carriers with triple-negative breast cancer could be due to a beneficial effect of risk-reducing surgery in *BRCA*

	All patients (n=2733)	*BRCA1*-positive (n=201)	*BRCA2*-positive (n=137)	*BRCA*-positive (n=338)	*BRCA*-negative (n=2395)	p value*
(Continued from previous page)						
Pathological N stage						*BRCA*-positive *vs* *BRCA*-negative p=0·013, *BRCA1*-positive *vs* *BRCA2*-positive p<0·0001
0	1304/2692 (48%)	129/201 (64%)	55/135 (41%)	184/336 (55%)	1120/2356 (48%)	
1	1388/2692 (52%)	72/201 (36%)	80/135 (59%)	152/336 (45%)	1236/2356 (53%)	
Axillary nodal involvement						*BRCA*-positive *vs* *BRCA*-negative p=0·019, *BRCA1*-positive *vs* *BRCA2*-positive p=0·00017
1–3	899/2692 (33%)	43/201 (21%)	51/135 (38%)	94/336 (28%)	805/2356 (34%)	
4–9	330/2692 (12%)	14/201 (7%)	19/135 (14%)	33/336 (10%)	297/2356 (13%)	
≥10	159/2692 (6%)	15/201 (8%)	10/135 (7%)	25/336 (7%)	134/2356 (6%)	
Missing	41 (2%)	0	2 (2%)	2 (<1%)	39 (2%)	
Lymphovascular invasion						*BRCA*-positive *vs* *BRCA*-negative p=0·23, *BRCA1*-positive *vs* *BRCA2*-positive p=0·013
Absent	1327/2539 (52%)	116/190 (61%)	58/124 (47%)	174/314 (55%)	1153/2225 (52%)	
Present	1212/2539 (48%)	74/190 (39%)	66/124 (53%)	140/314 (45%)	1072/2225 (48%)	
Missing	194 (7%)	11 (6%)	13 (10%)	24 (7%)	170 (7%)	
Chemotherapy						*BRCA*-positive *vs* *BRCA*-negative p=0·0058, *BRCA1*-positive *vs* *BRCA2*-positive p=0·016
None	294/2733 (11%)	9/201 (5%)	11/137 (8%)	20/338 (6%)	274/2395 (11%)	
Adjuvant	2027/2733 (74%)	171/201 (85%)	99/137 (72%)	270/338 (80%)	1757/2395 (73%)	
Neoadjuvant	412//2733 (15%)	21/201 (10%)	27/137 (20%)	48/338 (14%)	364/2395 (15%)	
Type of surgery						*BRCA*-positive *vs* *BRCA*-negative p=0·30, *BRCA1*-positive *vs* *BRCA2*-positive p=0·00040
Breast-conserving surgery	1337/2733 (49%)	106/201 (53%)	43/137 (31%)	149/338 (44%)	1188 (50%)	
Mastectomy	1373/2733 (50%)	94/201 (47%)	92/137 (67%)	186/338 (55%)	1187/2395 (50%)	
Nodal surgery only	7/2733 (<1%)	1/201 (<1%)	0	1/338 (<1%)	6/2395 (<1%)	
None	16/2733 (<1%)	0	2/137 (2%)	2/338 (<1%)	14/2395 (<1%)	
Chemotherapy regimen						*BRCA*-positive *vs* *BRCA*-negative p=0·015, *BRCA1*-positive *vs* *BRCA2*-positive p=0·38
None	294/2733 (11%)	9/201 (5%)	11/137 (8%)	20/338 (6%)	274/2395 (11%)	
Anthracyclines	1760/2733 (64%)	145/201 (72%)	89/137 (65%)	234/338 (69%)	1526/2395 (64%)	
Taxanes	24/2733 (<1%)	0	1/137 (<1%)	1/338 (<1%)	23/2395 (1%)	
Anthracyclines and taxanes	635/2733 (23%)	45/201 (22%)	34/137 (25%)	79/338 (23%)	556/2395 (23%)	
Other (including CMF)	20/2733 (<1%)	2/201 (1%)	2/137 (2%)	4/338 (1%)	16/2395 (<1%)	

Data are median (IQR, range) or n (%). Patients with missing data were not included in the p value calculation. BMI=body-mass index. CMF=cyclophosphamide plus methotrexate plus fluorouracil. *Test excluded patients with both *BRCA1* and *BRCA2* mutations. Mann-Whitney tests used for continuous variables and Pearson χ^2 tests for categorical variables, done on patients with complete data. †Defined as oestrogen-receptor-negative, HER2-negative, and progesterone-receptor-negative or unknown.

Table 1: **Baseline characteristics and clinicopathological information for all patients**

carriers, so we repeated the analysis in this subgroup excluding patients who underwent bilateral mastectomy within the first year after diagnosis. A further sensitivity analysis was done to compare the pattern of improved survival at an early timepoint with apparently worse survival in the long term by excluding patients who developed a new primary breast or ovarian cancer.

We did all analyses with Stata, version 14.2, and multiple imputation was incorporated in the multivariable analyses generated using the mi command.

Role of the funding source

The funders and their representatives had no role in study design, data collection, data analysis, data interpretation, or writing of the report or the decision to

	All patients (n=558)	*BRCA1*-positive (n=123)	*BRCA2*-positive (n=13)	*BRCA*-positive (n=136)	*BRCA*-negative (n=422)	p value†
Age at diagnosis (years)	36 (33–38, 19–40)	34 (32–37, 22–40)	33 (32–38, 30–40)	34 (32–37, 22–40)	36 (33–38, 19–40)	*BRCA*-positive *vs* *BRCA*-negative p=0·00056, *BRCA1*-positive *vs* *BRCA2*-positive p=0·79
BMI (kg/m²)						*BRCA*-positive *vs* *BRCA*-negative p=0·26, *BRCA1*-positive *vs* *BRCA2*-positive p=0·47
<25	274/546 (50%)	67/119 (56%)	5/13 (39%)	72/132 (55%)	202/414 (49%)	
≥25 to <30	149/546 (27%)	32/119 (27%)	5/13 (39%)	37/132 (28%)	112/414 (27%)	
≥30	123/546 (23%)	20/119 (17%)	3/13 (23%)	23/132 (18%)	100/414 (24%)	
Missing	12 (2%)	4 (3%)	0	4 (3%)	8 (2%)	
Ethnicity						*BRCA*-positive *vs* *BRCA*-negative p=0·52, *BRCA1*-positive *vs* *BRCA2*-positive p=0·052
White	500/550 (91%)	110/122 (90%)	9/13 (69%)	119/135 (88%)	381/415 (92%)	
Black	26/550 (5%)	7/122 (6%)	2/13 (15%)	9/135 (7%)	17/415 (4%)	
Asian	19/550 (4%)	3/122 (3%)	2/13 (15%)	5/135 (4%)	14/415 (3%)	
Other	5/550 (<1%)	2/122 (2%)	0	2/135 (2%)	3/415 (<1%)	
Missing	8 (1%)	1 (<1%)	0	1 (<1%)	7 (2%)	
Histological grade						*BRCA*-positive *vs* *BRCA*-negative p=0·49, *BRCA1*-positive *vs* *BRCA2*-positive p=0·41
1	3/541 (<1%)	0	0	0	3/406 (<1%)	
2	30/541 (6%)	6/122 (5%)	0	6/135 (4%)	24/406 (6%)	
3	508/541 (94%)	116/122 (95%)	13/13 (100%)	129/135 (96%)	379/406 (93%)	
Missing or not graded	17 (3%)	1 (<1%)	0	1 (<1%)	16 (4%)	
Maximum invasive tumour size (mm)	22 (15–31, 1–160)	21 (15–30, 4–140)	23 (16–30, 15–30)	21 (15–30, 4–140)	23 (15–32, 1–160)	*BRCA*-positive *vs* *BRCA*-negative p=0·17, *BRCA1*-positive *vs* *BRCA2*-positive p=0·72
Missing	35 (6%)	5 (4%)	3 (23%)	8 (6%)	27 (6%)	..
Pathological N stage						*BRCA*-positive *vs* *BRCA*-negative p=0·46, *BRCA1*-positive *vs* *BRCA2*-positive p=0·64
0	341/552 (62%)	80/123 (65%)	7/12 (58%)	87/135 (64%)	254/417 (61%)	
1	211/552 (38%)	43/123 (35%)	5/12 (42%)	48/135 (36%)	163/417 (39%)	
Axillary nodal involvement						*BRCA*-positive *vs* *BRCA*-negative p=0·044, *BRCA1*-positive *vs* *BRCA2*-positive p=0·68
1 to 3	141/552 (26%)	26/123 (21%)	4/12 (33%)	30/135 (22%)	111/417 (27%)	
4 to 9	45/552 (8%)	7/123 (6%)	0	7/135 (5%)	38/417 (9%)	
≥10	25/552 (5%)	10/123 (8%)	1/12 (8%)	11/135 (8%)	14 /417 (3%)	
Missing	6 (1%)	0	1 (8%)	1 (<1%)	5 (1%)	
Lymphovascular invasion						*BRCA*-positive *vs* *BRCA*-negative p=0·83, *BRCA1*-positive *vs* *BRCA2*-positive p=0·19
Absent	312/517 (60%)	71/116 (61%)	4/10 (40%)	75/126 (60%)	237/391 (61%)	
Present	205/517 (40%)	45/116 (39%)	6/10 (60%)	51/126 (41%)	154/391 (39%)	
Missing	41 (7%)	7 (6%)	3 (23%)	10 (7%)	31 (7%)	

(Table 2 continues on next page)

	All patients (n=558)	*BRCA1*-positive (n=123)	*BRCA2*-positive (n=13)	*BRCA*-positive (n=136)	*BRCA*-negative (n=422)	p value†
(Continued from previous page)						
Chemotherapy						*BRCA*-positive *vs* *BRCA*-negative p=0·17, *BRCA1*-positive *vs* *BRCA2*-positive, p=0·074
None	13/558 (2%)	3/123 (2%)	0	3/136 (2%)	10/422 (2%)	
Adjuvant	450/558 (81%)	108/123 (88%)	9/13 (69%)	117/136 (86%)	333/422 (79%)	
Neoadjuvant	95/558 (17%)	12/123 (10%)	4/13 (31%)	16/136 (12%)	79/422 (19%)	
Type of surgery						*BRCA*-positive *vs* *BRCA*-negative p=0·19, *BRCA1*-positive *vs* *BRCA2*-positive p=0·014
Breast-conserving surgery	331/558 (59%)	69/123 (56%)	5/13 (39%)	74/136 (54%)	257/422 (61%)	
Mastectomy	223/558 (40%)	53/123 (43%)	7/13 (54%)	60/136 (44%)	163/422 (39%)	
Nodal surgery only	1/558 (<1%)	1/123 (<1%)	0	1/136 (<1%)	0	
None	3/558 (<1%)	0	1/13 (8%)	1/136 (<1%)	2/422 (<1%)	
Chemotherapy regimen						*BRCA*-positive *vs* *BRCA*-negative p=0·097, *BRCA1*-positive *vs* *BRCA2*-positive p=0·086
None	13 (2%)	3 (2%)	0	3 (2%)	10 (2%)	
Anthracyclines	382/558 (69%)	91/123 (74%)	6/13 (46%)	97/136 (71%)	285/422 (68%)	
Taxanes	2/558 (<1%)	0	0	0	2/422 (<1%)	
Anthracyclines and taxanes	159/558 (29%)	27/123 (22%)	7/13 (54%)	34/136 (25%)	125/422 (30%)	
Other (includes CMF)	2/558 (<1%)	2/123 (2%)	0	2/136 (2%)	0	

Data are median (IQR, range) or n (%). Patients with missing data were not included in the p value calculation. BMI=body-mass index. CMF=cyclophosphamide plus methotrexate plus fluorouracil. *Defined as oestrogen-receptor-negative, HER2-negative, and progesterone-receptor-negative or unknown. †Test excluded patients with both *BRCA1* and *BRCA2* mutations. Mann-Whitney tests used for continuous variables and Pearson χ^2-tests for categorical variables, done on patients with complete data.

Table 2: **Baseline characteristics and clinicopathological information for patients with triple-negative breast cancer***

submit it for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Jan 24, 2000, and Jan 24, 2008, we recruited 3021 eligible women, of whom 2733 (91%) were included in the analysis population, and 288 (9%) were excluded (figure 1; appendix p 11). We included all data received until July 26, 2016. Of 2721 patients for whom presentation was recorded, 45 (2%) were recorded as being enrolled in a surveillance programme, and 33 (1%) were recorded as having screen-detected breast cancer. Screening was offered according to local protocols; national guidelines were not formally established until after recruitment ended.

338 (12%) of 2733 patients included in the analysis population had either a *BRCA1* or *BRCA2* mutation, of whom 44 (13%) had large-copy-number variants (appendix pp 3–7). 75 (22%) of 338 patients did not meet current family history or pathology based genetic-testing guidelines.[18] Referral for a clinical genetics consultation and *BRCA* testing occurred for 388 patients (14%), of whom 182 (47%) had a pathogenic mutation. Immunohistochemical staining of tissue microarrays in 1336 cases, during 2012 and 2016, enabled clinical source data for oestrogen-receptor, progesterone-receptor, and HER2-receptor statuses to be corroborated.

The median time from breast cancer diagnosis to study registration blood draw was 5·5 months (IQR 3·2–10·7). There were several significant clinicopathological differences between *BRCA*-positive and *BRCA*-negative patients, and between *BRCA1* mutation carriers and *BRCA2* mutation carriers (table 1). The most commonly used chemotherapy regimen was anthracycline with or without taxanes. Of the 2733 patients in the analysis population, 558 (20%) had triple-negative breast cancer. *BRCA* mutations were identified in 136 (24%) of patients with triple-negative breast cancer, of whom 123 (90%) had a *BRCA1* mutation. Differences in tumour characteristics between *BRCA1* and *BRCA2* mutation carriers were also noted in patients with triple-negative breast cancer (table 2).

Median follow-up was 8·2 years (IQR 6·0–9·9); 91 (3%) patients were lost to follow-up. Contralateral breast tumours occurred in 151 (6%) patients: in 37 (18%) of 201 *BRCA1* mutation carriers, 17 (12%) of 137 *BRCA2* mutation carriers, and 97 (4%) of 2395 *BRCA*-negative patients. Median time to contralateral breast cancer was 3·0 years (IQR 1·5–4·8) in *BRCA*-positive patients and 2·7 years (1·2–5·3) in *BRCA*-negative patients. 752 (28%) women developed a distant recurrence. Of 678 deaths, 651 (96%) were due to breast cancer. Deaths due to non-breast malignancies included

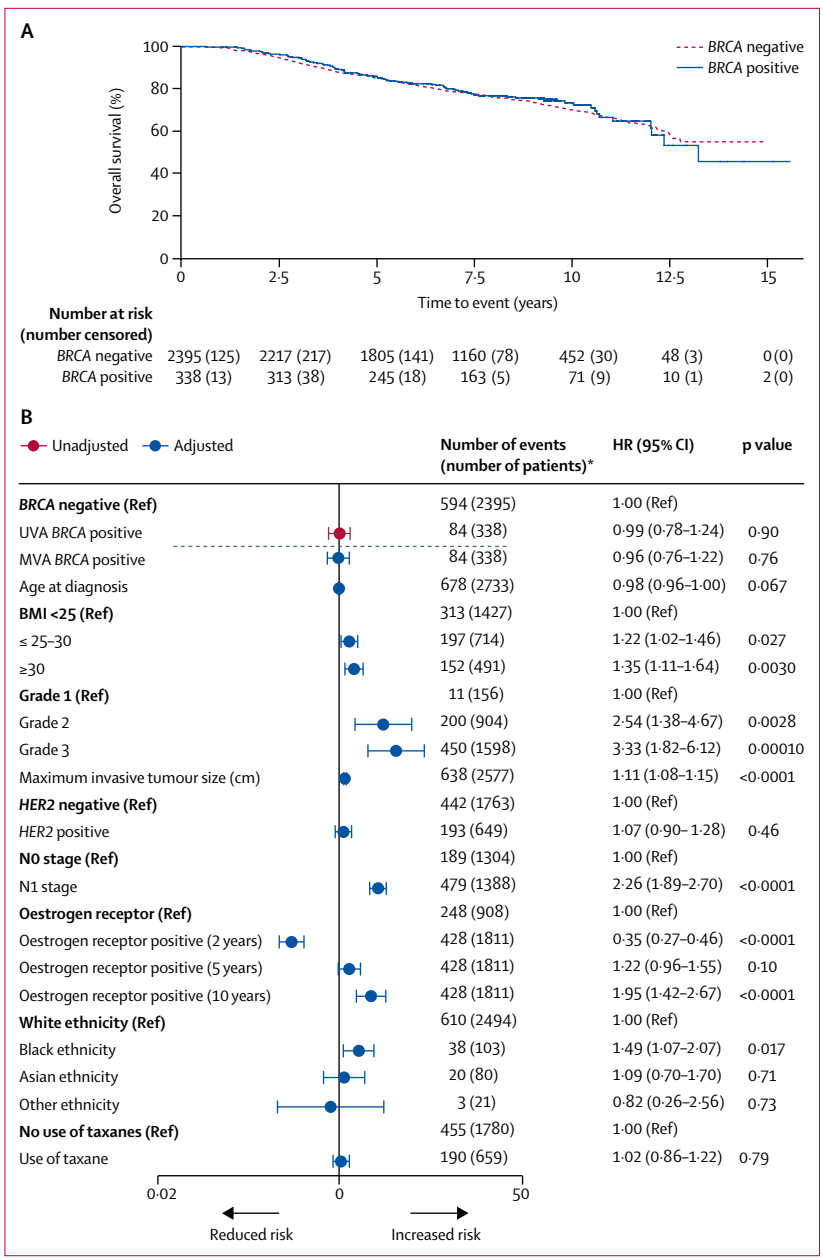

Figure 2: **Overall survival for all patients (analysis population) by *BRCA* mutation status**
(A) Kaplan-Meier plot and (B) forest plot of corresponding univariable and multivariable hazard ratios. In (B), multivariable analysis was adjusted for age, body-mass index (BMI; kg/m^2), grade, tumour size, HER2 status, oestrogen-receptor status, ethnicity, and use of taxane chemotherapy. Groups without a reference were assessed as a continuous variable. The dashed line separates the univariable analysis (UVA) from the multivariable analysis (MVA). Oestrogen-receptor-positive group assessed at 2, 5, and 10 years because the hazard ratio associated with oestrogen-positive status varies with time.[16] HR=hazard ratio. *Number of events (number of patients) from complete data obtained before multiple imputation.

six (3%) of 201 new primary cancers in *BRCA1* mutation carriers (three ovarian, one primary peritoneal, one oesophageal, and one pancreatic) and 12 (<1%) of 2395 malignancies in *BRCA*-negative patients (four haematological, three lung, and one each of brain, colorectal, gastric, pancreatic, and sarcoma; appendix p 8). There were no deaths attributed to second primary cancers among *BRCA2* mutation carriers.

Overall survival was 97·0% (95% CI 94·5–98·4) in *BRCA*-positive patients versus 96·6% (95·8–97·3) in *BRCA*-negative patients at 2 years; 83·8% (79·3–87·5) versus 85·0% (83·5–86·4) at 5 years; and 73·4% (67·4–78·5) versus 70·1% (67·7–72·3) at 10 years (figure 2). There was no difference in overall survival between groups either before or after adjusting for known prognostic factors, including adjustments for ethnicity and body-mass index (BMI; univariable analysis negative *vs* positive HR 0·99 [95% CI 0·78–1·24], p=0·90; multivariable analysis HR 0·96 [0·76–1·22], p=0·76). Similar results were noted when comparing distant disease-free survival between *BRCA*-positive and *BRCA*-negative groups (appendix p 12). Additionally, comparison of overall survival in *BRCA*-negative patients versus *BRCA1* or *BRCA2* carriers separately showed similar results (appendix pp 13–14).

In the subgroup of 558 patients with triple-negative breast cancer, 159 (28%) women developed a distant recurrence, 153 (27%) died, and all deaths were due to breast cancer. The estimated hazard for death after diagnosis of triple-negative breast cancer varied over time (appendix p 32). In the triple-negative breast cancer subgroup, overall survival was significantly better at 2 years for *BRCA*-positive patients than for *BRCA*-negative patients (95% [95% CI 89–97]) *vs* 91% [88–94]; multivariable analysis flexible parametric survival model HR 0·59 [95% CI 0·35–0·99], p=0·047). Overall survival at 5 years was 81% (95% CI 73–87) versus 74% (70–78; multivariable analysis flexible parametric survival model HR 1·13 [95% CI 0·70–1·84], p=0·62); and at 10 years was 72% (62–80) versus 69% (63–74; multivariable analysis flexible parametric survival model HR 2·12 [95% CI 0·82–5·49], p=0·12; figure 3). For distant disease-free survival, however, the difference between *BRCA*-positive and *BRCA*-negative patients was not significant (appendix p 15). Inclusion of time from diagnosis to registration blood draw in multivariable analyses did not affect the results (appendix p 16). For analyses of both the overall population and the subgroup of patients with triple-negative breast cancer, results with imputation were almost identical to complete case results (appendix pp 9–10). Results from tests of proportional hazards are also in the appendix (p 17).

A post-hoc, multivariable sensitivity analysis of overall survival in patients with triple-negative breast cancer excluding 31 (6%) patients (21 *BRCA*-positive and ten *BRCA*-negative) who underwent bilateral mastectomy within the first year after diagnosis showed a significant difference in overall survival at 2 years for *BRCA*-positive versus *BRCA*-negative patients (95% [95% CI 89–98] *vs* 91% [88–94]; HR 0·52 [95% CI 0·29–0·91], p=0·023). However, there was no significant difference for 5-year overall survival (83% [95% CI 74–89] *vs* 74% [69–78]; HR 0·98 [95% CI 0·58–1·65], p=0·94; appendix p 18).

We also repeated the primary analysis in patients with triple-negative breast cancer excluding 37 (7%) patients who developed a new primary breast or ovarian cancer. Overall survival at 10 years for *BRCA*-positive versus *BRCA*-negative patients was 78% (95% CI 69–85) versus 69% (64–74; HR 1·24 [95% CI 0·39–3·96], p=0·73; appendix p 19).

Discussion

The POSH prospective cohort study showed no significant difference in overall survival or distant disease-free survival between patients carrying a *BRCA1* or *BRCA2* mutation and patients without these mutations after a diagnosis of breast cancer. These results did not vary between unadjusted or adjusted analyses, including adjustments for ethnicity and BMI.[21,22] Following a diagnosis of early breast cancer, *BRCA* mutation carriers are frequently offered additional management options including bilateral mastectomy. Any prognostic implication of carrying a *BRCA* mutation for primary treatment is important to clarify to facilitate clinician and patient decisions around the optimum timing of additional surgery. Furthermore, clinical trials of treatments that are specifically targeted toward *BRCA* mutation carriers might need to take into account any effect of *BRCA* mutational status on primary treatment outcomes.

To our knowledge, this is the largest prospective study to report the prognostic implication of germline *BRCA* mutations and the only one with a preplanned analysis of patients presenting with triple-negative tumours. Our results are in broad agreement with more recent studies,[8–10,23] but others have reported conflicting results.[24–26] Ascertainment biases introduced by retrospective and selective identification of cases, incomplete genetic testing, small numbers, absence of adjustments for clinical variables including treatment, and short follow-up probably explain many discrepancies, although some studies have generally used stronger methods.[11–14]

The percentage of *BRCA*-positive patients in POSH (12%) was higher than anticipated from historical studies of patients diagnosed aged 40 years and younger, perhaps because of more sensitive mutation-testing options.[1] However, only 14% of all patients had clinical genetic testing. The ratio of patients with *BRCA1* to *BRCA*2 mutations was 1·5 to 1, which is similar to that reported in other large western population-based cohorts.[2,23] Deaths due to other malignancies were low in frequency in all groups reflecting the young age group; however, causes of deaths in patients who were *BRCA1*-positive included potentially preventable ovarian cancers at age 41–46 years. Bilateral risk-reducing mastectomy is not a necessary part of treating a unilateral breast cancer but unilateral mastectomy might enable breast radiotherapy to be omitted. Discussion about future primary cancer prevention during primary breast cancer treatment should take into account individual circumstances, including the likely tumour prognosis and the physical and psychological implications of more extensive surgery. In the POSH cohort, immediate bilateral mastectomy was not associated with improved survival, although the reported use of risk-reducing surgery was low; bilateral salpingo-oophorectomy was recorded in 32 patients and bilateral mastectomies in 107 patients.[27] This probably reflects the low level of clinical testing at the time of the study. Although risk-reducing bilateral salpingo-oophorectomy is highly effective at reducing ovarian cancer incidence, the risk of

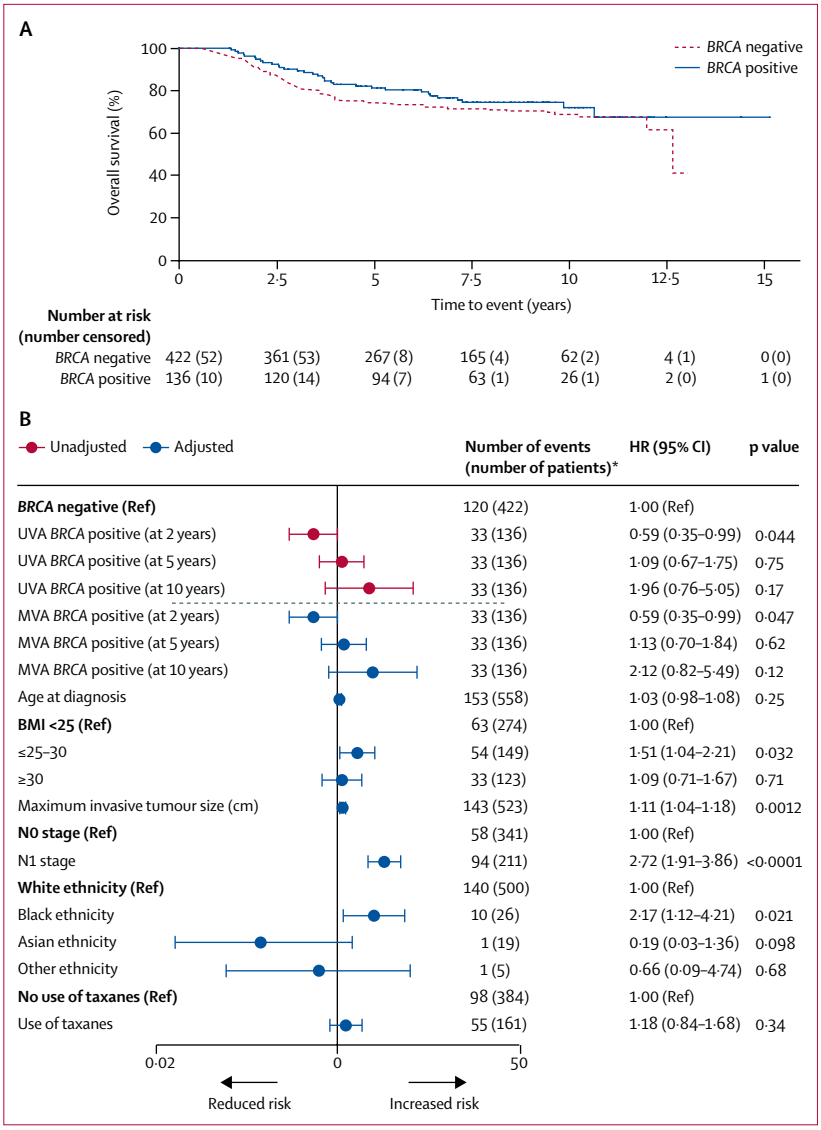

Figure 3: **Overall survival for all patients with triple-negative breast cancer* by *BRCA* mutation status**
(A) Kaplan-Meier plot and (B) forest plot of corresponding univariable and multivariable hazard ratios. In (B), multivariable analysis was adjusted for age, body-mass index (BMI; kg/m^2), grade, tumour size, HER2 status, oestrogen-receptor status, ethnicity, and use of taxane chemotherapy. Groups without a reference were assessed as a continuous variable. The dashed line separates the univariable analyses (UVA) from the multivariable analyses (MVA). HR=hazard ratio. *Number of events (number of patients) from complete data obtained before multiple imputation.

primary peritoneal cancer is not reduced and studies indicate that the previously reported effect of this procedure on future breast cancer risk in *BRCA1* and *BRCA2* mutation carriers might have been overestimated because of uncorrected bias.[28]

Our analysis of the 558 patients with triple-negative breast cancer in our cohort showed an intriguing difference in overall survival over the first few years after diagnosis. *BRCA* mutation carriers were less likely to die from early breast cancer than non-carriers. This early survival advantage has also been observed among patients with ovarian cancer who are *BRCA* mutation carriers.[29,30] If real, this advantage might reflect greater sensitivity of *BRCA*-mutant breast cancers to chemotherapy or the greater visibility of *BRCA*-mutant cancers to host immune attack.[31] One theory that could explain the slight survival advantage for *BRCA* mutation carriers not undergoing immediate bilateral mastectomy is that a major surgical intervention might compromise host immunity at a time when this is particularly important for eradicating micrometastases. This hypothesis would need further exploration due to the small number of patients in this subgroup.

Results from several published studies have suggested that the DNA repair deficiency associated with *BRCA* mutations results in enhanced sensitivity to many chemotherapy agents, particularly higher response rates to platinum-based drugs, have occurred in both metastatic and neoadjuvant settings.[4,7] Only 13 patients in our cohort were treated with platinum-based adjuvant regimens for early breast cancer, including one patient with a *BRCA1* mutation and one with *BRCA2*.

Our study illustrates the high breast cancer mortality in this unscreened young population and the effect of known tumour and patient-prognostic characteristics on mortality. Inevitably, there have been substantial changes in the management of *BRCA1* and *BRCA2* mutation carriers since the recruitment period of this study, including the exploration in trials of systemic therapies that exploit *BRCA*-null tumours, including platinum-based drugs and PARP inhibitors. The association of *BRCA* mutations with improved early outcomes related to breast cancer in patients with triple-negative breast cancer has the potential to affect early results from clinical trials. As advanced genomic investigations increasingly become a part of routine oncological care, many patients with breast cancer now learn their *BRCA* mutation status close to the time of diagnosis. In many cancer centres, immediate or post-chemotherapy bilateral mastectomy has become an almost routine recommendation for *BRCA1* and *BRCA2* mutation carriers regardless of the size or focality of the presenting tumour. In the longer term, risk-reducing surgery, particularly for *BRCA1* gene carriers is an appropriate management; in our analysis, the rising hazard for death in *BRCA* carriers over time was negated by removing from the analysis all patients who developed a second new primary breast or ovarian cancer during the follow-up period.

Clinicians need to consider short-term and long-term risks and benefits in discussing risk-reducing bilateral mastectomy with patients. The number of patients with triple-negative breast cancer who had immediate bilateral mastectomy in our cohort was small but our analysis suggests it is unlikely that the early bilateral mastectomy accounted for the early survival advantage in the *BRCA* mutation carriers with triple-negative breast cancer. With modern MRI-based breast screening, we conclude that patients who choose to delay additional surgery for 1 or 2 years until they are psychologically and physically recovered from their cancer treatment can be reassured that this choice is unlikely to lead to any substantial survival disadvantage. The importance of appropriately timed risk-reducing bilateral salpingo-oophorectomy, for *BRCA1* mutation carriers in particular, is clear, but should take plans for further pregnancy into account. Furthermore, risk-reducing bilateral salpingo-oophorectomy in very young women will have negative health consequences as a result of oestrogen deprivation from an early age.

The strengths of the POSH study include the large cohort size, few missing data, and inclusion of patients with young-onset breast cancer, which led to a large number of *BRCA1* and *BRCA2* mutation carriers and a high number of events, ensuring that the study was well powered for the main outcome analysis. Our study minimised many of the biases present in other studies by recruiting patients within the first year after diagnosis from oncology clinics nationally to minimise survival and selection bias and by establishing *BRCA* mutation status for all patients included in the analysis. POSH participants recruited from England represented 23% of the available population during the recruitment period and comparison with cancer registry data confirmed that the POSH cohort is representative of the wider population.[16] Comprehensive details of pathology enabled us to do a separate analysis of outcome in patients with triple-negative breast tumours; a unique contribution to this field. We have previously reported the significant and independent prognostic effects of obesity and ethnicity on long-term outcomes in this young patient group, and this study is the only prospective study to date to include these host factors in multivariable analyses.[21,22]

Limitations of this study included the non-universal use of multiplex ligation probe analysis; we therefore cannot exclude the possibility that some structural *BRCA* variants were not identified. However, even clinical diagnostic mutation testing is not 100% sensitive because of occult mutations not amenable to current methods (eg, deep intronic splice variants); the investigation of *BRCA1* and *BRCA2* gene sequences in this cohort was more comprehensive than in most other publications. All participants were tested for *TP53* mutations and

carriers were excluded from this analysis because of the high risk of non-breast malignancies. We acknowledge that other breast cancer susceptibility gene variants were not excluded; however, these were expected to be very low in frequency or low penetrance, and there is no evidence that they specifically affect prognosis. We had national outcome data up to a median 8·2 years. The treatments given reflected modern oncological practice with almost 90% of patients receiving neoadjuvant or adjuvant chemotherapy; in more than 95% of cases this was an anthracycline or anthracycline plus taxane combination regimen.

Other limitations of this study included restricting the main cohort to patients aged 40 years or younger at the time of diagnosis to enrich for *BRCA* mutation carriers. It is possible that observations in young-onset breast cancer patients might not translate to older ages at diagnosis. Progesterone-receptor testing was not done routinely in many UK centres during the period of recruitment and supplementary data were derived from tissue microarrays rather than full tumour sections. The relevance of triple-negative breast cancer in terms of biology and treatment has only become apparent since the POSH study was designed, so the study was not powered for this as the primary outcome; notably, the only difference in overall survival in this study was seen between mutation carriers and non-carriers in this subgroup. Recommendations for adjuvant treatment in the UK changed over the course of recruitment, with taxanes being recommended for node-positive disease from 2006 and adjuvant trastuzumab for HER2-positive breast cancer routinely available only from 2006. Although we specifically collected information at 5 years about risk-reducing surgery, we cannot exclude the possibility that risk-reducing mastectomy and oophorectomy might have been done at different hospitals from the recruiting cancer centre (eg, at specialist plastic surgery or gynaecological units).

This study confirmed that patients diagnosed with invasive breast cancer aged 18–40 years have a high breast-cancer-specific mortality, and a high proportion are *BRCA1* and *BRCA2* mutation carriers. We found no clear evidence that either *BRCA1* or *BRCA2* germline mutations significantly affect overall survival with breast cancer after adjusting for known prognostic factors. Decisions about timing of risk-reducing surgery should take into account primary tumour prognosis and patient preference. *BRCA* mutation carriers presenting with triple-negative breast cancer might have an improved survival during the first few years after diagnosis compared with non-carriers, although immediate bilateral mastectomy did not account for this advantage. Finally, analysis of early outcome data from trials exploring *BRCA*-deficient tumour treatment in patients with triple-negative breast cancer should be interpreted with caution in view of the possible early survival advantage for *BRCA* mutation carriers.

Contributors

The study was conceived and designed by DME, PS, and DGA, and planned and executed by DME, DGA, PS, DGE, AMT, PP, LJ, HH, SL, RE, AH, FJG, and SH. Data acquisition, management and curation was done by SG, LTD, ERC, TCM, WJT, RIC, SG-H, BE, LS, and DME. LJ was responsible for central pathology review, and AMD and DFE supervised the final research DNA sequencing. The statistical analysis plan was prepared by TCM, DGA, DME, ERC, and RIC. TCM did the statistical analysis and prepared the figures. DME, ERC, TCM, DGA, and RIC interpreted the data and ERC, TCM, and DME wrote the manuscript. All authors critically reviewed iterations of the manuscript and approved the final draft for submission.

Declaration of interests

ERC declares honoraria from Roche. RIC declares honoraria from GSK and Pfizer. DME declares honoraria from AstraZeneca and Pierre Fabre. All other authors declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Appendix - Tables

Appendix Table 1: Recruitment by active sites

List of recruitment number by all active sites in the reported cohort.

Recruiting Hospital	Principal Investigator	No. recruited
Guys Hospital	Mr. Hisham Hamed	126
Mount Vernon Hospital	Dr. Andreas Makris	108
Royal South Hants Hospital	Dr. Peter Simmonds	91
Weston Park Hospital	Lucy Birch	90
Maidstone Hospital	Dr. Rema Jyothirmayi	89
Royal Stoke University Hospital	Dr. Adrian Murray Brunt	87
Royal Cornwall Hospital	Dr. Duncan Wheatley	80
Royal Free Hospital	Dr. Jackie Newby	77
Queen Alexandra Hospital	Mr. Constantinos Yiangou	73
Ninewells Hospital	Professor A.M.Thompson	68
Southend Hospital	Dr Hafiz Algurafi	63
The Royal Surrey County Hospital	Avril Adams	59
Christie Hospital	Prof. Gareth Evans (Genetics) Dr. Andrew Wardley (Oncology)	53
Wexham Park (formerly Heatherwood & Wexham) Hospital	Dr. Marcia Hall	53
Royal Derby Hospital	Mr. Mark Sibbering	50
The James Cook University Hospital	Dr. John Hardman	50
Frenchay Hospital	Mr. Simon Cawthorn/Dr. Mike Shere	49
Velindre Hospital	Professor Peter Barrett-Lee	45
Belfast City Hospital	Dr. Seamus McAleer	44
Broomfield Hospital	Dr. Saad Tahir	43
Addenbrookes Hospital	Professor Helena Earl	41
The Great Western Hospital	Mr. Marcus Galea	40
Torbay Hospital	Dr. Peter Bliss	38
Countess of Chester Hospital NHS Trust	Mrs Claudia Harding-Mckean	37
Norfolk & Norwich University Hospital NHS Trust	Dr. Adrian Harnett	36
Milton Keynes Hospital NHS Trust	Miss Amanda Taylor	34
Withington Hospital	Dr. Anne Armstrong	32
Royal Marsden Hospital	Prof. Ros Eeles	31
Peterborough Hospital NHS Trust	Dr. Karen McAdam	30
Salisbury Healthcare NHS Trust	Dr. Clare Crowley	30
Manor Hospital	Dr. Inderajit Fernando	29
Royal Berkshire Hospital	Dr Madhumita Bhattachayya	29
The Hillingdon Hospital NHS Trust	Dr. Amy Guppy	29
Hope Hospital	Miss Zahida Saad	27
Macclesfield District General Hospital	Mr. Jalal Kokan	27
Nottingham City Hospital	Mr. R. Douglas Macmillan	27
Glan Clwyd Hospital	Dr. Jill Bishop	26
George Eliot Hospital NHS Trust	Dr. Susan Lupton	25
North Hampshire Hospital	Miss Anne Stebbing	25
Royal Devon and Exeter Hospital	Dr. Anne Hong	25
Royal Bournemouth Hospital	Mr. Anthony Skene	24
Stepping Hill Hospital	Mr. Mohammad Sharif	24
Wrexham Maelor Hospital	Dr Win Soe	24
Isle of Wight NHS Primary Care Trust	Dr. Jenny Marshall	23
Lister Hospital	Dr. Nihal Shah	22
Royal Victoria Infirmary	Dr. Radha Todd	22
Croydon University Hospital (Mayday Hospital)	Dr. Navita Somaiah	21
Royal Sussex County Hospital	Dr. David Bloomfield	21
Surrey & Sussex Heathcare NHS Trust	Miss Shamaela Waheed	21
Whittington Hospital	Prof. Jayant Vaidya	21
Yeovil District Hospital	Dr. G.E Sparrow	21
Barts & The London NHS Trust	Professor Peter Schmid	19
Derriford Hospital	Dr. Steve Kelly	19
Grantham & District Hospital	Mr. Jibril A. Jibril	19
Royal Hampshire County Hospital	Mr. D. Rainsbury	19
Walsgrave Hospital	Professor Robert J Grieve	19
Worthing Hospital	Mr. R. Bonomi	19
Queen's Hospital, Burton	Mr. Colin Rogers	18
St Georges' Hospital	Dr. Laura Assersohn	18
Huddersfield Royal Infirmary	Dr. Jonathan K Joffe	17
Kent & Canterbury Hospital	Dr. Natasha Mithal	17
Poole Hospital NHS Trust	Miss Abigail Evans	17
Stirling Royal Infirmary	Judith Fraser	17
Sunderland Royal Hospital	Mr Obiukwu Iwuchukwu (until 2015)	17
Dorset County Hospital	Sarah Williams	16
North Middlesex University Hospital	Dr. Fharat Raja	16
Royal Albert Edward Infirmary	Dr Elena Takeuchi	16
Solihull Hospital	Dr Medy Tsalic	16
Whipps Cross University Hospital	Mr. Peter Frecker	16

Recruiting Hospital	Principal Investigator	No. recruited
Frimley Park Hospital	Mr. Ian Laidlaw	15
New Cross Hospital	Dr. Rakesh Mehra	15
Royal Liverpool University Hospital	Mr. Chris Holcombe	15
University Hospital of Hartlepool	Mr. Pud Bhaskar	15
Withybush General Hospital	Dr. Gianfilippo Bertelli	15
Darlington Memorial Hospital	Dr. Alison Humphreys	14
Royal Preston Hospital	Dr. Elaine Young	14
Warwick Hospital	Dr. Nawaz Walji	14
William Harvey Hospital	Dr. Natasha Mithal	14
King George Hospital	Dr. Eliot Sims	13
Newham University Hospital NHS Trust	Professor Peter Schmid	13
Russells Hall Hospital	Dr. Rozenn Allerton	13
Charing Cross Hospital	Professor Charles Coombes	12
Darent Valley Hospital	Dr. Julia Hall	12
Friarage Hospital	Dr. Johannes Van Der Voet	12
North Devon District Hospital	Dr. Mark Napier	12
Cumberland Infirmary	Mr. M. Williams	11
The Shrewsbury & Telford Hospital (formerly Royal Shrewsbur)	Dr. Rajiv Agrawal	11
Stoke Mandeville Hospital	Dr. Ketan Shah	11
Wycombe Hospital	Dr. Ketan Shah	11
Kidderminster Hospital	Dr. Mark Churn	10
Queens Hospital (Oldchurch Hospital)	Dr. Mary Quigley	10
Sandwell Hospital	Dr. David Spooner	10
St. Richard's Hospital	Dr. Joanna Gale	10
Stafford General Hospital	Dr. Adrian Murray Brunt	10
Luton & Dunstable Hospital NHS Foundation Trust	Dr. Mei-Lin Ah-See	9
University College London	Dr. Grant Stewart (to 2012)	9
Homerton University Hospital NHS Foundation Trust (c/o Barts)	Professor Peter Schmid	8
James Paget Healthcare NHS Trust	Dr. Adrian Harnett	7
North Tyneside General Hospital	Mr. Mike Carr	7
Queen Elizabeth Hospital, Gateshead	Mr. David Browell	7
Royal Glamorgan Hospital	Dr. Jacinta Abraham	7
Royal Lancaster Infirmary	Dr. David Eaton	7
Royal Oldham	Dr. Juliette Loncaster	7
Birmingham City Hospital	Dr. David Spooner	6
Gwynedd Hospital (North West Wales)	Dr. Jill Bishop	6
Lincoln County Hospital	Mr. Jibril A. Jibril	6
South Tyneside District Hospital	Dr. Radha Todd	6
The Alexandra Hospital	Dr. Clive Irwin	6
The Leeds Teaching Hospital NHS Trust	Dr. Julian Adlard	6
Princess Royal University Hospital	Dr. Mark Harries	5
Wansbeck General Hospital	Mr. Mike Carr	5
West Suffolk Hospital	Dr. Margaret Moody	5
West Wales General	Dr. Margaret Wilkins	5
Conquest Hospital	Dr. Gillian Sadler	4
Royal Alexandra Hospital	Dr. Abdulla Al-hasso	4
Singleton Hospital	Dr. Gianfilippo Bertelli	4
Furness General Hospital	Dr. Geraldine Skailes	3
Queen Elizabeth The Queen Mother Hospital	Dr. Natasha Mithal	3
Bronglais Hospital	Sarah J Jones	2
Burnley General Hospital	Dr. Martin Hogg	2
Kings College London	Dr. Anne Rigg	2
University Hospital of North Tees	Mr. Colm Hennessy	2
Blackburn Royal Infirmary	Dr. Martin Hogg	1
Princess Elizabeth Hospital	Dr. Peter Gomes	1
Queen Elizabeth Hospital, Woolwich	Dr. Hartmut Kristeleit	1
Southern General Hospital	Dr. Abdulla Al-hasso	1

Appendix Table 2: List of BRCA1 and BRCA2 mutation annotation
List of 338 pathogenic BRCA1 and BRCA2 variants included in the BRCA+ group

GENE	Coding change	Protein change
BRCA1	c.514delC	p.Gln172fs
BRCA1	c.1961dupA	p.Lys654fs
BRCA1	c.3762_3763het_delGA	p.Cys1252fs
BRCA1	c.135-1G>T	
BRCA1	c.3400G>T	p.Glu1134X
BRCA1	c.3607C>T	p.Arg1203X
BRCA1	c.53T>C	p.Met18Thr
BRCA1	c.5153G>A	p.Trp1718X
BRCA1	c.302-1G>T	
BRCA1	c.4185+1G>T	
BRCA1	c.2680_2681del	p.Lys894fs
BRCA1	c.69_79del	p.Cys24fs
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.4185+1G>T	
BRCA1	c.4357+2T>G	
BRCA1	c.3967C>T	p.Gln1323X
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.4180delA	p.Thr1394fs
BRCA1	c.3668_3669insTCCC	p.Leu1223fs
BRCA1	c.1675delA	p.Lys519Argfs
BRCA1	c.427G>T	p.Glu143X
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.5503C>T	p.Arg1835X
BRCA1	c.427G>T	p.Glu143X
BRCA1	c.4357+6T>C	
BRCA1	c.1793T>G	p.Leu598X
BRCA1	c.5152+1G>T	
BRCA1	c.1954dupA	p.Lys652fs
BRCA1	c.5152+1G>T	
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.3768_3769del	p.Glu1257Glyfs
BRCA1	c.5152+1G>T,	
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.A4558T	p.R1520X
BRCA1	c.5194-12G>A	
BRCA1	c.4574_4575delAA	p.Gln1525Argfs
BRCA1	c.5194-12G>A	
BRCA1	c.5332+1G>A	
BRCA1	c.929delA	p.Gln310fs
BRCA1	c.427G>T	p.Glu143X
BRCA1	c.4574_4575delAA	p.Gln1525Argfs
BRCA1	c.5264dupC	p.Ser1755fs
BRCA1	c.1512dupT	p.Arg504fs
BRCA1	c.427G>T	p.Glu143X
BRCA1	c.1266T>G	p.Tyr422X
BRCA1	c.1A>G	p.Met1Val
BRCA1	c.5153G>A	p.Trp1718X
BRCA1	c.1823_1826delAGAA	pLys608fs
BRCA1	c.4586dupT	p.I1529fs
BRCA1	c.4327C>T	p.Arg1443X
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.547+2T>A	
BRCA1	c.2068delA	p.Lys690fs
BRCA1	c.2475delC	p.Asp825fs
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.3331_3334del	p.(Gln1111Asnfs*5)
BRCA1	c.2612_2613insT	p.Pro871fs
BRCA1	c.2074delC	p.His692fs
BRCA1	c.5264dupC	p.Ser1755fs
BRCA1	c.2676_2679del	p.Lys893fs
BRCA1	c.3718C>T	p.Gln1240X
BRCA1	c.5264dupC	p.Ser1755fs
BRCA1	c.1297_1298insCC	p.Ala433fs
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.181T>G	p.Cys61Gly
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.5193delG	p.E1731fs
BRCA1	Deletion exon 1-23	
BRCA1	Deletion exon 1-23	
BRCA1	c.4065_4068delTCAA	p.Asn1355fs

GENE	Coding change	Protein change
BRCA1	c.66dupA	p.Leu22fs
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	c.1141A>T	p.Lys381X
BRCA1	c.2125_2126insA	p.Phe709fs
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	c.5186delT	p.Leu1729fs
BRCA1	c.3228_3229del	p.(Gly1077Alafs*8)
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	c.2676_2679del	p.Lys893fs
BRCA1	Deletion exon 20	
BRCA1	c.4411delG	p.Gly1471fs
BRCA1	c.3331_3334del	p.(Gln1111Asnfs*5)
BRCA1	c.2704delG	p.Glu902fs
BRCA1	Deletion exon 21-24	
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	c.3331_3334delCAAG	p.Gln1111Asnfs
BRCA1	Deletion exon 21-24	
BRCA1	c.3002delA	p.Glu1001fs
BRCA1	c.5054C>T	p.Thr1685Ile
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.1012A>T	p.Lys338X
BRCA1	c.3064dupA	p.Thr1022fs
BRCA1	c.5363G>T	p.Gly1788Val
BRCA1	c.303T>G	p.Tyr101X
BRCA1	Deletion of exon 20	
BRCA1	c.69_79del	p.Cys24fs
BRCA1	c.5264dupC	p.Ser1755fs
BRCA1	Deletion of exon 24	
BRCA1	c.520delC	p.Gln174fs
BRCA1	c.2680_2681del	p.Lys894fs
BRCA1	c.427G>T	p.Glu143X
BRCA1	Deletion of exon 3	
BRCA1	c.2680_2681del	p.Lys894fs
BRCA1	Deletion of exon 3	
BRCA1	c.3228_3229del	p.(Gly1077Alafs*8)
BRCA1	c.3400G>T	p.Glu1134X
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.4357delG	p.A1453fs
BRCA1	Deletion of exons 1-17	
BRCA1	Deletion of exons 1-17	
BRCA1	Deletion of exons 1-17	
BRCA1	Deletion of exons 1-17	
BRCA1	c.181T>G	p.Cys61Gly
BRCA1	Deletion of exons 1-2	
BRCA1	c.1954dupA	p.Lys652fs
BRCA1	c.1961delA	p.Lys654fs
BRCA1	c.1326T>A	p.Cys442X
BRCA1	c.4354A>T	p.Lys1452X
BRCA1	Deletion of exons 1-2	
BRCA1	Deletion of exons 1-2	
BRCA1	c303T>G	p.Tyr101Ter
BRCA1	c.1954delA	p.Lys652fs
BRCA1	c.2475delC	p.Asp825fs
BRCA1	c.1471C>T	p.Gln491X
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.3869_3870delAA	p.Arg1290fs
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	c.5251C>T	p.Arg1751Ter
BRCA1	c.5153G>A	p.Trp1718X
BRCA1	c.5503C>T	p.Arg1835X
BRCA1	c.427G>T	p.Glu143X
BRCA1	c.4964_4982del	p.(Ser1655Tyrfs*16)
BRCA1	c.4574_4575delAA	p.Gln1525Argfs
BRCA1	Deletion of exons 14-17	
BRCA1	c.1961dupA	p.Lys654fs
BRCA1	c.1601_1602delAG	p.Gln534fs-X3
BRCA1	Deletion of exons 1a-1b	
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.427G>T	p.Glu143X
BRCA1	c.1749_1755del	p.(Lys583Asnfs*3)
BRCA1	Deletion of exons 1a-2	
BRCA1	c.1504_1508del	p.(Leu502Alafs*2)
BRCA1	c.2199delG	p.Glu733fs
BRCA1	Deletion of exons 1A-2	

GENE	Coding change	Protein change
BRCA1	c.5503C>T	p.Arg1835X
BRCA1	Deletion of exons 20	
BRCA1	c.5324T>G	p.Met1775Arg
BRCA1	Deletion of exons 21-24	
BRCA1	c.1949_1950delTA	p.Ile650fs]
BRCA1	c.5264dupC	p.Ser1755fs
BRCA1	c.2267delG	p.Arg756fs
BRCA1	c.5573delT	p.I1858fs
BRCA1	c.5324T>G	p.Met1775Arg
BRCA1	c.4574_4575delAA	p.Gln1525Argfs
BRCA1	Deletion of exons 8-13	
BRCA1	c.4349C>G	p.Ser1450X
BRCA1	c.4106delC	p.Ala1369fs
BRCA1	c.3046_3047insATGAG	p.Asn1016fs
BRCA1	c.3400G>T	p.Glu1134X
BRCA1	c.2953delC	p.Pro985fs
BRCA1	c.187_188delAG	p.Glu23Valfs
BRCA1	Duplication of exon 13	
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	c.4165_4166delAG	p.Ser1389X
BRCA1	c.3450_3453delCAAG	p.Gln1111fs
BRCA1	c.981_982del	p.Cys328Terfs
BRCA1	c.427G>T	p.Glu143X
BRCA1	Duplication of exon 13	
BRCA1	c.2068delA	p.Lys690fs
BRCA1	Duplication of exon 13	
BRCA1	c.3400G>T	p.Glu1134X
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.5503C>T	p.Arg1835X
BRCA1	c.797_798del	p.Val266fs
BRCA1	c.675delT	p.Ala225fs
BRCA1	Duplication of exon 13	
BRCA1	Duplication of exon 13	
BRCA1	c.929delA	p.Gln310fs
BRCA1	c.4065_4068delTCAA	p.Asn1355fs
BRCA1	c.1756delC	p.Pro586fs
BRCA1	c.181T>G	p.Cys61Gly
BRCA1	Duplication of exon 13	
BRCA1	Duplication of exon 13	
BRCA1	c.3331_3334del	p.(Gln1111Asnfs*5)
BRCA1	c.929delA	p.Gln310fs
BRCA1	c.1823_1826delAGAA	pLys608fs
BRCA1	Duplication of exon 13	
BRCA1	c.3751_3754delGTCT	p.Cys1252fs
BRCA1	c.68-69delAG	p..Glu23Valfs
BRCA1	Duplication of exon 13	
BRCA1	c.427G>T	p.Glu143X
BRCA1	c.5027T>A	p.Leu1676X
BRCA1	Duplication of exon 13	
BRCA1	Duplication of exon 5-8	
BRCA1	c.1823_1826delAGAA	p.Lys608fs
BRCA1	c.4065_4068delTCAA	p.Asn135Lysfs
BRCA1	c.5095C>T	p.Arg1699Trp
BRCA2	c.1813delA	p.Ile605fs
BRCA2	c.2330dupA	p.Asp777fs
BRCA2	c.1813delA	p.Ile605fs
BRCA2	c.5909C>A	p.Ser1970X
BRCA2	c.7762delA	p.Ile2588fs
BRCA2	c.4398_4402del	p.Leu1466Phefs
BRCA2	c.7757G>A	p.Trp2586X
BRCA2	c.7480C>T	p.Arg2494X
BRCA2	c.5946delT	p.Ser1982fs
BRCA2	c.9154C>T	p.Arg3052Trp
BRCA2	c.7542G>T	p.Gly2439X
BRCA2	c.8395delA	p.Arg2799fs
BRCA2	c.517-2A>G	
BRCA2	c.5130_5133del	p.Tyr1710fs-X
BRCA2	c.755_758del	p.Asp252Valfs
BRCA2	c.517-2A>G	
BRCA2	c.7988A>T	p.Glu2663Val
BRCA2	c.4416_4419del	p.(Asn1473Lysfs*5)
BRCA2	c.3785C>G	p.Ser1262X
BRCA2	c.4729G>T	p.Glu1577X
BRCA2	c.4972C>T	p.Gln1658X

GENE	Coding change	Protein change
BRCA2	c.5682C>G	p.Tyr1894X
BRCA2	c.274C>T	p.Gln92X
BRCA2	c.7654dupA	p.Ile2552fs
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	c.6405_6409del	p.(Asn2135Lysfs*3)
BRCA2	c.8940dupA	p.Glu2981Argfs
BRCA2	c.9382C>T	p.Arg3128X
BRCA2	c.5682C>G	p.Tyr1894X
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	c.7884dupA	p.Trp2629fs
BRCA2	c.1813dupA	p.Ile605fs
BRCA2	c.4478_4481delAAAG	p.Glu1493Valfs
BRCA2	c.4478_4481delAAAG	p.Glu1493Valfs
BRCA2	c.3847_3848delGT	p.Val1283fs
BRCA2	c.6757_6758del	p.(Leu2253Phefs*7)
BRCA2	c.9382C>T	p.Arg3128X
BRCA2	c.5303_5304delTT	p.Leu1768Argfs
BRCA2	c.7977-1G>C	
BRCA2	c.8755-1G>A	
BRCA2	c.1705_1706del	p.(Gln569Glufs*20)
BRCA2	c.9357_9360del	p.(Ile3120Leufs*42)
BRCA2	c.439C>T	p.Gln147X
BRCA2	c.9182delT	p.Leu3061X
BRCA2	c.7762delA	p.Ile2588fs
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	Deletion exon 21	
BRCA2	c.3969_3970insCAAA	p.Lys1323fs
BRCA2	c.4478_4481delAAAG	p.Glu1493Valfs
BRCA2	c.7737_7749delACAGTTGGCTGAT	p.(Ile2579Metfs*65)
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	c.6944_6947del	p.Ile2315Lysfs
BRCA2	Deletion exons 14-16	
BRCA2	c.1376T>G	p.Leu459X
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	Deletion of exon 17	
BRCA2	c.3847_3848delGT	p.Val1283fs
BRCA2	c.5577_5580del	p.(Lys1861*)
BRCA2	c.1296_1297del	p.(Asn433Glnfs*18)
BRCA2	c.1888dupA	p.Thr630fs
BRCA2	c.8813dup	p.(Asp2938Glufs*2)
BRCA2	c.5682C>G	p.Tyr1894X
BRCA2	c.3248delA	p.Asn1083fs
BRCA2	c.5722_5723del	p.Leu1908fs
BRCA2	c.4478_4481delAAAG	p.Glu1493Valfs
BRCA2	c.8904delC	p.Thr2968fs
BRCA2	c.7757G>A	p.Trp2586X
BRCA2	Deletion of exon 3a	
BRCA2	Deletion of exons 1-11	0
BRCA2	c.755_758del	p.Asp252Valfs
BRCA2	c.5864C>A	p.Ser1955X
BRCA2	c.8904delC	p.Thr2968fs
BRCA2	c.9196C>T	p.Gln3066X
BRCA2	Deletion of exons 1-2	
BRCA2	c.407delA	p.Asn136fs
BRCA2	c.5350_5351delAA	p.Asn1784Hisfs
BRCA2	Deletion of exons 14 - 16	
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	Deletion of exons 14-16	
BRCA2	c.3689delC	p.Ser1230fs
BRCA2	c.9435_9436del	p.Ser3147Cysfs
BRCA2	c.7069_7070del	p.Leu2357Valfs
BRCA2	c.5722_5723delCT	p.Leu1908fs
BRCA2	Deletion of exons 14-16	
BRCA2	c.8878C>T	p.Gln2960X
BRCA2	c.8297delC	p.Thr2766fs
BRCA2	c.1813delA	p.Ile605fs
BRCA2	c.5682C>G	p.Tyr1894X
BRCA2	c.6099delA	p.Ile2033fs
BRCA2	c.6079dupA	p.Arg2027fs
BRCA2	c.8297delC	p.Thr2766fs
BRCA2	c.539_540insAT	p.Ile180fs
BRCA2	c.2034_2038delTAATA	p.Asn678fs
BRCA2	c.9382C>T	p.Arg3128X
BRCA2	c.2836_2837del	p.(Asp946Phefs*12)
BRCA2	c.7069_7070del	p.Leu2357Valfs

GENE	Coding change	Protein change
BRCA2	c.8904delC	p.Thr2968fs
BRCA2	c.370dupA	p.Met124fs
BRCA2	c.7007G>A	p.Arg2336His
BRCA2	c.2808_2811del	p.(Ala938Profs*21)
BRCA2	c.5350_5353del	p.Asn1784Hisfs
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	c.5682C>G	p.Tyr1894X
BRCA2	c.5946delT	p.Ser1982fs
BRCA2	c.9945delA	p.Lys3315fs
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	Deletion of exons 8-10	
BRCA2	c.7480C>T	p.Arg2494X
BRCA2	c.8167G>C	p.Asp2723His
BRCA2	c.7934delG	p.Arg2645fs
BRCA2	c.6816_6820del	p.Gly2274fs
BRCA2	c.1189_1190insTTAG	p.Gln397fs
BRCA2	c.755_758del	p.Asp252Valfs
BRCA2	c.9117G>A	p.Pro3039Pro
BRCA2	c.5946delT	p.Ser1982fs
BRCA2	c.755_758del	p.Asp252Valfs
BRCA2	c.9972A>T	p.Lys3326X
BRCA2	c.3405C>A	p.Tyr1135X
BRCA2	c.4478_4481delAAAG	p.Glu1493Valfs
BRCA2	c.574_575del	p.(Met192Valfs*13)
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	c.5645C>A	p.Ser1882X
BRCA2	c.3785C>G	p.Ser1262X
BRCA2	c.9196C>T	p.Gln3066X
BRCA2	c.6643delT	p.Tyr2215fs
BRCA2	c.755_758del	p.Asp252Valfs
BRCA2	c.6275_6276del	p.Leu2093fs
BRCA2	c.4169delT	p.Leu1390fs
BRCA2	c.9382C>T	p.Arg3128X
BRCA2	c.5350_5351delAA	p.Asn1784Hisfs
BRCA2	c.396T>A	p.Cys132X
BRCA2	c.1389_1390del	p.463_464del
BRCA2	c.5350_5351delAA	p.Asn1784Hisfs
BRCA2	c.5682C>G	p.Tyr1894X
BRCA2	c.6333_6337del	p.(Arg2112Profs*15)
BRCA2	c.1459delA	p.Ile411Tyrfs

Appendix Table 3: Cause of death breakdown by BRCA status (analysis population who died)
List of all causes of death in the reported cohort.

Characteristic	All patients (n=678)	BRCA1+ (n=47)	BRCA2+ (n=37)	BRCA+ (n=84)	BRCA- (n=594)
Cause of death					
Breast Cancer	**651 (96·0%)**	**41 (87·2%)**	**36 (97·3%)**	**77 (91·7%)**	**574 (96·6%)**
Other Cancer	**18 (2·7%)**	**6 (12·8%)**	**0 (0%)**	**6 (7·1%)**	**12 (2%)**
Brain	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Colorectal	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Gastric	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Haematological	4 (0·6%)	0 (0%)	0 (0%)	0 (0%)	4 (0·7%)
Lung	3 (0·4%)	0 (0%)	0 (0%)	0 (0%)	3 (0·5%)
Oesophageal	1 (0·1%)	1 (2·1%)	0 (0%)	1 (1·2%)	0 (0%)
Ovarian	3 (0·4%)	3 (6·4%)	0 (0%)	3 (3·6%)	0 (0%)
Pancreas	1 (0·1%)	1 (2·1%)	0 (0%)	1 (1·2%)	0 (0%)
Pancreatic	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Peritoneal	1 (0·1%)	1 (2·1%)	0 (0%)	1 (1·2%)	0 (0%)
Sarcoma	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Other	**8 (1·2%)**	**0 (0%)**	**1 (2·7%)**	**1 (1·2%)**	**7 (1·2%)**
Accident	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Adrenal insufficiency	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Alcohol	2 (0·3%)	0 (0%)	0 (0%)	0 (0%)	2 (0·3%)
Alcohol, adrenal failure	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Cardiac	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Cerebal complication from Crohn's disease	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)
Infection	1 (0·1%)	0 (0%)	1 (2·7%)	1 (1·2%)	0 (0%)
Unknown	**1 (0·1%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**1 (0·2%)**
Died abroad	1 (0·1%)	0 (0%)	0 (0%)	0 (0%)	1 (0·2%)

Appendix Table 4: Multivariable Analyses - Complete-Case Results (analysis population)
Breakdown of compete-case results for each multivariable analysis carried out on the analysis population.

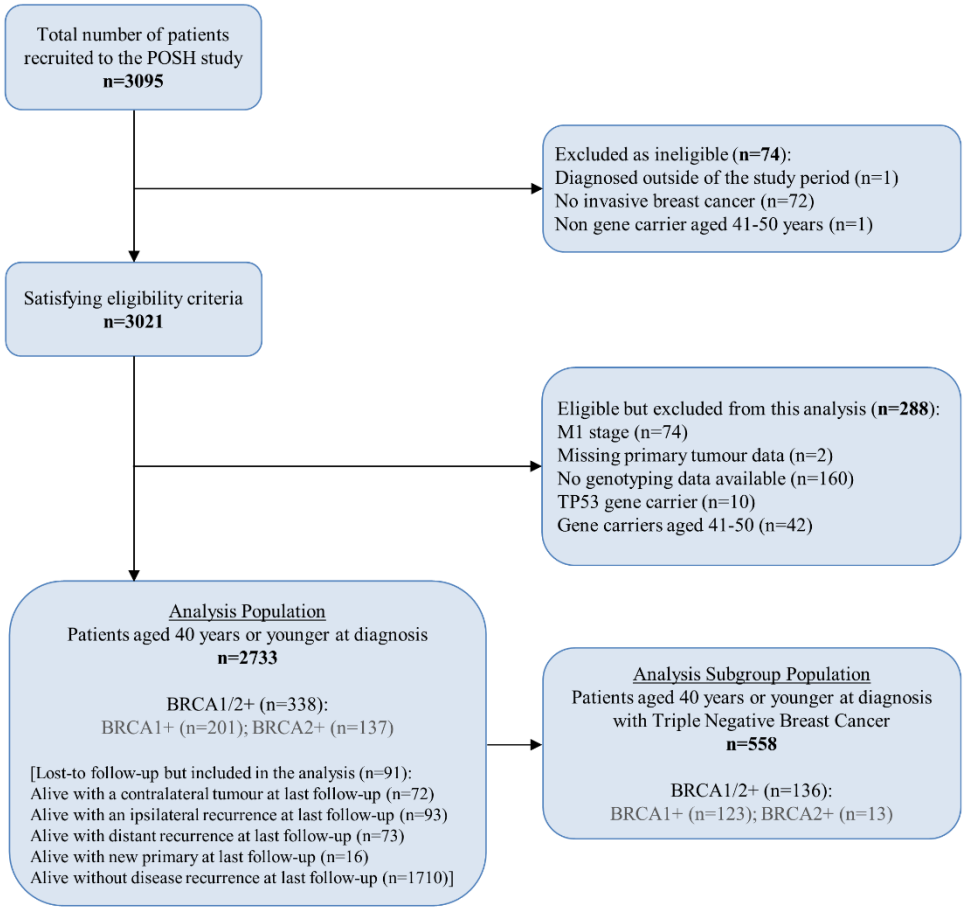
Characteristic	OS by BRCA		DDFS by BRCA		OS by BRCA1		OS by BRCA2		OS by BRCA (adjusted for time to blood draw)	
	# (events)	HR (95% CI), p-value}	# (events)	HR (95% CI), p-value}	# (events)	HR (95% CI), p-value}	# (events)	HR (95% CI), p-value}	# (events)	HR (95% CI), p-value}
BRCA- (Ref.)	2395 (594)	1.00 (Ref.)	2395 (659)	1.00 (Ref.)	2395 (594)	1.00 (Ref.)	2395 (594)	1.00 (Ref.)	2395 (594)	1.00 (Ref.)
UVA BRCA*+	338 (84)	0.99 (0.78, 1.24), 0.90	338 (93)	0.99 (0.80, 1.23), 0.94	201 (47)	0.93 (0.69, 1.25), 0.64	137 (37)	1.07 (0.76, 1.49), 0.71	338 (84)	1.01 (0.81, 1.27), 0.91
MVA BRCA*+	338 (84)	0.87 (0.66, 1.13), 0.29	338 (93)	0.91 (0.70, 1.17), 0.45	201 (47)	0.86 (0.61, 1.20), 0.37	137 (37)	0.86 (0.58, 1.29), 0.47	338 (84)	0.89 (0.68, 1.17), 0.41
Age at diagnosis	2733 (678)	0.97 (0.95, 1.00), 0.019	2733 (752)	0.97 (0.95, 0.99), 0.014	2596 (641)	0.97 (0.95, 1.00), 0.027	2532 (631)	0.97 (0.95, 1.00), 0.024	2733 (678)	0.97 (0.95, 1.00), 0.018
BMI<25 (Ref.)	1427 (313)	1.00 (Ref.)	1427 (359)	1.00 (Ref.)	1357 (298)	1.00 (Ref.)	1313 (294)	1.00 (Ref.)	1427 (313)	1.00 (Ref.)
25{&le}BMI<30	714 (197)	1.24 (1.02, 1.50), 0.032	714 (211)	1.17 (0.97, 1.41), 0.10	673 (183)	1.20 (0.98, 1.47), 0.077	667 (181)	1.18 (0.97, 1.45), 0.11	714 (197)	1.24 (1.02, 1.51), 0.028
BMI{&ge}30	491 (152)	1.28 (1.03, 1.60), 0.026	491 (166)	1.26 (1.02, 1.55), 0.031	469 (145)	1.26 (1.00, 1.57), 0.046	460 (142)	1.20 (0.96, 1.52), 0.11	491 (152)	1.28 (1.03, 1.60), 0.026
Grade 1 (Ref.)	156 (11)	1.00 (Ref.)	156 (18)	1.00 (Ref.)	156 (11)	1.00 (Ref.)	154 (10)	1.00 (Ref.)	156 (11)	1.00 (Ref.)
Grade 2	904 (200)	2.56 (1.05, 6.25), 0.040	904 (231)	1.67 (0.85, 3.28), 0.13	864 (185)	2.47 (1.01, 6.03), 0.048	888 (197)	2.54 (1.04, 6.21), 0.041	904 (200)	2.58 (1.06, 6.30), 0.038
Grade 3	1598 (450)	3.63 (1.49, 8.83), 0.0045	1598 (482)	2.25 (1.15, 4.39), 0.018	1509 (431)	3.65 (1.50, 8.90), 0.0043	1419 (408)	3.57 (1.47, 8.70), 0.0051	1598 (450)	3.63 (1.49, 8.83), 0.0045
Max. inv. size (cm)	2577 (638)	1.10 (1.06, 1.14), <0.0001	2577 (710)	1.11 (1.07, 1.15), <0.0001	2454 (607)	1.10 (1.06, 1.14), <0.0001	2386 (594)	1.10 (1.06, 1.14), <0.0001	2577 (638)	1.10 (1.06, 1.14), <0.0001
HER2- (Ref.)	1763 (442)	1.00 (Ref.)	1763 (484)	1.00 (Ref.)	1652 (414)	1.00 (Ref.)	1599 (400)	1.00 (Ref.)	1763 (442)	1.00 (Ref.)
HER2+	649 (193)	0.97 (0.80, 1.17), 0.74	649 (218)	1.07 (0.89, 1.28), 0.48	635 (185)	0.94 (0.78, 1.14), 0.56	637 (191)	0.97 (0.80, 1.18), 0.76	649 (193)	0.98 (0.81, 1.18), 0.81
N0 stage (Ref.)	1304 (189)	1.00 (Ref.)	1304 (212)	1.00 (Ref.)	1249 (179)	1.00 (Ref.)	1175 (166)	1.00 (Ref.)	1304 (189)	1.00 (Ref.)
N1 stage	1388 (479)	2.26 (1.84, 2.78), <0.0001	1388 (530)	2.30 (1.90, 2.80), <0.0001	1308 (452)	2.30 (1.86, 2.83), <0.0001	1316 (455)	2.27 (1.83, 2.81), <0.0001	1388 (479)	2.28 (1.86, 2.80), <0.0001
ER- (Ref.)	908 (248)	1.00 (Ref.)	908 (260)	1.00 (Ref.)	887 (245)	1.00 (Ref.)	757 (212)	1.00 (Ref.)	908 (248)	1.00 (Ref.)
ER+ (2 years)	1811 (428)	0.34 (0.25, 0.45), <0.0001	1811 (490)	0.63 (0.52, 0.78), <0.0001	1696 (394)	0.34 (0.25, 0.45), <0.0001	1762 (417)	0.32 (0.23, 0.43), <0.0001	1811 (428)	0.34 (0.25, 0.45), <0.0001
ER+ (5 years)	1811 (428)	1.27 (0.97, 1.67), 0.082	1811 (490)	1.61 (1.23, 2.10), 0.00048	1696 (394)	1.20 (0.93, 1.55), 0.17	1762 (417)	1.21 (0.92, 1.59), 0.17	1811 (428)	1.28 (0.97, 1.69), 0.076
ER+ (10 years)	1811 (428)	2.17 (1.50, 3.13), <0.0001	1811 (490)	3.46 (2.01, 5.95), <0.0001	1696 (394)	2.22 (1.52, 3.27), <0.0001	1762 (417)	2.39 (1.58, 3.61), <0.0001	1811 (428)	2.15 (1.49, 3.10), <0.0001
White ethnicity (Ref.)	2494 (610)	1.00 (Ref.)	2494 (672)	1.00 (Ref.)	2372 (577)	1.00 (Ref.)	2316 (566)	1.00 (Ref.)	2494 (610)	1.00 (Ref.)
Black ethnicity	103 (38)	1.36 (0.94, 1.98), 0.10	103 (44)	1.54 (1.09, 2.18), 0.014	97 (36)	1.41 (0.97, 2.06), 0.075	93 (36)	1.45 (1.00, 2.12), 0.053	103 (38)	1.36 (0.94, 1.97), 0.10
Asian ethnicity	80 (20)	1.01 (0.59, 1.72), 0.98	80 (24)	1.13 (0.70, 1.84), 0.61	76 (20)	1.03 (0.60, 1.76), 0.91	75 (19)	1.00 (0.57, 1.74), 01	80 (20)	0.99 (0.58, 1.69), 0.97
Other ethnicity	21 (3)	0.96 (0.31, 3.01), 0.95	21 (5)	1.18 (0.44, 3.17), 0.74	19 (2)	0.69 (0.17, 2.78), 0.60	18 (3)	1.01 (0.32, 3.17), 0.98	21 (3)	0.99 (0.32, 3.10), 0.99
No use of taxanes (Ref.)	1780 (455)	1.00 (Ref.)	1780 (507)	1.00 (Ref.)	1689 (436)	1.00 (Ref.)	1633 (422)	1.00 (Ref.)	1780 (455)	1.00 (Ref.)
Use of taxanes	659 (190)	1.02 (0.84, 1.23), 0.84	659 (205)	0.95 (0.79, 1.14), 0.56	624 (175)	1.00 (0.83, 1.22), 0.97	614 (177)	1.01 (0.83, 1.23), 0.94	659 (190)	1.01 (0.83, 1.22), 0.95

Appendix Table 5: Multivariable Analyses - Complete-Case Results (TNBC population)
Breakdown of compete-case results for each multivariable analysis carried out on the TNBC population.

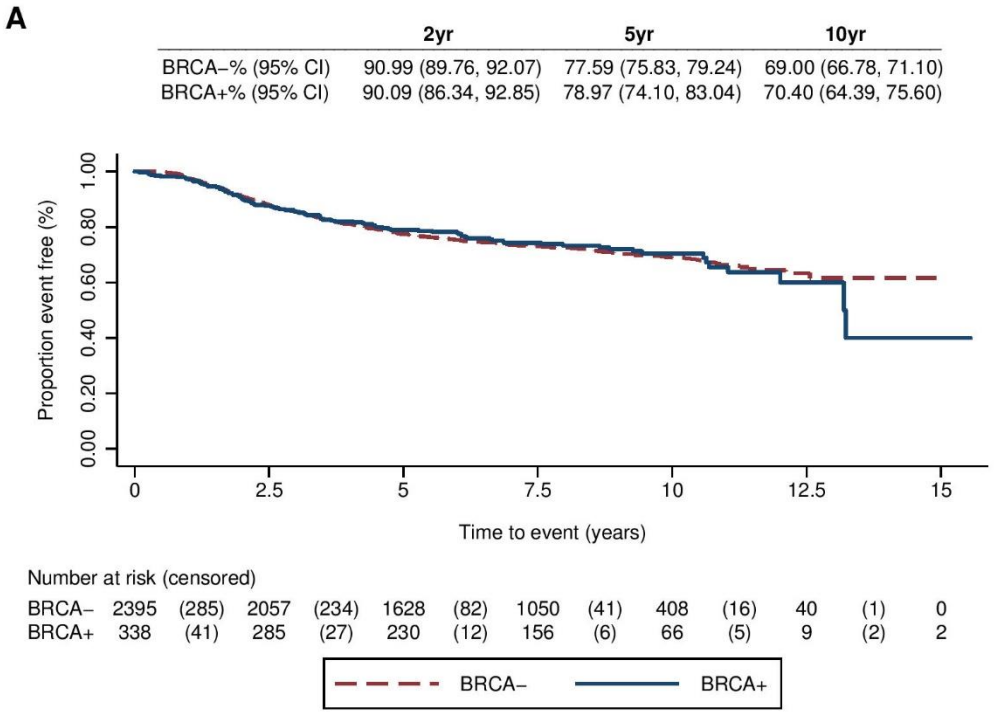
Characteristic	OS by BRCA		DDFS by BRCA		OS by BRCA (excluding bilateral mastectomies)		OS by BRCA (excluding new primary or ovarian cancers)	
	# (events)	HR (95% CI), p-value}	# (events)	HR (95% CI), p-value}	# (events)	HR (95% CI), p-value}	# (events)	HR (95% CI), p-value}
BRCA- (Ref.)	422 (120)	1.00 (Ref.)	422 (122)	1.00 (Ref.)	412 (119)	1.00 (Ref.)	407 (114)	1.00 (Ref.)
UVA BRCA+ (at 2 years)	136 (33)	0.59 (0.35, 0.99), 0.044	136 (37)	0.82 (0.55, 1.20), 0.31	115 (27)	0.55 (0.32, 0.97), 0.039	114 (23)	0.60 (0.34, 1.05), 0.071
UVA BRCA+ (at 5 years)	136 (33)	1.09 (0.67, 1.75), 0.75	136 (37)	1.46 (0.81, 2.64), 0.20	115 (27)	1.00 (0.60, 1.68), 0.99	114 (23)	0.80 (0.44, 1.43), 0.46
UVA BRCA+ (at 10 years)	136 (33)	1.96 (0.76, 5.05), 0.17	136 (37)	2.41 (0.83, 7.05), 0.11	115 (27)	1.72 (0.64, 4.63), 0.29	114 (23)	1.08 (0.34, 3.46), 0.90
MVA BRCA+ (at 2 years)	136 (33)	0.51 (0.29, 0.90), 0.019	136 (37)	0.94 (0.50, 1.75), 0.85	115 (27)	0.43 (0.22, 0.80), 0.0084	114 (23)	0.52 (0.28, 0.96), 0.037
MVA BRCA+ (at 5 years)	136 (33)	1.08 (0.65, 1.79), 0.79	136 (37)	1.27 (0.69, 2.35), 0.46	115 (27)	0.90 (0.52, 1.57), 0.73	114 (23)	0.87 (0.47, 1.60), 0.67
MVA BRCA+ (at 10 years)	136 (33)	2.10 (0.80, 5.54), 0.13	136 (37)	3.60 (0.89, 14.49),0 .071	115 (27)	1.72 (0.62, 4.81), 0.30	114 (23)	1.36 (0.44, 4.19), 0.60
Age at diagnosis	558 (153)	1.02 (0.97, 1.08), 0.36	558 (159)	1.02 (0.97, 1.07), 0.48	517 (143)	1.03 (0.98, 1.09), 0.22	521 (137)	1.04 (0.99, 1.10), 0.16
BMI<25 (Ref.)	274 (63)	1.00 (Ref.)	274 (68)	1.00 (Ref.)	257 (60)	1.00 (Ref.)	257 (57)	1.00 (Ref.)
25{&le}BMI<30	149 (54)	1.51 (1.02, 2.23), 0.038	149 (55)	1.41 (0.97, 2.06), 0.074	141 (50)	1.48 (0.99, 2.20), 0.055	139 (50)	1.59 (1.06, 2.37), 0.025
BMI{&ge}30	123 (33)	1.11 (0.71, 1.74), 0.63	123 (33)	0.97 (0.62, 1.50), 0.88	119 (33)	1.10 (0.70, 1.72), 0.68	113 (27)	1.07 (0.66, 1.72), 0.79
Max. inv. size (cm)	523 (143)	1.11 (1.04, 1.19), 0.0012	523 (149)	1.12 (1.05, 1.20), 0.0010	495 (137)	1.11 (1.04, 1.19), 0.0012	491 (130)	1.11 (1.04, 1.19), 0.0014
N0 stage (Ref.)	341 (58)	1.00 (Ref.)	341 (61)	1.00 (Ref.)	322 (55)	1.00 (Ref.)	322 (51)	1.00 (Ref.)
N1 stage	211 (94)	2.72 (1.88, 3.94), <0.0001	211 (97)	2.61 (1.82, 3.75), <0.0001	200 (90)	2.82 (1.93, 4.12), <0.0001	194 (86)	2.98 (2.01, 4.41), <0.0001
White ethnicity (Ref.)	500 (140)	1.00 (Ref.)	500 (145)	1.00 (Ref.)	474 (133)	1.00 (Ref.)	470 (128)	1.00 (Ref.)
Black ethnicity	26 (10)	2.12 (1.02, 4.39), 0.044	26 (11)	2.00 (1.00, 3.97), 0.049	24 (10)	2.52 (1.21, 5.24), 0.014	21 (6)	1.89 (0.82, 4.38), 0.13
Asian ethnicity	19 (1)	0.33 (0.05, 2.36), 0.27	19 (1)	0.28 (0.04, 2.04), 0.21	18 (1)	0.34 (0.05, 2.46), 0.29	18 (1)	0.35 (0.05, 2.49), 0.29
Other ethnicity	5 (1)	0.68 (0.09, 4.90), 0.70	5 (1)	0.96 (0.13, 6.97), 0.97	3 (1)	0.76 (0.10, 5.53), 0.79	5 (1)	0.70 (0.10, 5.08), 0.72
No use of taxanes (Ref.)	384 (98)	1.00 (Ref.)	384 (102)	1.00 (Ref.)	361 (94)	1.00 (Ref.)	357 (88)	1.00 (Ref.)
Use of taxanes	161 (55)	1.17 (0.81, 1.68), 0.41	161 (57)	1.19 (0.84, 1.71), 0.33	154 (52)	1.12 (0.77, 1.64), 0.55	152 (49)	1.12 (0.76, 1.64), 0.57

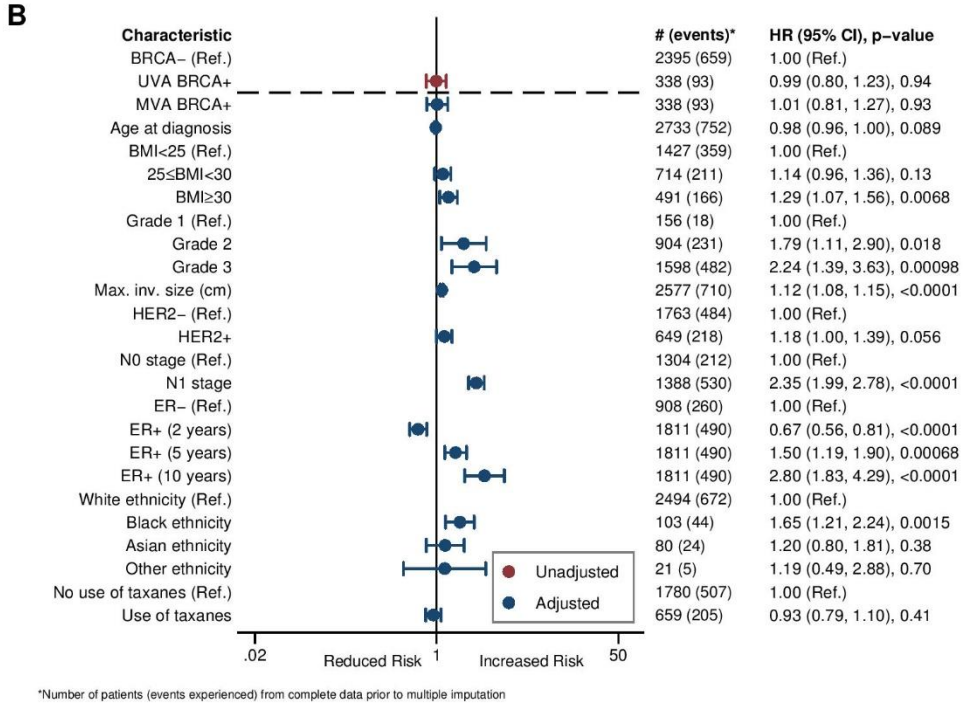
Appendix - Figures

Appendix Figure 1 – Flow diagram of the POSH cohort
Flow diagram of the POSH cohort.



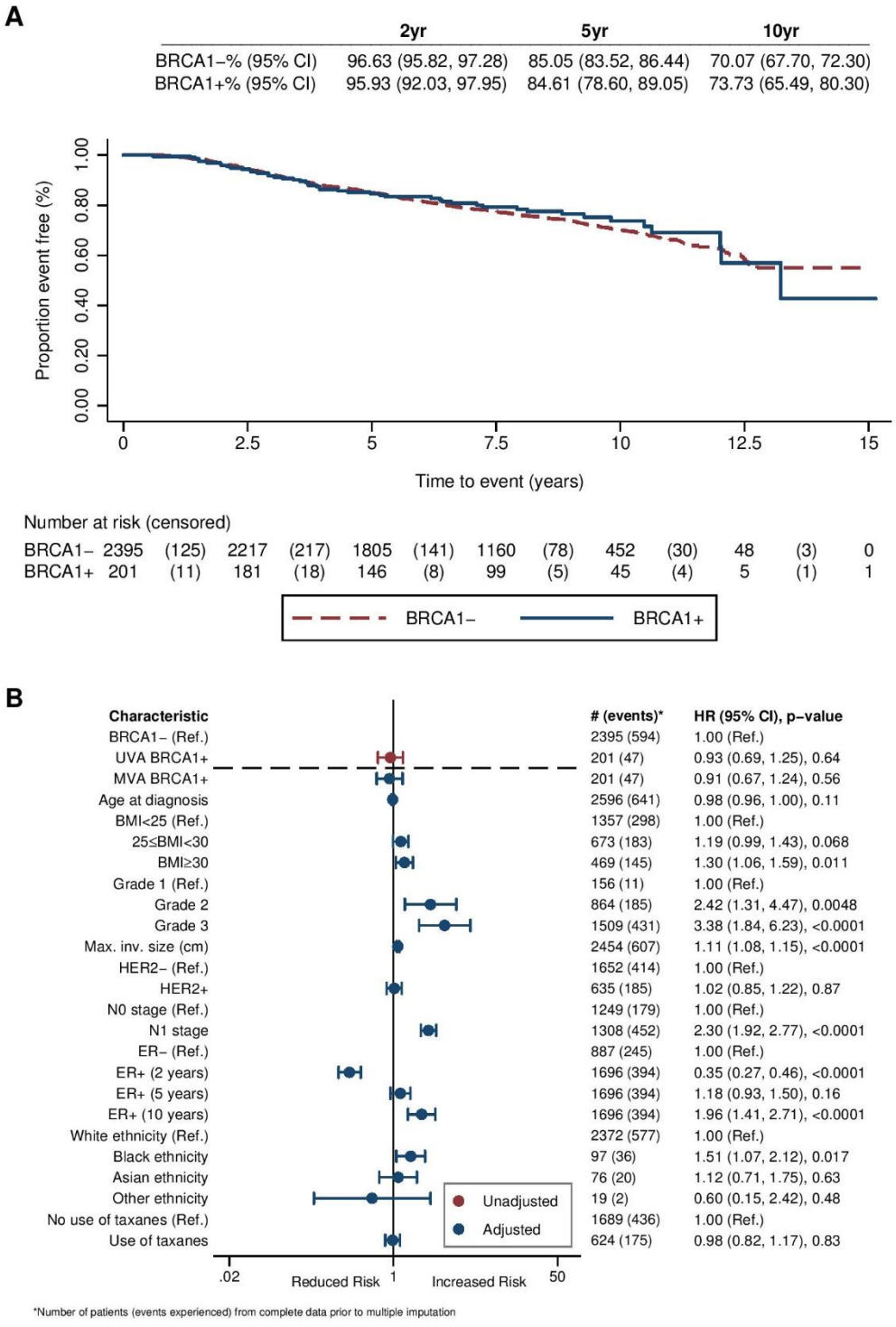
Appendix Figure 2 – Distant Disease Free Survival by *BRCA* status for all patients (analysis population)
Kaplan-Meier plot by *BRCA*1 and/or 2 status (*BRCA*+/-) for Distant Disease Free Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA*+/- status for Distant Disease Free (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.



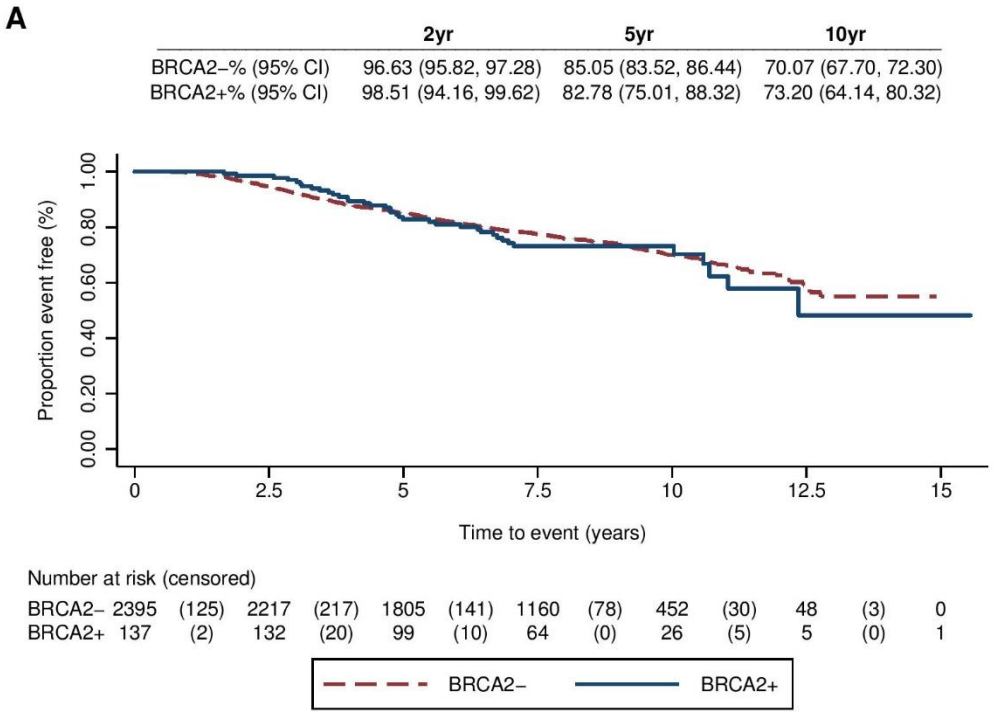


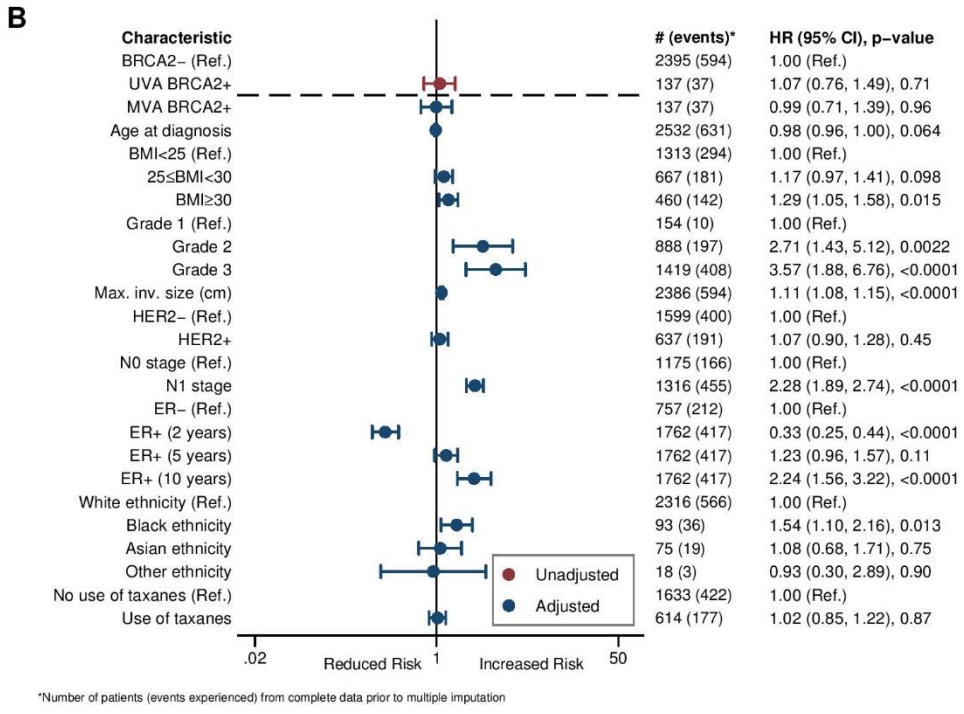
*Number of patients (events experienced) from complete data prior to multiple imputation

Appendix Figure 3 – Overall Survival by *BRCA1* status for all patients (analysis population)
Kaplan-Meier plot by *BRCA1* status (*BRCA1*+/-) for Overall Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA1*+/- status for Overall Survival (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.



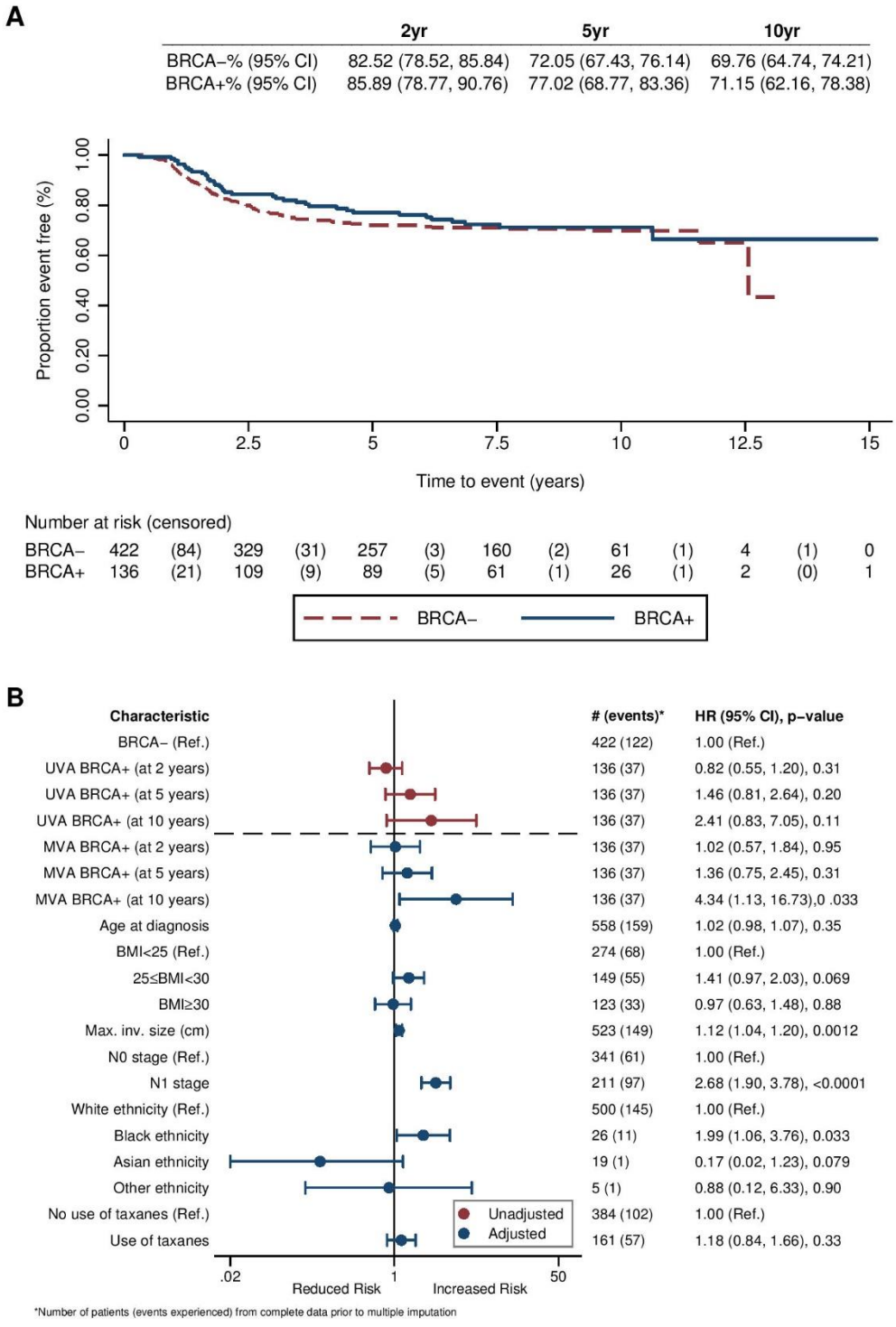
Appendix Figure 4 – Overall Survival by *BRCA2* status for all patients (analysis population)
Kaplan-Meier plot by *BRCA2* status (*BRCA2*+/-) for Overall Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA2*+/- status for Overall Survival (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.





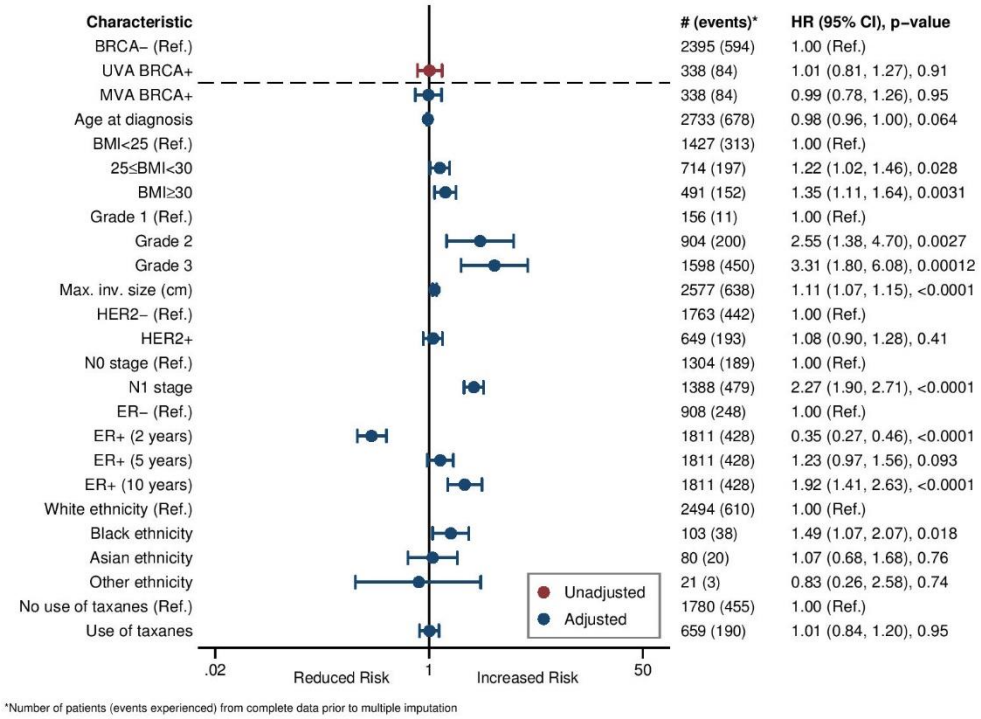
*Number of patients (events experienced) from complete data prior to multiple imputation

Appendix Figure 5 – Distant Disease Free Survival by *BRCA* status for all TNBC patients (TNBC population)
Kaplan-Meier plot by *BRCA*1 and/or 2 status (*BRCA*+/-) for Distant Disease Free Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA*+/- status for Distant Disease Free Survival (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, tumour size, ethnicity and use of taxane chemotherapy.



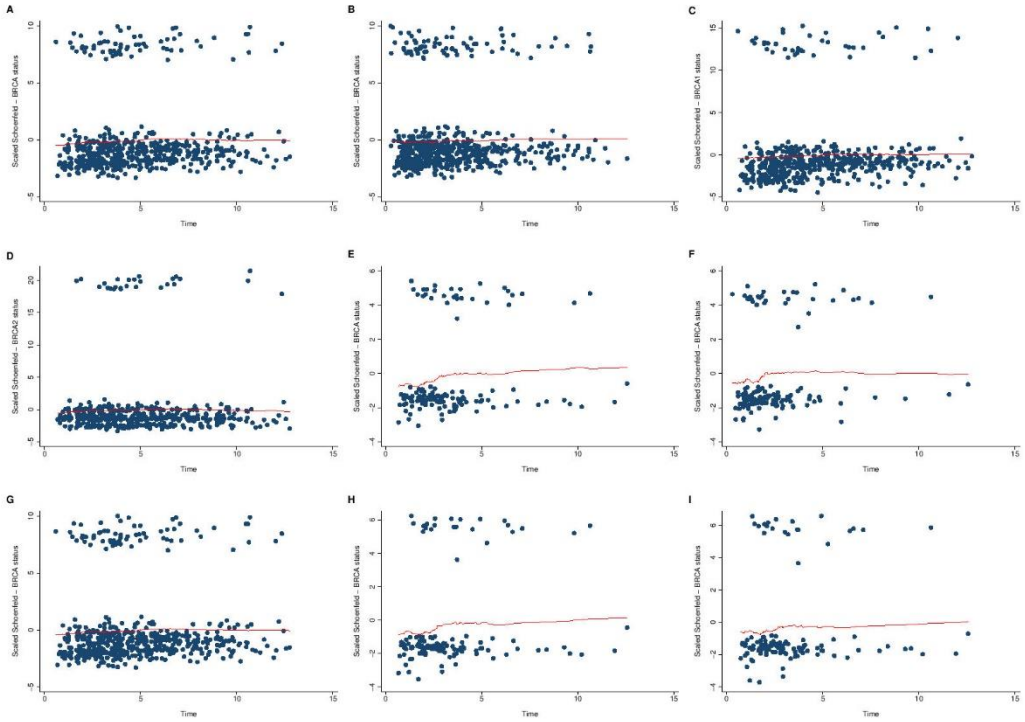
Appendix Figure 6 – Overall Survival by *BRCA* status for all patients, adjusting for time to blood draw (analysis population)

Forest Plot of univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (OS), adjusting for time to blood draw. Multivariable analysis is also adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.



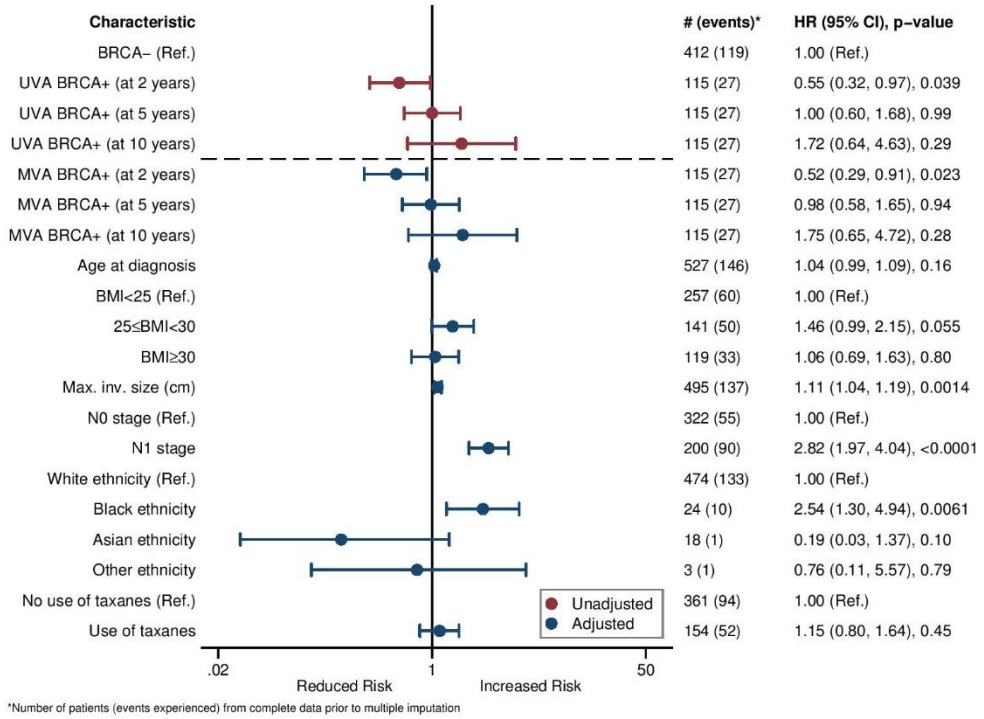
*Number of patients (events experienced) from complete data prior to multiple imputation

Appendix Figure 7 – Multivariable Analyses - Proportional hazards tests
Proportional hazards (PH) test results for the main comparators for: (A) Overall Survival (OS) by BRCA status – analysis population (PH assumption met); (B) Distant disease free survival (DDFS) by BRCA status – analysis population (PH assumption met); (C) OS by BRCA1 status – analysis population (PH assumption met); (D) OS by BRCA2 status – analysis population (PH assumption met); (E) OS by BRCA status – TNBC population (PH assumption not met); (F) DDFS by BRCA status – TNBC population (PH assumption not met); (G) OS by BRCA status, adjusted for time to blood draw – analysis population (PH assumption met); (H) OS by BRCA status - TNBC population, excluding patients not having immediate bilateral mastectomies (PH assumption not met); (I) OS by BRCA status - TNBC population, excluding patients who developed a new primary breast or ovarian cancer (PH assumption not met).

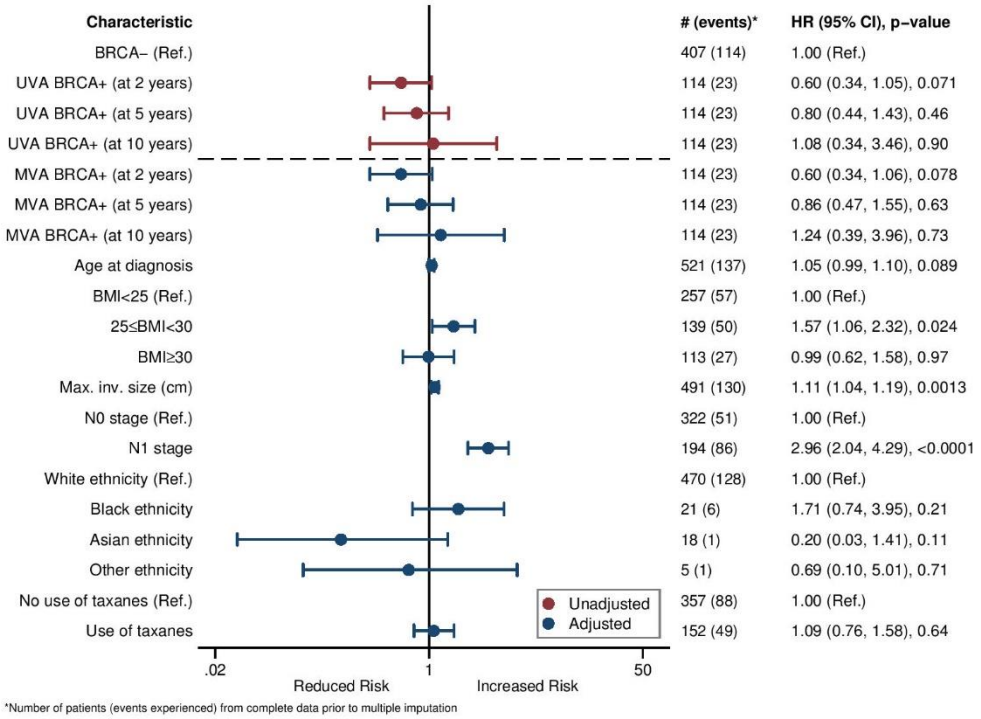


Appendix Figure 8 – Overall Survival by *BRCA* status for TNBC patients not having immediate bilateral mastectomies (TNBC population, excluding patients not having immediate bilateral mastectomies)

Forest Plot of univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (OS). Multivariable analysis is adjusted for age, body mass index, tumour size, ethnicity and use of taxane chemotherapy.



Appendix Figure 9 – Overall Survival by *BRCA* status for TNBC patients who did not develop a new primary breast or ovarian cancer (TNBC population, excluding patients who developed a new primary breast or ovarian cancer)
Forest Plot of univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (OS). Multivariable analysis is adjusted for age, body mass index, tumour size, ethnicity and use of taxane chemotherapy.



Appendix - Methods

Appendix Methods 1: BRCA1 and BRCA2 gene sequencing and variant calling
Details of sequencing methodology and annotation of variants.

Amplicon design, enrichment, sequencing, and variant calling:

All POSH study cases with a DNA sample submitted were included. Fluidigm targeted DNA amplification assay design software (Fluidigm, South San Francisco, California, USA) was used to select PCR ≤235bp amplicons covering all exons, splice junctions and UTRs of the BRCA1 and BRCA2 genes. These 261 amplicons were part of a larger multiplex panel of 1,122 amplicons covering 35 genes (manuscript in preparation). Using the Fluidigm software, primer pairs were multiplexed into 20 pools. The Fluidigm Juno Access Array 192.24 system was used for library preparation, according to the manufacturer's protocols (Fluidigm, South San Francisco, California, USA). Target sequences were amplified, then one of 1,536 unique sample barcodes and Illumina sequencing adaptors were ligated (supplied by Fluidigm, South San Francisco, California, USA). Liquid handling robotics and barcode plate identification were used in all steps of the library preparation process. Each library of 1,536 samples was quantified with the KAPA Library Quantification Kit (KapaBiosystems, Boston, Massachusetts, USA) and then sequenced in 150-base paired-end mode on a single lane of an Illumina Hi-Seq2000 instrument using v4 chemistry, according to the manufacturer's protocols (Illumina, San Diego, California, USA).

Raw sequence data were converted to FASTQ format and demultiplexed using the Illumina CASAVA v1.8 pipeline (Illumina, San Diego, California, USA. CutAdapt v1.5[1] was used for orientation-specific, end-wise primer sequence trimming, and untrimmed reads were discarded. Reads were aligned to the hg19 human reference sequence with BWA-MEM v0.7.[2]. Both SAMtools and GATK v3.3[3] was used for local insertion-deletion variant (indel) realignment and base quality score recalibration. Using intervals containing one or more full exons, GATK UnifiedGenotyper was used to perform SNP and indel discovery and variant calling across all samples simultaneously, according to the GATK best practice recommendations [4, 5]. We also called variants using a case by case approach which gave improved sensitivity and reduced specificity.

Sample and variant quality control (QC) filtering:

VCFtools[6] was used to first remove all variants with >20% missing calls, and then all samples with missing data for >20% of remaining variants. GATK was used to recalculate variant-level quality metrics for only the retained samples, and variant positions with quality by depth <3 or >25 were excluded. Genotypes with depth <20 or genotype quality <13 were recoded as no call using VCFtools. Finally, samples and then variants with >5% missing calls were excluded. After all filtering, 5,488/5,952 controls (92%) and 13,087/13,824 cases (95%) were retained for further analysis.
Indels with more than three alleles were removed. Potentially problematic variants, including indels longer than 1-bp in length, indels within 10-bp of one another, dinucleotide substitutions, and rare variants (defined by carrier frequency <0.1% in the ExAC Non-Finnish European dataset) for which one or more samples was called homozygous, were inspected manually in the Integrative Genome Viewer (IGV).[7] Where there were discrepancies between UnifiedGenotyper calls and the IGV inspection, the IGV-based variant call was used.

Functional prediction and variant frequency classification:

The Ensembl Variant Effect Predictor (VEP)[8] was used to assign the canonical transcript- and protein-level consequence for each variant. Frameshift, stop/gain, and canonical splice variants (i.e. positions -1,-2, +1 or +2) were considered as protein truncating. Missense variants were further annotated with effect predictions from CADD,[9] PolyPhen2,[10] SIFT,[11] and AlignGVGD,[12] a cancer gene-specific missense variant effect prediction tool. The consequences of the putative splice site variant CHEK2 c.320-5T>A were evaluated using the in silico prediction tools SpliceSiteFinder-like,[13] MaxEntScan,[14] NNSPLICE,[15] GeneSplicer,[16] and Human Splicing Finder.[17]

Coverage, quality, and variant call concordance metrics:

Per-sample and per-base mean sequence coverage were tabulated with BEDTools.[19]. For each sample, the GATK "callable loci" script was used to calculate the percentage of exonic bases with at least 20 reads and a minimum base quality of 20.
The accuracy of variant calling was assessed by Sanger sequencing to estimate the false positive rate (positive predictive value, PPV). Sanger sequencing primers with M13 sequence tags were designed. Sanger calls were checked against NGS results, and discrepancies were resolved via comparison of results and inspection of reads in IGV. Genotypes were successfully validated for 188/188 samples carrying SNVs (positive predictive value=100.0%) and 67/68 samples carrying indels (positive predictive value=98.5%).

Appendix Methods 1 – References:

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Appendix - Documents

Appendix Document 1: Statistical Analysis Plan
Statistical analysis plan (SAP), approved on 10-May-2016, and formatted for Lancet Oncology Appendix.

[Please note: Figures in this SAP are taken from the POSH data available up until June 2015, and thus only represent approximations of the new data due to be downloaded from the POSH database in 2016/2017.]

Please note: This statistical analysis plan has been written in the past tense because it will form the basis of a paper. The headings used in this document come from the STROBE reporting guideline for observational studies (see http://www.strobe-statement.org/ or http://www.annals.org/content/147/8/W-163.full.pdf+html).

Statistical Analysis Plan Version

Issue no	Revision History	Author	Date
0.1	First draft written based on discussion at meeting on 8th Oct 2010	Louise Stanton (née Dent)	20th Oct 2010
0.2	Additional comments and annotations	Diana Eccles, Sue Gerty	13th Oct 2010
0.3	Further notes on confounding factors and example figures for POSH cohort added	Diana Eccles	25th Nov 2010
0.4	Updated based on meeting with Diana Eccles and Sue Gerty on the 29th Oct 2010 and meeting with Sue Gerty on 9th December 2010	Louise Stanton (née Dent)	17th Dec 2010
0.5	Updated based on comments from Doug Altman	Louise Stanton (née Dent)	21st Feb 2011
0.6	Updated based on discussions	Diana Eccles, Louise Stanton (née Dent)	24th Feb 2011
0.7	Updated based on meeting with Louise Stanton (née Dent) on 21st March 2012	Tom Maishman	30th Mar 2012
0.8	Updated based on comments from Diana Eccles	Tom Maishman	2nd Apr 2012
0.9	Updated following a meeting with Doug Altman, Diana Eccles and Louise Stanton (née Dent)	Tom Maishman	18th Mar 2013
0.10	Updated following planned updates to obtain further BRCA testing information	Tom Maishman	30th Jun 2015
0.11	Updated following comments from Diana Eccles and Ellen Copson	Tom Maishman	14th Jul 2015
0.12	Updated following comments from Diana Eccles and Ellen Copson	Tom Maishman	28th Jul 2015
0.13	Updated following meeting with Doug Altman on 30th July 2015	Tom Maishman	7th Aug 2015
1	Finalised using v0.13	Tom Maishman	10th May 2016

1. Introduction

1.1 Background / Rationale

BRCA1 and BRCA2 are the most frequently reported highly penetrant monogenic factors that predispose to breast cancer. Both genes also predispose to ovarian cancer. Mutation in either gene has been shown to lead to higher grade breast cancer than average and to young age at onset (median age for BRCA1 is 43 years and for BRCA2 is 48 years compared to the population mean age at diagnosis of about 60 years). In addition for BRCA1 associated breast cancer, the proportion of oestrogen receptor negative cancers is much higher than average (80-90% compared to ~ 30% amongst breast cancers in women diagnosed < 50 years of age). There are conflicting conclusions in the literature exploring whether BRCA1 or BRCA2 mutation carriers develop breast cancers with a better or worse prognosis. Most reported studies are small, retrospective and with incomplete data on many of the factors known to influence breast cancer outcomes. Some of the early reports of better survival failed to recognise or adequately account for survival bias in many of the BRCA tested patients. Knowledge of a family history of breast cancer, even without genetic testing may lead to earlier diagnosis of breast cancer due to heightened awareness and early presentation and investigation; this bias may lead to observations of improved survival in BRCA gene carriers. The adverse pathological features associated with breast cancers diagnosed in BRCA gene carriers may account for observations of a worsened prognosis in gene carriers compared with the average.. A differentially better or worse response to adjuvant chemotherapy in relation to the underlying genetic predisposition may also affect prognosis. It is important to understand the overall effect of genetic predisposition factors on prognosis in order to better inform gene carriers making decisions about primary prevention and about cancer treatment and to help design more informative prospective clinical trials of both conventional and novel targeted treatments. The Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) is a large contemporary cohort study of breast cancer cases diagnosed before 41 years of age and designed to investigate the effect of genetic factors on breast cancer prognosis.

1.2 Objectives

This paper presents the results from analyses carried out on data collected from the POSH study.

The primary objective was:

- To investigate whether patients with early breast cancer and an inherited BRCA1 or BRCA2 gene mutation (BRCA-Positive [BRCA+]) have a superior Overall Survival (OS) than patients without a BRCA1 or BRCA 2 mutation (BRCA-Negative [BRCA-]).

Secondary objectives were:

- To investigate whether BRCA+ patients with early breast cancer have a superior Distant Disease Free Survival (DDFS) than BRCA- patients.
- To investigate whether BRCA+ patients with early breast cancer have a superior Post Distant Relapse Survival (PDRS) than BRCA- patients.

- To investigate whether patients with early breast cancer and an inherited BRCA1 gene mutation (BRCA1-Positive [BRCA1+]) have a superior OS than patients without a BRCA1 mutation (BRCA1-Negative [BRCA1-])[1].
- To investigate whether BRCA1+ patients with early breast cancer have a superior DDFS than BRCA1- patients.
- To investigate whether BRCA1+ patients with early breast cancer have a superior PDRS than BRCA1- patients.
- To investigate whether patients with early breast cancer and an inherited BRCA2 gene mutation (BRCA2-Positive [BRCA2+]) have a superior OS than patients without a BRCA2 mutation (BRCA2-Negative [BRCA2-])[2].
- To investigate whether BRCA2+ patients with early breast cancer have a superior DDFS than BRCA2- patients.
- To investigate whether BRCA2+ patients with early breast cancer have a superior PDRS than BRCA2- patients.

- To investigate whether Triple Negative (TNT)[3] BRCA+ patients with early breast cancer have a superior OS than TNT BRCA- patients.
- To investigate whether TNT BRCA+ patients with early breast cancer have a superior DDFS than TNT BRCA- patients.
- To investigate whether TNT BRCA+ patients with early breast cancer have a superior PDRS than TNT BRCA- patients.

- To investigate whether BRCA+ patients with early breast cancer have a superior DDFS than BRCA- patients when adjusting for chemotherapy.

[1] This comparison excludes patients with a BRCA2 positive gene mutation.
[2] This comparison excludes patients with a BRCA1 positive gene mutation.
[3] Triple Negative Patients defined as Patients with a HER2 negative status, ER negative status and either a PR negative status or PR missing/unknown status i.e. patients with a confirmed PR positive status are excluded.

2. Methods

2.1 Study Design

The POSH study is a prospective cohort study. The protocol for the study can be found in the following journal article http://www.biomedcentral.com/1471-2407/7/160.

2.2 Setting

The POSH study recruited women from breast cancer units across England, Scotland, Wales and Northern Island between 1st June 2001 to 31st January 2008.

2.3 Participants

The study recruited 3052 women aged 40 years or younger at breast cancer diagnosis. The women had to have been diagnosed with breast cancer between January 2000 and January 2008. In addition, 43 women aged 41-50 were also included if they had a known BRCA1 or BRCA2 gene mutation and were diagnosed with invasive breast cancer within the study period were excluded for this analysis. Women were excluded if they had a previous invasive malignancy (with the exception of non-melanomatous skin cancer), were not available for follow up or refused consent to retain diagnostic and follow up data. Genetic testing was performed on xxx women. Those not tested were excluded from the additional comparison. Patients with confirmed M1 stage (n=74) were also excluded. A total of 2925 women were included in the analysis population.

Clinical follow up data were obtained from the patient medical records by the clinical trials practitioner (CTP) at each recruiting centre. Data forms collecting information at diagnosis, 6 months, 12 months were completed by the CTP usually at 12 months from diagnosis. Annual data collection was continued from the date of definitive diagnosis until death, loss to follow up or until the end of the current phase of the study (mmm yyyy).

Family history data: patients in the POSH study completed a family history questionnaire (http://www.biomedcentral.com/1471-2407/7/160 supplementary figure). The web-based and validated genetic risk prediction software BOADICEA (Antoniou A, et al 2008. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. J Med Genet. Jul;45(7):425-31) was used to process pedigree data and generate a predicted likelihood that each patient might carry a BRCA1/2 mutation. No family history was provided for 106 of the 2956 patients. BOADICEA scores for the remaining 2850 patients were calculated from the family history of the proband at the time she presented with breast cancer. A total of 1939 (66%) scored below 0.05, 372 (13%) scored 0.05-0.099, 226 (8%) scored 0.10-0.199 and 314 (11%) scored 0.20 or over. BOADICEA scores for the xxx patients were calculated from the family history of the proband at the time she presented with breast cancer.
Genetic testing results for BRCA1/2 were already available through clinical test reports or other research sub-studies in xxx cases and these data were used to validate the sensitivity and specificity of the Fluidigm technology used across the cohort. Mutation testing was carried out on all patients recruited to the study for whom a DNA sample was available (n=xxx). A panel of genes was tested using Fluidigm targeted sequence capture and next generation sequencing with additional analysis using Multiple Ligation Probe Analysis (MLPA) to detect large exonic deletions or duplications where there was either a greater than 10% estimated probability of an underlying BRCA1/2 gene mutation (estimated using BOADICEA) or where there was evidence from the Fluidigm assay of a large deletion or duplication. Only mutations that were clearly pathogenic were used to assign gene carriers to the relevant group for analysis purposes.

2.4 Variables (data taken as of June 2015)

Variable	Type of data / categories	Amount of missing data (Analysis Group A – see Section 2.8, n=2873)	Amount of missing data (Analysis Group B – see Section 2.8, n=725)	Possible reasons for missing data
2.4.1 Primary outcome				
Time to death from any cause	Survival data Date of death from any cause – Date of invasive breast cancer diagnosis	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A
2.4.2 Secondary outcomes				
Time to distant relapse or death from any cause	Survival data Date of first distant relapse (or death from any cause) – Date of invasive breast cancer diagnosis	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit	N/A
Time from first relapse to death from any cause	Survival data Date of death from any cause – Date of first distant relapse	N/A, patients who haven't relapsed will not be included. Patients who have relapsed and haven't died will be censored at the date of their last follow up visit	N/A, patients who haven't relapsed will not be included. Patients who have relapsed and haven't died will be censored at the date of their last follow up visit	N/A
2.4.3 Candidate predictor				
Genetic status[1]	Categorical For the main comparison, each patient is assigned one of 3 categories: BRCA 1 gene carrier confirmed by genetic testing (n=xxx) BRCA 2 gene carrier confirmed by genetic testing (n=xxx) TP53 (n=xxx) No mutation found/variant unknown significance	TBA	TBA	TBA
2.4.4 Potential confounders / effect modifiers - measured at breast cancer diagnosis presentation				
1. Age at diagnosis	Continuous, in years	0 records	0 records	N/A
2. Body Mass Index (BMI)	Categorical Underweight/Healthy, Overweight, Obese, or missing/unknown	108 (3.8%) records	15 (2.1%) records	Consider MAR
3. Histological Tumour grade	Categorical 1, 2, 3, or not graded/missing/unknown	70 (2.4%) records not graded/missing/unknown	19 (2.6%) records not graded/missing/unknown	MCAR. Inadequate reporting by pathologist. If grade of core biopsy tumour not stated, and after neo-adjuvant chemotherapy there was a complete pathological response then no tumour to report on.
4. Maximum tumour diameter invasive (tumour size)	Continuous, in mm or Categorical <15mm, 15mm to 20mm, >20mm to 35mm, >35mm to	162 (5.6%) records	53 (7.3%) records	Missing for similar reasons as tumour grade (MCAR)

Variable	Type of data / categories	Amount of missing data (Analysis Group A – see Section 2.8, n=2873)	Amount of missing data (Analysis Group B – see Section 2.8, n=725)	Possible reasons for missing data
	50mm, >50mm, or missing/unknown			
5. Pathological N stage (lymph node status)	Categorical N0, N1 or missing/unknown	31 (1.1%) records	10 (1.4%) records	MCAR. No axillary surgery, no lymph nodes in resected specimen.
6. Number of positive Lymph nodes	Categorical 0, 1-3, 4-9, 10+, or missing/unknown	31 (1.1%) records	10 (1.4%) records	Same as above (MCAR)
7. Lymphovascular invasion	Categorical Present, absent or missing/unknown	203 (7.1%) records	58 (8.0%) records	Poor reporting. Consider as MCAR.
8. M stage	Categorical M0, M1 or missing/unknown	22 (0.8%) records	5 (0.7%) records	MCAR, likely to be M0 as only 2.1% of patients are M1.
9. Oestrogen receptor (ER)[1]	Categorical Negative, positive, or missing/unknown	11 (0.4%) records	0 records	N/A
10. HER2[2]	Categorical Negative, positive, or missing/unknown	352 (12.3%) records	0 records	Missing because diagnosis predated routine testing and patient has not suffered a further breast cancer event since initial diagnosis. Consider Missing At Random (MAR).
11. PR[3]	Categorical Negative, positive, or missing/unknown	564 (19.6%) records	85 (11.7%) records	MAR. Missing because specific centres don't do PR IHC.
12. Ethnicity	Categorical Caucasian/White, Black, Asian, Other, or missing/unknown	41 (1.4%) records	8 (1.1%) records	Consider MAR
Diagnosis Year	Categorical ≤2005 or >2005	0 records	0 records	N/A
Adjuvant or neo-adjuvant chemotherapy indicator	Categorical Yes or No/missing/unknown	0 records	0 records	N/A
Chemotherapy with taxane indicator	Categorical Yes or No/missing/unknown	0 records	0 records	N/A
17. Focality (distribution of tumour)	Categorical Multifocal, localised or missing/unknown	61 (8.0%) records	286 (9.7%) records	Missing for similar reasons as tumour grade (MCAR).
18. Definitive surgery	Categorical Breast Conserving Surgery (BCS), Mastectomy, No surgery, Nodal surgery only, or missing/unknown	0 records	0 records	N/A
19. Chemotherapy regimen	Categorical Anthracyclines, A&T, Taxanes, Other, or None	0 records	0 records	N/A
2.4.5 Additional (descriptive) variables				
13. Length of follow-up	Continuous, in months	0 records	0 records	N/A
Amount of missingness in the multivariable models				
No. of pts with at least 1 variable with missing data from the MV model 1 (see Section 2.8)		**596 (20.7%)**	**155 (21.4%)**	
No. of pts with at least 1 variable with missing data from the MV model 2 (see Section 2.8)		**610 (21.2%)**	**159 (21.9%)**	

[1] Not all patients in the POSH study had genetic testing (in the same way not all patients do currently in the NHS). BOADICEA scores were calculated purely based on family history data from the patient family history questionnaire; no information about mutation testing was included in the estimates. Patients with a combined (BRCA1 and BRCA2) score of <0.05 had no significant family history of cancer. Scores above 0.10 would be eligible for testing according to American Society of Oncology guidelines and scores above 0.10 are eligible for testing under the 2013 UK NICE guidelines.

[2] Oestrogen receptor allocation of result from POSH database to Oestrogen receptor category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative*
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

*For ER, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as ER negative, and an Allred socre of 3+ treated as ER positive. However it is possible that reviewers will disagree so we can reclassify this as positive if required.

[3] HER2 allocation of result from POSH database to a HER2 category:

Result	Category result assigned to
FISH/CISH positive	Positive**
3+	Positive
FISH/CISH borderline	Negative**
2+	Negative
FISH/CISH negative	Negative**
1+	Negative
0	Negative
Not done	Not done
Unknown	Missing/unknown

** FISH/CISH results take precedence i.e. a 2+ result which is later found to have a FISH/CISH positive result is categorised as Positive rather than Borderline.

[4] PR allocation of result from POSH database to a PR category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative***
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

***For PR, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as PR negative. However it is possible that reviewers will disagree so we can reclassify this as positive if required

2.5 Data sources/measurement

The tumour biopsy, definitive histopathological report, clinical and radiological reports were all submitted to the study. Pathological characteristics of the tumours were taken from the diagnostic histopathology report, clinical staging from the clinical and radiological reports.

National death data were obtained for patients in the cohort from the Medical Research Information Service (MRIS).

ER, PR and HER2 data were taken from pathology reports. Scoring systems varied as expected across contributing hospitals. Positive and Negative categories are straightforward however borderline results exist in all three IHC categories and were classified into a separate borderline group. The borderline category was merged with negative for the purposes of these analyses. Additional IHC data for these three markers was available from the Tissue Micro Arrays (TMAs) constructed from tumour pathology blocks for study participants which were used to populate these missing clinical data fields.

This paper presents the results of analyses conducted on follow up data available up until dd-mmm-yyyy.

2.6 Bias

Clinical data for all patients were collected via standard clinical research forms which were completed from the clinical notes by the Clinical Trials Practitioner in each centre.

HER2 data: There are concerns regarding the amount of missing HER2 data obtained. In addition:

- HER2 Testing was only widely introduced after 2006 (proportion tested prior to 2006 was 83% (1704/2041), proportion tested on/after 2006 was 98% (897/915)). Prior to 2006 HER2 testing was more likely to have been carried out in patients who had progressed (93% i.e. 520 tested out of 561 who progressed, compared to 80% i.e. 1184 tested out of 1480 who had not progressed). Therefore, patients for whom we knew their HER2 status were more likely to have had a worse prognosis. Hence, if we selected patients on the basis of HER2 testing and compared them to patients who may or may not have been HER2 tested this would have been biased as the patients who have been HER2 tested could look worse by comparison.

- In addition, any analyses that select any patients which have a known HER2 status (which includes patients diagnosed before 2006) will include more cases who had relapsed (and were therefore tested for HER2 amplification retrospectively) than the whole cohort which could potentially compromise the validity of results.

2.7 Study Size

This is covered in the BMC paper.

2.8 Statistical Methods

Patients excluded from the analyses
Patients were excluded from this analysis if we didn't have confirmation that they had invasive cancer from pathology results or were missing primary data (21 patients). Genetic testing was performed on xxx women. Those not tested were excluded from the additional comparison. Patients with confirmed M1 stage (n=74) were also excluded. A total of 2925 women were included in the analysis population, of which:

- n=2873 were aged 40 years or younger at diagnosis without a TP53 gene mutation **(Analysis Group A)**;
- n=725 were aged 40 years or younger at diagnosis without a TP53 gene mutation and had a TNT status **(Analysis Group B)**;
- n=43 were aged 41-50 years at diagnosis with a confirmed gene mutation **(Analysis Group C)**;
- n=9 were aged 40 years or younger at diagnosis and had a TP53 gene mutation **(Analysis Group D)**.

Primary outcome measure
Overall Survival (OS) where OS is defined as the time from the date of invasive breast cancer diagnosis to death from any cause. Patients who had not died will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the primary outcome analysis.

Secondary outcome measures
Distant Disease Free Survival (DDFS) where DDFS is defined as the time from the date of invasive breast cancer diagnosis to distant relapse or death from any cause. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died or relapsed at the time of analysis will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the secondary outcome analysis.

Post Distant Relapse Survival (PDRS) where PDRS is defined as the time from the date of distant relapse to death from any cause. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the secondary outcome analysis.

Univariate analyses
Where specified for analysis groups A, B, C and D above, we summarised patient and tumour characteristics by the following:

- All patients (Analysis **Groups A, B, C and D**)
- BRCA1+ patients (Analysis **Groups A, B and C** only)
- BRCA2+ patients (Analysis **Groups A, B and C** only)
- BRCA+ patients (Analysis **Groups A and B** only)
- BRCA- patients (Analysis **Groups A and B** only)

For analysis groups A and B, we summarised and produced Kaplan Meier survival curves of OS, DDFS, and PDRS and compared the survival curves using a log rank test for the following:

- BRCA+ versus BRCA-
- BRCA1+ versus BRCA1- (excluding BRCA2+ patients)
- BRCA2+ versus BRCA2- (excluding BRCA1+ patients)

For analysis group C, we summarised and produced Kaplan Meier survival curves of OS, DDFS, and PDRS and compared the survival curves using a log rank test for BRCA1+ versus BRCA2+patients.

Multivariable analyses
Comparison groups:

- BRCA+ versus BRCA- **(analysis Group A)**
- BRCA1+ versus BRCA1-(excluding BRCA2+ patients) **(analysis Group A)**
- BRCA2+ versus BRCA2- (excluding BRCA1+ patients) **(analysis Group A)**
- TNT BRCA+ versus TNT BRCA- **(analysis Group B)**

For the comparisons i) to iv) above, we fitted a multivariable model for OS and DDFS adjusting for the following covariates:

- Age at diagnosis, in years (fitted as a continuous covariate);
- Body Mass Index (BMI) (fitted as a categorical covariate [Underweight/Healthy, Overweight or Obese]);
- Histological Grade (fitted as a categorical covariate [1, 2 or 3]);
- Maximum invasive tumour size, in mm (fitted as a continuous covariate);
- N stage (fitted as a binary covariate [N0 or N1]);
- ER status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;
- HER2 status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;

For the comparisons i) to iv) above, we fitted a multivariable model for OS and DDFS, comparing BRCA+ versus BRCA-, adjusting for the following covariates:

- Age at diagnosis, in years (fitted as a continuous covariate);
- Body Mass Index (fitted as a categorical covariate [Underweight/Healthy, Overweight or Obese]);
- Histological Grade (fitted as a categorical covariate [1, 2 or 3]);
- Maximum invasive tumour size, in mm (fitted as a continuous covariate);
- N stage (fitted as a binary covariate [N0 or N1]);
- ER status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;
- HER2 status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;
- Ethnicity (fitted as a categorical covariate [Caucasian, Black or Asian]) – *where appropriate*;
- Diagnosis Year (fitted as a binary covariate [≤2005, or >2005]) – *where appropriate*;
- Adjuvant or neo-adjuvant chemotherapy indicator (fitted as a binary covariate [yes, or no/missing/unknown]) – *where appropriate*;
- Chemotherapy with taxane indicator (fitted as a binary covariate [yes-with taxane, or no-without taxane]) – *where appropriate*.

Hazard Ratios

Evidence suggests that the effect of ER status changes over time (Azzato, et al, 2009, Bellera et al, 2010)[1]. Indeed, this was evident after testing the proportional hazards assumption based on the Schoenfeld residuals and using the identity matrix for the time-scaling function[2] i.e. using the estat phtest command in STATA. This result provided strong evidence against the Cox proportional hazards assumption ($p<0.001$), which was also seen when plotting the scaled Schoenfeld residuals over time[2].

As a result of the time-varying effects of the ER status, a flexible parametric survival model was programmed in STATA using the stpm2 command (Lambert, Royston, 2009)[3] to model ER as a time-dependent covariate. The degrees of freedom for the restricted cubic spline function used for the hazard rate was set to the default setting of 3, whilst the degrees of freedom for the time-dependent effects was set so as to provide the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC). The time-varying hazard ratio and 95% confidence interval was plotted over time and 2-, 5-, and 8-year relative hazard ratios and survival estimates were produced.

[1]The Azzato, et al paper can be found at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2695697/. The Bellera et al paper can be found at http://www.biomedcentral.com/1471-2288/10/20.
[2]Results obtained from T Maishman's MSc Project analysis undertaken on POSH data downloaded in May 2011.
[3] The Lambert & Royston paper can be found at www.stata-journal.com/article.html?article=st0165 or http://www.pauldickman.com/cancerepi/handouts/handouts_survival/Lambert2009.pdf

Method used to handle missing data

The amount of missingess will be investigated and if deemed appropriate, methods of multiple imputation will be incorporated. Otherwise, a complete-case analysis approach will be incorporated.

To date, between 20-22% of patients have are missing data for at least 1 covariate in the multivariable models.

Appendix Document 2: STROBE Checklist

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist.

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1 (and 3)	Within the title (1) and abstract (3)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3	Within the abstract (Methods and Findings)
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5	Within the Background
Objectives	3	State specific objectives, including any prespecified hypotheses	5	Within the Background
Methods				
Study design	4	Present key elements of study design early in the paper	5	Within the Background and Methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-7	Within the Methods
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-6	Within the Methods
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-8	Within the Methods
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7	Within the Methods
Bias	9	Describe any efforts to address potential sources of bias	8	Within the Methods
Study size	10	Explain how the study size was arrived at	7	Within the Methods

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8	Within the Methods
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8	Within the Methods
		(*b*) Describe any methods used to examine subgroups and interactions	7-8	Within the Methods
		(*c*) Explain how missing data were addressed	8	Within the Methods
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	8	Within the Methods
		(*e*) Describe any sensitivity analyses	8	Within the Methods
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8-9 & Appendix Figure 1	Within the Results & Appendix Figure 1
		(b) Give reasons for non-participation at each stage	8-9 & Appendix Figure 1	Within the Results & Appendix Figure 1
		(c) Consider use of a flow diagram	Appendix Figure 1	Within Appendix Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9, Tables 1 & 2, Appendix Figure 1	Within the Results, Tables 1 & 2, & Appendix Figure 1
		(b) Indicate number of participants with missing data for each variable of interest	Tables 1 & 2	Within the Tables 1 & 2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	9	Within the Results
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	9-10, Figures 1 & 2, Appendix Figures 2, 3, 4, 5, 6, 8, & 9	Within the Results, Figures 1 & 2, & Appendix Figures 2, 3, 4, 5, 6, 8, & 9
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A	N/A
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11, Figures 1 & 2, Appendix Figures 2, 3, 4, 5, 6, 8, & 9	Within the Results, Figures 1 & 2, & Appendix Figures 2, 3, 4, 5, 6, 8, & 9
		(*b*) Report category boundaries when continuous variables were categorized	Tables 1 & 2	Within Tables 1 & 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	N/A

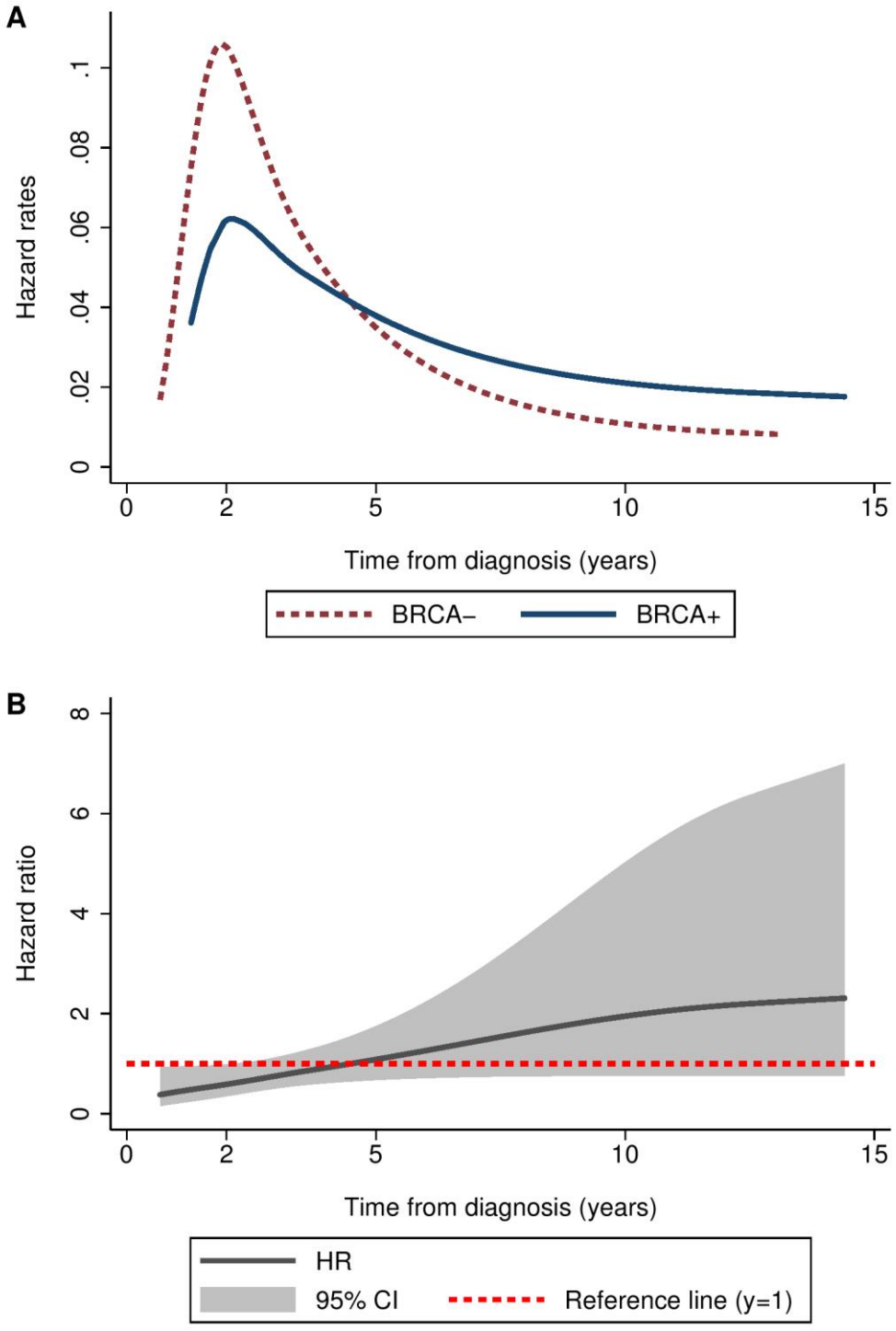
Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-11, Appendix Figures 8 & 9	Within the Results and Appendix Figures 8 & 9 for post-hoc analyses results
Discussion				
Key results	18	Summarise key results with reference to study objectives	11-13	Within the Discussion
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14-15	Within the Discussion
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15	Within the Discussion
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-15	Within the Discussion
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	4, 8, 16	Within the Funding section following the abstract, within the Methods and within Acknowledgements

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix Figure 10 – Time-varying effects of BRCA status on Overall Survival for all TNBC patients (TNBC population)
Time-varying hazard rates by BRCA1 and/or 2 status (BRCA+/-) for Overall Survival (OS) (Panel A); and corresponding time-varying hazard ratio for Overall Survival (Panel B).



Chapter 8: Paper 6 - Local recurrence and breast oncological surgery in young women with breast cancer

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8.1 Contribution

As the lead statistical methodologist, scientific lead at the SCTU and joint first author:

- Responsible for study methodology and research of all statistical methods to be implemented in the analyses, including the use of FPSMs for time-varying covariates;
- Assisted with the literature review;
- Developed and authored the SAP – including the organisation and participation of meetings to develop the SAP, and the creation of all draft and final versions of the SAP;
- Responsible for central data monitoring, data cleaning and data interpretation – liaising with the study team to identify data queries, and for the resolution of all data queries raised with data management;
- Developed and conducted all statistical programming and analyses – including the creation of all database and analysis programming required for the analyses;
- Designed, developed and produced all manuscript figures and tables – including the development of the time-varying hazard plots by surgical type, the transcription of all results, the creation of all draft and final versions of the manuscript figures and tables;
- Co-drafted and reviewed all versions of the manuscript – listed as joint 1st named author, co-drafted all versions of the manuscript, and extensively involved in the resolution of reviewer comments and responses;
- Jointly responsible for manuscript submission and administrative correspondence.

[7] Joint first authors

Professor Diana Eccles, chief investigator of the POSH study, chair of the POSH Study Steering Group, and senior last author of **Paper 6**, confirms the accuracy of contribution outlined above of Thomas Christopher Maishman.

Signed: .....

Date: .....

OPEN

Local Recurrence and Breast Oncological Surgery in Young Women With Breast Cancer

The POSH Observational Cohort Study

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Objective: To assess clinical and surgical factors affecting local recurrence and survival in young breast cancer patients in the Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH).

Background: Emerging data suggest young age is a predictor of increased local recurrence.

Methods: POSH is a prospective cohort of 3024 women of 18 to 40 years with breast cancer. Cohort characteristics were grouped by mastectomy or BCS. Endpoints were local-recurrence interval (LRI), distant disease-free interval (DDFI), and overall survival (OS); described using cumulative-hazard and Kaplan-Meier plots and multivariable analyses by Flexible Parametric and Cox regression models.

Results: Mastectomy was performed in 1464 patients and breast-conserving surgery (BCS) in 1395. Patients undergoing mastectomy had larger tumors and higher proportions of positive family history, estrogen receptor+, progesterone receptor+, and/or human epidermal growth factor receptor 2+ tumors. Local events accounted for 15% of recurrences. LRI by surgical type varied over time with LRI similar at 18 months (1.0% vs 1.0%, $P = 0.348$) but higher for BCS at 5 and 10 years (5.3% vs 2.6%, $P < 0.001$; and 11.7% vs 4.9%, $P < 0.001$, respectively). Similar results were found in the adjusted model. Conversely, distant-metastases and deaths were lower for BCS but not after adjusting for prognostic factors. After mastectomy chest-wall radiotherapy was associated with improved LRI (hazard ratio, HR = 0.46, $P = 0.015$). Positive surgical margins, and development of local recurrence predicted for reduced DDFI (HR = 0.50, $P < 0.001$; and HR = 0.29, $P = 0.001$, respectively).

Conclusions: Surgical extent appears less important for DDFI than completeness of excision or, where appropriate, chest-wall radiotherapy. Despite higher local-recurrence rates for BCS, surgical type does not influence DDFI or OS after adjusting for known prognostic factors in young breast cancer patients.

Keywords: breast cancer, breast conserving surgery, local recurrence, mastectomy, outcome, survival, young women

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Breast cancer is the most common cancer in young adult women (age, ≤40 years) in the UK, with over 2000 new cases annually.[1] Young women have been found to develop more aggressive tumors coupled with lower survival and higher local-recurrence rates (LRR) than older women,[2–9] and this may be a particular issue in the developing world where a greater proportion of breast cancer appears in women of young age.[7,8] The choice between mastectomy and breast-conserving surgery (BCS) in young women is not often a straightforward decision for clinician and/or patient.[10] BCS is associated with better quality of life but higher LRR,[4,11] although a meta-analysis of mostly registry and database studies in patients <40 years suggests equivalent disease-free and overall survival,[12] whereas very young age (<35 years) has been considered a relative contraindication to BCS.[13] Although randomized controlled trials (RCTs) suggest equivalent survival for mastectomy and BCS, very few young patients were included in these analyses.[6,14] Indeed, young women are not routinely analyzed or reported separately in individual RCTs of BCS versus mastectomy, and any that do have very few women ≤40 years presented.[12]

Emerging evidence suggests a possible survival advantage for mastectomy in BRCA gene-mutation carriers.[15] Young patients are more likely to be BRCA-mutation positive[5] and retrospective cohort studies suggest that LRR are higher for BCS compared with mastectomy.[6,10,11,16] Although family history does not affect clinical outcome in young patients, it appears to affect surgical type selection, and it is unknown if family history of breast cancer will influence local recurrence.[17]

The effect of radiotherapy plays a key role in the treatment of younger breast cancer patients. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) found that women <40 years undergoing BCS had the highest incidence of recurrence and the largest benefit from radiotherapy; with the 10-year recurrence rate (local or distant) significantly lower compared with those without radiotherapy (36.1% vs 60.7%, respectively, $P = 0.00009$).[18] Similar results were seen in an RCT investigating the benefit of radiotherapy boost after BCS, where the largest absolute benefit was seen in patients ≤40 years with a significant relative-risk reduction for boost ($P = 0.003$).[19] Likewise, an RCT of premenopausal women undergoing mastectomy

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Tom Maishman and Ramsey I Cutress contributed equally to this work and so are joint first authors.

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with/without radiotherapy showed that irradiation after mastectomy significantly improved outcomes, even after controlling for clinical and pathological factors,[20] and a Canadian population registry study of 588 women <35 years found that LRR were reduced for postmastectomy radiation.[21]

These differences observed in the effect of radiotherapy, and the trend towards young patients having bilateral mastectomy as part of their initial cancer treatment, demonstrate that an important question remains about surgical type and outcomes in this age group. Moreover, local recurrence is very important in young breast cancer patients as there are a fewer competing risks and, other than their breast cancer, their life expectation is longer. There are no large prospective cohort studies reporting local recurrence in this age group, and a dedicated RCT comparing mastectomy versus BCS in young women is unlikely. A large prospective cohort study may offer the best level of evidence, minimizing inclusion bias, to guide management, and enable a comparison of local recurrence with disease-free survival. The Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) is an observational cohort of 3000 young women with breast cancer, and is representative of the UK breast cancer population.[22] We have not previously reported local-recurrence outcomes, and the aim of this analysis was therefore to report breast ipsilateral LRR in the POSH study to determine whether acceptable rates are found in a large cohort of young patients, and what factors, including surgical type, affect these outcomes in this age group.

METHODS

Study Population

POSH (MREC: 00/06/69) is a multicenter prospective observational cohort study of 3000 young women diagnosed with breast cancer in the UK between 2000 and 2008 (http://www.southampton.ac.uk/medicine/research/posh.page). All patients received treatment according to local protocols. The detailed study protocol was published in 2007,[22] and the cohort previously described.[23]

For this analysis, type of surgery was defined as the final oncological surgery to the breast for example, if a patient had BCS followed by mastectomy ≤3 months; this was classed as a mastectomy. A mastectomy performed >3 months after primary treatment in the absence of local disease-recurrence was considered risk reducing rather than oncological. Analyses of risk-reducing surgery will be the subject of future work. Margin status was the final surgical margin after oncological operation(s), and a positive margin was defined according to American Society for Clinical Oncology (ASCO) guidance as tumor at the margin (ie, tumor on ink).[24] This article presents analyses conducted on follow-up data from the POSH cohort received until June 26, 2015.

Statistical Analysis

All analyses were conducted according to a prespecified plan in line with published guidance.[25] Patients with metastatic disease at presentation were excluded. Summary statistics were used to describe the cohort and key characteristics were compared by surgical type using Pearson χ^2 tests or Mann-Whitney U tests. All reported P-values were 2-sided.

Study endpoints were inbreast ipsilateral local-recurrence interval (LRI), distant disease-free interval (DDFI), and overall survival (OS). LRI was defined as time from date of diagnosis to date of local recurrence (either an ipsilateral recurrence or ipsilateral new primary, whichever event occurred first after BCS or chest-wall recurrence after mastectomy). The local-recurrence event was counted as an event if the date of the nonevent (death from breast cancer, distant metastases, ipsilateral local axillary recurrence, ipsilateral regional nodes recurrence, and/or contralateral recurrence, if/where applicable) was >3 months after the date of the local-recurrence event. If the date of the nonevent was ≤3 months after the local recurrence event then the patient was censored at the date of nonevent. Deaths from other cancers after local recurrence did not affect the event. DFFI was defined as time from breast cancer diagnosis to distant metastases or death from breast cancer; deaths from other causes were censored at the time of death. OS was defined as time from breast cancer diagnosis to death from any cause.

Nelson-Aalen cumulative-hazard plots were used to describe LRI and Kaplan-Meier plots were used to describe DDFI and OS. Univariable analyses (UVA) and multivariable analyses (MVA) were carried out using Cox proportional-hazards models, or Flexible Parametric Survival Models (FPSMs) for models which involved time-varying hazards.[26] Covariates included in the MVA models included age at diagnosis (fitted as a continuous variable), tumor size, focality, nodal (N) stage, histological grade, ER and HER2 tumor status, adjuvant radiotherapy, adjuvant hormone therapy, and surgical margins, regardless of significance. Patients treated with neoadjuvant chemotherapy were included in UVA but excluded from all MVA because of difficulties in classifying pathological T and N staging for these patients. For each FPSMs, we explored varying degrees of freedom for the baseline-hazard rate and time-dependent effect to obtain the best model fit.

All analyses were performed using STATA v13.1 (StataCorp, College Station, TX, USA) on records with complete data (levels of missing data were reported).

RESULTS

Patient Characteristics and Definitive Surgery Information

The POSH study recruited 3095 patients across the United Kingdom, and of 2882 included in this analysis (Fig. 1), 1464 (50.8%) underwent mastectomy and 1395 (48.4%) BCS. All patients included underwent surgery to the axilla (axillary dissection, sentinel node biopsy, or sample ± axillary dissection). Twenty-three (0.8%) patients underwent lymph node surgery only, with no surgery to the breast. Table 1 shows baseline demographics by surgical type. Median age at diagnosis was 36 years for mastectomy and BCS. Family history of breast cancer was reported significantly more for mastectomy compared with BCS (52.1% vs 48.1%, $P = 0.037$), and surveillance-detected tumors were more frequent for mastectomy than BCS (1.5% vs 0.6%). However, no significant differences were observed between surgical type for BMI and ethnicity.

Tumor Pathology

Significant differences in grade and focality were found between mastectomy and BCS ($P = 0.005$ and $P < 0.001$, respectively). Patients undergoing mastectomy had larger tumors were more likely to be human epidermal growth factor receptor 2+ (HER2+) and with a higher proportion of Extensive Intraductal Component positive (EIC+) compared with BCS ($P < 0.001$ in all cases).

Patients undergoing mastectomy had a significantly higher proportion of ER+ and/or PR+ tumors than BCS (estrogen receptor, ER: 69.3% vs 62.8%, $P < 0.001$; progesterone receptor, PR: 59.3% vs 53.9%, $P = 0.009$, respectively).

Treatment and Surgery Information

Patients undergoing mastectomy had a higher frequency of negative margins compared with BCS ($P < 0.001$). Specifically, the proportion of margins >5 mm was shown to be higher (42.7% vs 24.0%, respectively), whereas the proportion of margins 1 to 5 mm was lower for BCS (39.6% vs 55.0%, respectively).

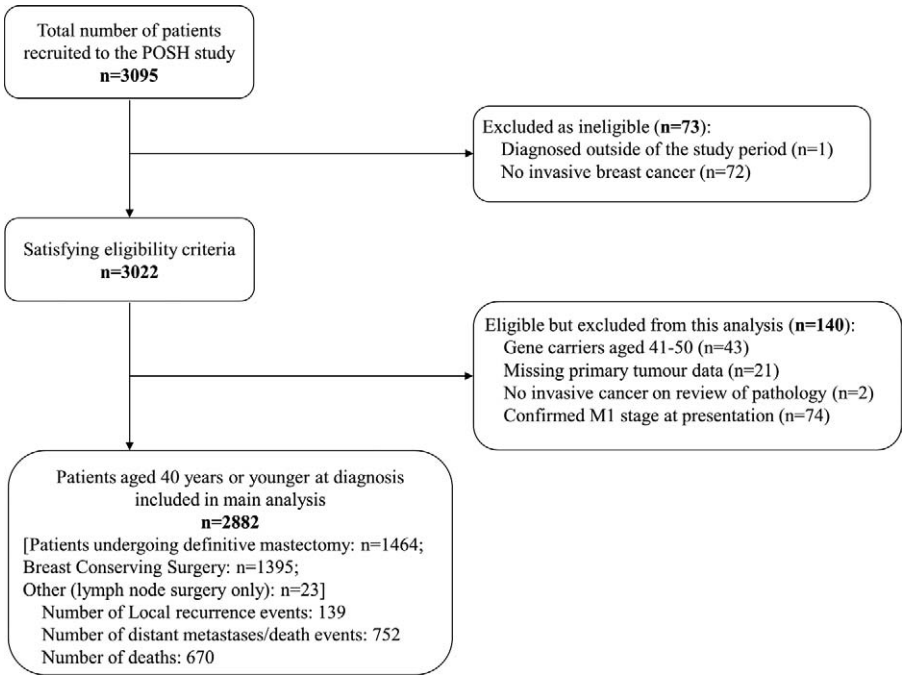

FIGURE 1. Flow chart for POSH study; local-recurrence analyses.

Although the median number of operations was one for mastectomy and BCS, the distribution was significantly different ($P < 0.001$), with a higher proportion of patients undergoing mastectomy having more than one surgery (28.5% vs 20.8%, $P < 0.001$). Only 11.1% of BCS patients underwent neoadjuvant chemotherapy compared with 18.8% for mastectomy. Adjuvant radiotherapy was given to 68.7% of patients undergoing a mastectomy. In 56 patients undergoing BCS, we were unable to confirm adjuvant breast radiotherapy. We cannot exclude that these patients had radiotherapy to the breast at a different center and that the information was not recorded, nor can we confirm that these patients did not receive adjuvant breast radiotherapy. However, these patients were analyzed as not having radiotherapy. Nine-hundred (61.5%) patients undergoing mastectomy had chest-wall radiotherapy (CWR-XRT) whereas 977 (70.0%) patients undergoing BCS had a radiotherapy boost. In patients undergoing BCS, no clear association was seen between margin status (>0/negative vs 0/positive) and provision of a radiotherapy boost (data not shown).

Missing data were similar across surgical types and low for most demographic information. Exceptions were PR, HER2, and surgical margin information, with up to 19.7%, 13.3%, and 24.5% missing, respectively.

Follow Up and Survival

Median follow up was 7.3 years for mastectomy, BCS, and overall. There were 139 local-recurrence events compared with 752 DDFI events overall, demonstrating that the majority of events experienced were because of distant metastases or death from breast cancer (Fig. 1). Ninety-five local-recurrence events were experienced for patients undergoing BCS (6.8% of these patients), compared with just 40 (2.7%) for mastectomy. Two-hundred and sixty DDFI events were experienced for BCS (18.6%), and 485 (33.1%) for mastectomy. Similar numbers were found for OS, with 232 (16.6%) and 431 (29.4%) for BCS and mastectomy, respectively. Figure 2A shows the Nelson-Aalen cumulative-hazard rates for LRI by surgical type. There was no significant difference in the estimated 18-month LRR between mastectomy and BCS (hazard ratio, HR: 1.43; 95% confidence interval, CI: 0.89–2.32; $P = 0.143$). However, patients undergoing BCS had a significantly higher LRR at 5 years (2.63% vs 5.33%; HR, 3.39; 95% CI, 2.03–5.66; $P < 0.001$) and at 10 years (4.93% vs 11.68%; HR, 5.27; 95% CI, 2.43–11.43; $P < 0.001$). The change in HR over time is illustrated in Fig. 2B, with the HR crossing one at 12 months. Similar results were found when excluding patients with a maximum overall (invasive + in situ) tumor diameter >30 mm (thus excluding patients with larger tumors who were likely to be treated with a mastectomy) (data not shown). MVA showed that patients undergoing BCS also had a significantly higher chance of a local recurrence at both 5 and 10 years, with only adjuvant radiotherapy significantly affecting the MVA (Table 2). When looking at LRI for mastectomy by CWR-XRT (Supplementary Figure 1A, http://links.lww.com/SLA/B64), patients without CWR-XRT had a significantly higher LRR compared with those with CWR-XRT (HR, 0.46; 95% CI, 0.24–0.86; $P = 0.015$). However, when assessing LRI for BCS patients by radiotherapy boost, no significant differences were found between patients with/without a boost (HR, 0.90; 95% CI, 0.58–1.38; $P = 0.614$) (Supplementary Figure 1B, http://links.lww.com/SLA/B64). There was also no difference for BCS for those with surgical margins of 0 mm versus >0 mm in terms of LRI (HR, 0.86; 95% CI, 0.41–1.78; $P = 0.680$) (Supplementary Figure 1C, http://links.lww.com/SLA/B64).

DDFI by surgical type showed that mastectomy patients had a significantly worse DDFI than BCS (HR, 0.51; 95% CI, 0.44–0.60; $P < 0.001$) (Fig. 3). However, in the MVA the difference was no longer significant (HR, 0.82; 95% CI 0.64–1.05; $P = 0.115$) (Supplementary Table 1, http://links.lww.com/SLA/B64). Factors affecting the MVA were maximum invasive tumor size, N stage, grade, and ER status. DDFI by patients experiencing versus not experiencing a local-recurrence event identified that DDFI was similar at 5 years but the hazards separated at 10 years (HR, 0.80; 95% CI 0.54–1.18; $P = 0.263$ and HR, 0.29; 95% CI 0.14–0.62; $P = 0.001$, respectively) (Supplementary Figure 2A, http://links.lww.com/SLA/B64). When assessing DDFI by BCS patients with surgical margins of 0 mm versus >0 mm, those with margins >0 mm had a significantly better DDFI compared with those with 0 mm

TABLE 1. Baseline Demographic Information for All Patients by Surgery Type

Characteristic	Mastectomy (n = 1464)	Breast Conserving Surgery (n = 1395)	Total* (n = 2882)	P†
Age at diagnosis, y				0.868
Median	36	36	36	
Range	18 to 40	19 to 40	18 to 40	
IQR	33 to 38	34 to 38	33 to 38	
Missing	0	0	0	
Body mass index				0.154
Median	24.5	24.8	24.6	
Range	16.5 to 59.5,	16.8 to 55.9,	16.5 to 59.5,	
IQR	22.0 to 28.1	22.1 to 28.4	22.1 to 28.4	
Missing	44 (3.0%)	64 (4.6%)	108 (3.7%)	
Race/ethnicity				0.436
White	1324 (91.9%)	1284 (93.2%)	2625 (92.4%)	
Black	63 (4.4%)	44 (3.2%)	112 (3.9%)	
Asian	43 (3.0%)	41 (3.0%)	84 (3.0%)	
Other	10 (0.7%)	9 (0.7%)	20 (0.7%)	
Missing	24 (1.6%)	17 (1.2%)	41 (1.4%)	
Family history				0.037
No	672 (47.9%)	690 (51.9%)	1378 (50.0%)	
Yes	731 (52.1%)	640 (48.1%)	1376 (50.0%)	
Missing	61 (4.2%)	65 (4.7%)	128 (4.4%)	
Presentation				<0.001
Symptomatic	1424 (97.7%)	1380 (99.4%)	2826 (98.5%)	
Screen detected	22 (1.5%)	8 (0.6%)	30 (1.0%)	
Other	12 (0.8%)	0	12 (0.4%)	
Missing	6 (0.4%)	7 (0.5%)	14 (0.5%)	
Histological grade				0.005
Grade 1	68 (4.8%)	93 (6.8%)	161 (5.7%)	
Grade 2	515 (36.2%)	429 (31.3%)	948 (33.7%)	
Grade 3	840 (59.0%)	848 (61.9%)	1703 (60.6%)	
Missing	41 (2.8%)	25 (1.8%)	70 (2.4%)	
Histological type				<0.001
Ductal	1246 (86.3%)	1230 (89.1%)	2494 (87.7%)	
Lobular	85 (5.9%)	44 (3.2%)	131 (4.6%)	
Ductal and lobular	50 (3.5%)	24 (1.7%)	74 (2.6%)	
Other	83 (5.7%)	97 (7.0%)	183 (6.4%)	
Missing	20 (1.4%)	15 (1.1%)	37 (1.3%)	
Surgical margin				<0.001
0	98 (8.9%)	113 (10.0%)	211 (9.4%)	
≥0 to <1	97 (8.8%)	126 (11.1%)	223 (10.0%)	
≥1 to ≤5	438 (39.6%)	624 (55.0%)	1062 (47.4%)	
>5	472 (42.7%)	272 (24.0%)	745 (33.2%)	
Missing	359 (24.5%)	260 (18.6%)	641 (22.2%)	
EIC‡				<0.001
Negative	1010 (72.8%)	1178 (86.8%)	2189 (79.7%)	
Positive	378 (27.2%)	179 (13.2%)	557 (20.3%)	
Missing	76 (5.2%)	38 (2.7%)	136 (4.7%)	
Lymphovascular invasion				<0.001
Absent	614 (45.1%)	784 (59.9%)	1402 (52.4%)	
Present	747 (54.9%)	524 (40.1%)	1276 (47.6%)	
Missing	103 (7.0%)	87 (6.2%)	204 (7.1%)	
Number of positive nodes				<0.001
0	549 (37.6%)	837 (60.5%)	1389 (48.7%)	
1–3	532 (36.5%)	404 (29.2%)	940 (33.0%)	
4–9	246 (16.9%)	99 (7.2%)	346 (12.1%)	
10+	132 (9.0%)	43 (3.1%)	175 (6.1%)	
Missing	5 (0.3%)	12 (0.9%)	32 (1.1%)	
ER status				<0.001
Negative	449 (30.7%)	516 (37.2%)	975 (34.0%)	
Positive	1013 (69.3%)	870 (62.8%)	1896 (66.0%)	
Missing	2 (0.1%)	9 (0.6%)	11 (0.4%)	
PR status				0.009
Negative	478 (40.7%)	519 (46.1%)	1008 (43.5%)	
Positive	697 (59.3%)	607 (53.9%)	1309 (56.5%)	
Missing	289 (19.7%)	269 (19.3%)	565 (19.6%)	

TABLE 1. (*Continued*)

Characteristic	Mastectomy (n = 1464)	Breast Conserving Surgery (n = 1395)	Total* (n = 2882)	P†
HER2 status				<0.001
Negative	906 (69.6%)	918 (75.9%)	1839 (72.7%)	
Positive	395 (30.4%)	292 (24.1%)	691 (27.3%)	
Missing	163 (11.1%)	185 (13.3%)	352 (12.2%)	
Focality				<0.001
Localised	707 (53.5%)	1136 (87.5%)	1845 (70.3%)	
Multifocal	615 (46.5%)	163 (12.5%)	779 (29.7%)	
Missing	142 (9.7%)	96 (6.9%)	258 (9.0%)	
Maximum invasive tumor size (mm)				<0.001
Median	28.5	19.0	22.0	
Range	0.0 to 199.0,	0.0 to 90.0,	0.0 to 199.0,	
IQR	19.0 to 43.0	14.0 to 25.0	15.0 to 33.0	
Missing	93 (6.4%)	45 (3.2%)	160 (5.6%)	
Maximum overall (invasive + in situ) tumor size, mm				<0.001
Median	37.0	20.5	27.0	
Range	0.0 to 199.0,	0.0 to 115.0,	0.0 to 199.0,	
IQR	25.0 to 55.0	15.0 to 27.0	18.0 to 40.0	
Missing	68 (4.6%)	38 (2.7%)	128 (4.4%)	
Number of operations, categorical				<0.001
1	1047 (71.5%)	1105 (79.2%)	2174 (75.4%)	
2	361 (24.7%)	279 (20.0%)	641 (22.2%)	
3	53 (3.6%)	11 (0.8%)	64 (2.2%)	
4	2 (0.1%)	0	2 (0.1%)	
5	1 (0.1%)	0	1 (0.0%)	
Missing	0	0	0	
Chemotherapy treatment period				<0.001
Adjuvant	1088 (74.3%)	1055 (75.6%)	2149 (74.6%)	
Neoadjuvant	275 (18.8%)	155 (11.1%)	447 (15.5%)	
Palliative	1 (0.1%)	0	1 (0.0%)	
Not applicable	100 (6.8%)	185 (13.3%)	285 (9.9%)	
Missing	0	0	0	
Adjuvant trastazumab				N/A
No/missing	1255 (85.7%)	1246 (89.3%)	2523 (87.5%)	
Yes	209 (14.3%)	149 (10.7%)	359 (12.5%)	
Missing	0	0	0	
Adjuvant radiotherapy				N/A
No/missing	458 (31.3%)	56 (4.0%)	525 (18.2%)	
Yes	1006 (68.7%)	1339 (96.0%)	2357 (81.8%)	
Missing	0	0	0	
Adjuvant hormone treatment				N/A
No/missing	490 (33.5%)	556 (39.9%)	1059 (36.7%)	
Yes	974 (66.5%)	839 (60.1%)	1823 (63.3%)	
Missing	0	0	0	

EIC indicates extensive intraductal component; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IQR, interquartile range; PR, progesterone receptor; U/H, underweight/healthy.

*Total column includes data from the whole cohort that is, BCS, mastectomy, and other (23 patients).

†*P*-value obtained using Pearson χ^2 test (for categorical variables) or Mann-Whitney test (for continuous variables).

‡EIC defined as positive where the total tumor in-situ size is ≥25% the size of the total tumor size (or where the total tumor invasive size is <75% the size of the total tumor size).

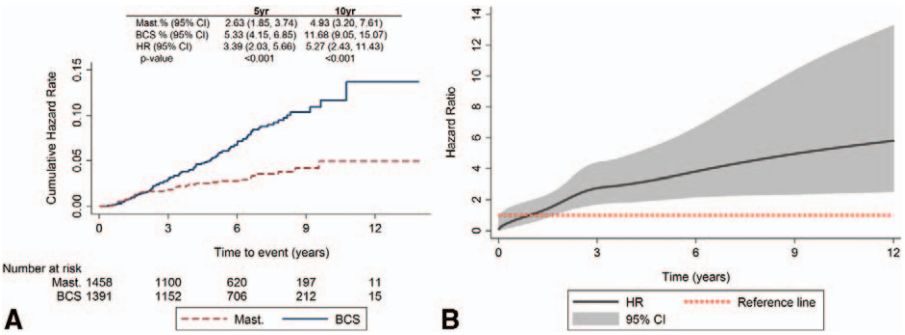

FIGURE 2. Local-recurrence interval for all patients by surgical type. A, Nelson-Aalen cumulative hazard plot. B, Flexible parametric survival model time-varying hazard over time.

TABLE 2. Local Recurrence Interval Flexible Parametric Survival Model Multivariable Analysis Results for all Patients (Excluding Those With Neoadjuvant Chemotherapy)

Covariate	HR*	95% CI	P
Surgical type at 5 years			
Mastectomy	1 (Ref. cat.)	—	—
BCS (unadjusted)	5.33	4.15 to 6.85	<0.001
BCS (adjusted)	5.00	3.57 to 23.69	<0.001
Surgical type at 10 years			
Mastectomy	1 (Ref. cat.)	—	—
BCS (unadjusted)	11.68	9.05 to 15.07	<0.001
BCS (adjusted)	6.06	1.29 to 28.40	0.022
Age at diagnosis, y, (continuous)	1.02	0.95 to 1.09	0.662
Maximum overall (invasive + in situ) tumor size, (mm) (continuous)	1.42	0.78 to 2.58	0.253
Focality			
Localized	1 (Ref. cat.)	—	—
Multifocal	1.15	0.58 to 2.30	0.688
N stage			
N0	1 (Ref. cat.)	—	—
N1	1.18	0.71 to 1.96	0.527
Histological grade			
1	1 (Ref. cat.)	—	—
2	1.63	0.38 to 7.02	0.514
3	1.42	0.33 to 6.16	0.636
ER status			
Negative	1 (Ref. cat.)	—	—
Positive	0.64	0.28 to 1.48	0.297
HER2 status			
Negative	1 (Ref. cat.)	—	—
Positive	1.33	0.77 to 2.30	0.306
Adjuvant radiotherapy			
No/unknown	1 (Ref. cat.)	—	—
Yes	0.32	0.16 to 0.64	0.001
Adjuvant hormone therapy			
No/unknown	1 (Ref. cat.)	—	—
Yes	0.64	0.28 to 1.47	0.295
Surgical margins, mm			
0	1 (Ref. cat.)	—	—
≥0 to <1	0.74	0.26 to 2.14	0.579
≥1 to ≤5	0.93	0.40 to 2.18	0.871
>5	0.89	0.36 to 2.22	0.803

CI indicates confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

*Unless otherwise stated, HR presented for the multivariable (adjusted) model.

margins (Supplementary Figure 2B, http://links.lww.com/SLA/B64). Similar results were also found in OS. UVA of OS by surgical type demonstrated that mastectomy patients had a significantly worse OS compared with BCS (HR, 0.53; 95% CI 0.45–0.62; $P < 0.001$) (Fig. 4), and in the MVA the difference was no longer significant (HR, 0.79; 95% CI 0.61–1.03; $P = 0.081$) (Supplementary Table 2, http://links.lww.com/SLA/B64). Excluding ER status, the same factors also affected the MVA for OS. Moreover, when looking at OS by local-recurrence event and by surgical margins, the findings matched those of the DDFI analyses (Supplementary Figure 3A and 3B, http://links.lww.com/SLA/B64).

DISCUSSION

Previous findings from the POSH study reported on the effects of ethnicity and obesity, both of which have affected outcome including DDFI in this young age group,[27,28] and family history,

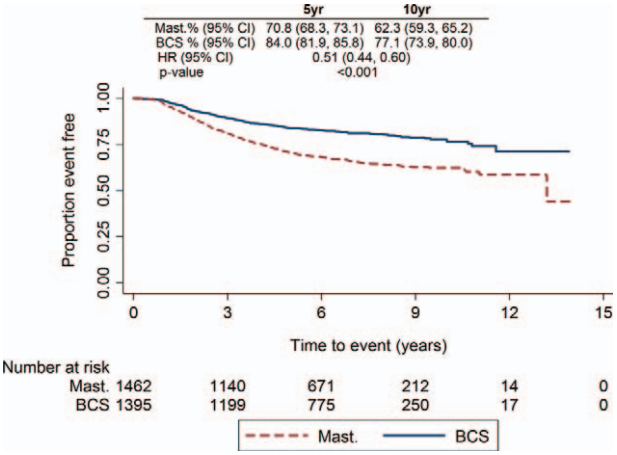

FIGURE 3. Distant disease free interval Kaplan-Meier plot for all patients by surgical type.

which has not.[17] This study has investigated the effect of surgery on LRI, DDFI, and OS, and the effect of local recurrence on DDFI and OS in young women with breast cancer.

A large number of studies use inconsistent definitions of local-recurrence, often not specifying which events have been included in the local-recurrence definition.[29] This study has therefore clearly described the definition of local recurrence in the methods with criteria outlining which events were included/excluded depending on the time of competing events. This study also incorporated the use of FPSMs to assess the time-varying effect of surgical type on LRI.

Previous findings from a meta-analysis of RCTs conducted by the EBCTCG[18] presented first recurrence rates (local and distant) which appeared much higher after BCS in younger women; 36.1% for women <40 years undergoing BCS with radiotherapy. However, the number analyzed was relatively small (n = 363) and there was no breakdown in the meta-analysis of local versus distant recurrences. In this study, the majority of events were found to be distant and not local (139 LRI vs 752 DDFI events) which demonstrates the predominant risk in these young patients is of distant and not local recurrence.

Although UVA demonstrated worse DDFI and OS for mastectomy compared with BCS, this is almost certainly because of

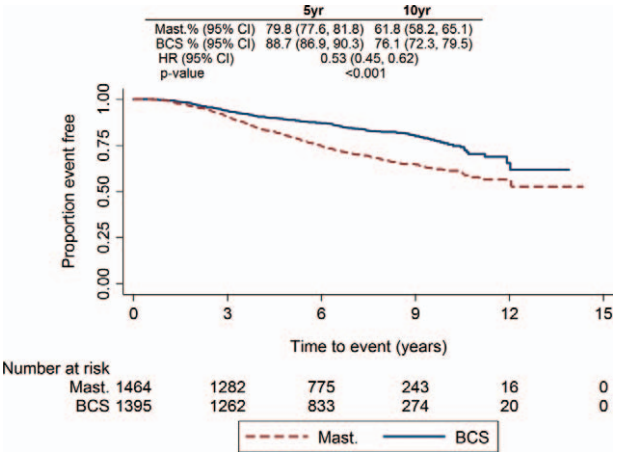

FIGURE 4. Overall survival Kaplan-Meier plot for all patients by surgical type.

imbalances in prognostic features between the groups. Patients undergoing mastectomy had significantly larger tumors than BCS, with a higher proportion of tumors EIC+, and ER+, PR+, and/or HER2+. Unsurprisingly, tumors of maximum invasive tumor size >30 mm, N1 stage, and grade 3 were significant factors in both DDFI and OS MVA, the differences between surgical type for DDFI and OS were no longer significant after correction for these factors. Interestingly, maximum overall (invasive + in situ) tumor size >30 mm was not a significant factor in either OS or DDFI MVA models, indicating that whereas overall tumor size influences surgical decision making, invasive tumor size is the relevant size parameter predicting DDFI and OS. A sensitivity analysis excluding patients with a maximum overall tumor size >30 mm showed similar results in a UVA of LRI comparing surgical type.

The results of this study support existing literature in regards to both OS and DDFI by surgical type, with no evidence of surgical type affecting survival or distant-disease in this age group. This is consistent with RCTs comparing mastectomy versus BCS in the breast cancer population as a whole[4–6,11] indicating that surgical choices for younger women can use accepted criteria without impacting outcome.

The results of this study also demonstrated similar LRR in the first 18 months for mastectomy and BCS, but a larger disparity is seen at 5 and 10 years, with significantly higher LRR for BCS. The clinical implication is that, at least initially, for local recurrence there is no disadvantage in treating young women surgically with BCS in general and no evidence that BCS leads to a disadvantage in DDFI or OS. It is not yet possible to comment beyond 10 years at this stage for this cohort. However, other studies suggest LRR continue to rise beyond 10 years after BCS.[19,30]

An interesting finding from this study is the effect of margin on outcomes. Although no effect was seen for LRI, differences were observed for both OS and DDFI; positive margins were associated with significantly worse OS and DDFI. This could be because of reduced power of LRI outcome because of a fewer local-recurrence events and missing data for margin status, or possibly because of patients with a positive margin being more likely to present with a distant relapse, or combination of distant disease and local recurrence, which would, as defined here, be considered a distant event, as these would not be surgically salvageable, isolated local recurrences. In this analysis, a positive margin was defined according to ASCO guidance as tumor at the margin.[24] Although 24.5% of margin status information was missing, sensitivity analyses of MVA models using multiple imputation were carried out and showed very similar results to the complete-case analyses. The finding that surgical margins are a factor in the development of distant disease would support the concept of the importance of surgical quality with attention to margins, with re-excision where appropriate. Taken together with the lack of evidence here that oncological surgical type influences distant-relapse it could be argued that completeness of excision is more important than the extent of surgery.

In regards to radiotherapy, patients treated with BCS who were not documented to have adjuvant radiotherapy unsurprisingly had higher LRR, implying that the data were correct (rather than data missing because of patients having radiotherapy elsewhere), and highlighting the importance of radiotherapy as part of breast conservation. Although no effect of radiotherapy boost was shown for patients undergoing BCS, it must be noted that this is not an RCT. Interestingly, provision for radiotherapy boost was not shown to be statistically correlated with margin status; however, the clinical implication we would draw is that, at least in this study, provision of a radiotherapy boost appears to be less important than attention to detail to surgical margins in terms of its effect on LRI and DDFI.

More than 60% of patients undergoing mastectomy received CWR-XRT and a clear association of benefit of CWR-XRT on LRI has been demonstrated here, despite potential confounding. Given results of the most recent Oxford overview[31] it is likely that thresholds for CWR-XRT after mastectomy are likely to fall further. Given that the majority of young patients are likely to receive radiotherapy, even if surgically treated with mastectomy, there are likely to be implications for reconstructive decision making.

These findings also support the message that avoiding local recurrence is important as increased local recurrence is associated with poorer DDFI and OS.[30,32,33] Our data suggest that valid strategies to reduce local recurrence might include avoidance of a positive margin after BCS and provision of CWR-XRT after mastectomy where indicated, but do not support mastectomy over BCS where both options are available.

In this analysis, the frequency of local recurrence is much lower than that of distant relapse indicating that the main hazard experienced by these patients, at least within the first 10 years, is of distant rather than local recurrence. We have not demonstrated an impact of tumor stage or biological type on local recurrence in this analysis, possibly because of reduced power because of a lower LRR. In addition to young age, factors recognized to influence local recurrence after BCS and mastectomy include axillary nodal status, margin status, and lack of systemic therapy.[34] When considering molecular subtype a greater proportion of young women appear to have luminal B tumors[2]; however, young age remains predictive of LRR independent of molecular subtype,[35] although there is a suggestion that molecular subtype may affect local recurrence.[36] Younger patients with breast cancer are also more likely to carry a germline BRCA-mutation and it is currently unknown whether this influences LRI or DDFI, although clearly it does increase second new primary breast tumors; and contralateral new primary events were not included in this analysis of local recurrence. Once final genotyping in this cohort has been completed, further analyses will also be performed by BRCA status to see if this has an effect.

Regardless of this, the current Association of Breast Surgery guidelines state that the target local-recurrence rate after surgery should be <3% and not >5% at 5 years.[37] This study has demonstrated that LRI in younger patients treated by mastectomy would fulfill this criterion (HR, 2.63; 95% CI 1.85–3.74), and that the lower 95% LRI limit for younger women undergoing BCS is within this range (HR, 5.33; 95% CI 4.15–6.85).[37] Furthermore, our findings are consistent with recommendations for breast surgery within recent consensus guidelines for the management of young women with breast cancer.[38]

A limitation of this study is that as this was not an RCT, any differences/lack of differences in LRI, OS, and/or DDFI were because of the surgical type alone could be the result of confounding. However, we have accounted as far as possible for biases and this is a large prospective cohort representative of cancer treatment in this age group in the United Kingdom.[23] It should be noted this analysis was performed according to a prespecified plan and LRI was clearly defined to address the inconsistency of reporting in a number of previous studies.[29]

In conclusion, there are no survival advantages for surgical type after adjusting for known prognostic factors. There is no difference in LRI between BCS and mastectomy in young women with breast cancer in the short-term but, beyond 18 months, LRR are higher after BCS. Local recurrence is associated with increased risk of distant relapse, and in patients undergoing BCS, a positive surgical margin increases the likelihood of a distant relapse. For those undergoing mastectomy, CWR-XRT reduces the likelihood of distant relapse. Surgical extent therefore appears less important for DDFI than completeness of excision or, where appropriate, CWR-XRT.

Future work will assess the impact of germline genotype on LRI, distant relapse, and OS.

ACKNOWLEDGMENTS

The authors would like to thank all the patients who participated in this study and the POSH collaborators including the POSH Steering group: Professor Diana Eccles, Dr. Peter Simmonds, Professor Douglas G. Altman, Professor Paul Pharoah, Professor Louise Jones, Professor Ros Eeles, Professor D. Gareth Evans, Professor Andrew Hanby, Professor Alistair M. Thompson, Professor Shirley Hodgson, Mr Hisham Hamed, Dr Fiona Gilbert, and Professor Sunil Lakhani. Participating principal investigators are listed on the study website: http://www.southampton.ac.uk/medicine/research/posh.-page. We thank the NIHR NCRN for supporting patient recruitment, and all patients participating in the POSH study.

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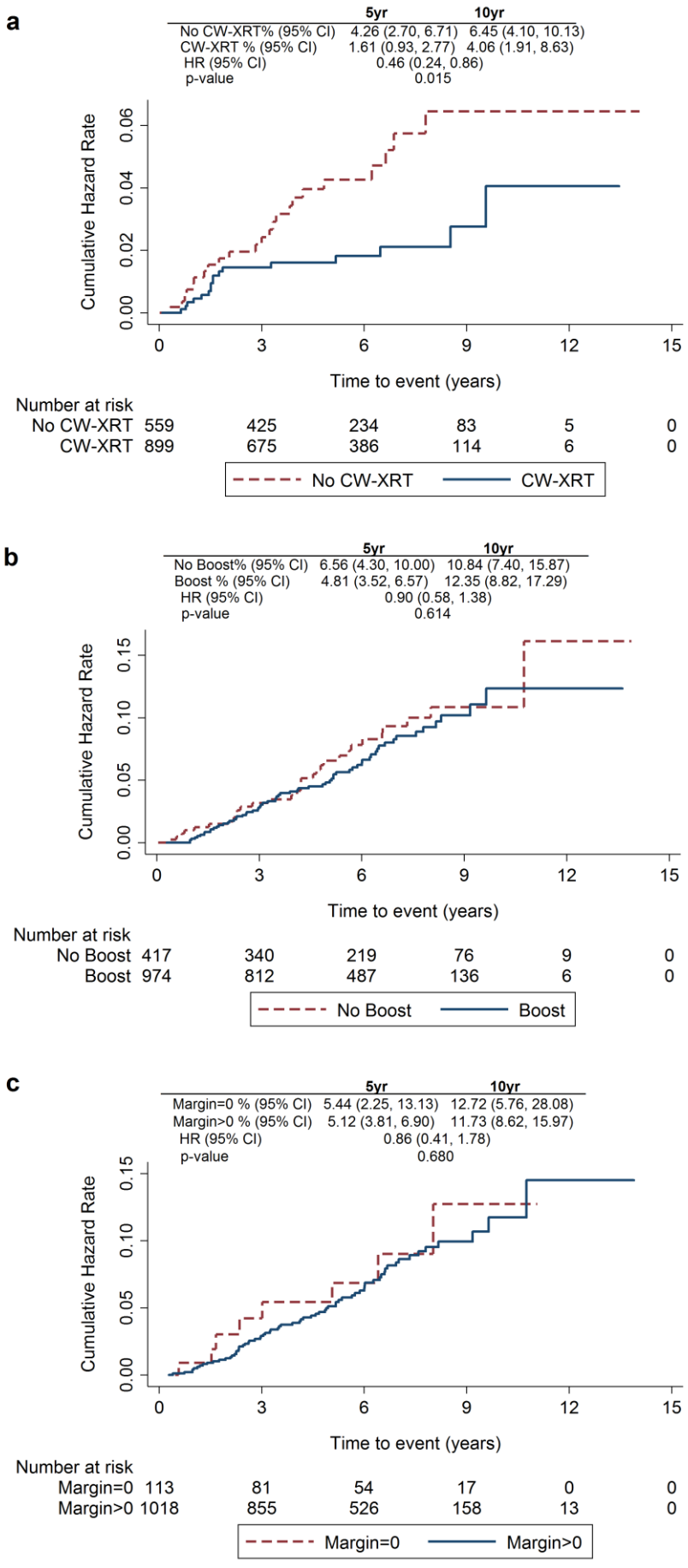
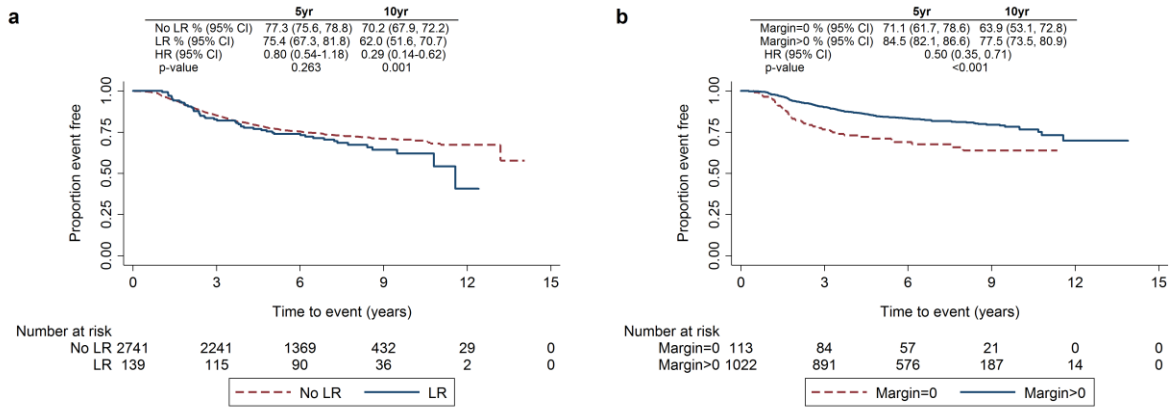
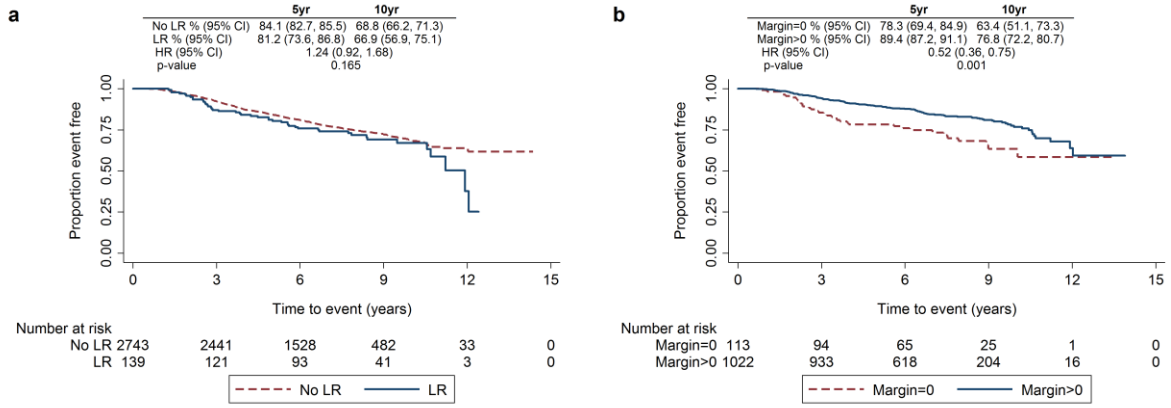
SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE 1. Local-recurrence interval Nelson-Aalen cumulative hazard plot for: a all patients undergoing mastectomy by chest-wall radiotherapy; b all patients undergoing breast conserving surgery by radiotherapy boost; and c all patients undergoing breast conserving surgery by surgical margin.



SUPPLEMENTARY FIGURE 2. Distant disease free interval Kaplan-Meier plot for: a all patients by Local-recurrence event; and b all patients undergoing breast conserving surgery by surgical margin.



SUPPLEMENTARY FIGURE 3. Overall survival Kaplan-Meier plot for: a all patients by Local-recurrence event; and b all patients undergoing breast conserving surgery by surgical margin.

SUPPLEMENTARY TABLE 1. Distant disease free interval Cox proportional hazards model multivariable analysis results for all patients (excluding those with neoadjuvant chemotherapy), stratified by adjuvant hormone therapy and surgical margin

Covariate	HR†	95% CI	p-value
Surgical type			
Mastectomy	1 (Ref. cat.)	-	-
BCS (unadjusted)	0.51	0.44 to 0.60	<0.001
BCS (adjusted)	0.82	0.64 to 1.05	0.115
Age at diagnosis, in years (continuous)	0.98	0.95 to 1.01	0.139
Maximum overall (invasive+*in situ*) tumour size			
≤30mm	1 (Ref. cat.)	-	-
>30mm	0.96	0.66 to 1.39	0.811
Maximum invasive tumour size			
≤30mm	1 (Ref. cat.)	-	-
>30mm	1.79	1.25 to 2.57	0.001
Focality			
Localised	1 (Ref. cat.)	-	-
Multifocal	0.99	0.79 to 1.26	0.963
N stage			
N0	1 (Ref. cat.)	-	-
N1	2.33	1.85 to 2.95	<0.001
Histological Grade			
1	1 (Ref. cat.)	-	-
2	2.07	0.96 to 4.45	0.064
3	2.69	1.25 to 5.76	0.011
ER Status			
Negative	1 (Ref. cat.)	-	-
Positive	1.42	1.01 to 2.00	0.042
HER2 Status			
Negative	1 (Ref. cat.)	-	-
Positive	1.19	0.96 to 1.48	0.114
Adjuvant radiotherapy			
No/unknown	1 (Ref. cat.)	-	-
Yes	0.86	0.63 to 1.17	0.335

HR=Hazard Ratio, CI=Confidence Interval, ER=Oestrogen Receptor, HER2=Human Epidermal growth factor Receptor 2.
† Unless otherwise stated, HR presented for the multivariable (adjusted) model.

SUPPLEMENTARY TABLE 2. Overall survival Cox proportional hazards model multivariable analysis results for all patients (excluding those with neoadjuvant chemotherapy), stratified by adjuvant hormone therapy and surgical margin

Covariate	HR†	95% CI	p-value
Surgical type			
Mastectomy	1 (Ref. cat.)	-	-
BCS (unadjusted)	0.53	0.45 to 0.62	<0.001
BCS (adjusted)	0.79	0.61 to 1.03	0.081
Age at diagnosis, in years (continuous)	0.98	0.95 to 1.01	0.147
Maximum overall (invasive+*in situ*) tumour size			
≤30mm	1 (Ref. cat.)	-	-
>30mm	0.94	0.63 to 1.39	0.740
Maximum invasive tumour size			
≤30mm	1 (Ref. cat.)	-	-
>30mm	1.66	1.14 to 2.43	0.009
Focality			
Localised	1 (Ref. cat.)	-	-
Multifocal	0.97	0.75 to 1.24	0.783
N stage			
N0	1 (Ref. cat.)	-	-
N1	2.45	1.91 to 3.15	<0.001
Histological Grade			
1	1 (Ref. cat.)	-	-
2	2.88	1.05 to 7.86	0.039
3	4.17	1.54 to 11.34	0.005
ER Status			
Negative	1 (Ref. cat.)	-	-
Positive	1.37	0.96 to 1.95	0.085
HER2 Status			
Negative	1 (Ref. cat.)	-	-
Positive	1.10	0.88 to 1.38	0.405
Adjuvant radiotherapy			
No/unknown	1 (Ref. cat.)	-	-
Yes	1.04	0.74 to 1.45	0.839

HR=Hazard Ratio, CI=Confidence Interval, ER=Oestrogen Receptor, HER2=Human Epidermal growth factor Receptor 2.

† Unless otherwise stated, HR presented for the multivariable (adjusted) model.

Chapter 9: Paper 7 - An evaluation of the prognostic model PREDICT using the POSH cohort of women aged ≤40 years at breast cancer diagnosis

Authors	**Maishman T**, Copson E, Stanton L, Gerty S, Dicks E, Durcan L, Wishart GC, Pharoah P; POSH Steering Group, Eccles D.
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Citations to date	9

9.1 Contribution

As the lead statistical methodologist, scientific lead at the SCTU, and first and corresponding author:

- Responsible for the choice of study methodology and research of all statistical methods to be implemented in the analyses, including the use of model calibration and discrimination methods;
- Performed the literature review;
- Responsible for data ascertainment, monitoring and data cleaning – liaising with both the POSH study team and PREDICT team to identify data queries, and for the resolution of all data queries raised with data management;
- Developed and conducted all statistical programming and analyses – including the creation of all database and analysis programming required for the analyses;
- Designed, developed and produced all manuscript figures and tables – including the development of the calibration plots and AUCs, the transcription of all results, the creation of all draft and final versions of the manuscript figures and tables;
- Drafted and reviewed all versions of the manuscript – listed as the 1st named author, drafted all versions of the manuscript, and involved in the resolution of reviewer comments and responses;
- Responsible for manuscript submission and administrative correspondence.

Professor Diana Eccles, chief investigator of the POSH study, chair of the POSH Study Steering Group, and senior last author of **Paper 7**, confirms the accuracy of contribution outlined above of Thomas Christopher Maishman.

Signed: .....

Date: .....

FULL PAPER

BJC

British Journal of Cancer (2015), 1–9 | doi: 10.1038/bjc.2015.57

Keywords: breast cancer; prognostic model; young onset; HER2

An evaluation of the prognostic model PREDICT using the POSH cohort of women aged ⩽40 years at breast cancer diagnosis

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Background: Breast cancer is the most common cancer in younger women (aged ⩽40 years) in the United Kingdom. PREDICT (http://www.predict.nhs.uk) is an online prognostic tool developed to help determine the best available treatment and outcome for early breast cancer. This study was conducted to establish how well PREDICT performs in estimating survival in a large cohort of younger women recruited to the UK POSH study.

Methods: The POSH cohort includes data from 3000 women aged ⩽40 years at breast cancer diagnosis. Study end points were overall and breast cancer-specific survival at 5, 8, and 10 years. Evaluation of PREDICT included model discrimination and comparison of the number of predicted versus observed events.

Results: PREDICT provided accurate long-term (8- and 10-year) survival estimates for younger women. Five-year estimates were less accurate, with the tool overestimating survival by 25% overall, and by 56% for patients with oestrogen receptor (ER)-positive tumours. PREDICT underestimated survival at 5 years among patients with ER-negative tumours.

Conclusions: PREDICT is a useful tool for providing reliable long-term (10-year) survival estimates for younger patients. However, for more accurate short-term estimates, the model requires further calibration using more data from young onset cases. Short-term prediction may be most relevant for the increasing number of women considering risk-reducing bilateral mastectomy.

Breast cancer is the most common cancer in women in the United Kingdom, with around 50 000 newly diagnosed cases each year (Cancer Research UK, 2014). Approximately 4% of cases are in younger women (aged ⩽40 years at diagnosis) yet it remains the most frequent malignancy in women of this age group (Cancer Research UK, 2014).

Determining the long-term outcome and potential benefits from systemic adjuvant treatments for early-stage breast cancer has been improved through the use of a number of currently available predictive tools, including the Nottingham Prognostic Index (NPI), Adjuvant! and PREDICT. These tools have become increasingly sophisticated; incorporating a growing number of prognostic factors, enabling a move from categorising patients into broad prognostic groups to providing survival estimates at a patient level. However, many existing prognostic tools have only been validated in a small number of younger women, in which an overestimation of up to 30% in overall survival (OS) is reported in younger women (Engelhardt *et al*, 2014).

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[4]See Appendix

revised 10 December 2014; accepted 18 January 2015

MATERIALS AND METHODS

Existing tools. The original NPI was based on 387 patients treated in a single institution and used tumour size, grade, and lymph node status to provide a prognostic index (Haybittle *et al*, 1982). The tool was later validated (Todd *et al*, 1987; D'Eredita *et al*, 2001) and updated to provide survival estimates by NPI group (Blamey *et al*, 2007a), and at a patient level (Blamey *et al*, 2007b).

Adjuvant! is an online decision aid tool, which was developed in 2001, based on 34 252 women aged 36–69 years from multiple institutions in the United States, and incorporated the patient's age, tumour size and grade, oestrogen receptor (ER) status, and number of positive nodes, to provide individual 10-year outcomes (Ravdin *et al*, 2001). The tool was later validated in patients from Canada (Olivotto *et al*, 2005) and the United Kingdom (Campbell *et al*, 2009). Adjuvant! makes an adjustment for women under 35 years, based on data from 415 patients; however, this applies only to patients with ER-positive tumours (Aebi *et al*, 2000).

The online breast cancer prognostic and treatment benefit tool, PREDICT, is used to estimate survival for individual patients based on known pathological prognostic factors, including age at diagnosis, mode of detection, tumour grade, tumour size, ER and nodal status, and type of therapy. The tool was developed in the United Kingdom, based on 5694 women aged 23–95 years (401 aged $\leqslant 40$ years), from multiple institutions in East Anglia, and validated in two further cohorts: 5468 women aged 22–93 years from the West Midlands Cancer Intelligence Unit (Wishart *et al*, 2010); and the same cohort used for the validation of Adjuvant! (Wishart *et al*, 2011).

An updated version of PREDICT (http://www.predict.nhs.uk) now incorporates human epidermal growth factor receptor 2 (HER2) status (Wishart *et al*, 2012). The updated tool was validated using 1653 patients with information on HER2 from the same cohort of 3140 women from Canada used in the previous validations of PREDICT and Adjuvant!, and was shown to provide accurate OS and breast cancer-specific survival (BCSS) estimates at 10 years. The results demonstrated that the incorporation of HER2 status improved the tool, with predicted overall and breast cancer-specific deaths within 8.4% and 2.5% of those observed ($P = 0.05$ and $P = 0.60$, respectively). However, the results indicated that PREDICT underestimated the number of deaths in women aged 20–35 years by 32% for both OS and BCSS (Wishart *et al*, 2012).

Although PREDICT was successfully validated in a large cohort, only 159 (9.6%) of these women from Canada were aged $\leqslant 40$ years at diagnosis. However, young age at diagnosis is associated with lower survival rates compared with older patients (El Saghir *et al*, 2006; Adami *et al*, 1986). The reasons for the differing effects remain unclear; studies have shown that lower survival rates coupled with higher relapse rates in younger patients are independent of other known prognostic factors (de la Rochefordiere *et al*, 1993; Nixon *et al*, 1994). Younger patients may receive less significant survival benefits from hormonal therapy after adjuvant chemotherapy than older patients (Ahn *et al*, 2007). Furthermore, studies of premenopausal and perimenopausal women with breast cancer have found that chemotherapy alone was insufficient for younger patients, and perhaps tailored treatments should be investigated (Aebi *et al*, 2000; Colleoni *et al*, 2006).

The aim of this study was to investigate the performance of the updated PREDICT tool in a large cohort of women aged $\leqslant 40$ years at breast cancer diagnosis in terms of model calibration and discrimination for both OS and BCSS.

Study population. POSH is a multicentre prospective observational cohort study of 3000 young women diagnosed with breast cancer in the United Kingdom between 2000 and 2008 (http://www.southampton.ac.uk/medicine/research/posh.page). The detailed study protocol was published in 2007 (Eccles *et al*, 2007), and the cohort has previously been described (Copson *et al*, 2013a).

Information obtained in the POSH cohort included age at diagnosis, ethnicity (Caucasian/white, black, Asian, other, missing/unknown), menopausal status (pre-, peri-, and post-menopausal, or missing/unknown), family history of breast cancer, ER, progesterone receptor, and HER2 status, histology, histological grade, tumour size, number of positive lymph nodes, lymphovascular invasion status, focality (localised, multifocal, missing/unknown), presentation, gene status (BRCA1, BRCA2, other, not tested/missing/unknown), and type of adjuvant therapy. This article presents analyses conducted on follow-up data from the POSH cohort received until 22 October 2013.

Study end points were OS and BCSS at 5, 8, and 10 years, where OS is defined as time from breast cancer diagnosis to death from any cause, and BCSS as time to death from breast cancer (deaths from other causes were censored at the time of last follow-up). The cohort includes data from 2827, 1843, and 597 patients with key prognostic information needed for the analyses for OS at 5, 8, and 10 years, respectively. Cause of death was missing for 5 patients leaving 2822, 1841, and 595 patients for analyses for BCSS, respectively.

Predicted OS and BCSS were calculated for each patient using PREDICT by investigators blinded to actual patient outcomes. Age at diagnosis, tumour size, and number of positive lymph nodes were entered as continuous variables. Categorical variables were used for presentation (screen-detected, symptomatic, or unknown), histological grade (1, 2, 3, or unknown), ER status (negative (ER−) or positive (ER+)), HER2 (negative (HER2−), positive (HER2+), or unknown), and chemotherapy regimen (second generation or third generation).

Model calibration and discrimination. Using a similar approach to the methods applied in previous validations of PREDICT (Wishart *et al*, 2011, 2012), predicted OS and BCSS were compared with the corresponding observed OS and BCSS at 5, 8, and 10 years. Model calibration, a comparison between the predicted and observed mortality, was evaluated for the complete data set, by quartiles of the predicted mortality and also within strata (Wishart *et al*, 2012). As evidence suggests that existing prognostic tools do not perform well in Asian patients (Engelhardt *et al*, 2014), and black ethnicity was found to be an independent risk factor for reduced survival in younger women (Copson *et al*, 2013b), model calibration was also evaluated across ethnic groups. Goodness-of-fit tests were performed using χ^2 tests based on the number of predicted and observed events (4 d.f.). Model discrimination was assessed by calculating the area under the receiver-operator characteristic curve (AUC) and corresponding 95% confidence intervals for 5-, 8-, and 10-year predicted all-cause mortality and breast cancer-specific mortality. The AUC is the probability that the predicted mortality of a randomly selected patient who died is higher than that of a randomly selected patient who survived; the higher the AUC, the better the model is at identifying patients with a worse survival (Wishart *et al*, 2012).

All analyses were performed using STATA v12.1 (StataCorp LP, College Station, TX, USA).

RESULTS

Model calibration. The demographic, tumour, and treatment information at baseline together with the predicted and observed all-cause OS at 5, 8, and 10 years are shown in Table 1. Overall, PREDICT did not perform well at 5 years, with an underestimation of the total number of deaths by 25% (455 *vs* 607, $P < 0.001$), and within most subgroups; most notably in patients with grade 2 tumours (58%; 67 *vs* 161, $P < 0.001$), 0–10 mm tumours (52%; 20 *vs* 42, $P = 0.001$), ER+ tumours (56%; 158 *vs* 362, $P < 0.001$), and

Table 1. Observed and predicted 5-, 8-, and 10-year all-cause mortality by demographical, tumour ,and treatment characteristics

	5 Years					8 Years					10 Years				
Characteristic	**N**	**O**	**P**	**D**	**%**	**N**	**O**	**P**	**D**	**%**	**N**	**O**	**P**	**D**	**%**
Total	2827	607	455	−152	−25	1843	454	430	−24	−5.3	597	152	164	12	7.9
Age at diagnosis (years)															
18–25	40	7	5	−2	−28.6	21	4	4	0	0	8	1	1	0	0
26–30	258	62	46	−16	−25.8	167	48	43	−5	−10.4	55	15	16	1	6.7
31–35	864	210	152	−58	−27.6	580	168	146	−22	−13.1	203	60	57	−3	−5
36–40	1665	328	252	−76	−23.2	1075	234	237	3	1.3	331	76	90	14	18.4
Menopausal status															
Premenopause	2771	591	444	−147	−24.9	1801	440	420	−20	−4.5	581	148	162	14	9.5
Post-menopause	7	2	1	−1	−50	4	2	1	−1	−50	1	1	0	−1	−100
Perimenopause	5	2	1	−1	−50	5	2	2	0	0	3	1	1	0	0
Unknown	44	12	9	−3	−25	33	10	8	−2	−20	12	2	2	0	0
Morphology															
Ductal	2447	531	410	−121	−22.8	1606	396	388	−8	−2	520	132	149	17	12.9
Lobular	129	27	10	−17	−63	79	21	12	−9	−42.9	30	8	4	−4	−50
Other	216	42	31	−11	−26.2	133	32	27	−5	−15.6	40	12	10	−2	−16.7
Unknown	35	7	4	−3	−42.9	25	5	4	−1	−20	7	0	1	1	.
Grade															
1	156	5	4	−1	−20	98	5	4	−1	−20	31	3	2	−1	−33
2	929	161	67	−94	−58.4	614	121	75	−46	−38	200	44	31	−13	−30
3	1676	429	378	−51	−11.9	1097	322	346	24	7.5	351	103	129	26	25.2
Not graded/missing	66	12	7	−5	−41.7	34	6	5	−1	−16.7	15	2	2	0	0
LV invasion															
Negative	1374	182	156	−26	−14.3	873	142	144	2	1.4	290	47	59	12	25.5
Positive	1253	385	272	−113	−29.4	845	287	264	−23	−8	270	96	99	3	3.1
Unknown	200	40	27	−13	−32.5	125	25	22	−3	−12	37	9	7	−2	−22
Node status															
Negative	1370	161	128	−33	−20.5	869	111	115	4	3.6	266	39	42	3	7.7
Positive	1431	439	324	−115	−26.2	959	339	312	−27	−8	327	112	121	9	8
Unknown	26	7	4	−3	−42.9	15	4	3	−1	−25	4	1	1	0	0
Tumour size															
0–10	265	42	20	−22	−52.4	161	29	17	−12	−41.4	48	14	7	−7	−50
11–20	930	125	100	−25	−20	629	99	99	0	0	221	41	45	4	9.8
21–50	1229	302	233	−69	−22.8	798	228	219	−9	−3.9	244	78	79	1	1.3
50+	244	99	85	−14	−14.1	171	75	83	8	10.7	54	13	28	15	115.4
Unknown	159	39	16	−23	−59	84	23	12	−11	−47.8	30	6	5	−1	−16.7
ER status															
Negative	965	245	297	52	21.2	642	183	242	59	32.2	231	64	94	30	46.9
Positive	1862	362	158	−204	−56.4	1201	271	188	−83	−30.6	366	88	70	−18	−20.5
Local Rx															
BCS + RT	1310	188	168	−20	−10.6	872	146	159	13	8.9	275	59	59	0	0
Mast + RT	1001	313	228	−85	−27.2	648	231	213	−18	−7.8	208	70	85	15	21.4
Mast alone	445	87	48	−39	−44.8	277	65	47	−18	−27.7	101	22	18	−4	−18.2
Other	71	19	12	−7	−36.8	46	12	10	−2	−16.7	13	1	3	2	200
Systemic Rx															
None	46	4	5	1	25	28	3	5	2	66.7	6	1	1	0	0
Hormone	227	16	6	−10	−62.5	157	15	8	−7	−46.7	50	6	3	−3	−50
Chemo	986	261	297	36	13.8	659	198	248	50	25.3	237	71	94	23	32.4
Both	1568	326	147	−179	−54.9	999	238	170	−68	−28.6	304	74	66	−8	−10.8
HER2 status															
Negative	1773	383	255	−128	−33.4	1059	273	234	−39	−14.3	327	82	83	1	1.2
Positive	679	183	159	−24	−13.1	434	141	141	0	0	140	50	53	3	6
Borderline	40	10	6	−4	−40	33	10	7	−3	−30	14	5	4	−1	−20
Unknown	335	31	35	4	12.9	317	30	49	19	63.3	116	15	24	9	60
Ethnicity															
Caucasian/White	2582	547	413	−134	−24.5	1692	411	392	−19	−4.6	557	144	156	12	8.3
Black	110	36	21	−15	−41.7	74	27	20	−7	−25.9	17	5	4	−1	−20
Asian	84	15	14	−1	−6.7	55	11	13	2	18.2	16	3	3	0	0
Other	19	3	3	0	0	12	3	3	0	0	3	0	0	0	.
Unknown	32	6	5	−1	−16.7	10	2	2	0	0	4	0	1	1	.

Abbreviations: BCS = breast-conserving surgery; D = difference; ER = oestrogen receptor status; HER2 = human epidermal growth factor receptor 2; LV=lymphovascular invasion; N = number of patients; O = number of observed events; P = number of predicted events; RT = radiotherapy; Rx = Treatment.

patients receiving both hormone and chemotherapy (55%; 147 *vs* 326, $P<0.001$). Conversely, PREDICT overestimated the number of deaths by 21% (297 *vs* 245, $P=0.0009$) in patients with ER− tumours and patients receiving adjuvant trastuzumab (data not shown) by 25% (86 *vs* 69, $P=0.041$). Across HER2 and ethnicity subgroups, PREDICT was found to underestimate all-cause mortality in HER2− tumours, Caucasian/white and Black ethnicity (33%; 255 *vs* 383, $P<0.001$, 25%; 413 *vs* 547, $P<0.001$, and 42%; 21 *vs* 36, $P=0.012$ respectively), and slightly underestimated for HER2+ and borderline tumours. In addition, when looking at chemotherapy regimen (data not shown) the tool was found to underestimate the number of deaths by 28% (297 *vs* 410, $P<0.001$) and by 18% (146 *vs* 177, $P=0.020$) for patients receiving second- and third-generation chemotherapy, respectively. Despite a poor performance across most subgroups at 5 years, the tool did perform well in certain subgroups; notably in patients with tumours >50 mm (14% underestimation; 85 *vs* 99, $P=0.159$), patients receiving both radiotherapy and breast conserving surgery (11% underestimation; 168 *vs* 188, $P=0.145$), and in Asian patients (7% underestimation; 14 *vs* 15, $P=0.796$).

At 8 years, the performance of PREDICT considerably improved, with the difference between the predicted deaths within 6% of those observed across the entire cohort (430 *vs* 454, $P=0.260$), and the tool performing well across most subgroups. Notable improvements to the predicted all-cause mortality from 5 to 8 years were found in patients aged 36–40 years (23%, $P<0.001$, to 1%, $P=0.845$), patients with a ductal morphology (23%, $P<0.001$, to 2%, $P=0.688$), negative nodal status (21%, $P=0.009$, to 4%, $P=0.704$), 21–50 mm tumours (23%, $P<0.001$, to 4%, $P=0.551$), patients who had radiotherapy and a mastectomy (27%, $P<0.001$, to 8%, $P=0.236$), and patients receiving second- or third-generation chemotherapy (28%, $P<0.001$, to 7%, $P=0.201$ and 18%, $P=0.020$, to 4%, $P=0.705$, respectively). The performance of the tool had also greatly improved across all ethnicity subgroups and in patients with HER2+ and borderline tumours. The number of predicted deaths remained much lower than those observed for patients with grade 2 tumours, 0–10 mm tumours, patients with a lobular morphology, and patients receiving both hormone and chemotherapy. There was also greater disparity in the predicted *vs* observed number of deaths at 8 years compared with 5 years when looking at patients receiving adjuvant trastuzumab and at ER subgroups, with all-cause mortality overestimated for patients receiving adjuvant trastuzumab by 58% (52 *vs* 33, $P=0.001$), and overestimated for ER− tumours by 32% (242 *vs* 183, $P<0.001$) and underestimated for ER+ tumours by 31% (188 *vs* 271, $P<0.001$).

In contrast to the underestimation of the total number of deaths at 5 and 8 years, PREDICT was found to overestimate the number of deaths at 10 years by 8% (164 *vs* 152, $P=0.330$). The number of deaths remained overestimated for patients with ER− tumours (47%; 94 *vs* 64, $P<0.001$), grade 3 tumours (25%; 129 *vs* 103, $P=0.010$), and tumours larger than 50 mm (115%; 28 *vs* 13, $P<0.001$). In addition, the tool was found to overestimate all-cause mortality in patients aged 36–40 years (18%; 90 *vs* 76, $P=0.108$), patients with grade 3 tumours (25%; 129 *vs* 103, $P=0.010$) and tumours without lymphovascular invasion (26%; 59 *vs* 47,

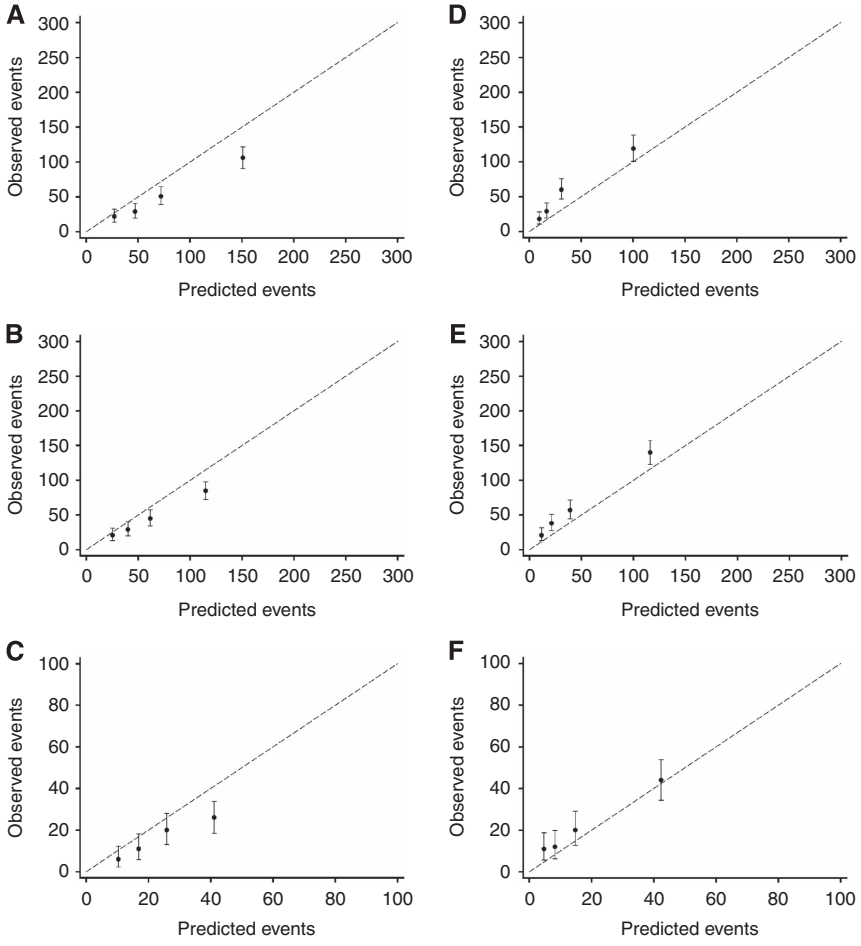

Figure 1. Calibrations plots of observed OS outcomes with 95% confidence intervals against predicted outcomes by quartiles of the predicted risk, by ER status. OS outcomes for patients with ER− tumours at (**A**) 5 years, (**B**) 8 years, and (**C**) 10 years, and OS outcomes for patients with ER+ tumours at (**D**) 5 years, (**E**) 8 years, and (**F**) 10 years.

$P = 0.080$), patients receiving chemotherapy alone (32%; 94 *vs* 71, $P = 0.006$), and patients receiving third-generation chemotherapy (31%; 42 *vs* 32, $P = 0.077$). Nevertheless, the 10-year estimates predicted by the tool had much improved across most subgroups, including ethnicity subgroups and HER2 subgroups.

The number of predicted *vs* observed breast cancer-specific deaths was very similar to the results for OS, with no notable differences between the OS and BCSS results when comparing these both by year (5, 8, and 10 years) and across subgroups (Supplementary Table 1).

The comparison between the predicted and observed all-cause mortality by quartiles of the predicted risk both across years and split by ER status is presented in Figure 1. PREDICT was found to overestimate the total number of deaths for patients with ER− tumours but underestimate for the ER+ subgroup. Goodness-of-fit test *P*-values identified that PREDICT performed better at 10 years compared with 5 and 8 years ($P < 0.001$, $P = 0.00284$, and $P = 0.0295$ for ER− tumours at 5, 8, and 10 years, respectively, and $P < 0.001$, $P < 0.001$ and $P = 0.0183$ for ER+ tumours).

The findings were almost identical when comparing the predicted and observed breast cancer-specific mortality by year and ER status (Supplementary Figure 1). Moreover, when comparing the predicted and observed all-cause mortality by ER status for patients with HER2− tumours only (Figure 2) and HER2+ tumours only (Figure 3), the pattern of overestimating mortality in patients with ER− tumours and underestimating mortality in ER+ tumours remained evident across years. The overestimation of the tool was also apparent when comparing the number of predicted *vs* observed all-cause deaths in patients with both ER− and HER2+ tumours (data not shown), with an overestimation at 5, 8, and 10 years of 38% (101 *vs* 73, $P = 0.001$), 39% (78 *vs* 56, $P = 0.003$), and 68% (32 *vs* 19, $P = 0.003$), respectively. For patients with both ER− and HER2− tumours, the overestimation was not as large (8% ($P = 0.301$), 18% ($P = 0.060$), and 22% ($P = 0.188$), respectively). For patients with both ER+ and HER2− tumours, large differences between the predicted and observed deaths could be seen at 5 and 8 years but not at 10 years. Underestimation was prominent in patients with both ER+ and HER2+ tumours, with an underestimation at 5, 8, and 10 years of 46% (59 *vs* 101, $P < 0.001$), 26% (63 *vs* 85, $P = 0.017$), and 32% (21 *vs* 31, $P = 0.072$), respectively.

Model discrimination. PREDICT provided a reasonably high degree of discrimination for OS across years (data not shown) and when splitting by ER and HER2 status (Figure 4). Model discrimination was slightly better for patients with ER+ tumours across all years (AUC: 0.718 *vs* 0.730, 0.709 *vs* 0.748, 0.694 *vs* 0.724 for ER− *vs* ER+ tumours at 5, 8, and 10 years, respectively). Conversely, model discrimination was better for patients with HER2− tumours, particularly at 10 years (AUC: 0.724 *vs* 0.592 for HER2− *vs* HER2+ tumours). Similar findings were apparent for BCSS (Supplementary Figure 2).

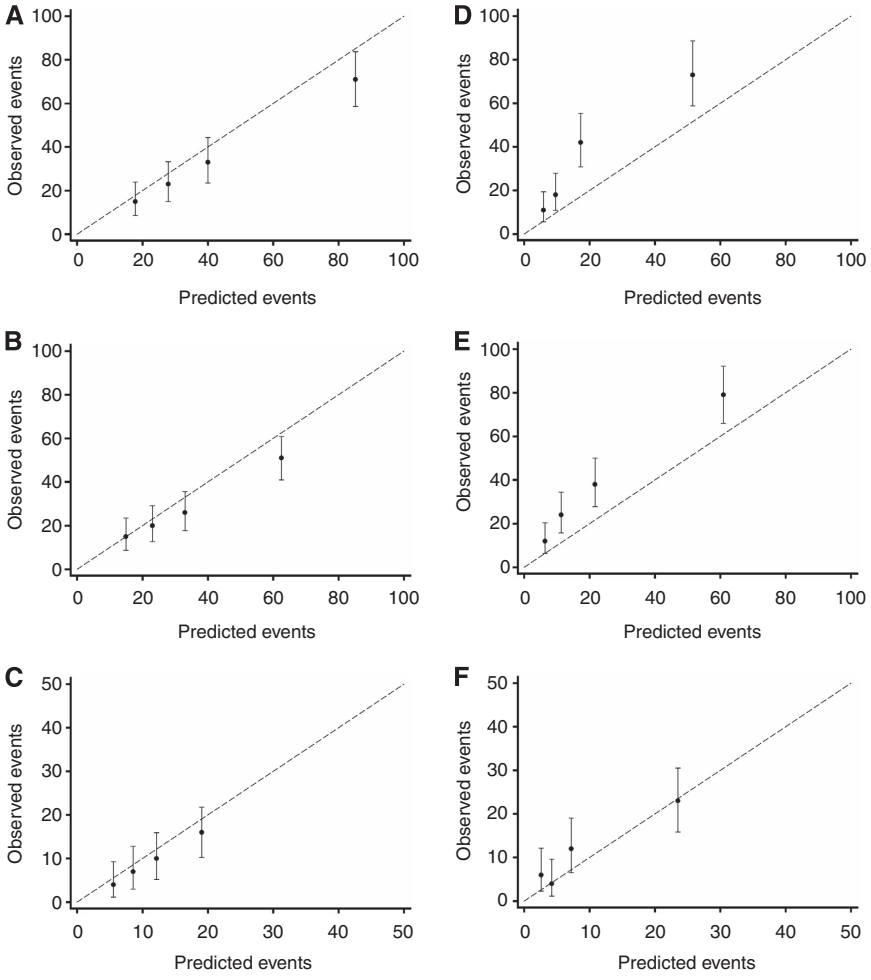

Figure 2. Calibrations plots of observed OS outcomes with 95% confidence intervals against predicted outcomes by quartiles of the predicted risk for HER2− patients only, by ER status. OS outcomes for patients with ER− tumours at (**A**) 5 years, (**B**) 8 years, and (**C**) 10 years, and OS outcomes for patients with ER+ tumours at (**D**) 5 years, (**E**) 8 years, and (**F**) 10 years.

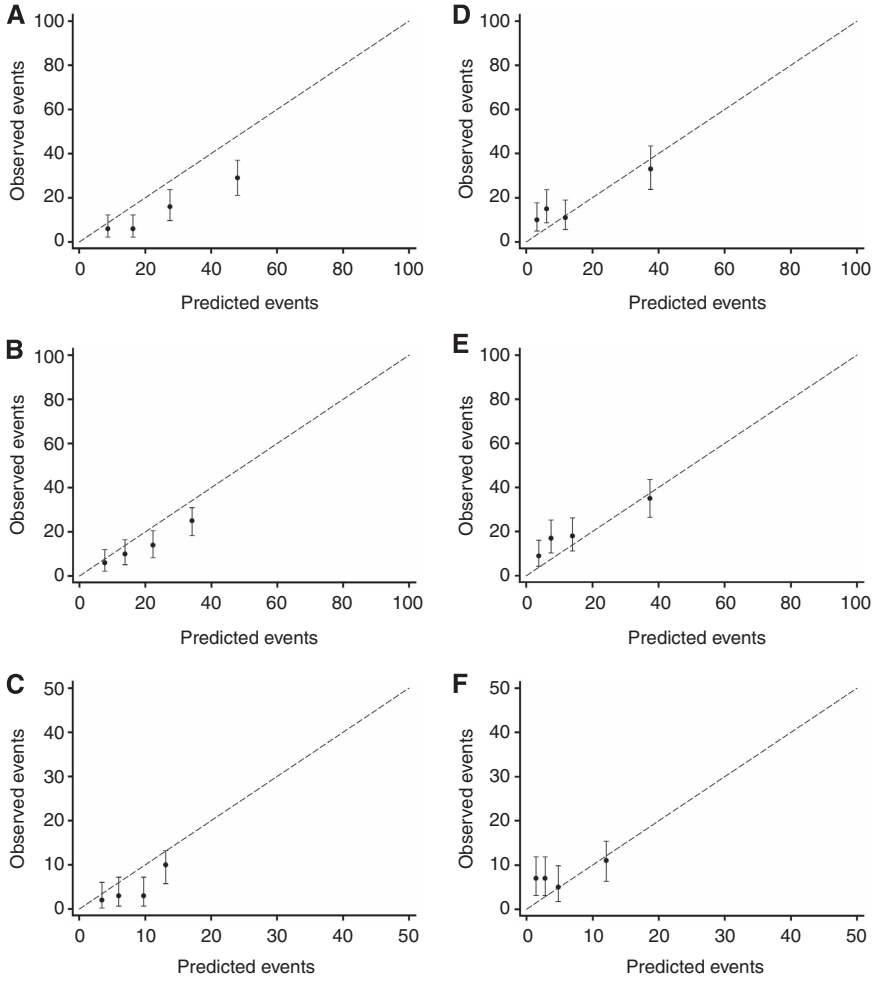

Figure 3. Calibrations plots of observed OS outcomes with 95% confidence intervals against predicted outcomes by quartiles of the predicted risk for HER2+ patients only, by ER status. OS outcomes for patients with ER− tumours at (**A**) 5 years, (**B**) 8 years, and (**C**) 10 years, and OS outcomes for patients with ER+ tumours at (**D**) 5 years, (**E**) 8 years, and (**F**) 10 years.

DISCUSSION

This study has demonstrated that the prognostic tool PREDICT is able to provide accurate long-term (8- and 10-year) outcomes for younger women with breast cancer but provides only limited accuracy regarding short-term (5-year) survival, for both OS and BCSS.

High performance in terms of accurate predictions in the number of deaths was prominent in a number of subgroups across both years and survival type. These subgroups included patients receiving both radiotherapy and breast conserving surgery, patients with grade 1 tumours, HER2+ tumours, and patients with both ER− and HER2− tumours. Although the number of Asian patients included in the analyses at 5, 8, and 10 years was small (84, 55, and 16, respectively), PREDICT performed well in this subgroup of the POSH cohort, which was contrary to previous findings on a number of prognostic tools (Engelhardt *et al*, 2014). Our findings also demonstrated that in Caucasian/white and Black ethnicity subgroups, PREDICT was able to provide accurate long-term, but not short-term, estimates.

A key area in which PREDICT could improve its prognostic ability is in patients with ER− tumours. The number of predicted deaths across both years and survival type was overly pessimistic in this subgroup as demonstrated by the calibration plots (Figure 1 and Supplementary Figure 1). These results were contrary to the previously published validation of PREDICT for patients aged 20–85 years, in which the tool was found to accurately predict 10-year OS and BCSS in the ER− subgroup (Wishart *et al*, 2012).

Splitting the ER− subgroup by HER2 status, it was evident that there is some disparity between the PREDICT estimates for HER2− and HER2+ patients in this subgroup, with the tool providing accurate predictions for patients with both ER− and HER2− tumours across years and survival type, while consistently overestimating the number of deaths for patients with both ER− and HER2+ tumours. In contrast, the tool underestimated the number of deaths in patients with ER+ tumours, when looking not only at the subgroup as a whole but also when further splitting by HER2 status.

In relation to HER2 subgroups overall, the tool was able to predict the number of deaths for both OS and BCSS to within 1% of those observed for the HER2− subgroup, with corresponding 10-year AUCs of 0.724 and 0.718, respectively. PREDICT was not quite as reliable in terms of identifying patients with a worse survival in the HER2+ subgroup (AUC = 0.592 for both 10-year OS and BCSS), although the predicted number of deaths for OS and BCSS were within 6% of those observed. Fewer patients with HER2+ tumours were available in the 10-year evaluation ($n = 140$ for both OS and BCSS) compared with HER2− tumours ($n = 327$ and $n = 325$, respectively), which might have contributed to the reduction in accuracy of PREDICT for patients with HER2+ tumours.

In terms of OS and BCSS overall, the findings of our study demonstrated that PREDICT performs equally well, whereas the

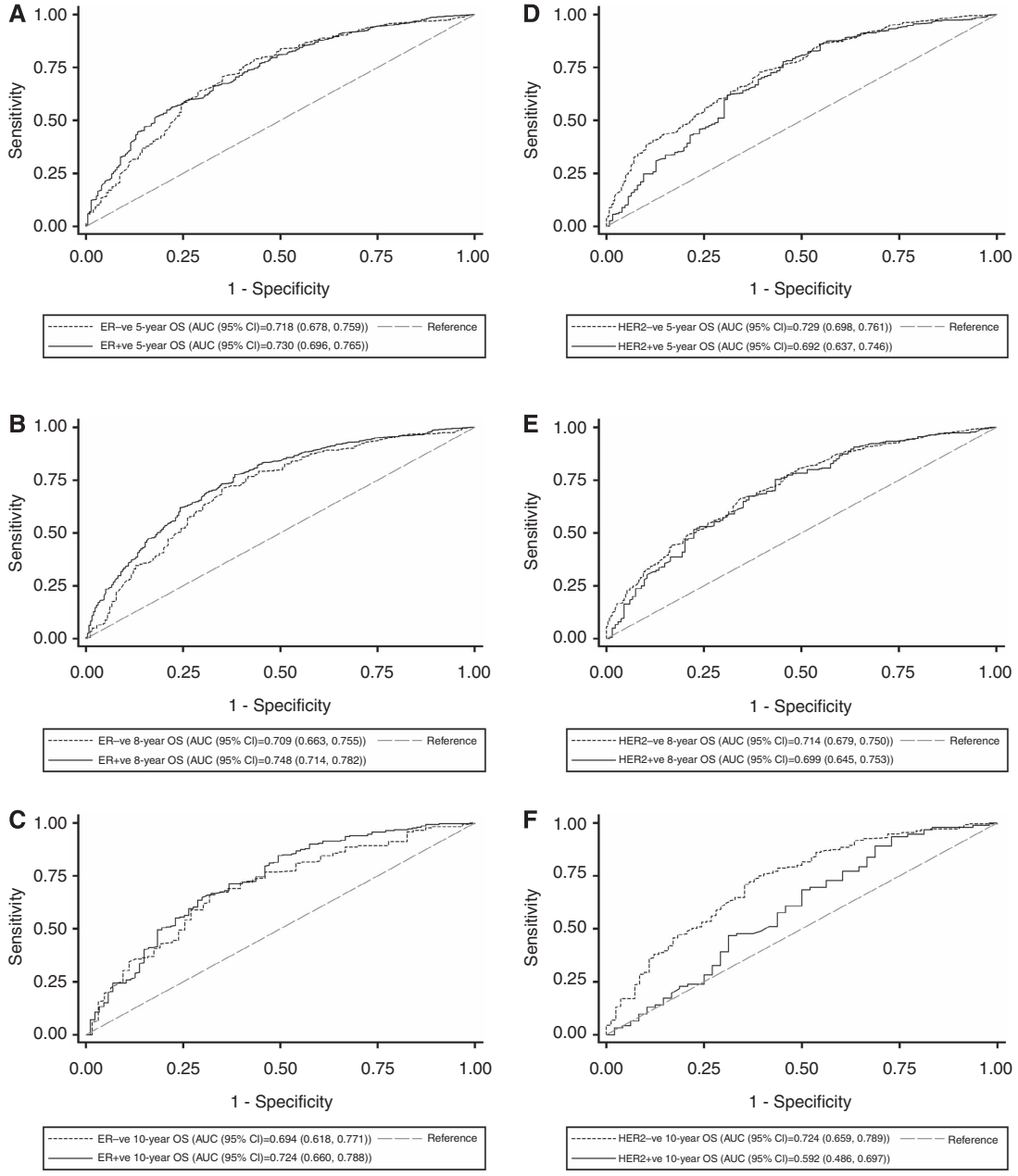

Figure 4. ROC curves for OS: split by ER status at (**A**) 5 years, (**B**) 8 years, and (**C**) 10 years; and split by HER2 status at (**D**) 5 years, (**E**) 8 years, and (**F**) 10 years.

Wishart *et al* validation showed that PREDICT is slightly better at providing BCSS estimates compared with OS estimates (Wishart *et al*, 2012). The validation also found that PREDICT underestimated the number of deaths at 10 years by 32% for patients aged 20–35 years (Wishart *et al*, 2012). In contrast to this, our study of outcomes for patients aged 18–40 years at diagnosis showed an overestimation in 10-year all-cause mortality of 8%. We explored this a little further by dichotomising age at diagnosis into two groups (data not shown), <35 and 35–40 years. Surprisingly, for the group aged <35 years, the number of deaths predicted (OS) was within 2% of those observed (60 *vs* 61, $P=0.898$) and within 14% (104 *vs* 91, $P=0.173$) for patients aged 35–40 years, indicating that differences in response to treatment between these two age groups could lead to some disparity between the performance of PREDICT between these two age groups.

There could be several reasons for the differences found in this study compared with other studies. The similarity of the OS and BCSS results of our study is likely due to the fact that competing mortality does not play as important a role within this age group; excluding five patients with a missing/unknown cause of death, over 96% of deaths were due to breast cancer. In addition, PREDICT was developed to provide long-term (10-year) outcomes, not short-term, which might explain the reduced accuracy of the 5-year estimates. A possible reason for poor performance in patients aged 20–35 years is the low numbers on which the model was based; only 401 women aged $\leqslant 40$ years were used in the development of PREDICT (131 patients had ER− and 270 ER+ tumours). Furthermore, the number of patients aged $\leqslant 40$ years evaluated in the validation of the enhanced PREDICT tool was relatively small ($n=159$; Wishart *et al*, 2012). It should be noted that poorer prediction in younger patients is a common finding across a number of other prognostic tools, which were found to overestimate OS by up to 30% in younger women (Engelhardt *et al*, 2014). As PREDICT did not perform well in a number of subgroups in our study, including the ER− subgroup, further

modifications to PREDICT using this larger data set could improve survival estimates for younger breast cancer patients. Furthermore, inclusion of the proliferation marker KI67 in the PREDICT model has led to a statistically significant improvement in function of the PREDICT model for ER+ patients (Wishart *et al*, 2014), which may also improve prognostication for younger patients.

There are some limitations to our study, which should be taken into account when interpreting the results. Less than half the patients from the POSH cohort had reached 10 years from diagnosis at the time of this analysis and so only a relatively small number could be included in the 10-year comparison ($n=607$). This is, however, still considerably larger than the number of women aged $\leqslant 40$ years in the validation of PREDICT ($n=159$; Wishart *et al*, 2012). Our study also demonstrated an over-estimation in OS at 10 years so arguably this longer follow-up may not improve the estimates. Our data confirm a need for caution in extrapolating data from older cohorts to inform management in young patients with breast cancer. It also confirms the need to investigate treatment approaches in trials involving sufficiently large numbers of younger women, which would allow independent analysis to determine whether there are major outcome differences and to understand why. Trials of more treatment approaches specifically directed to younger patients with breast cancer should be investigated (Aebi *et al*, 2000; Colleoni *et al*, 2006; Narod, 2012). An additional limitation is that adjuvant Herceptin has been used in the United Kingdom routinely since 2005 so patients diagnosed with HER2+ disease before this date will only have received Herceptin in the metastatic setting. It is therefore possible that the outcome of HER2 patients in the POSH cohort as a whole is inferior to HER2 patients diagnosed and treated in the United Kingdom since 2005. However, the impact of this is likely to be minimal as the number of patients with HER2+ tumours diagnosed before 2005 ($n=298$) and since 2005 ($n=381$) included in this analysis is relatively small.

In conclusion, this study has demonstrated that PREDICT, a web-based tool that is easy to navigate for both patients and users, is a valuable resource in providing accurate and reliable long-term outcomes for younger patients. Although caution should currently be used when interpreting the short-term survival estimates in younger patients and the long-term estimates of younger patients with ER− tumours, it is intended that future modifications of PREDICT will include the incorporation of the POSH data set to allow for more robust estimates for younger women with breast cancer. Accurate prediction of outcome at both short- and long-term time points may be particularly important to women trying to determine the optimal timing of risk-reducing mastectomies.

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CONFLICT OF INTEREST

EC has received honoraria from Roche. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)

APPENDIX

POSH Steering group: Professor Diana Eccles, Dr Peter Simmonds, Professor Douglas G Altman, Dr Paul Pharoah, Professor Louise Jones, Professor Ros Eeles, Professor D Gareth Evans, Professor Andrew Hanby, Professor Alistair M Thompson, Professor Shirley Hodgson, Mr Hisham Hamed, Dr Ruth Warren, and Professor Sunil Lakhani.

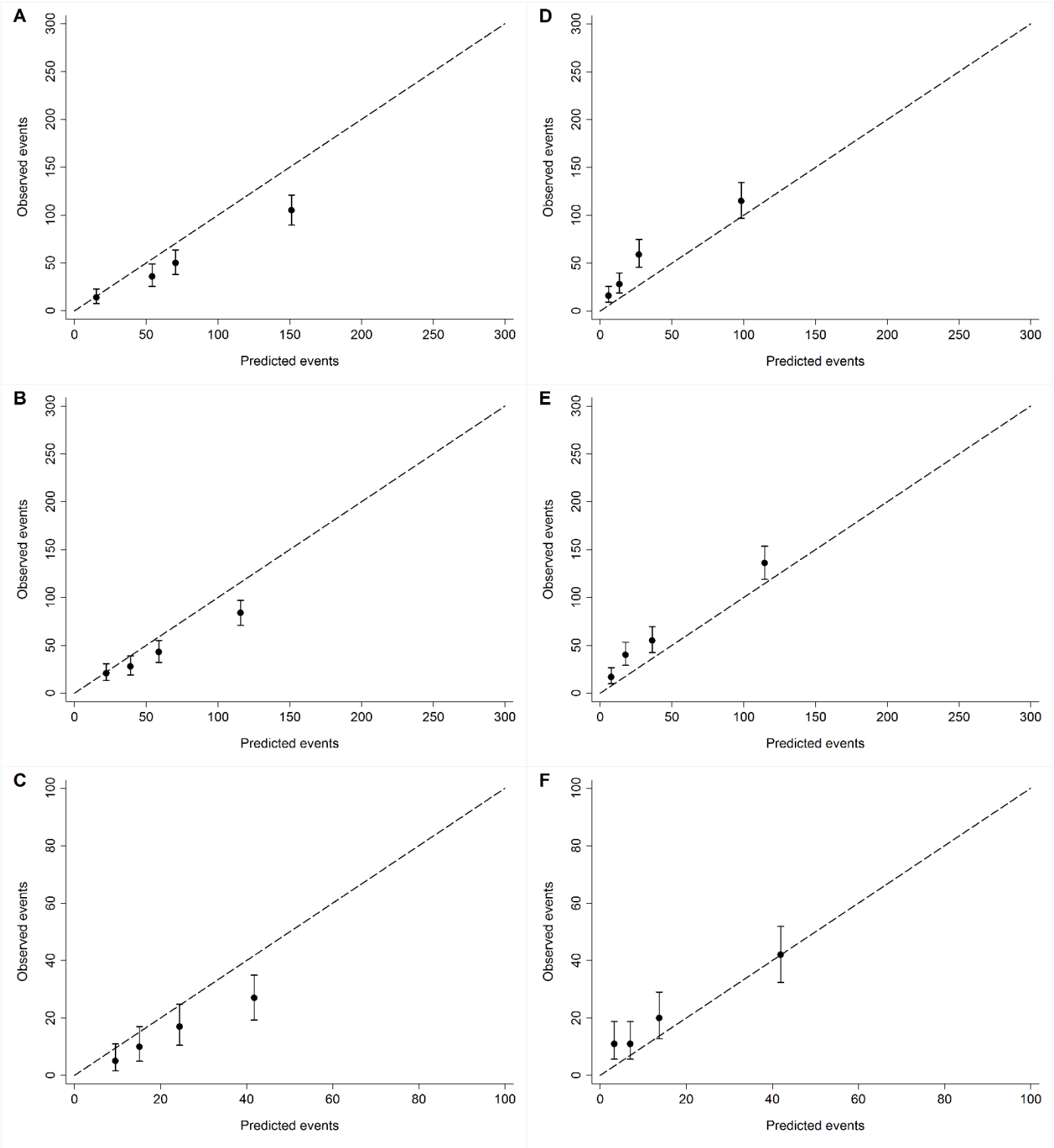
SUPPLEMENTARY MATERIAL

Supp Table 1. Observed and predicted 5-, 8- and 10-year breast cancer-specific mortality by demographical, tumour and treatment characteristics

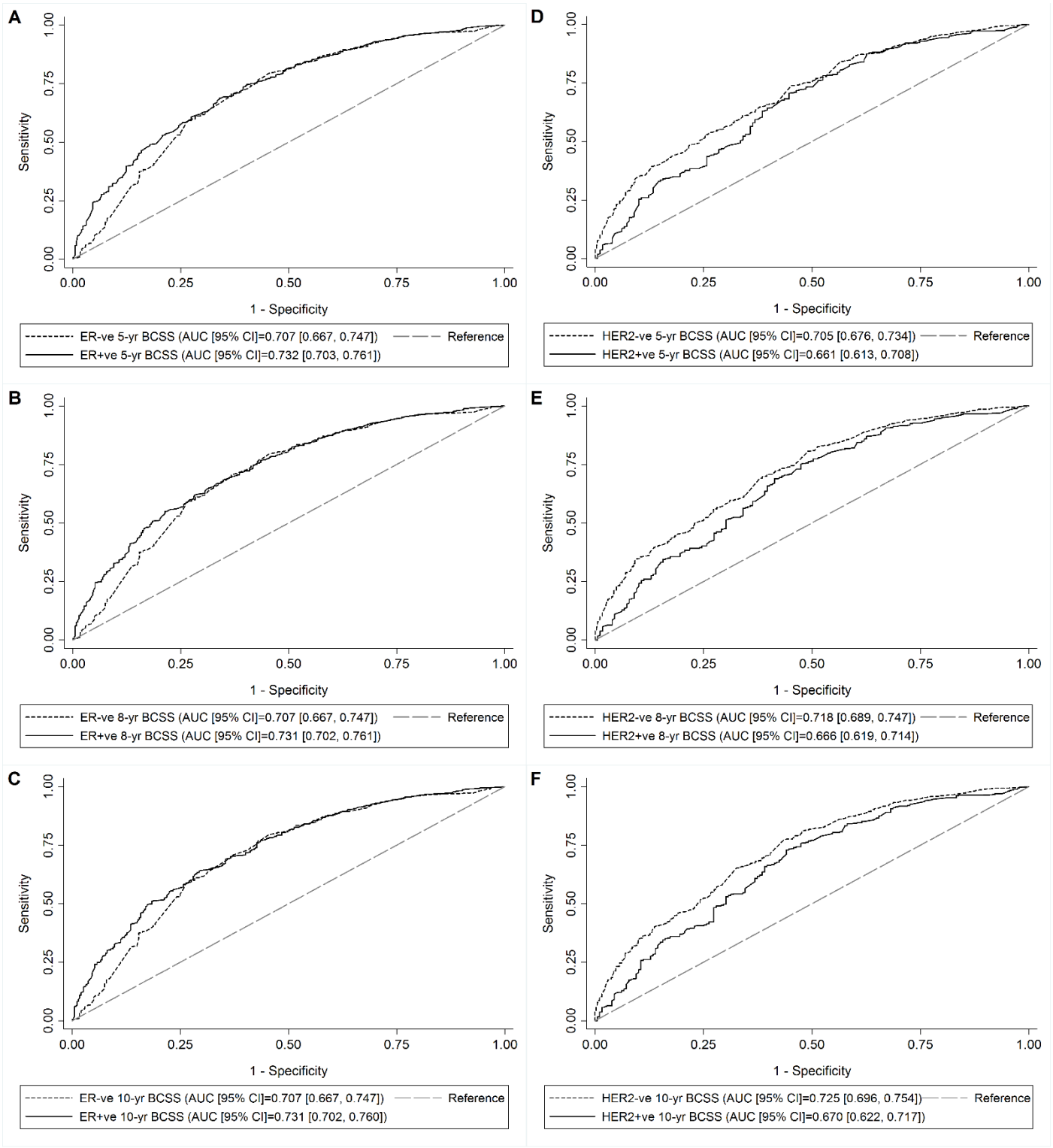
Characteristic	5-years					8-years					10-years				
	N	O	P	D	%	N	O	P	D	%	N	O	P	D	%
Total	2822	587	436	-151	-25.7	1841	441	412	-29	-6.6	595	145	157	12	8.3
Age at diagnosis															
18-25	40	7	5	-2	-28.6	21	4	4	0	0	8	1	1	0	0
26-30	257	61	44	-17	-27.9	167	48	41	-7	-14.6	55	15	16	1	6.7
31-35	863	206	147	-59	-28.6	579	164	141	-23	-14	202	59	54	-5	-8.5
36-40	1662	313	239	-74	-23.6	1074	225	225	0	0	330	70	86	16	22.9
Menopausal status															
Pre-	2766	573	426	-147	-25.7	1799	428	402	-26	-6.1	579	142	154	12	8.5
Post-	7	2	0	-2	-100	4	2	1	-1	-50	1	1	0	-1	-100
Peri-	5	1	1	0	0	5	1	2	1	100	3	0	1	1	.
Unknown	44	11	8	-3	-27.3	33	10	8	-2	-20	12	2	2	0	0
Morphology															
Ductal	2442	512	393	-119	-23.2	1604	383	372	-11	-2.9	518	125	143	18	14.4
Lobular	129	27	9	-18	-66.7	79	21	11	-10	-47.6	30	8	3	-5	-63
Other	216	41	30	-11	-26.8	133	32	25	-7	-21.9	40	12	10	-2	-17
Unknown	35	7	4	-3	-42.9	25	5	4	-1	-20	7	0	1	1	.
Grade															
1	156	4	2	-2	-50	98	4	3	-1	-25	31	2	1	-1	-50
2	927	153	60	-93	-60.8	614	117	68	-49	-41.9	200	41	29	-12	-29
3	1673	418	367	-51	-12.2	1095	314	337	23	7.3	349	100	125	25	25
Not graded/missing	66	12	6	-6	-50	34	6	4	-2	-33.3	15	2	2	0	0
LV invasion															
Negative	1374	175	146	-29	-16.6	873	137	134	-3	-2.2	290	44	55	11	25
Positive	1248	372	264	-108	-29	843	279	257	-22	-7.9	268	92	95	3	3.3
Unknown	200	40	25	-15	-37.5	125	25	21	-4	-16	37	9	7	-2	-22
Node status															
Negative	1369	153	118	-35	-22.9	869	106	105	-1	-0.9	266	38	39	1	2.6
Positive	1427	427	315	-112	-26.2	957	331	304	-27	-8.2	325	106	117	11	10.4
Unknown	26	7	4	-3	-42.9	15	4	3	-1	-25	4	1	1	0	0
Tumour size															
0-10	265	40	18	-22	-55	161	27	15	-12	-44.4	48	13	6	-7	-54
11-20	928	119	93	-26	-21.8	628	95	92	-3	-3.2	220	39	42	3	7.7
21-50	1226	292	225	-67	-22.9	797	221	211	-10	-4.5	243	74	76	2	2.7
50+	244	97	84	-13	-13.4	171	75	82	7	9.3	54	13	28	15	115
Unknown	159	39	15	-24	-61.5	84	23	11	-12	-52.2	30	6	5	-1	-17
ER status															
Negative	963	240	291	51	21.3	640	178	236	58	32.6	229	60	91	31	51.7
Positive	1859	347	145	-202	-58.2	1201	263	176	-87	-33.1	366	85	66	-19	-22
Local Rx															
BCS+RT	1308	182	158	-24	-13.2	871	142	150	8	5.6	274	57	55	-2	-3.5
Mast+RT	999	303	222	-81	-26.7	647	224	208	-16	-7.1	207	66	83	17	25.8
Mast alone	444	83	45	-38	-45.8	277	63	44	-19	-30.2	101	21	17	-4	-19
Other	71	19	11	-8	-42.1	46	12	10	-2	-16.7	13	1	2	1	100
Systemic Rx															
None	46	4	5	1	25	28	3	4	1	33.3	6	1	1	0	0
Hormone	227	14	5	-9	-64.3	157	13	6	-7	-53.8	50	5	3	-2	-40
Chemo	984	257	290	33	12.8	657	194	241	47	24.2	235	69	91	22	31.9
Both	1565	312	136	-176	-56.4	999	231	160	-71	-30.7	304	70	63	-7	-10
HER2 Status															
Negative	1769	371	242	-129	-34.8	1057	265	223	-42	-15.8	325	77	78	1	1.3
Positive	678	179	155	-24	-13.4	434	139	137	-2	-1.4	140	49	52	3	6.1
Borderline	40	10	5	-5	-50	33	10	7	-3	-30	14	5	4	-1	-20
Unknown	335	27	32	5	18.5	317	27	45	18	66.7	116	14	23	9	64.3
Ethnicity															
Caucasian/White	2577	528	395	-133	-25.2	1690	399	375	-24	-6	555	137	148	11	8
Black	110	36	20	-16	-44.4	74	27	19	-8	-29.6	17	5	4	-1	-20
Asian	84	14	13	-1	-7.1	55	10	13	3	30	16	3	3	0	0
Other	19	3	3	0	0	12	3	3	0	0	3	0	0	0	.
Unknown	32	6	4	-2	-33.3	10	2	2	0	0	4	0	1	1	.

Abbreviations: N=Number of patients, O=Number of observed events; P=Number of predicted events; D=Difference; ER=Oestrogen receptor status; HER2=Human epidermal growth factor receptor 2.

Supplementary Figure 1. Calibrations plots of observed BCSS outcomes with 95% confidence intervals against predicted outcomes by quartiles of the predicted risk, by ER status. BCSS outcomes for patients with ER negative tumours at (A) 5-years, (B) 8-years and (C) 10-years, and BCSS outcomes for patients with ER positive tumours at (D) 5-years, (E) 8-years and (F) 10-years.



Supplementary Figure 2. ROC curves for BCSS: split by ER status at (A) 5 years, (B) 8 years, and (C) 10 years; and split by HER2 status at (D) 5 years, (E) 8 years, and (F) 10 years.



Chapter 10: Discussion

As outlined in **Section 1.2**, the overarching aim of this thesis was to study factors affecting outcomes for young women with invasive breast cancer. This chapter provides a summary of the main findings of the seven papers, followed by a discussion of each of the seven papers, suggestions for future research and conclusions drawn from the research undertaken.

The discussion of individual papers includes the aim of the paper, key methods implemented in the analyses, together with a summary of the results found as well as conclusions drawn from the paper, and finally the strengths and limitations. As the publications spanned a five year period (first paper published in May 2013 and the most recent published in February 2018), the strengths and limitations of each paper are discussed in conjunction with the available literature at the time of publication.

10.1 Summary of findings

The POSH study recruited 3095 women with invasive breast cancer, with just under 3000 women aged 18 to 40 years of age included in all analyses. It represents one of (if not the largest) prospective studies of young breast cancer patients to date, and is representative of the wider population of young women with breast cancer in the UK. No selection of patients was made in the POSH study other than by young age, thus minimising potential inclusion bias, and the results are generalizable to the young British breast cancer population. Furthermore, unlike studies based in other countries, all patients in the POSH cohort were managed within the National Health Service (NHS) i.e. all patients had equal access to healthcare including diagnostic, surgical and oncology services.

Women in the POSH cohort were found to have large tumours with increased frequency of N1 stage tumours, and a high proportion of grade 3 and ER negative tumours. Median age at diagnosis for patients was 36 years, and survival was found to be lower compared to older patients, despite modern oncology treatment; particularly for young women with HER2 positive disease, who were demonstrated to have a worse prognosis irrespective of ER status. Over 96% of deaths were due to breast cancer in this young cohort, and subsequently, similar results were found between OS and BCSS outcome results. Outcomes were shown to vary over time with regards to ER tumour status and surgical type, and also BRCA mutation status for patients with triple negative breast cancer. Black ethnicity and obesity were found to be associated with inferior survival, independent of a number of prognostic factors and despite equal access to healthcare. Conversely, a reported family

history of breast or ovarian cancer, BRCA mutation status and surgical type were not found to impact on survival after adjusting for prognostic and/or potential confounding factors. The prognostic tool PREDICT was found to provide accurate survival estimates for young women with breast cancer in the long term but less accurate short-term estimates.

10.2 Discussion - Paper 1

10.2.1 Aim and background

This was the first paper to describe and present outcomes from the POSH cohort. The main aim of the paper was to describe the presenting characteristics, tumour pathology, treatment and survival of young women with invasive breast cancer from the cohort.

The paper provided a background of the POSH study: a multicentre prospective observational cohort study of women aged 18 to 40 years of age from the UK who were diagnosed with invasive breast cancer between 1st January 2000 and 31st January 2008. An additional subgroup of women aged 41-50 years of age from the UK were recruited if they had a known BRCA1 or BRCA2 gene mutation but were excluded from the analyses of this paper. Women were recruited from 127 hospitals from England, Wales, Scotland and Northern Ireland and received treatment according to local hospital protocols.

Sources of data collection were described, including medical records used to collect personal characteristics, tumour pathology, disease stage, treatment received, and clinical follow-up information. In addition, pathology and radiological reports from sites, diagnostic pathology test reports and tumour microarray (TMA) data, patient questionnaires for reported family history, and Medical Research Information Service for notification for date and cause of death were used.

The paper utilised data from 3095 women recruited from the POSH cohort, of whom 3022 satisfied the eligibility criteria. Women aged 41-50 with a known BRCA1 or BRCA2 gene mutation, patients with no invasive cancer on review of pathology, and with patients with missing primary tumour data, were excluded from the analyses. As a result, just under 3000 women (n=2956) were included in the analyses, including patients with metastatic disease at presentation (M1 stage patients). A further 11594 women from the lead national registry for breast cancer, the West Midlands Cancer Intelligence Unit (WMCIU), were used for comparison with patients only from England in the POSH cohort (n=2695) in order to rule out any systematic ascertainment bias.

10.2.2 Key statistical methods

Key statistical methods implemented included using summary statistics to describe the patient characteristics and risk factors of the cohort, as well as to compare the tumour characteristics and treatment information by ER status. An additional comparison on key data was made between information from the POSH cohort with the WMCIU.

In terms of survival, both OS and DDFI (as defined in Chapter 2) were described using Kaplan-Meier curves on information from the POSH cohort. As the effect of ER status on survival was found to vary over time, both in terms of OS and DDFI, FPSMs were used with ER status fitted as a time-varying covariate, both for the unadjusted and adjusted regression estimates. Multivariable models were adjusted for grade, maximum invasive tumour size and nodal status. The choice of degrees of freedom for both the baseline hazard rate and time-dependent effect of each FPSM produced were found using the AIC and overlaying the FPSM hazard rates onto the smoothed hazard rates to obtain the best model fit for each FSPM. The time-varying hazard rates and time-varying hazard ratios were then plotted over time to illustrate the effect of ER status over time more clearly. All statistical analyses were performed using Stata version 11.2[74] on records with complete data (complete-case analyses), and were conducted accordingly to a pre-specified analysis plan (see **Appendix A3**) on follow-up data received up to 11th April 2012.

10.2.3 Key findings

Of the 2956 patients included in the analysis population, information on ER status was available for 2944 patients. All 2956 patients were included in the descriptive tables, but summary statistics and univariable survival analyses were carried out on 2944 patients, of whom 997 (33.9%) had ER negative tumours and 1947 (66.1%) ER positive tumours. Information on grade, maximum invasive tumour size and nodal status, required for the MVA, was available for 2701 patients.

The median age of the analysis population was 36 years of age, ranging from 18 to 40 years of age at diagnosis. In terms of other patient characteristics and risk factors at diagnosis, summary statistics also identified that less than 1% of patients (n=12 [0.4%]) presented with screen-detected malignancies, whilst the majority were symptomatic at presentation (n=2900 [98.1%]). Almost all women were pre-menopausal (97.6%) at diagnosis, and median BMI was 24.6 kg/m^2 (a normal/healthy weight), ranging from 14.7 (underweight) to 59.5 kg/m^2 (obese). Over 70% of women (n=2097) had at least one child, with a median of two children amongst those with children. The majority of women (n=2598 [87.9%]) had used the contraceptive pill and approximately half of the women had smoked/were currently smokers. Just over 14% of women had a first degree relative and 18.7% a second degree relative with breast cancer.

Concerning tumour characteristics, most women had grade 3 tumours (58.9%), followed by grade 2 tumours (32.9%) and finally grade 1 tumours (5.5%), and the majority (86.5%) had a ductal histological tumour type. In terms of the distribution of the tumour, localised tumours were the most common (63.4%) compared to multifocal tumours (27.0%). The majority of tumours were ER positive (65.9%), PR positive (45.4%), and/or HER2 negative (62.2%). However, important to note is that of the proportion non-missing, these percentages were 66.1%, 56.5% and 70.7%, respectively; consistent with the fact that ER and PR are highly correlated. Almost all patients (96.8%) had M0 stage disease at presentation, and most patients had either T1 or T2 pathological T stage tumours, with the median maximum invasive tumour size of 22mm, ranging from 0 to 199mm. Approximately half of the women had N1 stage tumours, of whom the median number of positive axillary lymph nodes recovered was two, ranging from one to 50. In addition, comparing key tumour information from the POSH cohort with data from the WMCIU indicated that the POSH cohort is representative of the wider population of young women with breast cancer in the UK.

Regarding the treatment received, summary statistics identified that approximately half of the women underwent BCS, and the other half a mastectomy. The majority of chemotherapy was adjuvant (72.8%), with a small proportion of women undergoing neo-adjuvant chemotherapy (15.6%). Medical ovarian suppression in any treatment period was undertaken in 784 patients (26.5%), and oophorectomy in 398 patients (13.5%).

Genetic testing information was only collected from the medical records if conducted through referral to an NHS clinical genetic service for testing. As a result, only 763 patients (26% of the analysis population) were tested; 136 patients were found to have a BRCA1 mutation, 78 had a BRCA2 mutation and four had a TP53 mutation.

Finally, in terms of survival, median follow-up at the time of analyses was five years, ranging from one month to 11 years, with only a small proportion of patients lost to follow-up (n=72 [2.4%]). A total of 613 deaths (20.7%) had occurred, of which the vast majority (n=578 [94.3%]) were due to breast cancer. There were 712 patients (24%) who developed a distant relapse, of whom 149 were still alive at the time of analyses. An additional 17 patients who had not experienced a distant relapse but died from breast cancer were included as events in the DDFI analyses. As the number at risk was greatly reduced by ten years, five and eight year event rates were presented in the paper. The five year Kaplan-Meier estimate for all patients was 81.9% for OS and 76.6% for DDFI. By eight years, OS was reduced to 67.6% and DDFI to 68.3%. The five year survival rate of the entire cohort was also found to be similar to the 2005-2009 national statistics relative survival of 83.5% in women aged 15-39.

For patients with ER negative tumours, five year OS was significantly lower compared to ER positive tumours; 75.7% (95% CI: 72.8% to 78.4%) vs. 85.0% (95% CI: 83.2% to 86.7%), respectively. However by eight years, the difference was no longer significant; 67.7% (95% CI: 63.6% to 71.5%) vs. 67.5% (95% CI: 63.3% to 71.3%), respectively. Similar results at both five and eight years were also found when comparing ER status for DDFI. The time-varying nature of ER was further demonstrated in **Figures 2E** and **Supplementary Figure 3** of the paper. **Figure 2E** showed the FPSM hazard rates for ER negative and positive tumours, with the risk of death initially greater for patients with ER negative tumours but the hazards then crossed at approximately 4.5 years and the risk of death then greater for patients with ER positive tumours. **Supplementary Figure 3A** and **3B** illustrated the time-varying OS hazard ratio for ER status over time, both unadjusted (A) and when adjusted for key prognostic factors (B). The hazard ratios crossed the reference line of y=1 at approximately 4.5 years in both instances, demonstrating that the change in hazard ratios over time was still evident even after adjusting for other prognostic factors. The result of this time-varying hazard effect on survival was that by eight years, the survival estimates of both the ER negative and positives converged, as illustrated in **Figures 2D** and **2F** in the paper, which showed the Kaplan-Meier survival plot by ER status and survival estimates produced from the FPSM, respectively. The similarity of these two plots also demonstrated a good model fit of the FPSM.

When separating the survival curves by ER and HER2 status (**Figure 3** in the paper), it was evident that patients with HER2 positive tumours had an inferior survival compared to patients with HER2 negative tumours, both in terms of OS and DDFI, and irrespective of the ER tumour status. Patients with both ER positive and HER2 positive tumours were shown to have the lowest survival at five and eight years: 70.2% and 58.4% for OS; and 62.2% and 53.4% for DDFI, respectively. Patients with both ER negative and HER2 negative tumours, consistently had the highest survival rates at eight years for OS (68.3%) and DDFI (70.7%), but not at five years. The highest survival rates at five years were found in patients with tumours which were both ER positive and HER2 negative for OS (84.1%) and DDFI (78.3%).

10.2.4 Strengths

This was the first outcome analysis from the POSH cohort; representing one of, if not the largest, prospective cohort studies of young women with invasive breast cancer who were both diagnosed and treated in the 21st Century, and in whom long-term follow-up data were available. Despite no direct comparison with older patients, this paper was able to report results according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines, as well as all analyses being conducted according to a pre-specified analysis plan. As such, the results can

be presented in an accurate, clear and detailed manner to ensure transparency in the findings and future work planned.

This was made particularly evident when modelling the effects of ER status on mortality over time using an appropriate method; incorporating the use of FPSMs and presenting not only the modelled hazard rates over time, but also the unadjusted and adjusted modelled hazard ratios over time as well as the modelled survival rates. The hazard rates provided a clear illustration of how the risk of death for patients with ER negative tumours increased noticeably compared to ER positive tumours initially. However, this risk later decreased considerably, with a corresponding increase in the risk of death for patients with ER positive tumours. The unadjusted hazard ratio over time clearly demonstrated the magnitude of the change in risk over time by way of statistical significance; with the risk of death significantly greater in patients with ER negative tumours compared to ER positive tumours prior to 3.5 years, not significantly different between 3.5 to 6.5 years, and finally significantly less post 6.5 years. The corresponding adjusted hazard ratio over time reinforced this evident change in risk over time, even when adjusting for other key prognostic factors such as maximum invasive tumour size, grade and nodal status. Finally, comparing the modelled survival rates with the Kaplan-Meier plot and showing how close these estimates matched provided a visual confirmation of a good model fit with the data.

Another key strength of this paper was that it was able to show that the POSH cohort was also representative of the wider population, both in terms of tumour pathology and survival. This was found when comparing with data from the WMCIU, despite the fact that WMCIU did not record ER status information. In addition, the representativeness of the POSH cohort was also found in terms of similar biological characteristics found in young women in other published works, where a higher frequency of grade 3, N1 stage and ER negative tumours were evident as well as larger tumours[17,19,22,26,28,29,107]. This increased frequency of larger and N1 stage tumours found in POSH patients also provides possible reasons for the high rate of mastectomies carried out in the POSH cohort compared to non-age selected symptomatic and screen detected UK patients[108].

In terms of impact on treatment, approximately 10% of patients with ER positive tumours were found to have relapsed between five and eight years, raising the question regarding the duration of hormone therapy on selected women aged 18-40, which is normally prescribed for a five year period. Indeed, at the time of writing a recently published randomised trial, which included 1270 women aged 45 years or younger indicated that extending tamoxifen to ten years, as opposed to five years, reduced both the breast cancer recurrence and mortality rates in pre- and post-menopausal women[109].

10.2.5 Limitations

One of the limitations of the paper was that ten year survival estimates were based on somewhat immature data, as was evident from the low numbers at risk at ten years shown in the Kaplan-Meier plots in **Figures 2** and **3** of the paper. However, this paper was not only able to present hazard ratios at five years but also at eight years, which contained noticeably larger numbers at risk and thus more accurate results and indications of what the longer-term survival impact would be on younger women with invasive breast cancer.

Another potential limitation of this publication was the time at which patients were recruited and were thus treated. For example, at the time of publication, the benefits of ovarian suppression in addition to chemotherapy and tamoxifen were not proven[110]. However, patients recruited in the POSH cohort underwent treatment prior to the National Institute for Clinical Excellence (NICE) guidance to only use ovarian suppression in addition to chemotherapy and tamoxifen in a clinical trial setting[107], which is reflected by the use of ovarian suppression (whether medical, irradiation and/or oophorectomy) in 703 (36.1%) of POSH patients with ER positive tumours. However, in spite of this, the paper was able to demonstrate that over 88% of POSH patients received chemotherapy in addition to local treatments, which suggested general compliance with the current guidelines of the time[111].

Due to 53% of the cohort being diagnosed prior to 2005 when adjuvant trastuzumab became a routine treatment in the UK, less than 50% of patients were recorded as having adjuvant trastuzumab. In addition, although the proportion of HER2 positive tumours found in the cohort was 24%, HER2 status was not routinely tested in the UK before 2006. In fact, ER, PR and HER2 information was obtained from local pathology reports, with a variety of differing scoring systems in place at each site. As a result, the finding of HER2 positive tumours being associated with worse survival in the paper should be interpreted with caution, as these patients were not likely to be representative of modern treatment of HER2 positive disease and the true proportion of HER2 positive tumours in this cohort is uncertain. However, this concern was somewhat lessened following additional analyses on the cohort for 1336 tumours tested on TMA data, which was able to show that 18.2% of tumours were found to be HER2 positive, which is within the range for all breast tumours reported elsewhere.

In terms of systemic treatment, evidence from a recent meta-analysis at the time of publication found that women aged under 45 received the largest absolute benefit from systemic therapy but the proportional reduction was found to be independent of age[112]. Although most POSH patients received anthracycline-based chemotherapy, the age effect was beyond the scope of this publication and not evaluated. Furthermore, this leads to another potential limitation of the

publication which was that the upper age limit of 40 for the analysis population was below the minimum for the UK breast screening program and previous history of malignancy was excluded in this trial. The analysis population did not therefore include patients who were routinely screen-detected or had radiation-induced breast cancer. Indeed, this was reflected in the presentation information collected which consisted of just 30 patients (1.0%) who were screen-detected. However, the age limit of 40 has provided other advantages, such as the high proportion of breast cancer specific deaths as opposed to non-breast cancer deaths often observed in older women.

10.2.6 Conclusions

This initial publication was able to demonstrate that the POSH cohort was found to be representative of the wider population of young women with breast cancer in the UK. Young women with invasive breast cancer were shown to have large tumours with increased frequency of N1 stage, grade 3 and ER negative tumours. Young women with HER2 positive disease have been shown to have a worse prognosis, irrespective of ER status. In terms of ER risk of death and recurrence, this was shown to vary over time, with a higher risk of death for ER negative tumours prior to four years, and post four years the risk of death was higher for patients with ER positive tumours. In terms of survival, this corresponded to a reduced survival rate of almost 10% in patients with ER negative tumours comparted to ER positive tumours at five years (75.7% vs. 85.0%, respectively), but by eight years, the corresponding survival difference was negligible (67.7% vs. 67.5%, respectively).

The five year survival rate of the entire cohort was shown to be markedly similar to the 2005-2009 national statistics relative survival in women aged 15-39; thus laying emphasis on the fact that, despite modern oncology treatment, advances in survival are lower in young women compared to older patients with breast cancer[1]. Young women with invasive breast cancer have also been shown to have an increased risk of early disease recurrence, and this paper highlighted the need for new treatment approaches to be investigated in this young age group.

10.3 Discussion - Paper 2

10.3.1 Aim and background

This was the second paper to describe and present outcomes from the POSH cohort. The main aim of this paper was to describe the tumour pathology and treatment and compare outcomes of young women with invasive breast cancer according to their ethnic origin.

Similar to **Paper 1**, this paper described the cohort in addition to highlighting how all patients in the POSH cohort were managed within the NHS, and thus had equal access to healthcare, including diagnostic, surgical and oncology services. Sources of data collection were described, including how ethnic origin was self-reported, and patients were then categorised according to the National Institute of Health reporting guidelines[113] into either 'White/Caucasian', 'Black', 'Asian' or 'Other'.

The analysis population of 2956 patients from the POSH cohort used for **Paper 1** was also used as the analysis population for **Paper 2**.

10.3.2 Key statistical methods

Key statistical methods implemented included using summary statistics to describe the patient characteristics and risk factors of the cohort, as well as to compare the tumour characteristics and treatment information by ethnic origin; specifically White/Caucasian vs. Black, White/Caucasian vs. Asian, and Black vs. Asian.

Concerning survival, both OS and DDFI (labelled DRFS in this paper), were described using Kaplan-Meier curves. UVA and MVA were carried out using Cox regression, with MVA (for DDFI only) adjusting for grade, maximum overall tumour size[h], nodal status, BMI and ER status. As was demonstrated in the previous publication, the effect of ER status varied over time, however as it was not the comparator of interest and due to the FPSMs being computationally more complex and time-consuming, the multivariable models which adjusted for ER status were instead stratified by ER status for the analyses in this paper. Additional subgroup UVA and MVA analyses were carried out separately on patients with: ER negative tumours; ER positive tumours; and TNT tumours. For the subgroup analyses, all MVA (for DDFI only) were adjusted for grade, maximum overall tumour size, nodal status and BMI. Similar to **Paper 1**, all statistical analyses were performed using Stata version 11.2[74] on records with complete data, and conducted according to a pre-specified analysis plan (see **Appendix A3**) on follow-up data received up to 11th April 2012.

10.3.3 Key findings

Of the 2956 patients included in the analysis population, self-reported ethnicity was available for 2915 patients, of which 2902 reported a single ethnic/racial category. Thirteen patients reported mixed ethnicity: 12 were subsequently categorised as Black and one categorised as Asian. As a result, of the 2915 patients with self-reported ethnicity available: 2690 patients (92.3%) were categorised as White/Caucasian; 118 patients (4.0%) as Black; 87 patients (3.0%) as Asian; and 20

[h] Maximum overall tumour size includes the maximum invasive tumour size and ductal carcinoma *in situ*

patients (0.7%) were from 'Other' ethnic groups. All 2956 patients were included in the descriptive tables, but summary statistics and univariable survival analyses were only carried out on the 2895 patients who were made up of White/Caucasian, Black, or Asian patients. Information on grade, maximum overall tumour size, nodal status, ER status and BMI, required for the MVA, was available for 2581 patients.

In terms of patient characteristics, median age was statistically significantly lower in Asian patients compared to White/Caucasian patients (35 vs. 36, p<0.001) or Black patients (35 vs. 36, p=0.0472). Median BMI was also statistically significantly lower in Asian patients compared to White/Caucasian patients (24.1 kg/m^2 vs. 24.6 kg/m^2, p=0.0401) or Black patients (24.1 kg/m^2vs. 26.9 kg/m^2, p<0.001), and in turn BMI was statistically significantly lower in White/Caucasian patients compared to Black patients (24.6 kg/m^2 vs. 26.9 kg/m^2, p<0.001). Most women had at least one child, with the proportion of women with children not statistically significantly different across ethnic groups. Similarly, almost all women had breast cancer that was symptomatic at presentation and were premenopausal at diagnosis, irrespective of ethnic origin. Use of the contraceptive pill was similar in Black and Asian patients but significantly higher in White/Caucasian patients compared to Black patients (90.0% vs. 67.8%, p<0.001) and Asian patients (90.0% vs. 56.3%, p<0.001). The proportion of patients who ever smoked varied across all ethnic groups, with the largest proportion found in White/Caucasian patients (52.4%), followed by Black patients (41.2%), and finally Asian patients (18.8%). The number of patients with a reported first degree relative with breast cancer were relatively small across ethnic groups, with the corresponding proportion of patients similar between ethnic groups (White/Caucasian: 14.7% vs. Black: 15.8% vs. Asian: 6.0%).

Describing tumour information by ethnic origin identified that the majority of patients had grade 3 tumours, followed by grade 2 then grade 1 tumours. The distribution of grade by ethnic origin was statistically significantly different between White/Caucasian patients vs. Asian patients and between Black vs. Asian patients, with a smaller proportion of grade 1 tumours found in White/Caucasian and Black patients compared to Asian patients (5.6%, 0.9% 11.8% respectively). . Most White/Caucasian patients had localised tumours compared to Black and Asian patients (71.1%, 56.6% and 63.0%, respectively). The distribution of N and M stage were not statistically significantly different across ethnic groups. However, the median number of positive nodes recovered was statistically significantly higher in White/Caucasian patients compared to Asian patients (2 vs. 1, p=0.0143), and in Black patients compared to Asian patients (3 vs. 1, p=0.0169), but not statistically significantly different between White/Caucasian patients compared to Black patients (2 vs. 3, p=0.496). Although not statistically significant, when compared to other ethnic groups: White/Caucasian patients had a higher proportion of ER positive tumours; Black patients had a higher proportion of tumours which were PR positive and/or HER2 negative; and Asian

patients had a higher proportion of ER negative, PR negative and/or HER2 positive tumours. In terms of TNT status, White/Caucasian patients were statistically significantly less likely to have TNT tumours compared to Black patients (18.6% vs. 26.1%, p=0.043), and less likely, although not statistically significantly, compared to Asian patients (18.6% vs. 23.2%, p=0.291).

In terms of treatment information, the majority of patients had either BCS or mastectomy, with the rates of BCS higher in White/Caucasian patients (48.1%) compared to Black patients (39.8%), but slightly lower compared to Asian patients (48.3%). In White/Caucasian patients, the largest proportion of patients underwent adjuvant chemotherapy (73.8%), followed by neo-adjuvant chemotherapy (14.8%), no chemotherapy (9.7%) and palliative (1.7%). In contrast, the rate of adjuvant chemotherapy was lower in Black and Asian patients (65.3% and 69.0%, respectively), whilst the rate of neo-adjuvant chemotherapy was much higher (23.7% and 20.7%, respectively), and palliative chemotherapy was also slightly higher (4.2% and 3.5%, respectively). Use of anthracyclines and/or taxanes was similar across all ethnic groups. Formal statistical tests comparing adjuvant trastuzumab and radiotherapy by ethnic groups was not appropriate to undertake due to the amount and nature of missing data. However, rates of confirmed adjuvant radiotherapy appeared to be similar across ethnic groups. Hormone therapy information was presented by ethnic groups for patients with ER positive tumours only. However, once again formal statistical tests were precluded due to the nature of the missing data.

Regarding survival, the five year OS was significantly lower in Black patients compared to White/Caucasian patients (71.1% [95% CI: 61.0 to 79.1%] vs. 82.4% [95% CI 80.8 to 83.9%]), and lower in Black patients, although not significantly, compared to Asian patients (78.7% [95% CI 66.7 to 86.7%]), which was in turn higher, although not significantly, than White/Caucasian patients. Results were similar for DDFI[i], although Black patients had significantly lower DDFI at five years compared to both White/Caucasian and Asian patients (62.8% [95% CI: 52.1 to 71.8%], 77.0% [95%CI: 75.3 to 78.6%], and 77.0% [95%CI: 65.1 to 85.3%], respectively), with the DDFI at five years identical for White/Caucasian patients and Asian patients. Similar results were found when assessing patients with ER positive tumours, however in the ER negative and TNT subgroups, DDFI was much higher for Asian patients compared to both Black and Caucasian/White patients; notably so in the TNT subgroup (Caucasian/White: 72.8% [95% CI: 68.4% to 76.8%]; Black: 65.8% [95% CI: 43.9% to 80.8%]; Asian: 94.7% [95% CI: 68.1% to 99.2%]). Comparisons at eight years were not able to be made due to the small number of Black and Asian patients with longer-term follow-up information available.

[i] Termed 'DRFS' (Distant Relapse-Free Survival) in the publication.

When adjusting for tumour size, grade, nodal status, BMI and ER status in a MVA, Black ethnicity was found to be a significant prognostic factor for poorer DDFI compared to Caucasian/White patients (HR [95% CI]: 1.50 [1.06 to 2.13]). Similar results were found when restricting the analyses to patients with ER positive (HR [95% CI]: 1.60 [1.03 to 2.47]), ER negative (HR [95% CI]: 1.31 [0.73 to 2.36]) and TNT tumours (HR [95% CI]: 1.18 [0.52 to 2.69]), although the effect was only found to be statistically significant in the ER positive subgroup. In contrast, Asian patients were associated with slightly improved DDFI in the MVA overall and in the subgroup analyses, although these differences were not found to be statistically significant.

10.3.4 Strengths

Of the self-reported ethnicity, almost all patients (n=2902 [99.6%]) reported a single ethnic/racial category, with only 13 patients (0.4%) reporting a mixed ethnicity and needing further categorisation. This was reassuring as it showed that distinctive categorisations could be made, and therefore associations with ethnicity could be clearly interpreted. In addition, the self-reported ethnicity in the POSH cohort was also later found to be concordant with the genetic testing carried out by principle component analysis using genome-wide single-nucleotide polymorphism (SNP) typing[114], thus further highlighting the accuracy of the data.

Another key strength of this paper was that all patients in the POSH cohort were managed within the UK NHS i.e. all patients had equal access to healthcare including diagnostic, surgical and oncology services; thus removing important potential confounding social and economic factors. Moreover, in terms of treatment, receipt of chemotherapy and use of anthracylines/taxanes were similar across ethnic groups in this cohort, unlike other studies which reported a large disparity between Black and White/Caucasian patients[115,116]. Furthermore, a limitation highlighted in **Paper 1** was the fact that women below the age of 40 in the UK were not routinely screen detected. However, this worked as an advantage in this study as it was thus removed as a potential confounding factor.

Findings from this paper that Black patients have a significantly inferior survival, both in terms of OS and DDFI, are consistent with other studies[32,36-40,117], together with the finding that Asian and White/Caucasian patients have similar outcomes[117]. This study was also able to demonstrate the effect of ethnicity on outcome after adjusting for potential confounding factors in MVA and by carrying out a sensitivity MVA in patients with ER positive tumours separately. The effect of Black ethnicity on outcome was no longer statistically significant in the ER negative and TNT tumour subgroups, however this could have been due to a reduced number of events due to the reduced number of patients in these subgroups.

10.3.5 Limitations

Potential limitations of this paper in terms of the analyses undertaken were two-fold. Firstly, that, a closed testing approach was not used for the baseline comparisons by ethnic origin; a result of which was that additional tests may have been carried out unnecessarily. However, it should equally be noted that these comparisons were all pre-specified in the analysis plan, and did not form part of the primary outcome of the paper. Secondly, although the effect of ER status on survival was found to vary over time, Cox regression models stratified by ER status were used instead of FPSMs due to the FPSMs being computationally more complex and time-consuming to undertake in the required analyses time. However, as ER status was not the comparator of interest, stratified Cox regression models were still statistically appropriate and had the added benefit of being easy to interpret.

Another limitation of the study was that the majority of women in the POSH cohort were White/Caucasian (2690 [92.7%]), with only small numbers of Black (106 [3.7%]) and Asian (86 [3.0%]) patients. Subsequently, this led to reduced numbers in the comparisons, particularly evident when carrying out the MVA for the ER and TNT tumour subgroups. The number at risk was also very small, particularly at eight years for Black patients with ER negative tumours, which could contribute to the lack of statistical significance for Black ethnicity and DDFI in the subgroup MVA. Nevertheless, this does reinforce the inferior outcomes of Black patients compared to White/Caucasian patients, especially in patients with ER positive tumours, as the estimates were shown to be statistically significant, despite reduced numbers available for these comparisons.

At the time of the publication, there were insufficient data to assess chemotherapy dose intensity in the cohort. However, evidence has shown an increased mortality in Black patients despite similar relative adjuvant chemotherapy dose intensity[118]. Another potential limitation of this study was that gene expression profiling information was unavailable at the time of analyses so conclusions could not be drawn regarding the impact of this factor on outcomes with regards to ethnicity. Nevertheless, this study was able to demonstrate clear differences in DDFI between Black and White/Caucasian patients, after adjustment for a number of key prognostic and confounding factors. Moreover, although MVA were carried out on DDFI only and not OS, it is also equally important to note that little difference was observed between the DDFI and OS UVA results, with similar results shown at both five and eight years.

Another potential limitation of the study was that pregnancy was assessed as a risk factor for breast cancer as opposed to a prognostic factor and dates of second and subsequent pregnancies were not recorded; evidence suggests that breast cancers diagnosed during or within a year of pregnancy are associated with more aggressive tumours[119], which could explain the results found in this study

by a higher number of pregnancy associated (and more aggressive) tumours in Black patients. The fact that Black patients who had children by the time of their diagnosis were more likely to have more children than White/Caucasian patients suggests that Black patients had a greater period of time in which they were pregnant before the date of their diagnosis, and could therefore have been at a higher risk of pregnancy-related breast cancer.

10.3.6 Conclusions

This second publication of results from the POSH cohort was able to present outcomes according to ethnicity. Young women with invasive breast cancer and of Black ethnicity were shown to have more aggressive and larger tumours, and have an increased risk of breast cancer recurrence compared to Caucasian/White patients despite equal access to healthcare. Moreover, Black ethnicity has been shown to be an indicator for poorer distant disease-free survival in young women with invasive breast cancer, independent of other prognostic factors; suggesting that current treatment may not be as effective for Black patients and further research should be carried out to provide more optimal treatment approaches for this population.

10.4 Discussion - Paper 3

10.4.1 Aim and background

The aim of this third paper reporting outcomes of the POSH cohort was to explore the associations of obesity with tumour pathology, treatment and outcome in young women with invasive breast cancer.

As with **Papers 1** and **2**, this paper described the cohort, including diagnostic, surgical and treatment information, and was presented according to body mass index (BMI); calculated using height and weight, measured at trial registration. Patients were categorised into World Health Organisation (WHO) defined BMI categories according to the BMI value recorded at baseline: underweight (BMI≤18.5 kg/m^2); healthy-weight (18.5 kg/m^2<BMI<25 kg/m^2); overweight (Ov) (25 kg/m^2≤BMI<30 kg/m^2); and obese (Ob) (BMI≥30 kg/m^2)[120]. Due to small numbers of patients categorised as underweight, these were combined with patients categorised as healthy-weight to form an underweight/healthy-weight category (U/H).

The paper utilised data from 3095 women recruited from the POSH cohort, of whom 3022 satisfied the eligibility criteria. Women aged 41-50 with a known BRCA1 or BRCA2 gene mutation, together with patients with missing primary tumour data, and a further 113 patients with missing BMI

information were excluded from the analyses. This resulted in 2843 patients being included in the analyses, including patients with metastatic disease at presentation (M1 stage patients).

10.4.2 Key statistical methods

Summary statistics were used to describe the patient and tumour characteristics and treatment information of the cohort by BMI categories. Methods of analyses were improved for this paper compared to the previous outcome paper following gained knowledge and understanding, and a closed testing approach was used for the comparisons: binary/categorical and continuous variables were first compared by BMI categories overall (Underweight/Healthy [U/H] vs. Overweight [Ov] vs. Obese [Ob] patients) using Pearson $\chi 2$ tests and Kruskal-Wallis tests. If, however, a significant difference was found when comparing BMI categories overall, further tests comparing individual BMI categories were then carried out using Pearson $\chi 2$ tests and Mann-Whitney U tests.

In terms of survival, both OS and DDFI were described using Kaplan-Meier curves. UVA and MVA were carried out using Cox regression, with MVA (for both OS and DDFI) adjusting for age at diagnosis, grade, maximum overall tumour size[j], nodal status, HER2 status, and ER status. Similar to **Paper 2**, the effect of ER status varied over time but was not the comparator of interest and due to the FPSMs being computationally more complex and time-consuming to undertake, the multivariable models were therefore stratified by ER status for the analyses in this paper. Similar to **Papers 1** and **2**, all statistical analyses were performed using Stata version 11.2[74] on records with complete data, and conducted according to a pre-specified analysis plan (see **Appendix A3**). However, unlike **Papers 1** and **2**, follow-up information used was based on data taken at a later time-point of 22nd October 2013.

10.4.3 Key findings

Of the 2843 patients analysed, 1526 (53.7%) patients were categorised as U/H (BMI<25 kg/m^2) including 36 underweight patients (BMI of ≤18.5 kg/m^2), 784 (27.6%) patients categorised as Ov (25 kg/m^2≤BMI<30 kg/m^2), and 533 (18.7%) as Ob (BMI≥30 kg/m^2).

U/H patients were slightly younger than Ov and Ob patients (median age: 36, 37 and 37, respectively). Furthermore, the distribution of age at menarche and age at first birth was also different across BMI groups, despite having identical median age at menarche and age at first birth in each group, indicating a statistical but not clinical difference between groups. A significantly

[j] Maximum overall tumour size, or total tumour diameter, includes the maximum invasive tumour size and ductal carcinoma *in situ*

smaller proportion of U/H patients had children compared to Ov and Ob patients (69.7%, 75.6% and 74.0%, respectively), which could indicate that a higher proportion of women are overweight/obese as they gained weight following pregnancy. Approximately 50% of all patients had smoked at least once, irrespective of BMI. Furthermore, the majority of patients were symptomatic, premenopausal, and had used the contraceptive pill at least once, irrespective of BMI. However, ethnicity by BMI categories was significantly different, with a higher proportion of Black patients being Ob compared to U/H and Ob patients (U/H: 3.1%, Ov: 4.1% and Ob: 6.6%), and a higher proportion of White/Caucasian patients being U/H compared to Ov and Ob patients (U/H: 93.1%, Ov: 91.9%, and Ob: 91.7%).

In terms of treatment, the highest proportion of patients undergoing BCS was found in Ob patients (49.3%), followed by Ov (48.0%) and finally U/H patients (46.3%), whilst the proportion of patients undergoing adjuvant chemotherapy was largest amongst Ov patients (76.0%) compared to U/H patients (75.2%) and Ob patients (70.2%). Moreover, the smallest proportion of patients who received any neo-adjuvant chemotherapy was observed in U/H patients (12.9%), compared to 15.4% in Ov patients and 22.1% in Ob patients. The vast majority of patients received anthracyclines and/or taxanes and confirmed adjuvant radiotherapy, irrespective of BMI. In addition, for patients with ER positive tumours, similar proportions of confirmed adjuvant hormone treatment was observed in all BMI groups.

Tumour characteristic information presented demonstrated that U/H patients had significantly smaller tumours compared to Ov and Ob patients, with tumours in Ov patients also significantly smaller compared to Ob patients (median tumour size in mm: 20, 24 and 26, respectively). Histological grade was also significantly different across BMI groups, specifically with regards to U/H patients compared to Ob patients, with a smaller proportion of grade 3 tumours but higher proportion of grade 1 and 2 tumours, observed in U/H patients compared to Ov and Ob patients. Conversely, the distribution of histological grade in Ov patients was closely matched to that of Ob patients. The focality, M stage and HER2 status of the tumour were evenly distributed across BMI categories. However, a higher proportion of U/H patients had N1 stage tumours, which were ER positive, and/or PR positive compared to Ob patients.

In terms of survival, median follow-up was 5.87 years, and ranged from one month to 13 years. Despite longer follow-up, the proportion lost to follow-up was only 87 (3.1%) of patients. A total of 674 deaths had occurred (a further 61 deaths since 11th April 2012), and 801 distant relapse events or deaths [data not presented in publication]. Despite the increased number of events for both DDFI and OS, the number at risk was still greatly reduced by ten years, and so five and eight year event rates were presented in the paper.

When comparing survival by BMI categories, DDFI was significantly lower in Ov patients (HR [95% CI]: 1.29 [1.09 to 1.52]) and Ob patients (HR [95% CI]: 1.44 [1.20 to 1.72]) compared to U/H patients (five year DDFI% [95% CI]: U/H 78.6% [76.4% to 80.7%] vs. Ov 72.9% [69.5% to 75.9%] vs. Ob 71.3% [67.1% to 75.0%]; eight year DDFI% [95% CI]: U/H 73.0% [70.3% to 75.5%] vs. Ov 68.1% [64.3% to 71.5%] vs. Ob 63.5% [58.3% to 68.3%]). When splitting the cohort by ER status, DDFI for patients with ER negative tumours remained significantly lower in Ov patients (HR [95% CI]: 1.39 [1.06 to 1.82]) but not for Ob patients (HR [95% CI]: 1.16 [0.85 to 1.58]), compared to U/H patients. Conversely, DDFI for patients with ER positive tumours was no longer significantly lower in Ov patients (HR [95% CI]: 1.21 [0.98 to 1.50]) but was significantly lower for Ob patients (HR [95% CI]: 1.58 [1.26 to 1.98]). MVA of DDFI, adjusting for age, grade, size nodal status and HER2 status, and stratified by ER status provided similar results to the unadjusted HRs. However, when also adjusting for ethnicity, DDFI was no longer significantly lower for Ob patients compared to U/H patients (HR [95% CI]: 1.22 [1.00 to 1.51]). MVA of DDFI in patients with ER negative tumours found no significant difference between BMI categories irrespective of whether ethnicity was adjusted for or not. However, in patients with ER positive tumours, DDFI in Ob patients remained significantly lower compared to U/H patients in both MVA (HR [95% CI]: 1.37 [1.06 to 1.76]). Similar results to those observed for DDFI were found when comparing OS by BMI categories, with a key difference observed in the MVA adjusted for ethnicity, where Ob patients remained at significantly higher risk of death compared to U/H patients (HR [95% CI]: 1.35 [1.08 to 1.68]).

10.4.4 Strengths

A key strength of this paper was the amount and quality of data available for analyses. Of the 2956 patients analysed, BMI information was calculated using objective measurements of height and weight recorded at patient registration, and was only missing for 113 (3.8%) patients, which enabled 2843 patients to be available for the analyses. Conversely, a number of previous studies have relied on self-reported height and weight, or relied solely on the use of BMI data for patients who received chemotherapy only[121]. This paper also used the standard definitions of WHO BMI categories available at the time of analyses to categorise patients accordingly, ensuring both clarity and the ability to directly compare with other meta-analyses. Furthermore, analyses for this paper were based on data taken at a later time-point compared to **Papers 1** and **2**; and thus more events were available.

Another key strength of this paper was that a closed testing approach was used when comparing demographic, tumour and treatment information by BMI categories i.e. comparisons were first undertaken across BMI categories overall before individual comparisons were made. This was

statistically more appropriate than performing individual testing first as was the case in **Paper 2**, as it reduced possible multiple testing which might have arisen.

The finding from this paper confirmed evidence from previous studies and meta-analyses in the literature, in that obesity was found to be associated with inferior OS and DDFI compared to U/H patients, as demonstrated by the unadjusted HRs comparing Ov and Ob vs. U/H patients[44,45].

Significant differences observed in survival might have been due to adverse biological differences associated with obesity found in this study which, again, is consistent with previous literature. Indeed, compared to U/H patients, Ob patients were found to have more advanced disease[122] with biologically adverse tumours; with statistically significantly larger, higher grade tumours with an increased likelihood of having nodal involvement, being ER negative, and being TNT. The reasons for these differences are unclear, with evidence suggesting that either the micro-environment of the tumour may be affected as obesity is associated with raised levels of adipocytokines and results in inflammation and alteration of adipokine (e.g. leptin and adiponectin) signalling and insulin levels[123,124].

Inferior OS and DDFI was also demonstrated when adjusted for tumour grade, nodal status, HER2 status, age at diagnosis, and stratified by ER status in both Ov and Ob patients, compared to U/H patients, with the 95% lower HR CI for DDFI just falling below one (p=0.052) for Ob patients when also adjusted for ethnicity. Although the MVA result was not as conclusive for DDFI as that demonstrated in OS, the findings were nonetheless supportive of previous studies which found obesity associated with poorer OS and DDFI compared to U/H patients[122,125,126].

Other consistencies in the findings of this paper with the literature include the fact that obesity was shown to be an independent prognostic factor in patients with ER positive tumours but not for ER negative tumours. Despite smaller numbers in the ER negative subgroup, almost 1000 patients were nevertheless included in these analyses. This corroborates findings from data on 80,000 patients across 70 trials, which found a significant association between obesity and prognosis in pre- and peri-menopausal women with ER positive disease only[127].

10.4.5 Limitations

The ABCSG-12 trial in premenopausal women with breast cancer reported that BMI significantly influenced the efficacy of anastrozole plus goserelin but did not affect the prognosis of patients treated with tamoxifen plus goserelin[128]. Although 88.6% of patients with ER positive tumours in the POSH cohort received tamoxifen, limited use of ovarian suppression was reported in this subgroup. As a result, this paper could suggest that tamoxifen without use of ovarian suppression

might not be as effective in patients with a high BMI. One of the potential limitations of this paper is that tamoxifen adherence information was not collected, and as a result, exclusion of the possibility of an association between obesity and lower adherence of tamoxifen cannot be made.

Another potential limitation of this paper is that data on chemotherapy dose intensity for the entire study is incomplete. This limitation is highlighted when attempting to establish an association between reduced use of chemotherapy in Ob patients, which previous studies have inferred might lead to inferior survival[122]. Although chemotherapy regimen information from the POSH cohort conversely shows that the proportion of patients having anthracycline and/or taxanes was highest in Ob, followed by Ov and finally in U/H (93.1% vs. 92.9% vs. 88.9%, respectively), the majority of cytotoxics for breast cancer are dosed according to the patient's body surface area, which is not a formula designed for extreme ranges of weight. In addition, it was also reported in a 2012 review that almost 40% of obese cancer patients receive capped chemotherapy[129]. It is therefore difficult to determine an accurate amount of chemotherapy received in patients in the POSH cohort without complete dose intensity information. However, despite these limited data, a sensitivity analysis on 77 patients from the Southampton Oncology Centre indicated that obese patients were significantly more likely to receive a dose delay in adjuvant chemotherapy compared to healthy weight patients (33.3% vs. 5.9%, p=0.0068 [data presented in text within the paper only]).

Improved survival in U/H patients might be confounded by the fact that ethnicity by BMI categories was significantly different as demonstrated in **Paper 2**; with a higher proportion of Black patients being Ob (who were demonstrated as having inferior survival), and a higher proportion of both White/Caucasian and Asian patients being U/H (demonstrated as having improved survival)[130]. However, **Paper 2** was able to demonstrate ethnicity was an independent prognostic factor for OS and DDFI, and the MVA carried out in this paper adjusting for ethnicity was also able to demonstrate that obesity was an independent prognostic factor for OS (overall and in the ER positive subgroup), and in DDFI (ER positive subgroup). Moreover, although Cox regression models stratified by ER status (or analysed separately for each tumour status) were used instead of FPSMs (similar to **Paper 2**), this has not been highlighted as a limitation in this paper as it has allowed for a clear, easy and useful comparison between the results of these two papers, in addition to comparisons with previous literature.

10.4.6 Conclusions

This third publication of results from the POSH cohort presented outcomes according to BMI, and provided confirmatory evidence demonstrating that obesity is associated with inferior survival in

young women with invasive breast cancer, and is an independent prognostic factor in patients with ER positive tumours, indicating that further research into ways to optimise treatment is needed for this patient group.

10.5 Discussion - Paper 4

10.5.1 Aim and background

The aim of this fourth paper was to compare tumour pathology and outcomes in patients from the POSH cohort with (and without) a reported family history of breast cancer, to determine whether the presence, or degree, of family history is an independent prognostic factor in young women with invasive breast cancer.

This paper described the personal characteristics, tumour pathology, treatment and family history of breast/ovarian cancer. The paper described how family history of breast/ovarian cancer was recorded using a self-reported questionnaire provided at recruitment. The questionnaire comprised of details of all first and second degree relatives, including the current age (or age at death), any cancer diagnosis, age at cancer diagnosis and treatment type information of the relative(s). Additional details of any other family members with a history of malignancy were also requested. A pedigree was subsequently drawn using this information collected. Patients were then categorised as having a positive family history (FH+) if they reported at least one first or second degree relative with a reported diagnosis of breast and/or ovarian cancer. The remaining group of patients were categorised as having a negative family history (FH-), unless they did not complete a family history questionnaire (i.e. missing FH).

The analysis population of 2956 patients from the POSH cohort used for **Papers 1** and **2** was also used as the analysis population for **Paper 4**.

10.5.2 Key statistical methods

Key statistical methods implemented included using summary statistics to describe and compare patient characteristics, tumour pathology, and treatment information by reported family history status (FH- vs. FH+), as well as by patients with at least one reported first degree relative (FDR) versus patients with at least one reported second degree relative (SDR), with the SDR group excluding those patients with a reported FDR. In addition, patient characteristics and tumour pathology was also compared separately by reported family history status for patients with ER negative and ER positive tumours.

In terms of outcomes, only DDFI was analysed for this paper, and was described using Kaplan-Meier curves based on information from the POSH cohort. Similar to **Paper 1**, the effect of ER status on survival was found to vary over time for DDFI, and FPSMs were used with ER status fitted as a time-varying covariate in the adjusted regression estimates. Multivariable models were modelled by fitting reported family history as a binary covariate (FH- vs. FH+), and adjusted for the following covariates recorded at diagnosis, regardless of statistical significance to the model fit: age; tumour size (fitted as a log-transformed continuous covariate); grade; nodal status; lymphovascular invasion; focality (distribution of the tumour); ER status; PR status; and HER2 status of the tumour. As with **Paper 1**, the choice of degrees of freedom for both the baseline hazard rate and time-dependent effect of each FPSM produced were found using the lowest AIC to obtain the best model fit for each FSPM [not described in publication]. HRs for ER status were then reported over time. However, unlike **Papers 1** to **3**, methods of analyses were further improved for this paper following gained knowledge and understanding and this paper incorporated the use of multiple imputation so that even patients with missing FH were included in the MVA (imputed as either FH- or FH+). All missing data were assumed to be either MAR or MCAR, and censoring was assumed to be non-informative. A set of multiple imputed datasets were then generated using the 'ice' command in Stata, with MVA subsequently carried out using the 'mim' command. All statistical analyses were performed using Stata version 11.2[74], and were conducted accordingly to a pre-specified analysis plan (see **Appendix A3**) on follow-up data received up to 22nd October 2013 (similar to **Paper 3**).

10.5.3 Key findings

Of the 2956 patients analysed, family history patient questionnaires were returned by 2850 (96.4%) of patients, of which, 1878 (65.9%) of patients reported no family history (FH-) and 972 (34.1%) reported a positive family history (FH+).

Summary statistics and univariable survival analyses were carried out on the 2850 patients with family history information available. There was no statistically significant difference in age at diagnosis between FH- and FH+ patients, nor between FDR and SDR patients. However, the distribution of presentation was significantly different between groups, with a higher frequency of surveillance detected tumours in FH+ patients (29 [3.0%]); the majority of which were FDR patients (25 [6.0%]), whereas only 1 (0.1%) of FH- patients had tumours which were surveillance detected.

In terms of tumour pathology, histological grade was significantly different between FH- and FH+ patients, with a smaller proportion of grade 3 tumours in FH- patients compared to FH+ patients (58.9% vs. 63.3%). There were no statistically significant differences between FH- and FH+ patients in terms of tumour size, focality, N stage, M stage, ER, PR status or lymphovascular invasion.

However, a statistically significantly higher proportion of FH- patients had negative HER2 tumours compared to FH+ patients (28.8% vs. 24.7%). There were no statistically significant differences between patients in the FDR and SDR groups, except for tumour size, which were smaller in the FDR group (median 20mm vs. 23mm, respectively). Analysing patients with ER negative vs. positive tumours separately, presentation or tumour remained statistically significantly different between FH- and FH+ patients for both patients with ER negative and ER positive tumours. However, histological grade and HER2 status was no longer statistically significantly different between FH- and FH+ patients. Conversely, tumour and M stage were significantly different between FH- and FH+ patients in the ER negative subgroup. No other statistically significant differences were observed between FH- and FH+ patients in the ER positive subgroup.

Treatment information presented by FH- vs. FH+ patients identified significant differences in surgical type, in which a higher frequency of FH- patients were reported to have BCS compared to FH+ patients (49.8% vs. 44.7%), and a lower frequency of mastectomies performed in FH- compared to FH+ patients (48.5% vs. 54.4%, respectively). Comparing treatment information by FDR and SDR patients showed a significant difference in chemotherapy timing, with a higher proportion of adjuvant therapy being performed in FDR vs. SDR patients (78.2% vs. 70.9%, respectively). In a subgroup of patients with ER positive tumours, it was determined that over 90% received hormone therapy (and was similar across family history groups). A higher proportion of FH+ patients had an oophorectomy (170 [23.3%]), compared to 148 (13.6%) in FH- patients, of which: the minority, 26 (15.3%) and 31 (20.9%), respectively, had an oophorectomy within 12 months of diagnosis i.e. likely to be part of their primary treatment; whilst the majority, 144 (84.7%) and 117 (79.1%), respectively, had an oophorectomy beyond 12 months of diagnosis i.e. likely to be due to genetic testing [data not presented in publication].

Regarding survival, there was no significant difference in DDFI between FH- and FH+ patients (UVA HR [95% CI]: 0.88 [0.75 to 1.02]; five year DDFI% [95% CI]: 74.9% [72.9% to 76.9%] vs. 77.4% [74.5% to 79.9%], respectively; eight year DDFI% [95% CI]: 68.7% [66.2% to 71.1%] vs. 72.0% [68.5% to 75.1%], respectively;). This was also the case when analysing FH status in the ER positive subgroup (UVA HR [95% CI]: 0.98 [0.81 to 1.18]; five year DDFI% [95% CI]: 77.6% [75.1% to 79.9%] vs. 77.4% [73.8% to 80.6%], respectively; eight year DDFI% [95% CI]: 70.1% [67.0% to 73.0%] vs. 71.1% [66.6% to 75.0%], respectively). In the ER negative subgroup, however, FH+ patients were found to have significantly improved DDFI compared to FH- patients (UVA HR [95% CI]: 0.74 [0.57 to 0.96]; five year DDFI% [95% CI]: 69.7% [65.9% to 73.2%] vs. 77.2% [72.2% to 81.4%], respectively; eight year DDFI% [95% CI]: 66.1% [61.7% to 70.0%] vs. 73.6% [67.9% to 78.5%], respectively). Comparing FDR and SDR patients also showed no significant differences in DDFI (for all patients, and when assessing this in the ER positive and ER negative subgroups). When adjusting for other factors, MVA results

demonstrated no significant differences in DDFI between FH- and FH+ patients, even in the ER negative subgroup (MVA HR [95% CI] for all patients: 0.89 [0.76 to 1.03]; MVA HR [95% CI] for ER positive subgroup: 0.95 [0.78 to 1.15]; MVA HR [95% CI] for ER negative subgroup: 0.80 [0.62 to 1.04]). Significant factors affecting DDFI in the MVA models included age at diagnosis, tumour size, PR status, grade (grade 3 vs. 1 only), lymphovascular invasion, ER status (at five years and eight years only). An additional sensitivity analysis looking at whether the definition of FH+ (which included both FDR and SDR patients) might have affected the results was carried out by moving the SDR patients into the FH- group. However, no significant difference between FH+ and FH- in DDFI was found.

10.5.4 Strengths

A number of key strengths of this paper included the fact that almost all patients completed a family history questionnaire, with only 3.6% missing. Similar to **Papers 1** to **3**, there was also minimal loss to follow-up, thus increasing the number of events and power for comparisons. Equally, unlike **Papers 1** to **3**, the use of multiple imputation implemented in this paper was able to further account for any missing data in any covariates of the MVA. The statistical strengths of the MVA of this paper have also been highlighted by the additional incorporation of FPSMs in the MVA to account for the time-varying effect of ER status.

Another key strength of this paper was the contribution of its findings to the existing literature, which contained conflicting information with regards to outcome for patients with/without a positive reported family history of breast cancer[56-64]. Previous literature in support of the findings from this study that FH+ in patients did not significantly affect DDFI included: a study on a large prospective population-based study of 905 women with young-onset disease (aged <35 years)[62]; a subgroup of women aged ≤50 years from a Swedish population registry-based study of over 17,000 patients[64]; and a large population-based study of over 4,153 patients from their breast cancer registries (California, Melbourne and Ontario)[61]. Conversely, previous studies which found an improvement in survival in FH+ patients included Malone et al which used two retrospective studies of 1,260 young women aged ≤45 years which found a survival advantage in patients with an affected first-degree relative[56]. Although the Malone et al findings contained information on long-term follow-up, it was limited by being retrospective, with women enrolled between 1983 and 1992, and a number of oncological treatments have changed since this time; highlighted by the fact that only two-thirds of patients received chemotherapy[56]. Conversely, the majority of patients in the POSH study were treated with modern anthracycline- and/or taxane-based regimens, and with patients with HER2 positive tumours treated with trastuzumab and those with ER positive tumours treated with hormone therapy. The Malone et al study was also limited by being selected for ethnicity from

two North American studies; and as highlighted in **Paper 2**, access to equal healthcare and treatment might vary between different ethnic groups in North America[115]. Moreover, a sensitivity MVA carried out in **Paper 4** to allow for a more direct comparison with this study assessed whether FDR patients had a DDFI advantage over any FH- patients (including SDR patients), but did not show any statistically significant difference in DDFI.

10.5.5 Limitations

This paper also contained a number of potential limitations. Unlike **Paper 3**, for example, the comparator was based on self-reported information (family history questionnaire), not on data objectively taken. However, a number of studies have shown that self-reporting is fairly robust with regards to breast cancer in close relatives[57,61,62,131]. Family history is also a dynamic construct i.e. it is inevitable for this status to change over time as patients seek more information about other family members' medical and cancer history and as new diagnoses arise. However, unlike retrospective and registry studies, the POSH study was prospective, with family history status determined at the point of enrolment to the study, shortly after diagnosis, and therefore less vulnerable to biases in recall/knowledge of family history status.

Differences between FH- vs. FH+ patients and FDR vs. SDR patients should also be highlighted here. FDR patients had significantly smaller tumours compared to SDR and FH- patients, and, coupled with the fact that the majority of patients in whom breast cancer was screen-detected were in the FH+ group, most probably reflects an earlier presentation of the disease to medical services. However, it might also be indicated that this difference would be negated by the fact that there was a higher proportion of grade 3 tumours in the FH+ group.

Another potential limitation highlighted in this paper, which could also have potentially affected **Papers 1** to **3**, is of potential survival bias of the study, otherwise known as left-truncation[132]. Patients were enrolled up to one year after their diagnosis, and subsequently patients who were diagnosed and died within one year who might have otherwise joined the study (if enrolment was fixed to an earlier time-point) did not join because they died. Consequently, very early deaths (those within one year of diagnosis) might be under-represented in this study. However, as demonstrated in **Paper 1**, a similar proportion of patients who presented with M1 disease at presentation were found in this cohort compared to the national registry data, therefore reducing the impact of this potential limitation.

10.5.6 Conclusions

This fourth publication of results from the POSH cohort demonstrated that family history of breast cancer is not an independent prognostic factor for recurrence in young women with invasive breast cancer treated with modern oncological treatments for the disease. The importance of the findings from this paper is highlighted by the fact that evidence suggests that younger women have a greater fear of recurrence of breast cancer compared to older women[133], and that stronger family history of the disease may lead to greater anxiety about both recurrence and death from breast cancer after witnessing cancer within their family[134]. This study demonstrates that young women who have a positive family history of breast cancer, and are diagnosed with the disease, should be reassured by their surgeon that they are at no higher risk of recurrence or death from the disease compared to patients without a family history of the disease.

10.6 Discussion - Paper 5

10.6.1 Aim and background

This was the sixth paper to describe and present outcomes from the POSH cohort (and the fifth in the order of this thesis). The main aim of the paper was to determine the effect of a germline BRCA1 or BRCA2 mutation on breast cancer outcomes in young women with invasive breast cancer.

This paper presented the patient characteristics, clinicopathological and treatment information according to BRCA mutation status. The paper described how BRCA1 and BRCA2 genetic mutations were identified using blood DNA collected at the time of recruitment. A multiplex amplicon-based library preparation system was used to target a panel of breast-cancer-susceptibility genes (which included BRCA1, BRCA2, and TP53 genetic mutations) for sequencing. Variants unequivocally defined as pathogenic on the basis of multiple lines of evidence and expert review, and confirmed by Sanger sequencing, were assigned to the BRCA-mutation carrier group (BRCA+). All other patients who were tested were assigned to the 'no-mutation found' group (BRCA-), which included all patients with BRCA1 or BRCA2 variants of uncertain significance or very low penetrance, but excluded patients with a pathogenic variant of TP53. Patients with a confirmed BRCA1 mutation were also assigned to a separate group for analyses (the BRCA1+ group), with all other patients from the BRCA- group and patients with a confirmed BRCA2 mutation assigned to the corresponding BRCA1- group. Similarly, patients with a confirmed BRCA2 mutation were assigned the BRCA2+ group, and with all other patients from the BRCA- group and patients with a confirmed BRCA1 mutation assigned to the corresponding BRCA2- group.

The paper utilised data from 3095 women recruited from the POSH cohort, of whom 3021 satisfied the eligibility criteria. Women aged 41-50 with a known BRCA1 or 2 gene mutation[k], patients with M1 stage disease, patients with missing primary tumour data, patients with a confirmed TP53 mutation status, together with patients for whom no genotyping data was available, were excluded from the analyses. This resulted in 2733 patients being included in the analyses.

10.6.2 Key statistical methods

Two populations were analysed in this paper: the main analysis population of all 2733 patients; and a pre-specified subgroup of patients with triple-negative patients (TNBC population), formed of patients from the analysis population who had ER negative, HER2 negative and PR negative or PR unknown tumours. All analyses were carried out on both the analysis population and TNBC population, unless otherwise specified.

Summary statistics were used to compare patient characteristics information, clinicopathological information and treatment information by genetic status; with formal comparisons between BRCA+ vs. BRCA- patients and BRCA1+ vs. BRCA2+ patients.

With regard to survival outcomes, OS, DDFS and PDRS were analysed, and described using Kaplan-Meier plots, presented by BRCA+ vs. BRCA- patients, BRCA1+ vs. BRCA1- patients, and BRCA2+ vs. BRCA2- patients. UVA in the analysis population was performed using Cox regression models. However, similar to **Papers 1** and **4**, the effect of ER status varied over time and so FPSMs were used with ER status fitted as a time-varying covariate in the MVA. In addition, the effect of BRCA+/- status was also found to vary over time in the TNBC population, and was subsequently fitted as a time-varying covariate using FPSMs in both the UVA and MVA for this population. Similar to **Papers 1** and **4**, the choice of degrees of freedom for both the baseline hazard rate and time-dependent effect of each FPSM produced were found using the lowest AIC to obtain the best model fit for each FSPM. Multivariable models were adjusted for the following covariates recorded at diagnosis, regardless of statistical significance to the model fit: age at diagnosis; BMI (U/H, Ov and Ob); tumour size (in cm); N stage; ethnicity; use of taxanes; grade (analysis population only); HER2 status (analysis population only); and ER status (analysis population only). HRs for ER status (and BRCA status, where applicable) were reported over time at two, five and ten years. Similar to **Paper 4**, this paper also incorporated the use of multiple imputation in the MVA. All missing data were assumed to be either MAR or MCAR, and censoring was assumed to be non-informative. A set of

[k] Including one patient subsequently found not to have a confirmed BRCA1 or 2 mutation following the genetic testing performed for **Paper 5**, and therefore removed from 3022 patients who satisfied the eligibility criteria outlined in **Papers 1** to **4**.

multiple imputed datasets was then generated using the updated 'mi' command in Stata, with MVA subsequently carried out using the 'mi estimate' command.

Pre-specified sensitivity analyses included an adjustment to account for possible left-truncation; the MVA of the primary comparison comparing OS in BRCA+ patients with BRCA- patients was repeated in the analysis population only, with an additional adjustment of time from date of diagnosis to date of blood draw in order to investigate the degree of potential survival bias from time of diagnosis to entry into the study (date of blood draw). Another pre-specified analysis carried out for this paper was conditional imputation [not described in publication], which incorporated both the amount and timing of missing HER2 data in the POSH cohort; the MVA of the primary comparison comparing OS in BRCA+ patients with BRCA- patients was repeated in the analysis population only, whereby any existing HER2 values recorded for patients diagnosed prior to 2006 were initially removed. All missing HER2 data (including this removed data) were then imputed along with other missing data required for the MVA. Finally, where available, any of the newly imputed HER2 values for patients diagnosed prior to 2006 were replaced with the actual values recorded (which were initially removed). Pre-specified sensitivity analyses also included repeating all multiple-imputed MVA on corresponding complete-case data for each MVA model.

All statistical analyses were performed using Stata version 14.2[77], and were conducted according to a pre-specified analysis plan (see **Appendix A3**) (unless otherwise stated) on follow-up data received up to 26 July 2016.

10.6.3 Key findings

Of the 2733 patients in the analysis population, 338 (12%) were found to carry a BRCA1 (n=201) or BRCA2 (n=137) mutation. No patients carried both a BRCA1 and BRCA2 mutation. The TNBC population consisted of 558 patients (20% of the analysis population), of whom 136 (24%) carried a BRCA1 (n=123) or BRCA2 (n=13) mutation.

Summary statistics and univariable survival analyses were carried out on both the analysis and TNBC populations. In the analysis population, median time to blood draw from date of diagnosis was 5.5 months (IQR 3.2 to 10.7 months) for all patients. Several statistically significant clinicopathological differences were observed between both BRCA- vs. BRCA+ groups and BRCA1+ vs. BRCA2+ groups. BRCA1 carriers were younger (median age 36) compared to BRCA2 carriers and non-carriers (median age 37 in both groups). Moreover, a larger proportion of BRCA1 carriers were found to have tumours which were higher grade, ER negative, PR negative and/or HER2 negative compared to non-carriers, whilst BRCA2 carriers were found to have more ER and/or PR positive tumours. The frequency of BCS was also higher in BRCA1 carriers compared to both BRCA2 carriers and non-

carriers, with a higher proportion of mastectomies being carried out in BRCA2 carriers. In the TNBC population, grade was not significantly different between BRCA1 and BRCA2 carriers, in which almost all (94%) of TNBC patients had grade 3 tumours. Age at diagnosis remained significantly different between carriers and non-carriers but no longer between BRCA1 and BRCA2 carriers. The distribution of surgical type was also no longer significantly different between comparator groups in this population.

In terms of survival, median follow-up was 8.2 years, and ranged from 4.5 months to 15.5 years in the analysis population. Similar to **Papers 1** to **4**, only 91 (3%) of patients were lost to follow-up. A total of 678 deaths had occurred, of which 651 (96%) were due to breast cancer. There were 752 deaths or distant relapse events, of which 74 patients were still alive at last follow-up. With the increased length of follow-up since 2013, the number at risk remained high at ten years, and so two, five and ten year event rates were presented in this paper. In the TNBC population, there were 159 deaths or distant relapse events, of which 6 patients were still alive at last follow-up. All deaths in the TNBC population were due to breast cancer.

In the analysis population, there was no significant difference in OS between non-carriers and carriers (two year OS% [95% CI]: 96.6% [95.8% to 97.3%] vs. 97.0% [94.5% to 98.4%], respectively; five year OS% [95% CI]: 85.0% [83.5% to 86.4%] vs. 83.8% [79.3% to 87.5%], respectively; ten year OS% [95% CI]: 70.1% [67.7% to 72.3%] vs. 73.4% [67.4% to 78.5%], respectively; UVA HR [95% CI]: 0.99 [0.78 to 1.24]). There was also no difference between non-carriers and carriers even after adjusting for known prognostic factors including ethnicity and BMI (MVA HR [95% CI]: 0.96 [0.76 to 1.22]). Larger and higher grade tumours, obesity and Black ethnicity were associated with inferior OS, irrespective of BRCA+/- status. Similar to **Paper 1**, the effect of ER status was demonstrated to vary over time, with survival significantly higher for patients with ER positive tumours in the short term, but significantly inferior longer-term. Similar results were also found when assessing DDFS, and also when comparing outcomes of BRCA- with BRCA1+ or BRCA2+ separately. Moreover, the pre-specified sensitivity analyses adjusting for time to blood draw did not affect the results, nor did the incorporation of conditional multiple imputation of HER2 data [data not presented in publication]. Pre-specified sensitivity complete-case MVA also provided similar findings.

In the TNBC population, the effect of BRCA mutation status was found to vary over time, with a significant difference in OS between non-carriers and carriers observed in the short term but not in the longer-term (two year OS% [95% CI]: 91.4% [88.2% to 93.7%] vs. 94.8% [89.4% to 97.5%] respectively, UVA HR [95% CI]: 0.59 [0.35 to 0.99]; five year OS% [95% CI]: 74.2% [69.7% to 78.2%] vs. 81.3% [73.4% to 87.1%] respectively, UVA HR [95% CI]: 1.09 [0.67 to 1.75]; ten year OS% [95% CI]: 68.8% [63.4% to 73.7%] vs. 72.1% [61.9% to 79.9%] respectively, UVA HR [95% CI]: 1.96 [0.76

to 5.05]). Similar results were also found when adjusting for known prognostic factors including ethnicity and BMI (two-year MVA HR [95% CI]: 0.59, [0.35 to 0.99]; five-year MVA HR [95% CI]: 1.13 [0.70 to 1.84]; and ten-year MVA HR [95% CI]: 2.12 [0.82 to 5.49]). Although histological tumour grade was removed from the MVA due to small numbers of grade 1 and 2 tumours observed in the TNBC population, larger tumours, obesity and Black ethnicity were also associated with inferior OS, irrespective of BRCA+/- status in the TNBC population. Similar results were also found when assessing DDFS, except for the effect of BRCA status itself, which was no longer significant between non-carriers and carriers at two years. As with the analysis population, pre-specified sensitivity complete-case MVA also provided similar findings.

In order to determine whether the increased survival of BRCA+ patients with TNBC could be due to these patients undergoing risk-reducing surgery, a post-hoc sensitivity analysis was carried out for the MVA primary comparison comparing OS in BRCA+ patients with BRCA- patients in the TNBC population, but excluding those patients who underwent bilateral mastectomy within the first year of diagnosis. A total of 31 patients from the TNBC population underwent bilateral mastectomies and were excluded, and similar findings were observed in OS between BRCA non-carriers and carriers. To assess the pattern of improved survival followed by an apparently inferior survival in BRCA+ patients with TNBC, another post-hoc sensitivity analysis was carried out for the MVA primary comparison comparing OS in BRCA+ patients with BRCA- patients in the TNBC population, but excluding those patients who developed a new primary breast or ovarian cancer. A total of 37 patients from the TNBC population developed a new primary and were excluded, and once again, similar findings were observed in OS between BRCA non-carriers and carriers, although the difference observed at two years was no longer statistically significant, likely due to the reduced number of patients.

10.6.4 Strengths

Key strengths of this paper include the size of this prospective cohort. Few missing data and the inclusion of women with young onset breast cancer led to large numbers of both BRCA1 and BRCA2 mutation carriers, with a correspondingly high number of events in the comparator groups, enabling sufficient power for the main outcome analyses. Furthermore, as with **Paper 4**, the incorporation of both multiple imputation and FPSMs was able to further account for any missing data in any covariates of the MVA, as well as for the effect of any time-varying covariates in a clear and succinct manner.

The prospective cohort also minimised a number of biases and eliminated a number of weaknesses present in other studies, such as ascertainment biases introduced by retrospective studies which

select based on identification of cases, shorter follow-up and smaller number of patients, incomplete genetic testing, and insufficient adjustment for clinical variables. Genetic testing information was available for all 2733 patients analysed, and comprehensive pathology information enabled extensive analyses to be carried out on the pre-specified subgroup of patients with TNBC; which is a unique contribution to this field. Furthermore, analyses for this paper were carried out according to a pre-specified analysis plan, which was published in the supplementary appendix material, helping to ensure clarity of both the methods and results presented.

The findings of this study have also corroborated results in much of the recent literature[135-138], and although there are also a number of conflicting results available[139-141], many of these are likely due to the biases introduced from the study design and insufficient data. Furthermore, the ratio of BRCA1 to BRCA2 mutations found in this cohort was found to be similar to that of other western population based cohorts[138,142].

Another key strength of this paper includes the impact on future studies. The finding of this paper with regards to an early survival advantage for BRCA mutation carriers in the TNBC population has the potential to impact early results from clinical trials in this population; further highlighted by advances in genomic testing and investigations which enable many patients to learn their BRCA mutation status close to date of diagnosis.

The robustness of the findings of this paper was also highlighted in a number of places; not only did the UVA and MVA provide similar results, the numerous sensitivity analyses carried out also made little to no difference to the results.

10.6.5 Limitations

Potential limitations of this paper include the possibility that some BRCA mutations were not identified due to the method of using the non-universal multiplex ligation probe analysis incorporated for this paper. However, it should equally be noted that clinical diagnostic testing is not 100% sensitive, and genetic testing in this cohort to investigate BRCA1 and BRCA2 gene sequences was more comprehensive than in most other studies. Moreover, the identification and exclusion of TP53 mutations, which have a high risk of non-breast malignancies, proved an equal strength of this paper, despite other breast cancer susceptibility gene variants not being excluded from the analyses, which were expected to be low in both frequency and penetrance.

The percentage of BRCA+ carriers in the POSH cohort was somewhat higher than anticipated based on previous studies of younger women with breast cancer[143]. However, this may have been due to more sensitive and robust mutation testing carried out in this cohort.

Potential limitations of this paper also included the fact that management of BRCA1 and BRCA2 mutation has changed since the start of recruitment into this cohort study. Indeed, evidence suggests that DNA repair deficiency associated with BRCA mutations results in higher response rates to platinum-based drugs[144,145], and the POSH cohort consisted of only 13 patients who were treated with platinum-based adjuvant regimens, including one BRCA1 mutation carrier and one BRCA2 mutation carrier. Nevertheless, over 90% of patients in the POSH cohort received neo-adjuvant or adjuvant chemotherapy, and in over 95% of cases was incorporated with an anthracycline or anthracycline with taxane combination regimen; reflecting modern oncological treatment in the majority of patients.

A further potential limitation of this paper is the number of patients with TNBC, particularly evident when carrying out sensitivity analyses excluding patients with bilateral mastectomies or new primaries. However, the relevance of TNBC has only become apparent since the POSH was designed, and as a result, the study was not sufficiently powered for this as the primary outcome. Nevertheless, this study was able to demonstrate a short-term OS advantage in BRCA carriers with TNBC, despite these reduced numbers, which bilateral mastectomy was unable to account for; an important finding which could affect early results of clinical trials in this population.

10.6.6 Conclusions

This sixth publication of results from the POSH cohort (and the fifth in the order of this thesis), established that young women with invasive breast cancer who carry a BRCA mutation have similar survival to non-carriers, even after adjusting for known prognostic factors. However, BRCA mutation carriers with TNBC might have a short-term survival advantage compared with non-carriers. Future primary cancer prevention during primary breast cancer treatment should take into account the individual patient's circumstances, such as the tumour prognosis, as well as the timing and physical and psychological implications of further extensive surgery, and patient preferences. Clinicians should consider and discuss with patients both the short-term and long-term risks and benefits of risk-reducing bilateral mastectomy with patients.

10.7 Discussion - Paper 6

10.7.1 Aim and background

This was the fifth paper to describe and present outcomes from the POSH cohort (and the sixth in the order of this thesis), but the first in describing local recurrence outcomes. The main aim of the

paper was to assess clinical and surgical factors effecting both survival and also local recurrence in young women with invasive breast cancer.

This paper described the patient characteristics, clinicopathological and treatment information according to surgical type (mastectomy vs. breast conserving surgery [BCS]). The paper described how surgical type was categorised according to final oncological surgery to the breast i.e. patients undergoing BCS alone were categorised as a BCS, patients undergoing BCS followed by mastectomy after three months were categorised as a BCS, whilst patients undergoing BCS followed by mastectomy within three months were categorised as a mastectomy, and a mastectomy performed after three months following primary treatment in the absence of a local recurrence was considered risk-reducing as opposed to oncological.

The paper utilised data from 3095 women recruited from the POSH cohort, of whom 3022 satisfied the eligibility criteria. Women aged 41-50 with a known BRCA1 or 2 gene mutation, patients with M1 stage disease, patients with missing primary tumour data, together with patients with no invasive cancer on review of pathology, were excluded from the analyses. This resulted in 2882 patients being included in the analyses.

10.7.2 Key statistical methods

Summary statistics were used to describe and compare patient characteristic information, clinicopathological information and treatment information by surgical type. Study endpoints included inbreast ipsilateral local-recurrence interval (LRI)[l], DDFI, and OS. Cause-specific Nelson-Aalen plots were used to describe LRI, whilst Kaplan-Meier plots were used to describe DDFI and OS. UVA and MVA were carried out using Cox proportional hazards models, or FPSMs for models which involved time-varying hazards, which included the effect of surgical type on LRI (but not on DDFI or OS). Multivariable models adjusted for the following covariates measured at breast cancer diagnosis, regardless of statistical significance to the model fit: age at diagnosis; tumour size (in mm); focality; N stage; histological grade; ER and HER2 tumour status; adjuvant radiotherapy; adjuvant hormone therapy; and surgical margins[m,] (with OS and DDFI MVA models stratified by adjuvant hormone therapy and margin status due to the time-varying nature of these covariates for these specific outcome measures) MVA excluded patients treated with neo-adjuvant chemotherapy due to difficulties in classifying pathological T and N staging for these patients. As

[l] See **Section 2.3.6** for detailed definition of LRI.

[m] Categorised as either: 'negative' margins, where no cancer cells observed at the outer edge of the tissue removed; or 'positive' margins, where cancer cells are observed at the edge of the tissue removed (and further surgery is likely required).

with **Papers 1**, **4** and **5**, the choice of degrees of freedom for both the baseline hazard rate and time-dependent effect of each FPSM produced were found using the lowest AIC to obtain the best model fit for each FSPM.

Pre-specified analyses included carrying out the UVA and MVA, but excluding patients with a maximum overall tumour size greater than 30mm in order to minimise possible confounding as patients with larger tumours would be more likely to be treated with a mastectomy.

Pre-specified UVA also included: assessing DDFI and OS in all patients according to whether or not a LRI event occurred; assessing LRI, DDFI and OS in patients undergoing BCS according to margin status; assessing LRI in patients undergoing mastectomy according to whether or not chest wall radiotherapy was received; and assessing LRI in patients undergoing BCS according to whether or not a radiotherapy boost was received.

All statistical analyses were performed using Stata version 13.1[76], and were conducted accordingly to a pre-specified analysis plan (see **Appendix A3**) on follow-up data received up to 26 June 2015.

10.7.3 Key findings

Of the 2882 patients analysed, 1464 (50.8%) underwent mastectomy and 1395 (48.4%) BCS; all of whom underwent surgery to the axilla. Twenty-three patients underwent lymph node surgery only, with no surgery to the breast, and were excluded from the outcome analyses.

No statistically significant differences in age, BMI, and ethnicity were observed between surgical type. However, a statistically significantly larger proportion of patients undergoing mastectomy reported a positive family history compared to BCS (52.1% vs. 48.1%, respectively), and the vast majority (99.4%) of patients undergoing BCS had screen-detected tumours at presentation, compared to 97.7% of patients undergoing mastectomy.

In terms of tumour pathology and treatment, a number of statistically significant differences were observed between comparator groups. Patients undergoing mastectomy had a lower proportion of grade 3 tumours compared to BCS, but had larger tumours which were significantly more likely to be multifocal, ER, PR and HER2 and Extensive Intraductal Component (EIC)[n] positive. Patients undergoing mastectomy had a higher proportion of negative margins compared to BCS, specifically with regards to margins >5mm (42.7% vs. 24.0%). The distribution of the number of operations performed was significantly different, with 28.5% of patients undergoing mastectomy having more

[n] EIC is defined as positive where the total tumour in-situ size is ≥25% the size of the total tumour size (or where the total tumour invasive size is <75% the size of the total tumour size).

than one surgery compared to 20.8% of patients undergoing BCS. Although the proportion of patients undergoing adjuvant chemotherapy was similar between groups, the frequency of patients undergoing neo-adjuvant chemotherapy was higher for the mastectomy group compared to BCS (18.8% vs. 11.1%). Adjuvant radiotherapy was confirmed in 96% of patients undergoing BCS. The remaining 56 patients could have had radiotherapy to the breast at a different local centre, but the data did not enable confirmation of whether or not adjuvant radiotherapy was performed in these patients. Nevertheless, these patients were analysed as not receiving radiotherapy. Chest-wall radiotherapy was reported in 900 (61.5%) of patients undergoing mastectomy, and radiotherapy boost in 977 (70.0%) of patients undergoing BCS. However, no clear association was found between margin status (negative [>0mm] vs. positive [0mm]) and use of radiotherapy boost in patients undergoing BCS [data not presented in publication].

Median follow-up was 7.3 years and did not differ between mastectomy and BCS groups. Overall, 139 local recurrence events were observed, with a larger proportion of events observed in the BCS group (95 [6.8% of the mastectomy group], 40 [2.7% of the BCS group], and 4 [17.4% of the nodal surgery group]). However, the majority of events were DDFI with 752 observed; 260 (18.6%) and 458 (33.1%) for mastectomy and BCS groups, respectively. Similar proportions as those found for DDFI events were observed in terms of OS events: 670 overall; 232 (16.6% [compared to 18.6% for DDFI]); and 431 (29.4% [compared to 33.1% for DDFI]), respectively.

The effect of surgical type on LRI was found to vary considerably over time (**Figures 2A** and **2B** of the paper). Although no significant difference was observed between mastectomy and BCS in the short term (18-month LRI% [95% CI] & HR [95% CI]: 1.0% [0.6% to 1.7%] vs. 1.0% [0.6% to 1.7%] respectively & 1.43[0.89 to 2.32]), the risk of experiencing a local recurrence event was significantly higher for the BCS group compared to the mastectomy group following this time point (five year LRI% [95% CI] & HR [95% CI]: 5.3% [4.2% to 6.9%] vs. 2.6% [1.9% to 3.7%] & [3.39 [2.03 to 5.66]; ten year LRI% [95% CI] & HR [95% CI]: 11.7% [9.1% to 15.1%] vs. 4.9% [3.2% to 7.6%] & 5.27[2.43 to 11.43]). Similar findings were also observed when carrying out a sensitivity analysis excluding patients with larger tumours. When adjusting for other factors in the MVA, the risk of experiencing a local-recurrence event still remained significantly higher for BCS at both five and ten years, with only adjuvant radiotherapy a significant covariate in the model.

Conversely, this higher risk of a LRI event did not transfer to DDFI, in which not only was the effect no longer time-varying but patients undergoing mastectomy had a significantly higher risk of experiencing distant relapse, with a smaller proportion event-free over time, compared to BCS (HR [95% CI]: 0.51 [0.44 to 0.60]). However, in the adjusted model, the difference was no longer significant (HR [95% CI]: 0.82 [0.64 to 1.05]), and a number of significant covariates in the model

were found, including tumour size and grade[o]. For OS, the results were almost identical to that of DDFI, with patients undergoing mastectomy having a significantly worse survival compared to BCS; an effect which then disappeared when adjusting for other factors[o].

Pre-specified analyses assessing LRI by additional radiotherapy for mastectomy and BCS separately demonstrated that patients without chest-wall radiotherapy had significantly higher local recurrence rates compared to those with chest-wall radiotherapy in the mastectomy group (HR [95% CI]: 0.46 [0.24 to 0.86]). However, for BCS, no significant differences were found between patients with or without a radiotherapy boost (HR [95% CI]: 0.90 [0.58 to 1.38]). There was also no difference in LRI in the BCS group for those with positive vs. negative margins (HR [95% CI]: 0.86 [0.41 to 1.78]).

When assessing DDFI according to whether or not patients experienced an LRI event, the effect was found to be time-varying, with similar DDFI rates observed at five years (77.3% [no LRI event] vs. 75.4% [LRI event]; HR [95% CI]: 0.80 [0.54 to 1.18]) but significantly lower DDFI rates observed at ten years for patients who experienced an LRI event (70.2% [no LRI event] vs. 62.0% [LRI event]; HR [95% CI]: 0.29 [0.14 to 0.62]). However, no significant difference in OS between patients experiencing an LRI event or not was observed (HR [95% CI]: 1.24 [0.92 to 1.68]).

In patients undergoing BCS, DDFI was also found to be significantly lower in patients with a positive margin compared to a negative margin (HR [95% CI]: 0.50 [0.35 to 0.71]). Similar results were also found for the corresponding OS comparison (HR [95% CI]: 0.52 [0.36 to 0.75]), emphasising that quality of surgery remains important for outcome in young women undergoing BCS.

10.7.4 Strengths

Evidence from the literature demonstrates that a large number of studies have used inconsistent definitions of local recurrence, often failing to describe which events have been incorporated in their analyses[146]. A key strength of this paper was therefore highlighted by the clear definition of local recurrence used, together with criteria outlining which events were included and excluded from the analyses.

Previous evidence from RCTs in young women with invasive breast cancer remains limited, with only a small number of women included in these analyses[54]. Nevertheless, the findings of this study have corroborated evidence from existing literature including RCTs comparing mastectomy versus

[o] **Supplementary Tables 1 & 2** in **Paper 6** do not show results for adjuvant hormone therapy and surgical margin; models were stratified by these factors in the adjusted models and therefore estimates are not calculated.

BCS the breast cancer population, with no significant difference in DDFI or OS by surgical type found in this age group[10,12,14,16]. Furthermore, the results of this study support the message that avoiding local recurrence is important as it is associated with poorer outcomes[52,147,148].

Another key strength of this paper was that it was able to demonstrate that the POSH cohort had reasonably acceptable recurrence rates. Current Association of Breast Surgery (ABS) guidelines state that the target local recurrence rate following surgery should be less than 3% and no more than 5% at five years[149]. An adaptation of **Figure 2A** from the paper is shown in **Figure 7** of **Appendix A4**, which shows that this was met for mastectomy and that the lower 95% limit for BCS was within this range.

Sensitivity analyses assessing the effect of LRI, but excluding patients with larger tumours, also showed similar results to those shown for all patients. Moreover, although the proportion of patients with missing margin status information was quite high (24.5%), a sensitivity analysis of the MVA LRI model using multiple imputation showed very similar results to the complete-case analyses.

10.7.5 Limitations

One of the key limitations of this study was that it was not an RCT, and so the impact of surgical type on outcome (LRI, DDFI and OS) could potentially be due to confounding. However, this paper has equally demonstrated the ability to greatly minimise any biases in these analyses, including the use of a pre-specified analysis plan (see **Appendix A3**) and an analysis population which is representative of cancer treatment in this group in the UK (as demonstrated in **Paper 1**).

Another potential limitation of this study is that patients without confirmed use of adjuvant radiotherapy were treated as not receiving it. However, these patients were associated with significantly higher rates of local recurrence compared to patients with documented use of adjuvant radiotherapy, not only implying that the data are correct as opposed to the patients receiving radiotherapy elsewhere, but also demonstrating the importance of receiving adjuvant radiotherapy for patients undergoing BCS.

The reduced number of LRI events in these analyses also indicates a potential limitation of this paper of insufficient power for the comparison of LRI between patients undergoing BCS with positive vs. negative margins which showed no difference (**Supplementary Figure 1C** in the paper), whilst the comparison of both DDFI and OS demonstrated significant differences between patients undergoing BCS with positive vs. negative margins (**Supplementary Figure 2B** and **Supplementary Figure 3B**). Nevertheless, the difference observed in outcome between patients undergoing BCS

with positive vs. negative margins supports the message of the importance of surgical quality with attention to margins. Moreover, these results, together the lack of difference observed between surgical type and outcome indicates that surgical type appears less important for outcome than achieving clear surgical margins.

Due to the presence of competing risks for LRI (e.g. if a patient died before a local recurrence it would have been impossible to establish whether a local recurrence would have occurred if the patient had not died), cause-specific Nelson-Aalen plots were used to describe LRI. Competing risk regression models were not implemented in this paper to analyse LRI; instead FPSMs were used. However, although not presented in the publication, a post-hoc sensitivity analysis for the MVA on LRI was carried out which used a competing risk regression model, based on methods outlined by Fine and Gray[150] using the 'stccreg' command in Stata[151], and fitted surgical type as a time-varying covariate; the results obtained were almost identical to the FPSM results.

10.7.6 Conclusions

This publication from the POSH cohort established that reasonably acceptable recurrence rates were observed in the cohort, accepting a slightly higher rate than the 5% threshold for BCS. Despite higher local recurrence rates for BCS, surgical type does not influence survival after adjusting for known prognostic factors in young breast cancer patients, and appears less important for outcome than completeness of excision. Moreover, in patients undergoing a mastectomy, the addition of chest wall radiotherapy significantly reduces the likelihood of a local relapse in younger women.

10.8 Discussion - Paper 7

10.8.1 Aim and background

This seventh paper in the order of this thesis differs to the first six papers in that the aim of this paper was not to describe or compare outcomes of the POSH cohort by comparator groups. The main aim of this paper was instead to evaluate an existing prognostic model using the POSH cohort to determine how well the model performs in estimating survival in young women with invasive breast cancer. The results of this paper, together with the results of the first six papers of this thesis, were intended to identify and provide an overview of the key prognostic factors affecting outcomes for young women with breast cancer.

The paper described some of the existing prognostic tools available, including the Nottingham Prognostic Index (NPI), Adjuvant! Online and PREDICT. The paper outlined how these tools have been improved over the years; becoming increasingly sophisticated, incorporating a growing

number of prognostic factors in their models, and enabling a move away from broad prognostic groups into the ability to provide individual patient survival estimates. However, the paper also described the limitations of these tools, including the limited number of young women with breast cancer incorporated into these tools during both their development and validation[72].

The paper also included a brief description of the POSH cohort, with the incorporation of patient and clinicopathological information, together with treatment and follow-up information used to evaluate the PREDICT tool. The paper utilised data from 3095 women recruited from the POSH cohort, of whom 3022 satisfied the eligibility criteria of the POSH cohort. Women aged 41-50 with a known BRCA1 or BRCA2 gene mutation, together with patients with missing primary tumour data, patients with M1 stage disease, and a further 55 patients with missing key prognostic information required for the PREDICT tool were excluded from the analyses. This resulted in 2827 patients being included in the evaluation analyses.

10.8.2 Key statistical methods

Study endpoints were OS and BCSS at five, eight and ten years. Although the web-based PREDICT tool only provides five and ten year individual survival estimates, eight year survival estimates were obtained directly from the tool developers in order to provide more accurate long-term estimates as follow-up data was limited to data received up to 22nd October 2013 (similar to **Papers 3** and **4**). Of the 2827 patients included in the evaluation analyses, follow-up information was available for: all 2827 patients for the five year OS comparison; 1843 patients for the eight year OS comparison; and 597 for the ten year OS comparison. Cause of death was missing for five patients leaving: 2822; 1841; and 595 patients available for the corresponding BCSS comparisons.

Predicted OS and BCSS were calculated for each patient using PREDICT by investigators blind to the actual patient outcomes. Methods implemented to evaluate the tool were based on similar approaches applied previously to validate the tool[103,104]; the number of predicted deaths were compared against those observed at five, eight and ten years, and these were presented by key patient and tumour characteristics, including age at diagnosis, ethnicity, tumour size, number of positive lymph nodes, presentation, grade, ER and HER2 status, and chemotherapy regimen. The predicted and observed deaths were then compared using goodness-of-fit Pearson χ^2 tests[70,103-105], and illustrated using calibration plots by quartiles of the predicted risk.

Area under ROC curves were also produced to assess model discrimination for OS and BCSS, with AUCs calculated at five, eight and ten years, split by ER and HER2 status.

10.8.3 Key findings

For the five year OS comparisons, PREDICT did not perform well at five years, with a statistically significant underestimation of the total number of deaths by 25% (455 predicted vs. 607 observed) and within most sub-groups, including in: ER positive tumours (-56%; 158 predicted vs. 362 observed); HER2 negative tumours (-33%; 255 predicted vs. 383 observed); lobular morphology (-63%; 10 predicted vs. 17 observed); positive lymphovascular invasion (-29%; 272 predicted vs. 385 observed); grade 2 tumours (-58%; 67 predicted vs. 161 observed); tumour sizes 0-10mm (-52%; 20 predicted vs. 42 observed); black ethnicity (-42%; 21 predicted vs. 36 observed); underweight/health patients (-21%; 214 predicted vs. 271 observed) [data not presented in publication]; overweight patients (-26%; 134 predicted vs. 182 observed) [data not presented in publication]; obese patients (-31%; 95 predicted vs. 138 observed) [data not presented in publication]; patients undergoing a mastectomy with radiotherapy (-27%; 67 predicted vs. 161 observed), or a mastectomy alone (-45%; 48 predicted vs. 87 observed); and in patients receiving both hormone therapy and chemotherapy (-55%; 147 predicted vs. 326 observed). Conversely, PREDICT significantly overestimated the number of deaths in: patients with ER negative tumours (+21%; 297 predicted vs. 245 observed); patients receiving adjuvant trastuzumab (+25%; 86 predicted vs. 69 observed); and in patients receiving chemotherapy alone (+14%; 297 predicted vs. 261 observed).

A considerable improvement was observed when assessing the eight year OS comparison, with the estimated total number of deaths underestimated by only -5% (430 predicted vs. 454 observed). The predicted number of deaths were no longer statistically significantly different between: ethnicity; BMI [data not presented in publication]; and lymphovascular invasion subgroups. Subgroups for which a statistically significant underestimation remained included: ER positive tumours (-31%; 188 predicted vs. 271 observed); HER2 negative tumours (-14%; 234 predicted vs. 273 observed); grade 2 tumours (-38%; 75 predicted vs. 121 observed); tumour sizes 0-10mm (-41%; 17 predicted vs. 29 observed); patients undergoing a mastectomy alone (-28%; 47 predicted vs. 65 observed); and in patients receiving both hormone therapy and chemotherapy (-29%; 170 predicted vs. 238 observed). Compared to the five year OS comparisons, statistically significant overestimation actually increased in ER negative tumours (+32%; 242 predicted vs. 183 observed); in patients receiving adjuvant trastuzumab (+58%; 52 predicted vs. 33 observed), and in patients receiving chemotherapy alone (+25%; 248 predicted vs. 198 observed).

PREDICT provided more accurate ten year OS estimates for younger women, with the predicted total number of deaths of all patients slightly overestimated by +8% (164 predicted vs. 152 observed). The tool generally performed well across most sub-groups at ten years, including

ethnicity, BMI [data not presented in publication], lymphovascular invasion, morphology and HER2 subgroups. The number of predicted deaths remained underestimated in ER positive and grade 2 tumour subgroups (-21% [70 predicted vs. 88 observed] and -30% [31 predicted vs. 44 observed] respectively), but these were no longer statistically significant, likely due to smaller numbers. Indeed, possibly due to smaller numbers, no significant underestimation remained in any of the subgroups from the five and eight year comparisons. Conversely, subgroups which remained overestimated included: ER negative subgroups (+47%; 94 predicted vs. 64 observed); patients receiving adjuvant trastuzumab (+117%; 13 predicted vs. 6 observed); and patients receiving chemotherapy alone (+32%; 94 predicted vs. 71 observed). Subgroups which were initially underestimated at five years but were overestimated at eight and ten years included grade 3 tumours (-12% [378 predicted vs. 429 observed], +8% [346 predicted vs. 322 observed] and +25% [129 predicted vs. 103 observed] at five, eight and ten years respectively), and tumours larger than 50mm (-14% [85 predicted vs. 99 observed], +11% [83 predicted vs. 75 observed] and +115% [28 predicted vs. 13 observed], respectively).

The BCSS comparisons were very similar to the results for OS, with no notable differences between the OS and BCSS results when comparing these both by year (five, eight, and ten years) and across subgroups.

In terms of comparisons of all-cause mortality split by quartiles of the predicted risk, the performance of PREDICT improved markedly at ten years compared with five and eight years. When examining the results across years, a statistically significant overestimation was observed in ER negative tumours at five, eight and ten years (illustrated by values below the reference line in **Figures 1A**, **1B** and **1C** of the paper, respectively) and a statistically significant underestimation in ER positive tumours at five, eight and ten years (illustrated by values above the reference line in **Figures 1D**, **1E** and **1F** of the paper, respectively).

Once again, the comparisons of breast cancer specific mortality split by quartiles of the predicted risk were almost identical to those of the all-cause mortality results. Furthermore, the pattern of overestimation in ER negative tumours and underestimation in ER positive tumours remained even when splitting the data by HER2 negative and positive subgroups.

Model discrimination was reasonably high[152] across both years and survival outcomes. When assessing discrimination for OS by ER status subgroups, the highest AUC was found in the ER positive subgroups across all years (five year AUC [95% CI]: 0.718 [0.678 to 0.759] vs. 0.730 [0.696 to 0.765]; eight year AUC [95% CI]: 0.709 [0.663 to 0.755] vs. 0.748 [0.714 to 0.782]; ten year AUC [95% CI]: 0.694 [0.618 to 0.771] vs. 0.724 [0.660 to 0.788], for ER negative vs. ER positive subgroups respectively). Conversely, the highest AUC was found in the HER2 negative subgroups across all

years when assessing OS by HER2 status (five year AUC [95% CI]: 0.729 [0.698 to 0.761] vs. 0.692 [0.637 to 0.746]; eight year AUC [95% CI]: 0.714 [0.679 to 0.750] vs. 0.699 [0.645 to 0.753]; ten year AUC [95% CI]: 0.724 [0.659 to 0.789] vs. 0.592 [0.486 to 0.697], for HER2 negative vs. HER2 positive subgroups respectively). Unsurprising, the corresponding discrimination analyses for BCSS yielded similar results to that of OS.

10.8.4 Strengths

This paper was able to successfully evaluate the performance of PREDICT using a large cohort of young women with invasive breast cancer. The findings of this paper were able to clearly identify a number of areas requiring improvement to PREDICT's prognostic ability including overall short-term prediction, prediction of patients with ER negative and positive tumours, patients receiving chemotherapy alone and patients receiving adjuvant trastuzumab.

Findings of this paper identified a number of results which contradicted evidence from the existing literature, highlighting the importance of the contribution of this work to the existing literature. PREDICT was found to perform well in its prognostication of Asian patients for example, whilst a systematic review of prognostic models in women with early breast cancer found that existing prognostic tools performed poorly in this subgroup[72]. A previous validation of PREDICT found that its prediction of patients aged 20-85 years with ER negative tumours was accurate for ten year OS[104], whilst the results of this paper found PREDICT to overestimate OS by 47% at ten years in women ≤40 years, also demonstrated by the calibration plots (see **Figure 1** in the publication). Moreover, the results of this paper have demonstrated a considerable underestimation in the ER positive subgroup, irrespective of HER2 tumour status. Overall, a previous validation of PREDICT demonstrated an underestimation of the number of deaths at ten years by -32% in patients aged 20-35 years[104], whilst the results of this paper found an overestimation of +8%. Post-hoc exploratory analyses, which dichotomised age into <35 years and 35 to 40 years subgroups, was carried out to investigate this further. However, the differences in predicted vs. observed deaths was within 2% and 14% for the <35 years and 35 to 40 years subgroups respectively, indicating a possible difference in response to treatment between these two age groups could impact the performance of PREDICT in the prognostication of these two age groups. Furthermore, a previous validation of PREDICT also found that the tool performed better in predicting BCSS compared to OS[104], whereas the results of this study demonstrated almost identical findings between the two outcome measures.

There could be numerous reasons for the disparities observed between the findings of this study with that of the existing literature, such as the small number of patients aged ≤40 years included in

the comparisons in the literature and in the validation of PREDICT (n=159) compared to this evaluation. The similarity in results obtained when assessing the performance of PREDICT for OS and BCSS was perhaps unsurprising given that the vast majority of patients in the cohort died due to breast cancer, and thus competing mortality would not play as important a role within this young age group. Indeed, this result re-iterates the uniqueness of this young population compared to older women with invasive breast cancer. PREDICT was also developed to provide long-term (ten year), not short-term, survival estimates to patients and clinicians, and could therefore explain the disparity in accuracy between short- and long-term estimates.

10.8.5 Limitations

Limitations of this paper include the fact that BMI subgroup evaluations were not presented in this publication, and as demonstrated in **Papers 3** and **5**, obesity was found to be associated with inferior survival, and an independent prognostic factor in patients with ER positive tumours. However, post-hoc analyses on BMI subgroup demonstrated that only the short-term survival predicted estimates were found to differ significantly to that of the observed. By eight and ten years, no significant difference was found, indicating minimal impact on the conclusions of this paper.

Although the paper was able to demonstrate accurate long-term OS prediction in the HER2 subgroups overall, model discrimination was found to vary quite considerably for HER2 negative vs. HER2 positive subgroups (AUC: 0.724 vs. 0.592 respectively). However, fewer patients were available in the HER2 positive subgroup ten year evaluation (n=140) compared to the HER2 negative subgroup ten year evaluation (n=327) which was likely to have contributed to the lower discrimination in this subgroup. Moreover, patients recruited to the POSH cohort included patients diagnosed before 2005, for which Herceptin was only administered in the metastatic setting to patients with HER2 positive tumours. The outcomes of HER2 patients in the POSH cohort as a whole may therefore have been inferior to patients diagnosed since 2005. However, only 298 patients with HER2 positive tumours were diagnosed before 2005 and 381 patients with HER2 positive tumours diagnosed on/after 2005, and so the impact of this on this evaluation is likely to be minimal.

Another potential limitation of this paper was that only 597 patients, with key prognostic information required, were diagnosed at least ten years before the time of analyses and were thus available for the ten year comparisons. However, this number of women aged ≤40 years was nevertheless considerably larger than previously used in the literature or in the validation of PREDICT (n=159)[72,104]. Poorer prediction in this young age group was also found to be a common

finding in a number of other prognostic tools, with overestimates in the number of predicted all-cause deaths of up to 30% in this age group[72].

10.8.6 Conclusions

This paper demonstrated how PREDICT, a web-based tool that is easy to navigate for both patients and clinicians, was found to be a valuable resource in providing accurate and reliable long-term (ten year) survival estimates for younger patients, despite the lack of incorporation of ethnicity, BMI, or surgical type in its development. However, the tool was also found to require further calibration, and caution should be exercised when interpreting both the short-term survival estimates in younger patients, and the long-term estimates of those with ER negative tumours. Future modifications of the tool should be made including consideration for an adjustment of young age to further improve the tool and its accuracy.

The results of this paper re-iterate the need for caution when extrapolating data from older patient cohorts in order to determine the most appropriate treatment management options for younger women. Moreover, the findings of this paper also demonstrate the need for further investigation into treatment approaches targeted at younger women, including trials involving larger numbers of young women with invasive breast cancer.

10.9 Reflection

This thesis has successfully brought together a body of work in a cohesive manner to answer its research aim: to study factors affecting outcomes for young women with invasive breast cancer to provide additional data for clinicians, and their patients, to weigh up the optimum approach to reducing the risk of death in newly diagnosed young breast cancer patients. Through the findings of this research, factors found to affect clinical outcomes in young women included ER tumour status (**Papers 1, 5 & 7**), ethnicity (**Papers 2, 5 & 7**), and BMI (**Papers 3, 5 & 7**). Conversely, reported family history of breast cancer (**Papers 4 & 5**), BRCA mutation status (**Paper 5**), and surgical type (**Paper 6**) were not found to be in this research. Future research investigating new treatment approaches should take into account the findings of this research in order to provide clinicians with additional information to decide the optimum approach to improve outcomes in this population.

This thesis presents research based on data from a large prospective cohort study of 3000 young women with invasive breast cancer, and research which has made a significant contribution to learning in this field. This is evidenced by the journals in which the findings of this research have been published, the citations to date and also the impact of the research on the current national guidelines. For example, these articles have collectively been cited 200 times to date, including citations in 5 books and 19 reviews. Moreover, in terms of direct impact on current guidelines, **Paper 2** was used in the international consensus guidelines for breast cancer in young women in order to help identify research priorities and recommendations for management of breast cancer in young women[153-155]. Using data from the POSH study, a panel-based mutation screening of breast cancer predisposition genes of patients with TNBC to determine the best parameters for selection of patients with TNBC for BRCA testing was carried out[156]. Although not directly part of this research, but inked to the findings in **Paper 4**, this work contributed to the updated 2013 NICE guidelines for familial breast cancer which recommend testing patients aged <40 with TNBC for BRCA mutations even with no family history[157]. Furthermore, **Paper 6** was considered for use in the 2018 NICE guidelines for early and locally advanced breast cancer: diagnosis and management[158], however, was not included due to the paper not reporting on all of the required results. Finally, following the evaluation of the PREDICT tool in **Paper 7**, improvements of the tool were implemented and subsequently published in 2017[159]; thus highlighting the impact of this paper.

This work would not have been possible without contributions by a multidisciplinary team as it was essential to have individuals knowledgeable and experienced in a wide range of fields to undertake this work and take it forward. Nevertheless, my contribution to this work was extensive. I was the lead statistician and scientific lead at the Southampton Clinical Trial Unit (SCTU) for the research outlined in the seven papers of this thesis. I was also lead statistical methodologist on all but one

of the papers, joint first author on two papers and first and corresponding author on one paper. I carried out the research into the statistical methods implemented in the analyses for all papers, including research into Flexible Parametric Survival Models (FPSMs) to use for the time-varying covariates in a number of the papers. I also developed and authored the Statistical Analysis Plans (SAPs) for all six outcome papers. I was responsible for all central data monitoring, data cleaning and data interpretation, including liaising extensively with the study team to identify data queries with the POSH data, and for the resolution of all data queries raised with data management. I developed and conducted all statistical programming and analyses, including designing and producing all manuscript figures and tables. Finally, I co-authored all manuscripts and was heavily involved in the writing and finalisation of all manuscripts.

There are a number of limitations of this research, the majority of which have been highlighted in the individual discussions for each of the seven papers. However, one global limitation not discussed was the choice of alpha of 5% two-sided significance level. This level was chosen in the first six papers of this thesis describing outcomes as it was a deemed a standard and clinically relevant level to use. However, this level does not account for multiple testing, of which the six papers contain a large number of tests; with the more tests made, the more likely a statistically significant difference is found by chance. This, coupled with the large size of the POSH cohort study, meant that statistically significant differences were in some cases found which were not necessarily clinically relevant. For example, in **Paper 3**, the distribution of age at menarche and age at first birth was statistically significantly different across BMI groups, despite having identical median age at menarche (13) and age at first birth (27) in all three groups, indicating a statistical but not clinical difference between groups. This is a global limitation of this work. However, it should equally be noted that the choice of alpha was as stipulated in the protocol paper[73]. Moreover, although the choice of alpha was a limitation in these papers, all analyses were pre specified and clearly laid out as described in the corresponding SAPs; a key strength of this work (see **Appendix A3**).

10.10 Future research

Inferior survival observed in the POSH cohort has corroborated existing evidence that younger women with breast cancer have inferior survival compared to older patients. This finding highlights the need for further research into new treatment approaches for young women with invasive cancer, including extended adjuvant hormone therapy, as well as age-selected trials to provide sufficient evidence in this patient population. Moreover, the association of Black ethnicity and obesity with inferior survival also demonstrates the need for further research into ways to optimise treatment for these patient groups. Although it should be noted that of the clinically and statistically significant factors affecting outcomes, ER tumour status and ethnicity are not factors which may be

environmentally influenced, however BMI could be. Treatment approaches should therefore include investigating the incorporation of exercise and dietary programmes in studies to reduce BMI. Increased weight gain is also commonly experienced during adjuvant chemotherapy[160,161], and a trial currently in development within the SCTU which is trying to address this includes the PANACHE study, which aims to develop an effective lifestyle intervention for breast cancer chemotherapy patients that is deliverable within the breast cancer patient pathway, including analyses into health related quality of life measures. In addition, a trial which I have been directly involved with as lead statistical methodologist and scientific lead for the SCTU is the CANDO-3 trial, recently funded by Breast Cancer Now, which aims to assess the body composition of patients undergoing neo-adjuvant chemotherapy for early breast cancer to investigate associations between body composition, chemotherapy toxicity and tolerance using a prospective multicentre observational cohort study.

Future research should also investigate how physical and psychological implications of further extensive surgery, together with patient preferences and the implications of short- and long-term risks and benefits, can be utilised to determine the optimum timing for primary cancer prevention, including risk-reducing bilateral mastectomy for newly diagnosed young breast cancer patients. Psychological implications of treatments should also not be limited to further extensive surgery. As outlined above, the PANACHE study also aims to include extensive analyses on health related quality of life measures such as anxiety, depression, cancer worry, and fatigue. Further work using the POSH cohort specifically includes an extensive analysis to incorporate surgical, demographic and BRCA mutation status in order to investigate the impact of risk-reducing surgery on outcomes. However, due to the nature and impact of competing risks on local recurrence interval and loco-regional recurrence interval outcomes, competing risk regression methods will be implemented to assess this in a statistically appropriate manner. Also evident from the research undertaken in this thesis, the number of patients included in the multivariable analyses was markedly reduced due to the amount of missing data across a number of key prognostic variables in the POSH cohort. Subsequently, it is imperative that any multivariable analyses using the POSH cohort takes into account the amount of missing data accordingly using multiple imputation in order to provide sufficient power for the multivariable analyses. Other statistical considerations for future work using data from the POSH study are the use of appropriate methods to handle time-varying hazards. Although FPSMs have been used in numerous survival models in this research, it is not the only method available. For example, the extended Cox model which fits the time-varying covariate as a function of time can be used[162], or models which allow joint estimation of both the time-dependent and non-linear effects of continuous covariates on survival[163,164].

The evaluation of PREDICT further highlights the differences in this young age group compared to older women, and the need for further research into new treatment approaches. **Paper 7** demonstrated the need and intention for future modifications of PREDICT to incorporate the POSH cohort, including consideration for an adjustment of young age in the prognostic tool and including more up-to-date follow-up information. Following this evaluation, the aforementioned improvements of the tool were implemented and subsequently published in 2017[159]; thus highlighting the impact of this paper. Indeed, a later review of PREDICT, consisting of 254 women aged ≤35 years and 353 women aged 36 to 40, demonstrated a marked improvement in the tool, which only underestimated mortality by 6.6% (p=0.04) and 4.9% (p=0.06) respectively[165].

It should, however, be noted that this calibration of the tool was carried out prior to the analyses for **Paper 5** (the latest analyses of this thesis), which included more follow-up data and information on BRCA gene status. In addition, future competing risk regression analyses using the POSH cohort may lead to the identification of additional prognostic factors. As a result, further calibration may still be required for incorporation of ethnicity, BMI, and any additional prognostic factors in its model calibration to provide more accurate survival estimates for young women with invasive breast cancer.

A brief literature review of recent developments since the latest paper (**Paper 5**) was received on 31 August 2017, using the search terms "((((young women[Title]) OR under 40[Title]) OR <40[Title])) AND breast cancer[Title]" in PubMed, provided 57 articles, of which ten did not contain available abstracts and one presented findings on breast cancer in young men. Of the remaining 46 articles: 13 articles presented research carried out on the psychological implications of diagnosis and treatment management of breast cancer; eight presented findings on treatment approaches and their impact on outcomes, including an article highlighted previously which presented the international consensus guidelines for the recommendations for management of breast cancer in young women[155]; seven presented findings on screening approaches; seven on ER tumour status (1), age (6) and/or ethnicity (1) and other prognostic factors (2); seven on the family history and/or genetic factors affecting outcomes; three presented findings of risk factors associated with breast cancer; and one on surgical outcomes. No articles were found which reported findings on treatment or outcomes using BMI or weight. This brief literature review suggests that future research may be heading in the direction of psychological implications of breast cancer management and treatment, thus highlighting the importance of the PANACHE study. However, this review also highlights the gap in research on the relationship between treatment or outcomes and BMI/weight, and given the increasing incidence of obesity in England[166], for example, highlights the importance of the future CANDO-3 study which is due to start recruitment early in 2019.

10.11 Conclusion

In conclusion, the research within this thesis was undertaken to identify factors affecting the outcomes of young women with breast cancer using the POSH cohort study. The POSH cohort study consisted of over 3000 young women with invasive breast cancer and represents one of, if not the largest, prospective studies of young breast cancer patients to date, and is representative of the wider population of young women with breast cancer in the UK.

The findings from this research have made a significant contribution to learning in the field which is evidenced by the journals in which the collection of works has been published. Factors significantly affecting survival in young women, identified through this research, include ER tumour status, ethnicity, and BMI. Conversely, factors thought to be associated with inferior survival, including patient reported family history of breast cancer, surgical type and BRCA mutation status, have not been found to significantly affect outcomes in this research.

The evaluation of the PREDICT prognostic model in this research further demonstrated the impact of ER tumour status, ethnicity, and BMI on survival in this young age group. However, for this research to change routine practice for the better, the findings need to be circulated to all parties involved in the patient's care. Future research, including investigations into new treatment approaches, should take into account these prognostic factors to provide clinicians with additional information to decide the optimum approach to reduce the risk of death in young women with newly diagnosed invasive breast cancer.

In summary, through the published works submitted in this thesis and the future research planned, I have demonstrated my ability to inform the design, set-up, and oversee the running, analysis and interpretation of large observational studies in breast cancer. My contribution has supported the publication of papers in a number of high impact journals and made a significant contribution to the knowledge base in this research area.

Appendix A

A.1 Breast cancer T staging breakdown

Table 3 T stage categories and descriptions[4]

T stage	Description
TX	Tumour size cannot be assessed
Tis	Ductal carcinoma in situ (DCIS)
T1	Tumour size is ≤2cm
T2	Tumour size is >2cm and ≤5cm
T3	Tumour size is >5cm
T4a	Tumour has spread into the chest wall (structures surrounding and protecting the lungs)
T4b	Tumour has spread into the skin and the breast may be swollen
T4c	Tumour has spread to both the chest wall and the skin
T4d	Inflammatory breast cancer

A.2 Breast cancer grade

Table 4 Grade categories and descriptions[4]

Grade	Description
1	Low grade – slow growing cancer
2	Intermediate grade – intermediate growing cancer
3	High grade – fast growing cancer

A.3 Pre-Specified Analysis Plans (SAPs)

A.3.1 Statistical Analysis Plan – Paper 1

Statistical analysis plan (SAP), approved on 17-May-2012, and formatted for Lancet Oncology Appendix (as per SAP for Paper 5 for consistency purposes for this thesis).

[Please note: Figures in this SAP are taken from the POSH data available up until May 2011, and thus only represent approximations of the new data due to be downloaded from the POSH database in April 2012.]

Please note: This statistical analysis plan has been written in the past tense because it will form the basis of a paper. The headings used in this document come from the STROBE reporting guideline for observational studies (see http://www.strobe-statement.org/ or http://www.annals.org/content/147/8/W-163.full.pdf+html).

Statistical Analysis Plan Version

Issue no	Revision History	Author	Date
0.1	First draft written based on discussion at meeting on 24th Jan 2012	Tom Maishman	13th Mar 2012
0.2	Updated based on meeting on 21st March 2012 with Diana Eccles, Louise Dent, Bryony Eccles, Ellen Copson, and Sue Gerty.	Tom Maishman	30th Mar 2012
0.3	Updated based on comments from Diana Eccles	Tom Maishman	2nd April 2012
1	Updated based on meeting on 12th April 2012 with Doug Altman, Diana Eccles, Louise Dent, Bryony Eccles, Ramsay Cutress, Ellen Copson and Sue Gerty.	Tom Maishman	17th May 2012

1. Introduction

1.1 Background / Rationale
(Not included in SAP)

1.2 Objectives

This paper presents the results from analyses carried out on data collected from the POSH study.

The primary objective was:

- To describe the clinical presenting characteristics of breast cancer amongst younger women presenting over the last decade to UK hospitals.

Secondary objectives were:

- To make a comparison to assess how representative the POSH cohort is with the age equivalent UK population.
- To report on survival to date, and to compare survival according to Oestrogen Receptor (ER) status.

2. Methods

2.1 Study Design
The POSH study is a prospective cohort study. The protocol for the study can be found in the following journal article http://www.biomedcentral.com/1471-2407/7/160.

2.2 Setting
The POSH study recruited women from breast cancer units across England, Scotland, Wales and Northern Island between 1st June 2001 to 31st January 2008.

2.3 Participants
The study recruited 3052 women aged 40 years or younger at breast cancer diagnosis. The women had to have been diagnosed with breast cancer between January 2000 and January 2008. In addition, 43 women aged 41-50 were also included if they had a known BRCA1 or BRCA2 gene mutation and were diagnosed with invasive breast cancer within the study period were excluded for this analysis. Women were excluded if they had a previous invasive malignancy (with the exception of non-melanomatous skin cancer), were not available for follow up or refused consent to retain diagnostic and follow up data. A total of 2932 women were included in the analysis population.

Clinical follow up data were obtained from the patient medical records by the clinical trials practitioner (CTP) at each recruiting centre. Data forms collecting information at diagnosis, 6 months, 12 months were completed by the CTP usually at 12 months from diagnosis. Annual data collection was continued from the date of definitive diagnosis until death, loss to follow up or until the end of the current phase of the study (Apr 2012).

2.4 Variables (data taken as of May 2011)

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
2.4.1 Primary outcome			
Time to death from any cause	Survival data Date of death from any cause – Date of invasive breast cancer diagnosis	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A
2.4.2 Secondary outcomes			
Time to distant relapse or death from breast cancer	Survival data Date of first distant relapse (or death from breast cancer) – Date of invasive breast cancer diagnosis	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit. Patients who died from non-breast cancer deaths were censored at the date of death.	N/A
2.4.3 Candidate predictor			
ER status[1]	Categorical Negative, positive, or missing/unknown	12 (0.4%) records	MCAR (most people have ER done)
2.4.4 Potential confounders / effect modifiers - measured at breast cancer diagnosis presentation			
1. Age at diagnosis	Continuous, in years	0 records	N/A
2. Age when the first child was born	Continuous, in years	92 (3.1%) records missing (and 784 values not applicable)	Consider MAR
3. Age at menarche	Continuous, in years	0 records	N/A
4. Body Mass Index (BMI)	Categorical Underweight/Healthy, Overweight, Obese, or missing/unknown	114 (3.9%) records	Consider MAR
5. Number of children	Categorical 0, 1, …, or 8	25 (0.9%) records	Consider MCAR
6. Use of contraceptive pill	Categorical Ever, or never	0 records	N/A
7. Smoker	Categorical Ever, never, or missing/unknown	93 (3.2%) records	Consider MCAR
8. Menopausal status	Categorical Pre-, Peri-, or Post-menopausal, or missing/unknown.	59 (2.0%) records	Consider MCAR
9. Number of patients with at least one first or second degree relative with breast cancer	Categorical Yes, no, or missing/unknown.	106 (3.6%) records	Consider MCAR
10. Presentation	Categorical Symptomatic, screen-detected, other, or missing/unknown	14 (0.5%) records	Consider MCAR
11. Histological Tumour grade	Categorical 1, 2, 3, not graded, or missing/unknown	77 (2.6%) records missing/unknown, 2 (0.1%) not graded	MCAR. Inadequate reporting by pathologist. If grade of core biopsy tumour not stated, and after neo-adjuvant chemotherapy there was a complete pathological response then no tumour to report on.
12. Histological type	Categorical Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown	37 (1.3%) records	MCAR (Same as above for grade)
13. Focality (distribution of tumour)	Categorical Multifocal, localised, or missing/unknown	286 (9.8%) records	MCAR (Same as above for grade)
14. PR[2] status	Categorical Negative, positive, or missing/unknown	581 (19.8%) records	MAR - missing because specific centres don't do PR IHC.
15. HER2[3] status	Categorical Negative, positive, borderline, or missing/unknown	355 (12.1%) records	Missing when diagnosis predated routine testing. Potential bias towards missing in patients not experiencing disease recurrence.
16. M stage	Categorical M0, M1 or missing/unknown	22 (0.8%) records	MCAR
17. Pathological N stage (lymph node status)	Categorical N0, N1 or missing/unknown	65 (2.2%) records	MCAR. No axillary surgery, no lymph nodes in resected specimen.

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
18. Number of positive Lymph nodes	Categorical 0, 1-3, 4-9, 10+, or missing/unknown	59 (2.0%) records	Same as above (MCAR)
19. Number of axillary lymph nodes	Continuous (integer)	46 (1.6%) records	MCAR (Same as above for N stage)
20. Lymphovascular invasion	Categorical Present, absent or missing/unknown	222 (7.6%) records	MCAR (Same as above for grade)
21. Maximum tumour diameter invasive (tumour size)	Continuous, in mm or Categorical <15mm, 15mm to 20mm, >20mm to 35mm, >35mm to 50mm, >50mm, or missing/unknown	193 (6.6%) records	Missing for similar reasons as tumour grade (MCAR)
22. Maximum tumour diameter (including ductal carcinoma in-situ) (pathological)	Continuous, in mm	161 (5.5%) records	Missing for similar reasons as tumour grade (MCAR)
23. Clinical T stage (for patients receiving neo-adjuvant chemotherapy)[4]	Categorical T0, T1, T2, T3, T4 (not T4d/inflammatory), T4d/inflammatory, or missing/unknown	318 (10.8%) records	Clinical T stage recorded in patients receiving neo-adjuvant chemotherapy so bias likely – data recorded more likely in poorer prognosis cases
24. Pathological T stage (for patients receiving neo-adjuvant chemotherapy)	Categorical T0, T1, T2, T3, T4, Tis, Tx, or missing/unknown	12 (0.4%) records	MCAR. However pathological stage post neo-adjuvant chemotherapy is not comparable with primary resection
25. Definitive surgery	Categorical Breast Conserving Surgery (BCS), Mastectomy, No surgery, Nodal surgery only, or missing/unknown	2 (0.1%) records	Consider MCAR
26. Chemotherapy timing	Categorical Adjuvant, neo-adjuvant, palliative, not applicable, or missing/unknown	0 records	N/A
27. Chemotherapy regimen	Categorical Anthracyclines, A&T, Taxanes, Other, or None	0 records	N/A
28. Adjuvant trastuzumab	Categorical Yes, no/missing/unknown	2587 (88.2%) records no/missing/unknown	Consider MAR
29. Radiotherapy	Categorical Yes, no/missing/unknown	598 (20.4%) records no/missing/unknown	Consider MAR
30. Hormone treatment	Categorical Yes, no/missing/unknown	2156 (73.5%) records no/missing/unknown	Consider MAR
31. Oophorectomy	Categorical Yes, no/missing/unknown	2558 (87.2%) records no/missing/unknown	Consider MAR
32. Ovarian suppression	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2172 (74.1%) records no/missing/unknown	Consider MAR
33. LHRH	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2177 (74.2%) records no/not applicable/missing/unknown	Consider MAR
34. Diagnosis Year	Categorical 2000, 2001… , 2008	0 records	N/A
2.4.5 Additional (descriptive) variables			
Length of follow-up	Continuous, in months	0 records	N/A

[1] Oestrogen receptor allocation of result from POSH database to Oestrogen receptor category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative*
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

*For ER, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as ER negative, and an Allred score of 3+ treated as ER positive. However it is possible that reviewers will disagree so we can reclassify this as positive if required.

[2] PR allocation of result from POSH database to a PR category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative***
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

***For PR, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as PR negative. However it is possible that reviewers will disagree so we can reclassify this as positive if required

[3] HER2 allocation of result from POSH database to a HER2 category:

Result	Category result assigned to
FISH/CISH positive	Positive**
3+	Positive
FISH/CISH borderline	Negative**
2+	Negative
FISH/CISH negative	Negative**
1+	Negative
0	Negative
Not done	Not done
Unknown	Missing/unknown

** FISH/CISH results take precedence i.e. a 2+ result which is later found to have a FISH/CISH positive result is categorised as Positive rather than Borderline.

[4] An ultrasound measurement was used if available, followed by a mammogram if the ultrasound was unavailable or a clinical examination/description if the mammogram was unavailable.

2.5 Data sources/measurement

The tumour biopsy, definitive histopathological report, clinical and radiological reports were all submitted to the study. Pathological characteristics of the tumours were taken from the diagnostic histopathology report, clinical staging from the clinical and radiological reports.

National death data were obtained for patients in the cohort from the Medical Research Information Service (MRIS).

ER, PR and HER2 data were taken from pathology reports. Scoring systems varied as expected across contributing hospitals. Positive and Negative categories are straightforward however borderline results exist in all three IHC categories and were classified into a separate borderline group. The borderline category was merged with negative for the purposes of these analyses.

This paper presents the results of analyses conducted on follow up data available up until 11-Apr-2012.

2.6 Bias

Clinical data for all patients were collected via standard clinical research forms which were completed from the clinical notes by the Clinical Trials Practitioner in each centre.

HER2 data: There are concerns regarding the amount of missing HER2 data obtained. In addition:

- HER2 Testing was only widely introduced after 2006 (proportion tested prior to 2006 was 83% (1704/2041), proportion tested on/after 2006 was 98% (897/915)). Prior to 2006 HER2 testing was more likely to have been carried out in patients who had progressed (93% i.e. 520 tested out of 561 who progressed, compared to 80% i.e. 1184 tested out of 1480 who had not progressed). Therefore, patients for whom we knew their HER2 status were more likely to have had a worse prognosis. Hence, if we selected patients on the basis of HER2 testing and compared them to patients who may or may not have been HER2 tested this would have been biased as the patients who have been HER2 tested could look worse by comparison.

- In addition, any analyses that select any patients which have a known HER2 status (which includes patients diagnosed before 2006) will include more cases who had relapsed (and were therefore tested for HER2 amplification retrospectively) than the whole cohort which could potentially compromise the validity of results.

2.7 Study Size

This is covered in the BMC paper.

2.8 Statistical Methods

Patients excluded from the analyses
Patients were excluded from this analysis if they were 41 years of age or over at the date of invasive breast cancer diagnosis (43 patients) i.e. a patient born on 01-Jan-1960 would be included if she was diagnosed before 01-Jan-2001 and excluded if she was diagnosed on or after 01-Jan-2001. In addition, patients were excluded if we didn't have confirmation that they had invasive cancer from pathology results or were missing primary data (47 patients). As a result, a total of 2932 were included in the analysis population.

Primary outcome measure
Overall Survival (OS) where OS is defined as the time from the date of invasive breast cancer diagnosis to death from any cause. Patients who had not died will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the primary outcome analysis.

Secondary outcome measures
Distant Disease Free Interval (DDFI) where DDFI is defined as the time from the date of invasive breast cancer diagnosis to distant relapse or death from breast cancer. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died from breast cancer or relapsed at the time of analysis will be censored at their date of last follow up/death.

Univariate analyses
1. We summarised the following characteristics of the cohort:
    - Age at diagnosis, in years – median (range, IQR), n (%);
    - Duration of follow-up – median (range, IQR), n (%);
    - Age at first birth, in years – median (range, IQR), n (%), missing/unknown - n(%);
    - Age at menarche, in years – median (range, IQR), n (%),missing/unknown - n(%);
    - Body mass index, in kg/m^2 – median (range, IQR), n (%), missing/unknown - n(%);
    - Number with and without children – n (%)
        - Number of children (for those with children) – median (range, IQR);
    - Use of contraceptive pill (ever, never) – n(%);
    - Smoker (ever, never, missing/unknown) – n(%);
    - Menopausal status (pre, peri, post, or missing/unknown) – n (%);
    - Number of patients with at least one first or second degree relatives with breast cancer (Yes, no, or missing/unknown) – n (%);
    - Presentation (symptomatic, screen-detected, other, or missing/unknown) – n (%).

2. We summarised the following tumour characteristics of the cohort and compared these by Oestrogen receptor(ER) negative and positive status using Pearson Chi-squared tests for categorical variables and Wilcoxon Mann-Whitney tests for continuous variables:
    - Histological grade (1, 2, 3, not graded, or missing/unknown) – n (%);
    - Histological type (Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown) – n (%);
    - Location of the cancer (multifocal, localised, or missing/unknown) – n (%);
    - Progesterone receptor (PR) status (negative, positive, or missing/unknown) – n (%);
    - Human Epidermal growth factor receptor 2 (HER2) status (negative, positive, borderline, or missing/unknown) – n (%);
    - M stage (M0, M1, or missing/unknown) – n (%).

    The following excludes patients treated with neo-adjuvant chemotherapy:
    - Pathological T stage (T0, T1, T2, T3, T4, Tis, Tx, or missing/unknown) – n(%);
    - N stage (N0, N1, or missing/unknown) – n (%);
    - Number of axillary lymph nodes recovered – median(range, IQR), n(%), missing/unknown - n(%);
        - Number of positive axillary lymph nodes – median(range, IQR), n(%);
    - Maximum diameter invasive tumour, in mm - median (range, IQR), n(%), missing/unknown - n(%);
    - Maximum tumour diameter (including ductal carcinoma in-situ), in mm - median (range, IQR), n(%), missing/unknown - n(%).

3. For neo-adjuvant chemotherapy patients, we cross-tabulated and summarised the clinical and pathological T stages of the tumours. The following categories were included:
    - T0, T1, T2, T3, T4, Tis[1], Tx[1], T4d/inflammatory[2], or missing/unknown) – n (%).

[1] for Pathological T stage only.
[2] for Clinical T stage only.

4. We summarised the following primary treatment type of the cohort:
    - Definitive breast surgery for patients who have had/not had radiotherapy (mastectomy, breast conserving surgery, nodal surgery only, no surgery, or missing/unknown) – n (%);
    - Chemotherapy timing (adjuvant[1], neo-adjuvant, not applicable, or missing/unknown) – n (%);
    - Chemotherapy regimen (anthracycline, anthracycline & taxane, taxane, none, other[2], or missing/unknown) – n(%);
    - Adjuvant trastuzumab (yes, or no/missing/unknown) – n (%);
    - Hormone treatment (yes, or no/missing/unknown) – n (%);
    - Oophorectomy (yes, or no/missing/unknown);
    - Ovarian suppression (yes, or no/missing/unknown) – n(%);
    - LHRH (yes, or no/missing/unknown) – n(%).

[1] excluding any treatment for M1 disease.
[2] for example, CMF or anything not containing an anthracycline or taxane.

5. The following characteristics were summarised and compared against the West Midlands Cancer Intelligence Unit[3,4]:
    - Age at diagnosis (18 to 25, 26 to 30, 31 to 35, or 36 to 40) – n(%);
    - Year of diagnosis (2000, 2001, 2002, 2003, 2004, 2005, 2006, or 2007) – n (%);
    - Histological Grade (1, 2, 3, not graded, or missing/unknown) – n (%);
    - ER status (negative, positive, or missing/unknown);
    - PR status (negative, positive, or missing/unknown) – n (%);
    - HER2 status (negative, positive, borderline, or missing/unknown) – n (%);
    - Maximum diameter invasive tumour, in mm (<15mm, 15 to 20mm, >20 to 35mm, >35 to 50mm, >50mm, or missing/unknown) - n(%);
    - N stage (N0, N1, or missing/unknown) – n (%).

[3] Only patients diagnosed in England in the POSH cohort and the West Midlands Cancer Intelligence Unit were directly compared. Patients diagnosed in Wales, Scotland, and Northern Ireland were compared in a separate column.
[4] For each characteristic, the proportion of POSH cohort patients from the West Midlands Cancer Intelligence Unit was also calculated.

6. The following variables will be summarised in the text of the paper:
    - Imaging results (mammogram only; MRI only; ultrasound only; mammogram & MRI; mammogram & ultrasound; MRI & ultrasound; mammogram, MRI & ultrasound; or missing/unknown) – n (%);
    - Lymphovascular invasion (Absent, Present, or missing/unknown) – n(%);
    - Axillary surgery type (Axillary sampling[5], axillary clearance, no surgery, or missing/unknown) – n(%);
    - Family history (yes, no, or missing/unknown) – n(%)[6].

[5] including sentinel node sampling.
[6] only patients with a screen-detected presentation.

7. We produced Kaplan-Meier survival curves of OS and DDFI for all patients included in the analysis.
   In addition, OS and DDFI estimates with corresponding 95% confidence intervals were produced.

8. We produced Kaplan-Meier survival curves of OS and DDFI and compared the survival curves using an unadjusted univariate Cox model for patients with an ER negative and positive status (patients with an unknown/missing ER status were excluded from this comparison).
   In addition, OS and DDFI estimates with corresponding 95% confidence intervals were produced.

Hazard Ratios

Evidence suggests that the effect of ER status changes over time (Azzato, et al, 2009, Bellera et al, 2010)[1]. Indeed, this was evident after testing the proportional hazards assumption based on the Schoenfeld residuals and using the identity matrix for the time-scaling function[2] i.e. using the estat phtest command in STATA. This result provided strong evidence against the Cox proportional hazards assumption ($p<0.001$), which was also seen when plotting the scaled Schoenfeld residuals over time[2].

As a result of the time-varying effects of the ER status, a flexible parametric survival model was programmed in STATA using the stpm2 command (Lambert, Royston, 2009)[3] to model ER as a time-dependent covariate. The degrees of freedom for the restricted cubic spline function used for the hazard rate was set to the default setting of 3, whilst the degrees of freedom for the time-dependent effects was set so as to provide the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC). The time-varying hazard ratio and 95% confidence interval was plotted over time and 2-, 5-, and 8-year relative hazard ratios and survival estimates were produced.

[1]The Azzato, et al paper can be found at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2695697/. The Bellera et al paper can be found at http://www.biomedcentral.com/1471-2288/10/20.
[2]Results obtained from T Maishman's MSc Project analysis undertaken on POSH data downloaded in May 2011.
[3] The Lambert & Royston paper can be found at www.stata-journal.com/article.html?article=st0165 or http://www.pauldickman.com/cancerepi/handouts/handouts_survival/Lambert2009.pdf

Method used to handle missing data

This was a complete case analysis.

A.3.2 Statistical Analysis Plan – Paper 2

Statistical analysis plan (SAP), approved on 4-Dec-2012, and formatted for Lancet Oncology Appendix (as per SAP for Paper 5 for consistency purposes for this thesis).

[Please note: Figures in this SAP are taken from the POSH data available up until May 2011, and thus only represent approximations of the new data due to be downloaded from the POSH database in April 2012.]

Please note: This statistical analysis plan has been written in the past tense because it will form the basis of a paper. The headings used in this document come from the STROBE reporting guideline for observational studies (see http://www.strobe-statement.org/ or http://www.annals.org/content/147/8/W-163.full.pdf+html).

Statistical Analysis Plan Version

Issue no	Revision History	Author	Date
0.1	First draft written based on discussion at meeting on 12th April 2012 with Doug Altman, Diana Eccles, Louise Stanton (nee Dent), Tom Maishman, Ramsey Cutress, Ellen Copson, Bryony Eccles, and Sue Gerty.	Tom Maishman	10th May 2012
0.2	Updates made after meeting with Louise Stanton on 14-Nov-2012	Tom Maishman	14th Nov 2012
1	Updated based on comments from Ellen Copson and Diana Eccles.	Tom Maishman	4th Dec 2012

1. Introduction

1.1 Background / Rationale
(Not included in SAP)

1.2 Objectives

This paper presents the results from analyses carried out on data collected from the POSH study.

The primary objective was:

- To describe the pathology and treatment of young women with invasive breast cancer according to their ethnic origin,

Secondary objectives were:

- To compare survival outcome according to ethnic origin.

2. Methods

2.1 Study Design
The POSH study is a prospective cohort study. The protocol for the study can be found in the following journal article http://www.biomedcentral.com/1471-2407/7/160.

2.2 Setting
The POSH study recruited women from breast cancer units across England, Scotland, Wales and Northern Island between 1st June 2001 to 31st January 2008.

2.3 Participants
The study recruited 3052 women aged 40 years or younger at breast cancer diagnosis. The women had to have been diagnosed with breast cancer between January 2000 and January 2008. In addition, 43 women aged 41-50 were also included if they had a known BRCA1 or BRCA2 gene mutation and were diagnosed with invasive breast cancer within the study period were excluded for this analysis. Women were excluded if they had a previous invasive malignancy (with the exception of non-melanomatous skin cancer), were not available for follow up or refused consent to retain diagnostic and follow up data. A total of 2932 women were included in the analysis population.

Clinical follow up data were obtained from the patient medical records by the clinical trials practitioner (CTP) at each recruiting centre. Data forms collecting information at diagnosis, 6 months, 12 months were completed by the CTP usually at 12 months from diagnosis. Annual data collection was continued from the date of definitive diagnosis until death, loss to follow up or until the end of the current phase of the study (Apr 2012).

2.4 Variables (data taken as of May 2011)

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
2.4.1 Primary outcome			
Time to death from any cause	Survival data Date of death from any cause – Date of invasive breast cancer diagnosis	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A
2.4.2 Secondary outcomes			
Time to distant relapse or death from breast cancer	Survival data Date of first distant relapse (or death from breast cancer) – Date of invasive breast cancer diagnosis	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit. Patients who died from non-breast cancer deaths were censored at the date of death.	N/A
2.4.3 Candidate predictor			
Ethnicity	Categorical Caucasian/White, Black, Asian, Other, or missing/unknown	41 (1.4%) records	Consider MCAR
2.4.4 Potential confounders / effect modifiers - measured at breast cancer diagnosis presentation			
1. Age at diagnosis	Continuous, in years	0 records	N/A
2. Age when the first child was born	Continuous, in years	92 (3.1%) records missing (and 784 values not applicable)	Consider MAR
3. Age at menarche	Continuous, in years	0 records	N/A
4. Body Mass Index (BMI)	Categorical Underweight/Healthy, Overweight, Obese, or missing/unknown	114 (3.9%) records	Consider MAR
5. Number of children	Categorical 0, 1, …, or 8	25 (0.9%) records	Consider MCAR
6. Use of contraceptive pill	Categorical Ever, or never	0 records	N/A
7. Smoker	Categorical Ever, never, or missing/unknown	93 (3.2%) records	Consider MCAR
8. Menopausal status	Categorical Pre-, Peri-, or Post-menopausal, or missing/unknown.	59 (2.0%) records	Consider MCAR
9. Number of patients with at least one first or second degree relative with breast cancer	Categorical Yes, no, or missing/unknown.	106 (3.6%) records	Consider MCAR
10. Presentation	Categorical Symptomatic, screen-detected, other, or missing/unknown	14 (0.5%) records	Consider MCAR
11. Histological Tumour grade	Categorical 1, 2, 3, not graded, or missing/unknown	77 (2.6%) records missing/unknown, 2 (0.1%) not graded	MCAR. Inadequate reporting by pathologist. If grade of core biopsy tumour not stated, and after neo-adjuvant chemotherapy there was a complete pathological response then no tumour to report on.
12. Histological type	Categorical Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown	37 (1.3%) records	MCAR (Same as above for grade)
13. Focality (distribution of tumour)	Categorical Multifocal, localised, or missing/unknown	286 (9.8%) records	MCAR (Same as above for grade)
14. ER status[1]	Categorical Negative, positive, or missing/unknown	12 (0.4%) records	MCAR (most people have ER done)
15. PR[2] status	Categorical Negative, positive, or missing/unknown	581 (19.8%) records	MAR - missing because specific centres don't do PR IHC.
16. HER2[3] status	Categorical Negative, positive, or missing/unknown	355 (12.1%) records	Missing when diagnosis predated routine testing. Potential bias towards missing in patients not experiencing disease recurrence.

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
17. TNT status	Categorical Triple Negative (TNT), Not TNT, or missing/unknown	N/A (derived from ER, PR and HER2 above)	N/A
18. M stage	Categorical M0, M1 or missing/unknown	22 (0.8%) records	MCAR
19. Pathological N stage (lymph node status)	Categorical N0, N1 or missing/unknown	65 (2.2%) records	MCAR. No axillary surgery, no lymph nodes in resected specimen.
20. Number of positive Lymph nodes	Categorical 0, 1-3, 4-9, 10+, or missing/unknown	59 (2.0%) records	Same as above (MCAR)
21. Number of axillary lymph nodes	Continuous (integer)	46 (1.6%) records	MCAR (Same as above for N stage)
22. Lymphovascular invasion	Categorical Present, absent or missing/unknown	222 (7.6%) records	MCAR (Same as above for grade)
23. Maximum tumour diameter invasive (tumour size)	Continuous, in mm or Categorical <15mm, 15mm to 20mm, >20mm to 35mm, >35mm to 50mm, >50mm, or missing/unknown	193 (6.6%) records	Missing for similar reasons as tumour grade (MCAR)
24. Maximum tumour diameter (including ductal carcinoma in-situ) (pathological)	Continuous, in mm	161 (5.5%) records	Missing for similar reasons as tumour grade (MCAR)
25. Pathological T stage (for patients receiving neo-adjuvant chemotherapy)	Categorical T0, T1, T2, T3, T4, Tis, Tx, or missing/unknown	12 (0.4%) records	MCAR. However pathological stage post neo-adjuvant chemotherapy is not comparable with primary resection
26. Definitive surgery	Categorical Breast Conserving Surgery (BCS), Mastectomy, No surgery, Nodal surgery only, or missing/unknown	2 (0.1%) records	Consider MCAR
27. Chemotherapy timing	Categorical Adjuvant, neo-adjuvant, palliative, not applicable, or missing/unknown	0 records	N/A
28. Chemotherapy regimen	Categorical Anthracyclines, A&T, Taxanes, Other, or None	0 records	N/A
29. Adjuvant trastuzumab	Categorical Yes, no/missing/unknown	2587 (88.2%) records no/missing/unknown	Consider MAR
30. Radiotherapy	Categorical Yes, no/missing/unknown	598 (20.4%) records no/missing/unknown	Consider MAR
31. Hormone treatment	Categorical Yes, no/missing/unknown	2156 (73.5%) records no/missing/unknown	Consider MAR
32. Oophorectomy	Categorical Yes, no/missing/unknown	2558 (87.2%) records no/missing/unknown	Consider MAR
33. Ovarian suppression	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2172 (74.1%) records no/missing/unknown	Consider MAR
34. LHRH	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2177 (74.2%) records no/not applicable/missing/unknown	Consider MAR
35. Diagnosis Year	Categorical 2000, 2001… , 2008	0 records	N/A
2.4.5 Additional (descriptive) variables			
Length of follow-up	Continuous, in months	0 records	N/A

[1] Oestrogen receptor allocation of result from POSH database to Oestrogen receptor category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative*
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

*For ER, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as ER negative, and an Allred score of 3+ treated as ER positive. However it is possible that reviewers will disagree so we can reclassify this as positive if required.

[2] PR allocation of result from POSH database to a PR category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative***
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

***For PR, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as PR negative. However it is possible that reviewers will disagree so we can reclassify this as positive if required

[3] HER2 allocation of result from POSH database to a HER2 category:

Result	Category result assigned to
FISH/CISH positive	Positive**
3+	Positive
FISH/CISH borderline	Negative**
2+	Negative
FISH/CISH negative	Negative**
1+	Negative
0	Negative
Not done	Not done
Unknown	Missing/unknown

** FISH/CISH results take precedence i.e. a 2+ result which is later found to have a FISH/CISH positive result is categorised as Positive rather than Borderline.

2.5 Data sources/measurement

The tumour biopsy, definitive histopathological report, clinical and radiological reports were all submitted to the study. Pathological characteristics of the tumours were taken from the diagnostic histopathology report, clinical staging from the clinical and radiological reports.

National death data were obtained for patients in the cohort from the Medical Research Information Service (MRIS).

ER, PR and HER2 data were taken from pathology reports. Scoring systems varied as expected across contributing hospitals. Positive and Negative categories are straightforward however borderline results exist in all three IHC categories and were classified into a separate borderline group. The borderline category was merged with negative for the purposes of these analyses.

This paper presents the results of analyses conducted on follow up data available up until 11-Apr-2012.

2.6 Bias

Clinical data for all patients were collected via standard clinical research forms which were completed from the clinical notes by the Clinical Trials Practitioner in each centre.

HER2 data: There are concerns regarding the amount of missing HER2 data obtained. In addition:

- HER2 Testing was only widely introduced after 2006 (proportion tested prior to 2006 was 83% (1704/2041), proportion tested on/after 2006 was 98% (897/915)). Prior to 2006 HER2 testing was more likely to have been carried out in patients who had progressed (93% i.e. 520 tested out of 561 who progressed, compared to 80% i.e. 1184 tested out of 1480 who had not progressed). Therefore, patients for whom we knew their HER2 status were more likely to have had a worse prognosis. Hence, if we selected patients on the basis of HER2 testing and compared them to patients who may or may not have been HER2 tested this would have been biased as the patients who have been HER2 tested could look worse by comparison.

- In addition, any analyses that select any patients which have a known HER2 status (which includes patients diagnosed before 2006) will include more cases who had relapsed (and were therefore tested for HER2 amplification retrospectively) than the whole cohort which could potentially compromise the validity of results.

2.7 Study Size

This is covered in the BMC paper.

2.8 Statistical Methods

Patients excluded from the analyses
Patients were excluded from this analysis if they were 41 years of age or over at the date of invasive breast cancer diagnosis (43 patients) i.e. a patient born on 01-Jan-1960 would be included if she was diagnosed before 01-Jan-2001 and excluded if she was diagnosed on or after 01-Jan-2001. In addition, patients were excluded if we didn't have confirmation that they had invasive cancer from pathology results or were missing primary data (47 patients). As a result, a total of 2932 were included in the analysis population.

Primary outcome measure
Overall Survival (OS) where OS is defined as the time from the date of invasive breast cancer diagnosis to death from any cause. Patients who had not died will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the primary outcome analysis.

Secondary outcome measures
Distant Relapse-Free Survival (DRFS) where DRFS is defined as the time from the date of invasive breast cancer diagnosis to distant relapse or death from breast cancer. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died from breast cancer or relapsed at the time of analysis will be censored at their date of last follow up/death.

Univariate analyses
1. We summarised the following patient and tumour characteristics of the cohort and compared these by Ethnic categories (White/Caucasian vs. Black, White/Caucasian vs. Asian, and Black vs. Asian) using Pearson Chi-squared tests for categorical variables and Wilcoxon Mann-Whitney tests for continuous variables:
    - Age at diagnosis, in years – median (range, IQR), n (%);
    - Duration of follow-up – median (range, IQR), n (%);
    - Age at first birth, in years – median (range, IQR), n (%), missing/unknown - n(%);
    - Age at menarche, in years – median (range, IQR), n (%),missing/unknown - n(%);
    - Body mass index, in kg/m^2 – median (range, IQR), n (%), missing/unknown - n(%);
    - Number with and without children – n (%)
        - Number of children (for those with children) – median (range, IQR);
    - Use of contraceptive pill (ever, never) – n(%);
    - Smoker (ever, never, missing/unknown) – n(%);
    - Menopausal status (pre, peri, post, or missing/unknown) – n (%);
    - Number of patients with at least one first or second degree relatives with breast cancer (Yes, no, or missing/unknown) – n (%);
    - Number of relatives with breast cancer (0, 1, 2, >2, or missing/unknown) – n (%);
    - Presentation (symptomatic, screen-detected, other, or missing/unknown) – n (%).
    - Histological grade (1, 2, 3, not graded, or missing/unknown) – n (%);
    - Histological type (Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown) – n (%);
    - Location of the cancer (multifocal, localised, or missing/unknown) – n (%);
    - Progesterone receptor (PR) status (negative, positive, or missing/unknown) – n (%);
    - Human Epidermal growth factor receptor 2 (HER2) status (negative, positive, borderline, or missing/unknown) – n (%);
    - Triple Negative Tumour (TNT) Status (TNT, Not TNT, or missing/unknown) – n(%);
    - M stage (M0, M1, or missing/unknown) – n (%).
    - Pathological T stage (T0, T1, T2, T3, T4, Tis, Tx, or missing/unknown) – n(%);
    - N stage (N0, N1, or missing/unknown) – n (%);
    - Number of axillary lymph nodes recovered – median(range, IQR), n(%), missing/unknown - n(%);
        - Number of positive axillary lymph nodes – median(range, IQR), n(%);
    - Maximum diameter invasive tumour, in mm - median (range, IQR), n(%), missing/unknown - n(%);
    - Maximum tumour diameter (including ductal carcinoma in-situ), in mm - median (range, IQR), n(%), missing/unknown - n(%).

2. We summarised the following primary treatment type of the cohort by Ethnic categories:
    - Definitive breast surgery for patients who have had/not had radiotherapy (mastectomy, breast conserving surgery, nodal surgery only, no surgery, or missing/unknown) – n (%);
    - Chemotherapy timing (adjuvant[1], neo-adjuvant, not applicable, or missing/unknown) – n (%);
    - Chemotherapy regimen (anthracycline, anthracycline & taxane, taxane, none, other[2], or missing/unknown) – n(%);
    - Adjuvant trastuzumab (yes, or no/missing/unknown) – n (%);
    - Hormone treatment (yes, or no/missing/unknown) – n (%);
    - Oophorectomy (yes, or no/missing/unknown);
    - Ovarian suppression (yes, or no/missing/unknown) – n(%);
    - LHRH (yes, or no/missing/unknown) – n(%).

[1] excluding any treatment for M1 disease.
[2] for example, CMF or anything not containing an anthracycline or taxane.

3. We produced Kaplan-Meier survival curves of OS and DRFS and compared the survival curves of Ethnic categories using an unadjusted univariate Cox model (patients with an unknown/missing Ethnic category were excluded from this comparison). In addition, OS and DRFS estimates with corresponding 95% confidence intervals were produced.

Multivariable analyses
Comparison groups:

- White/Caucasian (reference category) versus Black version Asian (all patients)
- White/Caucasian (reference category) versus Black version Asian (patients with ER negative tumours only)
- White/Caucasian (reference category) versus Black version Asian (patients with ER positive tumours only)
- White/Caucasian (reference category) versus Black version Asian (patients with TNT tumours only)

For the comparisons i) to iv) above, we fitted a multivariable model for OS and DRFS adjusting for the following covariates:

- Body Mass Index (BMI) (fitted as a categorical covariate [Underweight/Healthy, Overweight or Obese]);
- Histological Grade (fitted as a categorical covariate [1, 2 or 3]);
- Maximum invasive tumour size, in mm (fitted as a continuous covariate);
- N stage (fitted as a binary covariate [N0 or N1]);
- ER status (stratified by this covariate which was fitted as a binary covariate [Negative or Positive]) **(all patients only)**.

Hazard Ratios
Evidence suggests that the effect of ER status changes over time (Azzato, et al, 2009, Bellera et al, 2010)[1]. Indeed, this was evident after testing the proportional hazards assumption based on the Schoenfeld residuals and using the identity matrix for the time-scaling function[2] i.e. using the estat phtest command in STATA. This result provided strong evidence against the Cox proportional hazards assumption ($p<0.001$), which was also seen when plotting the scaled Schoenfeld residuals over time[2].

As a result of the time-varying effects of the ER status, a stratified Cox model was programmed in STATA, stratified by ER status for the multivariable analyses.

[1]The Azzato, et al paper can be found at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2695697/. The Bellera et al paper can be found at http://www.biomedcentral.com/1471-2288/10/20.
[2]Results obtained from T Maishman's MSc Project analysis undertaken on POSH data downloaded in May 2011.

Method used to handle missing data
This was a complete case analysis.

A.3.3 Statistical Analysis Plan – Paper 3

Statistical analysis plan (SAP), approved on 5-Apr-2013, and formatted for Lancet Oncology Appendix (as per SAP for Paper 5 for consistency purposes for this thesis).

[Please note: Figures in this SAP are taken from the POSH data available up until May 2011, and thus only represent approximations of the new data due to be downloaded from the POSH database in October 2013.]

Please note: This statistical analysis plan has been written in the past tense because it will form the basis of a paper. The headings used in this document come from the STROBE reporting guideline for observational studies (see http://www.strobe-statement.org/ or http://www.annals.org/content/147/8/W-163.full.pdf+html).

Statistical Analysis Plan Version

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0.2	Updates made after meeting with Louise Stanton on 14-Nov-2012	Tom Maishman	14th Nov 2012
1	Updated based on comments from Ellen Copson and Diana Eccles.	Tom Maishman	5th Apr 2013

1. Introduction

1.1 Background / Rationale

(Not included in SAP)

1.2 Objectives

This paper presents the results from analyses carried out on data collected from the POSH study.

The primary objective was:

- To investigate whether a family history of breast cancer at presentation alters the prognosis of young onset breast cancer independent of known prognostic factors.

Secondary objectives were:

- To investigate whether the closeness (first v second degree) of breast cancer relatives at presentation alters the prognosis of young onset breast cancer independent of known prognostic factors.

2. Methods

2.1 Study Design

The POSH study is a prospective cohort study. The protocol for the study can be found in the following journal article http://www.biomedcentral.com/1471-2407/7/160.

2.2 Setting

The POSH study recruited women from breast cancer units across England, Scotland, Wales and Northern Island between 1st June 2001 to 31st January 2008.

2.3 Participants

The study recruited 3052 women aged 40 years or younger at breast cancer diagnosis. The women had to have been diagnosed with breast cancer between January 2000 and January 2008. Women were excluded if they had a previous invasive malignancy (with the exception of non-melanomatous skin cancer), were not available for follow up or refused consent to retain diagnostic and follow up data. A total of 2956 women were included in the analysis population.

Clinical follow up data were obtained from the patient medical records by the clinical trials practitioner (CTP) at each recruiting centre. Data forms collecting information at diagnosis, 6 months, 12 months were completed by the CTP usually at 12 months from diagnosis. Annual data collection was continued from the date of definitive diagnosis until death, loss to follow up or until the end of the current phase of the study (Oct 2013).

2.5 Variables (data taken as of May 2011)

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
2.4.1 Primary outcome			
Time to death from any cause	Survival data Date of death from any cause – Date of invasive breast cancer diagnosis	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A
2.4.2 Secondary outcomes			
Time to distant relapse or death from breast cancer	Survival data Date of first distant relapse (or death from breast cancer) – Date of invasive breast cancer diagnosis	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit. Patients who died from non-breast cancer deaths were censored at the date of death.	N/A
2.4.3 Candidate predictor			
Body Mass Index (BMI)	Categorical Underweight/Healthy, Overweight, Obese, or missing/unknown	114 (3.9%) records	Consider MAR
2.4.4 Potential confounders / effect modifiers - measured at breast cancer diagnosis presentation			
1. Age at diagnosis	Continuous, in years	0 records	N/A
2. Age when the first child was born	Continuous, in years	92 (3.1%) records missing (and 784 values not applicable)	Consider MAR
3. Age at menarche	Continuous, in years	0 records	N/A
4. Ethnicity	Categorical Caucasian/White, Black, Asian, Other, or missing/unknown	41 (1.4%) records	Consider MCAR
5. Number of children	Categorical 0, 1, …, or 8	25 (0.9%) records	Consider MCAR
6. Use of contraceptive pill	Categorical Ever, or never	0 records	N/A
7. Smoker	Categorical Ever, never, or missing/unknown	93 (3.2%) records	Consider MCAR
8. Menopausal status	Categorical Pre-, Peri-, or Post-menopausal, or missing/unknown.	59 (2.0%) records	Consider MCAR
9. Number of patients with at least one first or second degree relative with breast cancer	Categorical Yes, no, or missing/unknown.	106 (3.6%) records	Consider MCAR
10. Presentation	Categorical Symptomatic, screen-detected, other, or missing/unknown	14 (0.5%) records	Consider MCAR
11. Histological Tumour grade	Categorical 1, 2, 3, not graded, or missing/unknown	77 (2.6%) records missing/unknown, 2 (0.1%) not graded	MCAR. Inadequate reporting by pathologist. If grade of core biopsy tumour not stated, and after neo-adjuvant chemotherapy there was a complete pathological response then no tumour to report on.
12. Histological type	Categorical Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown	37 (1.3%) records	MCAR (Same as above for grade)
13. Focality (distribution of tumour)	Categorical Multifocal, localised, or missing/unknown	286 (9.8%) records	MCAR (Same as above for grade)
14. ER status[1]	Categorical Negative, positive, or missing/unknown	12 (0.4%) records	MCAR (most people have ER done)
15. PR[2] status	Categorical Negative, positive, or missing/unknown	581 (19.8%) records	MAR - missing because specific centres don't do PR IHC.
16. HER2[3] status	Categorical Negative, positive, or missing/unknown	355 (12.1%) records	Missing when diagnosis predated routine testing. Potential bias towards missing in patients not experiencing disease recurrence.

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
17. TNT status	Categorical Triple Negative (TNT), Not TNT, or missing/unknown	N/A (derived from ER, PR and HER2 above)	N/A
18. M stage	Categorical M0, M1 or missing/unknown	22 (0.8%) records	MCAR
19. Pathological N stage (lymph node status)	Categorical N0, N1 or missing/unknown	65 (2.2%) records	MCAR. No axillary surgery, no lymph nodes in resected specimen.
20. Maximum tumour diameter invasive (tumour size)	Continuous, in mm or Categorical <15mm, 15mm to 20mm, >20mm to 35mm, >35mm to 50mm, >50mm, or missing/unknown	193 (6.6%) records	Missing for similar reasons as tumour grade (MCAR)
21. Maximum tumour diameter (including ductal carcinoma in-situ) (pathological)	Continuous, in mm	161 (5.5%) records	Missing for similar reasons as tumour grade (MCAR)
22. Definitive surgery	Categorical Breast Conserving Surgery (BCS), Mastectomy, No surgery, Nodal surgery only, or missing/unknown	2 (0.1%) records	Consider MCAR
23. Chemotherapy timing	Categorical Adjuvant, neo-adjuvant, palliative, not applicable, or missing/unknown	0 records	N/A
24. Chemotherapy regimen	Categorical Anthracyclines, A&T, Taxanes, Other, or None	0 records	N/A
25. Adjuvant trastuzumab	Categorical Yes, no/missing/unknown	2587 (88.2%) records no/missing/unknown	Consider MAR
26. Radiotherapy	Categorical Yes, no/missing/unknown	598 (20.4%) records no/missing/unknown	Consider MAR
27. Hormone treatment	Categorical Yes, no/missing/unknown	2156 (73.5%) records no/missing/unknown	Consider MAR
28. Oophorectomy	Categorical Yes, no/missing/unknown	2558 (87.2%) records no/missing/unknown	Consider MAR
29. Ovarian suppression	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2172 (74.1%) records no/missing/unknown	Consider MAR
30. LHRH	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2177 (74.2%) records no/not applicable/missing/unknown	Consider MAR
2.4.5 Additional (descriptive) variables			
Length of follow-up	Continuous, in months	0 records	N/A

[1] Oestrogen receptor allocation of result from POSH database to Oestrogen receptor category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative*
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

*For ER, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as ER negative, and an Allred score of 3+ treated as ER positive. However it is possible that reviewers will disagree so we can reclassify this as positive if required.

[2] PR allocation of result from POSH database to a PR category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative***
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

***For PR, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as PR negative. However it is possible that reviewers will disagree so we can reclassify this as positive if required

[3] HER2 allocation of result from POSH database to a HER2 category:

Result	Category result assigned to
FISH/CISH positive	Positive**
3+	Positive
FISH/CISH borderline	Negative**
2+	Negative
FISH/CISH negative	Negative**
1+	Negative
0	Negative
Not done	Not done
Unknown	Missing/unknown

** FISH/CISH results take precedence i.e. a 2+ result which is later found to have a FISH/CISH positive result is categorised as Positive rather than Borderline.

2.5 Data sources/measurement

The tumour biopsy, definitive histopathological report, clinical and radiological reports were all submitted to the study. Pathological characteristics of the tumours were taken from the diagnostic histopathology report, clinical staging from the clinical and radiological reports.

National death data were obtained for patients in the cohort from the Medical Research Information Service (MRIS).

ER, PR and HER2 data were taken from pathology reports. Scoring systems varied as expected across contributing hospitals. Positive and Negative categories are straightforward however borderline results exist in all three IHC categories and were classified into a separate borderline group. The borderline category was merged with negative for the purposes of these analyses.

This paper presents the results of analyses conducted on follow up data available up until 22-Oct-2013.

2.6 Bias

Clinical data for all patients were collected via standard clinical research forms which were completed from the clinical notes by the Clinical Trials Practitioner in each centre.

HER2 data: There are concerns regarding the amount of missing HER2 data obtained. In addition:

- HER2 Testing was only widely introduced after 2006 (proportion tested prior to 2006 was 83% (1704/2041), proportion tested on/after 2006 was 98% (897/915)). Prior to 2006 HER2 testing was more likely to have been carried out in patients who had progressed (93% i.e. 520 tested out of 561 who progressed, compared to 80% i.e. 1184 tested out of 1480 who had not progressed). Therefore, patients for whom we knew their HER2 status were more likely to have had a worse prognosis. Hence, if we selected patients on the basis of HER2 testing and compared them to patients who may or may not have been HER2 tested this would have been biased as the patients who have been HER2 tested could look worse by comparison.

- In addition, any analyses that select any patients which have a known HER2 status (which includes patients diagnosed before 2006) will include more cases who had relapsed (and were therefore tested for HER2 amplification retrospectively) than the whole cohort which could potentially compromise the validity of results.

2.7 Study Size

This is covered in the BMC paper.

2.8 Statistical Methods

Patients excluded from the analyses
Patients were excluded from this analysis if they were 41 years of age or over at the date of invasive breast cancer diagnosis (43 patients) i.e. a patient born on 01-Jan-1960 would be included if she was diagnosed before 01-Jan-2001 and excluded if she was diagnosed on or after 01-Jan-2001. In addition, patients were excluded if we didn't have confirmation that they had invasive cancer from pathology results or were missing primary data (21 patients). As a result, a total of 2956 were included in the analysis population.

Primary outcome measure
Overall Survival (OS) where OS is defined as the time from the date of invasive breast cancer diagnosis to death from any cause. Patients who had not died will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the primary outcome analysis.

Secondary outcome measures
Distant Disease Free Interval (DDFI) where DDFI is defined as the time from the date of invasive breast cancer diagnosis to distant relapse or death from breast cancer. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died from breast cancer or relapsed at the time of analysis will be censored at their date of last follow up/death.

Univariate analyses

1. We summarised the following patient and tumour characteristics of the cohort and compared these by BMI categories[1]:
    - Age at diagnosis, in years – median (range, IQR), n (%);
    - Duration of follow-up – median (range, IQR), n (%);
    - Age at first birth, in years – median (range, IQR), n (%), missing/unknown - n(%);
    - Age at menarche, in years – median (range, IQR), n (%),missing/unknown - n(%);
    - Ethnicity (White/Caucasian, Black, Asian, Other, or missing/unknown) – n (%);
    - Number with and without children – n (%)
        - Number of children (for those with children) – median (range, IQR);
    - Use of contraceptive pill (ever, never) – n(%);
    - Smoker (ever, never, missing/unknown) – n(%);
    - Menopausal status (pre, peri, post, or missing/unknown) – n (%);
    - Number of patients with at least one first or second degree relatives with breast cancer (Yes, no, or missing/unknown) – n (%);
    - Number of relatives with breast cancer (0, 1, 2, >2, or missing/unknown) – n (%);
    - Presentation (symptomatic, screen-detected, other, or missing/unknown) – n (%).
    - Histological grade (1, 2, 3, not graded, or missing/unknown) – n (%);
    - Histological type (Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown) – n (%);
    - Location of the cancer (multifocal, localised, or missing/unknown) – n (%);
    - Progesterone receptor (PR) status (negative, positive, or missing/unknown) – n (%);
    - Human Epidermal growth factor receptor 2 (HER2) status (negative, positive, borderline, or missing/unknown) – n (%);
    - Triple Negative Tumour (TNT) Status (TNT, Not TNT, or missing/unknown) – n(%);
    - M stage (M0, M1, or missing/unknown) – n (%).
    - Pathological T stage (T0, T1, T2, T3, T4, Tis, Tx, or missing/unknown) – n(%);
    - N stage (N0, N1, or missing/unknown) – n (%);
    - Number of axillary lymph nodes recovered – median(range, IQR), n(%), missing/unknown - n(%);
        - Number of positive axillary lymph nodes – median(range, IQR), n(%);
    - Maximum diameter invasive tumour, in mm - median (range, IQR), n(%), missing/unknown - n(%);
    - Maximum tumour diameter (including ductal carcinoma in-situ), in mm - median (range, IQR), n(%), missing/unknown - n(%).

[1] Pearson Chi-squared tests for categorical variables and Kruskal-Wallis tests for continuous variables were first used to compare BMI (as a categorical variable). If any significant differences found between BMI categories were found, Pearson Chi-squared tests for categorical variables and Wilcoxon Mann-Whitney tests for continuous variables were used to compare BMI categories (Underweight/Healthy vs. Overweight, Underweight/Healthy vs. Obese, and Overweight vs. Obese).

2. We summarised the following primary treatment type of the cohort by BMI categories:
    - Definitive breast surgery for patients who have had/not had radiotherapy (mastectomy, breast conserving surgery, nodal surgery only, no surgery, or missing/unknown) – n (%);
    - Chemotherapy timing (adjuvant[1], neo-adjuvant, not applicable, or missing/unknown) – n (%);
    - Chemotherapy regimen (anthracycline, anthracycline & taxane, taxane, none, other[2], or missing/unknown) – n(%);
    - Adjuvant trastuzumab (yes, or no/missing/unknown) – n (%);
    - Hormone treatment (yes, or no/missing/unknown) – n (%);
    - Oophorectomy (yes, or no/missing/unknown);
    - Ovarian suppression (yes, or no/missing/unknown) – n(%);
    - LHRH (yes, or no/missing/unknown) – n(%).

[1] excluding any treatment for M1 disease.
[2] for example, CMF or anything not containing an anthracycline or taxane.

3. We produced Kaplan-Meier survival curves of OS and DDFI and compared the survival curves of BMI categories using an unadjusted univariate Cox model.
   In addition, OS and DDFI estimates with corresponding 95% confidence intervals were produced.

Multivariable analyses
Comparison groups:

- Underweight/Healthy (reference category) versus Overweight version Obese (all patients)
- Underweight/Healthy (reference category) versus Overweight version Obese (patients with ER negative tumours only)
- Underweight/Healthy (reference category) versus Overweight version Obese (patients with ER positive tumours only)

For the comparisons i) to iv) above, we fitted a multivariable model for OS and DDFI adjusting for the following covariates:

- Age at diagnosis, in years (fitted as a continuous covariate);
- Histological Grade (fitted as a categorical covariate [1, 2 or 3]);
- Maximum tumour size, in mm (fitted as a continuous covariate);
- N stage (fitted as a binary covariate [N0 or N1]);
- HER2 status (fitted as a binary covariate [Negative or Positive]);
- ER status (stratified by this covariate which was fitted as a binary covariate [Negative or Positive]) **(all patients only)**.

Hazard Ratios
Evidence suggests that the effect of ER status changes over time (Azzato, et al, 2009, Bellera et al, 2010)[1]. Indeed, this was evident after testing the proportional hazards assumption based on the Schoenfeld residuals and using the identity matrix for the time-scaling function[2] i.e. using the estat phtest command in STATA. This result provided strong evidence against the Cox proportional hazards assumption ($p<0.001$), which was also seen when plotting the scaled Schoenfeld residuals over time[2].

As a result of the time-varying effects of the ER status, a stratified Cox model was programmed in STATA, stratified by ER status for the multivariable analyses.

[1]The Azzato, et al paper can be found at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2695697/. The Bellera et al paper can be found at http://www.biomedcentral.com/1471-2288/10/20.
[2]Results obtained from T Maishman's MSc Project analysis undertaken on POSH data downloaded in May 2011.

Method used to handle missing data
This was a complete case analysis.

A.3.4 Statistical Analysis Plan – Paper 4

Statistical analysis plan (SAP), approved on 6-Aug-2013, and formatted for Lancet Oncology Appendix (as per SAP for Paper 5 for consistency purposes for this thesis).

[Please note: Figures in this SAP are taken from the POSH data available up until May 2011, and thus only represent approximations of the new data due to be downloaded from the POSH database in October 2013.]

Please note: This statistical analysis plan has been written in the past tense because it will form the basis of a paper. The headings used in this document come from the STROBE reporting guideline for observational studies (see http://www.strobe-statement.org/ or http://www.annals.org/content/147/8/W-163.full.pdf+html).

Statistical Analysis Plan Version

Issue no	Revision History	Author	Date
0.1	First draft written based on discussion at meeting on 12th April 2012 with Doug Altman, Diana Eccles, Louise Stanton (nee Dent), Tom Maishman, Ramsey Cutress, Ellen Copson, Bryony Eccles, and Sue Gerty.	Tom Maishman	10th May 2012
0.2	Updates made after meeting with Louise Stanton on 14-Nov-2012	Tom Maishman	14th Nov 2012
0.3	Updates made after meeting with Louise Stanton on 04-Jan-2013	Tom Maishman	28th Jan 2013
0.4	Updates made after comments from Louise Stanton on 31-Jan-2013	Tom Maishman	1st Feb 2013
0.5	Updates made after meeting with Louise Stanton on 05-Feb-2013	Tom Maishman	5th Feb 2013
0.6	Updates made after meeting with Diana Eccles on 07-Feb-2013	Tom Maishman	7th Feb 2013
0.7	Updates made after meeting with Doug Altman, Diana Eccles and Louise Stanton on 19-Feb-2013	Tom Maishman	19th Feb 2013
0.8	Updates made after exploration of multiple imputation assumptions on 15-Mar-13	Tom Maishman	15th Mar 2013
0.9	Updates made after meeting with Louise Stanton on 26-Mar-13	Tom Maishman	26th Mar 2013
0.10	Updates made after comments from Diana Eccles	Tom Maishman	4th Apr 2013
0.11	Updates made after comments from Doug Altman	Tom Maishman	17th Jul 2013
1	Finalised version created following v0.11 changes	Tom Maishman	6th Aug 2013

1. Introduction

1.1 Background / Rationale
(Not included in SAP)

1.2 Objectives

This paper presents the results from analyses carried out on data collected from the POSH study.

The primary objective was:

- To investigate whether a family history of breast cancer at presentation alters the prognosis of young onset breast cancer independent of known prognostic factors.

Secondary objectives were:

- To investigate whether the closeness (first v second degree) of breast cancer relatives at presentation alters the prognosis of young onset breast cancer independent of known prognostic factors.

2. Methods

2.1 Study Design
The POSH study is a prospective cohort study. The protocol for the study can be found in the following journal article http://www.biomedcentral.com/1471-2407/7/160.

2.2 Setting
The POSH study recruited women from breast cancer units across England, Scotland, Wales and Northern Island between 1st June 2001 to 31st January 2008.

2.3 Participants
The study recruited 3052 women aged 40 years or younger at breast cancer diagnosis. The women had to have been diagnosed with breast cancer between January 2000 and January 2008. Women were excluded if they had a previous invasive malignancy (with the exception of non-melanomatous skin cancer), were not available for follow up or refused consent to retain diagnostic and follow up data. A total of 2956 women were included in the analysis population.

Clinical follow up data were obtained from the patient medical records by the clinical trials practitioner (CTP) at each recruiting centre. Data forms collecting information at diagnosis, 6 months, 12 months were completed by the CTP usually at 12 months from diagnosis. Annual data collection was continued from the date of definitive diagnosis until death, loss to follow up or until the end of the current phase of the study (Oct 2013).

Family history data: patients in the POSH study completed a family history questionnaire (http://www.biomedcentral.com/1471-2407/7/160 supplementary figure).

2.4 Variables (data taken as of May 2011)

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
2.4.1 Primary outcome			
Time to distant relapse or death from breast cancer	Survival data Date of first distant relapse (or death from breast cancer) – Date of invasive breast cancer diagnosis	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit. Patients who died from non-breast cancer deaths were censored at the date of death.	N/A
2.4.2 Candidate predictor			
Family History of breast cancer	Categorical Yes, no, missing/unknown	106 (3.6%) records	Family history may not be provided because they could be adopted or family history unknown. Consider MCAR.
2.4.3 Candidate predictor (secondary endpoint)			
Type of breast cancer relative	Categorical First degree relatives (FDR), second degree relatives (SDR), no FDR/SDR, or missing/unknown	106 (3.6%) records	Same as above (MCAR)
2.4.4 Potential confounders / effect modifiers - measured at breast cancer diagnosis presentation			
1. Age at diagnosis	Continuous, in years	0 records	N/A
2. Presentation	Categorical Symptomatic, screen-detected, other, or missing/unknown	14 (0.5%) records	Consider MCAR
3. Histological Tumour grade	Categorical 1, 2, 3, not graded, or missing/unknown	77 (2.6%) records missing/unknown, 2 (0.1%) not graded	MCAR. Inadequate reporting by pathologist. If grade of core biopsy tumour not stated, and after neo-adjuvant chemotherapy there was a complete pathological response then no tumour to report on.
4. Focality (distribution of tumour)	Categorical Multifocal, localised, or missing/unknown	286 (9.8%) records	MCAR (Same as above for grade)
5. ER status[1]	Categorical Negative, positive, or missing/unknown	12 (0.4%) records	MCAR (most people have ER done)
6. PR[2] status	Categorical Negative, positive, or missing/unknown	581 (19.8%) records	MAR - missing because specific centres don't do PR IHC.
7. HER2[3] status	Categorical Negative, positive, borderline, or missing/unknown	355 (12.1%) records	Missing when diagnosis predated routine testing. Potential bias towards missing in patients not experiencing disease recurrence.
8. M stage	Categorical M0, M1 or missing/unknown	22 (0.8%) records	MCAR
9. Pathological N stage (lymph node status)	Categorical N0, N1 or missing/unknown	65 (2.2%) records	MCAR. No axillary surgery, no lymph nodes in resected specimen.
10. Number of positive Lymph nodes	Categorical 0, 1-3, 4-9, 10+, or missing/unknown	59 (2.0%) records	Same as above (MCAR)
11. Lymphovascular invasion	Categorical Present, absent or missing/unknown	222 (7.6%) records	MCAR (Same as above for grade)
12. Maximum tumour diameter invasive (tumour size)	Continuous, in mm or Categorical: <15mm, 15mm to 20mm, >20mm to 35mm, >35mm to 50mm, >50mm, or missing/unknown	193 (6.6%) records	Missing for similar reasons as tumour grade (MCAR)
13. Maximum tumour diameter (including ductal carcinoma in-situ) (pathological)	Continuous, in mm	161 (5.5%) records	Missing for similar reasons as tumour grade (MCAR)
14. Definitive surgery	Categorical Breast Conserving Surgery (BCS), Mastectomy, No surgery,	2 (0.1%) records	Consider MCAR

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
	Nodal surgery only, or missing/unknown		
15. Chemotherapy timing	Categorical Adjuvant, neo-adjuvant, palliative, not applicable, or missing/unknown	0 records	N/A
16. Chemotherapy regimen	Categorical Anthracyclines, A&T, Taxanes, Other, or None	0 records	N/A
17. Adjuvant trastuzumab	Categorical Yes, no/missing/unknown	2587 (88.2%) records no/missing/unknown	Consider MAR
18. Radiotherapy	Categorical Yes, no/missing/unknown	598 (20.4%) records no/missing/unknown	Consider MAR
19. Hormone treatment	Categorical Yes, no/missing/unknown	2156 (73.5%) records no/missing/unknown	Consider MAR
20. Oophorectomy	Categorical Yes, no/missing/unknown	2558 (87.2%) records no/missing/unknown	Consider MAR
21. Ovarian suppression	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2172 (74.1%) records no/missing/unknown	Consider MAR
22. LHRH	Categorical Yes-adjuvant, yes-metastatic, or no/not applicable/missing/unknown	2177 (74.2%) records no/not applicable/missing/unknown	Consider MAR
2.4.5 Additional (descriptive) variables			
Length of follow-up	Continuous, in months	0 records	N/A

[1] Oestrogen receptor allocation of result from POSH database to Oestrogen receptor category:

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[2] PR allocation of result from POSH database to a PR category:

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Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative***
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Unknown	Missing/unknown
Null	Missing/unknown

***For PR, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as PR negative. However it is possible that reviewers will disagree so we can reclassify this as positive if required

[3] HER2 allocation of result from POSH database to a HER2 category:

Result	Category result assigned to
FISH/CISH positive	Positive**
3+	Positive
FISH/CISH borderline	Negative**
2+	Negative
FISH/CISH negative	Negative**
1+	Negative
0	Negative
Not done	Not done
Unknown	Missing/unknown

** FISH/CISH results take precedence i.e. a 2+ result which is later found to have a FISH/CISH positive result is categorised as Positive rather than Borderline.

[4] An ultrasound measurement was used if available, followed by a mammogram if the ultrasound was unavailable or a clinical examination/description if the mammogram was unavailable.

2.5 Data sources/measurement

The tumour biopsy, definitive histopathological report, clinical and radiological reports were all submitted to the study. Pathological characteristics of the tumours were taken from the diagnostic histopathology report, clinical staging from the clinical and radiological reports.

National death data were obtained for patients in the cohort from the Medical Research Information Service (MRIS).

ER, PR and HER2 data were taken from pathology reports. Scoring systems varied as expected across contributing hospitals. Positive and Negative categories are straightforward however borderline results exist in all three IHC categories and were classified into a separate borderline group. The borderline category was merged with negative for the purposes of these analyses.

This paper presents the results of analyses conducted on follow up data available up until 22-Oct-2013.

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Clinical data for all patients were collected via standard clinical research forms which were completed from the clinical notes by the Clinical Trials Practitioner in each centre.

HER2 data: There are concerns regarding the amount of missing HER2 data obtained. In addition:

- HER2 Testing was only widely introduced after 2006 (proportion tested prior to 2006 was 83% (1704/2041), proportion tested on/after 2006 was 98% (897/915)). Prior to 2006 HER2 testing was more likely to have been carried out in patients who had progressed (93% i.e. 520 tested out of 561 who progressed, compared to 80% i.e. 1184 tested out of 1480 who had not progressed). Therefore, patients for whom we knew their HER2 status were more likely to have had a worse prognosis. Hence, if we selected patients on the basis of HER2 testing and compared them to patients who may or may not have been HER2 tested this would have been biased as the patients who have been HER2 tested could look worse by comparison.

- In addition, any analyses that select any patients which have a known HER2 status (which includes patients diagnosed before 2006) will include more cases who had relapsed (and were therefore tested for HER2 amplification retrospectively) than the whole cohort which could potentially compromise the validity of results.

2.7 Study Size

This is covered in the BMC paper.

2.8 Statistical Methods

Patients excluded from the analyses
Patients were excluded from this analysis if they were 41 years of age or over at the date of invasive breast cancer diagnosis (43 patients) i.e. a patient born on 01-Jan-1960 would be included if she was diagnosed before 01-Jan-2001 and excluded if she was diagnosed on or after 01-Jan-2001. In addition, patients were excluded if we didn't have confirmation that they had invasive cancer from pathology results or were missing primary data (21 patients). As a result, a total of 2956 were included in the analysis population.

Primary outcome measure
Distant Disease Free Interval (DDFI) where DDFI is defined as the time from the date of invasive breast cancer diagnosis to distant relapse or death from breast cancer. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died from breast cancer or relapsed at the time of analysis will be censored at their date of last follow up/death.

Univariate analyses
1. We summarised and compared the following patient and tumour characteristics of the cohort by patients with/without a family history of breast cancer; and also by FDR or SDR. Pearson Chi-squared tests were used to compare categorical variables and a Wilcoxon Mann-Whitney Test was used for continuous variables:
   - Duration of follow-up – median (range, IQR), n(%);
   - Age at diagnosis, in years – median (range, IQR), n(%);
   - Maximum diameter invasive tumour, in mm – median (range, IQR), n(%), missing/unknown – n(%);
   - Maximum diameter invasive tumour, in mm (<15mm, 15mm to 20mm, >20mm to
   - 35mm, >35mm to 50mm, >50mm, or missing/unknown) – n(%);
   - Maximum tumour DCIS, in mm – median (range, IQR), n(%), missing/unknown – n(%);
   - Maximum tumour DCIS, in mm (<15mm, 15mm to 20mm, >20mm to
   - 35mm, >35mm to 50mm, >50mm, or missing/unknown) – n(%);

- Number of positive axillary lymph nodes (0, 1-3, 4-9, 10+, or missing/unknown) – n(%);
- Histological grade (1, 2, 3, or not graded/missing/unknown) – n (%);
- N stage (N0, N1, or missing/unknown) – n (%);
- M stage (M0, M1, or missing/unknown) – n (%);
- Presentation (Symptomatic, screen-detected, other, or missing/unknown) – n (%);
- Lymphovascular invasion (Absent, Present, or missing/unknown) – n(%);
- Distribution of tumour (Multifocal, localised, or missing/unknown) – n (%);
- Oestrogen receptor (ER) status (negative, positive, or missing/unknown) – n (%);
- Progesterone receptor (PR) status (negative, positive, or missing/unknown) – n (%);
- Human Epidermal growth factor receptor 2 (HER2) status (negative, positive, or missing/unknown) – n (%).

2. We summarised the following treatment information of the cohort by patients with/without a family history of breast cancer; and by FDR or SDR:
   - Definitive breast surgery for patients who have had/not had radiotherapy (mastectomy, breast conserving surgery, nodal surgery only, no surgery, or missing/unknown) – n (%);
   - Chemotherapy timing (adjuvant[1], neo-adjuvant, not applicable, or missing/unknown) – n (%);
   - Chemotherapy regimen (anthracycline, anthracycline & taxane, taxane, none, other[2], or missing/unknown) – n(%);
   - Adjuvant trastuzumab (yes, or no/missing/unknown) – n (%);

   For patients with ER positive tumours only:
   - Hormone treatment (yes, or no/missing/unknown) – n (%);
   - Oophorectomy (yes, or no/missing/unknown);
   - Ovarian suppression (yes, or no/missing/unknown) – n(%);
   - LHRH (yes, or no/missing/unknown) – n(%).

[1] excluding any treatment for M1 disease.

3. We produced Kaplan-Meier survival curves of DDFI and compared the survival curves by patients with/without a family history of breast cancer; and by FDR or SDR using an unadjusted univariate Cox model.
   In addition, DDFI estimates with corresponding 95% confidence intervals were produced.

Multivariable analyses

To adjust for potential confounders, we fitted a flexible parametric survival model for DDFI, which was programmed in STATA using the stpm2 command (Lambert, Royston, 2009)[1]. This model included family history and the following covariates measured at breast cancer diagnosis presentation: age at diagnosis, tumour size, tumour grade, n stage, lymphovascular invasion, distribution of tumour, ER status, PR status, and HER2 status. See Section 2.4 above for a description of these fields and how they were categorised.

From this we estimated the hazard ratio and 95% confidence interval for family history of breast cancer. All covariates were included in the model regardless of whether they were statistically significant at the 5% level ($p<0.05$) or not.

As a result of the time-varying effects of the ER status (Azzato, et al, 2009, Bellera et al, 2010)[2,3], and possible time-varying effects of other variables included in the Multivariable (MV) analyses, we chose to fit a flexible parametric survival model as opposed to a Cox's regression model; enabling us to model these variables as time-varying covariates.

We explored varying degrees of freedom (df) for the baseline hazard rate (BHR) and time-dependent effect (TDE) using the Akaike Information Criterion and overlaying the flexible parametric model hazard curves onto the smoothed hazard rates. The smoothed hazard rates are an estimate of the hazard function based on a weighted kernel smooth of the estimated hazard contributions[4], and are obtained in STATA using the sts graph command and by specifying hazard in the main options. The df for the BHR and TDE were chosen by finding a model with as low as possible AIC without evidence of over-fitting .The time-varying hazard ratio and 95% confidence interval was plotted over time and 2-, 5-, and 8-year relative hazard ratios and survival estimates were produced. A Cox proportional hazards model was also fitted to determine if there were any key differences between the two models.

[1] The Lambert & Royston paper can be found at www.stata-journal.com/article.html?article=st0165 or http://www.pauldickman.com/cancerepi/handouts/handouts_survival/Lambert2009.pdf.
[2]The Azzato, et al paper can be found at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2695697/. The Bellera et al paper can be found at http://www.biomedcentral.com/1471-2288/10/20.
[3]Results obtained from T Maishman's MSc Project analysis undertaken on POSH data downloaded in May 2011.
[4] (Klein and Moeschberger 2003) Survival Analysis: Techniques for Censored and Truncated Data (Statistics for Biology and Health). 2nd Edition. Springer. pp167-168.

Exploration of Tumour Size

As tumour size could be modelled as either categorical or continuous variables, investigations were first made into which univariate model - fitting the covariate as either a categorical or continuous type of variable (including transformations) - provided the lower Akaike Information Criterion (AIC).

Method used to handle missing data
The amount of missing data and reasons for missingness were explored and are shown in section 2.4. All missing data were assumed to be either MAR or MCAR (see Section 2.4 for more details), and the censoring was assumed to be non-informative. Assessment of missingness across a) different hospital sites b) dates of diagnosis and c) relapse or disease free were used to explore potential reasons for missing data.

The largest proportion of missing data can be seen for HER2 (12.0%) and PR (19.7%). These were less frequently reported across the cohort and the time period of this study than ER which was more consistently available.

Multiple imputed datasets were generated using the ice command in STATA, which imputes missing values by using switching regression; an iterative multivariable regression technique (using chained equations). We created m=10 imputed datasets, each with 10 cycles of regression switching (White & Royston, 2009[4] and Vergouwe *et al*, 2009)[5]. All covariates we planned to use in the MV model (see MV section) were included in multiple imputation model together with site, year of diagnosis, D and the Nelson-Aalen (marginal) cumulative hazard estimator, where D is an indicator variable which is 1 if the patient had a distance relapse or died and 0 otherwise (indicates if observation is censored or not). The Nelson-Aalen (marginal) cumulative hazard estimator approximates $H_0(t)$. D and $H_0(t)$ were included in the multiple imputation model as recommended by White and Royston (White & Royston, 2009)[4].

The ice command imputes dichotomous covariates (HER2, N stage, lymphovascular invasion, distribution, ER status, and PR status) using multiple logistic regression. Polychotomous covariates (tumour grade and possibly maximum tumour diameter) were imputed using polytomous logistic regression. Continuous covariates were imputed using linear regression (possibly age at diagnosis and/or maximum tumour diameter).

The methods used for multiple imputation assume that any continuous covariates to be imputed are Normally distributed (White & Royston, 2009[4]). If it was found that tumour size was best fitted as a continuous variable in the multiple imputation model, this variable would first be transformed to approximate Normality before imputation. Exploration of the most appropriate transformation to use included using both the gladder and ladder commands in STATA.

For each model, the MV analyses of the multiply imputed datasets were then carried out using the mim command in STATA, which combines the results from the imputed data sets using Rubin's rules[4].

[4] The 'Imputing missing covariate values for the cox model' paper by White & Royston can be found at http://www.ncbi.nlm.nih.gov/pubmed/19452569.
[5] The Vergouwe *et al* paper can be found at http://www.ncbi.nlm.nih.gov/pubmed/19596181.

Sensitivity analyses – additional models
We performed the following sensitivity analyses by making the corresponding adjustments to the chosen model:

1. Excluding M1 stage patients;
2. Excluding non-Symptomatic patients (e.g. screen-detected patients, etc.);
3. Excluding both M1 stage patients and non-Symptomatic patients (e.g. screen-detected patients, etc.);
4. Fitting a Cox proportional hazards model instead of a flexible parametric model (no patients excluded);
5. Fitting a Cox proportional hazards model instead of a flexible parametric model, and excluding M1 stage patients;
6. Fitting a Cox proportional hazards model instead of a flexible parametric model, and excluding non-Symptomatic patients (e.g. screen-detected patients, etc.);
7. Fitting a Cox proportional hazards model instead of a flexible parametric model, and excluding M1 stage patients and excluding non-Symptomatic patients (e.g. screen-detected patients, etc.).

Closeness of breast cancer relatives
The methods outlined in the multivariable analyses section above were then repeated for closeness of breast cancer relatives.

A.3.5 Statistical Analysis Plan – Paper 5

Statistical analysis plan (SAP), approved on 10-May-2016, and formatted for Lancet Oncology Appendix.

[Please note: Figures in this SAP are taken from the POSH data available up until June 2015, and thus only represent approximations of the new data due to be downloaded from the POSH database in 2016/2017.]

Please note: This statistical analysis plan has been written in the past tense because it will form the basis of a paper. The headings used in this document come from the STROBE reporting guideline for observational studies (see http://www.strobe-statement.org/ or http://www.annals.org/content/147/8/W-163.full.pdf+html).

Statistical Analysis Plan Version

Issue no	Revision History	Author	Date
0.1	First draft written based on discussion at meeting on 8th Oct 2010	Louise Stanton (née Dent)	20th Oct 2010
0.2	Additional comments and annotations	Diana Eccles, Sue Gerty	13th Oct 2010
0.3	Further notes on confounding factors and example figures for POSH cohort added	Diana Eccles	25th Nov 2010
0.4	Updated based on meeting with Diana Eccles and Sue Gerty on the 29th Oct 2010 and meeting with Sue Gerty on 9th December 2010	Louise Stanton (née Dent)	17th Dec 2010
0.5	Updated based on comments from Doug Altman	Louise Stanton (née Dent)	21st Feb 2011
0.6	Updated based on discussions	Diana Eccles, Louise Stanton (née Dent)	24th Feb 2011
0.7	Updated based on meeting with Louise Stanton (née Dent) on 21st March 2012	Tom Maishman	30th Mar 2012
0.8	Updated based on comments from Diana Eccles	Tom Maishman	2nd Apr 2012
0.9	Updated following a meeting with Doug Altman, Diana Eccles and Louise Stanton (née Dent)	Tom Maishman	18th Mar 2013
0.10	Updated following planned updates to obtain further BRCA testing information	Tom Maishman	30th Jun 2015
0.11	Updated following comments from Diana Eccles and Ellen Copson	Tom Maishman	14th Jul 2015
0.12	Updated following comments from Diana Eccles and Ellen Copson	Tom Maishman	28th Jul 2015
0.13	Updated following meeting with Doug Altman on 30th July 2015	Tom Maishman	7th Aug 2015
1	Finalised using v0.13	Tom Maishman	10th May 2016

1. Introduction

1.1 Background / Rationale

BRCA1 and BRCA2 are the most frequently reported highly penetrant monogenic factors that predispose to breast cancer. Both genes also predispose to ovarian cancer. Mutation in either gene has been shown to lead to higher grade breast cancer than average and to young age at onset (median age for BRCA1 is 43 years and for BRCA2 is 48 years compared to the population mean age at diagnosis of about 60 years). In addition for BRCA1 associated breast cancer, the proportion of oestrogen receptor negative cancers is much higher than average (80-90% compared to ~ 30% amongst breast cancers in women diagnosed < 50 years of age). There are conflicting conclusions in the literature exploring whether BRCA1 or BRCA2 mutation carriers develop breast cancers with a better or worse prognosis. Most reported studies are small, retrospective and with incomplete data on many of the factors known to influence breast cancer outcomes. Some of the early reports of better survival failed to recognise or adequately account for survival bias in many of the BRCA tested patients. Knowledge of a family history of breast cancer, even without genetic testing may lead to earlier diagnosis of breast cancer due to heightened awareness and early presentation and investigation; this bias may lead to observations of improved survival in BRCA gene carriers. The adverse pathological features associated with breast cancers diagnosed in BRCA gene carriers may account for observations of a worsened prognosis in gene carriers compared with the average.. A differentially better or worse response to adjuvant chemotherapy in relation to the underlying genetic predisposition may also affect prognosis. It is important to understand the overall effect of genetic predisposition factors on prognosis in order to better inform gene carriers making decisions about primary prevention and about cancer treatment and to help design more informative prospective clinical trials of both conventional and novel targeted treatments. The Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) is a large contemporary cohort study of breast cancer cases diagnosed before 41 years of age and designed to investigate the effect of genetic factors on breast cancer prognosis.

1.2 Objectives

This paper presents the results from analyses carried out on data collected from the POSH study.

The primary objective was:

- To investigate whether patients with early breast cancer and an inherited BRCA1 or BRCA2 gene mutation (BRCA-Positive [BRCA+]) have a superior Overall Survival (OS) than patients without a BRCA1 or BRCA 2 mutation (BRCA-Negative [BRCA-]).

Secondary objectives were:

- To investigate whether BRCA+ patients with early breast cancer have a superior Distant Disease Free Survival (DDFS) than BRCA- patients.
- To investigate whether BRCA+ patients with early breast cancer have a superior Post Distant Relapse Survival (PDRS) than BRCA- patients.

- To investigate whether patients with early breast cancer and an inherited BRCA1 gene mutation (BRCA1-Positive [BRCA1+]) have a superior OS than patients without a BRCA1 mutation (BRCA1-Negative [BRCA1-])[1].
- To investigate whether BRCA1+ patients with early breast cancer have a superior DDFS than BRCA1- patients.
- To investigate whether BRCA1+ patients with early breast cancer have a superior PDRS than BRCA1- patients.
- To investigate whether patients with early breast cancer and an inherited BRCA2 gene mutation (BRCA2-Positive [BRCA2+]) have a superior OS than patients without a BRCA2 mutation (BRCA2-Negative [BRCA2-])[2].
- To investigate whether BRCA2+ patients with early breast cancer have a superior DDFS than BRCA2- patients.
- To investigate whether BRCA2+ patients with early breast cancer have a superior PDRS than BRCA2- patients.

- To investigate whether Triple Negative (TNT)[3] BRCA+ patients with early breast cancer have a superior OS than TNT BRCA- patients.
- To investigate whether TNT BRCA+ patients with early breast cancer have a superior DDFS than TNT BRCA- patients.
- To investigate whether TNT BRCA+ patients with early breast cancer have a superior PDRS than TNT BRCA- patients.

- To investigate whether BRCA+ patients with early breast cancer have a superior DDFS than BRCA- patients when adjusting for chemotherapy.

[1] This comparison excludes patients with a BRCA2 positive gene mutation.
[2] This comparison excludes patients with a BRCA1 positive gene mutation.
[3] Triple Negative Patients defined as Patients with a HER2 negative status, ER negative status and either a PR negative status or PR missing/unknown status i.e. patients with a confirmed PR positive status are excluded.

2. Methods

2.1 Study Design

The POSH study is a prospective cohort study. The protocol for the study can be found in the following journal article http://www.biomedcentral.com/1471-2407/7/160.

2.2 Setting

The POSH study recruited women from breast cancer units across England, Scotland, Wales and Northern Island between 1st June 2001 to 31st January 2008.

2.3 Participants

The study recruited 3052 women aged 40 years or younger at breast cancer diagnosis. The women had to have been diagnosed with breast cancer between January 2000 and January 2008. In addition, 43 women aged 41-50 were also included if they had a known BRCA1 or BRCA2 gene mutation and were diagnosed with invasive breast cancer within the study period were excluded for this analysis. Women were excluded if they had a previous invasive malignancy (with the exception of non-melanomatous skin cancer), were not available for follow up or refused consent to retain diagnostic and follow up data. Genetic testing was performed on xxx women. Those not tested were excluded from the additional comparison. Patients with confirmed M1 stage (n=74) were also excluded. A total of 2925 women were included in the analysis population.

Clinical follow up data were obtained from the patient medical records by the clinical trials practitioner (CTP) at each recruiting centre. Data forms collecting information at diagnosis, 6 months, 12 months were completed by the CTP usually at 12 months from diagnosis. Annual data collection was continued from the date of definitive diagnosis until death, loss to follow up or until the end of the current phase of the study (mmm yyyy).

Family history data: patients in the POSH study completed a family history questionnaire (http://www.biomedcentral.com/1471-2407/7/160 supplementary figure). The web-based and validated genetic risk prediction software BOADICEA (Antoniou A, et al 2008. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. J Med Genet. Jul;45(7):425-31) was used to process pedigree data and generate a predicted likelihood that each patient might carry a BRCA1/2 mutation. No family history was provided for 106 of the 2956 patients. BOADICEA scores for the remaining 2850 patients were calculated from the family history of the proband at the time she presented with breast cancer. A total of 1939 (66%) scored below 0.05, 372 (13%) scored 0.05-0.099, 226 (8%) scored 0.10-0.199 and 314 (11%) scored 0.20 or over. BOADICEA scores for the xxx patients were calculated from the family history of the proband at the time she presented with breast cancer.
Genetic testing results for BRCA1/2 were already available through clinical test reports or other research sub-studies in xxx cases and these data were used to validate the sensitivity and specificity of the Fluidigm technology used across the cohort. Mutation testing was carried out on all patients recruited to the study for whom a DNA sample was available (n=xxx). A panel of genes was tested using Fluidigm targeted sequence capture and next generation sequencing with additional analysis using Multiple Ligation

Probe Analysis (MLPA) to detect large exonic deletions or duplications where there was either a greater than 10% estimated probability of an underlying BRCA1/2 gene mutation (estimated using BOADICEA) or where there was evidence from the Fluidigm assay of a large deletion or duplication. Only mutations that were clearly pathogenic were used to assign gene carriers to the relevant group for analysis purposes.

2.4 Variables (data taken as of June 2015)

Variable	Type of data / categories	Amount of missing data (Analysis Group A – see Section 2.8, n=2873)	Amount of missing data (Analysis Group B – see Section 2.8, n=725)	Possible reasons for missing data
2.4.1 Primary outcome				
Time to death from any cause	Survival data Date of death from any cause – Date of invasive breast cancer diagnosis	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A
2.4.2 Secondary outcomes				
Time to distant relapse or death from any cause	Survival data Date of first distant relapse (or death from any cause) – Date of invasive breast cancer diagnosis	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit	N/A
Time from first relapse to death from any cause	Survival data Date of death from any cause – Date of first distant relapse	N/A, patients who haven't relapsed will not be included. Patients who have relapsed and haven't died will be censored at the date of their last follow up visit	N/A, patients who haven't relapsed will not be included. Patients who have relapsed and haven't died will be censored at the date of their last follow up visit	N/A
2.4.3 Candidate predictor				
Genetic status[1]	Categorical For the main comparison, each patient is assigned one of 3 categories: BRCA 1 gene carrier confirmed by genetic testing (n=xxx) BRCA 2 gene carrier confirmed by genetic testing (n=xxx) TP53 (n=xxx) No mutation found/variant unknown significance	TBA	TBA	TBA
2.4.4 Potential confounders / effect modifiers - measured at breast cancer diagnosis presentation				
1. Age at diagnosis	Continuous, in years	0 records	0 records	N/A
2. Body Mass Index (BMI)	Categorical Underweight/Healthy, Overweight, Obese, or missing/unknown	108 (3.8%) records	15 (2.1%) records	Consider MAR
3. Histological Tumour grade	Categorical 1, 2, 3, or not graded/missing/unknown	70 (2.4%) records not graded/missing/unknown	19 (2.6%) records not graded/missing/unknown	MCAR. Inadequate reporting by pathologist. If grade of core biopsy tumour not stated, and after neo-adjuvant chemotherapy there was a complete pathological response then no tumour to report on.
4. Maximum tumour diameter invasive (tumour size)	Continuous, in mm or Categorical <15mm, 15mm to 20mm, >20mm to 35mm, >35mm to 50mm, >50mm, or missing/unknown	162 (5.6%) records	53 (7.3%) records	Missing for similar reasons as tumour grade (MCAR)
5. Pathological N stage (lymph node status)	Categorical N0, N1 or missing/unknown	31 (1.1%) records	10 (1.4%) records	MCAR. No axillary surgery, no lymph nodes in resected specimen.
6. Number of positive Lymph nodes	Categorical 0, 1-3, 4-9, 10+, or missing/unknown	31 (1.1%) records	10 (1.4%) records	Same as above (MCAR)
7. Lymphovascular invasion	Categorical Present, absent or missing/unknown	203 (7.1%) records	58 (8.0%) records	Poor reporting. Consider as MCAR.
8. M stage	Categorical M0, M1 or missing/unknown	22 (0.8%) records	5 (0.7%) records	MCAR, likely to be M0 as only 2.1% of patients are M1.
9. Oestrogen receptor (ER)[1]	Categorical Negative, positive, or missing/unknown	11 (0.4%) records	0 records	N/A

Variable	Type of data / categories	Amount of missing data (Analysis Group A – see Section 2.8, n=2873)	Amount of missing data (Analysis Group B – see Section 2.8, n=725)	Possible reasons for missing data
10. HER2[2]	Categorical Negative, positive, or missing/unknown	352 (12.3%) records	0 records	Missing because diagnosis predated routine testing and patient has not suffered a further breast cancer event since initial diagnosis. Consider MAR.
11. PR[3]	Categorical Negative, positive, or missing/unknown	564 (19.6%) records	85 (11.7%) records	MAR. Missing because specific centres don't do PR IHC.
12. Ethnicity	Categorical Caucasian/White, Black, Asian, Other, or missing/unknown	41 (1.4%) records	8 (1.1%) records	Consider MAR
Diagnosis Year	Categorical ≤2005 or >2005	0 records	0 records	N/A
Adjuvant or neo-adjuvant chemotherapy indicator	Categorical Yes or No/missing/unknown	0 records	0 records	N/A
Chemotherapy with taxane indicator	Categorical Yes or No/missing/unknown	0 records	0 records	N/A
17. Focality (distribution of tumour)	Categorical Multifocal, localised or missing/unknown	61 (8.0%) records	286 (9.7%) records	Missing for similar reasons as tumour grade (MCAR).
18. Definitive surgery	Categorical Breast Conserving Surgery (BCS), Mastectomy, No surgery, Nodal surgery only, or missing/unknown	0 records	0 records	N/A
19. Chemotherapy regimen	Categorical Anthracyclines, A&T, Taxanes, Other, or None	0 records	0 records	N/A
2.4.5 Additional (descriptive) variables				
13. Length of follow-up	Continuous, in months	0 records	0 records	N/A
Amount of missingness in the multivariable models				
No. of pts with at least 1 variable with missing data from the MV model 1 (see Section 2.8)		**596 (20.7%)**	**155 (21.4%)**	
No. of pts with at least 1 variable with missing data from the MV model 2 (see Section 2.8)		**610 (21.2%)**	**159 (21.9%)**	

[1] Not all patients in the POSH study had genetic testing (in the same way not all patients do currently in the NHS). BOADICEA scores were calculated purely based on family history data from the patient family history questionnaire; no information about mutation testing was included in the estimates. Patients with a combined (BRCA1 and BRCA2) score of <0.05 had no significant family history of cancer. Scores above 0.10 would be eligible for testing according to American Society of Oncology guidelines and scores above 0.10 are eligible for testing under the 2013 UK NICE guidelines.

[2] Oestrogen receptor allocation of result from POSH database to Oestrogen receptor category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative*
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

*For ER, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as ER negative, and an Allred score of 3+ treated as ER positive. However it is possible that reviewers will disagree so we can reclassify this as positive if required.

[3] HER2 allocation of result from POSH database to a HER2 category:

Result	Category result assigned to
FISH/CISH positive	Positive**
3+	Positive
FISH/CISH borderline	Negative**
2+	Negative
FISH/CISH negative	Negative**
1+	Negative
0	Negative
Not done	Not done
Unknown	Missing/unknown

** FISH/CISH results take precedence i.e. a 2+ result which is later found to have a FISH/CISH positive result is categorised as Positive rather than Borderline.

[4] PR allocation of result from POSH database to a PR category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative***
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

***For PR, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as PR negative. However it is possible that reviewers will disagree so we can reclassify this as positive if required

2.5 Data sources/measurement

The tumour biopsy, definitive histopathological report, clinical and radiological reports were all submitted to the study. Pathological characteristics of the tumours were taken from the diagnostic histopathology report, clinical staging from the clinical and radiological reports.

National death data were obtained for patients in the cohort from the Medical Research Information Service (MRIS).

ER, PR and HER2 data were taken from pathology reports. Scoring systems varied as expected across contributing hospitals. Positive and Negative categories are straightforward however borderline results exist in all three IHC categories and were classified into a separate borderline group. The borderline category was merged with negative for the purposes of these analyses. Additional IHC data for these three markers was available from the Tissue Micro Arrays (TMAs) constructed from tumour pathology blocks for study participants which were used to populate these missing clinical data fields.

This paper presents the results of analyses conducted on follow up data available up until dd-mmm-yyyy.

2.6 Bias

Clinical data for all patients were collected via standard clinical research forms which were completed from the clinical notes by the Clinical Trials Practitioner in each centre.

HER2 data: There are concerns regarding the amount of missing HER2 data obtained. In addition:

- HER2 Testing was only widely introduced after 2006 (proportion tested prior to 2006 was 83% (1704/2041), proportion tested on/after 2006 was 98% (897/915)). Prior to 2006 HER2 testing was more likely to have been carried out in patients who had progressed (93% i.e. 520 tested out of 561 who progressed, compared to 80% i.e. 1184 tested out of 1480 who had not progressed). Therefore, patients for whom we knew their HER2 status were more likely to have had a worse prognosis. Hence, if we selected patients on the basis of HER2 testing and compared them to patients who may or may not have been HER2 tested this would have been biased as the patients who have been HER2 tested could look worse by comparison.

- In addition, any analyses that select any patients which have a known HER2 status (which includes patients diagnosed before 2006) will include more cases who had relapsed (and were therefore tested for HER2 amplification retrospectively) than the whole cohort which could potentially compromise the validity of results.

2.7 Study Size

This is covered in the BMC paper.

2.8 Statistical Methods

Patients excluded from the analyses
Patients were excluded from this analysis if we didn't have confirmation that they had invasive cancer from pathology results or were missing primary data (21 patients). Genetic testing was performed on xxx women. Those not tested were excluded from the additional comparison. Patients with confirmed M1 stage (n=74) were also excluded. A total of 2925 women were included in the analysis population, of which:

- n=2873 were aged 40 years or younger at diagnosis without a TP53 gene mutation **(Analysis Group A)**;
- n=725 were aged 40 years or younger at diagnosis without a TP53 gene mutation and had a TNT status **(Analysis Group B)**;
- n=43 were aged 41-50 years at diagnosis with a confirmed gene mutation **(Analysis Group C)**;
- n=9 were aged 40 years or younger at diagnosis and had a TP53 gene mutation **(Analysis Group D)**.

Primary outcome measure
Overall Survival (OS) where OS is defined as the time from the date of invasive breast cancer diagnosis to death from any cause. Patients who had not died will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the primary outcome analysis.

Secondary outcome measures
Distant Disease Free Survival (DDFS) where DDFS is defined as the time from the date of invasive breast cancer diagnosis to distant relapse or death from any cause. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died or relapsed at the time of analysis will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the secondary outcome analysis.

Post Distant Relapse Survival (PDRS) where PDRS is defined as the time from the date of distant relapse to death from any cause. Distant relapse is defined as breast cancer recurrence at distant sites including supraclavicular lymph nodes, visceral, CNS and bone metastases. Patients who had not died will be censored at their date of last follow up. For this analysis we will not include new primary breast cancer diagnoses as distant relapse events in the secondary outcome analysis.

Univariate analyses
Where specified for analysis groups A, B, C and D above, we summarised patient and tumour characteristics by the following:

- All patients (Analysis **Groups A, B, C and D**)
- BRCA1+ patients (Analysis **Groups A, B and C** only)
- BRCA2+ patients (Analysis **Groups A, B and C** only)
- BRCA+ patients (Analysis **Groups A and B** only)
- BRCA- patients (Analysis **Groups A and B** only)

For analysis groups A and B, we summarised and produced Kaplan Meier survival curves of OS, DDFS, and PDRS and compared the survival curves using a log rank test for the following:

- BRCA+ versus BRCA-
- BRCA1+ versus BRCA1- (excluding BRCA2+ patients)
- BRCA2+ versus BRCA2- (excluding BRCA1+ patients)

For analysis group C, we summarised and produced Kaplan Meier survival curves of OS, DDFS, and PDRS and compared the survival curves using a log rank test for BRCA1+ versus BRCA2+patients.

Multivariable analyses
Comparison groups:

- BRCA+ versus BRCA- **(analysis Group A)**
- BRCA1+ versus BRCA1-(excluding BRCA2+ patients) **(analysis Group A)**
- BRCA2+ versus BRCA2- (excluding BRCA1+ patients) **(analysis Group A)**
- TNT BRCA+ versus TNT BRCA- **(analysis Group B)**

For the comparisons i) to iv) above, we fitted a multivariable model for OS and DDFS adjusting for the following covariates:

- Age at diagnosis, in years (fitted as a continuous covariate);
- Body Mass Index (BMI) (fitted as a categorical covariate [Underweight/Healthy, Overweight or Obese]);
- Histological Grade (fitted as a categorical covariate [1, 2 or 3]);
- Maximum invasive tumour size, in mm (fitted as a continuous covariate);
- N stage (fitted as a binary covariate [N0 or N1]);
- ER status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;
- HER2 status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;

For the comparisons i) to iv) above, we fitted a multivariable model for OS and DDFS, comparing BRCA+ versus BRCA-, adjusting for the following covariates:

- Age at diagnosis, in years (fitted as a continuous covariate);
- Body Mass Index (fitted as a categorical covariate [Underweight/Healthy, Overweight or Obese]);
- Histological Grade (fitted as a categorical covariate [1, 2 or 3]);
- Maximum invasive tumour size, in mm (fitted as a continuous covariate);
- N stage (fitted as a binary covariate [N0 or N1]);
- ER status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;
- HER2 status (fitted as a binary covariate [Negative or Positive]) **(for analysis Group A only)**;
- Ethnicity (fitted as a categorical covariate [Caucasian, Black or Asian]) – *where appropriate*;
- Diagnosis Year (fitted as a binary covariate [≤2005, or >2005]) – *where appropriate*;
- Adjuvant or neo-adjuvant chemotherapy indicator (fitted as a binary covariate [yes, or no/missing/unknown]) – *where appropriate*;
- Chemotherapy with taxane indicator (fitted as a binary covariate [yes-with taxane, or no-without taxane]) – *where appropriate*.

Hazard Ratios
Evidence suggests that the effect of ER status changes over time (Azzato, et al, 2009, Bellera et al, 2010)[1]. Indeed, this was evident after testing the proportional hazards assumption based on the Schoenfeld residuals and using the identity matrix for the time-scaling function[2] i.e. using the estat phtest command in STATA. This result provided strong evidence against the Cox proportional hazards assumption ($p<0.001$), which was also seen when plotting the scaled Schoenfeld residuals over time[2].

As a result of the time-varying effects of the ER status, a flexible parametric survival model was programmed in STATA using the stpm2 command (Lambert, Royston, 2009)[3] to model ER as a time-dependent covariate. The degrees of freedom for the restricted cubic spline function used for the hazard rate was set to the default setting of 3, whilst the degrees of freedom for the time-dependent effects was set so as to provide the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC). The time-varying hazard ratio and 95% confidence interval was plotted over time and 2-, 5-, and 8-year relative hazard ratios and survival estimates were produced.

[1]The Azzato, et al paper can be found at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2695697/. The Bellera et al paper can be found at http://www.biomedcentral.com/1471-2288/10/20.
[2]Results obtained from T Maishman's MSc Project analysis undertaken on POSH data downloaded in May 2011.
[3] The Lambert & Royston paper can be found at www.stata-journal.com/article.html?article=st0165 or http://www.pauldickman.com/cancerepi/handouts/handouts_survival/Lambert2009.pdf

Method used to handle missing data
The amount of missingess will be investigated and if deemed appropriate, methods of multiple imputation will be incorporated. Otherwise, a complete-case analysis approach will be incorporated.

To date, between 20-22% of patients have are missing data for at least 1 covariate in the multivariable models.

A.3.6 Statistical Analysis Plan – Paper 6

Statistical analysis plan (SAP), approved on 31-Jul-2015, and formatted for Lancet Oncology Appendix (as per SAP for Paper 5 for consistency purposes for this thesis).

[Please note: Figures in this SAP are taken from the POSH data available up until May 2011, and thus only represent approximations of the new data due to be downloaded from the POSH database in 26 June 2015.]

Please note: This statistical analysis plan has been written in the past tense because it will form the basis of a paper. The headings used in this document come from the STROBE reporting guideline for observational studies (see http://www.strobe-statement.org/ or http://www.annals.org/content/147/8/W-163.full.pdf+html).

Statistical Analysis Plan Version

Issue no	Revision History	Author	Date
0.1	First draft written by Sue Gerty based on previous notes	Ramsey Cutress and Sue Gerty	18th Mar 2013
0.2	Revisions by Ramsey Cutress	Ramsey Cutress and Sue Gerty	2 April 2013
0.3	Revisions RIC and SG	Ramsey Cutress and Sue Gerty	30 April 2013
0.4	Revisions RIC	Ramsey Cutress	03 Mar 2014
0.5	Revisions TM and RIC	Tom Maishman and Ramsey Cutress	24 April 2014
0.6	Revisions TM and RIC	Tom Maishman and Ramsey Cutress	05 August 2015
0.7	Revisions TM and RIC	Tom Maishman and Ramsey Cutress	25 June 2015
0.8	Revisions TM and RIC	Tom Maishman, Ramsey Cutress and Aurea Hernandez	02 July 2015
0.9	Revisions TM, AH and RIC	Tom Maishman, Ramsey Cutress and Aurea Hernandez	28 July 2015
1	Revisions TM, AH and RIC	Tom Maishman, Ramsey Cutress and Aurea Hernandez	31 July 2015

1. Introduction

1.1 Background / Rationale

Local recurrence is very important in young patients with breast cancer as they may live for many years before local recurrence develops. There are no large prospective cohort studies reporting local recurrence in this young (≤40) age group. To compare with distant disease free interval and to compare the magnitude of local recurrence with distant recurrence needs a prospective study to avoid inclusion bias to long survivors.

1.2 Objectives

This paper presents the results from analyses carried out on data collected from the POSH study.

The primary objective was:

- Describe Local Recurrence Interval (LRI[1]) in a cohort of young women (aged ≤40 years at breast cancer diagnosis) with breast cancer and investigate whether LRI for young patients that have had a mastectomy[2] differs to that of young patients that have had breast conserving surgery (BCS)[2].

[1] See Section 2.8 for endpoint definitions.

[2] Mastectomy is surgical removal of the breast. BCS/Breast conservation is removal of part of the breast, partial mastectomy, lumpectomy or wide excision and usually with the intention of treatment of the remainder of the breast with breast radiotherapy (see Section 2d for the categorisation of the type of surgery in terms of BCS and Mastectomy).

Secondary objectives were to:

- Describe the patient baseline characteristics in this cohort with particular reference to factors known or suspected to influence local recurrence interval (LRI), including age at diagnosis, family history (FH), extensive in situ component (EIC[1]), surgical margins, focality of cancer, Pathological T stage, Pathological N stage, grade (histological grade), histological type, maximum diameter invasive tumour, maximum tumour diameter (including ductal carcinoma in-situ), axillary surgery indicator, ER, PR, HER2 and TNT status.

[1] EIC defined as positive where the total tumour in-situ size is ≥25% the size of the total tumour size (or where the total tumour invasive size is <75% the size of the total tumour size).

- Describe the local primary treatment (i.e. type of surgery) of the primary breast cancer in this group at diagnosis or after Neoadjuvant Chemotherapy (NAC) including breast and axillary (nodal) surgery, number of surgeries, radiotherapy.

- Describe systemic therapies including adjuvant chemotherapy and neoadjuvant chemotherapy (describe BCS rates between AC/NAC), hormone therapy, and oopherectomy.

- Describe Loco-Regional Recurrence Interval (LRRI[1]) in a cohort of young women with breast cancer and investigate whether LRRI for young patients that have had a mastectomy differs to that of young patients that have had BCS.

[1] See Section 2.8 for endpoint definitions.

- Describe Distant Disease Free Interval (DDFI[1]) in a cohort of young women with breast cancer and investigate whether:
  - DDFI for young patients that have had a mastectomy differs to that of young patients that have had BCS;
  - DDFI for young patients that have experienced an LRI event differs to that of young patients that have not experienced an LRI event;
  - DDFI for young patients that have experienced an LRRI event differs to that of young patients that have not experienced an LRRI event.

[1] See Section 2.8 for endpoint definitions.

- Describe Overall Survival (OS[1]) in a cohort of young women with breast cancer and investigate whether:
  - OS for young patients that have had a mastectomy differs to that of young patients that have had BCS;
  - OS for young patients that have experienced an LRI event differs to that of young patients that have not experienced an LRI event;
  - OS for young patients that have experienced an LRRI event differs to that of young patients that have not experienced an LRRI event.

[1] See Section 2.8 for endpoint definitions.

- Determine whether any key factors known or suspected to influence LRI and DDFI, including key patient and tumour characteristics, and locoregional and systemic treatments, affect LRI and DDFI. Specifically, to investigate whether any of these factors affect LRI and DDFI for:
  - young patients that have had a mastectomy compared to those that have had BCS;
  - young patients that have had a mastectomy with the addition of chest wall radiotherapy compared to those that have had a mastectomy without the addition of chest wall radiotherapy;
  - young patients that have had BCS with the addition of a radiotherapy boost compared to those that have had BCS without the addition of a radiotherapy boost.

2. Methods

2.1 Study Design

The POSH study is a prospective cohort study. The protocol for the study can be found in the following journal article http://www.biomedcentral.com/1471-2407/7/160.

2.2 Setting

The POSH study recruited women from breast cancer units across England, Scotland, Wales and Northern Island between 1st June 2001 to 31st January 2008.

2.3 Participants

The study recruited ≈3000 women aged 40 years or younger at breast cancer diagnosis. The women had to have been diagnosed with breast cancer between January 2000 and January 2008. In addition, 43 women aged 41-50 were included if they had a known BRCA1 or BRCA2 gene mutation and were diagnosed with invasive breast cancer within the study period but were excluded for this analysis. Women were excluded if they had a previous invasive malignancy (with the exception of non-melanomatous skin cancer), were not available for follow up or refused consent to retain diagnostic and follow up data. In addition, patients with confirmed M1 stage were excluded. A total of 2882 women were included in the analysis population.

Clinical follow up data were obtained from the patient medical records by the clinical trials practitioner (CTP) at each recruiting centre. Data forms collecting information at diagnosis, 6 months, 12 months were completed by the CTP usually at 12 months from diagnosis. Annual data collection was continued from the date of definitive diagnosis until death, loss to follow up or until the end of the current phase of the study (June 2016).

2.4 Variables (data taken as of May 2011)

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
2.4.1 Primary outcome			
Time to ipsilateral/chest wall/new primary recurrence (LRI) , in years	Survival data Date of ipsilateral/chest wall/new primary recurrence (If no other event happened before or three months after this date) – Date of diagnosis	N/A, patients who haven't experienced an event will be censored at the date of the first event different from ipsilateral recurrence or last follow-up.	N/A
2.4.2 Secondary outcomes			
Time to ipsilateral/chest wall/new primary/contralateral recurrence (LRRI) , in years	Survival data Date of ipsilateral/chest wall/new primary/contralateral recurrence (If no other event happened before or three months after this date) – Date of diagnosis	N/A, patients who haven't experienced an event will be censored at the date of the first event different from ipsilateral recurrence or last follow-up.	N/A
Time to distant relapse or death from breast cancer	Survival data Date of first distant relapse (or death from breast cancer) – Date of invasive breast cancer diagnosis	N/A, patients who haven't relapsed or died will be censored at the date of their last follow up visit. Patients who died from non-breast cancer deaths were censored at the date of death.	N/A
Time to death from any cause	Survival data Date of death from any cause – Date of invasive breast cancer diagnosis	N/A, patients who haven't died will be censored at the date of their last follow up visit	N/A
2.4.3 Candidate predictor(s)			
Definitive surgery type	Categorical (BCS. Mastectomy, No surgery, Axillary, Missing/unknown)	2 (0.1%) records	Consider MCAR
Adjuvant radiotherapy (chest wall) indicator	(Yes, no/missing/unknown)	598 (20.4%) records	Consider MAR
Adjuvant Radiotherapy boost indicator	(Yes, no/missing/unknown)	1885 (64.3%) records	Consider MAR
2.4.4 Potential confounders / effect modifiers - measured at breast cancer diagnosis presentation			
1. Age at diagnosis	Continuous, in years	0 records	N/A
2. Ethnicity	Categorical Caucasian/White, Black, Asian, Other, or missing/unknown	41 (1.4%) records	Consider MCAR
3. Body Mass Index (BMI)	Categorical Underweight/Healthy, Overweight, Obese, or missing/unknown	114 (3.9%) records	Consider MAR
4. Family History of breast cancer	Categorical Yes, no, missing/unknown	106 (3.6%) records	Family history may not be provided because they could be adopted or family history unknown. Consider MCAR.
5. Presentation	Categorical Symptomatic, screen-detected, other, or missing/unknown	14 (0.5%) records	Consider MCAR
6. Histological Tumour grade	Categorical 1, 2, 3, not graded, or missing/unknown	77 (2.6%) records missing/unknown, 2 (0.1%) not graded	MCAR. Inadequate reporting by pathologist. If grade of core biopsy tumour not stated, and after neo-adjuvant chemotherapy there was a complete pathological response then no tumour to report on.
7. Histological type	Categorical Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown	37 (1.3%) records	Consider MAR
8. Focality (distribution of tumour)	Categorical Multifocal, localised, or missing/unknown	286 (9.8%) records	MCAR (Same as above for grade)

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
9. ER status[1]	Categorical Negative, positive, or missing/unknown	12 (0.4%) records	MCAR (most people have ER done)
10. PR[2] status	Categorical Negative, positive, or missing/unknown	581 (19.8%) records	MAR - missing because specific centres don't do PR IHC.
11. HER2[3] status	Categorical Negative, positive, borderline, or missing/unknown	355 (12.1%) records	Missing when diagnosis predated routine testing. Potential bias towards missing in patients not experiencing disease recurrence.
12. M stage	Categorical M0, M1 or missing/unknown	22 (0.8%) records	MCAR
13. Pathological N stage (lymph node status)	Categorical N0, N1 or missing/unknown	65 (2.2%) records	MCAR. No axillary surgery, no lymph nodes in resected specimen.
14. Number of positive Lymph nodes	Categorical 0, 1-3, 4-9, 10+, or missing/unknown	59 (2.0%) records	Same as above (MCAR)
15. Lymphovascular invasion	Categorical Present, absent or missing/unknown	222 (7.6%) records	MCAR (Same as above for grade)
16. Maximum tumour diameter invasive (tumour size)	Continuous, in mm or Categorical <15mm, 15mm to 20mm, >20mm to 35mm, >35mm to 50mm, >50mm, or missing/unknown	193 (6.6%) records	Missing for similar reasons as tumour grade (MCAR)
17. Maximum tumour diameter overall (including ductal carcinoma in-situ) (pathological)	Continuous, in mm	161 (5.5%) records	Missing for similar reasons as tumour grade (MCAR)
18. Maximum tumour diameter in-situ, in mm	Continuous	2588 (88.3%) records	Consider MAR
19. Extensive in situ component (EIC)	Categorical (EIC positive, EIC negative, Missing/unknown): EIC defined as positive where the total tumour in-situ size is ≥25% the size of the total tumour size (or where the total tumour invasive size is <75% the size of the total tumour size), and EIC defined as negative otherwise (unless the tumour size information is missing).	165 (5.6%) records	Consider MAR
20. Surgical Margins (distance to margin), in mm	Categorical (0, >0 and <1, ≥1 and ≤5, >5, and Missing/unknown)	641 (21.9%) records	Consider MAR
21. Pathological T stage (for patients receiving neo-adjuvant chemotherapy)	Categorical T0, T1, T2, T3, T4, Tis, Tx, or missing/unknown	12 (0.4%) records	MCAR
22. Chemotherapy timing	Categorical Adjuvant, neo-adjuvant, palliative, not applicable, or missing/unknown	0 records	N/A
23. Adjuvant trastuzumab	Categorical Yes, no/missing/unknown	2587 (88.2%) records no/missing/unknown	Consider MAR
24. Radiotherapy	Categorical Yes, no/missing/unknown	598 (20.4%) records no/missing/unknown	Consider MAR
25. Hormone treatment	Categorical Yes, no/missing/unknown	2156 (73.5%) records no/missing/unknown	Consider MAR
26. Diagnosis Year	Categorical 2000, 2001… , 2008	0 records	N/A
27. Adjuvant radiotherapy (breast) indicator	(Yes, no/missing/unknown)	1519 (51.8%) records	Consider MAR

Variable	Type of data / categories	Amount of missing data (n=2932)	Possible reasons for missing data
28. Adjuvant radiotherapy (axillary nodes) indicator	(Yes, no/missing/unknown)	2269 (77.4%) records	Consider MAR
29. Axillary surgery indicator (if available at the time of analysis)	Categorical (yes, no, missing/unkown) Categorised as "Yes" where the type of surgery (breakdown) is Axillary Categorised as "No" where the type of surgery (breakdown) is not Axillary (and is not missing) Categorised as "No" where the type of surgery (breakdown) is missing/unknown	N/A (only if available at the time of analysis)	N/A (only if available at the time of analysis)
2.4.5 Additional (descriptive) variables			
Length of follow-up	Continuous, in months	0 records	N/A

[1] Oestrogen receptor allocation of result from POSH database to Oestrogen receptor category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative*
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

*For ER, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as ER negative, and an Allred score of 3+ treated as ER positive. However it is possible that reviewers will disagree so we can reclassify this as positive if required.

[2] PR allocation of result from POSH database to a PR category:

Result	Category result assigned to
Negative	Negative
Borderline	Negative
Strongly Positive	Positive
Positive	Positive
Weakly positive	Negative***
Not done	Not done
Unknown	Missing/unknown
Null	Missing/unknown

***For PR, weakly positive (which we assume equates to an Allred score of 1-2) has been treated as PR negative. However it is possible that reviewers will disagree so we can reclassify this as positive if required

[3] HER2 allocation of result from POSH database to a HER2 category:

Result	Category result assigned to
FISH/CISH positive	Positive**
3+	Positive
FISH/CISH borderline	Negative**
2+	Negative
FISH/CISH negative	Negative**
1+	Negative
0	Negative
Not done	Not done
Unknown	Missing/unknown

** FISH/CISH results take precedence i.e. a 2+ result which is later found to have a FISH/CISH positive result is categorised as Positive rather than Borderline.

[4] An ultrasound measurement was used if available, followed by a mammogram if the ultrasound was unavailable or a clinical examination/description if the mammogram was unavailable.

2.5 Data sources/measurement

The tumour biopsy, definitive histopathological report, clinical and radiological reports were all submitted to the study. Pathological characteristics of the tumours were taken from the diagnostic histopathology report, clinical staging from the clinical and radiological reports.

National death data were obtained for patients in the cohort from the Medical Research Information Service (MRIS).

ER, PR and HER2 data were taken from pathology reports. Scoring systems varied as expected across contributing hospitals. Positive and Negative categories are straightforward however borderline results exist in all three IHC categories and were classified into a separate borderline group. The borderline category was merged with negative for the purposes of these analyses.

This paper presents the results of analyses conducted on follow up data available up until 26-June-2015.

2.6 Bias

Clinical data for all patients were collected via standard clinical research forms which were completed from the clinical notes by the Clinical Trials Practitioner in each centre.

HER2 data: There are concerns regarding the amount of missing HER2 data obtained. In addition:

- HER2 Testing was only widely introduced after 2006 (proportion tested prior to 2006 was 83% (1704/2041), proportion tested on/after 2006 was 98% (897/915)). Prior to 2006 HER2 testing was more likely to have been carried out in patients who had progressed (93% i.e. 520 tested out of 561 who progressed, compared to 80% i.e. 1184 tested out of 1480 who had not progressed). Therefore, patients for whom we knew their HER2 status were more likely to have had a worse prognosis. Hence, if we selected patients on the basis of HER2 testing and compared them to patients who may or may not have been HER2 tested this would have been biased as the patients who have been HER2 tested could look worse by comparison.

- In addition, any analyses that select any patients which have a known HER2 status (which includes patients diagnosed before 2006) will include more cases who had relapsed (and were therefore tested for HER2 amplification retrospectively) than the whole cohort which could potentially compromise the validity of results.

2.7 Study Size

This is covered in the BMC paper.

2.8 Statistical Methods

Patients excluded from the analyses
Patients were excluded from this analysis if they were 41 years of age or over at the date of invasive breast cancer diagnosis (43 patients) i.e. a patient born on 01-Jan-1960 would be included if she was diagnosed before 01-Jan-2001 and excluded if she was diagnosed on or after 01-Jan-2001. Patients were also excluded if we didn't have confirmation that they had invasive cancer from pathology results or were missing primary data (47 patients). In addition, M1 patients were excluded from the analyses (74 patients). As a result, a total of 2882 were included in the analysis population.

Primary outcome measure
The primary endpoint is Local Recurrence Interval (LRI), which is defined as time from date of diagnosis to date of ipsilateral recurrence/ipsilateral new primary (whichever event occurs first)1 following BCS or date of chest wall recurrence1 following mastectomy (whichever event occurs first). All other events are treated as censored at the date of these events.

Secondary Endpoints
The secondary endpoints are:

1. Loco-Regional Recurrence Interval (LRRI), which is defined as time from date of diagnosis to date of ipsilateral recurrence (including axilla and regional recurrence)[1] following BCS, date of chest wall recurrence[1] following mastectomy, date of contralateral recurrence[1]/ipsilateral new primary[1] (whichever event occurs first). All other events are treated as censored at the date of these events.

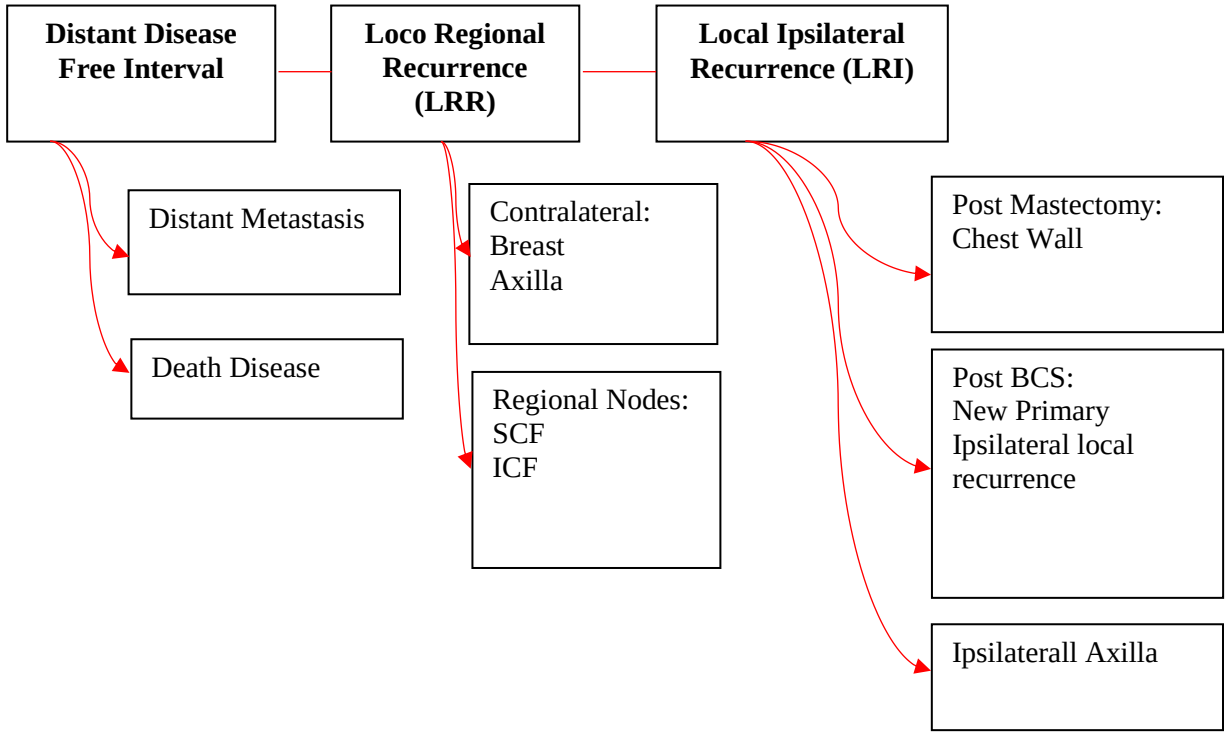
2. Distant Disease Free Interval (DDFI), where DDFI is defined as time from date of diagnosis to date of death from breast cancer or distant metastasis (whichever event occurs first). Deaths from other causes are censored at the date of death.

3. Overall Survival (OS), where OS is defined as time from date of diagnosis to date of death from any cause.

[1] See Image below. Where the date of recurrence (ipsilateral or chest wall, or ipsilateral new primary) is counted as an event provided that the date of death from breast cancer, date of distant recurrence, date of ipsilateral local axillary recurrence, date of ipsilateral regional nodes recurrence and/or date of contralateral recurrence (if/where applicable) is **>3 months after** the date of recurrence (ipsilateral or chest wall, or ipsilateral new primary). If the date of death from breast cancer, date of distant recurrence , date of ipsilateral local axillary recurrence, date of ipsilateral regional nodes recurrence, and/or date of contralateral recurrence (if/where applicable) is ≤**3 months after** the date of recurrence (ipsilateral or chest wall, or ipsilateral new primary), then the patient is censored at the date of death from breast cancer, date of distant recurrence, date of ipsilateral local axillary recurrence, date of ipsilateral regional nodes recurrence, and/or date of contralateral recurrence (if/where applicable). Deaths from other cancers following recurrence (ipsilateral or chest wall, or ipsilateral new primary) do not affect the event.

Definitions used for DDFI, LRRI and LRI

To differentiate between ipsilateral local recurrence and new primary (both components of In Breast Tumour Recurrence (IBTR) the ABS NCIN definition was used i.e. “to be classified as a new primary the recurrence had to occur within a different quadrant and/or be “histopathologically different” from the original primary in terms of tumour characteristics including morphology and/or receptor status”
(histological type, grade, receptors)



Univariate analyses

- We described the baseline patient and tumour characteristics of the cohort and compared these by definitive surgery type (BCS vs. Mastectomy) using Pearson Chi-squared tests for categorical variables and Wilcoxon Mann-Whitney tests for continuous variables:

  Patient characteristics:
  - Age at diagnosis, in years – median (range, IQR), n(%);
  - Age at diagnosis (18-25, 26-30, 31-35, 36-40) – n(%);
  - Ethnicity (Caucasian/White, Black, Asian, Other, or missing/unknown) – n(%);
  - Obesity (BMI) – median (range, IQR), n(%);
  - Family History (yes, no, or missing/unknown);
  - Presentation (Symptomatic, screen-detected, other, or missing/unknown) – n (%);

  Tumour characteristics:
  - Histological grade (1, 2, 3, not graded/missing/unknown) – n (%);
  - Histological type (Ductal, lobular, ductal & lobular, mixed, medullary, metaplastic, other, unclassified, not graded, or missing/unknown) – n (%);
  - Surgical margins (0, >0 and <1, ≥1 and ≤5, >5, or missing/unknown) – n (%);
  - Extensive in situ (EIC) component (EIC Positive, EIC Negative, or missing/unknown) – n (%);
  - Lymphovascular invasion (Absent, Present, or missing/unknown) – n(%);
  - Number of positive axillary lymph nodes (0, 1-3, 4-9, 10+, or missing/unknown) – n(%);
  - Oestrogen receptor (ER) status (negative, positive, or missing/unknown) – n (%);
  - Progesterone receptor (PR) status (negative, positive, or missing/unknown) – n (%);
  - Human Epidermal growth factor receptor 2 (HER2) status (negative, positive or missing/unknown) – n (%).
  - Focality of cancer (multifocal, localised, or missing/unknown) – n (%);
  - Pathological T stage (T0, T1, T2, T3, T4, Tis, Tx, or missing/unknown) – n(%);
  - Pathological N stage (N0, N1, or missing/unknown) – n (%);
  - Maximum diameter invasive tumour, in mm – median (range, IQR), n(%), missing/unknown – n(%);
  - Maximum tumour diameter overall (including ductal carcinoma in-situ), in mm – median (range, IQR), n(%), missing/unknown – n(%).

- We described the primary oncological treatment of the cohort by definitive surgery type i.e. by BCS or Mastecomy:
  a. Number of surgeries – median (range, IQR), n(%), missing/unknown – n(%);
  b. Chemotherapy timing (Adjuvant, neo-adjuvant, palliative or not applicable) – n(%);
  c. Adjuvant trastuzumab (yes or no/missing/unknown) – n(%);
  d. Adjuvant Radiotherapy (yes or no/missing/unknown) – n(%);
  e. Hormone treatment (yes or no/missing/unknown) – n(%);
  f. Adjuvant radiotherapy (chest wall) indicator (yes or no/missing/unknown) – n(%);
  g. Adjuvant radiotherapy (breast) indicator (yes or no/missing/unknown) – n(%);
  h. Adjuvant radiotherapy (axillary nodes) indicator (yes or no/missing/unknown) – n(%);
  i. Axillary clearance/sample details (only if available at the time of analysis) (yes or no/missing/unknown) – n(%).

- We produced cause-specific cumulative hazard plots for LRI and LRRI for the following groups and provided log-rank tests and Hazard Ratios (except for the all patients group):
  a. All patients;
  b. Patients with BCS **versus** Patients with a mastectomy;
  c. Patients with a mastectomy with the addition of chest wall radiotherapy **versus** Patients with a mastectomy without the addition of chest wall radiotherapy;
  d. Patients with BCS with the addition of radiotherapy boost **versus** Patients with BCS without the addition of radiotherapy boost.

- We produced Kaplan-Meier survival curves for DDFI and OS for the following groups and provided log-rank tests and Hazard Ratios (except for the all patients group):
  a. All patients;
  b. Patients with BCS **versus** Patients with a mastectomy;
  c. Patients with a mastectomy with the addition of chest wall radiotherapy **versus** Patients with a mastectomy without the addition of chest wall radiotherapy;
  d. Patients with BCS with the addition of radiotherapy boost **versus** Patients with BCS without the addition of radiotherapy boost;
  e. Patients with adjuvant chemotherapy **versus** Patients with neo-adjuvant chemotherapy;
  f. Patients that have experienced a local recurrence (LRI event) **versus** Patients that have not experienced a local recurrence (no LRI event);
  g. Patients that have experienced a locoregional recurrence (LRRI event) **versus** Patients that have not experienced a locoregional recurrence (no LRRI event).

Multivariable analyses

- **Excluding neo-adjuvant patients** (as we cannot be confident with the pathological T and N staging for these patients), we performed a MVA on LRI and DDFI, comparing young patients that have had a mastectomy compared to those that have had BCS, and adjusted for the following key variables:
    - Age at diagnosis (continuous variable);
    - Maximum diameter invasive tumour (continuous variable);
    - Maximum tumour diameter overall (continuous variable);
    - Surgical margins (categorical – and **excluding** missing/unknown);
    - Focality (categorical – and **excluding** missing/unknown);
    - Pathological N stage (categorical – and **excluding** missing/unknown);
    - histological grade (categorical – and **excluding** not graded/missing/unknown);
    - ER status (categorical – and **excluding** missing/unknown);
    - HER2 status (categorical – and **excluding** missing/unknown);
    - Adjuvant radiotherapy indicator (categorical – and **including** no/missing/unknown);
    - Hormone treatment indicator (categorical – and **including** no/missing/unknown);
    - Axillary surgery indicator (categorical – and **including** no/missing/unknown, but **only** if available at the time of analysis).

- **(If time available): Excluding neo-adjuvant patients and excluding patients with a maximum tumour diameter overall >30mm**, we performed a MVA on LRI and DDFI, comparing young patients that have had a mastectomy compared to those that have had BCS, and adjusted for the following key variables:
    - Age at diagnosis (continuous variable);
    - Maximum diameter invasive tumour (continuous variable);
    - Surgical margins (categorical – and **excluding** missing/unknown);
    - Focality (categorical – and **excluding** missing/unknown);
    - Pathological N stage (categorical – and **excluding** missing/unknown);
    - histological grade (categorical – and **excluding** not graded/missing/unknown);
    - ER status (categorical – and **excluding** missing/unknown);
    - HER2 status (categorical – and **excluding** missing/unknown);
    - Adjuvant radiotherapy indicator (categorical – and **including** no/missing/unknown);
    - Hormone treatment indicator (categorical – and **including** no/missing/unknown);
    - Axillary surgery indicator (categorical – and **including** no/missing/unknown, but **only** if available at the time of analysis).

  Hazard Ratios

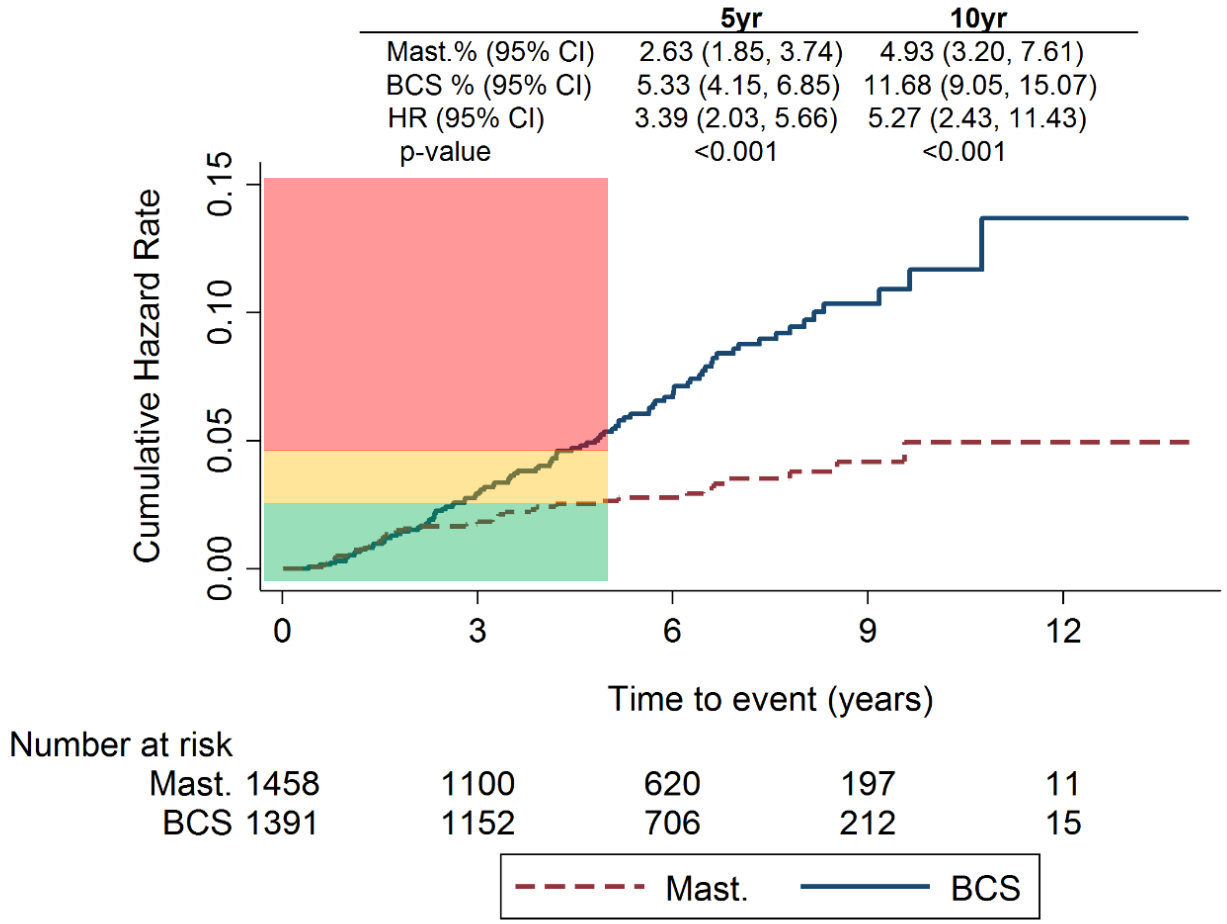
  Evidence suggests that the effect of ER status changes over time (Azzato, et al, 2009, Bellera et al, 2010)[1]. Indeed, this was evident after testing the proportional hazards assumption based on the Schoenfeld residuals and using the identity matrix for the time-scaling function[2]. This result provided strong evidence against the Cox proportional hazards assumption, which was also seen when plotting the scaled Schoenfeld residuals over time[2]. We therefore stratified any Cox models by ER status.

Method used to handle missing data

This was a complete case analysis.

A.4 Illustrative example of the ABS guidelines

Figure 7 Local recurrence interval for patients from the POSH cohort, incorporating the ABS guidelines[16]



[16] The red area correspondence to rates above 5% at five years, the orange area corresponds to rates between 3% and 5% at five years, and the red area corresponds to rates below 3% at five years.

Glossary of Terms

ABS	Association of Breast Surgery
AIC	Akaike Information Criterion
BCS	Breast Conserving Surgery
BCSS	Breast Cancer-Specific Survival
BMI	Body Mass Index
CI	Confidence Interval
DCIS	Ductal Carcinoma In Situ
DDFI	Distant Disease-Free Interval
DDFS	Distant Disease-Free Survival
DF	Degrees of Freedom
DNA	Deoxyribonucleic Acid
ER	Oestrogen Receptor
EIC	Extensive Intraductal Component
FDR	First Degree Relative
FH	Family History
FPSM	Flexible Parametric Survival Model
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hazard Ratio
IDC	Invasive Ductal Carcinoma
IQR	Inter-Quartile Range
LCIS	Lobular Carcinoma In Situ
LRI	Local-Recurrence Interval
MAR	Missing At Random
MCAR	Missing Completely At Random

Glossary of Terms

MNAR	Missing Not At Random
MRI	Magnetic Resonance Imaging
MVA	Multivariable Analyses
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NPI	Nottingham Prognostic Index
OS	Overall Survival
PDRS	Post Distant-Relapse Survival
POSH	Prospective Study of Outcomes in Sporadic and Hereditary Breast Cancer
PR	Progesterone Receptor
RCT	Randomised Controlled Trial
RNA	Ribonucleic Acid
SAP	Statistical Analysis Plan
SDR	Second Degree Relative
SNP	Single-Nucleotide Polymorphism
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TMA	Tumour Micro-Array
TNBC	Triple Negative Breast Cancer
TNT	Triple Negative
UK	United Kingdom
UVA	Univariable Analyses
WHO	World Health Organisation
WMCIU	West Midlands Cancer Intelligence Unit

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