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The clinical and pathological characteristics of breast cancer in young women and implications for genetic testing.

by

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ABSTRACT

FACULTY OF MEDICINE

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THE CLINICAL AND PATHOLOGICAL CHARACTERISITICS OF BREAST CANCER IN YOUNG WOMEN AND IMPLICATIONS FOR GENETIC TESTING.

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This thesis explores the way in which pathological parameters of breast cancer and family history affects outcomes and the probability of being a BRCA mutation carrier in a population of young onset UK breast cancer patients. This is set in the context of the mainstreaming genetic medicine agenda, VUS knowledge and working practises of UK genetics laboratories.

Descriptive characteristics of data from the UK Prospective study of Outcomes in Sporadic versus Hereditary breast cancer study (POSH) of 3000 patients were analysed with respect to distant disease free interval (DDFI) and overall survival (OS), stratified by Estrogen Receptor (ER) status and family history (FH). Patients with ER positive tumours compared to those with ER negative tumours had a better 5 year OS (85.0% vs 75.7%, $p<0.001$) that became non-significant by 8 years (67.5% v 67.7%, $p=0.931$). There was no difference in 5-year DDFI for FH + versus FH- patients (77.4% vs 74.9%, $p=0.001$).

Multifocal status significantly negatively correlated with BRCA1 mutation status and there was a non-significant positive association in BRCA2. In multivariate analysis multifocality was not an independent predictor for survival.

Analysis of common pathological factors (receptor status, grade and mutlifocality) in a subgroup with BRCA genetic testing results was used to

derive a new predictive algorithm for FH – patients to determining pathogenic BRCA 1/2 mutation carrier probabilities.

Knowledge of VUS results by UK breast cancer specialists and of laboratory practices reported by UK geneticists was assessed by two online questionnaire surveys. Overall 71.0% of 181 of breast cancer clinicians felt uncomfortable/ unequipped to interpret a genetics report with surgeons more confident than oncologists. To facilitate moving genetic BRCA testing into mainstream oncology, genetics laboratories need to standardise reporting and testing clinicians need additional training to interpret BRCA reports.

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DECLARATION OF AUTHORSHIP

I, Bryony Kathryn Elliott 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.

Clinical Implications of Genetic Testing in 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;

   The POSH study design and data collection occurred prior to my MD. I used the POSH database in conjunction with a statistian to analyse the data and interpret the results as shown in this MD. The statistical analysis plan for the descriptive paper (chapter 3), and family history paper (chapter 4) had been written by the POSH statistian; but I carried out the analysis and interpretation of results. The VUS chapter (5), multifocal chapter (6), and algorithm chapter (7) were conceived and carried out by myself. The exception was the geneticist survey (chapter 5) which was sent out by the ENIGMA consortium and I analysed the results.
7. Parts of this work have been published as:

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. 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 Jul 3;105(13):978-88. doi: 10.1093/jnci/djt134. Epub 2013 May 30

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Signed: .....

Date: .....

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Definitions and Abbreviations

ACGS	Association for Clinical Genetic Science
BOADICEA	Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm
CTC	Circulating Tumours Cells
DRFS	Distant Relapse Free Survival
ENIGMA	Evidence-based Network for the Interpretation of Germline Mutant Alleles
EORTC	European Organisation for Research and Treatment of Cancer
ER	Estrogen receptor
FH	Family History
FISH	Fluorescent in situ hybridisation
HER2	Human Epidermal Growth Factor receptor 2
HBOC	Hereditary Breast and ovarian cancer syndrome
IHC	Immunohistochemistry
MF	Multifocal
OS	Overall Survival
PR	Progesterone receptor
POSH	Prospective study of Outcomes in Sporadic versus Hereditary breast cancer
SEER	Surveillance, Epidemiology and End Results.
TFGT	Treatment focused genetic testing
UF	Unifocal
VUS	Variant of Unknown Significance
WMCIU	West Midlands Central Intelligence Unit
WT	Wild-type

Chapter 1: Introduction

1.1 Breast Cancer Statistics

In the UK breast cancer remains the most common cancer diagnosed accounting for 15% of the total cancer incidence and 30% of cancer cases in females. There were 49564 new female cases in 2010 with a lifetime risk of 1 in 8 for UK women. Since 2000 incidence rates have increased by 7%. Breast cancer like many solid organ cancers is more common with increasing age, with the peak incidence in the 60-64 age group. Only around 2057 cases of breast cancer are diagnosed in the 20-39 age group in the UK every year, but it is the most commonly diagnosed cancer in women aged under 39 (1).

Breast cancer is the second leading cause of cancer death accounting for 15% (2011 figures) but the mortality rate has decreased by 19% (between 2000-2002 to 2009-2011). Generally the outcome from breast cancer is good, with a 68.5-90.4% 5-year survival depending on the patient's age category. The youngest age group (15-39 year olds) account for very few cancers but 5 year survival is only 83.5% compared with the average. Breast cancer is the commonest cause of cancer death in women aged 15-49 and the number of life years lost is disproportionately higher than in older age groups (1).

1.2 Risk factors

There are many established factors both modifiable and non-modifiable that influence breast cancer risk, including reproductive factors, hormone use, breast density, lifestyle factors (BMI – obesity in post-menopausal women but low BMI in premenopausal (2-4), alcohol consumption (5), and radiation exposure. Women with breast and ovarian cancer affected relatives have a higher risk of developing breast cancer. For any individual, the more affected relatives she has and the younger the age at diagnosis in those affected, the higher her lifetime risk of developing breast cancer (6). Familial breast cancer is thought to account for 5-10% of all breast cancers, but of these familial breast cancers only 30% are found to be attributable to a known breast cancer predisposition gene mutation (7). In addition, most women with breast cancer

including women diagnosed at a young age do not have a positive family history (8). *BRCA1* and *BRCA2* mutations are the most common highly penetrant genes for monogenic breast cancer susceptibility yet account for only about 15% of familial breast cancer. They have been the most studied and with the clearest management recommendations for both primary and secondary cancer prevention (7).

Risk tools are available to clinicians to assist in identifying the degree of family history at which referral to genetics services is appropriate. These include the Manchester scoring system (9), Ontario Family History Assessment Tool, FHS-7, Referral Screening Tool and Pedigree Assessment Tool (10). These tools rely heavily on family history factors and specific ethnic groups such as Ashkenazi Jews (11). For the majority group of young women that do not have a positive family history, a small proportion will, nevertheless have a pathogenic mutation. How to identify those at risk and refer appropriately in the absence of a positive family history is not clear in national guidelines.

NICE Guidelines CG164 advise genetic testing in specialist genetic clinics for a person with breast or ovarian cancer if their combined *BRCA1* and *BRCA2* mutation carrier probability is 10% or more. ESMO guidelines state: “three or more breast and/or ovarian cancer cases, at least one <50 years; two breast cancer cases <40 years; male breast cancer and ovarian cancer or early onset female breast cancer; Ashkenazi Jew with breast cancer of <60 years; young onset bilateral breast cancer; and breast and ovarian cancer in the same patient”(12). In some countries, the criterion for testing is based on an a priori 10–20% probability of finding a mutation based on predictive models such as BRCAPRO, BOADICEA or Manchester Score, while less specific criteria include a potential benefit in the medical or surgical management of the individual or his/her relatives (12). These methods to determine carrier probability are discussed in more detail on chapter 7.

Certain tumour phenotypes also increase the chance a woman may be identified as a high risk gene carrier such as *BRCA1* or *BRCA2* mutations (13).

1.2.1 Genetics

The first gene to be associated with increased risk of breast cancer was *BRCA1* identified in 1994 (14). *BRCA1* acts as a tumour suppressor gene by maintaining genomic stability through the *BRCA1*-associated genome surveillance complex (15). It is a DNA-damage response protein resulting in DNA repair and checkpoint activation. *BRCA2* mediates homologous recombination (16). Pathogenic mutations in either of these genes lead to inadequate DNA damage repair and the accumulation of double strand (DS) breaks. Other DNA repair mechanisms have to be employed in the absence of functioning BRCA proteins. *BRCA1* is required for ER expression and supresses basal-like gene expression; so loss of *BRCA1* functionality by mutation or methylation results an ER negative, basal-like tumour in more than 50% of cases (17-19). *BRCA2* associated cancers resemble sporadic cancers (ER positive) but the precise link between *BRCA2* functional loss and the development of a luminal-type cancer is not understood (20).

BRCA1 and *BRCA2* are the most common genes known to date associated with an increased breast cancer risk and account for up to 15-20% of familial risk although less than 3% of all breast cancers arise as a result of inherited BRCA1/2 mutation (21). However there are many other breast cancer predisposition genes known and they can be grouped according to their penetrance. *BRCA1* and 2 are the most common highly penetrant predisposition genes and the associated clinical syndrome is called Hereditary Breast and Ovarian Cancer (HBOC). Patients with a strong family history but without a *BRCA1* or *2* mutation can still have HBOC. The other highly penetrant predisposition genes are *TP53, PTEN, STK11* and *CDH1*. *TP53* mutations are rare (0.1% of breast cancers) and with an 18-60 fold risk of early (<45yrs) breast cancer. *PTEN, STK11* and *CDH1* are also associated with a high breast cancer risk (21, 22). They all confer not only an increased breast cancer risk but also an increased risk of specific other non-breast cancers (15). Mutations in these genes along with other clinical features lead to a syndromic diagnosis for example PTEN and Cowden Syndrome; STK11 and Peutz-Jeuger syndrome and CDH1and Hereditary Diffuse Gastric Cancer. Moderate penetrance genes (*ATM, CHEK2, PALB2 BRIP1, RAD51C, XRCC2, BARD1, ABRAXAS, PPM1D and NBS1, RAD50 and MRE11* (MRN protein complex) are rarer accounting for 3% of

familial risk and confer a 2-4x increased risk but their place in clinical management is less clear (21, 23) and they are not tested for routinely.

There are many low penetrance genes that modify breast cancer risk most commonly SNP's (increasing or decreasing risk) which probably act in a polygenic model (a large number of genes each of which has a small effect on risk) (24). Low penetrance breast cancer loci that have been identified through genome wide association studies include 6 with a statistically significant association with breast cancer risk (MAP3K1, FGFR2, LSP1, TNRc19, CASP8 and H19) (15, 23, 25, 26).

In patients with a well characterised high risk gene mutation such as *BRCA1* and *BRCA2* the lifetime risk of breast cancer varies. Penetrance varies even between members of the same family with the same gene mutation. Modifiers of risk clustering within families may be either genetic factors or environmental (27). The CIMBA (Consortium of Investigators of Modifiers of *BRCA1*/2) investigating risk modifiers identified a SNP in RAD51which modified the breast cancer risk in *BRCA2* carriers (28). Further work identified 3 further SNPs in FGFR2, TNRC9 and MAP3K. These are associated with an increased risk of breast cancer in the general population and modify risk in *BRCA1*/2 mutated individuals (29). A recent meta-analysis of studies investigating modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers found some association with some lifestyle factors such as age at first live birth, (decreasing risk with increasing age in *BRCA1* carriers); and smoking (increased risk in *BRCA2* carriers) (30).

Breast cancer in younger age categories will include proportionally more BRCA mutation carriers. *BRCA1* mutations in particular are more commonly associated with an adverse biological phenotype, particularly triple negative and high grade disease. Once the negative prognostic markers are accounted for it is unclear whether mutation status per se may be an independent predictor of survival (31). The current benefits for a woman and her family of identifying a mutation in a high risk gene are primary and secondary cancer prevention. For the patient already with a cancer diagnosis, a further benefit includes tailoring their breast cancer management although at present this is in the context of a clinical trial.

The National Institute for Clinical Excellence (NICE) lowered the risk threshold to 10% likelihood of being a gene carrier (previously 20%) which is in keeping with other guidelines (ESMO, ASCO). However the selection emphasis remains on patients with a strongly positive family history as the driver for a genetics referral. There are a number of reasons why a gene carrier may not have a strong family history of cancer including adoption, estrangement and small families). Inherent in the development of any guideline is the trade-off between benefit to health care system at reducing disease and potential benefit to individual, versus cost and problems surrounding genetic test false positives (eg variants of uncertain significance), and uninformative negatives (a negative genetic test in a patient with a very strong family history).

1.3 Breast cancer pathology

The histopathological diagnosis of invasive breast carcinoma is dominated by ductal carcinoma (75%), then lobular carcinoma (10%) followed in frequency by rarer carcinomas of 'special type'. These include tubular, mucinous, cribriform, medullary-like, metaplastic, apocrine, invasive micropapillary and adenoid cystic (32). Immunohistochemistry (IHC) is used to assess estrogen (ER) and progesterone receptor status (PR). Her2 amplification is assessed by IHC and/or *in situ* hybridisation (ISH). Estrogen receptor and Her2 status is now routinely performed in all breast cancers and is an essential part of the diagnostic and treatment planning process. However, fourteen years ago the molecular classification of 42 patients' breast cancer into 4 subgroups (ER+/luminal-like, basal-like, Erb-B2+ and normal breast) by gene expression profiling demonstrated the complexity of breast cancer as heterogeneous diseases with differing outcomes (33). Further work refined this classification into Luminal A, Luminal B, Erb-B2 overexpression, 'Basal-like' and Normal Breast-like (34). There are a range of approaches to approximating the gene expression sub-types to clinico-pathological surrogate definitions based on receptor status, Ki-67 and recurrence score (based on multi gene-expression data if available). Luminal A subtype is often defined as as ER+, PR+, Her2-, Ki-67 'low' and recurrence risk low; Luminal B / Her2 neg subtype as ER+, Her2- and $\geq$ 1 of: Ki67 'high', PR 'neg/low' and recurrence risk high; Luminal B /Her2+ subtype as ER+, Her2+, any Ki67, any PR; Erb-B2 overexpression subtype as Her2+, ER and PR –; and Basal-like subtype as ER-, PR- and Her2-.

These are approximations, for example triple receptor negative breast cancer is not entirely synonymous with basal-like (35). There is an ~80% overlap between them, with triple negative including some special histological subtypes and some basal-like cancers have low-positive ER staining that cluster in the non-luminal molecular subtypes on gene expression profiling. The 2011 St Gallen International Breast Cancer Conference Consensus accepted that clinico-pathological factors can be used as a surrogate for subtype classification into the molecular subgroups defined by multi-gene molecular assays (36). One of these assays, Oncotype DX has been approved by NICE (Diagnostics Guidance 10) to guide cancer management in only specific circumstances but is not yet routinely used in NHS care due to funding considerations. Molecular classification can provide additional information to guide clinicians in prognosis particularly in the use of adjuvant cytotoxics.

1.4 Staging

Breast cancer is staged by conventional TNM staging as defined by the American Joint Committee on Cancer (AJCC) (37). T refers to primary tumour size, N to nodal status and M to presence or absence of distant metastases. Breast cancers can be unifocal, where there is one primary focus; or multifocal/multicentric where there is more than one focus of cancer in the breast. There is controversy around how to describe tumour size when more than one focus of cancer is present in the breast. Multifocality and multicentricity are discussed in detail in chapter 5 with their relationship to outcome.

1.5 Age as a prognostic outcome marker

It is accepted that young onset breast cancer patients have a worse survival than older onset patients. An excess of more aggressive biology such as high grade, ER negative cancers, large tumours, and lymph node positivity may account for the worse prognosis compared to middle aged women (3). Larger tumours in the <40 year old may be a reflection of the lack of screening in this age group.

There is a correlation between hormone status, age and prognosis. A pooled analysis of four large EORTC trials found that molecular subtype was strongly associated with overall survival and distant disease free survival in younger patients as with older age groups (38). A large population-based analysis of the SEER database found that young age (<30) was only associated with a poorer prognosis than older age in ER positive women. Young women with ER negative cancers had no different survival than all other age groups bar the elderly (>75 years) (39).

Inadequate treatment of the extremes of age has been postulated to account for this difference in survival. The International Breast Cancer Study Group (IBCSG) found that chemotherapy alone without endocrine blockade was insufficient in the very young (<35) who had worse survival than the women over >35years old (40). However, in a Korean study even with the addition of adjuvant hormonal therapy after chemotherapy survival was worse in the young age (<35) hormone receptor positive patients, again hormone receptor negative very young patients did not have worse survival (41). Azim et al looked at relapse free survival (RFS) by molecular subgroup of pooled cases from publically available datasets also finding younger patients <40 with luminal cancers had worse RFS than older patients with luminal cancers. This pattern was not found by the Anders group, with ER status not predictive of survival by univariate or multivariate analysis in young women defined as <45 (5). There are substantial differences between POSH and the Anders cohort which was a combination of data from 4 public datasets, only 200 of which were <45 years old. Adjuvant therapy information was missing from 1 of the 4 datasets and a second set was comprised on only lymph node negative patients who did not receive adjuvant therapy.

Many studies report age as an independent adverse prognostic factor (42-49) particularly in the <35 age group although most are population-based registry studies and retrospective in nature not using modern oncological management. In addition, in these studies there is less use of adjuvant chemotherapy, particularly modern combination chemotherapy with difference in use depending on age. There is often lack of knowledge of Her2 status and use of Her2 targeted therapies making interpretation difficult.

The use of adjuvant cytotoxic chemotherapy has changed with young age alone a recognised poor prognostic marker supporting the use of adjuvant chemotherapy irrespective of other prognostic factors in the 1998 St Gallen consensus without a good supporting evidence base (50). However at the 2013 St Gallen conference young age (<35) was a factor for inclusion of adjuvant chemotherapy in only 54% of expert panel responses (51).

To accurately compare outcomes for young onset breast cancer with middle/older age not only should there be adjustment for known prognostic variables such as T and N stage, grade, and receptor status but differences in adjuvant hormonal and cytotoxic chemotherapy should also be recognised.

1.6 Breast cancer in young women – POSH study

Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (the POSH study), is a prospective UK based cohort study designed to investigate factors affecting prognosis in a young patient age group of 18-40 years old (52). This MD uses data previously collected from the POSH study to explore the clinical questions outlined over the subsequent chapters.

The **primary aims** of the POSH study were as follows:

a) The prognosis of patients with breast cancer who harbour BRCA1 or BRCA2 gene mutation differs from non-carrier patients matched for age and other prognostic factors?
b) Breast cancers occurring in patients with different predisposing inherited gene mutations have a consistent and distinct phenotype?
c) There are difference in the pattern of distant breast cancer recurrence between patients with BRCA1 or BRCA2 gene mutations and matched non carrier patients?

Secondary aims were:

(d) To develop a validated set of pathological criteria that improve the specificity and sensitivity of methods for identifying carriers of germ-line BRCA1/2 mutations to facilitate future clinical trials based on genotype.

(e) To determine whether inherited genetic variants influence tumour biology (particularly metastatic potential)

(f) To describe the radiological features in a large cohort of young breast cancers and correlate these with genetic factors, tumour pathology and prognosis.

Objectives for this MD:

This MD does not cover all of these above aims set by the POSH chief investigator, but uses the POSH database to investigate the following:

Detail the design and methods of the POSH study.

Describe the POSH cohort characteristics with reference to the national dataset from the West Midlands Intelligence Unit.

Overall survival (OS) and distant disease free survival (DDFS) is analysed for the whole cohort and by ER status.

The influence of a positive or negative family history on distant disease free interval (DDFI) of the whole POSH cohort.

Explore and discuss issues arising from genetic testing particularly focusing on BRCA variants of unknown significance (VUS) from surveys performed of both UK breast cancer specialists and UK geneticists.

Look at the little researched area of multifocal breast cancer looking at a possible association with outcome and with BRCA mutation status.

Analyse pathological variables that may help to predict the genetic mutation status of the subgroup of patients without a family history of breast or ovarian cancer and develop a new predictive algorithm for use in the oncology clinic.

Chapter 2: The POSH Study Methods and Statistics

2.1 Patients

The POSH study recruited female patients from 127 UK hospitals (52). Patients were eligible if diagnosed with invasive breast cancer between 1st Jan 2000 and 31st Jan 2008 at an age of 40 years or younger. Additionally patients aged 41-50 with known BRCA 1 or BRCA2 mutations diagnosed with breast cancer were eligible. Exclusion criteria were patients with a previous invasive cancer (excepting non-melanomatous skin cancer), not available for follow-up or refused consent for retention of diagnostic tissue or follow-up data.
Potential recruits were identified within 12 months of initial breast cancer diagnosis. All patients received treatment according to local protocols. Written consent was obtained. All patients were informed that genetic testing may be carried out on their DNA sample.

The detailed study protocol was published in 2007 (52). This study received approval from the South West Multi-centre Research Ethics Committee (MREC 00/6/69).
I was not involved in the study design, recruitment phase or data collection.

2.2 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 neoadjuvant chemotherapy, initial tumour diameter was derived from radiological reports. Maximum invasive tumour was defined as the size in mm of invasive tumour by longest diameter excluding any DCIS, or if multifocal the sum of longest diameters. Family history and personal risk factors were collected using a questionnaire completed by participants at recruitment but not corroborated independently.

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 Health and Social Care Information Centre to facilitate automatic notification of date and cause of death.

2.3 Biological Analyses

Estrogen receptor (ER), progesterone receptor (PR) and HER2 receptor status of primary tumours were determined from diagnostic pathology test reports. ER positive was defined as an Allred score of 3-8 (53) as determined by immunohistochemistry (IHC) when the score was given in local pathology reports. Her2 positivity was defined as 3+ on IHC, or positive by ISH methodology. Tissue microarray data from 1336 cases were used to corroborate and supplement missing clinical data on receptor status. These cases were selected at random. TMAs were assembled by Linda Hayward and IHC staining analysed by Professor Louise Jones at St Bartholomew's Hospital. In a small number of cases where there was discrepancy between local pathology reports and TMA data, we took TMA data as most accurate and used this result. Where IHC and FISH contradicted each other we used ISH results. Genetic testing results have been recorded for trial participants who underwent formal genetic assessment and diagnostic *BRCA1*/2 screening at a regional clinical genetics centre following referral by the local oncological team. In addition research genetic testing for *BRCA1*/2 mutations for POSH participants has been carried out for part of the cohort and testing of the whole cohort will be completed by the end of 2015.

2.4 Statistics

The original sample size for the POSH study was 2000. This was based on an assumed prevalence rate of 10% of BRCA1 gene carriers, giving a 97% power to detect a difference of 10% in 2 year event rate (20% in gene carriers compared to 10% in sporadic cases). However pilot mutation testing of 120 study patients found a 3% BRCA1 mutation rate (1/38) in family history negative patients and 39.5% (32/81) in family history positive patients. Lower than assumed BRCA1 prevalence rate would have resulted in less statistical power to detect an event

rate difference with confidence, so the sample size was recalculated to 3000 (52).

The statistical analysis plans (SAP) for the POSH Descriptive Study (Chapter 3) and Family History Analysis (Chapter 4) were written by Mr Tom Maishman, was carried Statistician, University of Southampton Clinical Trials Unit; but the analysis and interpretation of the results was carried out by myself.

SAP's for the BRCA Variant Unknown Significance analyses (Chapter 5), Multifocal analyses (Chapter 6) and BRCA Algorithm development (Chapter 7) were conceived and written by Dr Bryony Eccles in conjunction with Mr Tom Maishman. Summary statistics were performed predominantly by Dr Bryony Eccles and checked by Mr Tom Maishman. 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 and key data were compared with information from the West Midlands Central Intelligence Unit (WMCIU).

Overall survival (OS) and distant disease free interval (DDFI) were assessed using Kaplan-Meier curves and performed by Mr Tom Maishman. 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 (54). Survival from ER negative tumours falls in the first two years after diagnosis and then plateaus whereas the fall in survival continues long term (>5 years with late relapses common) in ER positive tumours. *Therefore, to assess the effect of ER status a flexible parametric survival model was fitted to OS and DRFS using the STATA stpm2 command with ER as a time-dependent covariate (55). In each case, the degrees of freedom (df) was set to three and two for the baseline hazard rate and time-dependent effect respectively (to achieve the lowest Akaike Information Criterion- AIC). The lowest AIC implies the best model fit. 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 (Chapter 3).* *Mr Tom Maishman*

The Declaration of authorship details which of the following work is my own, or conceived by another.

Chapter 3: Young Breast Cancer Patients: The POSH study

3.1 Introduction

Although only a small number of women diagnosed with breast cancer are aged 40 years or under at diagnosis, the excess mortality in younger women translates to a greater number of life years lost resulting in a substantial burden to the family, to the health care system and to society (46, 56).

Young age at diagnosis is widely accepted as being associated with poorer prognosis compared with older onset, with 5 year survival 6.9% lower in the 15-39 age group (83.5%) compared to the 50-59 age group (90.4%) (1). Only the > 70 age group has inferior survival compared with the young and this is probably related to reduced systemic treatment given in the older woman because of comorbidities and age. Aggressive tumour biology is more common in the younger compared to middle aged woman and this may in part account for inferior survival (3, 5, 48, 57). More ER and Her2 receptor negative, inflammatory cancers, higher grade and a greater preponderance of lymphovascular invasion occurs in the young in comparison to older age groups. All of these are poor prognostic features. Historical studies frequently quote young age as an additional poor prognostic indicator (42, 48, 58-60). The St Gallen Consensus statement previously listed age <35 as a poor prognostic feature, with a requirement to consider adjuvant chemotherapy on this criterion alone; however this is absent from the most recent consensus published in 2013 (51). A recent publication compared the <40 with 40-49 year olds over two time periods (1986-1992 and 2004-2007) by breast cancer subtype as defined by IHC. As expected, overall relapse free survival improved in more modern times, but only in luminal subtypes did the inferior survival of the younger age category persist (61). There is a difference in relapse pattern and median overall survival between tumours of differing ER status (62) such that any survival analysis must be stratified by ER status to be interpretable.

Screening for breast cancer has been attributed along with adjuvant chemotherapy as the cause for falling mortality in western countries (63). In

the UK an independent review (64) published in 2012 concluded that breast cancer screening prevents 43 breast cancer deaths but at the cost of 129 over diagnoses per 10,000 women screened from age 50 -70. Lack of screening in the young (infrequent incidence makes screening unfeasible on a population level) may also account for the often larger, more node positive tumour in the young age onset breast cancer patient.

Young breast cancer patients also have more complex psychosocial needs (65). A young family adds a psychological burden with the fear of dying and leaving behind dependents. Chemotherapy affects fertility with preservation more complex and much harder to achieve in a woman (66, 67) than simple sperm banking for a man. Breast cancer management including surgery, chemotherapy and radiotherapy may affect body image and self-esteem with psychosexual issues more strongly present in the young woman (68, 69). Lastly younger women are more likely to be in the work force adding an additional financial personal and social burden. These additional stressors may influence a woman's decision in the type of surgery and whether to receive adjuvant chemotherapy.

POSH (Prospective study of Outcomes in Sporadic versus Hereditary breast cancer), a UK multicentre, prospective cohort study set up to investigate prognostic factors in the young breast cancer patient. This analysis describes the baseline characteristics of the patient, the tumour and management with reference to national statistics provided by the West Midlands Cancer Intelligence Unit (WMCIU). Distant relapse free survival (DRFS) and overall survival (OS) are looked at in the whole cohort and by ER status.

3.2 Methods

Collection of data on patient characteristics, study variables and data sources are detailed in Chapter 2: The Posh Study Methods. All patients recruited to POSH were included in the descriptive analysis, except those aged 41 or over with known BRCA gene mutations (n=43). This analysis was conducted on follow-up data received until 11th April 2012.

To rule out any systematic ascertainment bias, cohort characteristics were compared with WMCIU data. Data on all known invasive breast cancers

diagnosed within England for the same age range and time period were provided. WMCIU only provides data on women diagnosed in England. The POSH study recruited 23% of all eligible patients in England during the recruitment period.

3.2.1 Statistical analysis

As per section 2.44. The statistical analysis was performed according to a pre-specified plan as per STROBE (Strengthening the reporting of observational studies in epidemiology) guidelines (54). The STROBE checklist devised for cohort studies is designed to ensure adequate and complete reporting of studies in the literature in order to be able to assess their strengths and weakness.

3.3 Results

The POSH study recruited 3095 patients across England (n=2695), Scotland (n=86), Wales (n=131) and Northern Ireland (n=44). 139 trial participants were excluded due to ineligibility as they did not have invasive cancer (n=74), diagnosis was made outside study period (n=1), and 66 women excluded from this analysis due to missing primary tumour data (n=22), or from the older age and known gene mutation carrier group (n=43) (Figure 3-1). This analysis was performed on data from the remaining 2956 patients. Recruitment peaked in 2005 (Figure 3-2). According to WMCIU data, 11594 female patients aged 18-40 were registered with invasive breast cancer in England in during the main study period of 2000-2007. POSH participants recruited from England thus represent 23% of the available population during the recruitment period.

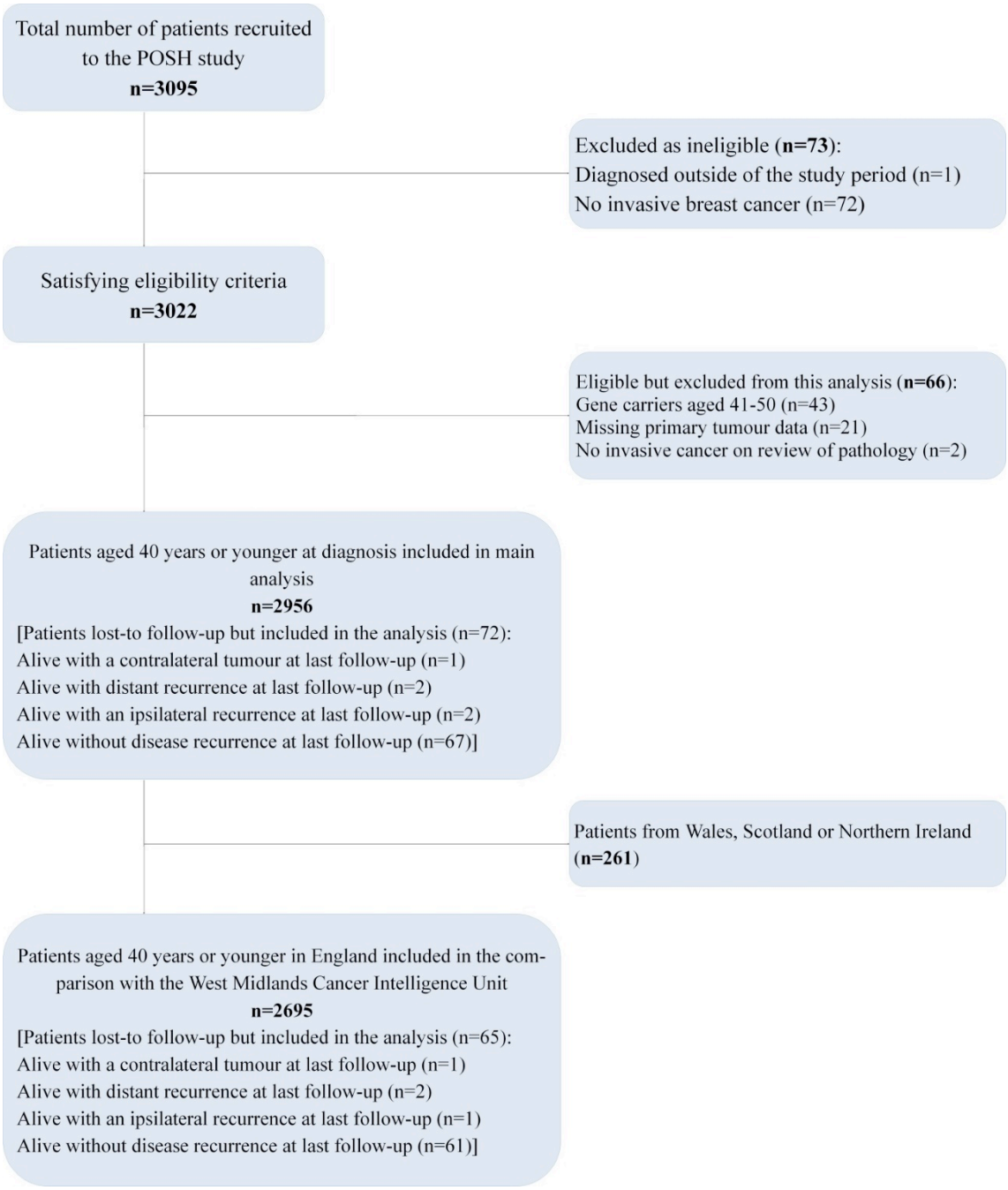


Figure 3-1 POSH Flow Diagram

3.3.1 Patient characteristics

Detailed patient demographics, method of presentation and breast cancer risk factors are shown in Table 3-1. Median age at diagnosis of breast cancer was 36 years (range 18-40). Only a very small minority of women were 25 or under

at diagnosis (1.6%). Most women had children (70.9%) and had used the oral contraceptive pill (87.9%).

Table 3-1 Patients' characteristics and risk factors

Characteristic	**Median (range, IQR), number of patients (%)**
Age at diagnosis, in years	36 (18 to 40, 33 to 38), n=2956 (100%)
18 to 25	46 (1.6%)
26 to 30	269 (9.1%)
31 to 35	900 (30.5%)
36 to 40	1741 (58.9%)
Duration of follow-up in months	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%)
(Risk factor characteristics)	
Age at menarche, in years	13 (8 to 18,12 to14), n=2956 (100%)
Body mass index, in 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, in years	27 (13 to 40, 23 to 30), n=2080 (70.4%)
Missing/unknown	92 (4.4%)
	Number of patients (%)
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%)

Characteristic	Median (range, IQR), number of patients (%)
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%)

3.3.2 Presentation and diagnostics

Symptomatic presentation accounted for 98% (n=2900) of the cohort. Thirty women presented with screen detected malignancies whilst on surveillance programs due to a previously identified *BRCA 1/2* mutation in the patient (n=3), 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%). 220 patients (7·4%) underwent magnetic resonance imaging of the breasts. No imaging modality data were available in 82 patients (2·8%).

3.3.3 Tumour pathology

The tumour pathological features have been analysed for the whole 2956 patients and by ER status. Tumours were reported as ER positive in 65·9% and ER negative in 33·7% of cases. HER2 overexpression was recorded in 24·3% (n=717) patients overall. However on randomly selected study specific TMA's 243 of 1336 patients (18·2%) had HER2 overexpression (Table 3-2). 588 (19·9%) patients were ER, HER2 and (if available) PR receptor negative and 148 (14·1%) on study TMA results. The most common histological subtype was ductal carcinoma (86.5%), followed by lobular (4.5%) and mixed ductal and lobular (2.6%). The distribution of subtypes was statistically different between ER positive and ER negative ($p<0.001$) (Table 3-2). Lobular carcinoma was more common in ER positive than ER negative (6.5% versus 0.7%), but medullary carcinoma less common (0.2% versus 2.8%) respectively. Grade, distribution of cancer, PR status and pathological T stage and nodal status were all significantly differently distributed between ER positive and ER negative

patients. A higher grade tumour was more common in ER negative than positive (grade3: 86.7% versus 44.7%). However ER negative tumours more commonly had localised tumours (71.1% versus 59.4%). Multifocality of tumours and implications is discussed further in Chapter 6.

Table 3-2 Tumour characteristics by ER status

Characteristic	ER Negative (n=997)	ER Positive (n=1947)	Total* (n=2956)	p value††
	Number of patients (33.7%)	Number of patients (65.9%)	Number of patients (100%)	
Histological Grade				
1	6 (0.6%)	155 (8.0%)	163 (5.5%)	
2	100 (10.0%)	871 (44.7%)	972 (32.9%)	p<0.001
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)	
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%)	p<0.001
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%)	p<0.001
Localised	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%)	p<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%)	
Positive	256 (25.7%)	461 (23.7%)	717 (24.3%)	p=0.431
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%)	p=0.350
M1	21 (2.1%)	52 (2.7%)	74 (2.5%)	
Missing/unknown	7 (0.7%)	15 (0.8%)	22 (0.7%)	

Characteristic	**ER Negative (n=997) Number of patients (33.7%)**	**ER Positive (n=1947) Number of patients (65.9%)**	**Total* (n=2956) Number of patients (100%)**	
Pathological T stage (all patients)				
T0	39 (3.9%)	33 (1.7%)	73 (2.5%)	
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%)	p=0.005
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%)	
T1	398(48.8%)	869 (51.9%)	1270 (50.9%)	
T2	352 (43.2%)	689 (41.2%)	1044 (41.8%)	p=0.703
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%)	
N stage (excluding neoadjuvant patients□)				
N0	456 (56.0%)			
N1	348 (42.7%)	753 (45.0%)	1213 (48.6%)	
1-3	220 (63.2%)	903 (54.0%)	1252 (50.2%)	
4-9	78 (22.4%)	597 (66.1%)	817 (65.3%)	p<0.001
10+Missing/unknown	49 (14.1%)	200 (22.2%)	279 (22.3%)	
	11 (1.4%)	106 (11.7%)	155 (12.4%)31 (1.2%)	
		17 (1.0%)		

	ER Negative (n=997)	ER Positive (n=1947)	Total* (n=2956)	p value‡‡
	Median (range, IQR), number of patients(%)	Median (range, IQR), number of patients(%)	Median (range, IQR),number of patients(%)	
Maximum diameter invasive tumour, in mm (all patients) Missing/unknown	22 (1-199,15-31) 912 (91.5%) 85 (8.5%)	22 (0-170,15-35) 1840 (94.5%) 107 (5.5%)	22 (0-199,15-33) 2763 (93.5%) 193 (6.5%)	p=0.156
Maximum diameter invasive □ tumour, in mm (exc neoadjuvant) Missing/unknown	22 (1.5-199, 15-30) 796 (79.8%) 19 (2.3%)	22 (1-150, 16-33) 1643 (84.4%) 30 (1.8%)	22 (1-199, 15-32) 2446 (82.8%) 50 (2.0%)	p=0.206
Maximum tumour diameter¥ in mm (all patients) Missing/unknown	26 (0.6-199, 18-37) 928 (93.1%) 69 (6.9%)	27 (0-190, 19-42) 1856 (95.3%) 91 (4.7%)	27 (0-199,18-40) 2795 (94.6%) 161 (5.5%)	p=0.005
Maximum tumour diameter¥□ in mm (exc neoadjuvant patients) Missing/unknown	26 (3-199, 18-35), 801 (80.3%) 14 (1.7%)	27 (1-190, 19-41) 1653 (84.9%) 20 (1.2%)	26 (1-199, 19-40) 2461 (83.3%) 35 (1.4%)	p=0.006
Number of axillary lymph nodes recovered (all patients) Missing/unknown	13 (0-46, 8-18) 981 (98.4%) 16 (1.6%)	12 (0-53, 7-17) 1920 (98.6%) 27 (1.4%)	12 (0-53, 8-17) 2910 (98.4%) 46 (1.6%)	p=0.022
Number of axillary lymph nodes recovered (exc neoadjuvant patients)□ Missing/unknown	13 (0-46, 8-18) 807 (99.0%) 8 (1.0%)	12 (0-53, 7-17) 1660 (99.2%) 13 (0.8%)	12 (0-53, 7-17) 2472 (99.0%) 24 (1.0%)	p=0.088
Number of positive axillary lymph nodes (all patients) Missing/unknown	3 (1-42, 1-6) 426 (42.7%) 22 (2.2%)	2 (1-50, 1-5) 1067 (54.8%) 34 (1.8%)	2 (1-50, 1-5) 1495 (50.6%) 59 (2.0%)	p=0.212
Number of positive axillary lymph nodes (exc neoadjuvant patients) □ Missing/unknown	2 (1-42, 1-5) 349 (35.0%) 13 (1.3%)	2 (1-50, 1-5) 910 (46.7%) 18 (0.9%)	2 (1-50, 1-5) 1260 (42.6%) 34 (1.2%)	p=0.708

*Total column includes data from the whole cohort i.e. ER+ve, ER-ve and ER status unknown (12 patients).
†Not graded as pathology from axillary node, no primary detected.
‡Includes data from TMA as well as primary POSH data.
□ total no of patients excluding neoadjuvant n=2496.
¥ Maximum tumour diameter includes DCIS (ductal carcinoma in-situ).
†† p-values obtained from the Pearson chi-squared test between ER status and each categorical variable (excluding missing/unknown data).
‡‡ p-values obtained from the Mann-Whitney test between ER status and each continuous variable (excluding missing/unknown data).

Despite similar pathological T stage, the difference in nodal status between ER positive and negative patients is significant (p<0.001). More ER positive than negative tumours were node positive (54.0% versus 42.7%). Although if node

positive, the median number of positive nodes was not significantly different between ER positive and negative tumours (median no. =2 in both ER positive and ER negative, p=0.708) (Table 3-2). For larger tumours downstaging between clinical and pathological T stage is demonstrated reflecting the more frequent use of neoadjuvant chemotherapy (Table 3-3).

Table 3-3 Clinical T stage and Pathological T stage of primary breast cancer cross tabulated

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

KEY: Grey highlight is where clinical T stage matched pathological T stage. All areas to the left of the highlight reflect downstaging.

Comparing the cohort with available English national data (WMCIU) demonstrates that POSH study patients are representative for available key patient characteristics and tumour variables, age, grade, tumour size and nodal status (Table 3-4). However there were much larger numbers of missing data in the WMCIU data, especially for ER, PR and Her2 receptor status (>80% missing) which make comparison of these variables uninterpretable.

Table 3-4 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	247 (9.2%)	1075 (9.3%)	23.0%
31 to 35	838 (31.1%)	3298 (28.4%)	25.4%
36 to 40	1565 (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	530 (21.0%)	1974 (23.5%)	26.9%
15 to 20	593 (23.6%)	2218 (26.4%)	26.7%
>20 to 35	871 (34.6%)	2567 (30.5%)	39.3%
>35 to 50	298 (11.8%)	934 (11.1%)	31.9%
>50	226 (9.0%)	715 (8.5%)	31.7%
Missing/unknown	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 3-4.

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

The BRCA mutation status of the POSH cohort was not looked at in this analysis. Available genetic testing results will be discussed in later chapters.

3.3.4 Treatment

Most patients 98·6% (2915) had surgical treatment and 27 had only surgery with no other modality of treatment (Table 3-5). There was little difference in treatments between ER positive and negative patients except for definitive surgery. Of ER positive patients the majority had a mastectomy (53.3%), however more patients had breast conserving surgery if ER negative (52.2%).

15·6% (460) received neoadjuvant chemotherapy. The majority of these (329) had T1/2 tumours, 57 had T3 tumours and 68 patients had T4 (including inflammatory cancer). More ER negative than ER positive patients received neadjuvant chemotherapy (18.2% versus 14.1%). Adjuvant chemotherapy was given to 72·8% (2152) patients and 1·8% (54) patients who presented with distant metastatic disease 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 (Figure 3-2 Number of patients recruited to POSH and to each chemotherapy regime by year Figure 3-3). Use of trastuzumab was reported in 12·3% (363) patients from the whole cohort, but 24.3% were Her2 positive. However most patients were recruited before trastuzumab became standard therapy.

In ER positive patients, tamoxifen use was recorded in 88·6% (1726) and an aromatase inhibitor in 2·8% (55). A small, but appreciable number of patients (16.7% of ER positive) underwent oophorectomy.

The data received for hormone therapy, trastuzumab, and radiotherapy is incomplete. No/missing/unknown are in the same category due to data collection methods.

Table 3-5 Treatment details

Characteristic	ER Negative (n=997) (33.7%)	ER Positive (n=1947) (65.9%)	Total* (n=2956) (100%)
Definitive breast surgery			
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			
Adjuvant[1]	772 (77.4%)	1378 (70.8%)	2152 (72.8%)
Neo-adjuvant	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			
Anthracycline based	690 (69.2%)	1245 (63.9%)	1938 (65.6%)
Anthracycline & taxane	264 (26.5%)	416 (21.4%)	684 (23.1%)
Taxane based	13 (1.3%)	7 (0.4%)	20 (0.7%)
Other[2]	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			
Yes	129 (12.9%)	234 (12.0%)	363 (12.3%)
Other treatment period/no/missing/unknown[3]	868 (87.1%)	1713 (88.0%)	2593 (87.7%)
Adjuvant radiotherapy			
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[3]	167 (16.8%)	367 (18.8%)	598 (20.2%)
Adjuvant Hormone treatment			
Yes	98 (9.8%)	1790 (91.9%)	1823 (61.7%)
No/missing/unknown[3]	899 (90.2%)	157 (8.1%)	1133 (38.3%)
Ovarian suppression (in any treatment period)			
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%)

[1] excluding any treatment for M1 disease.
[2] for example, CMF or anything not containing an anthracycline or taxane.
[3]Due to the data collection methods and emerging knowledge of Her2 and guidance through the study this is likely to be inaccurate.
*Includes ER unknown = 12 patients

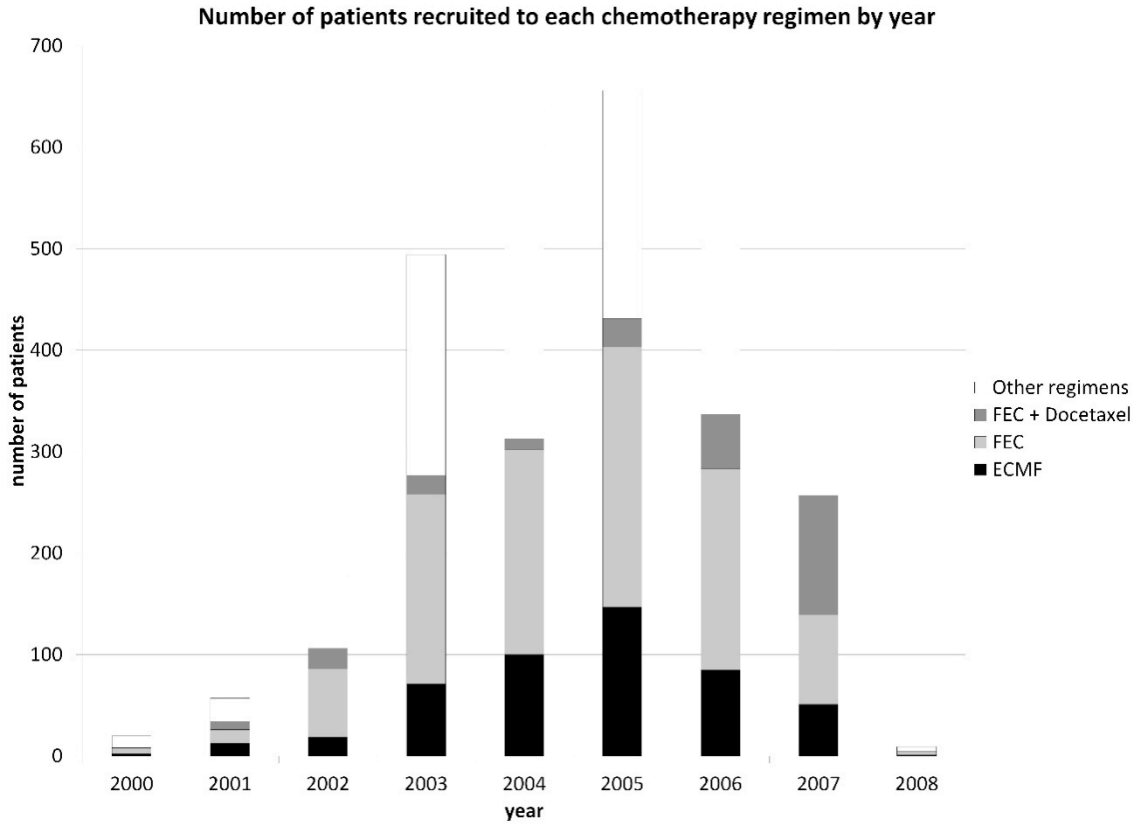


Figure 3-2 Number of patients recruited to POSH and to each chemotherapy regime by year

3.3.5 *BRCA1/BRCA2* testing

To date, 26% (763) patients have had genetic testing. BRCA tested cases 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 pre-specified characteristics (436), such as tumour pathology. Further testing of the cohort is ongoing as part of the study. A pathogenic mutation has been found in 218 patients (*BRCA1* in 136, *BRCA2* in 78, *TP53* in 4). More detailed analysis of the BRCA mutations identified in POSH is discussed in Chapter 4:

3.3.6 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, 5 non-breast cancer deaths and 6 non-cancer deaths, with missing data in 24

patients. A total of 14 non-breast cancer malignancies have been reported: melanoma (2), choroidal melanoma (1), thyroid adenocarcinoma (1), cervical squamous cell carcinoma (2), ovarian adenocarcinoma (1), endometrial carcinoma (1), borderline endometroid carcinoma, non-Hodgkin's lymphoma (1), basal cell carcinoma (1), acute myeloid leukaemia (2), and anaplastic oligodendroglioma (1).

712 women (24%) have developed a distant recurrence of which 149 are still alive. Kaplan Meier (KM) survival curves are plotted in Figure 3-3a-d. Median survival from date of first distant relapse to death was longer in ER positive than ER negative patients (23.4 vs 10.8 months). Isolated local relapse events were few (89 ipsilateral, 63 contralateral). The estimated 5 year OS for the entire POSH cohort is 81·9% and DRFS 76·6%. Patients with ER positive tumours had an estimated 5 year OS of 85·0% compared with 75·7% for those with ER negative tumours ($p<0·001$). DRFS at 5 years was 78·5% for patients with ER positive tumours and 72·7% for patients with ER negative tumours ($p<0·001$). At 8 years OS for the whole cohort is 67·6% and by this time there is no difference in survival (67·5% v 67·7%, $p=0·931$) or DRFS (68·3% vs 68·1%, $p=0·965$) between patients with ER positive and ER negative tumours. The flexible parametric survival model (Figure 3-3 e,f) shows the hazard ratio and hazard and survival rates over time by ER status. It graphically illustrates that the risk of death prior to 5 years is greater for ER negative patients and after 5 years is greater for ER positive patients. The result is that by 8 years the survival curves converge. The model OS estimates closely match the Kaplan-Meier estimates indicating a good model fit (Figures 3-3d compared to 3-3f). The observed difference in survival over time by ER status is still apparent even after adjustment for tumour size, grade and nodal status in a multivariate model.

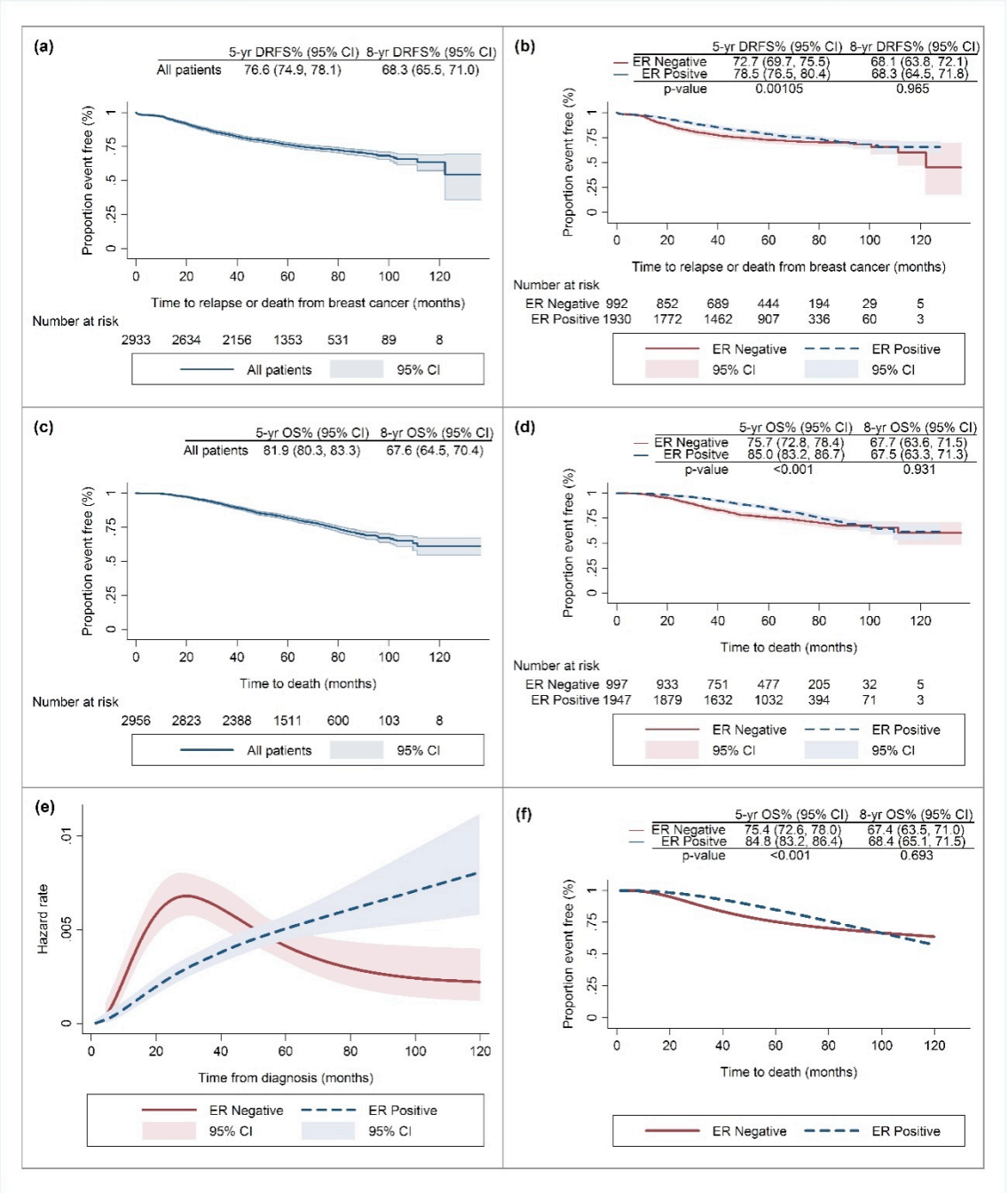


Figure 3-3 KM Distant Relapse Free Survival estimates for (a) all patients, (b) ER negative and positive patients. KM OS estimates for (c) all patients and (d) ER negative and positive patients. Time-varying hazard OS for ER negative and ER positive patients, showing the time-varying (e) hazard rates by ER status, and (f) survival rate by ER status.

The data from POSH has not been categorised into molecular subtypes and a clinico-pathologic surrogate definition on the current data set would be inaccurate due to the inconsistent reporting of Ki-67 and PR status. However looking at DRFS and OS by known ER and Her2 status (Figure 3-4), differences

in both relapse free and overall survival can be seen between the different subgroups. ER positive Her2 negative (mostly Luminal A tumours) have the best relapse free survival, with ER negative Her2 positive the worst. The DDFI curve for ER and Her2 negative cases (these will be predominantly triple negative) plateaus after an initial fall in the first 2 years. HER2-positive compared to HER2-negative tumours showed lower OS and DDFI at all time-points in patients with either ER-positive or ER negative tumours. However the difference in survival between Her2 positive and Her2 negative was statistically significant in ER positive tumours for DDFI at only at five years (five-year DDFI: 71.4% vs 78.3%, p = 0.008; eight-year DDFI: 60.3% vs 66.3%, p = 0.227; five-year OS: 81.4% vs 84.1%, p= 0.206; eight-year OS: 57.6% vs 65.4%, p= 0.159). For patients with ER-negative tumours, the difference in DDFI between Her2 positive and Her2 negative was statistically significant at five and eight years (five-year DDFI: 62.2% vs 73.9%, p= 0.001; eight-year DDFI: 53.4% vs 70.7%, p= 0.004); and OS was statistically significantly lower at 8 years (five-year OS: 70.2% vs 75.2%, p= 0.154; eight-year OS: 58.4% vs 68.3%, p= 0.047) (Figure 3-4)

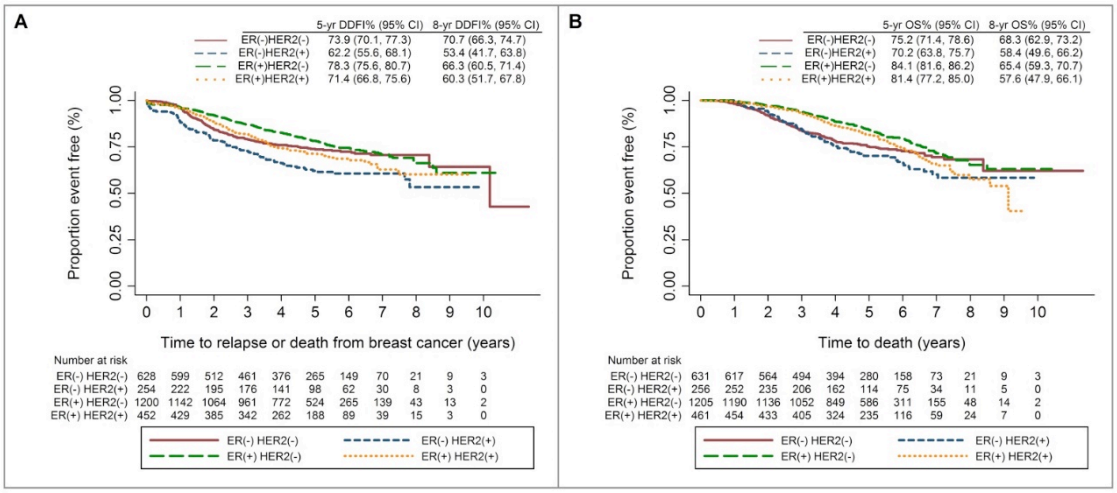


Figure 3-4 Kaplan- Meier (A) Distant Relapse Free Survival and (B) Overall Survival estimates, by ER and HER2 Status.

3.4 Discussion

Analysis of survival of the POSH cohort, a large cohort study in young women with breast cancer has confirmed in predominantly premenopausal women (aged 18-40) overall survival is 81.9% at 5 years, very similar to the 83.5% 5

year relative survival of similarly aged women (15-39 years old) from 2005-2009 data published by CRUK (1).

ER negative tumours at all ages have a worse 5 years DDFI and OS in comparison to ER positive tumours which our data in young women corroborates. Multivariate analysis (MVA) found this difference was maintained when adjusting for size, grade, and nodal status. In our ER positive group there were a greater number of lobular carcinomas (6.5% versus 0.7%) which have a better overall survival in the first 10 years (70). Our MVA did not take special morphological types into account. The 10% survival difference between ER positive and ER negative at 5 years is negated to 0.2% by 8 years as the KM curves converge. Looking at the survival curves in more detail by Her2 as well as by ER status it is clear that the different subtypes (as defined by receptor status) show a different pattern over time of relapse and death. The ER negative cases tend to have an initial fall in survival over the first couple of years, then plateauing out by 5 years. ER positive cases show a more constant fall in survival that continues on past 5 years, such that the survival curves meet at 8 years and there is no significant difference. Figure 3-3e shows the effect of time dependent on ER status on overall survival hazard ratio illustrating the continuing risk in ER positive disease.

Her2 positive cases have the worst prognosis both in ER negative and ER positive disease which may reflect that trastuzumab only became widely used for early Her2 positive breast cancer in the UK in 2006. 53.3% of the POSH cohort had been diagnosed before 2005. Our Her2 positive status was high compared to other reported series at 24%, but only 18.2% on the 1336 tumours tested on TMA were IHC positive. This inflation in the proportion of HER2+ cases reported is likely at least in part due to retrospective testing of primary tumour at relapse leading to a systematic bias in the cases where testing was recorded towards those who had experienced a relapse. Therefore both the Her2 positive survival and apparent low use of adjuvant trastuzumab given the high Her2 positive rate recorded is reflects the practice at the time of the study period but not of current oncological management.

The treatment pathway for ER positive and ER negative tumours is very different with total treatment duration of 6-18 months (surgery, chemotherapy +/- trastuzumab) for ER negative tumours and 5 years + for ER positive

tumours (surgery, chemotherapy +/- trastuzumab + endocrine therapy). At the time of this study the recommended treatment duration for endocrine therapy was 5 years. Hence a patient with an ER negative tumour will finish adjuvant therapy ~4 years earlier than an ER positive patient. Those ER negative patients not 'cured' by surgery and chemotherapy tend to relapse early. ER negative tumours are more likely to have other aggressive biological features such as high grade. Evidence shows that the protective effect of hormone therapy on breast cancer mortality lasts not only the 5 years on treatment but for an additional 10 years (71). Following this observation, the pivotal trials ATLAS (72) and aTTom (73), investigating prolonged endocrine therapy looked at 10 versus 5 years of hormone therapy. They demonstrated the superiority of 10 years treatment with tamoxifen. ATLAS found a decreased DFS during treatment (HR 0.95, (95% CI 0.79-10.2), but this was more evident after therapy ceased (HR 0.75, 95% CI 0.62-0.90). The exact mechanism behind this delayed anti-cancer effect is not fully understood. One theory is that selective pressure on cancer cells during the years of tamoxifen therapy results in cancer cells growing independent of estrogen which then become vulnerable once estrogen blockade has ceased. At this point the woman's own endogenous estrogen paradoxically acts to kill those tumour cells (74).

Late relapse is an interesting phenomenon not unique to ER positive breast cancer. Dormant breast cancer cells may be present in these ER positive patients and at some point due to an unknown trigger become active to cause recurrent, clinically relevant and detectable disease. A small study (75) in 36 patients 7-22 years after their breast cancer diagnosis and definitive treatment, without evidence of active disease compared to matched controls found 36% had detectable circulating tumours cells (CTC) (n=36). There were similar percentages of ER positive patients in the CTC +ve group (n=13, 73% ER+) and CTC -ve group (n=23, 76% ER+). The authors postulated a source of subclinical micrometastases present in these patients that was being kept under check. However the full reason why it is that ER positive tumours have a greater tendency to late relapse is not fully understood.

Both the long duration of hormone therapy as well as the side effects can lead to poor adherence over time. A meta-analysis of extended tamoxifen for early breast cancer found 38% of patients discontinued therapy before completion of 5 years extended therapy (76). In the ATLAS trial at 7 years 16% of those

allocated to the extended 10 years of therapy had prematurely discontinued. Poor compliance and development of tumour resistance to hormone therapy may both contribute to relapse (77).

The excess of mastectomies in the ER positive compared with ER negative group may be a reflection of the higher number of invasive lobular carcinomas (ILC) (6.5% ILC in ER positive versus 0.7% ILC in ER negative). Presentation of a diffuse rather than discrete mass, higher rate of multifocal tumours and higher conversion rates of BCS to mastectomy due to positive margins with ILC compared to IDC may contribute to this trend (78). In addition the median maximum tumour diameter (invasive + insitu) was significantly larger in ER positive patients.

Limitations of this analysis of the POSH study are of potentially inconsistent receptor categorisation. Just over a third of samples have undergone central review to check receptor status. Particularly when considering the known tendency for late relapse of ER positive tumours we have a relatively short duration of follow-up at present. Lastly, it is well known that molecular subtyping carries prognostic information and we are unable on current information to categorise patients molecularly or using surrogate techniques due to the lack of ki-67 status or tumour gene expression data in most cases.

POSH is a large multicentre prospective cohort study that recruited 23% of the available English population of women aged 40 or under diagnosed with breast cancer during the recruiting time period. This data has minimal loss to follow-up and is representative of the population demonstrated by comparison to national data from WMCIU and as such gives strength to the conclusions drawn.

3.5 Conclusion

The POSH cohort is a large prospective cohort representative of young UK women with breast cancer with minimal loss to follow-up. ER negative breast cancers in young UK women (aged 40 or under) have a worse survival than women with ER positive cancers in the first 5 years after diagnosis. This remains evident in multivariate analysis. However DDFI and OS in ER positive cancers continues to fall over time to meet that of ER negative tumours at 8

years. Prolonged adjuvant endocrine therapy may change this pattern in future studies. In the POSH study prolonged follow-up and molecular subtyping of the cohort would facilitate more detailed analysis of tumour characteristics influencing recurrence and survival.

Chapter 4: Family History

4.1 Introduction

Approximately 25% 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 (79). At the time of diagnosis, 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.

A positive family history is a known risk factor for developing breast cancer particularly at a younger age (80, 81). Other recognised risk factors often relate to oestrogen exposure, lifestyle and environmental factors that modify risk. Studies since the 1990's of outcome with selection based on family history (rather than mutation selected) have shown inconsistent results with improved (82, 83), worse (84, 85), and unchanged survival (81, 85-88). Inconsistent definitions of familial breast cancer, hereditary breast cancer, differing study populations and the known heterogeneous nature of breast cancer may be partly the cause of conflicting results.

Breast cancer genetics is complex. The majority of breast cancer in the young (80%) is not related to *BRCA1* or *BRCA2* (1, 21, 23, 42, 89-93). Familial risk is influenced by multiple genes including high (*BRCA1*, *BRCA2*, TP53) and moderate penetrant genes (*PTEN, STK11, CDH1, ATM, CHEK2, PALB1, BRIP1, RAD51C, XRCC2, MRE11, RAD50* and *MRE11*) and low penetrance variants acting together in a polygenic model (23, 42) modified by lifestyle and environmental factors.

Studies looking at family history as a prognostic marker are usually unselected for age, often retrospective and using different comparisons and endpoints with variable gene testing results. A positive family history is more likely in younger women who develop breast cancer and so this population is enriched for breast cancer predisposition mutation carriers including *BRCA1* and *BRCA2*. Breast cancer risk is known to be highest for these two genes. But as the impact of BRCA on mutations on prognosis is uncertain (80, 94, 95) and the majority of familial breast cancer is not attributable to pathogenic mutations BRCA genes (93, 96, 97), the impact of a positive family history per se is important to understand. Identification

of known prognostic factors helps to guide decisions on adjuvant therapy but also is important for counselling a patient and the family.

The purpose of this analysis was to investigate the association of family history and prognosis specifically in young women by analysing pathological and phenotypic features in a large prospective cohort, the POSH study.

4.2 Methods:

4.2.1 Study variables and data sources

All POSH Study participants were included in this analysis on follow-up data received until 22nd October 2013. Patients were asked to complete a family history questionnaire at recruitment, comprising details of all first degree (FDR) and second degree relatives (SDR) 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 categorised as having a positive family history (FH+) if they reported at least one affected first (FDR) or second degree relative (SDR), 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.

4.2.2 Statistical analysis – written by Mr Tom Maishman

The statistical analysis was conducted according to a pre-specified plan as per published guidance (15). Summary statistics were used to describe the cohort and tumour characteristics were compared between family history categories using chi-squared or Mann-Whitney 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 their date of last follow-up. As a result of the time-varying effects of the ER status (98) and to adjust for potential confounders, multivariate analyses (MVA) were based on the flexible parametric survival model (FPSM) (50). This model included family history and all of the following covariates

measured at breast cancer diagnosis regardless of statistical significance: age, tumour size (fitted as a log transformed continuous covariate as distribution skewed), tumour grade, axillary nodal stage, lymphovascular invasion, distribution of tumour, ER status, PR status, and HER2 status.

Multiple imputed datasets were generated using the ice command and MVA of these carried out using the mim command in STATA v11.2.

Sensitivity analyses were performed by fitting multivariate models which excluded M1 and/or non-symptomatic patients (results not shown). A further MVA, stratified by ER status, was also performed.

The statistical analysis was performed by Dr Bryony Eccles and Mr Tom Maishman.

4.3 Results

4.3.1 Patient characteristics and family history

Of the 2956 patients (see Chapter 2: Methods), family history data were available for 2850 (96.4%). 1878 (65.9%) reported no family history of breast/ ovarian malignancies (FH-) and 972 (34.1%) reported breast/ ovarian cancer in one or more first or second degree relative (FH+). Median age of diagnosis was 36 years, range 18-40 FH- and range 21-40 FH+. There was no significant difference between family history groups. Surveillance detected tumours were more frequent in women with a FH+ compared with FH- (29, 3.0% vs 1, 0.1%) and in patients reporting an affected FDR compared with those with an affected SDR (25, 6.0% vs 4, 0.7%) ( ).

Table 4-1 Patient and tumour characteristics by family history of breast cancer and by patients with a First degree relative (FDR) or Second degree relative (SDR).

Characteristic	FH-[†] n=1878 (65.9%)	FH+[†] n=972 (34.1%)	p-value	FDR[‡] n=418 (43.0%)	SDR[‡] n=554 (57.0%)	p-value
	Median (range, IQR), number of patients (%)			Median (range, IQR), number of patients (%)		
Maximum invasive tumour size, in mm	22 (0 to 199, 15 to 34), 1761 (93.8%)	22 (1 to 170, 15 to 32), 912 (93.8%)	p=0.745	20 (1 to 100, 15 to 30), 393 (94.0%)	23.4 (1 to 170, 16 to 35), 519 (93.7%)	p=0.004
Missing/	117 (6.2%)	60 (6.2%)		25 (6.0%)	35 (6.3%)	

Characteristic	FH-† n=1878 (65.9%)	FH+† n=972 (34.1%)	p-value	FDR‡ n=418 (43.0%)	SDR‡ n=554 (57.0%)	p-value
	Median (range, IQR), number of patients (%)			Median (range, IQR), number of patients (%)		
unknown						
Maximum tumour DCIS, in mm	27 (0 to 199, 19 to 40), 1778 (94.7%)	26 (1 to 180, 18 to 40), 925 (95.2%)	p=0.417	25 (1 to 180, 17 to 39.5), 398 (95.2%)	27 (1 to 170, 18 to 41), 527 (95.1%)	p=0.098
Missing/ unknown	100 (5.3%)	47 (4.8%)		20 (4.8%)	27 (4.9%)	
	Number of patients (%)		p-value	Number of patients (%)		p-value
Presentation						
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%)	p<0.001	7 (1.7%)	0 (0%)	p<0.001
Missing/ unknown	3 (0.2%)	5 (0.5%)		2 (0.5%)	3 (0.5%)	
Distribution of tumour						
Localised	1211 (71.5%)	615 (68.9%)		266 (68.9%)	349 (68.8%)	
Multifocal	483 (28.5%)	278 (31.1%)	p=0.165	120 (31.1%)	158 (31.2%)	p=0.981
Missing/ unknown	184 (9.8%)	79 (8.1%)		32 (7.7%)	47 (8.5%)	
Tumour grade						
1	113 (6.2%)	43 (4.5%)		17 (4.2%)	26 (7.8%)	
2	637 (34.9%)	306 (32.2%)		121 (29.7%)	185 (34.1%)	
3	1076 (58.9%)	602 (63.3%)	p=0.040	270 (66.2%)	332 (61.1%)	p=0.281
Not graded/ missing/ unknown	52 (2.8%)	21 (2.2%)		10 (2.4%)	11 (2.0%)	
Pathological N stage						
N0	898 (48.7%)	473 (49.3%)	p=0.774	215 (52.2%)	258 (47.1%)	p=0.117
N1-3	946 (51.3%)	487 (50.7%)		197 (47.8%)	290 (52.9%)	
Missing/ unknown	34 (1.8%)	12 (1.2%)		6 (1.4%)	6 (1.1%)	
M stage						
M0	1824 (97.5%)	942 (97.6%)		408 (98.1%)	534 (97.3%)	
M1	46 (2.5%)	23 (2.4%)	p=0.900	8 (1.9%)	15 (2.7%)	p=0.414
Missing/ unknown	8 (0.4%)	7 (0.7%)		2 (0.5%)	5 (0.9%)	
ER status #						
Negative	625 (33.4%)	333 (34.4%)		146 (35.0%)	187 (33.9%)	
Positive	1246 (66.6%)	636 (65.6%)	p=0.608	271 (65.0%)	365 (66.1%)	p=0.713
Missing/ unknown	7 (0.4%)	3 (0.3%)		1 (0.2%)	2 (0.4%)	
PR status #						
Negative	658 (43.6%)	335 (43.2%)		136 (40.5%)	199 (45.2%)	
Positive	852 (56.4%)	441 (56.8%)	p=0.853	200 (59.5%)	241 (54.8%)	p=0.186
Missing/ unknown	368 (19.6%)	196 (20.2%)		82 (19.6%)	114 (20.6%)	
HER2 status #						
Negative	1172 (71.2%)	648 (75.3%)		276 (77.5%)	372 (73.7%)	
Positive	474 (28.8%)	213 (24.7%)	p=0.031	80 (22.5%)	133 (26.3%)	p=0.196
Missing/ unknown	232 (12.4%)	111 (11.4%)		62 (14.8%)	49 (8.8%)	
*TNT status			p=0.579			p=0.590

Characteristic	FH-† n=1878 (65.9%)	FH+† n=972 (34.1%)	p-value	FDR‡ n=418 (43.0%)	SDR‡ n=554 (57.0%)	p-value
	Median (range, IQR), number of patients (%)			Median (range, IQR), number of patients (%)		
TNT	343 (19.2%)	183 (20.0%)		82 (20.9%)	101 (19.4%)	
Not TNT	1448 (80.9%)	730 (80.0%)		311 (79.1%)	419 (80.6%)	
Missing/ unknown	87 (4.6%)	59 (6.1%)		25 (6.0%)	34 (6.1%)	
Lymphovascular invasion						
Absent	903 (52.0%)	471 (52.0%)		206 (53.1%)	265 (51.2%)	
Present	833 (48.0%)	435 (48.0%)	p=0.989	182 (46.9%)	253 (48.8%)	p=0.564
Missing/ unknown	142 (7.6%)	66 (6.8%)		30 (7.2%)	36 (6.5%)	

† n=106 (3.6%) with missing/unknown family history excluded from these analyses.
‡ n=1878 (63.5%) with no family history tumour and n=106 (3.6%) with missing/unknown family history excluded from these analyses.
*(TNT) includes patients with an ER Negative, HER2 Negative and PR Negative Status.
Includes data from TMA as well as primary POSH data.

4.3.2 Tumour pathology

The distribution of grade was significantly different between FH+ and FH- groups (p=0.040) with patients with a positive family history more likely to have a grade 3 tumour than patients with a negative family history (63.3% vs. 58.9%) (p=0.040). There were no significant differences in median tumour diameter, presence of lymphovascular invasion (LVI), incidence of nodal involvement or presence of metastases at diagnosis between the positive and negative family history groups. The frequency of ER and PR positive tumours did not vary significantly between family history groups. Patients with no family history were significantly more likely to have a HER2 positive tumour than those with a positive family history (28.8% vs. 24.7%, p=0.031). There were no significant differences in any of the pathological features between patients with first degree or second degree affected relatives other than the median maximum invasive tumour size which was smaller in FDR (20mm) than SDR (23.4mm), p=0.004. When the small number of surveillance detected cases are excluded the median invasive tumour is still smaller in FDR than SDR (20 vs 23.7 mm, p=0.016). Patients with FH- had a median size of invasive tumour of 22mm (FH- vs FDR p=0.0295 and FH- versus SDR p 0.1694).

When ER positive and negative patients were analysed separately (Table 4-2), grade and Her2 status were no longer significantly differently distributed for FH+ and FH- patients. In ER negative patients the distribution of M0 and M1 was also significantly different with more metastatic patients in the FH- group than in the

FH+ group (2.6% vs 0.6%, p=0.033). In the ER negative subgroup only tumour diameter (invasive, p=0.0251 and including DCIS, p=0.0350) was significantly different between family history groups with a median invasive tumour diameter of 23mm in FH- and 20mm in FH+ group.

Table 4-2 Patient and tumour characteristics by family history of breast cancer and ER status

Characteristic	ER Negative patients only #			ER Positive patients only #		
	FH-† n=625 (65.2%)	FH+ † n=333 (34.8%)	p-value	FH-‡ n=1246 (66.2%)	FH+ ‡ n=636 (33.8%)	p-value
	Median (range, IQR), number of patients (%)			Median (range, IQR), number of patients (%)		
Maximum invasive tumour size, in mm	23 (1 to 199, 15 to 32), 567 (90.7%)	20 (1 to 117, 15 to 30), 315 (94.6%)	p=0.025	22 (0 to 150, 15 to 35), 1187 (95.3%)	23 (1 to 170, 16 to 35), 595 (93.6%)	p=0.269
Missing/ Unknown	58 (9.3%)	18 (5.4%)		59 (4.7%)	41 (6.5%)	
Maximum tumour DCIS, in mm	27 (0.6 to 199, 18 to 40), 576 (92.2%)	25 (1 to 131, 17 to 34.5), 320 (96.1%)	p=0.035	27 (0 to 190, 19 to 41), 1195 (95.9%)	28 (1 to 180, 18 to 43), 603 (94.8%)	p=0.609
Missing/ Unknown	49 (7.8%)	13 (3.9%)		51 (4.1%)	33 (5.2%)	
	Number of patients (%)		p-value	Number of patients (%)		p-value
Presentation						
Symptomatic	623 (99.8%)	318 (96.1%)		1240 (99.7%)	610 (96.4%)	
Surveillance	1 (0.2%)	10 (3.0%)		0	19 (3.0%)	
Other	0	3 (0.9%)	p<0.001	4 (0.3%)	4 (0.6%)	p<0.001
Missing/ Unknown	1 (0.2%)	2 (0.6%)		2 (0.2%)	3 (0.5%)	
Distribution of tumour						
Localised	443 (80.6%)	245 (79.8%)		761 (66.9%)	369 (63.2%)	
Multifocal	107 (19.5%)	62 (20.2%)	p=0.794	376 (33.1%)	215 (36.8%)	p=0.121
Missing/ Unknown	75 (12.0%)	26 (7.8%)		109 (8.8%)	52 (8.2%)	
Tumour grade						
1	6 (1.0%)	0		106 (8.7%)	42 (6.8%)	
2	64 (10.6%)	32 (9.8%)	p=0.175	572 (47.0%)	274 (44.1%)	p=0.089
3	534 (88.4%)	296 (90.2%)		538 (44.2%)	305 (49.1%)	
Not graded/ missing/ Unknown	21 (3.4%)	5 (1.5%)		30 (2.4%)	15 (2.4%)	
Pathological N stage						
N0	333 (54.4%)	196 (59.2%)		560 (45.7%)	277 (44.1%)	
N1-3	279 (45.6%)	135 (40.8%)	p=0.156	666 (54.3%)	351 (55.9%)	p=0.521
Missing/ Unknown	13 (2.1%)	2 (0.6%)		20 (1.6%)	8 (1.3%)	
M stage						
M0	605 (97.4%)	330 (99.4%)		1212 (97.6%)	610 (96.8%)	
M1	16 (2.6%)	2 (0.6%)	p=0.033	30 (2.4%)	20 (3.2%)	p=0.336
Missing/	4 (0.6%)	1 (0.3%)		4 (0.3%)	6 (0.9%)	

Characteristic	ER Negative patients only #			ER Positive patients only #		
	FH-† n=625 (65.2%)	FH+ † n=333 (34.8%)	p-value	FH-‡ n=1246 (66.2%)	FH+ ‡ n=636 (33.8%)	p-value
	Median (range, IQR), number of patients (%)			Median (range, IQR), number of patients (%)		
Unknown						
PR status #						
Negative	516 (91.0%)	263 (90.7%)		141 (15.0%)	72 (14.8%)	
Positive	51 (9.0%)	27 (9.3%)	p=0.879	800 (85.0%)	414 (85.2%)	p=0.932
Missing/ Unknown	58 (9.3%)	43 (12.9%)		305 (24.5%)	150 (23.6%)	
HER2 status #						
Negative	395 (70.0%)	225 (75.3%)		774 (71.7%)	423 (75.3%)	
Positive	169 (30.0%)	74 (24.8%)	p=0.105	305 (28.3%)	139 (24.7%)	p=0.126
Missing/ Unknown	61 (9.8%)	34 (10.2%)		167 (13.4%)	74 (11.6%)	

† n=38 (3.8%) ER Negative patients with missing/unknown family history excluded from these analyses.
‡ n=66 (3.4%) ER Positive patients with missing/unknown family history excluded from these analyses.
# Includes data from TMA as well as primary POSH data.

4.3.3 Treatment

Detailed information on multimodal treatment is given in Table 4-3 as stratified by family history groups. The distribution of primary surgery was statistically different between FH- and FH+ groups, but not between FDR and SDR. More women in the FH+ group had a mastectomy (54.4%) than in FH- (48.5%). Chemotherapy timing and regime were similar in both FH groups; the vast majority having anthracycline based and or taxane based adjuvant chemotherapy. However there was a statistical difference in the distribution of chemotherapy timing in FDR compared to SDR patients. More women with a FDR compared to women with a SDR had adjuvant chemotherapy (78.2% vs 70.9%), and less had neo-adjuvant chemotherapy (11.7% vs 17.7%). For women with ER positive cancers the vast majority (>90%) received hormonal therapy with little difference between the family history groups.

Table 4-3 Treatment details by family history of breast cancer and by patients with a First degree relative (FDR) or Second degree relative (SDR)

Characteristic	FH-†	FH+†	p-value	FDR‡	SDR‡	p-value
	n=1878 (65.9%)	n=972 (34.1%)		n=418 (43.0%)	n=554 (57.0%)	
	Number of patients (%)			Number of patients (%)		
Definitive surgery						
Breast conserving surgery	932 (49.6%)	434 (44.7%)	p=0.007	181 (43.3%)	253 (45.7%)	p=0.484
Mastectomy	910 (48.5%)	529 (54.4%)		232 (55.5%)	297 (53.6%)	
Nodal surgery only	8 (0.4%)	1 (0.1%)		0	1 (0.2%)	

Characteristic	FH-[†]	FH+[†]	p-value	FDR[‡]	SDR[‡]	p-value
	n=1878 (65.9%)	n=972 (34.1%)		n=418 (43.0%)	n=554 (57.0%)	
	Number of patients (%)			Number of patients (%)		
No surgery	28 (1.5%)	8 (0.8%)		5 (1.2%)	3 (0.5%)	
Chemotherapy timing						
Adjuvant	1370 (73.0%)	720 (74.1%)		327 (78.2%)	393 (70.9%)	
Neo-adjuvant	284 (15.1%)	147 (15.1%)	**p=0.806**	49 (11.7%)	98 (17.7%)	**p=0.030**
Palliative	36 (1.9%)	15 (1.5%)		38 (9.1%)	52 (9.4%)	
Not applicable	188 (10.0%)	90 (9.3%)		4 (1.0%)	11 (2.0%)	
Chemotherapy regimen						
Anthracycline &/or taxane	1677 (89.3%)	871 (89.6%)	**p=0.397**	375 (89.7%)	496 (89.5%)	**p=0.975**
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 (12.3%)	109 (11.2%)		36 (8.6%)	73 (13.2%)	
Other treatment period /no/missing/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/missing/unknown #	346 (18.4%)	214 (22.0%)		102 (24.4%)	112 (20.2%)	
ER Positive patients only	**(n=1246)**	**(n=636)**		**(n=271)**	**(n=365)**	
Ovarian suppression (in any treatment period) Hormone therapy						
Tamoxifen or AI	1142 (91.7%)	592 (93.1%)	-	255 (94.1%)	337 (92.3%)	-
No/missing/unknown	104 (8.3%)	44 (6.9%)		16 (5.9%)	28 (7.7%)	
Medical (LHRH agonist)						
Yes	374 (30.0%)	262 (41.2%)	-	120 (44.3%)	142 (38.9%)	-
No/missing/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/missing/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/missing/unknown	1076 (86.4%)	488 (76.7%)		199 (73.4%)	289 (79.2%)	

4.3.4 Follow-up and recurrence

Patients were followed up for a median of 5.9 years. DDFI was similar between women with and without a family history. Estimated 5-year DDFI rates were 74.9% for FH- vs 77.4% for FH+, and 8 year DDFI 68.7% and 72.0%, but this was non-significant (HR 0.879, 95% CI 0.754-1.025, p=0.100) (Figure 4-1A). When the analysis was repeated for ER-ve and ER+ve cases separately, the hazard ratio for DDFI remained non-significant in ER+ve cases (HR 0.976, 95% CI 0.807-1.180, p=0.813) (Figure 4-1D) but was significant for ER-ve patients with patients with a positive family history having a superior outcome (HR 0.736, 95% CI 0.567-0.955, p=0.021), 5 year DDFI for ER-FH- 69.7% vs ER-FH+ 77.2% (Figure 4-C). There was no significant difference between DDFI in patients with either a FDR or SDR for the entire cohort (HR 1.171, 95% CI 0.904-1.519, p=0.234) (Figure 4-1D) or when stratified by ER status (ER+ve patients: HR 1.053, 95% CI 0.768-1.445, p=0.761), (ER-ve patients: HR 1.440, 95% CI 0.913-2.271, p=0.117) (results not shown graphically).

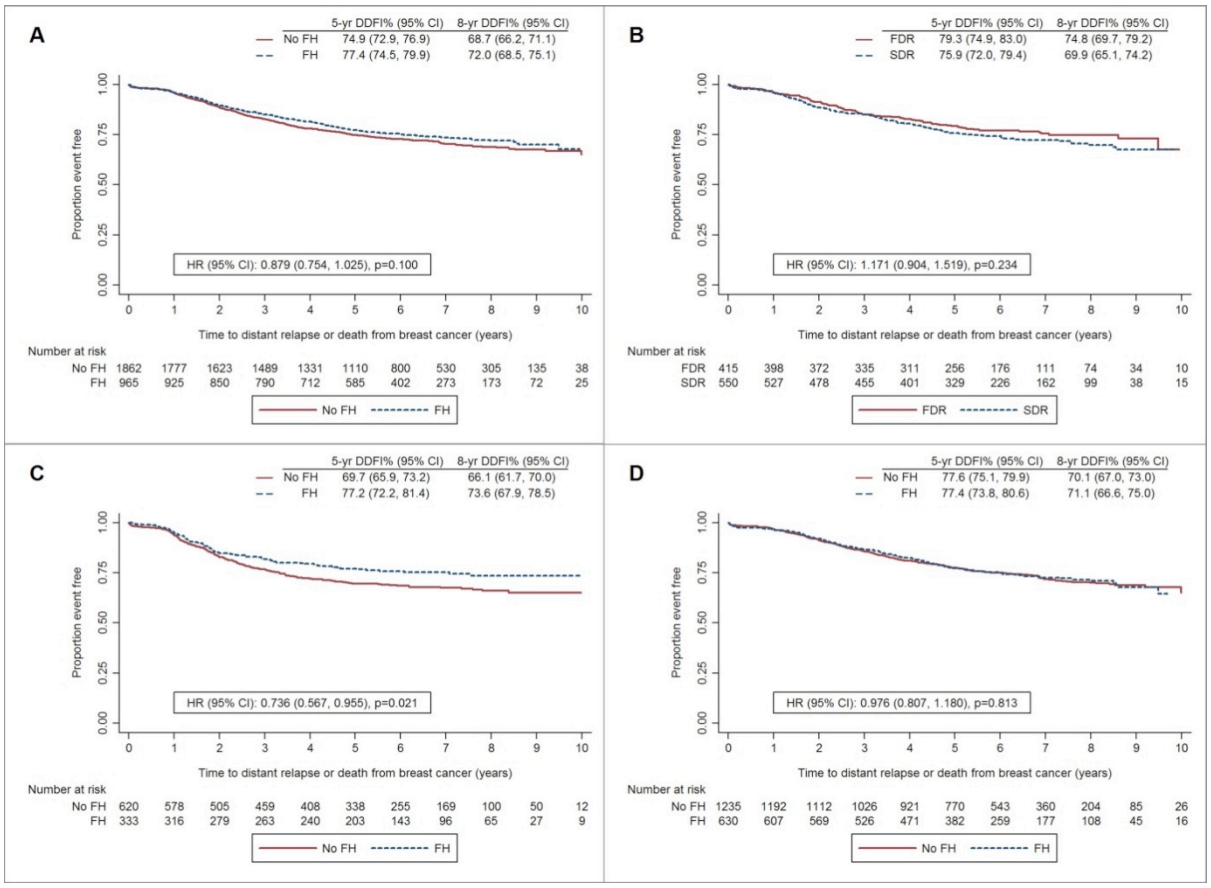


Figure 4-1 5-yr and 8-yr DDFI for by FH (Figure A), by FDR or SDR (Figure B), by FH for ER negative patients only (Figure C) and by FH for ER positive patients only (Figure D).

In MVA adjusting for receptor status, age at diagnosis, grade, tumour size, N stage, distribution of cancer and LVI, DDFI was similar between FH- and FH+ patients (HR 0.89, p=0.120) for all patients and when categorised by ER status (ER- HR 0.80, p=0.101 and ER+ HR 0.95, p=0.589) (Table 4-5). The variables independently contributing significantly to DDFI were age at diagnosis, grade, size, nodal disease, LVA and PR; with FH not a contributing variable in a range of tested models (Table 4-4). No changes in direction of HR estimates were observed when performing sensitivity analyses.

Table 4-4 DDFI HRs for all patients (unadjusted Cox model and adjusted flexible parametric model)

Variable	Category	Unadjusted† HR (95% CI), p-value			Adjusted†† HR (95% CI), p-value		
		Non time-varying			Non time-varying		
Age at diagnosis	N/A (continuous)	0.97 (0.95, 0.99), p=0.002			0.98 (0.96, 0.99), p=0.009		
Max invasive tumour	N/A (continuous, log transformed)	1.96 (1.74, 2.21), p<0.001			1.37 (1.19, 1.56), p<0.001		
FH	Negative	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	Positive	0.88 (0.75, 1.02), p=0.100			0.89 (0.76, 1.03), p=0.120		
HER2	Negative	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	Positive	1.38 (1.18, 1.61), p<0.001			1.04 (0.89, 1.22), p=0.627		
PR	Negative	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	Positive	0.68 (0.58, 0.79), p<0.001			0.68 (0.52, 0.88), p=0.005		
Grade	1	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	2	2.20 (1.36 (3.56), p=0.001			1.37 (0.84, 2.23), p=0.205		
	3	3.12 (1.95, 4.99), p<0.001			1.71 (1.05, 2.78), p=0.030		
N stage	N0	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	N1	2.82 (2.40, 3.30), p<0.001			1.09 (0.40, 2.99), p=0.860		
Number of positive lymph nodes	0	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	1-3	2.10 (1.75, 2.52), p<0.001			1.56 (0.57, 4.27), p=0.389		
	4-9	3.67 (2.98, 4.51), p<0.001			2.29 (0.82, 6.40), p=0.112		
	10+	6.56 (5.22, 8.26), p<0.001			3.58 (1.27, 10.04), p=0.016		
Lymph invasion	Absent	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	Present	2.56 (2.19, 3.00), p<0.001			1.51 (1.27, 1.80), p<0.001		
Distribution	Localised	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	Multifocal	1.38 (1.18, 1.61), p<0.001			1.09 (0.93, 1.28), p=0.262		
		Time-varying			Time-varying		
		2 years	5 years	8 years	2 years	5 years	8 years
ER	Negative	*1 (Ref. cat.)*			*1 (Ref. cat.)*		
	Positive	0.71 (0.60, 0.83), p<0.001	0.93 (0.74, 1.16), p=0.522	2.06 (1.51, 2.81), p<0.001	0,93 (0.74, 0.16), p=0.522	2.06 (1.51, 2.81). p<0.001	3.69 (2.11, 6.47), p<0.001

† Unadjusted Cox regression analyses performed using a complete case analyses
†† Adjusted flexible parametric survival analyses performed using multiply imputed2 data

Table 4-5 Multivariate Analyses (by ER status)

		Unadjusted		Adjusted	
		N†	Complete Case HR (95% CI), p value	N††	Multiple imputed data HR (95% CI), p value
All patients	**FH-**	2827	1 (ref. cat.)	2932	1 (ref. cat.)
	FH+		0.88 (0.75, 1.02), p=0.100		0.89 (0.76, 1.03), p=0.120
ER Negative	**FH-**	953	1 (ref. cat.)	993	1 (ref. cat.)
	FH+		0.74 (0.57, 0.96), p=0.021		0.80 (0.62, 1.04), p=0.101
ER Positive	**FH-**	1865	1 (ref. cat.)	1935	1 (ref. cat.)
	FH+		0.98 (0.81, 1.18), p=0.801		0.95 (0.78, 1.15), p=0.589

4.4 Discussion

Our data indicates that a positive family history for breast or ovarian cancer does not provide any independent contribution to the risk of DDFI in young breast cancer patients after adjustment for known prognostic factors.
Univariate analysis of patients stratified by ER status showed that ER negative patients with a positive family history did have a significantly superior outcome than ER negative patients with no family history. FH+ patients had higher grade tumours and more likely to be Her2 negative (at the 5% significance level) reflecting the likely dominance of *BRCA1* mutations as the genetic cause in this subgroup particularly amongst patients presenting with ER-ve disease. However background genetic aetiology is likely to be accounted for by a wide spectrum of genes and modes of inheritance, particularly amongst the FH+ER+ group. The superior outcome of FH+/ER- patients over FH-/ER-patients seen in univariate analyses was no longer present when adjusting for other prognostic variables.
More women with a positive family history underwent a mastectomy rather than BCS. Given the median size of the tumour was the same in both groups and there were not statistically more multifocal cancers in the FH+ group the presence of a FH may have influenced the surgeon and or woman to choose mastectomy. A recently published study of patients eligible for BCS treated by a single surgeon found that family history was not independently associated with womens choice for mastectomy (99). From the POSH data the majority of patients were treated with modern anthracyline and or taxane based regimes, trastuzumab if HER2 amplified and endocrine therapy if ER postitive.

Most previously reported studies have also found no clear evidence of a prognostic effect of family history although the evidence is inconclusive, with a paucity of large prospective studies in young onset women (90, 100-103). The largest investigation to date of the effect of family history on prognosis is a Swedish population-registry based study of over 17000 patients (88) which found a non-significant trend towards improved survival for patients with FH+ in the subgroup of women aged <50 years old with a HR of 0.82 (95% CI 0.60-1.12). Another contemporary and prospective population based study of 905 women selecting for a high percentage of young (<35) and "high genetic risk" from the Ontario Familial Breast cancer registry (81) found overall no significant association between family history and recurrence or overall survival. A further large population-based study using pooled data of 4153 cases from 3 breast cancer family registries (California, Ontario, Melbourne) also found no mortality differences between cases with and without a family history (86). The two largest of these studies did not report the use of breast cancer screening and the largest did not include data about stage or tumour type (86, 88).

The POSH study specifically recruited young women to enrich for genetic factors. Two studies specifically looking at young women found longer survival in FH+ patients that is contrary to our findings (90, 100). Malone et al (90) analysed results from 1260 women from two retrospective population-based American studies of women with breast cancer aged under 46, finding that women with a FDR had a significant reduction in mortality (HR 0.7 95% CI 0.5-0.9) independent of other prognostic factors. They found similar results when excluding mutation carriers from the analyses (genotype information on 77% of patients with a FDR). While this study focused on young women and had a very long follow-up of 19 years, it was retrospective, enrolling women from 1983-1992 and selected for ethnicity using pooled data from two studies in one part of the US. In addition, many patients (~30%) did not receive chemotherapy. In univariate analysis we found a greater difference in DDFI HR when comparing FH+ with FH- than looking at FDR verses non FDR. A much smaller study (100) of 95 breast cancer families (proband aged <46 years old) comparing to 329 matched controls also found a survival advantage in women with FH+ in MVA with a relative risk of survival of 6.11 (95% CI 2.81-13.28). Differences in study population, treatment and design and small size of the second study may

account for the differing results to our findings. Alternatively differing definitions of FH positivity, i.e. number and degree of relatives affected may account for differing results.

Younger women may have a greater fear of breast cancer recurrence than older women perhaps due to concern on the impact on young children. Furthermore patients with a strong family history may have high anxiety about recurrence and death from their breast cancer due to witnessing cancer within their family (104). Patients who present with breast cancer in the context of a personal family history of the disease may seek reassurance from their oncologist that they are at no higher risk from recurrence or death from this breast cancer than similar patients with no family history. This study provides further evidence that family history per se is not an independent prognostic feature for recurrence in young onset breast cancer treated in the modern era.

Our study used family history defined at a single time point at enrolment into the study. It is important to note that despite the young age of this cohort nearly two thirds did not have a positive family history. However family history is dynamic and changes over time as patients seek more information about other family member's medical history and new diagnoses arise.

Patients with a known positive family history are more likely to be known to genetics services and dependent on the level of risk may be attending surveillance programmes. Very few tumours from patients included 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 except for patients who fit into the highest risk group defined by a very strong FH or a known *BRCA1*/2 mutation. Moderate risk (based on family history but not known to be a BRCA1/2 gene in the family) are recommended in current UK guidelines (NICE CG164), to start screening at 40 years but this is still not widely implemented. Unsurprisingly the majority of patients with surveillance detected tumours were in the FH+ group but there was no difference in median tumour size in comparison to FH- patients. However, we found that patients with a close affected relative (FDR compared to SDR and FDR compared to FH-) had significantly smaller tumours both for all patients and when excluding the small number patients detected by surveillance. This likely reflects an earlier self-presentation to medical services in those patients. Earlier presentation may

be offset by the higher grade tumours in the FH+ (particularly FDR subgroup) negating any outcome difference.

The major strengths of the POSH study and this analysis are the prospective nature of the study and large sample size with no selection of patients other than by young age. We had minimal loss to follow-up and very small amounts of missing data. The greatest amount of missing data was in PR and Her2 status, reflecting historical local pathology reporting practices. Equity in missing data in both family history groups would be expected. Other than by age we have not selected for high-risk individuals or breast cancer families so the results should be generalisable to the young breast cancer population (8).

Potential limitations of this study include survival bias. Patients were enrolled up to one year after diagnosis. Very early deaths may therefore be under represented although in breast cancer death within 12 months of first presentation is uncommon and the proportion of patients presenting with distant metastases was similar to the same age group data from all of England in the same time period (WMCIU data). Our study did not exclude patients presenting with metastatic disease. This represented a very small proportion of the whole cohort and analyses were repeated excluding M1 patients with no change to the results. A further limitation was that self-reporting of family history was not independently confirmed, however this represents a real-world scenario where an oncologist does not generally seek independent confirmation and reliability of self-reporting is fairly robust for breast cancer in close relatives (83, 86, 105).

4.4.1 Conclusion

The outlook for breast cancer overall in this young age group is relatively poor and efforts to clarify the reasons for this and to improve cancer outcomes overall are needed. The results from this study show that in young women presenting with breast cancer and undergoing modern oncological treatment, once all tumour characteristics are accounted for, outcome is similar whether there is a family history of the disease or not.

Chapter 5: BRCA Variants of Unknown Significance (VUS)

5.1 Introduction

Pathogenic mutations in the *BRCA1* and *BRCA2* genes confer a high lifetime risk of breast (and ovarian) cancers. Over the 19 years since the identification of the *BRCA1* gene (14, 106) genetic testing requests to identify pathogenic mutations in *BRCA1* and 2 have risen steadily. NHS BRCA testing was introduced in the 1990's delivered through specialist genetics services referred from secondary or primary care. Increased demand is due to a growing knowledge by the general public, fuelled by high profile figures in the media such as Angelina Jolie (107) and academic interest in targeted drug treatment in triple negative breast cancers (108, 109). This phenotype, particularly in young women is associated with an increased risk of BRCA mutations. There is a great deal of research into the development of novel drugs for this population. Advances in laboratory techniques have facilitated the development of rapid treatment focused genetic testing at the point of diagnosis. These advances, combined with the rise of test requests and the "Genetics in Mainstream Medicine" agenda (110) may result in more non-geneticists being required to interpret and communicate a BRCA genetics test result.

Broadly a BRCA genetic test can yield 3 possible types of results: positive (a pathogenic mutation is found), negative (no mutation found or polymorphism only) or variant of uncertain significance (VUS). This is an alteration in the gene sequence with unknown consequences on the function of the gene product or risk of causing disease. A VUS is usually inconsequential, that is, has no or little effect on cancer risk, but a few can be pathogenic, for example by altering a splice site or causing a change in tertiary protein structure in a highly conserved domain. An expert working group convened by the International Agency for Research into Cancer, IARC in 2008 (111) proposed a 5 stage classification for Sequence Variants from Class 5 (definitely pathogenic) to Class 1 (not pathogenic or of no clinical significance) along with recommendations for clinical testing, surveillance and research testing of

family members. Class 4 and 5 can be used for predictive testing in unaffected relative. A class 3 variant (VUS) has a 0.05-0.949 probability of being pathogenic and so causes particular difficulties for the geneticist, the breast specialist and the patient as by definition an alteration has been found that is communicated to the patient but with unknown cancer predisposition implications. The closer to 95% the probability reaches the more useful further studies may become to determine pathogenicity. However some variants may behave as low penetrance mutation and not as a highly penetrant variant such as *BRCA1* or *BRCA2* and consequent management should not be the same (112). This concept is difficult for patients to understand when a clear answer to the question: "Is my cancer caused by a genetic problem?" cannot be answered.

The frequency in which laboratories report a VUS varies worldwide depending on the ancestry of the population it serves. In African-American populations the rate can be up to 21%, whilst it is 5-6% in individuals of European ancestry in the USA, and 15% in European laboratories (113, 114). Most VUSs are missense or splice site mutations and a record of the over 1500 VUS results (115) (as well as *BRCA1* and *BRCA2* coding variants) is held publicly by the Breast Cancer Information Core Database (http://research.nhgri.nih.gov/bic/). However reporting is voluntary.

NHS genetic testing laboratories have a national quality assurance scheme and observe the Association for Clinical Genetics Science guidelines in assessing pathogenicity of variants but these are not BRCA gene specific. There is no standard reporting template or classification scheme.

Testing breast cancer patients as opposed to testing unaffected individuals brings unique challenges. The timing of the test in relation to the patient's cancer treatment, which patients without a family history to test, and the management of a patient with a very strong family history but a negative BRCA genetic test result are all complex issues requiring careful thought and a multidisciplinary approach.

Rapid testing at breast cancer diagnosis (treatment focused genetic testing-TFGT) made possible by next generation sequencing has some strong advocates (89, 116) and is viewed positively by some patients (117, 118). Increased use of neo-adjuvant chemotherapy can mean there is time for a

genetic testing to be completed before definitive cancer surgery. Advocates maintain this gives more choice and the option for one stop radical surgery, rather than for example breast conserving cancer surgery followed by a prophylactic mastectomy later on once a positive genetic test result is known. But what if the test is uninformative and a VUS is found? In the time pressured situation of TFGT this may result in misinterpretation by physicians or patients. Current standard practise of genetic testing in patients with breast cancer is to look for the high penetrance, high risk pathogenic mutations in *BRCA1* and *BRCA2* and *TP53.* There is no widespread screening for the known intermediate penetrance genes or low penetrance alleles. 65%-70% of familial breast cancer is currently unexplained and even within the context of a known mutation, risk modifying factors including lifestyle factors are not taken in to account or indeed fully understood (119). A patient or relative with a strong family history of breast and ovarian cancer but negative for a *BRCA1* or *BRCA2* mutation still has an elevated risk and in some cases risk reducing options may be appropriate. With gene panel sequencing becoming cheaper than looking at a single gene, 'routine' testing for multiple mutations on different genes may become common but without the robust evidence based knowledge of how to manage the result.

In the UK, 'Mainstreaming Genetic Testing' is piloting the move of breast and ovarian BRCA genetic testing away from the genetic clinics to a joint oncology-genetics model (110). The advantages of more patients having access to genetic tests and a more streamlined process must be balanced with the concerns about whether oncologists are prepared or equipped to take on not only the 'easy' results but what to tell the patient when the significance of the result is uncertain.

Previous work has looked at patient knowledge of BRCA genetic testing (120), of GP's (89, 121) and medical specialists (122, 123) including oncologists (124) but there is little known about the knowledge of specialists who treat breast cancer (nor geneticists) particularly with regard to a BRCA VUS result. The only studies to our knowledge specifically looking at BRCA VUS knowledge is a survey of genetic counsellors in the US (125) where significant variation in personal interpretation and management recommendations existed and a second study of patients with a VUS, referring family physicians and genetic counsellors concluded national VUS-related guidelines were required (126).

In this study we aimed to explore the knowledge among breast oncologists and surgeons at specialist training/registrar and consultant level UK wide of interpretation and initial management of a VUS result. To place this in context we also approached medical geneticists to gain an insight into the variations in laboratory practice in reporting VUS and in methods currently used to clarify pathogenicity within the UK.

5.2 Methods

A questionnaire was sent electronically in September 2013 to all members of 3 large national organisations, the UK Breast Intergroup, the Association of Cancer Physicians and the Association of Breast Surgeons. Members of these mailing lists included medical and clinical oncologists specialising in breast cancer and breast surgeons at a specialist trainee or consultant level practising within the UK. This questionnaire was designed and distributed by the author, B Eccles. The second survey was also sent electronically but designed and sent from the ENIGMA group (Evidence-based Network for the Interpretation of Germline Mutant Alleles) to Medical Geneticists in December 2012- January 2013. UK responses only have been included in this work.

5.2.1 Questionnaires:

The breast specialists questionnaire was designed by the authors with 10 questions and included demographics questions, level of genetics training, referral practice for genetic testing, general knowledge of VUS and the interpretation and communication of two real (anonymised) genetic test results reporting a VUS from different UK laboratories. Report 1 summary: A missense mutation in exon 11 *BRCA2* gene (unclassified variant) and change in exon 13 of *BRCA2* (rare polymorphism). Report 2 summary: Heterozygous for *BRCA2* c.9098C>T, p.Thr3033Ile (clinical significance unknown) (full reports given in questionnaire: Supplementary 1). The questions were close-ended with limitations on multiple responses except for the communication to patients of the two genetics reports. Free text boxes were included to capture further responses and to allow for qualitative comments.

The term VUS was deliberately not included in the communication of report to patient questions so respondents could not just intelligently guess the answer in the knowledge this was a survey about VUS. The six responses (and Other-free text box) were further categorised into appropriate, inappropriate and don't know responses to allow significance testing between specialities response. Appropriate responses were "Explain there may be a hereditary cause and discuss further tests" and or "Refer patients to a genetics consultant". Inappropriate responses were "Reassure the patient that there is no hereditary cause for her breast cancer", "Explain a *BRCA2* mutation contributed to causing her breast cancer", "Explain she has a *BRCA2* mutation discuss risk reducing options". Blank responses were classified as "Don't know." Responses from the free text were reviewed and classified into the appropriate category.

The geneticist's questionnaire also included study participants demographics questions and level of clinical experience, referral patterns, laboratory workload, BRCA and VUS reporting proportions, actions to clarify clinical significance of a VUS and usage of classification systems.

5.2.2 Statistical Analysis:

Analysis was performed in STATA ver. 11.0. Descriptive statistics were used to describe the study population characteristics. Differences between disciplines (oncologist versus surgeon) in Table 2 were tested by Pearson chi squared test. Communication of report to patients were categorised in dichotomous variables appropriate / inappropriate and 'don't know.' Fishers exact test was used to test differences in communication of report to patients by speciality excluding don't know responses. Free text comments were scored manually and results recorded thematically.

5.3 Results

5.3.1 Breast Cancer Specialists Survey

The breast cancer specialists' questionnaire was sent to 800 medical and clinical oncologists and breast surgeons including registrar and consultant

grade in September 2013 with 181 (22.5%) responses. Responses from allied professionals (nurses/geneticist/radiologists/pathologist/trials staff) were excluded leaving 155 eligible respondents. The most frequent age category was 40-50 years old (34.8%). Three quarters of respondents were consultants, and three quarters oncologists.

Most specialists (74.2%) had received genetics training in medical school or as part of their postgraduate exams, with 11.6% stating they had received no genetics training (Table 5-1). The majority (95.3%) had directly referred patients to a genetics service for genetic testing. There was no significant difference between the specialities in terms of genetics training or referral to genetics departments. Overall 71.0% of respondents felt unsure or uncomfortable/unequipped to interpret a genetics report and the distribution of responses was significantly different between surgeons and medical oncologists (p=0.030), but non-significant between surgeons and clinical oncologists (p=0.066) and clinical and medical oncologists (p=0.831). The surgeons were also more confident (50.0%) than oncologists (24.1% clinical oncologists and 38.1% medical oncologists) in perceived understanding of a VUS (surgeons vs clinical oncologist p=0.003, surgeons vs medical oncologists p=0.073) but with no difference between oncology specialities (p=0.234). When asked how common a VUS is reported all three specialities gave 10-20% as the most frequent response.

Table 5-1 Genetics training, referrals and VUS knowledge by breast cancer specialists.

	Total No. (%)	**Medical Oncologist No. (%)**	**Clinical Oncologist No. (%)**	**Surgeon No. (%)**
	155 (100%)	63 (40.7%)	54 (34.8%)	38 (24.5%)
Genetics training				
None	18 (11.6%)	9 (14.3%)	5 (9.3%)	4 (10.5%)
Medical School/postgrad exams	115 (74.2%)	47 (74.6%)	44 (81.5%)	24 (63.2%)
Genetics course	4 (3.6%)	2 (3.2%)	1 (1.9%)	1 (2.6%)
Module with genetics service	1 (0.7%)	0 (0%)	1 (1.9%)	0 (0%)
Specialist interest (no formal training)	9 (5.8%)	2 (3.2%)	1 (1.9%)	6 (15.8%)
Higher degree	5 (3.2%)	2 (3.2%)	2 (3.7%)	1 (2.6%)
Other	3 (1.9%)	1 (1.6%)	0 (0%)	2 (5.3%)
Direct genetics service referral				
Yes	143 (95.3%)	59 (93.7%)	49 (96.1%)	35 (97.2%)
No	7 (4.7%)	4 (6.4%)	2 (3.9%)	1 (2.8%)
Missing	5 (3.2%)	0 (0%)	3 (5.3%)	2 (5.6%)
Ability to interpret genetics report				

	Total No. (%)	**Medical Oncologist No. (%)**	**Clinical Oncologist No. (%)**	**Surgeon No. (%)**
	155 (100%)	63 (40.7%)	54 (34.8%)	38 (24.5%)
Yes	45 (29.0%)	14 (22.2%)	13 (24.1%)	18 (47.4%)
No	64 (41.3%)	27 (42.9%)	25 (46.3%)	12 (31.6%)
Unsure†	46 (29.7%)	22 (34.9%)	16 (29.6%)	8 (21.1%)
VUS understanding				
Fully understand	56 (36.1%)	24 (38.1%)	13 (24.1%)	19 (50.0%)
Don't fully understand	66 (42.6%)	25 (39.7%)	24 (44.4%)	17 (44.7%)
Not heard/don't understand	33 (21.3%)	14 (22.2%)	17 (31.5%)	2 (5.3%)
How common is a VUS?				
<10%	26 (21.1%)	9 (17.0%)	13 (31.0%)	5 (15.2%)
10-20%	61 (47.7%)	29 (54.7%)	15 (35.7%)	17 (51.5%)
20-30%	24 (18.8%)	11 (20.8%)	8 (19.1%)	5 (15.2%)
30-40%	11 (8.6%)	4 (7.6%)	3 (7.1%)	4 (12.1%)
40-50%	2 (1.6%)	0 (0%)	2 (4.8%)	0 (0%)
>50%	3 (2.3%)	0 (0%)	1 (2.4%)	2 (6.1%)
Don't' know/no answer	27 (17.4%)	10 (15.9%)	12 (22.2%)	5 (13.2%)

Genetics report 1 (a female breast cancer patient with a strong family history) provided the reader with the following summary "missense mutation in exon 11 of the *BRCA2* gene". In the more detailed interpretation it states that it "has previously been reported as an unclassified variant", that "this sequence change reduces RAD51C binding activity" and "it may not be appropriate to offer pre-symptomatic testing until pathogenicity has been confirmed" and screening of at risk relatives may help to clarify whether this is a disease-causing mutation in this family". Only one interpretation of this report was allowed in the questionnaire and the response option most consistent with a VUS was "a gene mutation found but unknown if causing her breast cancer." A clear majority (83.9%) chose this response, only 13.6% responded they "didn't know" and there were no responses that either a pathogenic *BRCA2* mutation was found or that no pathogenic mutation was found (Table 5-2).

Any number of responses were allowed when asked how report 1 would be communicated to the patient and the future management options (Table 5-2). Most specialists (61.2%) would have explained to the patient that there may be a hereditary cause and discuss further tests, as consistent with the laboratory report interpretation provided. Most would refer the patient to a genetics consultant (52.2%) and only 4.5% said they "didn't know" what cause of action to take. After categorising the responses of communication to patients for report 1 (Table 5-3), 94.2% of specialists gave appropriate responses with

statistical significance between speciality responses (surgeon versus medical oncologists p=0.024, surgeon versus clinical oncologists p=0.675, clinical oncologists versus medical oncologists p=0.171).

Table 5-2 Interpretation of two genetics reports by breast cancer specialists

	Total No. (%) 155 (100%)	**Medical Oncologist No. (%)** 63 (40.7%)	**Clinical Oncologist No. (%)** 54 (34.8%)	**Surgeon No. (%)** 38 (24.5%)
Interpretation of report 1				
No pathogenic mutation	0 (0%)	0 (0%)	0 (0%)	0 (0%)
A gene mutation found but unknown if causing her breast cancer	130 (83.9%)	57 (90.5%)	45 (83.3%)	28 (73.7%)
A pathogenic *BRCA2* gene mutation	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Don't know	21 (13.6%)	5 (7.9%)	8 (14.8%)	8 (21.1%)
Other	4 (2.6%)	1 (1.6%)	1 (1.9%)	2 (5.3%)
Communication to patient report 1*				
Reassure the patient that there is no hereditary cause for her breast cancer.	1 (0.6%)	0 (0%)	1 (1.9%)	0 (0%)
Explain there may be a hereditary cause and discuss further tests.	95 (61.2%)	45 (71.4%)	29 (53.7%)	21 (55.3%)
Explain *BRCA2* mutation contributed to causing her breast cancer.	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Explain she has a *BRCA2* mutation discuss risk reducing options.	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Refer patients to a genetics consultant.	81 (52.2%)	36 (57.1%)	30 (55.6%)	15 (39.5%)
Don't know.	7 (4.5%)	0 (0%)	3 (5.6%)	4 (10.5%)
Other (free text)	11 (7.1%)	4 (6.3%)	2 (3.7%)	5 (13.2%)
Interpretation of report 2				
No pathogenic mutation	35 (22.6%)	18 (28.6%)	10 (18.5%)	7 (18.4%)
A gene mutation found but unknown if causing her breast cancer	71 (45.8%)	31 (49.2%)	23 (42.6%)	17 (44.7%)
A pathogenic *BRCA2* gene mutation	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Don't know	49 (31.6%)	14 (22.2%)	21 (38.9%)	14 (36.8%)
Communication to patient report 2*				
Reassure the patient that there is no hereditary cause for her breast cancer.	10 (6.5%)	2 (3.2%)	2 (3.7%)	6 (15.8%)
Explain there may be a hereditary cause and discuss further tests.	13 (8.4%)	7 (11.1%)	3 (5.6%)	3 (7.9%)
Explain *BRCA2* mutation contributed to causing her breast cancer.	1 (0.6%)	0 (0%)	1 (1.9%)	0 (0%)
Explain she has a *BRCA2* mutation discuss risk reducing options.	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Refer patients to a genetics consultant.	68 (43.8%)	24 (38.1%)	24 (44.4%)	14 (36.8%)
Don't know / no answer	58 (37.4%)	25 (39.7%)	22 (40.7%)	11 (28.9%)
Other (free text)	12 (7.7%)	5 (7.9%)	2 (3.7%)	5 (13.2%)

Genetics report 2 (from a female breast cancer patient with negative family history) was summarised as: "Heterozygous for *BRCA2* c.9098C>T". Further information in the report included statements "not previously reported" and

"significance unknown", with no guidance on management. The BCS interpretation of this report was more mixed with 45.8% stating "a gene mutation found but unknown if causing her breast cancer", 22.6% stating no pathogenic mutation found and 31.6% don't know (Table 5-2).

In terms of communication and possible future management of the patient, 43.8% of BCS would refer her to a genetics consultant and 37.4% responded "don't know or no response given." 6.5% would reassure the patient there was no hereditary cause for her breast cancer and 8.4% would say there was a hereditary cause and discuss further tests (Table 5-2).

Again after all the communication responses were categorised, only 53.5% were appropriate responses and 39.4% don't know (No significant differences between response by specialty were found (surgeon versus medical oncologists, p=0.084, surgeon versus clinical oncologists p=0.222, and clinical oncologists versus medical oncologists p=0.810). 7.1% of specialists communicated an inappropriate interpretation to patients. 6.5% were overly reassuring and 0.6% would have said a *BRCA2* mutation contributed to causing the breast cancer.

There was more uncertainty around the interpretation, communication and management of genetics report 2 in comparison to report 1. Most free text responses for reports 1 and 2 were themed around whether a geneticist would/should be interpreting these results rather than breast cancer specialist as this was a 'complicated result'. One specialist discussed the pre-test risk being high in report 1 and therefore considering surveillance and risk reducing options despite no clear pathogenic mutation. For report 2 one surgeon said he/she would not have referred this patient without a family history.

Table 5-3 Categorising communication to patient responses by breast cancer specialists

	Total No. (%) 155 (100%)	**Medical Oncologist No. (%)** 63 (40.7%)	**Clinical Oncologist No. (%)** 54 (34.8%)	**Surgeon No. (%)** 38 (24.5%)
Report 1				
Appropriate	146 (94.2%)	62 (98.4%)	50 (92.6%)	34 (89.5%)
Inappropriate	2 (1.3%)	1 (1.6%)*	1 (1.9%)	0 (0%)
Don't know	7 (4.5%)	0 (0%)	3 (5.6%)	4 (10.5%)
Report 2				
Appropriate	83 (53.5%)	35 (55.6%)	28 (51.9%)	20 (52.6%)
Inappropriate	11 (7.1%)	2 (3.2%)	3 (5.6%)	6 (15.8%)
Don't know	61 (39.4%)#	26 (41.3%)	23 (42.6%)	12 (31.6%)

* 'Other: Probable pathogenic mutation so advice is on assumption that this is pathogenic ....'

Includes: Other (n=3): "These questions are hard to answer when we don't deal with interpreting these reports day to day" (n=1); "just read about it before seeing patient" (n=1), "why did I send her for genetic testing?? I wouldn't have done so with no FH!!!" (n=1).

5.3.2 Geneticists Survey

Geneticists Survey – Questionnaire 2

The geneticist's survey was sent to the members of the ENIGMA consortium who further distributed the survey and 175 responses were received from 21 countries. Of the 61 medical geneticists who responded we included only UK practising doctors leaving 31 eligible respondents (Table 5-4). Most (51.6%) geneticists surveyed were aged 40-49 and had been working in genetics for >10 years and discuss test results directly with patients on one or more occasion per week. Two thirds showed a copy of the test report to the patient. Geneticists reported using multiple methods to clarify VUS significance (median 5, range 0-11). They mainly worked with busy NHS diagnostic genetic laboratories with a 4-8 week time from blood draw to report. Pathogenic mutations were reported more commonly (10-20%) than VUS (1-10%) in these UK laboratories. A specific classification system was used in 11/31 (35.5%) of laboratory reports (for internal use) and 12/31 (38.7%) of clinical reports (report received by the requesting clinician). The same respondents reported that multiple systems of classification were used by their diagnostic laboratories, the most common was a word based system (pathogenic, uncertain, polymorphic) in both laboratory and clinical reports (32%), next most frequently used was a 4 point system based on a review of in-silico and literature evidence (in 13% laboratory and 3% clinical reports) and then the 5

point IARC system based on the multifactorial likelihood of pathogenicity, (10% laboratory and 6% clinical reports). BRCA test requests were accepted from clinicians in a genetics clinic or from allied professions with genetics qualifications. Very few were accepted from clinicians in oncology clinics or from patients.

Table 5-4 Medical Geneticists Questionnaire Results (n=31)

	Number	Percentage
Age category		
30-39	5	16.1%
40-49	16	51.6%
50-59	8	25.8%
60+	2	6.5%
Length of time working in genetics		
< 5 years	1	3.1%
5-10 years	8	25.8%
>10 years	22	71.0%
Patient Contact		
Do you discuss test results directly with patients?		
Never	2	6.5%
Rarely (1-2/yr)	0	0%
Sometimes (1-2/month)	4	12.9%
Regularly (≥1 per week)	25	80.6%
Do patients see a copy of the BRCA testing report?		
Yes	8	25.8%
Sometimes	11	35.5%
No	3	9.7%
Not sure/missing	9	29.0%
Acceptance of BRCA test requests*	**Yes**	**No**
Clinicians in genetics clinic	26 (83.9%)	0 (0%)
Clinicians in oncology clinic	3 (9.7%)	19 (61.3%)
Primary care/family doctor	1 (3.2%)	23 (74.2%)
Allied professionals with genetics qualification	23 (74.2%)	2 (6.5%)
Patients	1 (3.2%)	22 (71.0%)
Lab capacity and reporting	**Most common response**	**No. (%)**
No. of patient samples tested per year	100-500	17 (54.8%)
Proportion reporting clearly pathogenic mutation	10-20%	17 (54.8%)
Proportion reporting a VUS	1-10%	12 (38.7%)
Length of test time (blood draw- report)	4-8 weeks	13 (41.0%)
Methods to clarify significance of a VUS		
Colleague discussion	23	74.2%
Information from other lab/clinical expert	16	51.6%
Co-segregation (additional blood from family)	23	74.2%
Literature search	20	64.5%
Mutation database search	15	48.4%
Google search	9	29.0%
Splicing prediction software	7	22.6%
Conservation database	7	22.6%
Tumour pathology report	7	22.6%
Tumour DNA	5	16.1%
Other: RNA studies	2	6.5%

*Numbers exceed 100% as multiple responses allowed

5.4 Discussion

This survey was designed to gain a snapshot of the broad understanding of VUS's in the non-geneticist breast cancer specialist community in the UK. The aim was to test their interpretation and anticipated patient communication of two real anonymised patient reports of a BRCA VUS. Our study demonstrates that most breast cancer specialists directly refer their patients to genetics service for a consideration of a BRCA test. Perceived genetics knowledge is not uniform over differing specialities and genetics training is mainly limited to undergraduate level or as part of postgraduate exams. Critically overall the majority of survey respondents felt uncertain over their ability to interpret a genetics report or understand a VUS with a significant difference between all three specialities ($p=0.030$) and with surgeons feeling more confident than oncologists. The results from report 2 highlight the wide variation in interpretation of genetics reports by non-geneticists and the uncertainty generated around management particularly in a patient without a family history of breast or ovarian cancer. However this group of family history negative patients are increasingly being referred for testing particularly if young and if their breast cancer is triple receptor negative (127).

The geneticists that responded to the survey reported their labs reported lower VUS rates (1-10%) than expected from the literature or by the breast cancer specialists surveyed (10-20%). This may be as a result of recall bias, or it may be that such experienced geneticist's council their patients away from testing if there is a greater chance of VUS result than a clearly pathogenic mutation. The most likely explanation for the reduced number of VUS reports compared to the historic literature is that with increasing volumes of genetic tests over years a variant previously reported as a VUS may now be classified as polymorphism based on the laboratory experience and increasing information from online databases.

Two real genetics reports were deliberately chosen with differing presentation style reflecting the variation in UK Genetics laboratories that still exists. There is no national reporting template and not all laboratories give guidance on interpretation and management. Equally a classification method for VUSs is not agreed by the genetics community (128, 129) as further demonstrated by the results of our geneticists' survey where multiple classification systems were

used with no clear majority leader. This can lead to further confusion by non-geneticists who may see the reports. The ENIGMA consortium was initiated to research and develop algorithms for the classification of variants in *BRCA1* and *BRCA2* (130).

We did not explore the understanding by specialists in the complexities of further methodology to attempt to elucidate the likely pathogenicity of a VUS result. Multiple methods exists: segregation analysis within the family (is the VUS inherited with the cancer phenotype), co-occurrence in Trans (a VUS and pathogenic mutation found but on different copies of the gene – trans, rather than on the same copy –cis), in-silico methods (computer programs that look at the how conserved a gene sequence is through evolution or protein conformational modelling), functional assays, and the pathology profile of the tumour in a VUS carrier in comparison to a sporadic tumour and a tumour in someone with a pathogenic BRCA mutation (115, 128, 131). The Association for Clinical Genetic Science gives guidelines on how to approach understanding the pathogenicity of a variant (132). From this survey it can be seen that UK geneticists adhere to guidelines and use multiple methods (median 5). These independent lines of evidence are more powerful if combined and can be used to give a posterior probability of pathogenicity of a BRCA VUS with resulting guidelines for testing relatives and surveillance recommendations (113, 128). For a non-geneticist clinician faced with VUS result, the strength of the family history and breast cancer pathological features must be taken into account. Referral to genetics services or discussion with a cancer genetics consultant should be considered. Referring specialists need to convey to patients that most VUS are not pathogenic and remain of unclear significance.

Communicating to the patient the complexities of the result and the uncertainty surrounding it is a challenging task. Many surveyed oncologists and surgeons are largely untrained and unconfident in fully understanding these complex results. Other studies have found varying knowledge of breast cancer genetics by physicians. A survey looking at US oncologist's general BRCA knowledge found only 40% answered all 4 questions correctly (124) but a Dutch survey reported better knowledge on hereditary breast cancer by surgeons, medical and radiation oncologists and radiologists with average score of 6.1/7 questions (123). These studies asked different questions and neither specifically looked at BRCA VUS knowledge. Certainly there is

considerable scope for physician misinterpretation of BRCA results including VUSs leading to inappropriate patient care (133, 134) and uncertainty of clinical management (111, 135). In our study most breast cancer specialists correctly interpreted report 1 but found report 2 more challenging with a greater degree of uncertainty. This could be to do with the differing style of the reports and clinical guidance provided or the differing family history.

How to manage a VUS result is challenging. Only 1% of specialists would have communicated to a patient that a *BRCA2* mutation contributed to causing her cancer in report 2. But studies have shown that rate of risk reducing surgery amongst patients receiving a VUS result vary but can be high, 7-10% RRM (120, 136), 24% BLM (117), 12-21% BSO (120, 136). Moreover a Dutch study (137) found very high rates (42%) of prophylactic surgery within one year after receiving a VUS result. In contrast a recent US survey of patients who received counselling after a VUS or an uninformative negative result acted appropriately in their choice of surgery uptake and in line with their level of risk. Patients find genetic counselling helpful (>90%) and reduces cancer distress (120). Removing or reducing the formal counselling process by moving genetic testing into the oncology clinics may be detrimental to the psychological health of patients. Practical aspects such as increased time pressure in a busy oncology clinic compared to a genetics clinic are also relevant.

Genetic testing at the time of cancer diagnosis, “treatment focused genetic testing” is already part of the mainstreaming agenda for more personalised treatment and is being implemented routinely in some areas of the UK particularly with a view to facilitating entry into clinical trials. The GTEOC study (138) is a pilot study looking at feasibility and acceptability of upfront genetic testing at time of ovarian cancer diagnosis irrespective of family history. The OlympiAD trial (139) a phase III randomised trial is recruiting BRCA gene carriers with metastatic breast cancer to olaparib versus physician choice chemotherapy.

Limitations of this study include response bias. Individuals with a greater interest and therefore knowledge in breast cancer genetics may be more likely to respond, overestimating the knowledge of breast cancer specialists. Recall bias in the second survey by geneticist’s may also have occurred.

5.4.1 Conclusion

Mainstreaming genetics services to the oncology clinic may eventually lead to a change in referrals to specialists genetics. More of the caseload will be future risk management advice to discuss and implement family cascade testing and for complex cases that require additional counselling or investigation such as a VUS result, an uninformative negative result (in the case of a strong family history but negative BRCA screening result) or the highly anxious patient. Whether oncologists or surgeons delivering genetics results will lead to a change in the care pathway of the patient and early tests or interventions such as treatment focused genetic testing (genetic testing at the point of breast cancer diagnosis), and or more early risk reducing surgery which has no proven survival advantage over delayed surgery is unknown. In the Netherlands most breast cancer specialists felt the best time for genetics referral was during adjuvant therapy or follow-up (123). The Mainstreaming Genetic Medicine pilots in the UK are not currently giving guidance on the timing of genetic testing within the patient's care pathway. Possible additional cancer worry and distress caused by a change in the care pathway and removing the formal genetic counselling process delivered by trained counsellors to all patients receiving genetic testing in the genetics service in the UK is a real concern. From this study it can be concluded that at present UK non-genetics trained breast cancer specialists require additional training should they need to facilitate an fully informed decision by patients to undergo genetic testing and in particular to deliver a VUS result to a patient. Should genetic medicine become mainstream these complex results might best be communicated to the patient by geneticists or genetic counsellors. In Newcastle, a filter system occurs with the oncologists requesting the test, the geneticists reviewing the result, and then writing to both the oncologist and the patient (personal communication). Moreover the adoption of all genetics laboratories to use one variant classification method in an agreed reporting style would greatly assist understanding when presented with un-definitive BRCA test result.

Chapter 6: Multifocal Breast Cancer in Young Women

6.1 Introduction

There is no internationally agreed definition of the terms multifocal (MF) and multicentric (MC) with reference to breast carcinoma. A common definition of multifocal tumours are those with two or more tumour loci within a single breast quadrant (<5cm apart and within same duct collecting system) (140, 141),or <4cm apart (142). Multicentricity is defined as two or more tumour foci within different quadrants of the same breast (>5cm apart) (140, 141), or 'arising from more than one site of origin' (143). However Fish et al (144) and the Danish Breast Cancer Cooperative Group (145) use a broader definition using the term multifocal and multicentric interchangeably to mean more than one focus of invasive breast cancer separated by benign tissue in one breast.

Multifocal and multicentric breast cancers are common, but reported rates vary widely due to different definitions used, the imaging method used and the type of breast surgery and pathological sampling methods. The incidence in contemporary studies ranges from 1.8%-24% (24, 146, 147). Multifocal disease is also more common in younger women than older women (28, 146, 148).

Whether multifocal cancer is a result of local intramammary spread or contemporaneous development of multiple malignancies within one breast on a background of a field change is an area of debate. Several studies using differing techniques have tried to resolve the issue of clonality (149-153) but the debate continues.

The reported prevalence depends on the definition used for which there is consensus and is necessary for accurate staging. Definition, stage and clonality all have implications for decisions regarding surgery and adjuvant chemotherapy and radiotherapy.

6.1.1 Staging and Outcomes

There are two possible methods purported to stage multifocal / multicentric (MF/MC) disease. The most commonly used is to assign the relevant T value to the largest focus with an (m) suffix (AJCC and International Union for cancer control (UICC) recommendations)(37). An alternative suggested in the literature is to aggregate the size of the tumour foci (sum the largest diameters) or to estimate the total tumour volume to give a picture of tumour burden.

MF/MC disease is associated with a higher risk of axillary lymph node positivity than unifocal disease, either due to the current methodology understaging the disease (by using only the largest tumour focus dimension) or due to inherent greater biological aggressiveness.

A retrospective Australian study (154) compared two methods (diameter of largest focus and sum of diameters of all foci) to determine which gave a more accurate prediction of nodal status and interpreted that to indicate tumour behaviour. They found that aggregating diameters gave a better prediction of nodal status than single largest focus diameter, and that some patients were upstaged by this method. However some studies have shown that when aggregation methods are used for staging there is no increased risk of lymph node positivity compared to unifocal cancers (UF) (154-156).

Is MF/MC disease an independent poor prognostic marker? The evidence so far is conflicting and depends on the outcome measured. Studies have used lymph node positivity as a surrogate outcome for survival, local recurrence, disease free survival and overall survival.

6.1.2 Multifocality association with BRCA pathological mutations

Certain tumour phenotypes are more common in patients with BRCA mutations and both BRCA mutations and multifocal disease are more common in younger breast cancer patients; yet only one study has looked specifically at multifocality and BRCA mutation status. This retrospective case-control Danish study used tissue from 119 young patients with multifocal or bilateral breast cancer identified from the Danish Breast Cancer Cooperative Group registry and tested tumour samples for BRCA status (140). Pathogenic mutations were found in 19% patients with multifocal disease. More *BRCA2* carriers than *BRCA1*

carriers had multifocal cancers but no statistical conclusion could be drawn due to small patient numbers. Other than family history little other detail was given in this study on patient demographic or tumour pathological characteristics.

6.1.3 Aims of this study:

1. To describe the characteristics of multifocal disease (MF) compared with unifocal disease (UF) in the POSH cohort and the relationship of multifocality with Distant Disease Free Interval (DDFI) and Overall Survival (OS).
2. To explore a possible association between multifocality and *BRCA1* and *BRCA2* pathological mutations.

6.2 Methods

Cases were taken from the prospective cohort study POSH of women diagnosed with breast cancer $\leq$ 40 years old. This study analyses follow-up data received until 22nd October 2013.

6.2.1 Study variables

Study variables are as described in detail in Chapter 2: The Posh Study Methods. Multifocal disease was defined in the POSH study as when more than one focus of invasive tumour was reported or when the term multifocal or multi-centric was used in the pathology report. Maximum tumour diameter for multifocal tumours was calculated by summing the reported diameters of individual tumours. Multifocal DCIS was classified separately as “Multifocal insitu”. DCIS without any invasive cancer on pathology specimen was an exclusion criteria for the POSH study. The general term ‘multifocal’ used in this study therefore includes multifocal and multicentric disease. Central review of pathology specimens for multifocality/ multicentricity is beyond the scope of this study and has not been performed and our data is based on copies of local pathology diagnostic reports.

6.2.2 Statistical Analysis

Analyses were performed in STATA v11.2 on records with complete data (levels of missingness were reported). Summary statistics were used to describe key data of patient and tumour characteristics and treatment details. Pearson chi-squared test for categorical variables and Mann Whitney test for continuous variables were used to identify any differences between distribution of cancer (multifocal or unifocal) and another variable. P-values reported are two-sided.

Multiple logistic regression was performed to explore the association between factors (TNT or ER and Her2, grade, multifocality and family history) for *BRCA1* or *BRCA2* status.

Overall survival (OS) and distant disease-free interval (DDFI) were assessed using Kaplan-Meier curves comparing multifocal and unifocal cases. 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). Local relapse data has not been analysed. Patients who had not experienced an event at the time of analysis were censored at their date of last follow-up. Multivariate Cox regression was used to assess the effect of multifocality on OS and DDFI, adjusting for known prognostic factors (ER, Her2, Grade, T stage (by largest max diameter and by aggregate max diameters) and N stage, ethnicity, BMI) on OS and DDFI in multifocal and unifocal cases. Chemotherapy type, ethnicity and T stage 4 were initially included in the model but excluded due to too small numbers in one category. Calculated T stage (aggregate tumour foci diameters) gave a better model fit than pathological T stage (longest single tumour diameter). The Cox model was split into ER positive and ER negative because of the differential effect of ER status over time.

6.3 Results

6.3.1 Patient characteristics

The median age of all patients was 36 years old (range 18-40), with the majority (58.9%) of patients in the 36-40 age category (Table 6-1). Data on tumour distribution (multifocal or unifocal) was present on 2670 patients (missing in 286). The distribution of age in patients with multifocal and

unifocal cancer was not significantly different (p=0.126). Patients with MF versus UF disease were well matched for BMI, age at menarche and first birth, use of the contraceptive pill, and smoking (Table 6-1). Significant differences between MF and UF patients were found in distribution of ethnicity (p=0.009), and number of children (p<0.001). More patients with MF cancer (76.2%) had children compared to patients with UF cancer (69.1%). Of those with children, patients with MF cancer had a statistically greater number of children (n=2) than patients with UF cancer (n=1), p<0.001. Despite the young age of this cohort nearly two-thirds of patients did not have a family history of breast or ovarian cancer with no statistical difference between MF and UF cases (63.5% vs 66.3%, p=0.381).

Table 6-1 Patients' characteristics and risk factors with Multifocal and Unifocal Breast cancer

Characteristic	Unifocal (n=1873, 70.0%)	Multifocal (n=797, 29.8%)	Total (n=2956)	p-value
Age at diagnosis (yrs) median (range, IQR) no	36 (18-40, 34-38)	36 (24-40, 33-39)	36 (18-40, 33-38)	
Age at diagnosis (yrs)				
18 to 25	30 (1.6%)	5 (0.6%)	46 (1.6%)	
26 to 30	160 (8.5%)	81 (10.2%)	270 (9.1%)	p=0.126* (NS)
31 to 35	572 (30.5%)	244 (30.6%)	900 (30.5%)	
36 to 40	1111 (59.3%)	467 (58.6%)	1740 (58.9%)	
Duration of follow-up (yrs) median (range, IQR) no	5.89 (0.6-13.2, 4.5-7.6)	5.79 (0.54-12.0, 4.1-7.3)	5.82 (0.1-13.2, 4.3-7.4)	p=0.043†
Presentation:				
Symptomatic	1838 (98.1%)	782 (98%)	2900 (98.1%)	p=0.421* (NS)
Screen detected	22 (1.2%)	7 (0.9%)	30 (1.0%)	
Other	6 (0.3%)	5 (0.6%)	12 (0.4%)	
Missing/unknown	7 (0.4%)	3 (0.4%)	14 (0.5%)	
Body mass index – median (range, IQR) no	24.7 (16.8-58.1, 22.1-28.4) n=1808	24.5 (14.7-59.5, 22.9-27.8) n=772	24.6 (14.7-59.5, 22.1-28.4), n=2843 (96.1%)	
Underweight / normal	957 (51.1%)	422 (53.0%)	1526 (51.6%)	
Overweight	503 (26.9%)	220 (27.6%)	784 (26.5%)	p=0.352* (NS)
Obese	348 (18.6%)	130 (16.3%)	533 (18.0%)	
Missing/unknown	65 (3.5%)	25 (3.1%)	113 (3.8%)	
Ethnicity				
White/Caucasian	1741 (94.0%)	707 (90.4%)	2690 (92.3%)	p=0.009*
Black	56 (3.0%)	43 (5.5%)	118 (4.05%)	
Asian	46 (2.5%)	27 (3.5%)	87 (3.0%)	
Other	10 (0.5%)	5 (0.6%)	20 (0.7%)	
Age at menarche (yrs) median (range, IQR)	13 (8-18, 12-14)	13 (8-18, 12-14)	13 (8-18, 12 -14)	p=0.242† (NS)

Characteristic	Unifocal (n=1873, 70.0%)	Multifocal (n=797, 29.8%)	Total (n=2956)	p-value
Age at first birth (yrs) median (range, IQR) no Missing/unknown	27 (14-40, 22-30), n=1290 (68.9%) 583 (331.1%)	26 (13-39, 23-30), n=603 (75.7%) 194 (24.3%)	27 (13-40, 23-30), n=2080 (70.4%) 876 (29.6%)	p=0.964† (NS)
Number with children				
Yes (Para>1)	1306 (69.7%)	607 (76.2%)	2097 (70.9%)	
No (Para 0)	558 (29.8%)	184 (23.1%)	834 (28.2%)	p=0.001*
Missing	9 (0.5%)	6 (0.8%)	25 (0.9%)	
Number of children median (range, IQR)	1 (0-8, 0-2) n=1864	2 (0-7, 0-2) n=791	2 (0-8, 0-2) n=2931	p<0.001†
Use of contraceptive pill				
Ever	1664 (88.8%)	693 (87.0%)	2598 (87.9%)	p=0.165*
Never	209 (11.2%)	104 (13.1%)	358 (12.1%)	(NS)
Missing/unknown	0 (0%)	0 (0%)	0 (0%)	
Smoker				
Ever	950 (52.3%)	376 (48.3%)	1326 (51.1%)	p=0.067*
Never	868 (47.7%)	402 (51.7%)	1270 (48.9%)	(NS)
Missing/unknown	55 (2.9%)	19 (2.4%)	19 (6.6%)	
Menopausal status				
Premenopausal	1832 (97.8%)	778 (97.6%)	2885 (97.6%)	p=0.868*
Perimenopausal	3 (0.2%)	2 (0.3%)	5 (0.2%)	(NS)
Postmenopausal	4 (0.2%)	2 (0.3%)	7 (0.2%)	
Missing/unknown	34 (1.8%)	15 (1.9%)	59 (2.0%)	
No. with first or second degree relatives with cancer¥				
First degree	266 (14.7%)	120 (15.8%)	386 (14.9%)	
Second degree	349 (19.1%)	158 (20.8%)	507 (19.6%)	
No family history	1211 (66.3%)	483 (63.5%)	1694 (65.5%)	
Number of affected relatives¥				
0	1209 (64.6%)	483 (60.6%)	1874 (63.4%)	
1	443 (23.7%)	207 (26.0%)	702 (23.8%)	p=0.381*
2	127 (6.8%)	51 (6.4%)	199 (6.7%)	(NS)
>2	47 (2.5%)	20 (2.5%)	75 (2.5%)	
Missing/unknown	47 (2.5%)	36 (4.5%)	106 (3.6%)	

Missing data on unifocal / multifocal in 286 patients.
*Significance testing of categorical variables using Pearson Chi-squared test
†Significance testing of continuous variables using Mann-Whitney test
¥Family history Questionnaire asked about breast, ovarian and prostate cancer

6.3.2 Tumour pathology

Histological type, grade, receptor status and N stage were all significantly differently distributed between MF and UF patients (Table 6-2). Patients with MF cancers had more lobular carcinomas compared with patients with UF disease (6.0% vs 3.8%, p<0.001), lower grade disease (Grade 3: 52.3% vs 63.8%,

p<0.001), less ER negative disease (22.1% vs 38.0%, p<0.001) and higher Her2 expression (31.4% vs 24.7%, p<0.001). There is a large amount of missing data for Her2 (overall 12.0%). As expected with increased tumour burden, patients with multifocal disease significantly more (p<0.001) were node positive (62.4%) compared to unifocal patients (46.1%). There was no difference in the small numbers of patients with metastatic disease between MF and UF patients (Table 6-2).

Table 6-2 Multifocal and Unifocal Breast cancer characteristics

Characteristic	Unifocal (n=1873) (70%)	Multifocal (n=797) (29.8%)	Total† (n=2956) (100%)	p value††
BRCA status				
BRCA1 mutation positive	110 (21.9%)	17 (8.7%)	136 (17.9%)	
BRCA2 mutation positive	40 (8.0%)	30 (15.4%)	78 (10.3%)	
VUS	59 (11.7%)	19 (9.7%)	83 (10.9%)	p<0.001
Wild-type (no mutation)	294 (58.4%)	130 (66.3%)	463 (60.9%)	
Not tested/unknown	1370 (73.1%)	601 (75.4%)	2196 (74.3%)	
Histological type				
Ductal	1652 (89.2%)	663 (84.1%)	2556 (87.6%)	
Lobular	70 (3.8%)	47 (6.0%)	134 (4.6%)	
Ductal and Lobular	32 (1.7%)	41 (5.2%)	78 (2.7%)	
Medullary	28 (1.5%)	1 (0.1%)	31 (1.1%)	
Metaplastic	7 (0.4%)	2 (0.3%)	11 (0.4%)	p<0.001
Mixed	14 (0.8%)	10 (1.3%)	26 (0.9%)	
Other	41 (2.2%)	19 (2.4%)	64 (2.2%)	
Unclassified adenocarcinoma	8 (0.4%)	5 (0.6%)	17 (0.6%)	
Missing/unknown	21 (1.1%)	9 (1.1%)	39 (1.3%)	
Histological Grade				
1	105 (5.7%)	50 (6.4%)	163 (5.7%)	
2	562 (30.5%)	325 (41.4%)	972 (33.8%)	p<0.001
3	1175 (63.8%)	411 (52.3%)	1742 (60.5%)	
Missing/unknown	31 (1.7%)	11 (1.4%)	79 (2.7%)	
ER Status				
Negative	709 (38.0%)	176 (22.1%)	997 (33.9%)	p<0.001
Positive	1156 (62.0%)	620 (77.9%)	1947 (66.1%)	
Missing/unknown	8 (0.4%)	1 (0.1%)	12 (0.4%)	
PR Status‡				
Negative	715 (47.7%)	206 (31.9%)	1033 (43.5%)	p<0.001
Positive	785 (52.3%)	440 (68.1%)	1342 (56.5%)	
Missing/unknown	373 (19.9%)	151 (19.0%)	581 (19.7%)	
HER2 Status‡				
Negative	1238 (75.3%)	480 (68.6%)	1884 (72.4%)	p=0.001
Positive	407 (24.7%)	220 (31.4%)	717 (27.6%)	
Missing/unknown	228 (12.2%)	97 (12.2%)	355 (12.0%)	

Characteristic	Unifocal (n=1873) (70%)	Multifocal (n=797) (29.8%)	Total† (n=2956) (100%)	p value††
Pathological T stage (all patients)				
T0	13 (0.7%)	6 (0.8%)	73 (2.5%)	
T1	935 (49.9%)	397 (49.9%)	1411 (47.9%)	
T2	781 (41.7%)	326 (41.0%)	1167 (39.6%)	p=0.972
T3	121 (6.5%)	57 (7.2%)	189 (6.4%)	(NS)
T4	4 (0.2%)	2 (0.3%)	6 (0.2%)	
Tis	10 (0.5%)	3 (0.4%)	21 (0.7%)	
Tx	8 (0.4%)	5 (0.6%)	77 (2.6%)	
Missing/unknown	1 (0.1%)	1 (0.1%)	12 (0.4%)	
Pathological T stage (excluding neoadjuvant patients□)				
T0	2 (0.1%)	1 (0.2%)	6 (0.2%)	
T1	860 (51.3%)	357 (52.3%)	1270 (51.0%)	
T2	716 (42.7%)	283 (41.5%)	1044 (41.9%)	p=0.711
T3	91 (5.4%)	40 (5.9%)	137 (5.5%)	(NS)
T4	1 (0.1%)	1 (0.1%)	2 (0.1%)	
Tis	2 (0.1%)	0 (0%)	2 (0.1%)	
Tx	5 (0.3%)	0 (0%)	29 (1.2%)	
Missing/unknown	1 (0.1%)	1 (0.1%)	6 (0.2%)	
N stage (excluding neoadjuvant patients□)				p<0.001
N0	893 (53.2%)	255 (37.4%)	1213 (48,7%)	
N1	773 (46.1%)	426 (62.5%)	1252 (50.3%)	
Missing/unknown	12 (0.7%)	2 (0.3%)	31 (1.2%)	
M stage				
M0	1830 (98.5%)	776 (97.7%)	2860 (97.5%)	p=0.170
M1	28 (1.5%)	18 (2.3%)	74 (2.5%)	(NS)
Missing/unknown	15 (0.8%)	3 (0.4%)	22 (1.9%)	

Column Percentages exclude missing/unknown data
†Missing distribution in 286 patients
‡Includes data from TMA as well as primary POSH data.
□ Total no of patients excluding neoadjuvant n=2496.
†† p-values obtained from the Pearson chi-squared test between distribution and each categorical variable; and from the Mann-Whitney test between distribution and each continuous variable (excluding missing/unknown data).

Pathological T stage as determined by the longest diameter of the tumour of largest focus of tumour in multifocal disease (as per AJCC) was not significantly different between UF and MF cases, whether comparing all patients or excluding neoadjuvant patients. The median aggregate of longest diameters of all foci was 29mm for multifocal patients (range 0-150, IQR 19-43) and the median longest diameter for unifocal patients was 20mm (range 0.15-199, IQR 15-30). The aggregates of the longest diameters were then reclassified into a Tstage (T stage calc). The two methodologies of calculating T stage were then

compared in both UF and MF cases (Table 3-1). For unifocal patients as expected, there was little difference using the two methodologies. For multifocal patients using the aggregate diameter method (T stage calc) resulted in reclassifying 107 patients as T2 and 64 patients as T3.

Table 6-3 T stage for Unifocal and Multifocal cases using two methodologies (longest single focus diameter method and aggregate diameters)

	Unifocal (n, %)			Multifocal (n, %)		
	T stage (longest diameter) †	**T stage calc (aggregate diameters)**	**Change in T stage**	**T stage (longest diameter)** †	**T stage calc (aggregate diameters)**	**Change in T stage**
T1	935(49.9%)	930 (49.7%)	-5	397 (49.8%)	218 (27.3%)	-179
T2	781 (41.7%)	782 (41.8%)	+1	326 (40.9%)	433 (54.3%)	+107
T3	121(6.5%)	120 (6.4%)	-1	57 (7.2%)	121 (15.2%)	+64
T4	4 (0.2%)	4 (0.2%)	0	2 (0.3%)	2 (0.3%)	0
Missing	1 (0.0%)	37 (2.0%)	-	1 (0.1%)	23 (2.9%)	-
Total	**1873**	**1873**	-	**797**	**797**	-

† T0, Tis and Tx omitted from table (see Table 6-2 for details)

6.3.3 Treatment

All treatment characteristics were significantly different between MF and UF cases. In keeping with more widespread disease most patients with MF disease had mastectomy (78.8%), but the more minimal surgery option of breast conservation was preferred in UF disease (61.0%) ($p<0.001$) with consequently higher rates of adjuvant radiotherapy (MF: 25.3% vs UF 66.3%, $p<0.001$) (Table 6-4). In this young cohort most patients had adjuvant chemotherapy (72.8%) and this was mainly anthracycline or anthracycline and taxane based. More multifocal cases than unifocal cases received neo-adjuvant chemotherapy (14.3% versus 10.4%). The data for adjuvant trastuzumab has a high degree of missing/unknown data due to the identification and targeting of Her2 midway during the course of the POSH cohort study. Similarly due to case report form design and site response there is less comprehensive data on adjuvant

radiotherapy and hormone treatment. It was not always clear from site response whether the information was missing or they did not have treatment.

Table 6-4 Treatment Details of patients with multifocal and unifocal breast cancer.

Characteristic	Localised n=1873 (70%)	Multifocal n=797 (29.8%)	Total n=2956 (100%)	p value
Definitive breast surgery				
Breast conserving surgery	1143 (61.0%)	168 (21.1%)	1409 (47.7%)	
Mastectomy	725 (38.7%)	628 (78.8%)	1497 (50.6%)	p<0.001
Nodal surgery only	3 (0.2%)	0 (0%)	6 (2.1%)	
No surgery	2 (0.1%)	1 (0.1%)	36 (12.6%)	
Missing/unknown	0 (0%)	0 (0%)	2 (0.7%)	
Adjuvant radiotherapy				
Yes	1561(66.3%)	596 (25.3%)	2358 (100%)	
BCS + Adjuvant RT	1091 (69.9%)	158 (26.5%)	1339 (56.8%)	p<0.001
Mastectomy + adjuvant RT	469 (30.0%)	438 (73.5%)	1007 (42.7%)	
Chemotherapy timing				
Neo-adjuvant	195 (10.4%)	114 (14.3%)	460 (15.6%)	
Adjuvant	1450 (77.4%)	599 (75.2%)	2152 (72.8%)	p=0.004
Palliative	207 (11.1%)	69 (8.7%)	290 (9.8%)	
Not applicable	21 (1.1%)	15 (1.9%)	54 (1.8%)	
Missing/unknown	0 (0%)	0 (0%)	0 (0%)	
Chemotherapy regimen				
Anthracycline based	375 (20.0%)	202 (25.4%)	684 (23.1%)	
Anthracycline & taxane	1257 (67.1%)	519 (65.1%)	1938 (65.6%)	
Taxane based	207 (11.1%)	69 (8.7%)	290 (9.8%)	p=0.006
Other[1]	18 (1.0%)	5 (0.6%)	24 (0.8%)	
None	16 (0.9%)	2 (0.3%)	20 (0.7%)	
Missing/unknown	0 (0%)	0 (0%)	0 (0%)	
Adjuvant trastuzumab				
Yes	203 (10.8%)	683 (85.7%)	2593 (87.7%)	p=0.011
Other treatment period/no/missing/unknown[2]	1670 (89.2%)	114 (14.3%)	363 (12.3%)	
Adjuvant Hormone treatment				
Yes	487 (26.0%)	240 (30.1%)	800 (27.1%)	p=0.029
No/missing/unknown[2]	1386 (74.0 %)	557 (69.9%)	2156 (72.9%)	

[1] Any regimen not containing an anthracycline or taxane eg CMF.
[2] Due to the data collection methods and emerging knowledge of Her2 and guidance through the study these fields are likely to be inaccurate.

6.3.4 BRCA results

Using available data from the whole POSH cohort (n=2695) 4 TP53 mutations were recorded. However as this clinical genetic testing was highly selected these 4 patients have been excluded from this analysis. The total for analysis is therefore 756 patients. Of these, 136 (18.0%) patients were positive for a

BRCA1 mutation, 78 (10.3%) patients had a *BRCA2* mutation and 459 (60.7%) were wild-type (Table 6-5). 83 (11.0%) patients had a VUS.

During development of the BRCA predictive algorithms for family history negative patients (Chapter 4: it was noted that most patients with a *BRCA1* pathological mutation had unifocal disease (86.6%). Most *BRCA2* carriers also had UF disease, but much smaller proportion (57.1%). but with a higher ratio of multifocal/unifocal disease in *BRCA2* mutation carriers. Patients with sporadic cancers had an in-between proportion of UF disease (69.3%) which was similar to the whole POSH cohort (63%). To further characterise the tumour pathological phenotype depending on BRCA status the pathological variables distribution, grade, ER status and T and N stage for *BRCA1*, *BRCA2* and wild-type (WT- no pathogenic BRCA mutation and no VUS found) (Table 6-5) were analysed using all available genetic testing results for *BRCA1*, *BRCA2* and WT patients (n=673). All of these characteristics with the exception of pathological T stage were significantly different between *BRCA1*, *BRCA2* and WT and specifically between *BRCA1* and *BRCA2* patients. There was a significant difference (p<0.001) in the distribution (MF or UF) between *BRCA1* and *BRCA2*; with only 13.4% (17/127) of *BRCA1* patients having a MF cancer compared to 42.9% (30/70) of *BRCA2* patients. Moreover *BRCA2* patients had significantly higher rates of node positivity (60.3%) than *BRCA1* (34.5%), p<0.001, which may be a reflection of the higher incidence of multifocal and ER positive disease in *BRCA2* than *BRCA1*.

As expected in patients with *BRCA1* and *BRCA2* mutations the majority had a positive family history (74.1% and 84.6% respectively) but with no significant difference between *BRCA1* and *BRCA2* (p=0.191).

Table 6-5 *BRCA1*, *BRCA2* and wild-type tumour characteristics (n=673)

Characteristic	*BRCA1* n=136 No. (%)	*BRCA2* n=78 No. (%)	Wild-type (WT) n=459 No. (%)	P-value
Distribution				p<0.001†
Multifocal	17 (13.7%)	30 (42.9%)	129 (30.6%)	*BRCA1 vs BRCA2* p<0.001
Unifocal	110 (86.6%)	40 (57.1%)	292 (69.9%)	*BRCA1 vs* WT p<0.001
Missing/unknown	12 (8.8%)	8 (10.3%)	37 (8.1%)	*BRCA2 vs* WT p=0.045
Grade				p<.001†
Grade 1	3 (2.2%)	0 (0%)	25 (5.6%)	*BRCA1 vs BRCA2* p<0.001
Grade 2	9 (6.5%)	23 (31.1%)	143 (31.8%)	*BRCA1 vs* WT p<0.001
Grade 3	126 (91.3%)	51 (68.9%)	282 (62.7%)	*BRCA2 vs* WT p=0.105

Characteristic	*BRCA1* n=136 No. (%)	*BRCA2* n=78 No. (%)	Wild-type (WT) n=459 No. (%)	P-value
Missing/unknown	1 (0.7%)	4 (5.1%)	8 (1.8%)	
ER status				p<0.001†
Positive	29 (20.9%)	66 (84.6%)	296 (64.6%)	*BRCA1 vs BRCA2* p<0.001
Negative	110 (79.1%)	12 (15.4%)	162 (35.4%)	*BRCA1 vs* WT p<0.001
Missing/unknown	0 (0%)	0 (0%)	0 (0%)	*BRCA2 vs* WT p<0.001
Path T stage (all patients)				
T0	2 (1.5%)	1 (1.3%)	7 (1.6%)	
T1	78 (56.5%)	39 (52.0%)	226 (51.5%)	p=0.743 (NS) †
T2	53 (38.4%)	28 (37.3%)	178 (40.6%)	
T3	5 (3.6%)	7 (9.3%)	28 (6.4%)	
T4	0 (0%)	0 (0%)	0 (0%)	
Missing/unknown	0 (0%)	0 (0%)	1 (0.0%)	
N stage at presentation				p<0.001†
N0	91 (65.5%)	30 (39.0%)	218 (48.8%)	*BRCA1 vs BRCA2* p<0.001
N1	48 (34.5%)	47 (61.0%)	229 (51.2%)	*BRCA1 vs* WT p=0.001
Missing/unknown	0 (0%)	1 (1.3%)	11 (2.4%)	*BRCA2 vs* WT p=0.111
Family history				
None	34 (24.8%)	11 (14.3%)	219 (48.1%)	p<0.001†
One relative	47 (34.3%)	36 (46.8%)	146 (32.1%)	*BRCA1 vs BRCA2* p=0.191
Two relatives	34 (24.8%)	17 (22.1%)	71 (15.6%)	*BRCA1 vs* WT p<0.001
>Two relatives	22 (16.1%)	13 (16.9%)	19 (4.2%)	*BRCA2 vs* WT p<0.001
Missing	2 (1.4%)	1 (1.3%)	3 (0.7%)	

†Pearson Chi-squared test between *BRCA1*, *BRCA2* and Wild-type.

VUS results excluded

When stratified by ER status the increased proportion of MF to UF disease remains; (ER+ve MF vs UF: 45.0% vs 20.7%; ER-ve MF vs UF: 30.0% vs 11.2%) (Table 6-6 and Figure 6-1). However is should be noted that the absolute number of *BRCA2* carriers with ER negative disease is small (n=10).

Table 6-6 Multifocality in BRCA1, BRCA2 and Wild-type ER positive and ER negative breast cancers (n=673)

	ER Positive			ER Negative		
	BRCA1	***BRCA2***	**Wild-type**	***BRCA1***	***BRCA2***	**Wild-type**
Multifocal	6 (20.7%)	27 (45.0%)	96 (35.6%)	11 (11.2%)	3 (30.0%)	33 (22.0%)
Unifocal	23 (79.3%)	33 (55.0%)	174 (64.4%)	87 (88.8%)	7 (70.0%)	118 (78.7%)
Missing/	0 (0%)	6 (9.1%)	26 (8.8%)	12 (10.9%)	2 (16.7%)	11 (6.8%)

Unknown						
Total	29 (100%)	66 (100%)	296 (100%)	110 (100%)	12 (100%)	162 (100%)

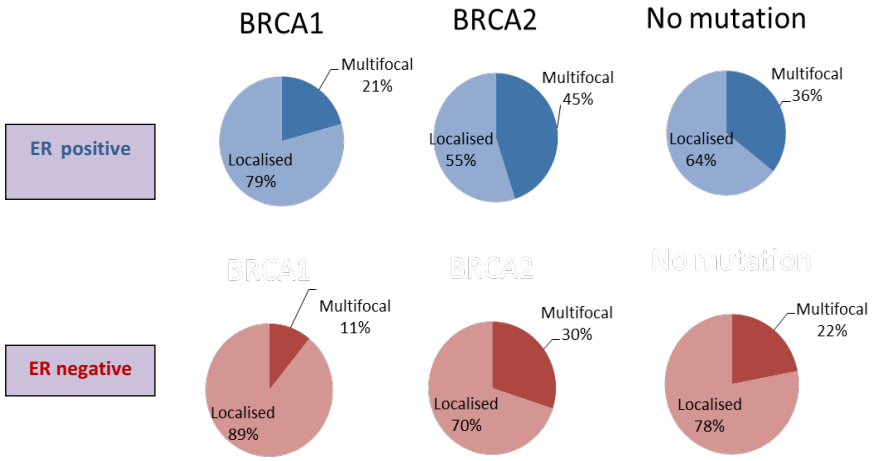


Figure 6-1 Proportion of Multifocal disease in each BRCA status and by ER status

Multiple logistic regression was performed on results from the randomly sampled patients (n=560, Table 7-4 and repeated using all patients with genetic testing results (n=756, Table 6-7) from the updated data from 22nd Oct 2013. Multifocality is significantly negatively associated with *BRCA1* mutations when adjusted for other variables, odds ratio 0.29 (p=0.009) and positively associated with *BRCA2* mutations OR 1.87 but not reaching significance (p=0.084).

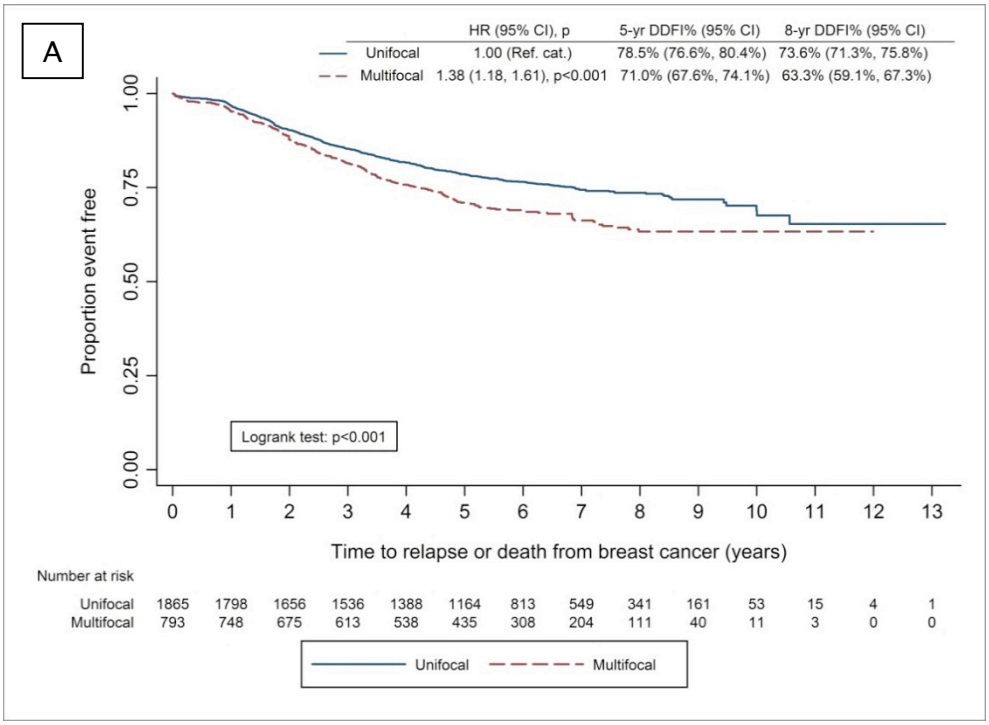
Table 6-7 Odds Ratios for variables associated with BRCA1 and BRCA2 by Multiple Logistic Regression (MLR)

	BRCA1		***BRCA2***	
	Adjusted OR (95% C.I.)	**p value**	**Adjusted OR (95% C.I.)**	**p value**
Multifocal	0.29 (0.12-0.74)	p=0.009	1.87 (0.92-3.79)	p=0.084
ER negative	4.70 (2.37-9.32)	p<0.001	0.11 (0.35-0.32)	p<0.001
Her2 negative	3.02 (1.19-7.63)	p=0.020	3.34 (1.12-10.0)	p=0.031
Grade 3 vs G1/2	2.87 (1.12-7.37)	p=0.029	2.51 (1.2-5.4)	p=0.019
Family history positive	4.89 (2.23-10.69)	p<0.001	10.55 (3.14-35.34)	p<0.001

There is more detailed discussion of the other factors associated with *BRCA1* and *BRCA2* from MLR in Chapter 4:

6.3.5 Follow-Up and Survival

The median follow-up at time of analysis was 5 years 9 months. DDFI was significantly worse for multifocal disease HR 1.38 (1.18-1.61, p<0.001, Figure 6-2A). At 5 years the DDFI for MF disease was 71.0% (95% CI 67.6%-74.1%) compared with 78.5% (95% CI: 76.6%-80.4) for UF disease. By 8 years there is 10.3% difference in DDFI between MF and UF cases. Similarly the 5 and 8 year OS was worse overall for MF patients (HR 1.36 95% CI 1.15-1.61, p<0.001, Figure 6-2B) with 9.7% difference in survival by 8 years between the two groups (65.5% multifocal vs 75.2% unifocal).



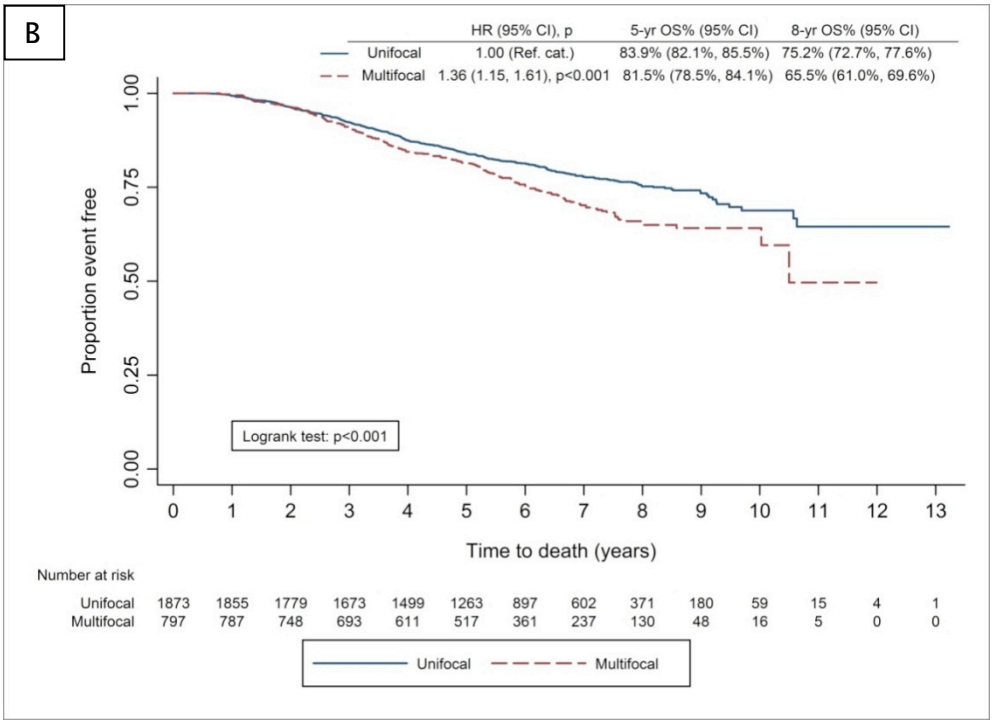


Figure 6-2 Distant Disease Free Interval (DDFI) (Fig A) and Overall Survival (OS) (Fig B) by Multifocal / Unifocal disease in whole POSH cohort (n=2956)

Previous studies have noted an excess in node positivity in MF cases, and nodal status is well known to be strongly correlated with survival so a separate analysis of DDFI and OS looking just at node negative and node positive patients by multifocality was performed (Figure 6-3). Node positive patients do worse than node negative regardless of focality of tumour in both 5yr and 8yr DDFI and OS. DDFI in node negative patients was significantly worse, HR 1.4 (1.02-1.93, p=0.001) (Figure 6-3A) for multifocal cases compared to unifocal cases. However there was no significance difference in DDFI for node positive disease (Figure 6-3A&B), and overall survival for node negative and node positive (Figure 6-3C&D). Interestingly the Kaplan Meier curve for OS in node negative patient shows a divergence of the curves after 5 years.

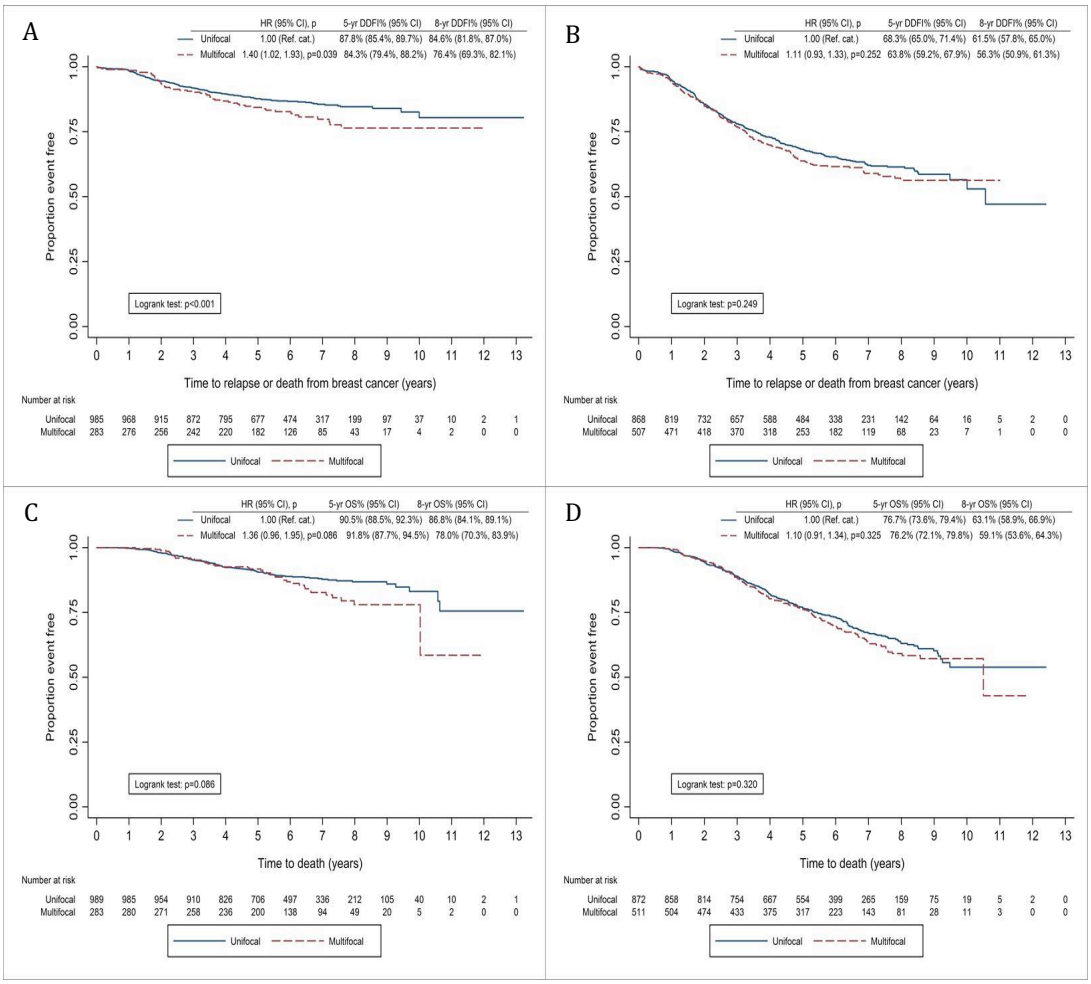


Figure 6-3 Distant Disease Free Interval (DDFI) and Overall Survival (OS) by Multifocal / Unifocal disease

A: 5 and 8-yr DDFI for Node Negative (N0) patients
B: 5 and 8-yr DDFI for Node Positive (N1) patients
C: 5 and 8-yr OS for Node Negative (N0) patients
D: 5 and 8-yr OS for Node Positive (N1) patients

The effect of multifocality on DDFI and OS was assessed using a multivariate (MV) cox model, adjusting for known prognostic factors. The MV model was stratified by ER status to account for the time-varying nature of ER over time.

Multifocality in both ER positive and ER negative patients was not a significant independent variable for DDFI (ER+ve: HR 1.1, 95% CI 0.89-1.36, p=0.392; ER-ve: HR 0.98, 95% CI 0.71-1.36, p=0.899) or OS (ER+ve: HR 1.07, 95% CI 0.85-1.35, p=0.570; ER-ve: HR 0.99, 95% CI 0.71-1.39, p=0.959) (Table 6-8).

In multivariate analysis only N stage and T stage (by aggregate score) had significantly raised hazard ratios for risk of distant metastases or death from breast cancer (DDFI) and death from any cause (OS) for both ER negative and

ER positive patients. In addition ER positive obese (HR1.42, p=0.08) and black (HR1.57, p=0.04) patients had a significantly higher hazard ratio for DDFI and obese patients for OS (1.52, p=0.004).

Table 6-8 Multivariate Cox Regression for DDFI and OS

ER Negative				
Variable (Prognostic factor)	**DDFI**		**OS**	
	HR (95% CI)	**P value**	**HR (95% CI)**	**P value**
Multifocal	0.98 (0.71-1.36)	0.899 (NS)	0.99 (0.71-1.39)	0.959 (NS)
Her2	0.75 (0.56-1.00)	0.052(NS)	0.89 (0.66-1.21)	0.464 (NS)
Grade	1.31 (0.81-2.10)	0.268 (NS)	1.42 (0.85-2.39)	0.177 (NS)
BMI				
Overweight	1.22 (0.90-1.67)	0.19 (NS)	1.34 (0.97-1.85)	0.073(NS)
Obese	1.04 (0.73-1.50)	0.79 (NS)	1.26 (0.88-1.81)	0.211 (NS)
Ethnicity				
Black	1.33 (0.739-2.41)	0.340 (NS)	1.58 (0.89-2.8)	0.117(NS)
Asian	0.62 (0.23-1.68)	0.23 (NS)	0.52 (0.17-1.64)	0.266 (NS)
T Stage				
T2	1.53 (1.13-2.10)	0.007	1.63 (1.18-2.25)	0.003
T3	2.28 (1.50-3.46)	<0.001	2.23 (1.46-3.43)	<0.001
N Stage	2.50 (1.84-3.39)	<0.001	2.79 (2.03-3.84)	<0.001
ER Positive				
Variable (Prognostic factor)	**DDFI**		**OS**	
	HR (95% CI)	**P value**	**HR (95% CI)**	**P value**
Multifocal	1.10 (0.88-1.36)	0.392 (NS)	1.08 (0.85-1.35)	0.570 (NS)
Her2	0.85 (0.688-1.06)	0.144 (NS)	0.92 (0.73-1.18)	0.527 (NS)
Grade	1.63 (1.32-2.00)	<0.001	1.83 (1.45-2.31)	<0.001
BMI				
Overweight	1.11 (0.88-1.41)	0.385(NS)	1.22 (0.94-1.58)	0.136 (NS)
Obese	1.42 (1.09-1.84)	0.008	1.52 (1.15-2.02)	0.004
Ethnicity				
Black	1.57 (1.02-2.42)	0.04	1.27 (0.78-2.06)	0.336 (NS)
Asian	1.64 (0.87-3.9)	0.13 (NS)	1.90 (0.9703.70)	0.060 (NS)
T Stage				
T2	1.35 (1.061-1.72)	0.015	1.48 (1.13-1.93)	0.004
T3	1.82 (1.31-2.52)	<0.001	1.87 (1.31-2.67)	0.001
N Stage	2.16 (1.69-2.75)	<0.001	2.14 (1.63-2.81)	<0.001

6.4 Discussion

6.4.1 BRCA mutations and phenotypes

There is detailed description in the literature about tumour phenotypes commonly associated with *BRCA1* and *BRCA2* pathological mutations, particularly with regard to receptor status (157). Atypical medullary cancers are common in *BRCA1* carriers with syncytial growth pattern and pushing margin of tumour growth (32). *BRCA1* associated tumours are more likely to be high grade. Whereas *BRCA2* associated tumours have distinct histological differences from *BRCA1* cancers; more pleomorphic lobular carcinomas, extensive intraductal carcinoma, lower grade, and lower mitotic rate, absence of pushing margins and fewer features of atypical medullary carcinomas (32, 158). *BRCA1* cancers tend to be triple receptor negative (57% in one study), while *BRCA2* can be triple receptor negative (23%) they are more commonly ER positive (17, 18, 157). Triple negative cancers often have a basaloid-like phenotype expressing high levels of stratified epithelial cytokeratins 5, 6, 14, 15 and 17. This is distinct from the luminal keratins CK7, 8,18 and 19 (159). However prediction of *BRCA1* status by cytokeratin expression in patients with a triple negative basal cancer has not proved successful (160).

Multifocal disease is common in breast cancer. Analysis of the 6134 patients in the GeparTrio, GeparQuattro, and GeparQuinto trials found 13.4% had multifocal (defined by the authors as ≥2 lesions in 1 quadrant), and 9.5% multicentric (≥1 lesion in ≥2 quadrants) disease (161). However there is little published literature on any possible association with multifocality and BRCA pathological mutations. The only published study specifically investigating this (140) concludes that bilateral or multifocal breast cancer in combination with age and family history is predictive of *BRCA1* and *BRCA2* mutation status. This Danish retrospective study of 119 young patients (61 multifocal) found that 82% (50/61) of the multifocal patients were BRCA wild-type; and of the 11 pathological mutations identified, 7 were in *BRCA2* (11%) and 4 in *BRCA1* (7%) (140). Patients had the same median age of diagnosis (36 yrs) but with a higher upper age limit (46 yrs) than the POSH study (40 yrs). No other pathological information was given on the multifocal tumours. From the POSH study we similarly found more *BRCA2* mutations (n=30, 42.9%)) than *BRCA1*

mutations (n=17, 13.7%) in patients with multifocal disease. The ratio was reversed for unifocal disease, with *BRCA1* mutations (n=110, 86.6%) more frequent than *BRCA2* (n=40, 57.1%). Patients with multifocal cancer compared to patients with unifocal cancer were also more likely to have ER positive disease (n=621, 78%, versus n=1156, 62%) a phenotype more commonly associated with *BRCA2* than *BRCA1*. The higher proportion of multifocality persisted when looking at either ER positive or ER negative disease and was independently (negative) associated with *BRCA1*. The positive odds ratio for multifocality and *BRCA2* was not significant in MVA but this could be due to much smaller numbers. The association of *BRCA1* mutations with unifocal cancers and *BRCA2* mutations with multifocal tumours suggests a field change with *BRCA2* mutations, contributing to formation of multiple tumours that is less commonly seen in *BRCA1* mutation carriers or in young women with wild-type BRCA. There is no definitive consensus whether multifocal tumours per se are clonal and the few studies performed are very small and not specifically on BRCA associated tumours (141, 149). A Japanese study of 30 patients, only 3 of which had multiple cancer foci, found all were monoclonal; concluding that multifocal ductal carcinomas are secondary deposits within the breast from one primary (149). *BRCA2* is a mediator of the core mechanism of homologous recombination. When deficient, attempts at DNA repair are inadequate resulting in accumulation of mutations and the utilisation of other, more error prone repair mechanisms. *BRCA2* mutation-associated tumours are far more phenotypically heterogeneous than *BRCA1* tumours. It may be that the multifocal BRCA2 tumours are distinct from unifocal *BRCA2* tumours and behave differently. More than one mutation contributes to the phenotype of any breast cancer. It is not known at what stage in cancer development is the contribution of BRCA mutations critical (162) and how they influence the end phenotype. In addition the influence of the stroma on the cancer cells and of epigenetics can result in phenotypic diversity (163). Very little previous published work has explored multifocality and BRCA mutations and this work provides new prospectively collected data in this area specifically in young women affected by breast cancer.

6.4.2 Tumour distribution and Survival

There is an ongoing debate in the literature over whether multifocality is an independent prognostic factor in breast cancer. Prognostic factors are important to take into account when considering adjuvant therapy options. Univariate analysis of data from the POSH study found an inferior DDFI and survival overall in multifocal patients compared to unifocal patients. However when adjusted for the most important prognostic factor, N stage, only multifocal node negative patients had a significantly worse DDFI in comparison to unifocal node negative patients that (HR 1.4, p=0.039). This could be explained by the reduced use of neoadjuvant and adjuvant chemotherapy in node negative patients versus node positive patients (81.6% versus 96.2%); assuming multifocal disease confers worse biology and a higher propensity for distant micrometastatic spread. However in Multivariate Cox regression multifocality was not an independent factor for DDFI or overall survival when adjusted for all other prognostic variables.

To put this analysis in context with other published data a systematic review published by Bendifallah (164) of studies from 1980-2010 was supplemented by a search of Pubmed to include papers published up to 10th Nov 2013 using the search terms “multifocal”, “multicentric” “multiple” and “breast”. 14 studies assessed the effect of multifocality on prognosis either in terms of overall survival, breast cancer specific survival or recurrence (progression free survival (PFS) / disease free survival (DFS) / locoregional recurrence (LRR) / distant recurrence (DR) (Table 6-9). 12 of the 13 relevant studies used multivariate analysis to adjust for other prognostic variables. Three studies found multifocality was independently associated with worse BCSS (165-167), with one study finding no association (143). Boyages et al (167) looked in more detail by size of tumour (< or > 20mm) and by methodology used to calculate size of tumour (largest diameter of largest single focus or aggregate diameters). The only situation in which multifocality was independently associated with poorer survival was in tumours >20mm in size using the largest diameter method (p=0.012). Only one study (168) found an independent association with overall survival with six studies finding no association (79, 145, 148, 169-171). Lastly when looking at recurrence, three

studies were positive for an independent association and five found no association.

Different methodologies were used including use of different factors in MVA. Only two of the 14 were prospective studies (148, 166) including the largest study by far of 25320 patients (166), 6.1% of which had multifocal/multicentric disease (defined as two or more lesions in the same breast). Any patient with Stage I-III breast cancer was included, with the median age at diagnosis of 59 (UF) and 56 (MF). No statistical difference in OS was found but a worse BCSS by 3.4% at 10 years in the MF group.

For non-age selected patients the weight of evidence from the published literature to date is in favour of multifocality associated with a poorer breast cancer specific survival but less so for overall survival or recurrence. However direct comparison of these studies is fraught with difficulties due to heterogeneity of population, methods and definition of MF. All studies except Litton et al (169) did not select for age. This single centre study from the University of Texas M.D. Anderson Cancer Centre looked at 300 women aged 35 or less at diagnosis of breast cancer. No difference was found in overall survival or recurrence free survival between unifocal and multifocal disease. Since 2013 a systematic review has been published of 22 studies comprising 67,557 patients that concluded a worse overall survival in multivariable analysis, (HR 1.65, p=0.02) but non-significant trend towards worse disease free survival (HR1.96, p=0.07) (172). Again this systematic review represents all age ranges of patients with marked variability in the studies.

Our prospectively collected data supports the observation by Litton et al (169) that tumour multifocality is not an independent prognostic marker in young women with breast cancer.

Table 6-9 Summary of Studies looking at impact of Multifocality on Prognosis

Author & Year	R / P ¥	Total No. of patients. (MF No, %)	Survival		Recurrence	
			Unadjusted	Adjusted (MVA)	Unadjusted	Adjusted (MVA)
Vera-Badillo 2014	R	67,557 (9.5%) SR		OS HR 1.65 p=0.002		DFS HR 1.96, p=0.07
Pekar 2013	R	444 (111, 25%)	-	BCSS HR 2.5 (1.40-4.46) p=0.0016	-	-

Author & Year	R / P ¥	Total No. of patients. (MF No, %)	Survival		Recurrence	
			Unadjusted	Adjusted (MVA)	Unadjusted	Adjusted (MVA)
Ustaaliogl u 2012	R	697 (107, 15%)	-	OS: p=0.06	DFS: 137 v 55 months, p<0.001	DFS: 0.33 (HR 0.18-0.58, p=0.001)
Lynch 2012	R	3722 (906, 24%)	-	-	LRR p=0.44	LRR p=0.88
Rezo 2011	P	812 (141)	-	OS: Not independent		PFS: Not independent
Tot 2011	R	499 (122)	"Survival" HR 1.96 (1.11-3.48), p=0.02	-	-	-
Boyages 2010	R	848 (94, 11.1%)	OS T >20mm: 72.1% v 54.7%, p=0.002 (LD) 72.1% v 69.5%, p=0.275 (AD)	BCSS T>20mm p=0.012 (LD), p=0.267 (AD) T<20mm p=0.695 (LD), p=1.0 (AD)	-	-
Weissenb acher 2010	R	576 (matched pair analysis from 5691)	OS: 221.6 v 203.3 mnths (p<0.001)	OS: p=0.016	DR p<0.003 LR p<0.001	p=0.038 p=0.001
Yerushal mi 2009	P	25320 (6.1%)	**5 yr OS** 85.9 v 85.4 No diff **10 yr OS** 70.2 v 68.4% No diff **5 Yr BCSS** 91% v 89.7% **10yr BCSS** 82.1% v 78.7%	**BCSS:** p=0.045 RR 1.17 (1.004-1.372	-	-
Joergense n 2008	R	7024 (945)	OS: p=0.43	OS: HR 1.05, p=0.43	DFS: p=0.013	DFS HR 1.16, p=0.013
Litton 2007	R	300 (58, 19%)	5 yr OS 69.7% v 67.3%, p=0.7	5 yr OS HR 1.57 p=0.34	5yr RFS 44.4% v 57.1%, p=0.36	5yr RFS HR 0.87 (0.42-1.78), p=0.7
Pedersen 2004	R	929 (158, 17%)	OS p=0.0001	OS HR 1.03, p=0.7741	-	-
Vlastos 2000	R	284 (21%)	-	OS Not independent	10yr DFS 84% v 83% No diff	Not independent
Rakowsk y 1992	R (LN+)	214 (15%)	-	Not independent	-	Not independent
Egan 1982	R	156 (69%)	Worse	-	-	-

¥ **R- Retrospective; P- Prospective**

Differences in outcome may be due to difference in adjuvant management for example in surgery and chemotherapy regimens. But 69.7% (438/628) of patients who had a mastectomy for multifocal disease had adjuvant radiotherapy and a similar number (64.7%, 469/725) of unifocal disease mastectomy patients. Chemotherapy regime was initially included in the Cox model but removed due to too small numbers in one category as the overwhelming majority of these patients received adjuvant chemotherapy.

Despite allowing for the time varying effect of ER status on prognosis we found no independent association of multifocality on outcome. Division of breast tumours into subtypes by molecular profiling however may find differing results. Pekar et al found MF an independent prognostic factor in Luminal A, Her2 post and basal like tumours (173). Pathology specimens in POSH have not undergone formal molecular subtyping so this analysis was not possible.

6.4.3 T stage methodology discussion

Using aggregate diameter to assign T stage resulted in a large number of patients in being upstaged in this analysis. Although multifocality was not an independent marker of worse prognosis, T stage was and adjuvant chemotherapy decisions are based in part on the size of the tumour. However in the POSH cohort most received adjuvant chemotherapy anyway. In a patient population where adjuvant chemotherapy is based more heavily on T stage (amongst other factors) rather than predominantly young age it could be argued that a more accurate assessment of T stage is required. If adjuvant chemotherapy is recommended or not on the basis of the T stage calculated from the largest focus this may well exclude patients with a much larger burden of disease that may benefit from adjuvant chemotherapy. Again there is no clear consensus in the literature. From retrospective analyses of the NCIC MA. 5 (pre and peri-menopausal lymph node positive only patients) and MA.12 trial results (pre-menopausal patients) (174), uni, bi or tri-dimensional tumour size measurements were calculated from review of pathological data. In both these trials all patients received adjuvant chemotherapy. However they are now outdated and when looking at the results from patients with central pathology review data only, no size evaluation method significantly affected survival in multivariate analysis. In another study, when different T stages were looked at

using different staging methodology, for larger tumours (over 20mm) 10 year survival was different depending on methodology used (54.7% largest focus diameter vs 69.5% aggregate size) and the significance disappeared (p=0.49) when aggregate size was compared to unifocal 10 year survival (72.1%) (167). A prospective cohort study using MF defined as multiple foci greater than 4mm apart (excluding women with > 10 foci or unmeasurable foci). Largest diameter, aggregate diameters and aggregate volumes were compared and using a Wald test found that largest diameter gave the best correlation with PFS and OS (148).

6.4.4 Limitations

The whole cohort has not been tested for BRCA mutations. This will be completed by the end of 2015. The genetic testing results that are available are for a selected group of patients (eg mostly family history positive) and therefore may not be representative of the whole cohort (as discussed in Chapter 7). However there was no significant difference in the degree of positive family history between unifocal and multifocal patients (p=0.381) and between *BRCA1* and *BRCA2* positive patients (p=0.191). Further analysis and discussion of the effect of family history was discussed in in Chapter 4. The identified possible BRCA multifocal association needs further demonstration in another cohort and will be replicated in the POSH cohort once all genetic testing has been completed so removing any possible selection bias.

6.4.5 Conclusion

Unifocal cancers are more common than multifocal cancers regardless of genetic status in this cohort of young breast cancer patients. However *BRCA2* mutation associated tumours have a much higher proportion of multifocal cancers than *BRCA1* or wild-type cancers. The positive odds ratio becomes non-significant in MVA possibly due to small numbers of *BRCA2* cancers. Multifocality is significantly negatively associated with *BRCA1* mutation tumours.

Multifocal disease is associated with a significantly worse 5 and 8 year DDFI and 5 and 8 year overall survival. For node negative patients DDFI is significantly worse in multifocal compared to unifocal patients; and there is a

late divergence of the overall survival curves between multifocal and unifocal node negative patients but this is non-significant. However, when controlling for known prognostic variables no independent association of multifocality was found with DDFI or OS.

Chapter 7: Genetic testing in Oncology Practice. Should BRCA testing be extended to young women with breast cancer who do not have a family history?

7.1 Introduction

Although only a small number of women (4%) diagnosed with breast cancer are aged 40 years or under at diagnosis (CRUK), younger age is associated with a higher chance of carrying a mutated high risk breast cancer predisposition gene such as *BRCA1* and *BRCA2* (92). Current genetics referral guidelines are aimed at patients with a significant family history of breast and ovarian cancer. However most young women with breast cancer do not have a positive family history (6, 8) and a proportion of these patients will also be mutation positive. This group of patients are not currently well recognised within the guidelines.

7.1.1 Hereditary Breast and Ovarian Cancer Syndrome

Familial breast cancer was first reported in 1866 by Paul Broca, referring to his wife's family pedigree with four generations affected by breast cancer (175). 129 years later the *BRCA1* and 2 genes were cloned (14). The Hereditary Breast and Ovarian Cancer Syndrome refers to a syndrome caused by a germline mutation in *BRCA1* or *2* and confers and highly increased risk of breast and ovarian cancer and increased risk of prostate and pancreatic cancer with high penetrance (14, 176). The population prevalence of deleterious mutations in non-Ashkenazi Jewish populations is estimated at about 0.12% for *BRCA1* and 0.044% for *BRCA2* (177). It is well recognised that only a minority of familial breast cancer is related to pathogenic mutations in *BRCA1* and *BRCA2*. Pathogenic mutations in these two large genes account for approximately 18% (7-10% *BRCA1* and 10% *BRCA2*) of familial breast cancer caused by known cancer predisposition genes (178).

Young age at onset of breast cancer is associated with a higher chance of carrying a germline mutation. However even in the very young (<35 years old) the majority do not have a pathogenic *BRCA1* or 2 mutation (179).

Pathogenic mutations in BRCA genes are the most common and best studied Many countries use clinical guidelines for referral, testing and management of BRCA carriers. The other highly penetrant mutations, moderate and low penetrant genes at present are not routinely tested. However next generation sequencing panels allow analysis of multiple genes simultaneously that will result in more 'positive' results but not necessarily with the genetic epidemiological evidence available yet to associate these genetic findings with evidence based clinical actions.

7.1.2 Threshold for Genetic Testing

Guidelines on referral for genetic counselling and mutation testing vary internationally (12, 180-182) and the methods for calculating the likelihood of carrying a high risk gene are not standardised between or even within countries. 2013 USA National Comprehensive Cancer Network guidelines recommend referring any woman with a personal history of breast cancer diagnosed aged 45 years or younger for genetic assessment irrespective of family history (183). It is generally accepted in European countries that a prior probability of >10% of a *BRCA1/BRCA2* mutation is high enough to warrant genetic referral and testing (181). The rationale for a testing threshold is partly economic (taking account of the time required for adequate pre-test genetic counselling in all cases) and partly because all people in the population have some chance of being a BRCA gene carrier (even if that is < 1%). The BRCA genes are large and as for any part of the genome there is a degree of genetic variability throughout the sequence such that rare variants in the expected sequence are quite frequent findings (~10%) even in a DNA sample tested from someone in the general population. A Variant of Unknown Significance (VUS) is the term used to describe a rare change to the expected gene sequence that is not clearly predicted to disrupt the gene or protein product and without clear evidence of pathogenicity. Reporting of genetic test results is still not well standardised between testing laboratories even in the NHS and reports can lead to the clinician can lead inadvertent over-interpretation of the significance

of the genetic result as discussed in chapter 5. Estimating when a patient has reached the 10% threshold of probability for carrying a pathogenic mutation is a complex challenge in a time-pressured oncology clinic. Using a pedigree to estimate risk is complex and not just based on the number of cancer affected individuals but the age of onset, type of cancer, numbers of unaffected members and relationships between members. Referral criteria are based on strength of family history, including multiple or bilateral breast/ovarian cancers, male breast cancer or Ashkenazi Jewish ancestry (ESMO, NICE). In women under 40 without a family history the BRCA mutation rate has been documented at between 4.9% - 23% (91, 184).

Reasons why a patient may have a high risk breast cancer gene such as *BRCA1* or *BRCA2* in the absence of a strong family history include small families, adoption, unknown family history, male dominance in a family, new sporadic mutations and modifiers of penetrance may all mask a positive family history.

Certain cancer phenotypes are more commonly associated with a *BRCA1* or *BRCA2* mutations (43, 180, 185). Most (~61%) breast cancers in women with *BRCA1* mutations are basal-like subtype in which the receptor status is usually triple negative (TN) (45, 159, 185). Furthermore a retrospective study of TN tumours showed 66-69% had a *BRCA1*-like Comparative Genomic Hybridisation (aCGH) profile and 27-37% *BRCA1* promoter hypermethylation (186). Studies of young women (<31, <41 years old, selected for no/minimal family history) with TN breast cancer have found a *BRCA1* detection rate of between 9-37% (46, 48, 187). A number of publications have called for all patients with a TN phenotype under various age categories to be offered genetic testing regardless of their family history (182). In contrast, predicting who is a *BRCA2* carrier from tumour subtype is harder (43), but Luminal B subtype cancers are most commonly described (185).

7.1.3 Methods of assessing gene carrier probability

The process of calculating risk should take into account both family history and tumour phenotype. Within the UK a referral from the hospital clinician or General Practitioner to the regional genetics clinic is based on the clinician's perception of risk in the clinic with reference to National guidelines (127). Secondary and tertiary care have a range of tools such as computer models or

algorithms to assess the probability that an individual having genetic testing will carry a *BRCA1*/2 gene. These tools all have their flaws with no single method taking into account all the factors of both family history and tumour subtype (9, 188, 189).

The Gail model and Claus tables were both developed prior to the discovery of the *BRCA1* and *BRCA2* genes. They are based on family history and are applicable to people unaffected by breast or ovarian cancer.

Tyrer-Cuzick is a computerised model to predict individual risk of BRCA mutation status and thus of developing breast cancer using family history and personal factors (such as age, height, BMI) and with the Bayes Theorem (190).

The Manchester Scoring system is a simple paper-based system that assigns a score to the number of family members with female breast cancer, male breast cancer, ovarian, prostate or pancreatic cancer in direct lineage (9, 191). The higher the score, the greater predicted risk of *BRCA1/BRCA2* mutation status. Again the updated model takes some tumour characteristics such as triple negative receptor status into account.

BOADICEA is a validated, freely available online, simple to use program; which does not require specialist knowledge and was also developed using UK data. It utilises age, family history of cancer (breast, ovarian, prostate and pancreatic), age at any cancer diagnosis, Ashkenazi Jewish origin, known genetic testing results and cohort information to estimate *BRCA1/BRCA2* mutation carrier probabilities (188, 192-194). This tool is recommended by NICE in the latest guidance and has been updated recently to incorporate now tumour pathology information.

BRCAPRO (189) is a computer based algorithm using family and personal history of breast and ovarian cancer to provide a probability an individual is carrying a *BRCA1* or *BRCA2* mutation. It is based on a US population including a large percentage of Ashkenazi Jews and uses Mendelian inheritance and Bayesian updating (40).

The Myriad model from Myriad Genetics, Inc. uses logistic regression but is only applicable to women with young onset breast cancer (<50) with a positive family history (195).

All these methods predominantly use family history, supplemented with age at onset, and in more recent iterations tumour oestrogen receptor status to estimate carrier probability. However most are impractical for use in a busy oncology clinic. For an oncologist a quick estimate of the probability that testing will reveal a high risk gene would facilitate discussions with the patient particularly regarding the appropriateness of a genetics referral.

7.1.4 Rational for Genetic Testing

Information that a person carriers a pathogenic gene mutation predisposing to cancer, screening available for some of these cancers and the inheritance of the mutation known; allows the opportunity for primary and secondary prevention. It also allows the wider family to be informed and tested for the mutation, primary or secondary prevention measures offered and cascaded to multiple family members.

7.1.4.1 Primary Cancer Prevention / early detection

Cancer prevention/early detection methods include chemical or surgical. Breast cancer screening guidelines in the UK are annual mammographic or MRI screening depending on risk, age, and genetic status (http://www.nice.org.uk/guidance/cg164). Tamoxifen or raloxifene chemoprevention for 5 years has also been introduced into the NICE familial breast cancer guidelines (CG164) for premenopausal high risk patients without a personal history of breast cancer at moderate or high risk postmenopausal women (127, 181). Some women elect to have risk reducing surgery as a method of cancer prevention. The benefits of bilateral salpingoophorectomy in a woman close to menopause once she has completed her family are substantial, particularly as there is no good screening method for ovarian cancer (196). Bilateral salpingoophorectomy can reduce breast cancer risk by up to 50% and ovarian cancer risk by 85-95% (197, 198). More controversial is prophylactic bilateral mastectomy. Highly effective, reducing breast cancer risk by up to 95% (199, 200) but with psychological and sexual implications, it needs careful and prolonged specialist counselling beforehand.

7.1.4.2 Secondary Cancer Prevention

Knowledge of BRCA mutation carrier status in a breast cancer patient presents the opportunity for potential risk reduction of second primary breast tumours and primary prevention of an ovarian malignancy (201). As in primary prevention, risk reduction options include breast screening and risk reducing surgery.

Next generation sequencing provides BRCA mutation results in as little as 2 weeks giving the potential for contemporaneous genetic testing during the course of management of the newly diagnosed breast cancer. This is at odds with the previous standard patient pathway of referral many months or years after their initial breast cancer diagnosis, usually after initial surgical management is complete. Identification of a gene mutation may be taken into consideration in planning the primary surgical management. More radical surgery; that is a mastectomy rather than BSC and, or contralateral prophylactic mastectomy may be offered. However care should be taken when offering radical surgery at a vulnerable time immediately after diagnosis without evidence of improvement in survival outcomes in comparison to delayed surgery or surveillance. The competing risk of death from the index primary (particularly if TN) may be greater in the short term than the risk of a new primary. The smaller the probability of a high risk pathogenic *BRCA1*/2 mutation being present, the greater the relative chance that an uninformative negative or a VUS (variant of uncertain significance/pathogenicity) is found.

Should early testing at the point of diagnosis become routine then the need for specialists to be able to use a simple, fast algorithm that gives an estimation of likelihood that a patient might be carrying a pathogenic BRCA gene mutation becomes more relevant still.

Outside clinical trials the recommendations for cancer treatment for the primary breast cancer currently are no different in patients with or without a BRCA mutation (ASCO, ESMO, NICE). However over 20 intervention trials are registered with ClinicalTrials.gov specifying BRCA mutation carrier patients in the inclusion criteria so this is likely to change in the future with management aimed specifically at this patient group.

7.1.5 Aim

Using data from a subset of patients recruited to the POSH study, we aimed to produce estimates of carrier probabilities for young women without a family history (negative family history- FH -) using pathological variables available in an oncology clinic to develop an algorithm. This study differs from others previously reported as it uses data from a breast cancer population selected only on the basis of young age at diagnosis and followed prospectively.

7.2 Methods

Description of the methods of the POSH study is in Chapter 2. A proportion of POSH patients (26%) had available genetic testing results from one of two sources (clinical testing or research testing). This analysis was performed on data received up to 11th April 2012.

Family history was obtained at recruitment from a patient completed questionnaire. Reported diagnoses in relatives have not been independently confirmed. A positive family history was defined as any first or second degree relative diagnosed with breast or ovarian cancer at any age. This very basic definition was deliberately chosen to make it as simple and user friendly as possible.

7.2.1 BRCA Genetic testing

Where diagnostic *BRCA1/2* screening test had been undertaken as part of the patient's management we collected copies of original genetics reports. Patients for research testing were non-randomly selected to complete a pre-specified range of tumour characteristics (e.g. TN tumours, FH -). DNA from a peripheral blood sample was tested at Wessex Regional Genetics laboratory using a highly sensitive mutation analysis technique. This consists of a combination of Conformation Sensitive Capillary Electrophoresis (CSCE) and Multiplex Ligation- dependent Probe Analysis (MLPA) which has similar sensitivity to full gene sequencing. The genetic testing detects all point mutations, insertion/deletion mutations and large exonic deletions or duplications predicted to be pathogenic.

7.2.2 Statistical Analysis

The study population was the POSH cohort. At the time of analysis BRCA testing the whole cohort was incomplete, although it is expected to finish by the end of 2015. Available BRCA genetic testing results were from clinical records for some patients within the cohort and were specifically tested for others as part of the POSH research study, using various referral criteria. Because of this, the total sample with available genetic testing results was biased towards specific subgroups of patients (eg TN, FH+) according to the criteria used for referral for testing.

A stratified sampling method was used to ensure representativeness to the whole POSH cohort and minimise selection bias. This was achieved by taking a random sample of the 760 patients with genetic testing information and matching the proportions of the key patient and tumour characteristics to those from the entire POSH cohort. Stratification variables that were matched in the sampling process included triple negative status, multifocal status, histological grade and family history status. Using this method a sample size of 560 patients was selected which was representative of the whole POSH cohort in terms of the key patient and tumour characteristics.

Analyses were performed in STATA v11·2 on records with complete data (levels of missingness were reported), excluding family history which was dichotomised as either yes or no/missing/unknown. Summary statistics were used to describe the cohort. Associations between independent variables were assessed by dichotomising each variable and using odds ratios (ORs). Potential predictors of *BRCA1* and *BRCA2* mutation carriers were tested separately using univariate logistic regression, and ORs with 95% confidence intervals (CI) calculated. Multiple logistic regression (MLR) was used to assess the combined influence of grade 3, multifocality and either TN status or Her2 and ER status of the cancer on the probability of a patient with no/unknown family history being a *BRCA1* carrier versus not a carrier. For *BRCA2* versus non *BRCA2* carriers MLR was used to assess the combined influence of grade 3, multifocality, Her2 and ER status on carrier probability. In each case, the best fitting MLR model was found using the lowest Akaike information criterion (202) and forward selection and backward elimination methods. A ten-fold

stratified cross-validation approach (203, 204) was used to assess the performance of each model (Appendix).

7.3 Results

The POSH study recruited 2956 patients aged 40 years or under and 760 patients (25.8%) had genetic testing results available at the time of this analysis, including 436 patients who underwent research testing and with the rest tested as part of their clinical management. After random sampling using the matrix method described above, data from 560 patients were analysed.

7.3.1 Patient Characteristics

Median age of patients in the whole POSH cohort and genetic testing subgroup was 36 (Table 7-1). The age range of patients recruited into POSH was 18-25, but most patients in the whole POSH cohort, the genetic testing group, *BRCA1* mutation patients and *BRCA2* mutation patients were in the 35-40 age bracket.

93.1% of patients self-reported white/Caucasian ethnicity and 1.1% Jewish, (1% missing data). Most patients (51%) did not report a family history of breast or ovarian cancer. The sampling method ensured that patient and tumour demographics and treatment were broadly comparable for the genetic testing subgroup and the whole POSH cohort (Table 7-1). Exceptions are the number of patients with an affected first degree relative (FDR) and number with a triple negative cancer, where there were more in the genetic testing group compared to the whole cohort (FDR: 29.0% versus 14.7%; and (TNT: 24.5% versus 19.6%).

Detailed information on the characteristics and outcomes of the POSH cohort including comparison to national data is described in Chapter 1: (8).

Table 7-1 Demographics from whole POSH cohort and genetic testing group included in the analysis

Characteristic	Whole POSH cohort (n=2956, 100%)	Genetic testing group analysed (n=560, 18.9%)
Age at diagnosis (median, range, IQR) 18-25 26-30 31-35 35-40	36 (18 to 40, 33 to 38) 46 (1.6%) 270 (9.1%) 900 (30.5%) 1740 (58.9%)	36 (22 to 40, 33 to 38) 18 (3.2%) 73 (13.0%) 154 (27.5%) 315 (56.3%)

Characteristic	Whole POSH cohort (n=2956, 100%)	Genetic testing group analysed (n=560, 18.9%)
Duration of follow-up in months (median, range, IQR)	60 (1 to 136, 45 to 75)	63 (4 to 127, 45 to 82)
No. of relatives with history of breast cancer		
0	1874 (65.8%)	283 (51.0%)
1	702 (24.6%)	166 (29.9%)
2	199 (7.0%)	72 (13.0%)
>2	75 (2.6%)	34 (6.1%)
Missing/unknown	106 (3.6%)	5 (0.9%)
Family history of breast cancer		
First degree	418 (14.7%)	161 (29.0%)
Second Degree	554 (19.4%)	111 (20.0%)
Histological Grade		
1	163 (5.7%)	22 (4.0%)
2	972 (33.8%)	166 (30.4%)
3	1742 (60.6%)	359 (65.6%)
Missing/unknown	79 (2.7%)	13 (2.3%)
Histological Type		
Ductal	2556 (87.6%)	497 (89.4%)
Lobular	134 (4.6%)	27 (4.9%)
Ductal and lobular	78 (2.7%)	7 (1.3%)
Medullary	31 (1.1%)	8 (1.4%)
Other	118 (4.1%)	17 (3.1%)
Missing/unknown	39 (1.3%)	4 (0.7%)
Distribution of cancer		
Localised	1873 (70.2%)	356 (71.3%)
Multifocal	797 (29.9%)	143 (28.7%)
Missing/unknown	286 (9.7%)	61 (10.9%)
ER Status		
Positive	1947 (66.1%)	344 (61.4%)
Negative	997 (33.9%)	216 (38.6%)
Missing/unknown	12 (0.4%)	0 (0%)
PR Status		
Positive	1342 (56.5%)	260 (54.5%)
Negative	1033 (43.5%)	217 (45.5%)
Missing/unknown	581 (19.7%)	83 (14.8%)
Her 2 Status		
Positive	717 (27.6%)	128 (25.3%)
Negative	1884 (72.4%)	378 (74.7%)
Missing/unknown	355 (12.0%)	54 (9.6%)
Triple Negative Tumour (TNT)		
Yes	549 (19.6%)	137 (24.5%)
No	2256 (80.4)	403 (72.0%)
Missing/unknown	151 (5.1%)	22 (3.6%)
Pathological T stage		
T0	73 (2.5%)	11 (2.0%)
T1	1411 (47.9%)	283 (50.6%)
T2	1167 (39.6%)	209 (37.4%)
T3	189 (6.4%)	33 (5.9%)
T4	6 (0.2%)	1 (0.2%)
Tis	21 (0.7%)	3 (0.5%)
Tx	77 (2.6%)	19 (3.4%)
Missing/unknown	12 (0.4%)	1 (0.2%)
N Stage		
N0	1417 (48.9%)	263 (48.0%)
N1	1484 (51.2%)	285 (52.0%)
Missing/unknown	55 (1.9%)	12 (2.1%)
M Stage		
M0	2860 (97.5%)	541 (97.5%)
M1	74 (2.5%)	14 (2.5%)
Missing/unknown	22 (0.7%)	5 (0.9%)
Definitive breast surgery		

Characteristic	Whole POSH cohort (n=2956, 100%)	Genetic testing group analysed (n=560, 18.9%)
Breast conserving surgery	1409 (47.7%)	261 (46.7%)
Mastectomy	1497 (50.7%)	288 (51.5%)
No surgery	39 (1.3%)	9 (1.6%)
Nodal surgery only	9 (0.3%)	1 (0.2%)
Missing /unknown	2 (0.1%)	1 (0.2%)
Primary Chemotherapy timing		
Adjuvant	2152 (72.8%)	419 (74.8%)
Neo-adjuvant	460 (15.6%)	79 (14.1%)
Palliative	54 (1.8%)	12 (2.1%)
Not applicable	290 (9.8%)	50 (8.9%)
Missing/unknown	0 (0%)	0 (0%)
Chemotherapy regimen		
Anthracyclines	1938 (65.6%)	363 (64.8%)
Anthracylines and Taxanes	684 (23.1%)	139 (24.8%)
Taxanes	20 (0.7%)	2 (0.4%)
Other	24 (0.8%)	6 (1.1%)
None	290 (9.8%)	50 (8.9%)
Adjuvant Radiotherapy		
Yes	2358 (79.8%)	433 (77.3%)
Missing/unknown	598 (20.2%)	127 (22.7%)

7.3.2 Pathology variables and *BRCA1* and *BRCA2* Analysis

Of the 560 patients analysed pathogenic mutations were identified in the *BRCA1* gene in 84 patients (15.0%), *BRCA2* in 55 patients (9.8%), variants of unknown significance (VUS) in 65 (11.6%) and no mutation in 352 (62.8%). Four patients had p53 mutations.

The distribution of age between *BRCA1* and *BRCA2* mutation carriers was significantly different (p=0.042); with *BRCA2* mutation carriers older (36 years) at diagnosis of breast cancer than *BRCA1* carriers (34 years) (Table 7-2).

Table 7-2 Age at diagnosis from whole cohort and genetic testing group

Age at diagnosis, in years	Whole POSH cohort No. (%) (n=2956)	Genetic testing group No. (%) (n=560)	BRCA 1 No. (%) (n=84)	BRCA 2 No. (%) (n=55)
18-25	46 (1.6%)	18 (3.2%)	2 (2.4%)	2 (3.6%)
26-30	270 (9.1%)	73 (13.0%)	15 (17.9%)	7 (12.7%)
31-35	900 (30.5%)	154 (27.5%)	31 (36.9%)	13 (23.6%)
35-40	1740 (58.9%)	315 (56.3%)	36 (42.9%)	33 (60.0%)

The cancers in BRCA 1 carriers were predominantly grade 3 (88.0%) and TN (64.0%); contrasting with grade 3 (70.6%), ER positive (87.3%), Her2 negative (78.6%) in tumours most commonly found in *BRCA2* carriers, VUS carriers and sporadic cancers (where the patient was tested but no mutation found). Of the

137 TN cancers in the genetic testing group, 48 (35.0%) were associated with a BRCA1 mutation (Table 7-3).

Nearly two thirds of tumours in this young age group of 40 or under are grade 3. However when looking at the patients who carried a *BRCA1* or 2 mutation a much higher proportion had grade 3 tumours (88% and 71% respectively) reflecting increased biological aggressiveness.

Multifocal tumours were rare in BRCA 1 carriers (9.3%) and negatively associated with the predictive risk of a *BRCA1* mutation (OR 0.22, p<0.001) and most common in *BRCA2* carriers (46.8%) and had a positive association of prediction of risk for BRCA 2 mutation (OR 2.41, p<0.01).

Table 7-3 Genetic status by family history, tumour receptor, grade and multifocality of genetic testing group included in analysis

	BRCA1 **(*n*=84)**	***BRCA2*** **(*n*=55)**	**VUS (*n*=65)**	**No mutation (*n*=352)**	**Total (*n*=560)***
Family History					
Yes	58 (69.1%)	44 (80.0%)	19 (29.2%)	149 (42.3%)	272 (48.6%)
No/missing	26 (31.0%)	11 (20.0%)	46 (70.8%)	203 (57.7%)	288 (51.4%)
Total	**84 (100%)**	**55 (100%)**	**65 (100%)**	**352 (100%)**	**560 (100%)**
TNT status					
TNT	48 (64.0%)	4 (7.3%)	13 (21.3%)	72 (20.9%)	137 (25.4%)
Not TNT	27 (36.0%)	51 (92.7%)	48 (78.7%)	273 (79.1%)	403 (74.6%)
Missing/unknown	9 (10.7%)	0% (0%)	4 (6.2%)	7 (2.0%)	20 (3.6%)
Total	**75 (100%)**	**55 (100%)**	**61 (100%)**	**345 (100%)**	**540 (100%)**
ER status					
Negative	66 (78.6%)	7 (12.7%)	23 (35.4%)	118 (33.5%)	216 (38.6%)
Positive	18 (21.4%)	48 (87.3%)	42 (64.6%)	234 (66.5%)	344 (61.4%)
Total	**84 (100%)**	**55 (100%)**	**65 (100%)**	**352 (100%)**	**560 (100%)**
Her2 status					
Negative	65 (91.6%)	45 (88.2%)	44 (78.6%)	224 (69.1%)	378 (74.7%)
Positive	6 (8.5%)	6 (11.8%)	12 (21.4%)	100 (30.9%)	128 (25.3%)
Missing/unknown	13 (15.5%)	4 (7.3%)	9 (13.9%)	28 (8.0%)	54 (9.6%)
Total	**71 (100%)**	**51 (100%)**	**56 (100%)**	**324 (100%)**	**506 (100%)**
Grade					
Grade 3	73 (88.0%)	36 (70.6%)	39 (60.0%)	207 (60.2%)	359 (65.6%)
Grade 1 or 2	10 (12.1%)	15 (29.4%)	26 (40.0%)	137 (39.8%)	188 (34.4%)
Missing/unknown	1 (1.2%)	4 (7.3%)	0 (0%)	8 (2.3%)	13 (2.3%)
Total	**83 (100%)**	**51 (100%)**	**65 (100%)**	**344 (100%)**	**547 (100%)**
Multifocality					
Multifocal	7 (9.3%)	22 (46.8%)	15 (25.0%)	98 (31.2%)	143 (28.7%)
Localised	68 (90.7%)	25 (53.2%)	45 (75.0%)	216 (68.8%)	356 (71.3%)
Missing/unknown	9 (10.7%)	8 (14.6%)	5 (7.7%)	38 (10.8%)	61 (10.9%)
Total	**75 (100%)**	**47 (100%)**	**60 (100%)**	**314 (100%)**	**499 (100%)**

*TP53 mutations found in 4 patients.

7.3.3 Results for Multiple Logistic Regression for BRCA 1 and 2

For *BRCA1* the odds ratios for all variables (TN, ER negative, Her 2 negative Grade 3, and Multifocal) were significant when unadjusted and adjusted for

other variables. ER negative and TN had the strongest association with *BRCA1* (adjusted OR 4.74 and 3.99 respectively). For *BRCA2*, Her 2 negativity had the highest odds ratio of 3.37 (1.02-4.65, p=0.029) followed by Grade 3 and multifocal disease (Table 7-4).

Table 7-4 Results for Multiple Logistic Regression (MLR) for BRCA1 and BRCA2

	Odds Ratio (95% Confidence Interval), *p*-value		
	Unadjusted (*n*=560)	**Adjusted (*BRCA1* TN Algorithm)† (*n*=475)**	**Adjusted (*BRCA1* ER Algorithm)†† (*n*=444)**
BRCA1			
Triple Negative	7.51 (4.44, 12.70), *p*<0.001	3.99 (2.19, 7.26), *p*<0.001	*(Not applicable)*
ER Negative	7.97 (4.57, 13.89), *p*<0.001	*(Not applicable)*	4.74 (2.41, 9.31), *p*<0.001
HER2 Negative	4.22 (1.78, 10.00), *p*=0.001	*(Not applicable)*	2.91 (1.15, 7.35), *p*=0.024
Grade 3	4.54 (2.29, 9.03), *p*<0.001	2.44 (1.11, 5.39), *p*=0.027	2.95 (1.16, 7.51), *p*=0.024
Multifocal	0.22 (0.10, 0.49), *p*<0.001	0.33 (0.14, 0.77), *p*=0..011	0.30 (0.12, 0.76), i=0.011
	Odds Ratio (95% Confidence Interval) , *p*-value		
	Unadjusted (*n*=560)	**Adjusted (*BRCA2* Algorithm)‡ (*n*=444)**	
BRCA2			
ER Negative	0.21 (0.09, 0.47), *p*<0.001	0.11 (0.04, 0.33), *p*<0.001	
HER2 Negative	2.75 (1.14, 6.60), *p*=0.024	3.37 (1.13, 10.03), *p*=0.029	
Grade 3	1.29 (0.68, 2.41), *p*=0.435	2.17 (1.02, 4.65), *p*=0.045	
Multifocal	2.41 (1.31, 4.43), *p*=0.005	2.12 (1.05, 4.30), *p*=0.036	

† Odds ratios from MLR model adjusted for family history status, TNT status, Grade 3 status, and multifocality. 85 patients did not have complete information.
†† Odds ratios from MLR model adjusted for family history status, ER status, HER2 status, Grade 3 status and multifocality. 116 patients did not have complete information.
‡ Odds ratios from MLR model adjusted for family history status, ER status, HER2 status, Grade 3 status and multifocality. 116 patients did not have complete information

7.3.4 Probability Algorithm

Using the variables discussed above an algorithm was derived (Figures 7-1 and 7-2) as a guide to the probability of finding a *BRCA1* or *BRCA2* pathogenic mutation in a young individual presenting with a breast cancer based on a combination of pathology variables. If a patient had a positive family history (as defined in the methods) the probability of *BRCA1* was 21.3% (16.5-26.2, p<0.001) and of *BRCA2* 16.2% (11.8-20.6%, p<0.001) unadjusted for other variables, based on this data. Therefore all patients aged 40 or under when

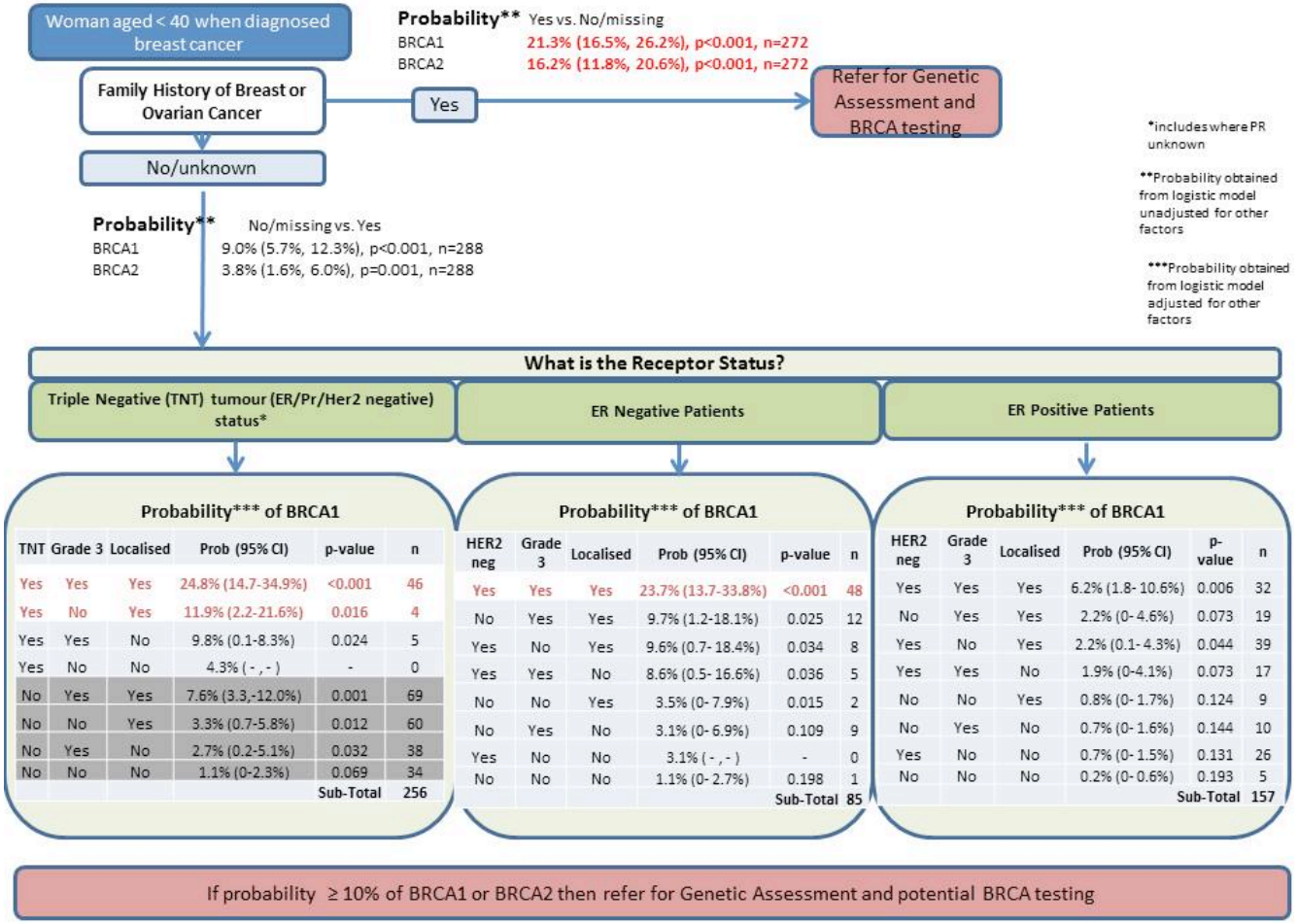
diagnosed with breast cancer and with any first or second degree relative diagnosed with breast or ovarian cancer would meet the 10% threshold for referral for genetic assessment and BRCA testing.

Following the algorithm for patients with no/unknown family history it is then split into two: Figure 7-1 the probability of a patient being a *BRCA1* pathogenic mutation carrier and Figure 7-2 the probability of a patient being a *BRCA2* pathogenic mutation carrier.

For *BRCA1* (Figure 7-1), three probability tables are given depending on whether the patient has i) triple negative tumour, ii) ER negative (PR+/-/unknown), iii) ER positive (PR+/-/unknown). A probability value is then given along with 95% confidence interval, p value and number of patients from which this was derived for each combination of pathology variables. For *BRCA1* these variables are TN/ER, Her 2, Grade 3 and localised disease. The combinations exceeding the 10% threshold are TN, Grade 3 and localised (24.8%), ER-ve, Her 2-ve, grade 3 and localised (23.7%) and TN and localised but not grade <3 (11.9%). However 3 other combinations are within 0.5 % of the 10% threshold and given the wide confidence intervals it maybe that with greater numbers with these combinations will exceed the threshold and a genetics service referral may be considered.

For *BRCA2* (Figure 7-2), the only combination exceeding the 10% probability was Her 2 neg, grade 3, and multifocal disease (14.7%). For ER negative patients all combinations were less than 2

Figure 7-1 BRCA1 Algorithm

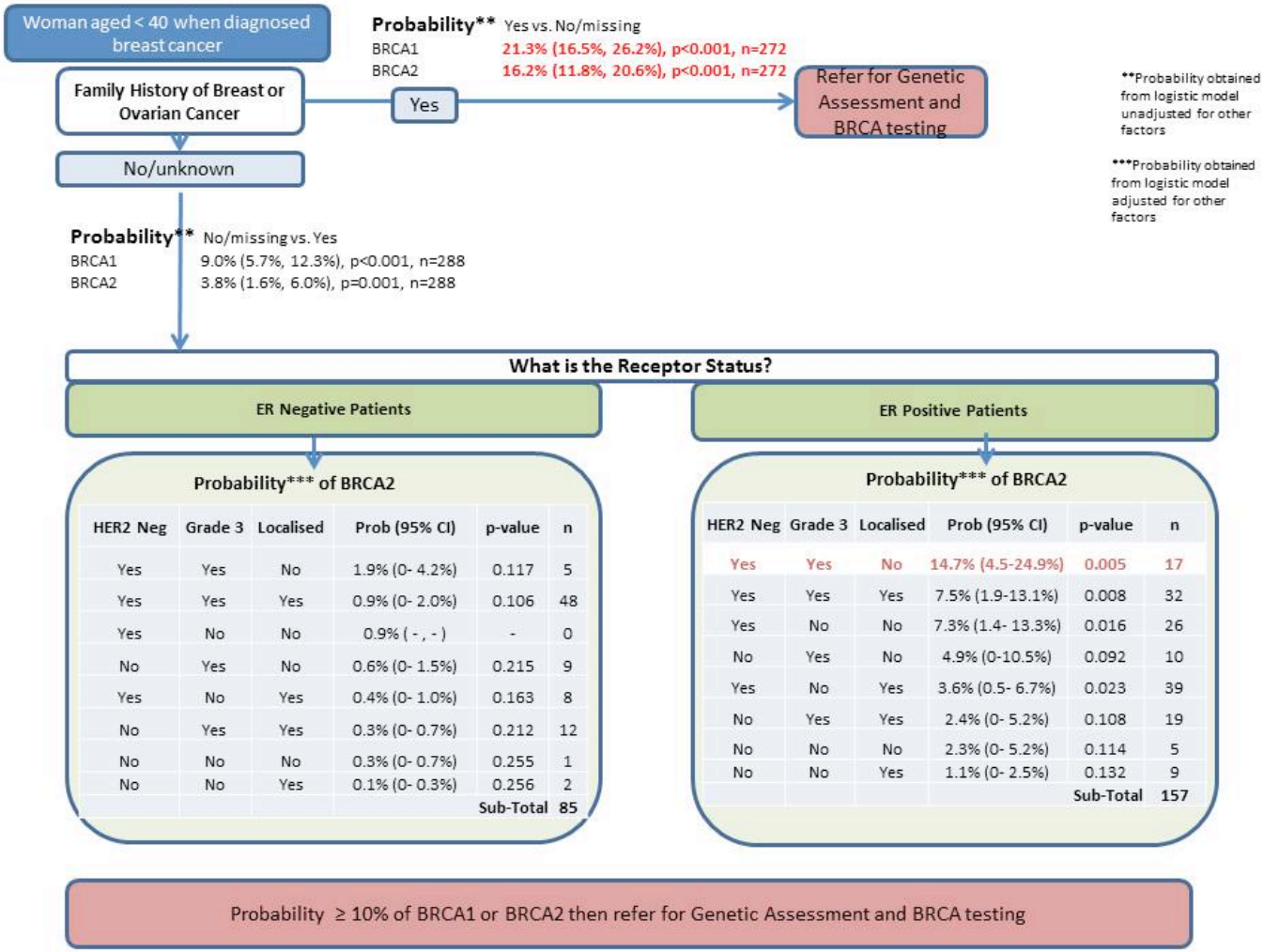


TNT	Grade 3	Localised	Prob (95% CI)	p-value	n
Yes	Yes	Yes	24.8% (14.7-34.9%)	<0.001	46
Yes	No	Yes	11.9% (2.2-21.6%)	0.016	4
Yes	Yes	No	9.8% (0.1-8.3%)	0.024	5
Yes	No	No	4.3% (- , -)	-	0
No	Yes	Yes	7.6% (3.3,-12.0%)	0.001	69
No	No	Yes	3.3% (0.7-5.8%)	0.012	60
No	Yes	No	2.7% (0.2-5.1%)	0.032	38
No	No	No	1.1% (0-2.3%)	0.069	34
				Sub-Total	256

HER2 neg	Grade 3	Localised	Prob (95% CI)	p-value	n
Yes	Yes	Yes	23.7% (13.7-33.8%)	<0.001	48
No	Yes	Yes	9.7% (1.2-18.1%)	0.025	12
Yes	No	Yes	9.6% (0.7- 18.4%)	0.034	8
Yes	Yes	No	8.6% (0.5- 16.6%)	0.036	5
No	No	Yes	3.5% (0- 7.9%)	0.015	2
No	Yes	No	3.1% (0- 6.9%)	0.109	9
Yes	No	No	3.1% (- , -)	-	0
No	No	No	1.1% (0- 2.7%)	0.198	1
				Sub-Total	85

HER2 neg	Grade 3	Localised	Prob (95% CI)	p-value	n
Yes	Yes	Yes	6.2% (1.8- 10.6%)	0.006	32
No	Yes	Yes	2.2% (0- 4.6%)	0.073	19
Yes	No	Yes	2.2% (0.1- 4.3%)	0.044	39
Yes	Yes	No	1.9% (0-4.1%)	0.073	17
No	No	Yes	0.8% (0- 1.7%)	0.124	9
No	Yes	No	0.7% (0- 1.6%)	0.144	10
Yes	No	No	0.7% (0- 1.5%)	0.131	26
No	No	No	0.2% (0- 0.6%)	0.193	5
				Sub-Total	157

Figure 7-2 BRCA2 Algorithm



7.4 Discussion

In the POSH study overall 63% of women aged 40 or under at diagnosis of breast cancer do not have a family history of breast or ovarian cancer. Referral of all patients under 40 years at diagnosis is impractical for publically funded health care systems, and testing all breast cancer patients will lead to a larger number of cases with VUS results than cases with clearly pathogenic results. We have aimed to address the understanding of mutation probabilities from a pragmatic oncology based clinic perspective by use of our data to identify variables predictive of gene mutation.

Results from the POSH study, support previous reports that TN disease strongly predicts for a pathogenic *BRCA1* mutation (adjusted odds ratio of 3.99 (2.19-7.26, p<0.001). We further conclude that any woman diagnosed with breast cancer at age 40 or under with any family history of breast or ovarian cancer affecting parents, siblings, aunts and grandparents (on mother or father's side of the family) should be referred to specialist genetics services (unadjusted probability of *BRCA1* 21.3%, 16.5%-26.2%, p<0.001 and for *BRCA2* 16.2%, 11.8%-20.6%, p<0.001). We acknowledge the very broad and inclusive definition of 'positive family history' deliberately used in this study. Nevertheless, it should be noted that even a young patient aged 40 years or under with a positive family history has a less than 50% chance of carrying a pathogenic BRCA mutation.

Our *BRCA1* mutation rates in TN cancers (9-24%) were comparable to other reports in series with FH - (182, 205). The two largest UK population based studies looking at young breast cancer patients (aged <45 and <55 respectively) and unselected for family history have found rates of 0.7-2.6% *BRCA1* and 1.3-2.4% *BRCA2* mutations (93, 97). A Swedish population based study of women diagnosed under 41 found *BRCA1* mutation rates of 6.8% and *BRCA2* in 2.1% (96). Both these studies were retrospective and while looking in detail at prevalence by age category and degree of family history did not examine tumour phenotype and its correlation to BRCA status. The Australian breast cancer family registry has identified morphological features predictive of mutation status including ER and PR (206). Their best predictive model comprised 3 variables: trabecular growth pattern, high mitotic index and

number of first degree relatives diagnosed under age 60 (area under the ROC curve of 0.87) but they did not include Her2 status and tumour multifocality. A young age-selected Danish study found a mutation frequency of 18% in 61 multifocal cancers (four *BRCA1* and seven *BRCA2* mutations) (140). Our study also identified a higher proportion of *BRCA2* (15.4%) than *BRCA1* (4.9%) mutation carriers in patients with multifocal cancers. We have also demonstrated that the distribution of cancer is a predictor of risk of *BRCA2* mutation status with an odds ratio unadjusted for other variables of 2.42 ($p<0.01$) (Table 3) but was not statistically significant when looking at FH - patients alone. While multifocal breast cancers are relatively common (~9.5%) (Table 6-9 (172), they are not mentioned in national (NICE or ESMO) guidelines for genetic testing.

In all public funded healthcare systems there must be a balance between cost (~*BRCA* test £550 in NHS laboratory) and benefit. If all breast cancer cases were offered testing under an age threshold rather than a genetic likelihood threshold, current laboratory and clinical services would rapidly be overwhelmed. Most patients referred to a genetics clinic and tested will not carry a pathogenic mutation, carrying both patient and resource implications (207). A US paper looking at the incremental cost-effectiveness in BRCA testing of testing various groups of women diagnosed with breast cancer under 50 found that testing women with TN cancers aged less than 50 was cost effective ($9,084 per quality-adjusted life-year) and could reduce breast and ovarian cancer risks by 23% and 41% respectively compared to a reference standard of no testing (208). Noted in this paper was the absence of a direct comparison of different criteria for BRCA testing in subgroups of women aged under 50.

Also rarely mentioned in European and US guidelines is the management of VUS (12, 209). Non-informative genetic test results such as VUS are common (10-20%) (113), and their interpretation and subsequent counselling can be particularly complex. Our study found VUS rates between 7-16%, with higher rates inversely associated with positive family history. Non-selectively lowering the threshold of genetic testing can result in a greater chance of a identifying a VUS result than a pathogenic mutation. Management of the resulting information is complex and requires expert genetics advice with associated expense to the health service and time in hospital appointments for the

patients and it can still cause additional worry and uncertainty for the patient that may lead to inappropriate treatment choices.

7.4.1 Limitations

We recognise that the main limitation of this study is incomplete genetic testing of the whole POSH cohort and a selection bias for some of the tested cases. A sampling method was used to try and ensure representativeness of the whole POSH cohort for the cases with genetic testing results included in this analysis. Further research testing is planned with the aim of completing testing in the entire 2956 patient cohort. Once complete this will provide a data set to validate the algorithm. External validation will also be required in the future. Small numbers resulting in wide confidence intervals necessitate caution in interpretation near the 10% boundary. Inter-laboratory variation in receptor results is an acknowledged limitation and the POSH study has completed central pathology review in one third of patients. A possible further limitation of the study is that family history verification was not performed; however it could be argued that these latter two limitations represent a more real-world scenario. Our whole cohort was UK only aged 40 or under and predominantly white Caucasian as such our external generalizability outside this demographic is limited.

Reducing the numbers analysed from 758 to 560 using the random sampling method as performed in Chapter 3 for algorithm development changed little compared to using all the available data with regards to significance but with consequently wider confidence intervals.

7.4.2 Conclusion and future work

Both the UK publication ‘Genetics in Mainstream Medicine’(110) and the ASCO cancer prevention and Risk Assessment Statement (181) note that there will be an increased burden on oncologists to become involved in explaining risk, counselling and interpreting genetic test results in the future. The growing trend towards rapid genetic testing at the point of breast cancer diagnosis, the increased role of oncologists and the complexities of interpreting both positive, negative and an uninformative test result on the background of the

psychological trauma of a new breast cancer diagnosis will require additional training.

Computer based tools formally calculating mutation carrier probabilities based on detailed family history and incorporating aspects of tumour phenotype are impractical for use by the non-geneticist within the oncology clinic. This work focused on the under-researched but majority group of patients without a family history. Our aim was to provide breast cancer specialists with a simple way of estimating the probability their young patient has a BRCA 1 or 2 mutation prior to any specialist genetic services involvement and anticipate future refinement and validation of this algorithm.

Chapter 8: Discussion

Breast cancer in young women is more likely to have adverse biological features than older women (8, 42, 210). There is no doubt that women diagnosed with breast cancer while young fare worse than their older counterparts. The mortality tables give evidence to the fact. The POSH study analysis found overall survival of 81.9% at 5years (CRUK statistics 83.5% in 18-39 years old), which represents 9.1% premature deaths averaging 53 life-years lost per woman. To investigate whether young onset cancers are biologically distinct, Azim et al found 2 themes that were associated with young age at diagnosis - immature mammary cell populations (RANKL, c-kit, BRCA-1 mutated, mammary cell populations and luminal progenitors) and growth factor signalling (MAPK and PI3K related) (3). Anders et al looked at genomic analysis and clinico-pathologic variables finding an independent age effect on disease-free survival when comparing <40 to 40-45 years only but not < 40 to >65 (5). They also found over 300 gene sets that uniquely distinguished young women's tumours from older women's tumours, including gene sets regulating multiple potentially drugable targets. A large Danish population based retrospective study concluded that the young age negative effect was only for women with 'low risk' disease who did not receive adjuvant cytotoxic treatment (185). This was historical in terms of modern oncology adjuvant management with patients included from 1978-1996. The vast majority of patients in POSH received chemotherapy (92.4% neoadjuvant or adjuvant intent). Another retrospective historical study found that the negative impact of young age in univariate analysis was no longer present once adjustment was made for other poor prognostic features such as tumour size and nodal involvement (187).

A large population-based analysis of the SEER database found that young age (<30) was only associated with a poorer prognosis compared with older age in ER positive women. Young women with ER negative cancers had no different survival than all other age groups bar the elderly (>75 years) (39). POSH did not have a reference older age group for comparison but found that ER negative women initially did worse than ER positive women (75.7% versus 85.0% 5 year survival) but by 8 years the rate of death had slowed in ER negative cancer patients (67.5%) but accelerated in ER positive women (67.7%) such that there is no statistical difference between the two groups. Is this difference in survival

pattern because women with ER negative tumours are treated more aggressively with chemotherapy-based adjuvant therapies than ER positive tumours 95.7% of ER negative women in POSH had neoadjuvant or adjuvant chemotherapy versus 84.8% of ER positive women. ER positive and ER negative breast cancers are not the same disease. For a premenopausal woman who does not enter the menopause after chemotherapy an estrogen driven cancer has the opportunity to relapse as the oestrogen drive continues until menopause. Prolonged endocrine therapy is now widely considered the standard of care after the ATLAS and aTTom trials (211). However a very small proportion of women in these large trials were premenopausal (8-10%). Complete oestrogen blockade has long been postulated as an effective means of preventing recurrence (212). The recently reported phase 3 trials, SOFT and TEXT found a 91.1% disease free survival for exemestane + ovarian suppression and 87.3% for tamoxifen + ovarian suppression (213). The greatest benefit from combined endocrine blockade versus tamoxifen alone was in the very young (<35 years) (214). POSH patients predominantly received single modality endocrine therapy for 5 years (33.8% ER positive patients received LHRH agonist and 16.7% had an oophorectomy).

Her2 overexpression also has prognostic importance and is the target of multiple biological therapies. Probably the biggest limitation of POSH in terms of analysing outcome is the incomplete picture of routine treatment with Her2 targeted treatments due to timing of the study with the introduction of trastuzumab over halfway through recruitment. The missing Her2 data seems balanced between groups and so little bias is expected to have been introduced. Further to the immunohistochemical classification of breast cancers is molecular subtyping into “intrinsic” groups (34). Microarray gene expression profiling based on a 50 gene classifier (prediction analysis of microarray –PAM50) and corresponding prognostic model for relapse (ROR-S) is one commercially available profiling system. Current evidence does not fully support the routine use of intrinsic molecular profiling to drive decision making on when to use adjuvant chemotherapy in all populations (215). However for the intermediate risk groups such as ER+ve, Her2-ve, LN-ve cancers with an intermediate Nottingham Prognostic Index score; Oncotype DX is a recommended option for aiding decision making on use of adjuvant chemotherapy (NICE Diagnostics Guidance (DG10). A future direction for POSH

could be to intrinsically classify the whole cohort for further recurrence and survival analysis.

Cancer medicine is increasingly all about both 'personalising' treatment and minimising necessary treatment but without a negative impact on survival. In breast cancer the first minimisation of radical management was the step from radical mastectomy to breast conserving surgery with wide local excision and radiotherapy. Multifocal cancers often necessitate a larger operation and they are more common in the young patient. Varying terminology and definition makes assessment of the impact on multifocal cancer on outcome difficult. POSH patients with node negative and multifocal disease had both a worse DDFI and OS than node negative unifocal cancers. But in node positive patients there was no difference in outcome depending on multifocality. Node positive patients are generally treated more aggressively with adjuvant chemotherapy. However in MVA multifocality was not an independent predictor for survival. A literature review found no clear weight of evidence for multifocality as an independent marker for recurrence, breast cancer specific or overall survival 6.4.2).

The overarching aim of POSH was to investigate the outcome of sporadic versus hereditary breast cancers in the young. With this in mind a detailed family history questionnaire was given to all POSH participants. Analysis of the impact of any positive family history (as defined by a family member with breast or ovarian cancer at any age), on outcome, found no independent effect on overall distant disease free interval. Familial risk is influenced by multiple genes in a population, as such findings in relation to family history are likely to be skewed by a combination of the most extreme influences such as the high lifetime risk associated with *BRCA1* and *BRCA2* mutations and the most common gene variant, providing it contributes sufficiently to risk. Breast cancer genetics is complex with multiple identified genes increasing risk, but only a handful currently being routinely tested. Many women have unanswered questions about the hereditary component of their breast cancer. High risk genes are tested for more commonly in the young patient, yet the overwhelming majority of young women presenting with breast cancer do not have a family history of breast cancer, even at the broadest definition. However knowledge of a high risk gene such as *BRCA1* and *BRCA2* may alter the management of the incident breast cancer, future cancer risk recurrence in the

individual and counselling and primary cancer risk recurrence in the family. The timing of when such a genetic test should take place in the cancer pathway has implications for the management of the patient but with possible psychological implications. The aim of Chapter 7 was to construct an algorithm using commonly known pathological factors to predict who may be at a sufficiently high risk of carrying a BRCA mutation in the *absence* of a known family history to be offered genetic testing. Small numbers have resulted in wide confidence intervals, particularly for the rare combinations in the matrix. Future testing of the whole POSH cohort will allow this algorithm to be refined and the results published. Investigation of multifocality as a predictive factor for pathogenic *BRCA1* and *BRCA2* mutations regardless of family history found a trend towards more multifocal disease in *BRCA2* patients and a smaller but significant negative odds ratio with *BRCA1* patients. There is a paucity of literature on BRCA mutations and multifocal disease and this analysis adds information. Again, this association will be investigated further once the whole POSH cohort has been genetically tested.

At present, triple receptor negative patients <40 years old are commonly referred for testing within the NHS regardless of family history (NICE guidance CG164). Otherwise recommendations for referral for genetic testing are strongly based on family history. The Royal Marsden "Mainstreaming Cancer Medicine" undertook a pilot research programme for direct oncologist requests for BRCA testing for breast and ovarian cancer patients without the normal referral to specialist genetics services. Publication is awaited from this pilot. Details from their website list the suggested criteria for direct BRCA testing as non-mucinous ovarian cancer at any age, ovarian and other primary cancer at any age, bilateral breast cancers diagnosed <50 years old, triple negative breast cancer diagnosed <50 years old, breast and ovarian cancer at any age. Oncologists are trained to inform and consent patients via online learning modules. Other patients who may meet testing criteria as set out by NICE guidelines, should be referred to specialist genetics services as per the normal route. Results from this direct access test come back to the oncologist and patient with information sheets for the patient regarding their result. Positive results also generate a referral to genetics services. The aim of this 'oncogenetic model' is to streamline testing for cancer patients (216).

The Mainstreaming Cancer Genetics programme has less focus on the timing of testing. The latter is currently left to physician choice. Treatment focused genetic testing (TFGT), testing at time of cancer diagnosis already performed in some centres has potential drawbacks as well as possible advantages. Concerns raised surround the psychological pressure and stress at time of diagnosis, concerns around understanding the test results and the implications for management including a possible variant of unknown significance result. Some health professionals view TFGT as a positive step and see it as the future for high risk patients (217). In ovarian cancer TFGT is deemed acceptable by patients and necessary to making treatment option decisions (218). Similarly women recently diagnosed with breast cancer felt that TFGT was relevant to decisions about surgery and did not add to their psychological burden (118). By virtue of the necessarily expedited patient pathway it may be usual for the surgeon to broach genetic testing with a patient unless neoadjuvant chemotherapy is being proposed. Work in Australia is trying to understand if standard genetic counselling can be replaced by an education leaflet given to patients by their surgeon (219). In chapter 5 exploration of UK breast cancer specialists including surgeons into the most difficult to interpret genetic testing results, variants of unknown significance, was explored by a national questionnaire. The majority of respondents (71%) were unsure/unconfident in interpreting a genetic test result and while the surgeons were the most sure of all 3 specialities still only 50% were confident in their perceived understanding of a VUS. However issued genetic testing results are not standardised in the UK and methods to investigate and report details given to clinicians on a VUS vary from laboratory. Improved genetics training such as the online learning modules from the Royal Marsden for existing clinicians at consultant level or standardisation of difficult results including management guidance such as referral to a genetics department will be a mandatory part of an integrated oncology- genetics programme.

8.1 Future work

POSH is a unique cohort of young breast cancer patients with extensive data collection and minimal missing data or loss to follow-up. POSH already has many international collaborations and ongoing work. A large piece of future analysis could include intrinsic molecular subtyping of the POSH cohort and

correlation with treatment received and outcome. While it is known that adverse biological features are associated with *BRCA1* carriers it is unknown whether BRCA mutations per se confer a differing prognosis compared to sporadic cancer (80, 94, 95). Once BRCA testing of the whole POSH cohort has been undertaken later in 2015 the main outcome of the POSH study, the independent impact of BRCA mutation on survival will be analysed.

Specifically I will also look to repeat the analysis of multifocal and unifocal disease in BRCA patients to look for an association independent of other prognostic markers. As pathological specimens have been stored from virtually all POSH patients, further investigation could include a more detailed analysis of patients with multifocal disease. This would be particularly interesting in those patients with a BRCA mutation. Work could include investigating whether multifocal cancers in BRCA carriers are monoclonal. Lastly, the BRCA algorithm for family history negative patients will be refined, internally validated with data from the rest of the cohort and disseminated through publication.

Appendices

Appendix 1 – Genetic Reports for VUS Questionnaire

Interpretation and communication to patient of BRCA test report

Report 1: *You sent your female breast cancer patient who has a strong family history (mother and aunt) of breast cancer for genetic testing. She returns to clinic and you have to explain this report to her.*

"Missense mutation (c.4813G>A; p.Gly1529Arg) identified in exon 11 of the *BRCA2* gene.

Report interpretation:
Sequencing analysis of exon 11 of the *BRCA2* mutation gene identified a G to A base substitution at nucleotide position 4813 (c.4813G>A) resulting in the substitution of the amino acid glycine for an arginine at codon 1529 (p.Gly1529Arg). This sequence change has previously been reported as an unclassified variant on the BIC database. A report by Chen et al. (J. Biol.Chem., 274, 32931) has shown that this sequence change reduces RAD51 binding activity. It may not be appropriate to offer presymptomatic testing to at-risk relatives until pathogenicity has been confirmed. Screening of affected relatives may help to clarify whether this is a disease-causing mutation in this family. No other mutation was identified in with BRCA 1 or *BRCA2*. Please note it has only been possible to screen fragment 11.5 of *BRCA2* in one direction. For information a synonymous change has been identified in exon 13 of *BRCA2* (c.7206T>G, p.SEr2326Ser). This change has not been reported in the literature of on the ENSEMBL or Genbank databases, but is likely to be a rare polymorphism."

Report 2: *You sent your female breast cancer patient who has NO family history of breast cancer for genetic testing. She returns to clinic and you have to explain this report to her.*

"*BRCA1* and *BRCA2* dosage analysis:
Normal

BRCA1 and *BRCA2* gene analysis:
Heterozygous for *BRCA2* c.9098C>T, p.Thr3033Ile

Patient X has been screened for mutations in all exons of the *BRCA1* and *BRCA2* genes. A C>T substitution at nucleotide 9098, c.9098C>T, was detected in exon 23 of the *BRCA2* gene. This substitution changes codon 3033 from a threonine residue to a isoleucine, p.Thr3033Ile. This amino acid changes has not been previously reported on the BIC or HGMD databases therefore the clinical significance of it is unknown. The splice site predictor program (www.fruitfly.org/cgi-bin/seq_tools/splice.pl) did not predict any donor or acceptor site alteration. No other mutation was detected and there was no evidence of a whole exon deletion or duplication of the *BRCA1* or *BRCA2* gene using MLPA dosage analysis."

Appendix 2 – Statistical Tables

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

10-fold cross-validation estimates of the areas under ROC curves for each algorithm

	Median (range, IQR)		
	***BRCA1* TN Algorithm†** **(*n*=475)**	***BRCA1* ER Algorithm††** **(*n*=444)**	***BRCA2* Algorithm‡** **(*n*=444)**
All patients	0.781 (0.770 to 0.790, 0.771 to 0.783)	0.809 (0.781 to 0.815, 0.804 to 0.812)	0.779 (0.763 to 0.788, 0.772 to 0.784)
Family History Negative	0.747 (0.725 to 0.761, 0.745 to 0.749)	0.812 (0.786 to 0.827, 0.804 to 0.826)	0.714 (0.678 to 0.751, 0.692 to 0.725)
Triple Negative	0.516 (0.494 to 0.551, 0.511 to 0.546)	*(Not applicable)*	*(Not applicable)*
ER Negative	*(Not applicable)*	0.616 (0.568 to 0.627, 0.609 to 0.621)	0.632 (0.535 to 0.671, 0.622 to 0.639)
HER2 Negative	*(Not applicable)*	0.780 (0.745 to 0.790, 0.776 to 0.785)	0.757 (0.742 to 0.764, 0.750 to 0.760)
Multifocal patients	0.798 (0.775 to 0.832, 0.786 to 0.809)	0.905 (0.852 to 0.922, 0.886 to 0.911)	0.789 (0.756 to 0.799, 0.780 to 0.793)
Grade 3 patients	0.710 (0.691 to 0.724, 0.707 to 0.716)	0.736 (0.708 to 0.744, 0.731 to 0.739)	0.809 (0.787 to 0.818, 0.800 to 0.814)

† *BRCA1* Algorithm A: MLR model adjusted for family history status, TNT status, Grade 3 status, and multifocality. 85 patients did not have complete information.

†† *BRCA1* Algorithm B: MLR model adjusted for family history status, ER status, HER2 status, Grade 3 status and multifocality. 116 patients did not have complete information.

‡ *BRCA2* Algorithm: MLR model adjusted for family history status, ER status, HER2 status, Grade 3 status and multifocality. 116 patients did not have complete information.

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Supporting Documentation – Published Work