Risk factors for anogenital cancers in postmenopausal women: the Million Women Study

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Thesis submitted for the degree of

Doctor of Philosophy

Trinity term 2015

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Abstract

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Background:

Anal, vulval and vaginal cancers predominantly affect postmenopausal women. Over 85% of registrations occur after the age of 50. Risk factors for these cancers, other than high-risk human papillomaviruses, are not well defined.

Methods:

1.3 million UK women, mostly aged 50-65 at recruitment, were followed for incident anogenital cancer. Cox regression models with age as the underlying time variable were used to calculate adjusted relative risks associated with various lifetime exposures.

Results:

570 anal, 898 vulval, and 170 vaginal cancers were registered over an average 13.8 years of follow-up. History of cervical intraepithelial neoplasia grade 3 (CIN 3) prior to recruitment was associated with a 4-fold increase in risk of anal cancer, a doubling of risk of vulval cancer, and a 7-fold increase in risk of vaginal cancer. Significant associations were also seen for past cervical cytological abnormalities, with an increase in risk of anal cancer for low-grade, and an increase in risk of all three cancers associated with high-grade abnormalities.

Anal cancer risk was also associated with smoking, prior use of oral contraceptives, nulliparity, tubal ligation, and not living with a husband/partner. Risk of vulval cancer was increased in overweight, obese women, and those with a menopause prior to age 50. Risk of vaginal cancer was increased amongst women who were nulliparous, overweight or obese, who had a hysterectomy prior to recruitment, or who were not married or living with a partner.

Conclusions:

Despite anatomical proximity and histological similarities of the anogenital tissues, anal, vulval and vaginal cancers have heterogeneous associations with many lifetime exposures, suggesting differences in aetiology. Past high-grade cervical abnormalities are a marker of increased risk of subsequent anogenital cancer, but only a small proportion of women with such a history go on to develop anal, vulval or vaginal cancer later in life.

Role of the author in the preparation of the thesis

This thesis is all my own work, with the exceptions outlined below.

The Million Women Study (MWS) is a large, ongoing prospective cohort study with over 1.3 million participants. The study was conceived of and started by Professor Dame Valerie Beral, with the assistance and support of the Million Women Study Co-ordinating Centre staff (Appendix A-3). Data collection, cleaning, and updating is undertaken by a large number of people without whom this work would not have been possible.

In 1999 I worked as part of the Million Women Study team for a year, assisting with the processing of the first resurvey; some of this data has formed the basis for some exposure variables in this work.

A small number of Million Women Study variables are derived from information from more than one questionnaire, and were created by study team data analysts for use in analyses related to the study. Apart from these I have coded all of my own variables for use in these analyses directly from the self-reported questionnaire values held in the study database. I have written and performed all the statistical analyses used in this thesis using STATA 13 (1), with the exception of the competing hazards model used for the vulval cancer histological subgroup analysis, which was initially written by my co-supervisor Dr Barnes. All plots are my own, with the exception of the Forest plots, which have been done in R by Dr Barnes.

Dr Kezia Gaitskell has assisted with histopathological classification of the various anogenital cancers, providing initial suggestions of pathological groupings, and checking the ICDO-3 tables for me.

The National Health Service Cervical Screening Programme data linkage occurred during my time at the Cancer Epidemiology Unit. I was involved with checking the pilot data, which included linked records for 10,000 women. Anna Brown performed initial checks on this data, and the final linkage was managed by Rupert Alison and Roger Blanks. An enormous amount of preliminary work went into getting the screening data into usable form, and most of this was done by Rupert Alison. A group including Professor Dame Beral, Professor Julietta Patnick, Professor Jane Green, Dr Gillian Reeves, Dr Isobel Barnes, Dr Roger Blanks, Rupert Alison and myself met several times to co-ordinate the linkage and preparation of the screening data.

Included as Appendix C-2 is a publication relating to another study I worked on during my DPhil. This was an examination of risk factors for uterine fibroids (a benign uterine muscle tumour) in postmenopausal women. This work further developed a project started some years earlier by a colleague, Dr Eva Sommer. She performed the initial analyses and wrote an early draft of the paper. I updated the work to include two further years of Hospital Episode Statistics data on cases, and rewrote the paper to publication standard, in collaboration with Dr Sommer and several other co-authors.

Professor Dame Valerie Beral and Dr Isobel Barnes have overseen and supported my work throughout the DPhil, assisting with planning the analyses, checking results, and reading drafts of published work and chapters. Any residual errors are my own.

Acknowledgements

This research would not have been possible without the generosity of more than 1.3 million women in the United Kingdom who took part in The Million Women Study, freely giving their time and information to assist research into breast cancer and other important health conditions affecting women later in life. The Million Women Study was conceived of and has been led by Professor Dame Valerie Beral, whose interest in women's health has driven her, and those of us mentored by her, to keep asking questions and trying to answer them. I thank her for the opportunity to work on the project, and for her supervision throughout the DPhil.

I would also like to thank Dr Isobel Barnes, who has been my co-supervisor, providing statistical support, encouragement and who undertook the final preparation of the screening data with grace and speed. Professor Jane Green, Professor Gillian Reeves, Professor Tim Key, and Dr Ben Cairns, whilst not officially my supervisors, have provided assistance on multiple occasions, with proof-reading, ideas, and thought-provoking discussion.

Professor Max Parkin, Dr Toral Gathani, Professor Terry Dwyer, and Associate Professor Ruth Travis have been my Examiners for Transfer and Confirmation of Status, and provided criticism, insight and helpful suggestions that have helped to guide me in writing this DPhil thesis.

The Million Women Study is run as a team effort. This research study, amongst many others based on the data that has been collected, scanned, checked, entered, and checked again, would not have been possible without the co-ordinating centre staff, who are too numerous to list here, but each play a pivotal role in running the study on a day-to-day basis. Hayley Abbiss, Professor Beral's PA, has been a life-saver on numerous occasions and has earned my eternal gratitude a number of times over for her efficiency and kindness.

Professor Julietta Patnick, Dr Roger Blanks, and Rupert Alison are part of the Cancer Screening Programmes Research Group, and have played an important part in the acquisition and preparation of the NHS Cervical Cancer Screening Programme data which we received relatively late in the day. Their hard work has been invaluable.

Dr Kezia Gaitskell provided much-needed assistance with the categorization of the cancers into histopathological subtypes, introduced me to the 'Blue book', and in general has been an inspiring and patient office-mate, with the eyes of an eagle when it comes to typographical errors. Kirstin Pirie has provided my data 'views' and been an inspiration in terms of coding. Angela Balkwill, Anna Brown, Barbara Crossley, Dave Ewart and Andrew Chadwick have cheerfully and patiently helped me in all manner of ways with data, Stata, and IT needs. Barabara kindly read an early version of the methods chapter.

Gabriele Price at the Oxford Cancer Registry met with me very early on in the DPhil and took me around the registry, giving me an understanding of how the registry collects data and deals with it. Kathryn Bradbury, Julie Schmidt , Sarah Floud Lewis Rowett, and Mary Foulkes are friends and colleagues and have made work like home. Dr Anne Edwards, my colposcopy trainer, and Drs Celia Devenish, Wayne Gillett, Helen Paterson in New Zealand have been my clinical inspiration for this work, and have supported me from afar.

This work would not have been possible without an enormous amount of support on the home front. Bethan Foulkes has been a fantastic and loving nanny to my children at very short notice. Anna Adsetts and Barnaby Kemp stepped and took the evacuees at the 9th hour, as well as providing us with friendship, laughter and white Russians for two years. My mother Marilyn Murphy flew half-way around the world to help us in 2009, and stayed: thank you mum. Finally I would like to thank Sean, Ciara, Ashling and Oscar, who are the best family one could wish for and are an inspiration to me always. Maureen Watson provided me with office space and cups of tea for my revisions, thank you!

This thesis is dedicated to Dr Bill Clow my first Consultant on labour ward as a registrar, who always had time to answer questions, and was always asking new questions well into his 70s. Bill sadly died of non-Hodgkin's lymphoma on 21 November 2014, but will not be forgotten.

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Abbreviations

BMI	Body mass index
CIN	Cervical intraepithelial neoplasia
CIN 1/2/3	Cervical intraepithelial neoplasia grade 1, 2 or 3
CI	Confidence interval
FIGO	International Federation of Gynaecology and Obstetrics
GP	General Practitioner
HR	Hazard ratio
HT	(Menopausal) hormone therapy
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HrHPV	High-risk human papillomavirus
ICD-10	International Classification of Diseases, version 10
ICDO-3	International Classification of Diseases for Oncology, version 3
IRR	Incidence rate ratio
LBC	Liquid-based cytology
MSM	Men who have sex with men
MWS	Million Women Study
NHAIS	National Health Application and Infrastructure Services
NHS	National Health Service

NCIN	National Cancer Intelligence Network
NCR	National Cancer Registry
NHSBSP	National Health Service Breast Screening Programme
NHSCSP	National Health Service Cervical Screening Programme
OC	Oral contraceptive pill
OR	Odds ratio
ONS	Office of National Statistics
RR	Relative risk
SD	Standard deviation
SIR	Standardised incidence ratio
STI	Sexually transmitted infection
UK	United Kingdom
US	United States of America
WHO	World Health Organisation

Introduction

1.1 Rationale and outline of the thesis

Anal, vulval and vaginal cancers are rare malignancies that have strong anatomical, histological, and aetiological relationships with cervical cancer. Anal, vulval and vaginal cancers occur uncommonly in premenopausal women, with over 85% of registrations seen after 50 years of age. Risk factors for these cancers, other than high-risk human papillomavirus, are not well defined in older women.

This thesis focuses on the non-cervical anogenital cancers of the anus, vulva and vagina, which have not previously been examined in large-scale prospective studies in postmenopausal women.

The understanding that high-risk strains of the human papillomavirus (HrHPVs) are a 'necessary but not sufficient' (2) cause of cervical cancer has gained wide acceptance. Anal, vulval and vaginal cancers share persistent HrHPV infection as an aetiological precursor, with varying degrees of association; it is generally accepted that the majority of cases of anal and vaginal cancer are likely to be caused by HrHPVs, while around half of vulval cancer cases have commonly also been attributed to the virus (3).

This thesis firstly examines the incidence of anal, vulval and vaginal cancer in the context of gynaecological (female reproductive system) cancers and all cancers in the United Kingdom (Chapter 2). Next, in chapters 4, 5 and 6 the incidence by cancer type is calculated within the Million Women Study cohort and associations between the

individual cancers and various lifestyle, reproductive and other lifetime risk factors are investigated using prospectively collected self-reported exposure data in adjusted multivariate Cox regression models. Thirdly, using routinely captured National Health Service Cervical Cancer Screening Programme (NHSCSP) data, an examination of the association between cervical screening history, prior registration of cervical carcinoma *in situ* (or cervical intraepithelial neoplasia grade 3, hereafter referred to as CIN 3) and anogenital cancer risk at older ages was carried out (Chapter 7).

Broadly, the aims of this thesis were to answer the following questions:

1. What are the incidences of anal, vulval and vaginal cancers in women over the age of 50 in the United Kingdom, and within the Million Women Study cohort?
2. What exposures are significantly associated with risk of incident anal, vulval and vaginal cancer in women aged over 50 in the Million Women study cohort? Do the associations vary by cancer type and histological subtypes within cancers?
3. Are there different associations between risk of anal, vulval and vaginal cancer and attendance or non-attendance for cervical cancer screening? Can nationally collected data on previous cervical smear abnormalities and prior registrations of cervical intraepithelial neoplasia grade 3 (CIN 3) be used to identify women at an increased risk of subsequent non-cervical anogenital cancers in the cohort?

1.2 Publications relating to this thesis

During the preparation of this thesis, I wrote two publications and co-wrote a third. The two first-author publications relate directly to the work presented here, and the third which I co-wrote with Dr Eva Sommer examined uterine fibroids in the Million Women Study cohort.

These are included in appendix C:

Coffey K, Beral V, Green J, Reeves G, Barnes I. Lifestyle and reproductive risk factors associated with anal cancer in women aged over 50 years. *Br J Cancer*. 2015; 112(9): 1568-74. doi: 10.1038/bjc.2015.89

Sommer EM, Balkwill A, Reeves G, Green J, Beral V, Coffey K. Effects of obesity and hormone therapy on surgically-confirmed fibroids in postmenopausal women. *Eur J Epidemiol*. 2015 Jun;30(6):493-9. doi: 10.1007/s10654-015-0016-7.

Coffey K, Gaitskell K, Beral V, Canfell K, Green J, Reeves G, Barnes I. Risk factors for vulval cancer in postmenopausal women vary by tumour histopathological subtype. [Accepted with revisions to British Journal of Cancer].

Chapter 2 Female anogenital cancers in England: background & epidemiology

2.1 Introduction

Anogenital cancers include anal, vulval, vaginal and cervical cancers. These anatomically and aetiologically-related cancers affect women throughout their lives with varying age-specific incidences. Cervical cancer is now most common during the reproductive years. Its incidence later in life is probably altered by screening, whereas anal, vulval and vaginal cancers are uncommon in younger women, but increase in incidence with age, rarely occurring before the age of fifty.

As all four cancers are known to be associated to a greater or lesser extent with high-risk human papillomavirus infection, it is notable that there are differences not only in their age distribution, but also in the exposures with which they are associated. This will be discussed in further detail in subsequent chapters, and is the main focus of this thesis. Because cervical cancer has been well-studied, with a natural history and epidemiology that have been the subject of a considerable amount of research over the past fifty years, I have chosen to examine the less well-investigated anogenital cancers, those of the anus, vulva and vagina.

As a background for the rest of the thesis, this chapter provides a review of the anogenital cancers examined in this work, with a brief summary of anogenital anatomy and histopathology, an introduction to the specific cancers of interest, and a survey of the descriptive epidemiology of anal, vulval and vaginal cancers in the context of female cancer registrations in England for 2012, using publicly available data from the Office of National Statistics.

2.2 The anogenital cancers

An outline of the regional anatomy and histology, and an introduction to the cancers discussed in this thesis follow below. Apart from their physical proximity, the anal, vulval and vaginal regions share many histological features which are important to be aware of when considering the similarities and potential differences in the aetiology of these cancers.

2.2.1 Anal anatomy and histology

The anal canal extends from the perianal skin externally, passes through the anorectal ring, and ends in the terminal part of the large intestine. The histological anal canal is usually around three centimetres long, beginning at the anorectal junction (Fig 2.1), and ends where the anal margin meets the perianal skin (4).

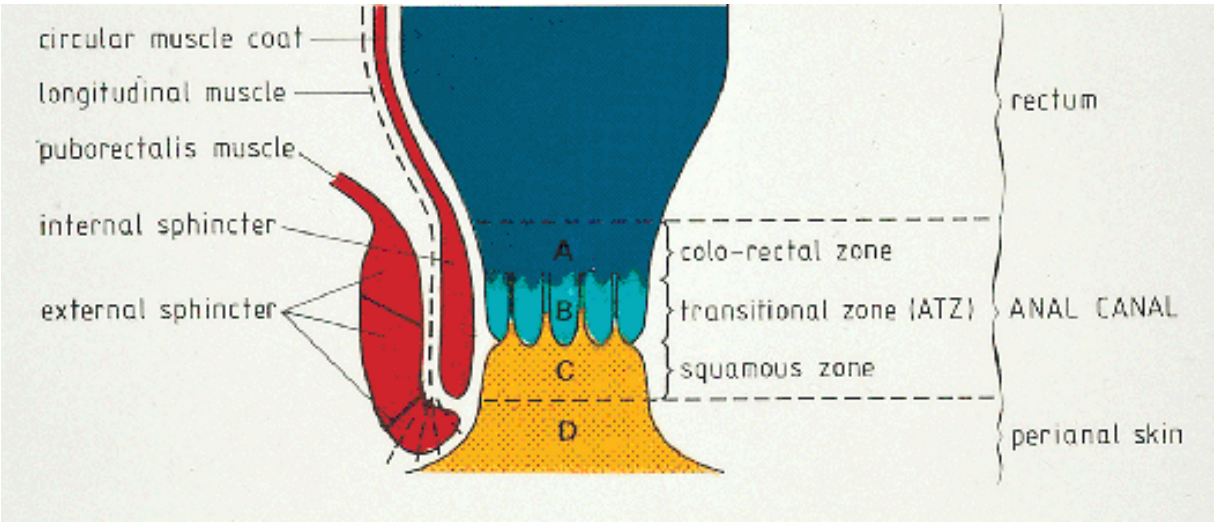


Figure 2-1 Schematic drawing of anal canal showing relationship with rectum and anal skin (5)

Histologically, the anal canal can be divided into three zones, which have different epithelial linings. The uppermost zone is covered by colorectal mucosa, which is glandular epithelium. The lower zone is covered with non-keratinised squamous epithelium, similar to that seen on the ectocervix and in the vestibule of the vulva. Between these two zones is the anal transitional zone (ATZ), which is a transitional zone between the borders of the squamous and glandular epithelia (6). The skin of the anal margin is in close proximity to, but not continuous with the skin of the vulva, being separated by the perineum.

2.2.2 Anal cancer

Anal cancer is a rare malignancy that is more common in women than in men, and is increasing in incidence in the UK (7). Anal cancers make up around 4% of lower gastrointestinal malignancies (8), and tumours in the anal canal are most commonly squamous cell carcinomas, with 80-85% of anal cancers reported to be of squamous cell morphology. Anal cancer of any histological subtype that is included within International Classification of Diseases version 10 code C21 (9) is examined in this work.

The anal canal is physically continuous with the rectum above, and the perianal skin below. Tumours in those areas are classified separately in ICD-10: those involving the anorectal junction are classified as rectal tumours (C20) if the epicentre of the tumour is more than 2 centimetres above (cranial to) the dentate line, whereas cancers arising in the perianal skin are biologically more similar to skin cancers, and are staged according to the classification for cancers of the skin (C44.5) (4). Rectal and anal skin cancers are not examined in this thesis.

HrHPV has been shown to be the most important aetiological factor in the development of anal cancer (3,10), and is reported to be responsible for 80-90% of anal cancers, with a natural history analogous to that seen in the cervical epithelium. Anal HrHPV infection causes anal intraepithelial neoplasia (AIN), a spectrum of disease including low-grade lesions, which are not thought to be precancerous, and high-grade lesions that may progress to invasive disease (8).

Anal lesions, like cervical lesions, may be sampled cytologically and treated at a pre-cancerous stage. There has been interest internationally in the concept of anal cancer screening, and it has been used in selected high-risk populations and in a research context (11), however there is currently no anal cancer screening programme in the UK. *In situ* anal cancers are not considered further here, but the parallels with cervical cancer and its preinvasive spectrum of disease are interesting and worth bearing in mind.

2.2.3 Vulval anatomy and histology

The vulva comprises the external female genitalia including the labia major, labia minora, prepuce, frenulum, clitoris and vestibule (Fig. 2.2). It also houses two sets of paired glands, the paraurethral (formerly 'Skene's duct') glands, and the Bartholin glands, as well as the minor vestibular glands. The urethra (urinary tract) opens into the vulval vestibule. The vulva is distal to vagina, and opens into the vagina. It is also in close proximity to the anus posteriorly, being separated from the anus by the perineal body. The close proximity of the vulva, vagina and anus can be appreciated in the illustration below.

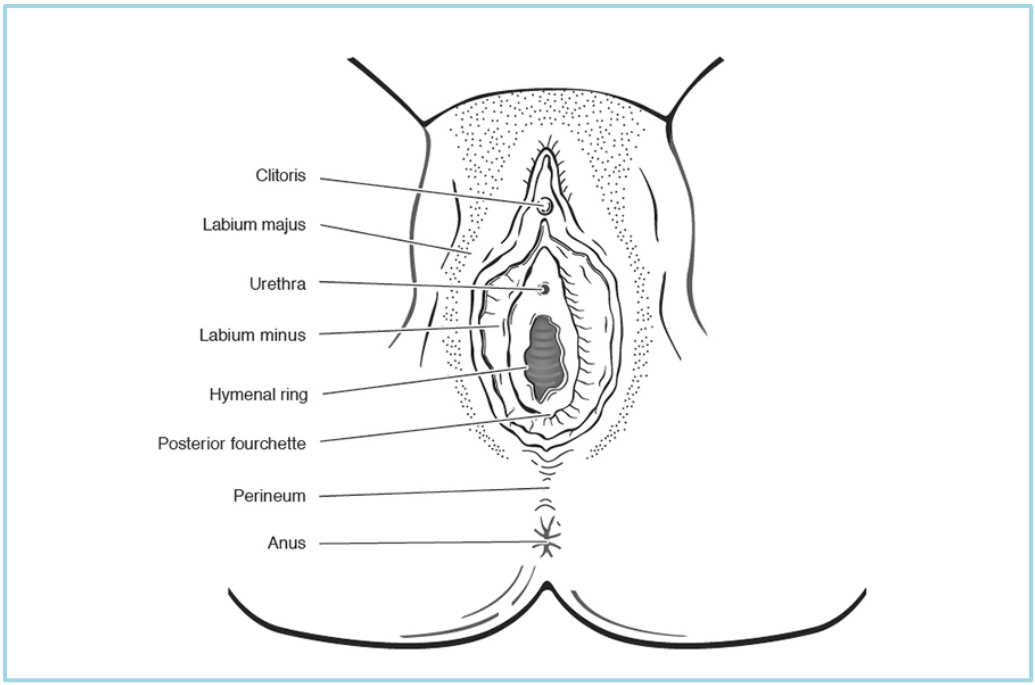


Figure 2-2 Anatomy of the vulva

(from www.rcpa.edu.au/getattachment/b1e7ad91-c0d5-46c4-9532-9173fd9ed9c1/Vulva-1.aspx, Reproduced here with permission of the Royal College of Pathologists of Australasia)

Histologically, like the anus and cervix, the vulva has a mix of squamous and glandular tissues. The external portions of the vulva are covered with keratinised, stratified squamous epithelium. The labia majora contain adipose tissue and smooth muscle, while the labia minora contain elastic tissue and blood vessels. The non-keratinised squamous epithelium of the vestibule meets transitional epithelium at the urethral meatus, and glandular epithelium at the duct openings of the vulval glands (12). The mix of squamous and glandular tissues means that, like the anus, the vulva can be the site of several histological subtypes of cancer, most commonly squamous cell carcinomas, but also glandular tumours as well as other, rarer tumour morphologies.

2.2.4 Vulval neoplasia

Vulval neoplasia includes pre-invasive and invasive lesions, similar to the disease seen elsewhere in the anogenital region. This thesis focusses on invasive cancers, some of which arise from or in a background of vulval intraepithelial neoplasia (VIN).

2.2.4.1 Vulval intraepithelial neoplasia

There are three histological sub-types of VIN: warty (condylomatous), basaloid, and differentiated. The first two are typically considered to be related to human papillomavirus infection, and are histologically very similar to the intraepithelial lesions caused by HPV seen elsewhere in the genital tract (i.e. CIN and AIN). Differentiated VIN is associated with a thickening of the epithelium and other skin changes; it is often found in association with a chronic vulval skin condition known as lichen sclerosis(13).

There are thought to be two aetiological pathways to vulval cancer, the first is HrHPV-associated, and is considered to parallel other anogenital HPV-related malignancies, arising within areas of HrHPV-related warty or basaloid VIN. The second, more common pathway is independent of HPV, and is considered to be associated with vulval dermatoses such as lichen sclerosis and differentiated VIN; this pathway is thought to predominate at older ages (14).

2.2.4.2 Vulval cancer

Vulval cancer is typically thought of as a disease of older women, and the incidence of vulval cancer markedly increases with age; but it has been reported that in some settings vulval cancer is also becoming more common at younger ages (15). Women with vulval cancer often present symptomatically, most commonly with pain, bleeding or pruritus (itching). Squamous tumours may present as 'exophytic', warty masses, or 'endophytic', ulcerated lesions. The labia majora and labia minora are the most common tumour sites, with the clitoris less commonly involved (in 5-15% of cases). Most vulval cancers are solitary, with fewer than 10% involving multiple foci (13).

The majority of vulval tumours are of squamous cell morphology; the UK National Cancer Intelligence Network (NCIN) reported that 83% of vulval cancers registered over the 2007-2010 triennium were of squamous cell morphology (16). Glandular tumours are the next most commonly seen subtype, and these arise mainly from the Bartholin glands or other skin appendages; melanocytic tumours, sarcomas, and other even rarer subtypes are also infrequently seen.

2.2.5 Vaginal anatomy and histology

Anatomically, the vagina is an orifice extending from the vulva to the cervix (Figure 2.3). Circumferentially fused to the distal portion of the uterine cervix, the anterior, posterior and lateral fornices of the vagina abut the *portio vaginalis* of the cervix. The vagina is a midline tubular structure, which in its resting state is generally partially collapsed. It measures approximately 9 cm in length in the adult female. The anterior and posterior walls of the vagina are in contact with each other, apart from the cranial (highest) portion of the vagina, in the region of the vaginal vault. The vagina is in close proximity to the urethra, urinary bladder, the vulva, the uterine cervix, and the rectum posteriorly (17).

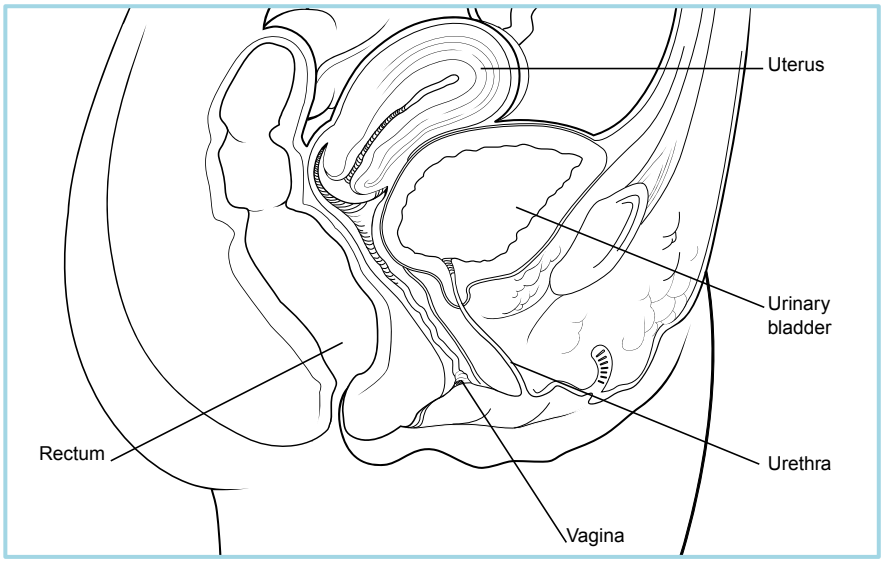


Figure 2-3 Sagittal view of the female reproductive organs

(from http://images.medscape.com/pi/features/ald/repro/reft-s.pdf.

© WebMD, image reproduced with permission from Medscape, 2015)

Histologically, the vaginal wall has three layers: the mucosa, which is a non-keratinising stratified squamous epithelium (like that covering the ectocervix, and the vulval vestibule); the muscularis, consisting of inner circular, and outer longitudinal layers of smooth muscle; and the adventitia, a thin layer of dense connective tissue containing the nerve bundles, lymphatics, and venous plexuses (17). The mature vaginal squamous epithelium is very similar to the squamous epithelium of the anus, vulva and cervix, and shows progressive maturation of the epithelial cells as they rise through the epithelium, from the basal layer to the surface (see Fig 2.4 below).

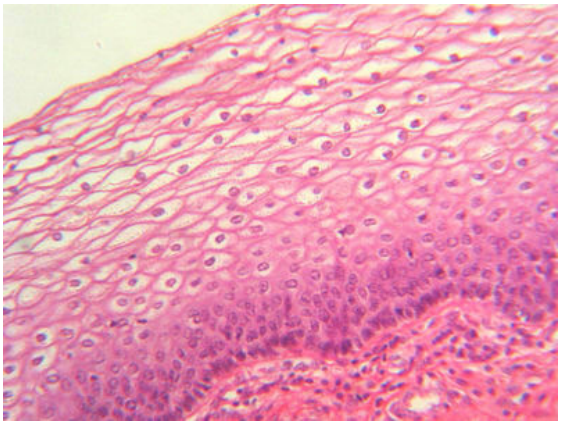


Figure 2-4 Normal vaginal squamous epithelium (courtesy of Valory Thatcher, Ph.D., Instructor of Anatomy & Physiology - Microbiology, Mt. Hood Community College)

2.2.6 Vaginal cancer

Vaginal neoplasia is rarer than cervical or vulval disease, despite a common aetiopathology; the reason for this discrepancy is not known (17). HrHPV-related vaginal epithelial lesions have a similar appearance and nomenclature to those of the other anogenital neoplasias, and are known as vaginal intraepithelial neoplasia (VAIN). Similarly, they are graded 1-3 depending upon the thickness of the epithelial involvement. Some vaginal cancers are preceded by VAIN lesions, however the natural history and malignant potential of VAIN are not certain (18), and the natural history of VAIN is not as well-understood as intraepithelial neoplasia at other anogenital sites. Interest is confined to invasive cancer in this study, and *in situ* disease of the vagina is not further explored.

Primary vaginal cancers account for around 1-2% of all gynaecological cancers (18–20). The majority of tumours that arise in the vagina are in fact metastatic disease--either direct spread from other genital sites, or distant metastasis of non-gynaecological tumours e.g. bladder, urethra, rectum, and more unusually from the breast, lung, or other more distant primary sites.

The examination of vaginal cancers to here is confined to primary registrations, International Classification of Diseases version 10 (ICD-10) C52. FIGO (International Federation of Gynaecology and Obstetrics) guidelines are quite restrictive, and mandate that vaginal cancers involving the cervix or vulva are classified as primary cervical or vulval cancer, respectively (18), so these are not included amongst our cases.

It is commonly stated that 80-90% of vaginal cancers are of squamous cell morphology (17,18,21), however a somewhat different distribution is reported later in this study, and these findings accord well with recently published national vaginal cancer morphology figures for England (16). Much of the literature in recent years that has examined anogenital cancers has concentrated on HrHPV as a risk factor, therefore it is possible that there has been over-selection of squamous tumours in published case series and studies. Adenocarcinomas are the next most frequently occurring tumour type.

Most women with vaginal cancers present with abnormal vaginal bleeding and discharge (20). In the recent past, there was a group of women who were at high risk of vaginal cancer, and these were women exposed to diethylstilboestrol (DES) *in utero*. DES is an exogenous hormone, and is associated with clear cell carcinoma of the vagina in young women with a reported mean age of 26 years at diagnosis (22). It was given to pregnant women to provide hormonal (luteal) support in pregnancies affected by threatened miscarriage, or where there was a poor reproductive history (23). Women who are known to have been exposed may request lifelong follow-up with annual pelvic examination and colposcopy in the United Kingdom, as they have around a 1 in 1000 risk of vaginal cancer. There were 3 cases of clear cell carcinoma of the vagina in the cohort; however our participants are older than the age at which DES-related malignancy commonly presents. It is possible that there are a small number of exposed women in the cohort, as the widespread use of DES began in the 1940s (24), and was not discontinued until the 1970s.

Vaginal cancer is a disease of older women; its incidence peaks in the 6th and 7th decades of life. Previously reported risk factors for squamous cell vaginal cancer are similar to those seen in cervical and anal cancer: multiple sexual partners, early age at first intercourse, low socioeconomic status, a history of genital warts or other HrHPV-related abnormality (25,26). Hysterectomy, chronic irritation, physical trauma to the vaginal epithelium by pessaries (used to conservatively manage vaginal and uterine prolapse), and cervical irradiation have also been suggested as risk factors for vaginal cancer (27).

2.3 Anogenital and gynaecological cancer registrations in England

The cancer registration system in England, which aims to collect standardised information on all cancers, is both well-organised and long-standing. The mechanics of data collection by the regional cancer registries are complex, but are briefly discussed further in the methods chapter (Chapter 3).

To provide a background for the studies of risk factors for individual anogenital cancers undertaken in this thesis, the descriptive epidemiology of anal, vulval, vaginal and cervical cancer in England is first described. The incidence of cancer at these sites is reviewed by age, looking at anogenital cancer registrations relative to other gynaecological cancers, and as a subset of all female cancers. The nationally collated dataset on cancer registrations used here is updated annually and is publicly available (19).

Within female cancer registrations, gynaecological cancers make up a greater proportion of all cancers during the reproductive years due to the relatively large number of cervical cancers (and a slightly lesser but still significant number of ovarian cancers) that occur in women under 50 years of age, when other types of cancer are still relatively rare (Table 2.1).

There were 16,931 gynaecological cancers registered at all ages in England in 2012, making up 12.3% of female cancer registrations (n=137,712, excluding non-melanoma skin cancers, C44). At younger ages, gynaecological cancers are a more important contributor to total cancer registrations, accounting for 16.4% of total registrations (3,313 of 20,197) in women under the age of 50. In women over the age of 50, when cancers at other sites become more

common, although the absolute number of gynaecological cancer registrations increase, they make up just under 12% of the total cancer burden (13,618 of 117,515 cancers).

An examination of the proportion of anogenital cancers shows that while around three-fifths of cervical cancers are registered prior to age 50, for anal, vulval and vaginal cancers, the great majority of cases, more than 85% of registrations, happen after the age of 50 (Table 2-1, final column). The detection and treatment of cervical pre-cancers means that treated women almost certainly do not go on to develop the same number of cervical cancers in later life that they would in the absence of organised screening. However, it is also possible that the natural histories of these related cancers differ to a certain extent.

Table 2-1 Female cancer registrations, England 2012: by ICD-10 code and site

from Office of National Statistics. Cancer Statistics Registrations, England, Series MB1, 2014 (19).

ICD-10 code	Site description	All ages	All <50	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85+	Cases > 50 (n)	% cases in >50s
C00-C97	**All cancers**	**172,563**	**23,248**	**11,411**	**12,829**	**17,772**	**21,955**	**20,247**	**21,172**	**19,919**	**24,010**	**149,315**	**86.5%**
C00-C97 (excl. C44)	**All cancers excluding NMSC**	**137,712**	**20,197**	**9,784**	**10,859**	**14,807**	**17,792**	**15,953**	**16,311**	**14,937**	**17,072**	**117,515**	**85.3%**
C21	**Malignant neoplasm of anus**	**665**	81	66	82	84	74	78	76	63	61	**584**	**87.8%**
C51	**Malignant neoplasm of vulva**	**1,052**	143	69	68	98	93	139	147	127	168	**909**	**86.4%**
C52	**Malignant neoplasm of vagina**	**210**	24	14	21	26	21	24	27	27	26	**186**	**88.6%**
C53	**Malignant neoplasm of cervix uteri**	**2,482**	1517	164	179	120	126	104	106	73	93	**965**	**38.9%**
C21, C51-53	**All anogenital cancers**	**4,409**	**1765**	**313**	**350**	**328**	**314**	**345**	**356**	**290**	**348**	**2,644**	**60.0%**
	% of gynaecological cancers	**26%**	**53.3%**	**24.2%**	**21.1**	**16.4**	**13.7**	**17.4**	**19.5**	**21.5**	**28.5**	**19.4**	**-**
C54	**Malignant neoplasm of corpus uteri**	**6,946**	450	537	839	1,061	1,197	990	872	562	438	**6,496**	**93.5%**
C55	**Malignant neoplasm of uterus , NOS**	**246**	46	25	18	20	28	26	27	24	32	**200**	**81.3%**
C56	**Malignant neoplasm of ovary**	**5,582**	1091	445	499	629	736	645	601	499	437	**4,491**	**80.5%**
C57	**Malignant neoplasm of other female genital organs**	**402**	32	37	32	48	86	54	48	36	29	**370**	**92.0%**
C58	**Malignant neoplasm of placenta**	**11**	10	1	0	0	0	0	0	0	0	**1**	**9.1%**
C51-C58	**All gynaecological cancers**	**16,931**	**3,313**	**1,292**	**1,656**	**2,002**	**2,287**	**1,982**	**1,828**	**1,348**	**1,223**	13,618	80.4%

2.4 Anogenital cancers as a subset of gynaecological cancer registrations

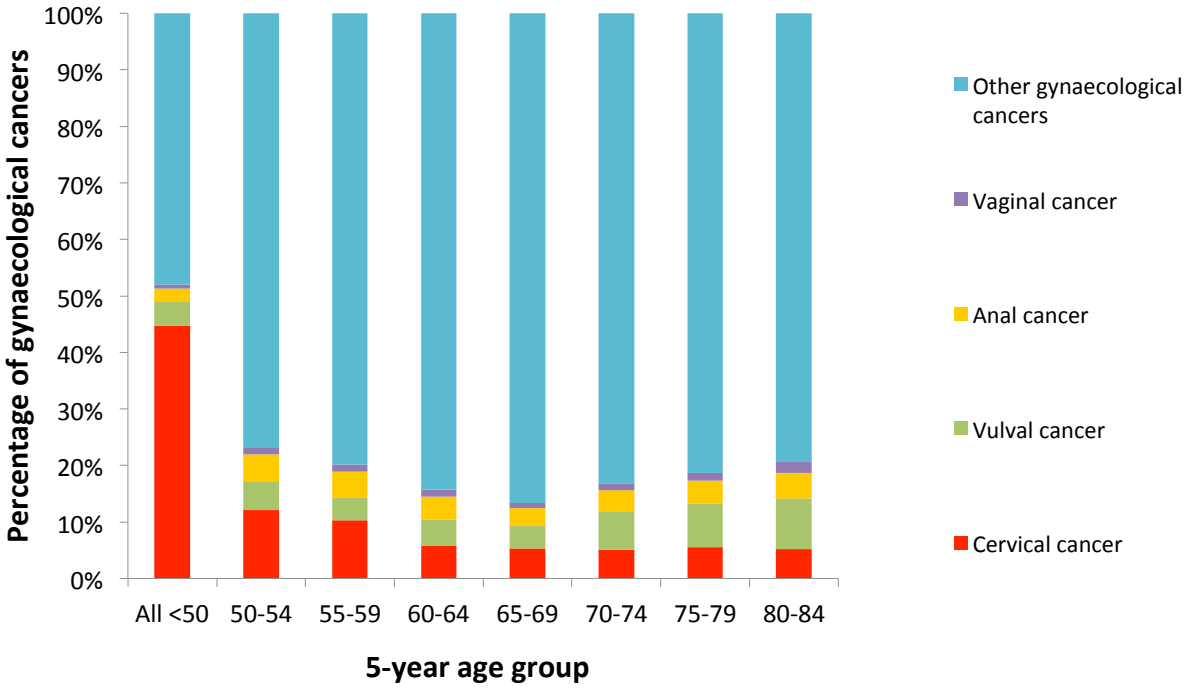


Figure 2-5 Anogenital cancers as a proportion of total gynaecological cancer registrations by 5-year age group, England 2012.

In England in 2012, anogenital cancers accounted for 2% of all female cancers registered, but made up over a quarter (26.1%) of total gynaecological cancer registrations at all ages (Figure 2.5). At younger ages, anogenital cancers accounted for just over half of the gynaecological cancers. This is chiefly due to the high incidence of cervical cancer in women under 50; in the over 50 age groups anogenital cancers make up 20% or fewer of the gynaecological cancers.

2.5 Age-specific incidence of anogenital cancers by site

Figure 2.6 below shows the age-specific incidences of the four anogenital cancers by 5-year age groups, starting at age 25. The incidences of these cancers prior to age 40 are almost nil, apart from cervical cancer, which falls from an age-specific rate of just over 20 per 100,000 between ages 25-29, more than halving in incidence to around 8 per 100,000 between ages 60-64. Cervical cancer remains the most common anogenital malignancy until age 70, when vulval cancer overtakes it. Anal, vulval and vaginal cancer incidence begins to rise around age 40, and these increase almost linearly in incidence until age 65-69, when there is a striking increase in vulval cancer cases.

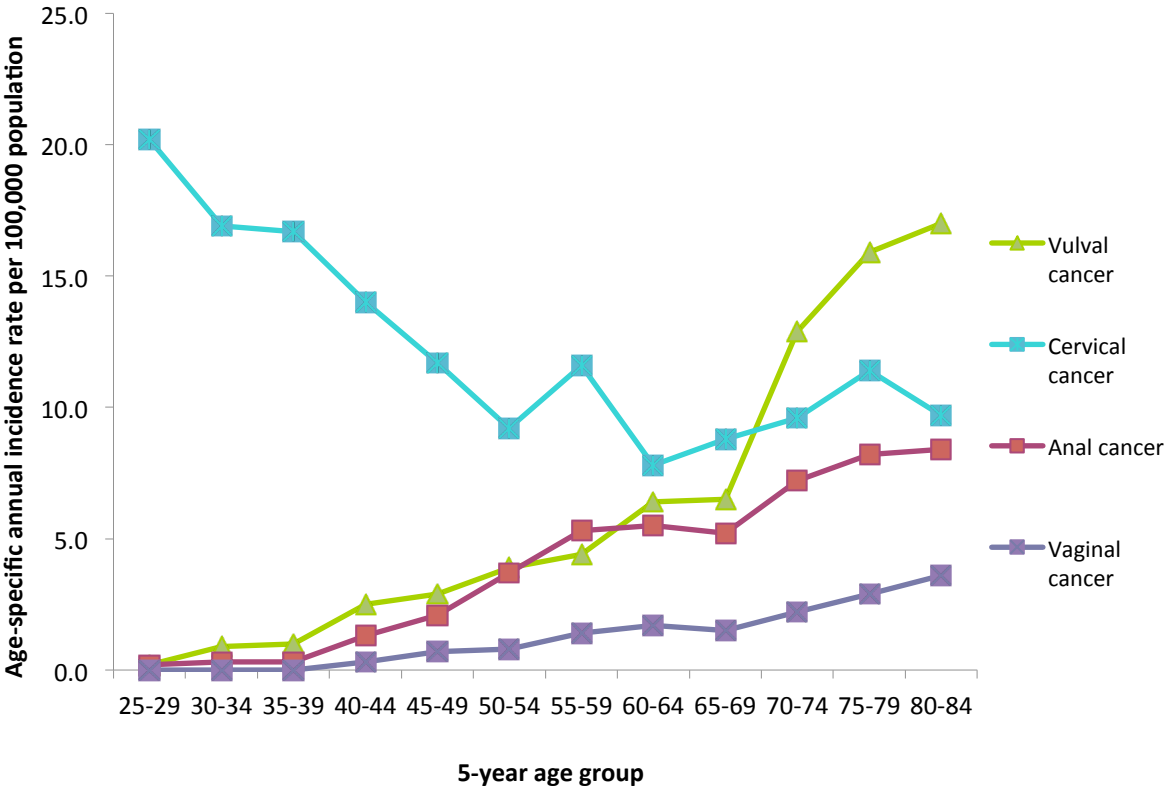


Figure 2-6 Age-specific annual incidence of anogenital cancers per 100,000 population, England, 2012, by 5-year age group starting at age 25

2.6 Trends in anal, vulval and vaginal cancer incidence over time

Figure 2.7 below shows female anal, vulval and vaginal cancer rates for England 1979-2012 (19). Anal cancer incidence has increased from 0.8 per 100,000 to 2.5 per 100,000 over three decades, which is over a three-fold increase in incidence. Male rates (not shown here) have also increased, albeit more slowly. The reason for the marked increase in anal cancer incidence is not fully understood, but the strong relationship between anal cancer and HrHPV, which is further discussed below and in chapter 4, may be the reason, along with changes in the prevalence of female smoking, and changes in sexual behaviour. The incidence of vulval and vaginal cancer, in contrast, has remained relatively stable.

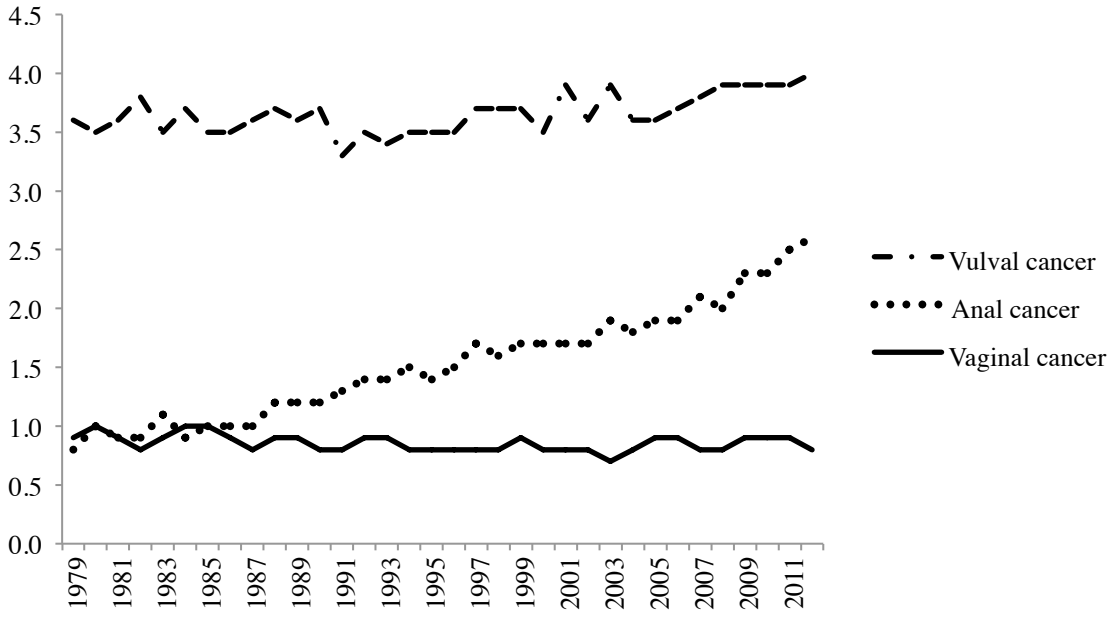


Figure 2.7 Age-standardised incidence of female anal, vulval and vaginal cancers in England 1979-2012 (19).

2.7 Conclusion

In the following chapters the epidemiology of anal, vulval and vaginal cancers in women over the age of 50 in the United Kingdom will be reviewed, and, using data from the Million Women Study cohort, associations between various reproductive and lifestyle exposures and these rare cancers will be examined.

Though all are related to high-risk human papillomavirus (HrHPV) infection, and thus share common relationships with some exposures related to HrHPV, there are striking differences between the cancers which will be explored further.

Chapter 3 Materials and Methods

3.1 Background to the Million Women Study

The Million Women Study is a population-based cohort study, which began recruiting women via the National Health Service Breast Screening Programme (NHSBSP) in May 1996 (28). The study was established by Professor Valerie Beral and a team of collaborators based at the Cancer Epidemiology Unit in Oxford, who wished to examine whether exogenous hormones, in the form of menopausal hormone therapy (HT), affected the risk of breast cancer in a screened population. The group also planned to look at the effect of HT on other outcomes that affected health in older women.

The study was designed to have 80% power to detect a relative risk of 1.1 of breast cancer in current HT users and current users of more than 5 years duration, when compared with women who had never used HT. In order to achieve this, the aim was to recruit 1 million participants by the year 2000.

3.2 Pilot study

Women were invited to participate in the study via a postal questionnaire, which was included with their routine breast screening invitation. A pilot study (29), confirmed that inclusion of the study questionnaire with the postal screening invitation did not affect attendance at breast screening.

This small randomised study included 6400 women in the Oxfordshire and West London regions, and found that screening attendance was 71% in both groups, those who received the study questionnaire with their invitation, and those who did not. Acceptability of

recruitment, questionnaire content, and how the questionnaire should be returned to the study centre were also examined. 77% of women who attended for screening returned a completed questionnaire; this was not affected by method of return.

3.3 Recruitment

Participants were recruited via their local NHS Breast Screening centre. England and Scotland are split into 10 cancer registry regions, each of which has several screening centres. Questionnaires were posted to women with their screening invitation. For practical reasons, some centres did not participate in the study; principally this was due to logistical issues surrounding the posting of questionnaires; some centres had difficulty including the questionnaire with the invitation and other information normally posted to women for their routine screening.

Recruitment began in 1996, and continued until the end of 2001. Women could bring their completed questionnaire with them to their breast screening appointment, or post it back to the Cancer Epidemiology Unit. Ultimately, over 1.3 million women returned a completed questionnaire.

Participating centres are shown on the map seen in Figure 3.1 overleaf, and include the centres listed in Table 3.1.

Figure 3-1 Map of the United Kingdom showing National Health Service breast screening centres* that participated in Million Women Study recruitment.

*** Wirral, East Sussex and Kent & Canterbury are not shown.**

Table 3-1 NHS Breast Screening Centres involved in recruitment to the study, including centre names, and number of participants recruited by centre

Region	Percent (n)	Centres	Participants
Oxford	6.3% (86,319)	Aylesbury (KAY)	0.5 (6,973)
		Wycombe (KHW)	1.1 (14,646)
		Milton Keynes (KMK)	0.6 (8,161)
		Oxford (KOX)	2.1 (27,970)
		West Berkshire/Reading (KRG)	1.0 (13,041)
		East Berkshire (KWI)	1.1 (15,528)
East Anglia	5.1% (69,372)	Cambridge (DCB)	1.6 (21,447)
		Great Yarmouth (DGY)	0.7 (9,885)
		Kings Lynn (DKL)	0.7 (9,269)
		East Suffolk (DSU)	1.2 (15,714)
		West Suffolk (DSW)	0.8 (10,799)
		Befordshire & Hertfordshire (ELD)	0.2 (2,258)
South West	20.8% (283,629)	Basingstoke (JBA)	0.8 (10,571)
		Dorset (JDO)	2.7 (36,325)
		Portsmouth (JPO)	1.8 (23,937)
		Swindon (JSW)	1.7 (22,987)
		Winchester (JWB)	0.8 (10,485)
		Avon (LAV)	3.5 (48,017)
		Cornwall (LCO)	1.8 (24,680)
		East Devon (LED)	2.1 (28,217)
		Gloucester (LGL)	2.0 (26,841)
		West Devon (LPL)	2.1 (28,255)
		Somerset/Bridgwater (LSO)	1.7 (23,314)
Thames	13.5% (183,490)	West London (ECX)	3.1 (41,576)
		Chelmsford & Colchester (FCO)	2.5 (33,966)
		North Middlesex (FNM)	0.9 (11,834)
		South Essex (FSO)	2.4 (32,424)
		Surrey (HGU)	1.2 (16,408)
		West Sussex (Worthing) (HWO)	3.1 (41,845)
		East Sussex (GBR)	0.2 (2,743)
		Kent/Canterbury (GCT)	0.2 (2,694)

West Midlands	8.7% (119,260)	North Birmingham (MBD) South Birmingham (MBS) South East Staffordshire (MBU) Coventry (MCO) Hereford & Worcester (MHW) Shropshire [FHSA] (MSH)	0.7 (9,283) 0.9 (12,048) 1.0 (13,234) 2.4 (32,741) 2.5 (34,026) 1.3 (17,928
Northern & Yorkshire	11.1% (151,968)	Gateshead (AGA) Newcastle (ANE) North Tees (ANT) Cumbria (AWC) North Yorkshire (BYO)	2.8 (38,339) 2.8 (38,766) 2.6 (35,762) 0.2 (2,334) 2.7 (36,767)
Trent	11.7% (159,154)	Barnsley (CBA) Doncaster (CDO) South Derbyshire (CDS) Leicestershire (CLE) North Nottingham (CNN) Nottingham (CNO) Rotherham (CRO) Sheffield (FHSA) (CSH)	0.6 (8,199) 0.9 (11,863) 1.9 (25,582) 2.8 (38,187) 0.8 (10,266) 2.6 (34,979) 0.8 (10,183) 1.5 (19,895)
North West (Mersey)	6.8% (92,314)	Chester (NCH) Crewe (NCR) Liverpool (NLI) Macclesfield (NMA) Warrington/Halton/St Helens (NWA) Wirral (NWI)	0.5 (7,357) 0.9 (12,252) 2.4 (32,466) 0.7 (9,150) 1.5 (20,699) 0.8 (10,390)
North West	7.5% (101,625)	North Lancashire (PLN) Manchester (PMA) Wigan (PWI)	1.8 (24,495) 3.8 (51,957) 1.9 (25,173)
Scotland	8.6% (117,164)	South West Scotland (Irvine) (SAA) North East Scotland (Aberdeen) (SNE) East Scotland (Dundee) (SOE) North Scotland (Inverness) (SOH) South East Scotland (Edinburth) (SSE)	1.4 (19,092) 1.8 (24,850) 1.5 (20,501) 0.9 (12,485) 3.0 (40,236)
Total	**1,364,295**	**All**	**100%**

3.4 Using the Million Women Study

The Million Women Study cohort has been well-studied over the past 16 years. The first major publication by the study group found that amongst the first 1,084,110 UK women recruited into the study, women who reported currently using HT at recruitment were 1.66 times more likely to develop breast cancer than never users (95% CI 1.58-1.75) (30). Subsequently, the group have investigated associations between HT use, lifestyle, reproductive and other exposures, and various cancer and non-cancer related outcomes (a small number of those studies are referenced here) (31–35).

A previous doctoral thesis examined HT use and cervical cancer in the Million Women Study. However, because cervical cancer is a rarer outcome than breast cancer, in 2004 there were an insufficient number of cases to look at invasive cervical cancer registrations as an outcome. The author used study data to perform a nested case-control study in order to assess the relationship between use of HT and attendance for cervical screening, the accuracy of self-reported cervical abnormalities in the Million Women Study, and the relationship between the use of HT and the risk of cervical disease (36). This is the first use of the cohort to look at incident anal, vulval and vaginal cancer registrations as primary outcome measures.

3.5 The Questionnaires

The analyses performed in this doctoral project are based on data and variables derived from the first survey, and a resurvey issued to participants approximately three years after recruitment as part of the Million Women Study.

3.5.1 The recruitment questionnaire:

The recruitment questionnaire asked women for information about demographics, schooling, qualifications, cigarette smoking, alcohol use, exercise and their current height and weight. They were asked whether they had ever had children, and if so, were asked to give details on date of birth and breastfeeding for each child (see Appendix B-1).

Because the study was set up to examine the relationship between hormone replacement therapy use and breast cancer risk as the primary outcome, women were asked about breast screening attendance, history of breast lumps, and personal or family history of breast cancer. General health questions focussed on cardiovascular disease and other common illnesses such as asthma, arthritis, thyroid disease and depression.

Questions on reproductive history asked about menarche, oral contraception, tubal ligation, hysterectomy and oophorectomy, menopausal status and HT use (ever use, timing of use with relation to the menopause, age at starting, type of HT and dose, time of stopping if relevant). There were no questions on marital status or cervical screening in the recruitment questionnaire, though some women reported 'abnormal cells' on the cervix as a common response to Q.21 'Have you ever had any other cancer?'

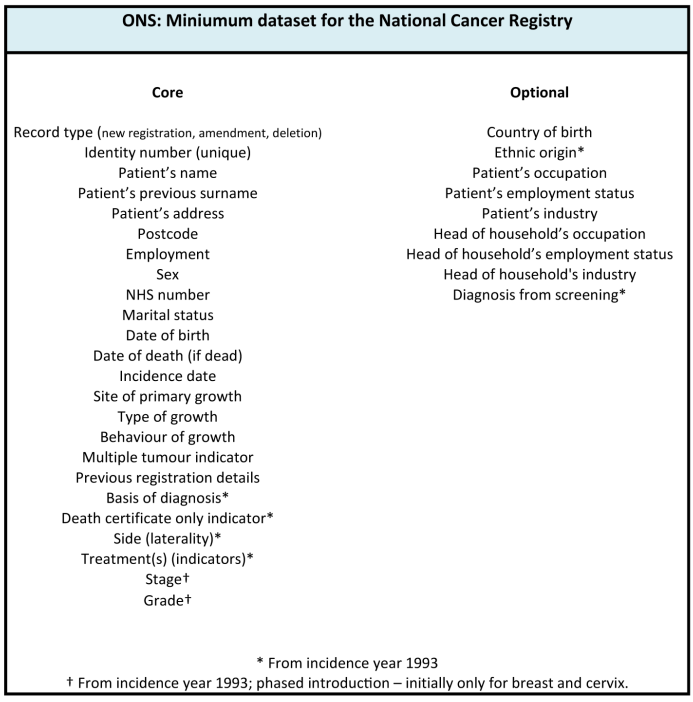
3.5.2 The first resurvey

The first resurvey questionnaire was sent to Million Women Study participants between 1999 and 2004. Several versions of the questionnaire were issued, the first, known as the '9909' version (see Appendix B-2), was sent to 205,702 women in 1999-2000. The second version was the '0012' (Appendix B-3).

The first resurvey questionnaire was sent out around three years after recruitment. 866,512 women completed this follow-up, with a response rate of 63.5%. Participants were asked about recently occurring health problems ('for the first time in the last 5 years'), principally serious illnesses such as cardiovascular disease, venous thromboembolism, breast and other cancers. On the second version of the questionnaire (0012) several questions were added about cervical cancer screening, including whether they had ever had a cervical smear test, if yes, when the last test was, and how many smears they had had in the past 10 years. Finally, women were asked if they had had an abnormal cervical smear test in the past 10 years. Women were also asked whether they were currently married or living with a partner.

3.6 Cancer registration

ONS (Office of National Statistics) National Cancer Registry database information was used to identify incident cancer cases in the cohort. Million Women Study participants are flagged on the National Health Service Central Registries, which include the cancer registry and the Office of National Statistics' mortality register. Cancer registrations and deaths are notified to the study co-ordinating centre approximately twice a year. The most recent update was to Dec 31, 2013. The NCR collects standard information on cancer cases throughout England (see Fig. 3.2 below). Million Women Study participants are linked to the ONS by name, date of birth and NHS number.



ONS: Miniumum dataset for the National Cancer Registry

Core	Optional
Record type (new registration, amendment, deletion)	Country of birth
Identity number (unique)	Ethnic origin*
Patient's name	Patient's occupation
Patient's previous surname	Patient's employment status
Patient's address	Patient's industry
Postcode	Head of household's occupation
Employment	Head of household's employment status
Sex	Head of household's industry
NHS number	Diagnosis from screening*
Marital status	
Date of birth	
Date of death (if dead)	
Incidence date	
Site of primary growth	
Type of growth	
Behaviour of growth	
Multiple tumour indicator	
Previous registration details	
Basis of diagnosis*	
Death certificate only indicator*	
Side (laterality)*	
Treatment(s) (indicators)*	
Stage†	
Grade†	

* From incidence year 1993
† From incidence year 1993; phased introduction – initially only for breast and cervix.

Figure 3-2 Minimum dataset for the National Cancer Registration System

From Cancer Registration Statistics, England (Series MB1), No. 42, 2011 Release (ONS, 2013)

The cancer registry receives information on cases from multiple sources, which increases the likelihood of an individual cancer being captured by the registry. Information may come from pathology laboratories, oncology treatment centres, and ultimately from death certificates in the case of patient death. The flow of information is outlined in Figure 3.3 below.

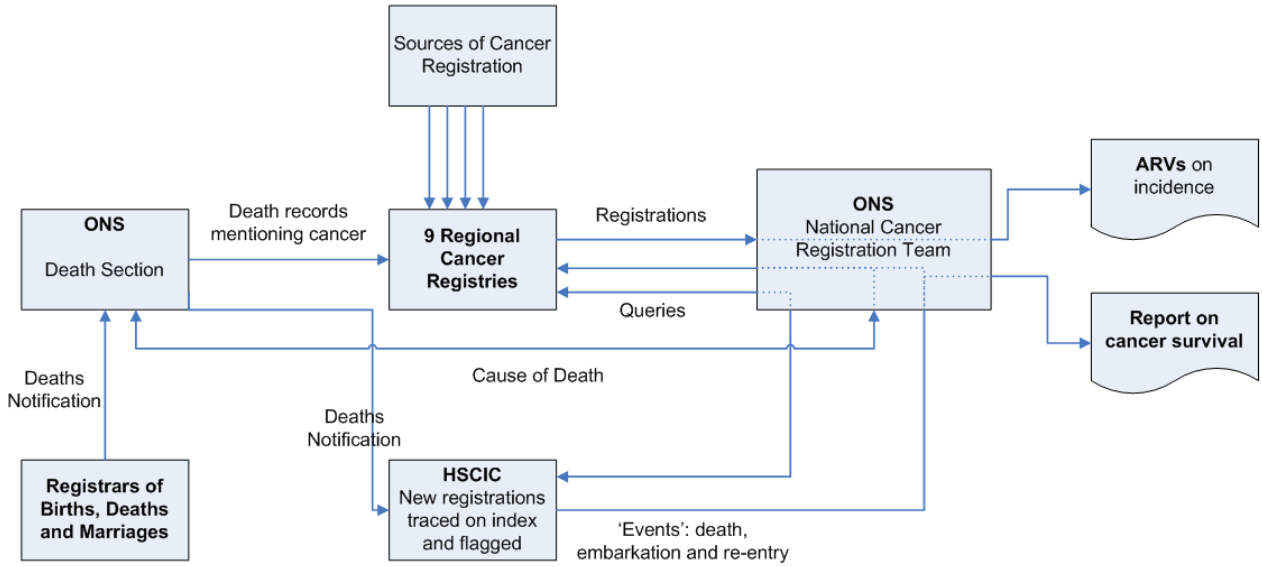


Figure 3-3 Sources of data in the cancer registration system in England (ONS 2013)

Participants with a cancer registration prior to recruitment were excluded from the analyses, apart from non-melanoma skin cancer (C44), which is known to be subject to likely under-registration. Potential non-melanoma skin cancers may be treated and excised entirely in a primary care or outpatient setting, and are often not pathologically verified, meaning they may not be picked up by the cancer registry. Under-ascertainment of around 15% has been reported previously (37).

3.7 ICD coding

ICD-10 (International Classification of Diseases version 10) codes were used to identify incident cases of anal (C21.0-21.8), vulval (C51.0-51.9), vaginal (C52) and cervical (C53.0-53.9) cancer (see Table 3.2 below). The ICD is a standardised set of codes used to classify diseases, including malignancies, for clinical, health management and epidemiological purposes. It is maintained by the World Health Organization (WHO), and is available online at http://www.who.int/classifications/icd/en/. The 10th version of the ICD replaced the ICD-9 in 1994.

Our recruitment began in 1996, so any incident cancers should be captured within the ICD-10 codes, except where cases were notified to the cancer registry solely on the basis of death certification, so-called 'DCO' cases (Death Certificate Only). The Office of National Statistics (ONS) had a lag of several years before adopting ICD-10 coding for mortality data in England and Wales, and were still using ICD-9 codes until the year 2000 (38), therefore both ICD-9 and ICD-10 codes have been used to define the variables for incident cancer cases.

The ICD-9 codes used are as follows: anal cancer (154.2, 154.3), vulval cancer (184.1-184.4), vaginal cancer (184.0), cervical cancer (180.0-180.9) and CIN 3 (233.1, 'Carcinoma in situ of cervix uteri').

Table 3-2 International Classification of Diseases version 10 (ICD-10) codes for invasive anogenital cancers, and in situ cervical neoplasia

International Classification of Diseases version 10 codes for anogenital malignancies and in situ disease	
C21: Malignant neoplasms of anus and anal canal	
C21.0	**Anus, unspecified** Excludes: Anal margin (C43.5, C44.5), anal skin (C43.5, C44.5), perianal skin (C43.5, C44.5)
C21.1	**Anal canal** Anal sphincter
C21.2	**Cloacogenic zone**
C21.8	**Overlapping lesion of rectum, anus and anal canal** Anorectal junction; anorectum; malignant neoplasm of rectum, anus and anal canal whose point of origin cannot be classified to anyone of the categories C21.0-C21.2
C51: Malignant neoplasm of vulva	
C51.0	**Labium majus** Bartholin [greater vestibular] gland
C51.1	**Labium minus**
C51.2	**Clitoris**
C51.8	**Overlapping lesion of vulva**
C51.9	**Vulva, unspecified** External female genitalia NOS; pudendum
C52: Malignant neoplasm of vagina	
C53: Malignant neoplasm of cervix uteri	
C53.0	**Endocervix**
C53.1	**Exocervix**
C53.8	**Overlapping lesion of cervix uteri**
C53.9	**Cervix uteri, unspecified**
D06: Carcinoma in situ of cervix uteri	
D06.0	**Endocervix**
D06.1	**Exocervix**
D06.7	**Other parts of cervix**
D06.9	**Cervix, unspecified**

When identifying anogenital malignancy or in situ disease (CIN 3) registrations which occurred prior to recruitment, ICD-9 codes were also used. See table 3.3 below.

Table 3-3 International Classification of Diseases version 9 (ICD-9) codes for invasive anogenital cancers and in situ cervical neoplasia.

International Classification of Diseases version 9 codes for anogenital cancers and in situ disease	
Anal cancer	
154.2	**Malignant neoplasm of anal canal**
154.3	**Malignant neoplasm of anus, unspecified site**
Cervical cancer	
180.0	**Malignant neoplasm of endocervix**
180.1	**Malignant neoplasm of exocervix**
180.8	**Malignant neoplasm of other specified sites of cervix**
180.9	**Malignant neoplasm of cervix uteri, unspecified site**
Vaginal cancer	
184.0	**Malignant neoplasm of vagina**
Vulval cancer	
184.1	**Malignant neoplasm of labia majora**
184.2	**Malignant neoplasm of labia minora**
184.3	**Malignant neoplasm of clitoris**
184.4	**Malignant neoplasm of vulva, unspecified site**
Cervical carcinoma in situ	
233.1	**Carcinoma in situ of cervix uteri**

ICD 9 and 10 codes give the anatomical location of malignant and in-situ disease, but do not tell us about histopathological subtypes. In order to be able to examine the range of tumour subtypes in the cohort, International Classification of Diseases for Oncology version 3 (39), ICD-O3 morphology codes were used in some analyses to stratify the cancers by histological subtype. Information on ICDO-3 coding of individual cancers appears in the relevant methods section of each chapter.

3.8 Statistical analysis

Cox proportional hazards (40) models were used to examine the relationship between exposures and outcome variables. The survival analyses used attained age as the underlying time variable, with incident cancer registrations (anal, vulval or vaginal) as the outcome of interest. Participants entered the analysis from the date they reported the relevant exposure, which was the date they filled in the questionnaire, either at recruitment or subsequent resurvey. The small number of women (3%) who were under the age of 50 at recruitment were entered in to the analysis at age 50. Women were followed to the first of these end-points: first cancer registration, death, emigration or other loss to follow-up, or end of the study period (based on the date of the relevant ONS follow-up period).

Using a stratified baseline risk, all analyses were stratified by recruitment region, consisting of 10 geographical areas which correspond to the cancer registry regions: Oxford, East Anglia, South West, Thames, West Midlands, Northern & Yorkshire, Trent, North West (Mersey), North West (Manchester/Lancashire), Scotland), and adjusted for deprivation. Deprivation quintiles were based on the Townsend Deprivation Index score (41).

Women with missing data (fewer than 5% for each adjustment variable) were included in most of the multivariate analyses in a separate category for the relevant variable (with the exception of the competing hazards models). It has been the practice in the Million Women Study research group to include the small number of participants with missing values for adjustment variables in our models because they make up a small proportion of the overall number, and past work has concluded that including them makes no material difference to the risk estimates obtained in our cohort, due to the large number of participants (42).

Hazard Ratios were calculated for each exposure in the model, which for simplicity have been termed relative risks in the following analyses, and are reported with 95% confidence intervals. All statistical analyses were done in Stata 13 (StataCorp 2013) by the author, with supervision by Dr Isobel Barnes and Professor Valerie Beral. Age-specific rates, where used, are derived from publicly available ONS cancer registration statistics data tables (19).

In order to examine associations between exposures and histopathological subtypes of each cancer, enabling comparison of all three cancers (anal, vulval and vaginal) or between several different subtypes of the same cancer in one model as separate outcomes, competing hazards models were used in several places; these are highlighted in the relevant methods section of each chapter.

Competing hazards models are used when we wish to consider several different possible 'events' (failures) in the same survival analysis (43–45): for instance, whether women who smoke have a different risk of squamous cell cancer vs adenocarcinoma. In a traditional survival analysis, participants are followed through time until the occurrence of a single event (failure); participants who have not experienced the event by the end of follow-up are said to be censored. They may also be censored during follow-up if they die, emigrate or are lost to follow-up (e.g. withdrawal from participation in the study). In a competing hazards model participants are at risk of multiple defined outcomes until they are censored, and censoring is considered to be informative (compared with an assumption of non-informative censoring for traditional survival analyses).

3.9 Exposure variables

Most data used to code the exposure variables used in the analyses in this study are taken directly from participant-reported values from the recruitment questionnaire (apart from screening variables and past CIN 3, see below). A few exposures were only ascertained on the first follow-up questionnaire, for example whether women had ever attended for a cervical screening smear test, and whether they were married or living with a partner 'in the past ten years'. In some cases, continuous variables (e.g. age at menarche, age at menopause, number of cigarettes smoked per day) were recoded into categorical ones.

A few variables were derived by the Million Women Study group, for example variables were created for both smoking and HT use, classifying never, past or current smoking/HT use

based on information taken from both the recruitment and the first follow-up questionnaire. Deprivation quintile was calculated based on postcode and 1991 census information.

The table overleaf (3.4) describes the categories used for the exposure variables, and gives frequencies and percentages for the whole cohort (n=1,364,295), including all women who returned a baseline questionnaire (a number of whom are excluded from the analyses due to past cancer). This table includes variables based on data from the recruitment questionnaire.

Table 3-4 Participant characteristics at baseline, taken from responses to the recruitment questionnaire

Exposure	Categories	%	n
Smoking	Never	48.0	655,445
	Past	26.8	366,058
	Current < 15/day	9.9	134,699
	Current > 15/day	9.4	127, 962
Alcohol consumption	0-2 units/week	59.2	807,203
	3-7.9 units/week	21.0	286,477
	8+ units/week	19.1	259,928
Body Mass Index	<25	43.8	597,746
	25-29	33.9	462,885
	30+	17.1	232,584
Age at menarche	≤12	37.4	509, 694
	13	23.9	325,044
	14+	36.8	501,448
Oral contraceptive pill use	None	45.3	618,139
	< 5 years	18.6	253,664
	5+ years	32.5	443,938
Parity	Nulliparous	10.9	148,703
	Parous	89.1	1,215,592
Age at first birth	≤20	21.8	264,433
	21-29	66.1	803,257
	30+	9.6	116,125
Number of children	1-2	66.9	913,301
	3-4	32.9	448,604
	5+	0.2	2,390
Tubal ligation	Yes	22.3	303,930
	No	74.6	1,017,566
Hysterectomy	Yes	25.4	346, 176
	No	73.9	1,008,218
Deprivation	Least deprived	20.2	272,362
	Quintile 2	19.9	271,737
	Quintile 3	19.8	270,127
	Quintile 4	19.8	270,374
	Most deprived	19.8	269,669
Age at menopause	<45	18.0	245,073
	45-50	30.0	408,663
	51+	23.6	322,022
Hormone therapy use	Ever	49.4	674,068
	Never	49.4	673,641

Several analyses in the thesis use exposure data that was gathered at resurvey, on average three years after recruitment. Participant characteristics based on responses to the first resurvey are given below in Table 3-5.

Table 3-5 Participant characteristics based on information gathered at the first resurvey, on average three years after recruitment

Exposure	Categories	%	n
Married or living with a partner in the past 10 years*	Yes	78.8	682,599
	No	19.3	167,089
Ever had a cervical smear test*	Yes	68.4	592,325
	No	3.0	25,607
Abnormal smear in the past 5 years*	Yes	5.9	51,439
	No	51.6	447,051

3.10 Cervical Cancer Screening Programme data

The organised call/recall system for cervical cancer screening began in 1988 in the UK, but the 'Exeter' database, formally known as the NHAIS (National Health Authority Information System) database, holds information on cervical screening programme invitations, screen results, and planned follow-up, as well as information on opportunistic cervical screening tests and those done in private healthcare settings in some cases.

In 2013, permission was obtained to link screening records to women in the Million Women Study. This was done in 2014, when screening records were linked by name, date of birth, and NHS number to Million Women Study participant identification numbers.

The NHAIS database is essentially used for administrative purposes, and as such the data it holds has some limitations. While smear results and the subsequent 'action' code (R – routine recall; S – suspended, generally meaning sent to colposcopy for further investigation of an abnormality; C – ceased, following hysterectomy or 65th birthday) are captured by the database, there is no information held on colposcopy, treatment or other outcomes (46). The database currently only holds records for women living in England, which meant that participants recruited in Scotland were excluded from the analyses using the screening data (see Chapter 7).

The linkage provided a large amount of data, which were organised into a screening hierarchy to give a single' worst' screening result per woman. After identifying women with a screening record, Million Women Study participants were divided into those who had a record of ever attending for a cervical screening test, and those who did not. Women who had ever attended were then categorised by their most abnormal result, negative (always normal smears), low-grade (inadequate, borderline or mild abnormalities), high-grade (moderate, severe or glandular dyskaryosis).

The creation of the screening hierarchy took an enormous amount of work, and much of the early work which permitted the creation of the variable was done by Rupert Alison and Dr Roger Blanks, who cleaned the data and amalgamated the screening records into a 'per woman' format. Dr Isobel Barnes and I then agreed how to create the hierarchy, as I wanted to be able to identify a woman's most severe abnormality prior to recruitment. This is discussed further in Chapter 7.

3.11 CIN 3

Cervical intraepithelial neoplasia grade 3 is the only grade of cervical preinvasive disease that is captured by the UK cancer registry, and the cancer registry is the only source of identification of women with CIN 3 (other than individual pathology laboratory results).

Cases of CIN 3 that were registered prior to recruitment were identified from ONS records using ICD-10 code D06, or ICD-9 code 233.1, 'carcinoma *in situ* of cervix uteri'. CIN 3 registrations were available for England and Scotland, and Cancer Registry data is used to identify the CIN 3 cases used in all of the following analyses.

3.12 The outcomes

Incident anogenital cancer registrations were the outcomes of interest in this study. The ONS cancer registry data to December 31, 2012 was used to identify cases of anal cancer. The anal cancer analyses were the first I undertook, and these were included in the thesis as published (47). Registry data to December 31, 2013 were used in all the other analyses. Cases of CIN 3 were also identified using ICD-9 and ICD-10 codes. The table below (3.5) gives the number of cases in the whole Million Women Study cohort. Numbers of cases in the analyses vary due to exclusion of women with previous cancers, and women lost to follow-up in the analyses restricted to those who answered the first follow-up questionnaire.

Women with prior breast or any invasive cancer were excluded from all analyses. The main rationale for this is because women who have been diagnosed with a cancer prior to recruitment may subsequently alter their behaviour (either their screening attendance) or

their lifestyle, making subsequent associations less reliable. There is also the potential for recall bias in participants who have already had a cancer. With anogenital cancers in particular, it is known that in those related to HrHPV, diagnosis of one cancer is associated with an increased risk of subsequent cancer at another anogenital site (58). Excluding participants with previous anogenital cancer at any site means that there is less of a risk of misclassification of cancer site in these physically proximal anatomical regions (e.g. misclassification of a cervical cancer as a vaginal cancer; or a vaginal cancer as a vulval cancer), however it does mean that there are cases of anogenital cancer diagnosed after another invasive cancer that are not included in our analyses.

Table 3-6 Incident anogenital cancer and cervical intraepithelial neoplasia grade 3 registrations in the Million Women Study cohort, after exclusion of women with prior cancers (excluding non-melanoma skin cancers).

Neoplasia	ICD-9 code	ICD-10 code	Cases	Mean age at registration
Anus	154.2, 154.3	C21.0-C21.8	570	65.9 (50.1-87.4)
Vulva	184.1-184.4	C51.0-C51.9	898	66.1 (50.4-85.2)
Vagina	184.0	C52	170	64.7 (52.9-88.3)
CIN 3 prior to recruitment	233.1	D06.0-D06.9	12,531	43.3 (21.3-79.4)

Finally, we have included a description of the Million Women Study cohort by age at recruitment, examining the number of person years from recruitment by 5-year age groups, giving the mean time from diagnosis of CIN 3 to recruitment, and the number of cases and rates per 100,000 by 5-year time period since recruitment by cancer site (anal, vulval and vaginal), see table 3.7 overleaf.

Table 3-7 Selected Million Women Study cohort characteristics including age at recruitment by 5-year age group, person-years at risk by 5-year age group, time from CIN 3 diagnosis to recruitment, and time to cancer diagnosis by cancer site

Selected Million Women Study cohort characteristics			
Age at recruitment			
5-year age group	n	%	
50-54	569,502	43.8	
55-59	373,185	28.7	
60-64	313,638	24.1	
65+	43,717	3.4	
Person-years at risk (by 5-year age group)			
<55	1,587,181		
55-60	3,680,338		
60-65	5,138,497		
65-70	4,330,983		
70+	3,205,135		
Mean time from CIN 3 diagnosis to recruitment (years)			
11.08 (SD 5.37)	Range (0-36 years)		
Time to cancer diagnosis and rates by cancer site, by 5-year group since recruitment			
Time since recruitment (years)	**Cases**	**Rate / 100,000**	**Person time (years)**
Anal cancer			
<5	126	1.99 (1.67-2.37)	6,342,722
5-9	173	2.89 (2.49-3.36)	5,981,188
10-14	248	4.83 (4.27-5.47)	5,132,744
15+	23	4.74 (3.15-7.13)	485,480
Vulval cancer			
<5	198	3.12 (2.72-3.59)	6,342,722
5-9	293	4.90 (4.37-5.49)	5,981,188
10-14	370	7.21 (6.51-7.98)	5,132,744
15+	37	7.62 (5.52-10.52)	485,480
Vaginal cancer			
<5	46	0.73 (0.54-0.97)	6,342,722
5-9	66	1.10 (0.87-1.40)	5,981,188
10-14	54	1.05 (0.81-1.37)	5,132,744
15+	4	0.82 (0.31-2.20)	485,480

Chapter 4 Anal cancer

4.1 Abstract:

Objective: To examine the incidence and histopathology of incident anal cancers in the Million Women Study cohort, and to establish the relationship between reproductive and lifestyle exposures and risk of anal cancer in women aged 50 and over.

Population: 1.3 million women in the UK, aged 50 and over.

Methods: Adjusted Cox regression models

Main Outcome Measures: Registrations of incident anal cancer (ICD-10 C21), classified by ICDO-3 cancer morphology code into groups by histological subtype.

Results: 517 incident anal cancers were registered over 13 years of follow-up. The greatest relative risk was associated with a prior registration of cervical intraepithelial neoplasia grade 3 (CIN 3; RR= 4.03, 95% CI 2.59-6.28). Other factors associated with significantly increased risks in multivariate analyses were: ever smoking (RR = 1.49, 1.24-1.80); previous use of oral contraceptives (RR= 1.51, 1.24-1.83); nulliparity (RR= 1.61, 1.24-2.07); tubal ligation (RR= 1.39, 1.13-1.70) and not living with a partner (RR= 1.82, 1.40-2.38). There was significant heterogeneity seen in the association with smoking, which was greater for squamous cell carcinoma than adenocarcinoma of the anus (RR 1.66 vs. 0.89, p for heterogeneity = 0.04).

Conclusion: Previous cancer registration for CIN 3, smoking, past oral contraceptive use, nulliparity, tubal ligation and not living with a partner are risk factors for anal cancer in women. The increase in risk associated with smoking varies by tumour morphology, and is seen for squamous cell anal cancers, but not adenocarcinomas.

4.2 Introduction

There has been a 126% increase in anal cancer registrations in women over the past 20 years in the United Kingdom; the rise being most marked in middle-aged and older women (7). Women have higher rates of anal cancer than men overall (48), with age-standardised incidence rates 20-30% higher in women over 50 than those seen in men of the same age (see Figure 4.1 below). In England in 2012 more than half (64%) of the 1043 registered anal cancer cases occurred in women, and most of those were in women over the age of 50 years (19).

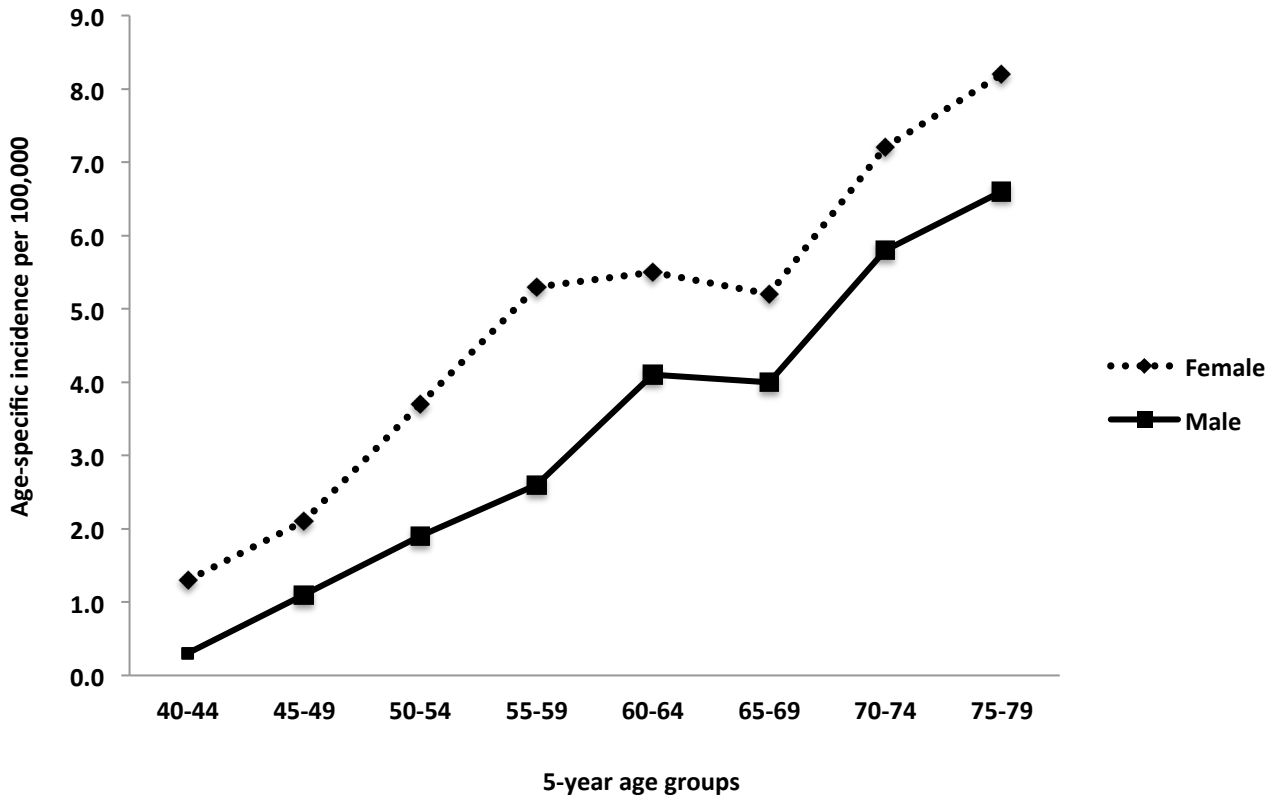


Figure 4-1 Age-specific rates of anal cancer per 100,000 population, by 5-year age-group starting at age 40, England 2012

This rise in the incidence of anal cancer is observed in many nations who capture cancer registration statistics, with similar increases seen in developed countries including the United States (49) and Denmark (50). A recent review paper from the Netherlands suggests that this increase has occurred in all Western countries over the past decade (51). Although older women account for a large proportion of anal cancer registrations, they have not been the focus of previous large-scale epidemiological research, which has instead focussed on several high-risk populations for anal cancer, including HIV-positive individuals (52), organ transplant recipients (53), men who have sex with men (MSM) (54), and women with a history of preinvasive or invasive cervical or vulval lesions (55). Most published research has concentrated HIV positive MSM (56).

It is not clear why more anal cancer registrations are seen in women than in men. The physical proximity of the anus to other epithelial regions that carry HPV in women may be partly to blame. The global rise in anal cancer incidence has been attributed to an increase in numbers of HIV positive MSM and renal transplant patients (51), however, this is not likely to be the explanation for the increase in incidence in older women, where changes in sexual behaviour including number of sexual partners and acceptance of sexual relationships outside of marriage have probably increased many women's lifetime exposure to HrHPV infection.

The literature on anal HPV infection in non-HIV infected individuals has also mostly examined risk in men, with twice as many published studies involving male data as those looking at females (57). Risk factors for anal cancer in older women, other than high-risk human papillomavirus (HrHPV) infection, are not well-established. This is a gap in the literature, as

most anal cancer registrations are in older women, despite the very high age-standardised incidence rates in the previously mentioned high-risk groups.

4.3 Methods:

Full details of the study design and methods are described in chapter 3 (Methods) of this thesis, and have been published in the literature (30), but in brief the Million Women Study is a population-based prospective cohort study, which recruited women via the National Health Service Breast Screening Programme (NHSBSP) from 1996-2001. Women were invited to participate in the study via a postal questionnaire, which was distributed with their usual breast screening invitation.

Cases of incident anal cancer, coded as C21 "malignant neoplasm of anus and anal canal" according to the International Classification of Diseases, 10th revision (ICD-10)(9) were identified from the National Cancer Registry; these include all primary anal cancers of any histological subtype. Further characterisation of tumour histology using ICDO-3 (International Classification of Diseases for Oncology version 3)(39) coding was performed for the analysis looking at the relationship between histological subtype and three of the exposures with sufficient cases to examine in a separate subgroup analysis.

Cervical intraepithelial neoplasia grade 3 (CIN 3) registrations (ICD-10 D06) which occurred prior to recruitment were identified from the Office of National Statistics (ONS) National Cancer Registry database. The main anal cancer analysis includes 1.3 million participants, and examines associations with lifestyle, reproductive and other risk factors as reported at recruitment. Participants were excluded from analyses if they had invasive breast or any other invasive cancer registered prior to recruitment, with the exception of non-melanoma

skin cancer (ICD-10 C44). Women with prevalent cancers, including other anogenital cancers are excluded from all analyses.

Two analyses were restricted to postmenopausal participants: one looking at the association between age at menopause and risk of anal cancer in women who reported a natural menopause or bilateral oophorectomy at baseline and had never used HT; and the second examining the association between HT use and anal cancer risk which excluded women who reported being pre- or peri-menopausal at recruitment. The effect of marital status and cervical screening history were also examined using data from the resurvey questionnaire sent to participants on average three years after recruitment.

Finally, the main risk factors were examined separately in the two most common histological subtypes of anal cancer: squamous cell carcinoma and adenocarcinoma. These were identified using ICDO-3 morphology codes. Squamous tumours included: M8010/3 (carcinoma NOS), M8070/3 (squamous cell carcinoma, NOS), M8071/3 (squamous cell carcinoma, keratinizing, NOS), M8072/3 (squamous cell carcinoma, large cell, non-keratinizing), M8083/3 (basaloid squamous cell carcinoma), M8094/3 (basosquamous carcinoma), M8123/3 (basaloid carcinoma), and M8124/3 (cloacogenic carcinoma). Adenocarcinomas included M8140/3 (adenocarcinoma NOS), M8210/3 (adenocarcinoma in adenomatous polyp), M8263/3 (adenocarcinoma in tubulovillous adenoma), M8480/3 (mucinous adenocarcinoma), and M8481/3 (mucin-producing adenocarcinoma).

4.3.1 Statistical analyses:

Cox proportional hazards models were used to examine the relationship between exposures and subsequent risk of anal cancer. Participants entered the analysis from the date they reported the relevant exposure (or at age 50 for the 3% who were younger than 50 at recruitment—almost all of whom were aged 49). Women were followed to the earliest of: date of first cancer registration, death, emigration, or 31 December 2012. As this was the first analysis completed as part of the series of anogenital cancers examined, the follow-up is slightly shorter than that seen in subsequent chapters, which use the most recent update of ONS data.

Analyses were stratified by region of residence (Oxford, East Anglia, South West, Thames, West Midlands, Northern & Yorkshire, Trent, North West (Mersey), North West (Manchester/Lancashire), Scotland), and adjusted where appropriate for: previous registration of CIN 3 (yes/no); smoking (never, past, current < 15 cigarettes per day, current > 15 cigarettes per day and never *vs.* ever); oral contraceptive pill use (none, <5 years, 5+ years and never *vs*. ever); parity (parous vs. nulliparous); tubal ligation (no/yes), deprivation (quintile of Townsend Deprivation Index); age at menarche (≤12, 13, 14+); alcohol use (0-2 units/week, 3-7 units/week, 8+ units/week); and body mass index (<25, 25-30, 30+).

In participants who responded to the first resurvey, impact of being married or living with a partner (yes/no), and self-reported attendance for cervical cancer screening (ever/never) were also examined. Age at menopause (50+, 45-49, <45) and HT use (never/ever) were examined as exposures in postmenopausal women only.

The small number of women with missing data (fewer than 2% for each variable) were included in a separate category for each variable of interest and included in the analysis, but these data are not shown. Relative risks are reported with 95% confidence intervals. Analyses were performed in Stata 13 (Statacorp 2013). The interactions between anal cancer histological sub-type and past CIN 3, smoking and oral contraceptive use were examined using a competing hazards model.

4.4 Results

1.3 million women were followed for an average of 13 years, giving 16.9 million person-years of follow-up overall (range 0-17 years). During follow-up, 517 women had an anal cancer registration, and anal cancer rates increased with time since recruitment, and age (Table 4.1 overleaf).

Age-specific incidence rates of anal cancer were lower in the Million Women Study cohort at all ages than those reported nationally in England in 2012 (19). The lowest rate was seen at ages 50-59 (there were no cases in the <50 age group), and this was 1.88 per 100,000 (95% CI1.54-2.29). This rate was lower in Million Women Study participants than the national rates, which were 3.7 per 100,000 at ages 50-54, and 5.3 for women aged 55-59. We saw 3.31 cases per 100,000 (95% CI 2.96-3.69) at ages 60-69, compared with national rates of 5.5 & 5.2 per 100,000 at 60-64 and 64-69. Finally, in women aged over 70 we saw a rate of 4.93 cases per 100,000 (95% CI 4.22-5.76), compared with rates of 7.2 per 100,000 at 70-74, and 8.2 per 100,000 at 75-79 (see Fig 4.1 above for national rates). The under-representation of smokers in our cohort compared to national smoking rates may in part explain the difference between anal cancer incidence in our cohort compared with national incidence rates.

Table 4-1 Participant characteristics and anal cancer incidence by time since recruitment and age

Number of women	1,300,101	
Age in years – mean (SD)	56.1 (± 4.9)	
Baseline characteristics:	**n**	**%**
Ever smokers	596,284	45.9
Ever used oral contraceptives	761,728	58.6
Ever used hormone therapy	644,831	49.6
Body mass index > 30	220,986	17.0
Parous	1,157,243	89.0
Hysterectomy	316,944	24.4
Most deprived quintile	255,619	19.7
History of tubal ligation	289,634	22.3
Postmenopausal, never HT user	424,872	32.7
Prior CIN 3 registration	12,531	1.0
Anal cancer cases in the cohort by time since recruitment and by 10-year age group		
Time since recruitment (years)	**Cases**	**Rate / 100,000**	**Person time (years)**
<5	126	1.99 (1.67-2.37)	6,342,722
5-9	173	2.89 (2.49-3.36)	5,981,188
10-14	248	4.83 (4.27-5.47)	5,132,744
15+	23	4.74 (3.15-7.13)	485,480
Age (by 10-year group)	**Cases**	**Rate / 100,000**	**Person time (years)**
<50	0	0	37.94
50-59	99	1.88 (1.54-2.29)	5,267,481
60-69	313	3.31 (2.96-3.69)	9,469,480
70+	158	4.93 (4.22-5.76)	3,205, 135

Mean age at recruitment was 56.1 (SD 4.9). 46% of the cohort reported ever having smoked, with 19% currently smoking at recruitment. 50% of women reported ever having used HT, with 33% currently using HT at recruitment. Most women had given birth at least once, with 11% reporting being nulliparous. Past use of the oral contraceptive pill was common, with 59% reporting ever having used oral contraceptives, and 33% reporting more than 5 years of use. 24% of participants reported having had a hysterectomy, and 22% had been sterilised.

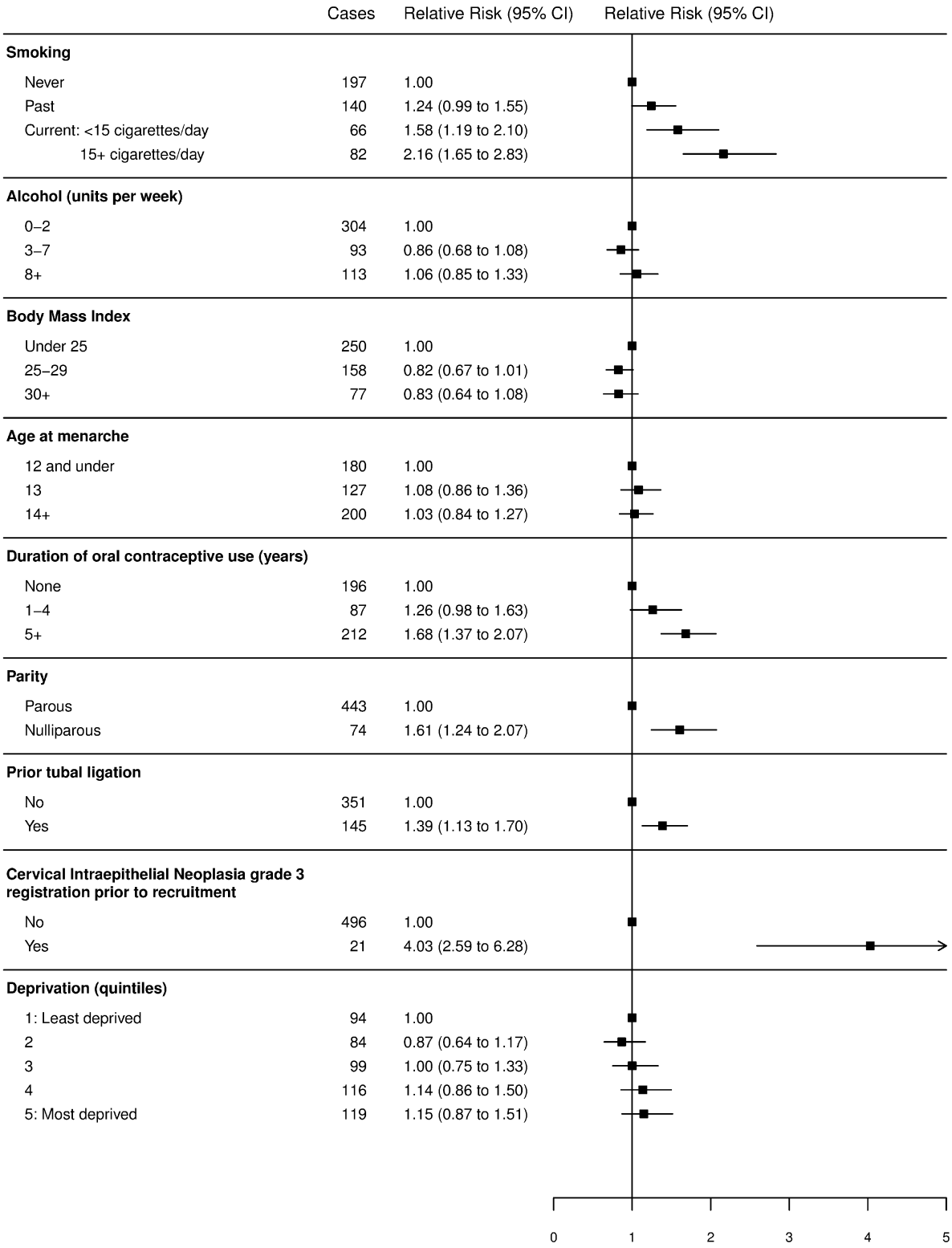
12,531 (1%) of the women in the cohort had a registration of CIN 3 prior to recruitment, and this was the strongest predictor of risk of anal cancer after the age of 50 (RR = 4.03, 95% CI 2.59-6.28), after adjustment for smoking, alcohol use, BMI, age at menarche, ever use of the oral contraceptive pill, parity, tubal ligation and deprivation (see Figure 4.2 below).

Ever having smoked was associated with just under 50% increase in risk of subsequent anal cancer, with a RR of 1.49 (95% CI 1.24-1.80) in women who reported past or current smoking, versus never smokers. When risk was stratified by amount smoked, those who reported smoking more than 15 cigarettes per day at recruitment had a RR of 2.16 (95% CI 1.65-2.83) (Figure 4.2). No significant increase in risk was seen with alcohol use, raised body mass index, age at menarche, or deprivation.

Overall, nulliparous women had a 60% greater risk of anal cancer when compared with parous women, with a RR of 1.61 (95% CI 1.24-2.07). Women who reported ever having given birth had a lower risk of developing anal cancer, irrespective of the number of children

they had, with a RR of 0.58 (95% CI 0.44-0.75) for those reporting 1-2 children, and a RR of 0.69 (95% CI 0.52-0.91) for women with 3+ children, compared with the nulliparous group.

Past use of the oral contraceptive pill was significantly associated with anal cancer risk, RR = 1.51 (95% CI 1.24-1.83) for ever vs. never use. When stratified by years of use, women who reported 5 or more years of oral contraceptive use had a significantly increased risk of anal cancer, RR = 1.68 (95% CI 1.37-2.07) (see Figure 4.2). Female sterilisation was also associated with an increased risk of anal cancer; women who reported having had a bilateral tubal ligation were significantly more likely to develop an anal cancer, RR 1.39 (95% CI 1.13-1.70).



RRs adjusted for smoking, BMI, alcohol use, age at menarche, oral contraceptive use, tubal ligation, parity, prior CIN 3, and deprivation

Figure 4-2 Forest plot showing association between anal cancer and various lifestyle and other factors in an adjusted multivariate model.

Younger age at menopause was not associated with an increased risk of anal cancer in women who had undergone a natural menopause or bilateral oophorectomy at recruitment and had never used HT (n = 424,855, 33%; see Table 4.2). Compared with women whose menopause occurred at age 50 or older, those who went through menopause at 45-49 had a RR of incident anal cancer of 1.18 (95% CI 0.83-1.68), and those whose menopause was before the age of 45 had a RR of 0.95 (95% CI 045-1.59).

Table 4-2 Association between age at menopause and anal cancer in postmenopausal women who have never used HT

Exposure	Cases/participants	RR	95% confidence interval
Age at menopause in never users of menopausal hormone therapy			
50+	80/237,157	**1.00**	-
45-49	52/126,008	**1.18**	0.83-1.68
<45	18/49,489	**0.95**	0.45-1.59

RR adjusted for smoking, BMI, alcohol use, age at menarche, oral contraceptive use, tubal ligation, parity, prior CIN 3, and deprivation

Similarly, ever use of HT did not significantly increase risk of subsequent anal cancer in postmenopausal women (Table 4.3) with a RR of 1.19 (95% CI 0.98-1.44) in those who reported ever having used HT, compared with never users.

Table 4-3 Risk of anal cancer by history of menopausal hormone therapy use

Exposure	Cases/participants	RR	95% confidence interval
Use of menopausal hormone therapy at baseline, postmenopausal participants			
Never	155/424,872	**1.00**	-
Ever	119/285,240	**1.19**	(0.98-1.44)

RRs adjusted for smoking, BMI, alcohol use, age at menarche, oral contraceptive use, tubal ligation, parity, prior CIN 3, and deprivation

At resurvey, on average three years after recruitment, women were asked whether they were currently married or living with a partner. The majority of women reported currently co-habiting; of the 807,602 respondents, 79% (n=638,170) reported living with a husband or partner. The risk of anal cancer was increased in the minority of women who reported not living with a husband or partner, with a RR of 1.82 (95% CI 1.40-2.38), compared to women who were co-habiting (Table 4.4). At the same resurvey, Million Women Study participants were asked about their cervical screening history, and whether they had ever attended for a cervical screening test (cervical pap smear). Nearly all respondents reported having attended for cervical cancer screening, with just 3% never having had a cervical screening test (n=23,997). The risk of anal cancer was not elevated in never-attenders (see Table 4.4).

Table 4-4 Association between living with a partner, and participation in cervical screening, on incidence of anal cancer in respondents to resurvey 3 years after recruitment.

Exposure	Cases/Population at risk	RR	95% confidence interval
Married or living with partner			
Yes	178/652,497	**1.00**	-
No	88/173,710	**1.82**	1.40-2.38
Ever had a cervical smear test?			
Yes	177/801,582	**1.00**	-
No	7/24,625	**0.80**	0.37-1.72

RRs adjusted for smoking, BMI, alcohol use, age at menarche, oral contraceptive use, tubal ligation, parity, prior CIN 3, and deprivation

Finally, risk was examined by tumour histology in women with anal squamous cell cancers (SCC) versus those with adenocarcinomas. The majority of the anal cancers registered in the cohort (82%) were squamous cell carcinomas (n= 425). Glandular tumours were less common, with 62 adenocarcinomas registered. The remaining 5% (n= 27) were rarer histological subtypes, including anal melanoma; this group had insufficient numbers to examine separately (see Table 4.5).

Table 4-5 Anal cancer registrations in the study, by ICDO-3 code and histopathological subtype

	ICDO-3 code	Histopathological subtype	Number (%)
Squamous tumours	M8070/3	Squamous cell carcinoma, NOS	319 (75)
	M8123/3	Basaloid carcinoma	38 (9)
	M8071/3	Squamous cell carcinoma, keratinizing, NOS	34 (8)
	M8010/3	Carcinoma, NOS (Epithelial tumour, malignant)	11 (3)
	M8072/3	Squamous cell carcinoma, lg. cell, non-keratinizing	7 (2)
	M8083/3	Basaloid squamous cell carcinoma	6 (1)
	M8094/3	Basosquamous carcinoma	5 (1)
	M8124/3	Cloacogenic carcinoma	5 (1)
		Total squamous tumours (% of all anal cancers)	**425** (82)
Glandular tumours	M8140/3	Adenocarcinoma NOS	45 (73)
	M8480/3	Mucinous adenocarcinoma	8 (13)
	M8210/3	Adenocarcinoma in adenomatous polyp	3 (5)
	M8263/3	Adenocarcinoma in tubulovillous adenoma	3 (5)
	M8481/3	Mucin-producing adenocarcinoma	3 (5)
		Total glandular tumours (% of all anal cancers)	**62** (12)
Other tumours	M8000/3, M8246/3 M8560/3	Neoplasm, malignant / Neuroendocrine carcinoma / Adenosquamous carcinoma	15 (56)
	M8720/3, 8721/3, M8743/3	Malignant melanoma NOS / Nodular melanoma / Superficial spreading melanoma	11 (41)
	M8542/3	Paget's disease (except of bone)	2 (7)
		Total 'other' tumours (% of all anal cancers)	**27** (5)
TOTAL INCIDENT ANAL CANCERS			**517** (100)

In women with a squamous cell anal cancer, associations between past CIN 3 registration, ever smoking or using the oral contraceptive pill were similar to those seen in the whole cohort (see Table 4. 6). The RR of adenocarcinoma in women who had ever smoked was 0.89 (95% CI 0.51-1.54), which was significantly different from the risk in women with squamous cell tumours (RR 1.66, 95% CI 1.35-2.05), p for heterogeneity = 0.04. (See Table 4.6).

Table 4-6 Association between anal cancer risk and past cervical intraepithelial neoplasia grade 3 (CIN 3), smoking and oral contraceptive use

	Squamous cell cancer			Adenocarcinomas			Heterogeneity between tumour types
Exposure	**Cases**	**RR**	**95% CI**	**Cases**	**RR**	**95% CI**	**p**
Past CIN 3 registration							
No	406	**1.00**	-	61	**1.00**	-	0.51
Yes	19	**4.43**	2.79-7.05	1	**2.12**	0.29-15.42	
Ever smoked							
Never	151	**1.00**	-	32	**1.00**	-	0.04
Ever	253	**1.66**	1.35-2.04	23	**0.89**	0.51-1.54	
Oral contraceptive use							
Never	138	**1.00**	-	24	**1.00**	-	0.74
Ever	282	**1.52**	1.23-1.89	38	**1.68**	0.98-2.88	

RRs adjusted for smoking, BMI, alcohol use, age at menarche, oral contraceptive use, tubal ligation, parity, prior CIN 3, and deprivation

4.5 Discussion

Lifestyle and reproductive risk factors for incident anal cancer were examined in a large cohort of UK women over 50 years of age, who were for the most part postmenopausal, and these findings add to the growing literature on anal cancer. The greatest increase in risk was seen in women with a registration of high-grade preinvasive cervical disease (CIN 3) prior to recruitment. Other factors associated with an increased risk of anal cancer were ever having smoked, with current smokers of more than 15 cigarettes a day at greatest risk; ever having used the oral contraceptive pill, with evidence of a stronger effect in women who had used the pill for 5 or more years; nulliparity; a history of tubal ligation; and not currently living with a husband or partner.

When the association between three of the strongest risk factors (past CIN 3, smoking, and oral contraceptive use) and anal cancer were examined by histological subtype, the risk of squamous cell anal carcinoma differed significantly from the risk of adenocarcinoma among smokers, with a significantly elevated risk of squamous cell tumours, but not of adenocarcinomas. Risks amongst past users of the oral contraceptive pill and women with a prior registration of CIN 3 did not differ significantly by histological subtype.

4.5.1 Past CIN 3

The strongest association with risk of anal cancer in our study was a past history of CIN 3. This is a well-studied relationship, and is perhaps unsurprising. Women with HrHPV-associated neoplasia in one part of the anogenital tract are more likely to have it elsewhere (58). After cervical cancer, anal cancer has the closest association with HrHPV infection (59), with an estimated 80-90% of anal cancers caused by oncogenic papillomaviruses (3,10,60). In a recent American study, nearly 80% of the anal cancers tested positive for HPV subtypes 16 or 18 (10), which are high-risk types that similarly have a strong causal relationship with cervical cancer.

Anal cancer, and other HPV-related anogenital cancers are increased in women with a history of anogenital warts (61), cervical dysplasia (62,63), and previous anogenital malignancy at another site (64–66). Past CIN 3 registration was chosen as an exposure and adjustment variable because in the UK, CIN 3 cases (but not other grades of cervical dysplasia) are captured by the National Cancer Registry, providing a registered disease episode which was independent of self-report. The strength of the association between past CIN 3 and anal cancer later in life, after adjustment for many other risk factors including smoking and deprivation, highlights the importance of this exposure as a marker of risk.

4.5.2 Smoking

Though smoking has been found to be a risk factor for anal cancer in some studies (67–70), not all studies have reported an association (55). A recent Brazilian case-control study attempted to measure the magnitude of the association between smoking and several cancers, including anal cancer (71). Their case-control study including 231,102 patients identified from the national Cancer Hospitals Registry in Brazil between 1998 and 2011. Controls were patients with non-melanoma skin cancer (n=26,971) while cases included all the major cancer sites (oropharyngeal, gastrointestinal tract, lung, bladder etc.) Smoking was defined as 'the report of habitual tobacco use…at the time of hospital enrolment'. They included 1498 anal cancer cases (75% of these were in women), and found a 'moderate' relationship between smoking and anal cancer, with an attributable factor of 23.4%. The crude OR for smoking and anal cancer in women in their study was 2.7 (95% CI 2.4-3.1), with an adjusted OR of 2.1 (95% CI 1.8-2.4), $p< 0.001$.

A retrospective study from Denmark and Sweden (69) with 417 anal cancer patients (324 women and 93 men) found an increased risk of anal cancer in female smokers, with an odds ratio of 1.9 (95% CI 1.3-2.8) when compared with never-smokers. When they stratified the results by menopausal status, they found a very strong association in premenopausal current smokers, OR 5.6 (95% CI 2.4-12.7), which was absent in postmenopausal women. They suggested that this finding could be due to an anti-oestrogenic effect of tobacco smoke on anal epithelium. These findings have not been replicated elsewhere, and are not supported by our findings.

Few other studies have looked at risk factors by histological subtype. Squamous cell anal cancers are by far the most common, and account for over 80% of the registered anal

cancers in our cohort. When risk associated with smoking was examined by histological subtype, there was a significant increase seen for risk of anal squamous cell carcinomas, but not adenocarcinomas, in women who have ever smoked.

Anal cancer is not the only HrHPV-related anogenital malignancy with a relationship to smoking; smoking has been well-studied as a risk factor for pre-invasive and invasive cervical neoplasia (72,73). However, there has been considerable debate over how much of the relationship is due to a direct biological effect, with cigarette constituents acting as direct carcinogens, or affecting the risk of HrHPV persistence/immune clearance, and how much is due to confounding with high risk sexual behaviours, such as a higher number of sexual partners and early initiation of sexual behaviour. Our finding of an increased risk of incident tumours with squamous cell histology, but not adenocarcinoma, argues against confounding as the causative mechanism in the relationship between risk of anal cancer and smoking.

Laboratory studies have found increased concentrations of nicotine and other metabolites in the cervical mucus of smokers (74–76), suggesting a potential biological pathway for the increase in risk. Similar to our findings in anal cancer, smoking has been established as a risk factor for cervical squamous cell carcinomas, but is not associated with a significantly increased risk of cervical adenocarcinoma (72). The heterogeneity in risk by histological subtype now observed both in cervical and anal cancers argues against confounding as the main underlying pathway for the increased risk seen in smokers, but it is not clear whether nicotine or other metabolites are exerting a direct biological effect, are interacting with HrHPV, or are acting through another mechanism that has as yet to be uncovered.

4.5.3 Oral contraceptive pill use

Past use of oral contraceptives was a strong predictor of risk of subsequent anal cancer in our study, particularly for women with a history of longer-term use of 5 or more years. This is not a well-studied association. Only one previous study of anal cancer was identified which mentioned oral contraceptive use, and they found no significant association (69) Oral contraceptive use has been shown to be associated with an increased risk of cervical cancer during use and for a period of time after cessation (77). It has been proposed that sex hormones may stimulate HrHPV gene expression or exert an effect on the immune microenvironment in the cervical epithelium (78), and it is possible that a similar effect could occur in anal epithelium.

Like with smoking, if the oral contraceptive pill is exerting a biological effect it could act directly, or indirectly by increasing the susceptibility to or carcinogenic potential of HrHPV. It is also possible that there is an element of behavioural confounding, and that women using oral contraceptives in the past had different sexual behaviour than their peers, or are more likely to have had more than one sexual partner.

4.5.4 Tubal ligation

The increased risk of anal cancer in women who reported a history of tubal ligation has not to our knowledge previously been reported. Tubal ligation is highly correlated with parity; over 97% of the women in our cohort who reported being sterilised had given birth to at least one child. Sterilised women had more children, with 49% reporting three or more children, compared with 33% of women in the cohort overall. They were also more likely to have given birth at a younger age, with 31% of sterilised women reporting an age at first birth ≤ 20 years of age, compared with 19% of the full cohort. Given that in

our analysis the risk of anal cancer was decreased in parous women, the relationship between tubal ligation and increased risk was unexpected. Adjusting for parity, age at first birth, or number of children did not affect the association.

An association between tubal ligation and cervical cancer has been reported in the literature, however the small effect seen has been attributed to a possible link with decreased likelihood of attendance for screening (79,80). There is also an increased incidence of sterilisation in women from a more deprived background and higher parity (81), which in cervical cancer are both associated with an increased risk of disease. However, these are unlikely to play a role in the relationship with anal cancer; in this analysis there was no association between deprivation and risk of anal cancer, and risk was significantly decreased in parous women, with nulliparous women 50% more likely to develop an anal cancer compared with women who had ever given birth. The association between tubal ligation and anal cancer risk is somewhat puzzling and merits further study.

4.5.5 Current or recent co-habitation with a husband or partner

Women who reported not currently being married or living with a partner at resurvey three years after recruitment had an increased risk of subsequent anal cancer. While this might seem counter-intuitive, this group is likely to include a large number of women who are widowed, separated, or divorced (34). Women who reported cohabiting or being married are likely to be in a stable relationship with a longer term partner, women in the non-cohabiting group may not be celibate. Early epidemiological studies on anal cancer which looked at marital status found a strong increase in risk in single men (but not women), most likely due to an increased number of homosexual men in this demographic (82,83).

When other marital groups were examined in both sexes, being separated or divorced was associated with an increased risk of anal cancer, with a risk ratio of 2.0 for squamous cell anal cancers in separated or divorced women compared with those who were married (84); the association of anal cancer with marital status was reported as being stronger at older ages, with a larger increase in risk associated with being separated or divorced in women over the age of 60. Our findings support this. Marital instability may be associated with an increased number of sexual partners, either as a cause, or an effect of marital dissolution. HPV infections are sexually transmitted; the lack of direct information on sexual behaviour from the women in the Million Women Study cohort is recognised as a limitation. We have used marital status and reported cohabitation as a proxy measure of being in a longer-term relationship, which may be associated with having had fewer sexual partners.

4.6 Conclusion

Anal cancer incidence has been reported to be rising in several developed countries (7,49,50). Identification of other risk factors that play a role in HPV-related anogenital carcinogenesis is clearly important in order to identify women at increased risk and implement preventative strategies.

In a large cohort of over a million women with prospectively collected information on exposures, with an average 13 years of follow-up, a registration of CIN 3 prior to recruitment, smoking, past use of the oral contraceptive pill, nulliparity, tubal ligation, and not currently living with a husband or partner were risk factors for incident anal cancer in women over 50 years of age.

When the two main histological subtypes of anal cancer were examined separately, a significant difference in risk was associated with smoking for tumours with squamous cell morphology compared to adenocarcinomas. Age at menarche, alcohol use, deprivation, age at menopause and postmenopausal HT use were not significantly associated with an increased risk of anal cancer in this study.

These results have been published in the British Journal of Cancer in 2015 (47). The exposures with significant associations to anal cancer risk later in life are strikingly similar to those seen for cervical cancer in other studies, providing further evidence for the relationship between HrHPV infection and anal cancer in women over the age of 50, who have, until now, been neglected in epidemiological investigations of this rare cancer.

Chapter 5 Vulval cancer

5.1 Abstract:

Objective: To examine the incidence and histopathology of incident vulval cancers in the Million Women Study cohort, and to establish the relationship between reproductive and lifestyle exposures and risk of vulval cancer in women aged 50 and over.

Population: 1.3 million UK women aged 50 and over.

Methods: Adjusted Cox regression models

Main Outcome Measures: Registrations of incident vulval cancer (ICD-10 C51), classified by ICDO-3 cancer morphology code into groups by histological subtype.

Results: Participants were followed on average for 14 years from recruitment. 898 vulval cancers were registered: 68% were squamous cell carcinomas (SCC) (n= 609); 10% were basal cell tumours (n= 92); 11% were glandular tumours (n=96);and 6% were melanocytic tumours (n=55). The remaining 5% were unspecified and sarcomatous tumours (n=46). Within the squamous cell cancers, there was an under-representation of typically high-risk human papillomavirus (HrHPV)-associated tumour morphologies.

Factors associated with an increased risk of incident vulval cancer in the cohort included prior registration of cervical intraepithelial neoplasia grade 3 (CIN 3) prior to recruitment, RR 2.68 (95% CI 1.71-4.18; p<0.0001); being overweight or obese, with overweight women (BMI 25-29) 19% more likely (RR 1.19, 95% CI 1.02-1.39; p<0.0001), and obese women (BMI 30+) 71% more likely (RR 1.71, 95% CI 1.44-2.04; p<0.0001) to develop vulval cancer than normal weight women. Finally, women in our cohort who reported a natural menopause or oophorectomy before the age of 50 had a 50% increase in vulval cancer risk (RR 1.52, 95% CI 1.22-1.89; p<0.0001) compared with those whose

menopause occurred at 50 or later. Diabetes, smoking, past use of the oral contraceptive pill, hysterectomy, and menopausal hormone therapy use were not significantly associated with an increased risk of vulval cancer.

When the effects of BMI, age at menopause, and smoking were examined by tumour histological subtype, the association between adiposity and vulval cancer was significantly greater for squamous cell carcinomas compared with other tumour morphologies (p for heterogeneity = 0.01). Similarly, being under 50 at menopause had a stronger association with risk of squamous cell and glandular tumours than those of basal cell or melanocytic subtypes (p = 0.03). Smoking and menopausal hormone therapy did not show any significant heterogeneity of effect.

Conclusions: Squamous cell carcinomas predominate amongst vulval cancer registrations in women over 50. Vulval cancers are more common in women who have had a registration of CIN 3 in the past, are overweight or obese, or who have undergone a natural or surgical menopause prior to age 50. The relationship between adiposity and vulval cancer is restricted to squamous cell vulval tumours, and the additional risk seen in women with an earlier menopause was confined to those with squamous cell and glandular tumours.

The exposures associated with risk of vulval cancer in our study, apart from past CIN 3, are not those typically see in other anogenital cancers which have a closer relationship with HrHPV infection.

5.2 Introduction

Vulval cancer is the fourth most common gynaecological cancer in the United Kingdom (UK) (19). In 2012, there were 1,052 vulval cancers registered in England, and the majority of these, 86% (909 cases), occurred in women aged over 50 years.

A rare malignancy, vulval cancer accounts for fewer than 1% of all cancer registrations in women. Its incidence increases markedly with age, rising from 2.5 cases per 100,000 women at ages 40-44, to 17.0 per 100,000 at ages 80-84, so that it overtakes cervical cancer to become the most common anogenital cancer in women over 70 (see figure 5.1 below).

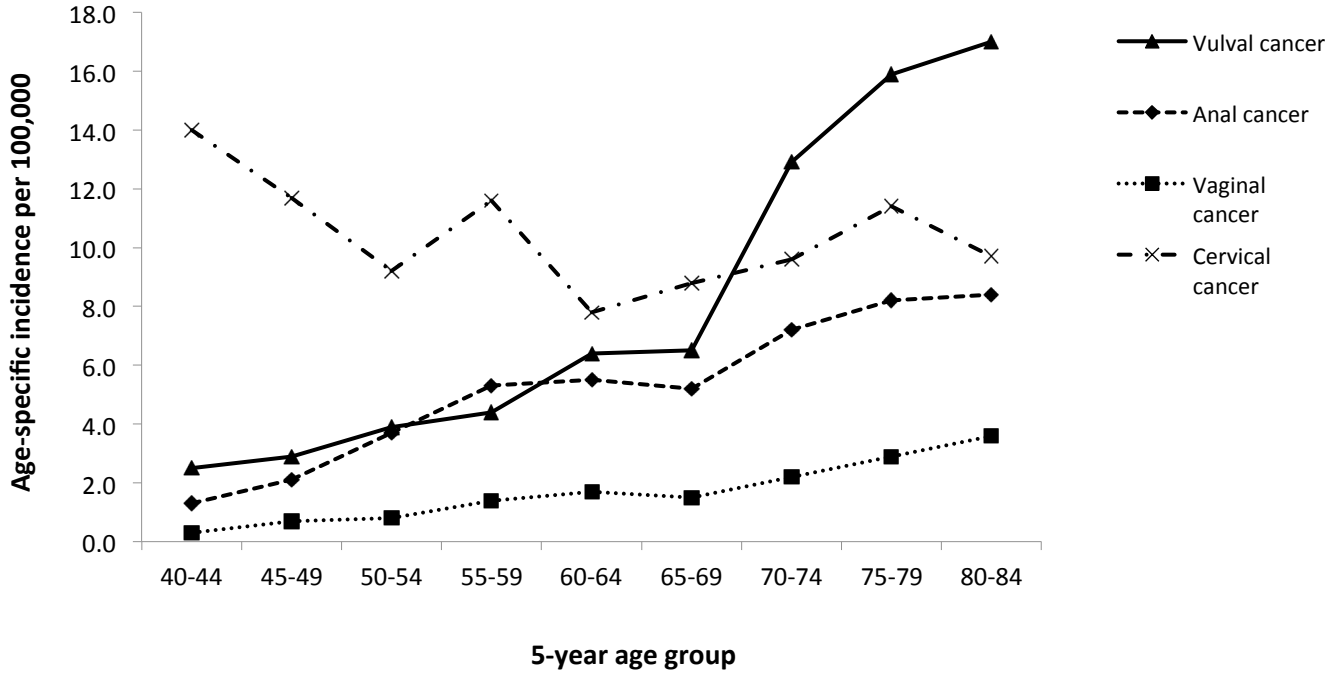


Figure 5-1 Age-specific rates of vulval cancer (ICD-10 C51), compared with cervical (C53), anal (C21), and vaginal (C52) cancer, England 2012. Rates are per 100,000 women by 5-year age groups, starting at age 40.

Until recently, it was commonly suggested that around half of vulval cancer cases were attributable to infection with high-risk human papillomaviruses (HrHPVs), with aetiopathological similarities to the other HrHPV-related anogenital cancers (cervical, anal and vaginal cancers) (86). In fact, it seems likely that far fewer, around a quarter of all vulval cancers, are causally associated with HrHPV infection, with a diminishing association seen with increasing age--a recent large international collaboration estimated that only around 15-17% of cases of vulval cancer in women 67 years of age and older were likely to be attributable to HrHPV (15). Vulval cancers in older women are more likely to arise in a background of vulval dermatosis (87), and are less likely to be HrHPV-driven.

Risk factors for this rare cancer were examined in a large cohort of women aged 50 and over, with the hypothesis that exposures related to increased risk might be different from those seen in other anogenital cancers (anal, cervical and vaginal cancer), which are known to be more strongly related to infection with HrHPVs (2,3). The aetiology of vulval cancer is variable, and two causal pathways have been described: the first occurs in older women and is most strongly associated with keratinising squamous cell carcinomas arising in a background of vulval dermatosis; the second occurs in younger women, and is related to HrHPV-driven tumours, generally with warty or basaloid tumour morphology (14,87,88). Unfortunately, we do not have any information on vulval dermatoses in Million Women Study participants, so it was not possible to examine them as an exposure.

In this study, vulval cancer incidence and its relationship with various lifestyle and reproductive risk factors are examined, including smoking, oral contraceptive use, parity, obesity, diabetes, past registration of CIN 3, and menopausal hormone therapy use (HT). Differences in risk by histological subtype are assessed where possible.

5.3 Methods:

As elsewhere, participants were excluded from the analysis if they had any invasive cancer registered prior to recruitment, other than non-melanoma skin cancer (ICD-10 C44). Self-reported data from the recruitment and subsequent resurvey questionnaires were used to define most exposures, apart from Townsend deprivation index, which was calculated using post-code and 1991 census data (41), and cervical intraepithelial neoplasia grade 3 (CIN 3) registrations prior to recruitment, which were derived from National Cancer Registry data (ICD-10 D06).

Cases of incident vulval cancer were identified from the UK Cancer Registry: "malignant neoplasm of vulva", ICD-10 C51. Body mass index (kg/m^2) was calculated from self-reported weight and height measurements at recruitment, which have been shown to correlate closely with measured variables in Million Women Study participants (31). BMI categories were based on standard World Health Organisation definitions (89), and grouped women with a BMI of less than 25 kg/m^2 as 'normal', 25 to 29.9 kg/m^2 as 'overweight', and 30 kg/m^2 or more as 'obese'.

Postmenopausal women were identified based on self-reported menopausal status at recruitment, and were limited to those who reported having undergone a natural menopause or having had a bilateral oophorectomy prior to recruitment. The analysis looking at the effect of age at menopause was further restricted to postmenopausal

women who reported never having used HT. Menopausal hormone therapy use, smoking, and alcohol use were categorised as they were reported at baseline and were not updated. Excellent agreement has been found between HT use as reported by Million Women Study participants and general practice prescription records in a past validation study (90).

The main risk factors were examined separately for the commonest histological subtype, squamous cell carcinoma (SCC), and three other groups: basal cell carcinomas (BCCs), glandular tumours, and melanocytic tumours. Histological subtypes were identified using ICDO-3 (International Classification of Diseases for Oncology version 3) morphology codes (22). The exposures examined in the subgroup analysis were BMI, age at menopause, and smoking, due to insufficient numbers of cases within some tumour morphology types for the other exposures.

Squamous tumours included the following ICDO-3 codes: M8051/3, M8070/3, M8071/3, M8072/3, M8074/3, and M8075/3; microinvasive SCCs were included in this group: M8070/5 and M8076/3. Basal cell carcinomas included: M8090/3, M091/3, M8094/3, and M8097/3. Glandular tumours (including Paget's disease, Bartholin gland tumours, and adenocarcinomas) were: M8542/3, M8140/3, M8200/3, M8480/3, M8560/3, M8390/3, M8400/3. Finally, melanocytic tumours included: M8720/3, M8721/3, M8743/3, M8744/3 and M8746/3. 'Other' rarer tumour types were excluded from the analysis, and these included unclassified malignancies and sarcomas (see the results section for further enumeration of 'other' tumour histologies).

5.3.1 Statistical analyses

Cox proportional hazards models were used to examine the relationship between reproductive and lifestyle factors, and risk of vulval cancer. Hazard ratios were estimated with attained age as the underlying time variable, and are referred to hereafter as relative risks (RRs). Participants entered the analysis from recruitment or at the age of 50 for the small number (3%) who were younger than 50 at recruitment (who were, for the most part, 49 years old). Follow-up continued until the date of first cancer registration; death; emigration; or 31 December 2013.

World Health Organization definitions were used to categorise participants into three groups by BMI: normal (BMI ≤ 25 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI ≥ 30 kg/m^2). Analyses were adjusted for: age at menarche (≤13, 14+); past oral contraceptive pill use (never *vs*. ever); parity (parous *vs*. nulliparous); smoking (never, past, current); alcohol use (0-2 units/week, 2+ units/week); tubal ligation (no/yes); hysterectomy (no / yes, with oophorectomy / yes, without oophorectomy); diabetes mellitus (no/yes); deprivation tertile (derived using the Townsend Deprivation Index, from least to most deprived); and registration of CIN 3 prior to recruitment (no/yes). Age at menopause (50+/<50) and HT use (never/ever) were examined separately in postmenopausal women. In order to avoid confounding, age at menopause was examined only in those who reported a natural menopause, or the small number of women reporting a bilateral oophorectomy, and who had never used HT. All analyses were stratified by region of residence, consisting of 10 geographical areas corresponding to the cancer registry regions.

Using data from the first resurvey, sent out on average three years after recruitment, associations between current marital status and self-reported cervical screening attendance, and risk of vulval cancer were examined in fully adjusted models. Women were asked about being married or living with a partner (yes/no), and whether they had ever attended for cervical cancer screening (ever/never).

The few women with missing data were included in a separate category for each variable of interest and included in the analysis, but these data are not shown. Relative risks are reported with 95% confidence intervals. Analyses were performed in Stata 13 (1).

5.4 Results:

Over 1.3 million women were followed for 18 million person-years, and there were 898 vulval cancers registered in the cohort during that period. Rates of vulval cancer registration in the cohort increased over that time, from 3.12 (95% CI 2.72-3.59) per 100,000 in the first five years after recruitment, to 7.62 (95% CI 5.52-10.52) 15 or more years after the study began (Table 5.1 overleaf). The age specific rates we observed also increased linearly with age. There was an increase in incidence after age 70 in the cohort, with 9.27 cases per 100,000 (95% CI 8.27-10.38); the rates we observed were not substantially different from national rates.

Characteristics of participants were as follows: most women were parous, with 89% of participants reporting ever having given birth. Nearly 60% had a history of oral contraceptive use, while 46% reported ever having smoked. Just over half of the cohort were overweight or obese, with 34% of women having a BMI of 25 to 29.9, putting them in the overweight range, and 17% with a BMI of 30 or more, who were in the obese group. Just under half of the cohort (49%) reported undergoing a natural menopause. Around half of all participants had ever used menopausal hormone therapy, these included pre-, peri-, and postmenopausal women; just over a third of the cohort were postmenopausal and had never used HT (see Table 5.1).

Table 5-1 Patient characteristics at recruitment, and details of cancer incidence by time since recruitment (in 5 year groups) and age (in 10 year groups): Vulval cancer

Characteristic		
Number of women (%)	1,300,042	(100.0)
Mean age at recruitment (SD)	56.1	(4.9)
Parous % (n)	89.0	(1157242)
Oral contraceptive user % (n)	58.6	(761,728)
Current or past smoker % (n)	45.9	(596,284)
Obese (body mass index >30)	17.0	(220,976)
Most deprived tertile % (n)	19.8	(257,552)
Postmenopausal % (n)	86.3	(1,122,569)
Ever hormone therapy user % (n)	49.6	(644,831)
Hysterectomy % (n)	24.4	(316,944)
Registration of CIN 3 prior to recruitment % (n)	1.0	(12,531)

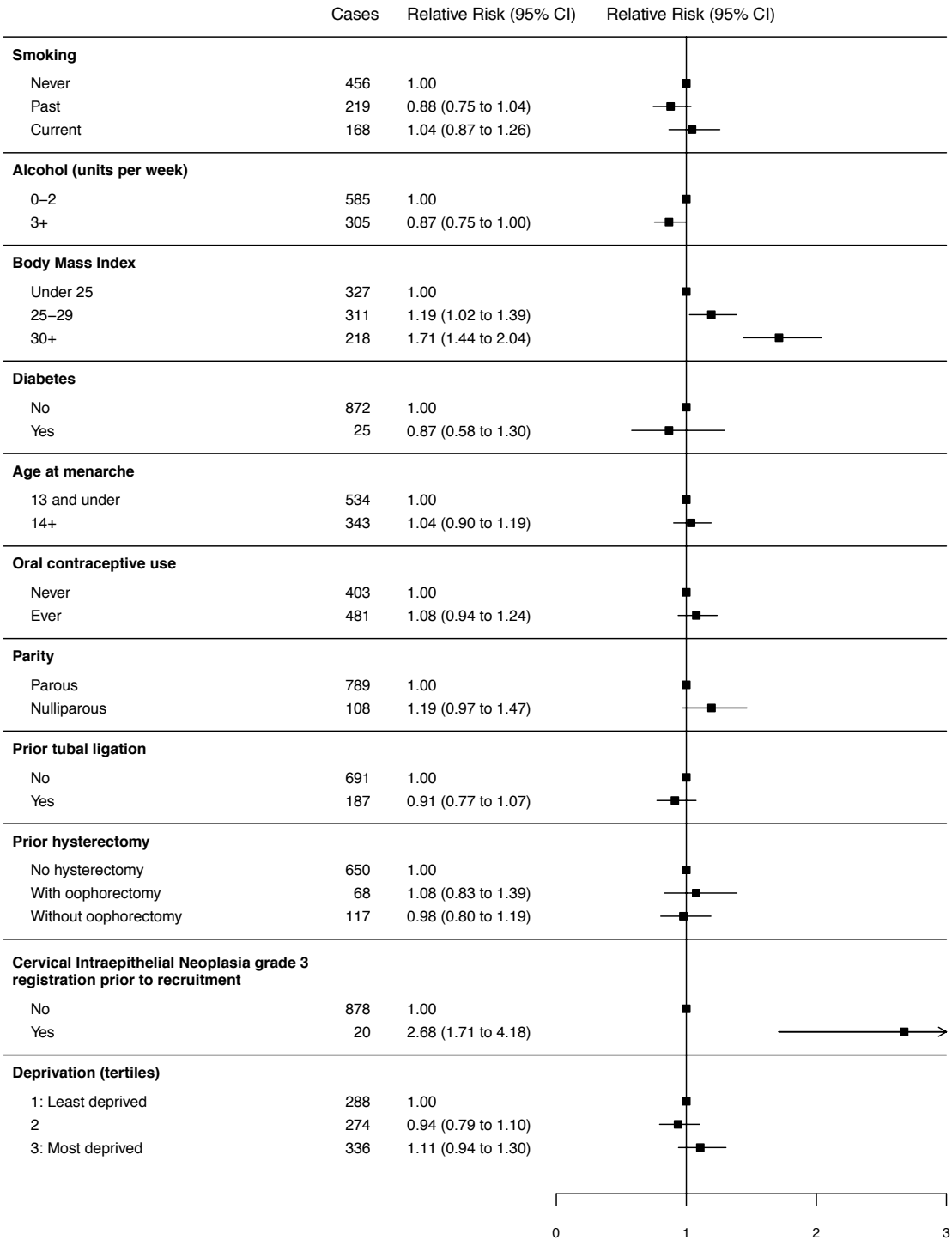
Vulval cancer cases in the cohort by time since recruitment and by 10-year age group

Time since recruitment (years)	Cases	Rate / 100,000	Person-time (years)
<5	198	3.12 (2.72-3.59)	6,342,722
5-9	293	4.90 (4.37-5.49)	5,981,188
10-14	370	7.21 (6.51-7.98)	5,132,744
15+	37	7.62 (5.52-10.52)	485,480
10-year age groups	**Cases**	**Rate/100,000**	**Person-time (years)**
<50	0	0	37.94
50-59	142	2.70 (2.29-3.18)	5,267,481
60-69	459	4.85 (4.42-5.31)	9,469,480
70+	297	9.27 (8.27-10.38)	3,205,135

The strongest predictor of risk of vulval cancer in our study was a registration of CIN 3 prior to recruitment (Figure 5.2). The 12,531 women with a CIN 3 lesion registered before entering the study made up 1% of the cohort, and these women had a RR of vulval cancer of 2.68 (95% CI 1.71-4.18, p= 0.0002) compared with women without such a history.

Being overweight or obese was associated with an increased risk of vulval cancer, compared with women with a normal BMI, and rising adiposity was associated with increasing risk ($p<0.0001$). Overweight women (BMI of 25-29) had a 19% increase in risk of incident vulval cancer, with a RR of 1.19 (95% CI 1.02-1.39; $p = 0.03$), and this rose further in women classed as obese, (BMI of 30 and over), who had a RR of 1.71 (95% CI 1.44-2.04; $p<0.0001$). There was no additional increase in risk seen in women with diabetes in a multivariate model after adjustment for BMI and various other risk factors.

There was no significant association between smoking status and vulval cancer risk; women who reported ever having smoked had a RR of 0.98 (95% CI 0.82-1.17) compared with never smokers. No significant increase in risk was seen with alcohol use, age at menarche, oral contraceptive pill use, parity, or tubal ligation. Hysterectomy with or without oophorectomy was also not significantly associated with an increased risk of vulval cancer.



RRs adjusted for smoking, alcohol use, BMI, diabetes, age at menarche, oral contraceptive use, parity, tubal ligation, hysterectomy/oophorectomy, prior CIN 3, and deprivation

Figure 5-1 Forest plot showing results of an adjusted multivariate analysis of lifestyle and reproductive exposures and their association with vulval cancer risk.

Risk associated with age at menopause was examined in women who had never used menopausal hormone therapy (HT) and reported a natural menopause or bilateral oophorectomy. While the majority of our participants (86%) were postmenopausal at recruitment, only 38% of these reported never having used HT. Selection of this group was in order to avoid any confusion over age at cessation of menses, which can be masked by HT use, and in order to avoid confounding with HT use itself.

Earlier age at menopause was associated with a significantly increased risk of vulval cancer later in life, with a RR of 1.52 (95% CI 1.22-1.89, p<0.001) for women with natural or surgical menopause before age 50, compared with those whose menopause occurred after 50. Use of HT was not associated with a significant alteration in risk of subsequent vulval cancer in participants who reported being postmenopausal at baseline (Table 5.2).

Table 5-2 Associations between vulval cancer and risk factors associated with menopause

Exposure	Cases/population at risk	RR	95% confidence interval	p-value
Age at menopause in women with natural menopause or oophorectomy who have never used hormone therapy				
50+	158/237,144	**1.00**	(reference)	<0.001
<50	167/175,489	**1.52**	(1.22-1.89)	
Use of menopausal hormone therapy at baseline, all postmenopausal participants				
Never	401/493,167	**1.00**	(reference)	0.63
Past	142/192,727	**0.94**	(0.77-1.14)	
Current: <5 years	94/174,251	**0.88**	(0.69-1.12)	
5 years +	151/242,043	**0.85**	(0.70-1.03)	

RRs adjusted for smoking, alcohol use, BMI, age at menarche, oral contraceptive use, parity, tubal ligation, hysterectomy/oophorectomy, prior CIN 3, age at menopause, and deprivation

At resurvey, on average three years after recruitment, women were asked whether they were currently married or living with a partner and about their prior cervical screening attendance. There was no difference in risk of vulval cancer associated with marital status or self-reported past cervical cancer screening attendance (Table 5-3).

Table 5-3 Living with a partner and participation in cervical screening: associations with incidence of vulval cancer in respondents to resurvey 3 years after recruitment

Exposure	Cases/population at risk	RR	95% confidence interval	p-value
Married or living with a partner				
Yes	332/637,848	**1.00**	(reference)	0.29
No	104/153,669	**1.19**	(0.95-1.50)	
At least one prior cervical smear test?				
Yes	307/557,908	**1.00**	(reference)	0.23
No	16/23,980	**0.96**	(0.57-1.62)	

RRs adjusted for smoking, alcohol use, BMI, diabetes, age at menarche, oral contraceptive use, parity, tubal ligation, hysterectomy/oophorectomy, prior CIN 3, and deprivation

Table 5-4 International Classification of Diseases for Oncology version 3 morphology codes of the vulval cancers registered in the cohort

ICDO-3 morphology code	Histological subtype	Number (%)
	Squamous tumours	
M8070/3	Squamous cell carcinoma, NOS	447 (50.0)
M8071/3	Squamous cell carcinoma, keratinizing, NOS	131 (14.6)
M8070/5, M8076/3	Squamous cell carcinoma,microinvasive	16 (1.8)
M8051/3	Verrucous carcinoma, NOS	8 (0.9)
M8072/3, 8074/3, 8075/3	Squamous cell carcinoma: large cell, non-keratinizing/spindle cell/ adenoid	7 (0.8)
	Total squamous cell tumours	**609 (67.8)**
	Glandular tumours	
M8542/3	Paget's disease	73 (8.1)
M8480/3,M8400/3, M8140/3	Adenocarcinoma: mucinous/sweat gland/ NOS	13 (1.4)
M8200/3,M8390/3, M8560/3	Adenoid cystic/skin appendage/adenoquamous carcinoma	10 (1.1)
	Total glandular tumours	**96 (10.7)**
	Basal cell tumours	
M8090/3	Basal cell carcinoma, NOS	80 (8.9)
M8091/3	Multifocal superficial basal cell carcinoma	6 (0.7)
M8094/3, M8097/3	Basosquamous carcinoma, basal cell carcinoma (nodular)	6 (0.7)
	Total basal cell carcinomas	**92 (10.2)**
	Melanocytic tumours	
M8720/3	Malignant melanoma NOS	21 (2.3)
M8721/3	Nodular melanoma	16 (1.8)
M8743/3	Superficial spreading melanoma	14 (1.6)
M8744/3, M8746/3	Acral lentiginous melanoma, malignant / mucosal lentiginous melanoma	4 (0.4)
	Total melanocytic tumours	**55 (6.1)**
	Other tumours: unspecified and sarcomatous	
M8000/3, 8010/3	Neoplasm/epithelial tumour, malignant	37 (4.0)
M8032/3, M8033/3, M8800/3, M8832/3, M8851/3, M8890/3, M8891/3	Spindle cell carcinoma, NOS/ Pseudosarcomatous carcinoma/ Sarcoma, NOS/ Dermatofibrosarcoma, NOS/ Liposarcoma, well-differentiated / Leiomyosarcoma / Epithelioid leiomyosarcoma	9 (0.9)
	Total 'other' tumours	**46 (5.1)**
	Total incident vulval cancers	**898** (100.0)

There were insufficient numbers of cases to examine all exposures by histological subtype, but it was possible to look at body mass index, smoking, age at menopause and menopausal HT use in a multivariate competing hazards model stratified by tumour histology (Table 5.5). Obese women, with BMI of 30 and above, had a significantly elevated risk of squamous cell carcinoma, but not basal cell, glandular, or melanocytic tumours (test for heterogeneity p = 0.01).

Similarly, when the risk associated with age at menopause was examined, the relationship was strongest for tumours with glandular and squamous cell morphology (p for heterogeneity = 0.03). There was no significant heterogeneity in the effect of smoking or menopausal hormone therapy on the risk of vulval cancer stratified by histological subtype.

Table 5-1 Associations between body mass index, smoking, and vulval cancer

	Squamous cell carcinoma			Basal cell carcinoma			Glandular tumours			Melanocytic tumours			p
Exposure	Cases/population at risk	RR	95% CI	Cases/population at risk	RR	95% CI	Cases/population at risk	RR	95% CI	Cases/population at risk	RR	95% CI	
BMI													
<30	406/1,010,993	**1.00**	-	76/1,010,993	**1.00**	-	86/1,010,993	**1.00**	-	43/1,0110,993	**1.00**	-	**0.01**
30+	168/220,976	**1.83**	(1.52,2.20)	16/220,976	**1.04**	(0.60,1.79)	17/220,976	**0.88**	(0.52,1.50)	11/220,976	**1.22**	(0.62,2.38)	
Smoking													
Never	294/627,145	**1.00**	-	52/627,145	**1.00**	-	55/627,145	**1.00**	-	36/627,145	**1.00**	-	
Ever	276/596,249	**1.02**	(0.86,1.21)	34/596,249	**0.76**	(0.49,1.18)	48/596,249	**1.01**	(0.68,1.50)	15/596,249	**0.48**	(0.26,0.89)	**0.07**
Age at menopause													
50+	208/508,813	**1.00**	-	42/508,813	**1.00**	-	34/508,813	**1.00**	-	24/508,813	**1.00**	-	
<50	299/532,286	**1.49**	(1.23,1.81)	35/532,286	**0.97**	(0.60,1.59)	52/532,286	**1.70**	(1.07,2.72)	20/532,286	**0.58**	(0.28,1.19)	**0.03**
Menopausal hormone therapy use													
Never	288/493,167	**1.00**	-	34/493,167	**1.00**	-	41/493,167	**1.00**	-	20/493,167	**1.00**	-	
Past	87/192,727	**0.81**	(0.64,1.04)	20/192,727	**1.64**	(0.93,2.90)	22/192,727	**1.42**	(0.83,2.43)	6/192,727	**0.71**	(0.28,1.80)	**0.28**
Current	159/424,544	**0.79**	(0.63,0.98)	25/424,544	**1.08**	(0.61,1.93)	35/424,54	**1.14**	(0.69,1.89)	24/424,544	**1.31**	(0.67,2.56)	

RRs adjusted for smoking, alcohol use, BMI, diabetes, age at menarche, oral contraceptive use, parity, tubal ligation, hysterectomy/oophorectomy, prior CIN 3, and deprivation; menopausal HT use is also adjusted by age at menopause

5.5 Discussion:

The dual aetiology of vulval cancer is reflected in the associations observed in this study. The strongest relationship was seen for previous registration of CIN 3, which was similar to our findings for anal cancer, however the magnitude of the effect seen is much smaller. Strikingly, none of the other exposures that are typically associated with HrHPV-related cancers (smoking, oral contraceptive use, marital status) appear to have a significant association with vulval cancer in our analysis.

As suggested previously, adiposity has a significant association with subsequent risk of vulval cancer in our study. A 70% increase in risk of vulval cancer was associated with obesity, while women who are overweight have a lesser, but still increased risk. Having diabetes was not associated with risk in a multivariate model which included BMI. The effect of BMI appears to be confined to squamous cell carcinomas, and does not seem to be associated with an increased risk of other types of vulval cancer.

Younger age at menopause was also associated with an increased risk of vulval cancer at older ages in our cohort, with women who reported a natural or surgical menopause having just over a 50% increase in incidence of vulval cancer after the age of 50.

Risk was not affected by self-reported smoking; alcohol use; marital status; parity; hysterectomy; attendance at cervical cancer screening; or use of exogenous hormones, either during the reproductive years, in the form of oral contraceptive use, or during the peri-menopausal or menopausal years, using menopausal hormone therapy.

5.5.1 CIN 3:

Despite the relatively small number of cases with a history of CIN 3 (n= 20), having a registration of the highest grade of HrHPV-associated cervical dysplasia was the strongest predictor of risk of subsequent vulval cancer identified in the cohort. Given that that only around 1 in 5 vulval cancers in older women are thought to be HrHPV-related, the relative risks reported here for vulval cancer after CIN 3 are much lower than those seen for anal cancer, which has a closer association with HrHPV infection. The risk of anal cancer associated with previous CIN 3 was greater, with a RR of 4.03 (95% CI 2.59–6.28, p<0.0001), compared with the RR of 2.68 (95% CI 1.71-4.18, p< 0.0001) seen here for vulval cancer.

Despite the fact that the non-viral pathway is more common in older women, clearly there are still a number of cases in the cohort aetiologically related to HrHPV infection. A retrospective study from Denmark (91) included 116 women with vulval SCC, and found that around 50% of the vulval tumours (compared with 89% of vaginal tumours in the same study), were HrHPV positive (p<0.001). They did not look at HPV-positivity by age, but only 17% (20/119) of their vulval cancer cases were under 50 at diagnosis. Overall they found a significant relationship between vulval cancer risk and number of lifetime partners, history of anogenital warts, pre-invasive or invasive cervical disease, smoking, alcohol consumption, and years at school. When they compared HrHPV-positive with HrHPV-negative tumours, there was significant heterogeneity with respect to the increase in risk seen in women with a history of smoking and cervical neoplasia, with only

HrHPV-positive cases at elevated risk. Being unmarried was associated with an increased risk of HrHPV-negative vulval cancer.

Several groups have examined the risk of subsequent anogenital cancer after a diagnosis of CIN or other primary anogenital cancer (55,62,92–94) . A Finnish study (62) followed 7564 women treated for CIN 1-3 between 1974-2001, with information on incident cancers to 2003. They found an increased risk of several cancers, predominantly at anogenital sites, as well as smoking-related cancers. They reported a standardised incidence ratio (SIR) of 4.1 for vulval cancer (95% confidence interval 1.5-8.9) after treatment of any grade of CIN, which was higher than our estimate, however, participants in their cohort were younger than our participants, and vulval cancer at younger ages appears to be more likely to be HrHPV-related.

It would have been helpful to be able to distinguish between the squamous cell cancers in the cohort that were likely to be related to HrHPV, and those that were not, based on their morphology coding. Vulval cancers in older women are more commonly keratinising SCCs, which are less likely to be HPV-related, whereas those with warty or basaloid morphology are commoner in younger women and more likely to be related to HPV infection (14). Ultimately, our ability to distinguish between histopathological subtypes was severely limited. There were only 8 registrations of tumours with warty (verrucous) morphology, and no basaloid SCCs. Of 609 squamous cell cancers, 131 (22%) were registered as 'keratinising' SCCs, and 447 (73%) were 'squamous cell carcinoma NOS' with no further information on morphology.

Given the age of Million Women Study participants, it is likely that the majority (probably at least 80%) of the SCC NOS group were keratinising SCCs, and the remainder were other subtypes including warty and basaloid tumours. In a recent large international study, which examined over 1700 vulval cancers (15), 72% of the SCCs were keratinising, and 19% had warty/basaloid morphology. Tumours of warty or basaloid morphology had a high incidence of HPV positivity, regardless of age at diagnosis. In contrast, keratinising tumours in women younger than 56 years of age in their study were more likely to be HPV positive than the same morphological variant when it occurred in older women. Under age 56, they found nearly 50% of vulval cancers of any histological subtype to be HPV positive; this dropped to around 20% in women aged 67 and over.

The under-representation of warty and basaloid SCCs in our cohort is likely to be multifactorial. Study participants were recruited via the NHS Breast Screening programme, and women in the UK are invited to participate in breast screening from the age of 50 onwards. It is reported that tumours with warty or basaloid morphology are commoner at younger ages, so it may be that there are simply fewer HrHPV-related tumours in our cohort due to the ages of participants. However it seems likely that the large number of tumours with unspecified ('SCC NOS') morphology, which made up half (50%) of all the cancers registered, and nearly three-quarters (73%) of the SCCs, may also be a factor.

The Royal College of Pathologists currently uses standardised guidelines for the histopathological reporting of vulval neoplasms (30). This document includes a pro-forma, which subdivides vulval cancers into: 'squamous (usual)', 'verrucous',

'adenocarcinoma', 'basal cell', and 'other (please specify)'. Basaloid tumours are not included in this categorisation, and therefore despite being mentioned elsewhere in the guidelines, there may be systematic under-reporting of tumours with basaloid morphology if pathologists are using this form for their reporting directly, or using it as an 'aide memoire' it to help them produce free text written reports.

There was no evidence of an association between self-reported cervical cancer screening attendance and vulval cancer, which has previously been shown to be associated with a 70-80% lower risk of vulval cancer in one small study (95); however, very few women reported no cervical screening attendance at all. There was also no apparent relationship with marital status.

Due to the paucity of 'classically' HrHPV-related tumours in our cohort (warty/basaloid subtypes) it was not possible to identify a group of SCCs that were more likely to be related to HrHPV infection, and directly compare associations by histological subtype within the squamous cell carcinomas, which may be why, apart from history of CIN 3 registration, significant relationships were not seen between HrHPV-related exposures and risk of vulval cancer in this study.

5.5.2 Obesity:

Some of the earliest studies looking at the epidemiology of what was then known as epidermoid carcinoma of the vulva described a high incidence of obesity in women with invasive vulvar tumours (96,97), however these were descriptive studies, mainly case series, and did not use population control groups or any formal statistical testing.

An early case series from Johns Hopkins Hospital (98), which looked at nearly 40 years of data, included women seen between 1935 and 1972, and found an overall incidence of obesity of 64% amongst women with vulval cancer, with 'on many occasions…obesity…described as massive and weights of well over 200 lbs…". Their cases included 246 SCCs (75%), 30 Paget's disease (9%), 16 melanomas (5%) and 8 sarcomas (2%), similar to the histopathological distribution in our study. Their study, along with several others (97,99), also suggested a relationship between diabetes and vulval cancer, which has not been confirmed here.

An Italian case-control study (95) found the risk of primary vulval cancer increased with BMI amongst 73 women with vulval cancer compared with 572 hospital controls. They found an odds ratio (OR) of 1.3 (95% CI 0.7-2.4) in overweight women (BMI between 25 and 30), and 2.3 (95% CI 1.1-4.5) in the obese group (BMI 30+), p for trend =0.05. These risks were higher than those seen here, however the relationship was not statistically significant in a multivariate model adjusted for age, parity, smoking, sexual partners, education, number of pap smears, and years since last pap smear.

Two other case-control studies similarly found either a non-significant (99), or no increase in risk of vulval cancer associated with increased weight or BMI (100). Kirschner et al (101) looked at survival in vulval SCC using obesity and smoking (amongst other exposures) as prognostic factors. 47% of their patients were reported to be obese, with a BMI of 27 or more. Smoking, but not obesity, was associated with a relative decrease in survival.

A recent review which looked at obesity and risk of gynaecological cancer concluded that while there may be a relationship between adiposity and vulval cancer, studies have been too small in size and number to definitively answer the question (102).

The vulval cancers which predominate at older ages and are not caused by HPV are said to be more likely to be related to chronic and inflammatory vulval skin conditions, such as lichen sclerosis (LS), lichen simplex chronicus, spongiotic dermatitis, or lichen planus (103). Though women with LS have a very high relative risk of vulval cancer, quoted at around 200 times the risk seen in the general population (104), the absolute risk in this group remains small. It is thought that around 5% of women with symptomatic vulval lichen sclerosis go on to develop squamous cell carcinomas; however, a literature review by Carlson et al (103) suggested that around 22% of women presenting with vulval SCCs were found to have previously undiagnosed 'clinically silent' LS adjacent to their tumours. They estimated that in women with symptomatic LS, there was an interval of approximately 20 years between mean age at diagnosis of LS (54 years) and mean age of development of SCC (74 years), which is close to the age at which a sharp rise in incidence of vulval tumours is seen in the UK (Figure 5.1, above).

Obesity has been found to have a significant association with genital lichen sclerosis in men; a recent case-control study found significantly higher BMI amongst male LS cases compared with age and ethnicity-matched controls (105). LS-affected men had a mean BMI of 31.0 (range 18.9-52.6) vs. 28.1 (16.8-64.1) in controls (p=0.001). I was unable to find reports of similar findings in women; lichen sclerosis is more common in women, but like many chronic conditions, is poorly studied.

One early paper on lichen sclerosis (106) hypothesized that obese females might be particularly at risk of lichen sclerosis due to local irritation of the skin or the effect of 'sugar-laden…urine'. A relatively large population-based case-control study from the UK published in 2012 (107) examined risk factors for vulval lichen sclerosis. They did not examine obesity, but did find a strong association between risk of LS and family history of diabetes (OR 7.0, 95% CI 1.54-31.84, p=0.012). They had insufficient numbers to assess personal history of diabetes, as there were 6 cases, but no controls with the disease.

An increase in risk of vulval squamous cell carcinoma was associated with rising adiposity in this study, but no additional risk was associated with diabetes. BMI did not appear to have an effect on the risk of other histological subtypes of vulval cancer. The increase in risk of vulval cancer associated with increased BMI in older women may have a mechanical mechanism, where irritation of the vulval skin leads to inflammation and carcinogenesis; there may be local immunological or other factors underlying the association, or there could be a hormonal explanation, though this seems less likely as we did not see an association between vulval cancer and menopausal HT use.

5.5.3 Age at menopause:

Early age at menopause was another risk factor noted in the earliest epidemiological studies of vulval cancer (96,97). In a case review of 249 patients with vulval cancer conducted in the United States of America in 1972, Franklin and Rutledge (97) noted that 19% of their cases had undergone menopause (natural or surgical) before the age of 41, and 54% by the age of 45, which was much higher than expected based on a normal population. They also commented on a high incidence of nulliparous women amongst their vulval cancer cases, though parity was not significantly associated with risk in the Million Women cohort.

Not everyone has found an elevated risk associated with earlier age at menopause; a retrospective study from the United States (100), including 209 vulval cancer cases and 348 controls, found an increased risk amongst women with early ages at natural or surgical menopause, RR 1.89 (95% CI 0.9-3.8) for surgical menopause < 40 years, and RR 1.75 (95% CI 0.8-4.1) for natural menopause <45 years, but this association did not reach statistical significance. They found no alteration in risk associated with HT use.

Parazzini et al (95) found no significant variation in vulval cancer risk associated with menopause aged 50-52 (OR 0.8, 95% CI 0.4-1.5), or 52 and over (OR 1.0 (95% CI 0.3-2.0) compared with women under 50 years of age at menopause. Menopausal status, age at menopause, and HT use were also examined in a case-control study by Newcomb et al (99), who did not find any difference in risk associated with any of these exposures, however this was a small study with only 22 cases of invasive vulval cancer.

There was a significant increase in risk associated with younger age at menopause in the Million Women Study cohort, with just over a 50% increase in risk of vulval cancer in women who reported undergoing a natural or surgical menopause (oophorectomy) prior to the age of 50 compared with a later menopause at 50 years or older. When the analysis was stratified by histological subtype of tumour, the increased risk associated with younger age at menopause showed significant heterogeneity by tumour subtype, with an increase in risk seen for squamous cell (RR 1.49, 95% CI 1.23-1.81) and glandular tumours (RR 1.70, 95% CI 1.07-2.72), but not basal cell carcinomas or melanocytic tumours (test for heterogeneity p = 0.03).

5.6 Conclusion:

By the age of seventy, vulval cancer overtakes cervical cancer to become the commonest female anogenital cancer in the United Kingdom. Using data from over a million women with prospectively collected information on exposures, and an average of 14 years of follow-up, a history of CIN 3, being overweight or obese, and having a menopause prior to age 50 were associated with an increased risk of incident vulval cancer in women over 50 years of age.

When risk was examined by histological subtype, the association between adiposity and risk of vulval cancer was restricted to squamous cell tumours, and was not seen for tumours with glandular, basal cell, or melanocytic morphologies. The effect associated with younger age at menopause was similarly confined to squamous and glandular tumours, and was not seen for basal cell or melanocytic tumours.

There was no significant heterogeneity of effect for smoking by tumour subtype, and there were too few cases to examine any of the other exposures in this type of subgroup analysis.

The risk factors associated with an increased risk of vulval cancer in this study are for the most part different to those seen in cervical and anal cancer (47,72,77), notably the lack of an increased risk associated with smoking or past oral contraceptive use, which probably reflects the smaller contribution of high-risk HPV infection to the aetiopathology of vulval cancer in women over the age of 50.

Chapter 6 Vaginal cancer

6.1 Abstract

Objective: To examine associations between reproductive and lifestyle exposures, including past carcinoma *in situ* of the uterine cervix, and incident vaginal cancer in women over 50 from the Million Women Study cohort.

Population: 1.3 million UK women, aged 50 years and over.

Methods: Adjusted Cox regression models

Main Outcome Measures: Registrations of incident vaginal cancer (ICD-10 C52), classified by ICDO-3 cancer morphology code into groups by histological subtype.

Results: 170 incident vaginal cancers were registered in the cohort over 14 years of follow-up. Just over half of the registered cancers (52%, n=89) had squamous cell morphology, while the next most common histological subtype was adenocarcinoma (n=31, 18%).

The greatest relative increase in risk of vaginal cancer was seen in women with a history of cervical intraepithelial neoplasia grade 3, RR 7.62 (95% 4.19-13.86, p< 0.00001). Women who reported having had a hysterectomy with oophorectomy prior to recruitment had a RR of 3.76 (95% CI 2.53-5.59) compared to women without hysterectomy, while those who reported a hysterectomy without oophorectomy had a lower, but still nearly doubled risk of 1.92 (95% CI 1.29-2.87), p <0.00001. Other factors associated with a significantly increased risk of vaginal cancer were adiposity (RR 1.49, 95% CI 1.08-2.08, p = 0.02) and nulliparity (RR 1.64, 95% CI 1.08-2.50, p = 0.03). Finally,

women who reported not living with a husband or partner had around a 60% increase in risk of vaginal cancer, RR 1.63 (95% CI 1.02-2.59, p = 0.045).

Smoking, alcohol use, age at menarche, oral contraceptive pill use, non-attendance for cervical screening, age at menopause, and use of menopausal hormone therapy were not significantly associated with risk of vaginal cancer in women over 50.

When associations were examined by histopathological subtype, there was insufficient evidence to support heterogeneity of risk associated with smoking, BMI, past use of the oral contraceptive pill, hysterectomy or parity between vaginal tumours with squamous cell vs. glandular (adenocarcinoma) morphology.

Conclusion: Having had a registration of CIN 3, a hysterectomy (with or without oophorectomy), being overweight or obese, nulliparity, and not living with a husband or partner are associated with an increased risk of vaginal cancer after the age of 50, but it remains a rare cancer with a low incidence. Although prior CIN 3 has a strong relationship with subsequent vaginal cancer, 93% of cases occurred in women without a history of CIN 3, meaning that the absolute risk of vaginal cancer in women with a prior CIN 3 registration is still very low, at around 1 in 1000.

6.2 Introduction

"Vaginal cancer occurs relatively infrequently as a presenting disease…[t]he mean age of patients affected with primary vaginal cancer is greater than that of patients with cancer in other genital sites (except the vulva)" (108). Of the anogenital cancers, vaginal cancer is the rarest, and occurs in around 200 women a year in England (19,109). Globally, it is similarly uncommon, with an estimated 13,000 cases diagnosed internationally in 2008 (110). Like anal and vulval cancer, vaginal cancer occurs most frequently in women over the age of 50, with 89% of registered cases in England in 2012 occurring in that age group (19). For reasons that are not entirely clear, vaginal cancer appears to be an aggressive malignancy with a poor prognosis, possibly due at least in part to late stage at presentation in many cases (111).

Vaginal cancers make up 1-2% of gynaecological cancers. Although vaginal tumours can occur as secondary disease following upward spread of a primary vulval lesion, or downward and lateral spread of a cervical cancer (or as a distant metastasis of other genital or non-genital cancers), tumours are only classed as primary vaginal cancers if the primary site of growth is the vagina—FIGO (International Federation of Gynaecology and Obstetrics) guidelines stipulate that tumours with cervical or vulval involvement must be counted as cervical or vulval primaries, respectively (18).

Around three-quarters of vaginal cancers are thought to be related to HrHPV infection (112). 80-90% of vaginal cancers have squamous cell morphology (113), and the risk factors for these are thought to be similar to those for cervical cancer (26), including smoking, HPV, other STIs, low socio-economic status, and multiple sexual partners.

In addition, vaginal trauma related to uterine prolapse and pessary use (27), hysterectomy, diethylstilboestrol exposure, and prior pelvic irradiation have been suggested as risk factors for vaginal cancer that are not commonly associated with cervical cancer (114).

Previous work looking at risk factors for vaginal cancer has been limited by the small numbers of affected women. There have been a few reviews (115,116) and retrospective studies (25,26,68,117,118), which have examined associations for *in situ* and invasive vaginal tumours, but no previous prospective cohort studies with collection of self-reported exposure data. Here, associations between lifestyle and reproductive exposures (including nationally collected cervical intraepithelial neoplasia grade 3 (CIN 3) registrations) and risk of incident primary vaginal cancer are examined in a large cohort of UK women over 50 years of age.

6.3 Methods:

Full methods are reported above in Chapter 3. In brief, as elsewhere, women were excluded from the analysis if they had any invasive cancer registered prior to recruitment, other than non-melanoma skin cancer (ICD-10 C44). Exposures were defined using self-reported data from the recruitment and subsequent resurvey questionnaires, apart from deprivation which was calculated using the Townsend Deprivation Index. Cervical intraepithelial neoplasia grade 3 (CIN 3) registrations prior to recruitment were derived from National Cancer Registry data (ICD-10 D06).

Postmenopausal women were identified based on self-reported menopausal status at recruitment. Analyses examining associations with HT use included all postmenopausal participants. To avoid confounding, the analysis looking at the effect of age at menopause was further restricted to postmenopausal women who had undergone a natural menopause or had a bilateral oophorectomy prior to recruitment, and who had never used HT. Menopausal hormone therapy use, smoking, and alcohol use were categorised as they were reported at baseline.

Risk factors with adequate numbers of cases for separate examination were assessed by histopathological subtype for squamous cell carcinomas and adenocarcinomas, and these were identified using ICDO-3 (International Classification of Diseases for Oncology version 3) morphology codes (22).

Squamous tumours included: M8052/3, M8070/3, M8071/3, M8072/3, M8123/3. Adenocarcinomas included: M8140/3, M8263/3, M8310/3, M8380/3, M8460/3, M8480/3, M8481/3. Other morphologies, including melanocytic tumours, sarcomas, and a small number of 'other' or unspecified tumour types had insufficient numbers to

examine separately. Vaginal tumours are enumerated by morphological group in a table in the results section.

6.3.1 Statistical analyses

The relationship between several reproductive and lifestyle factors, and risk of vaginal cancer was examined using Cox proportional hazards models. Age was included in the model as the underlying time variable, and all analyses were adjusted for deprivation and stratified by recruitment region. Hazard ratios were estimated for multiple mutually adjusted exposures, with incident vaginal cancer as the outcome in all analyses. For clarity, hazard ratios are referred to hereafter as relative risks (RRs).

Participants entered the analysis from the date of recruitment, or at the age of 50 for the small number (3%) who were younger than 50 when they joined the study. Follow-up continued until the first occurrence of any one of the following end-points: date of first cancer registration; death; emigration or other loss to follow-up; or 31 December 2013.

Analyses were adjusted for: age at menarche (≤13, 14+); body mass index (<25, 25-29, 30+); past oral contraceptive pill use (never *vs*. ever); parity (parous *vs*. nulliparous); smoking (never, past, current); alcohol use (0-2 units/week, 2+ units/week); tubal ligation (no/yes); hysterectomy (no/yes); deprivation quintile (from 1 [least] to 5 [most deprived]); and cervical intraepithelial neoplasia grade 3 prior to recruitment (no/yes). Age at menopause (50+/<50) and HT use (never/ever) were examined separately in postmenopausal women. Using data from the first resurvey, sent out on average three years after recruitment, the impact of current marital status: married or living with a

partner (yes/no), and self-reported cervical smear attendance (yes/no) on risk of vaginal cancer were also examined.

The few women with missing data (fewer than 5% for each variable) were included in a separate category for adjustment variables and included in the analysis, but these data are not shown. Relative risks are reported with 95% confidence intervals. Analyses were performed in Stata 13 (1).

6.4 Results

170 primary vaginal cancers were registered in the cohort during an average of 14 years of follow-up (Table 6-1). Mean age at recruitment was 56, and this was similar for the whole cohort, and the small number of women who went on to develop vaginal cancer.

The mean age at vaginal cancer registration was 64.7 (SD). Women with squamous cell carcinomas were on average two years older at cancer registration than those with adenocarcinomas; women with squamous cell tumours were on average aged 66 at registration (65.6, SD 6.7), those with adenocarcinoma were 64 years of age (63.5, SD 6.6).

Participant characteristics were similar to those in the previous two chapters. When we calculated the rates of vaginal cancer registrations in the cohort, unlike for anal and vulval cancer, we did not observe a marked increase in incidence over time (Table 6.1). In the first five years after recruitment there were 0.73 vaginal cancer cases per 100,000 women (95% CI 0.54-0.97), which rose to 1.10 at 5-9 years (95% CI 0.87-1.40), and remained similar at 10-14 years and 15+.

However, when we looked at incidence of vaginal cancer by age in 10-year age groups, there was a steady increase in incidence from 0.76 cases per 100,000 (95% CI 0.56-1.04) at ages 50-59, to 1.22 per 100,000 at ages 70+ (95% CI 0.89-1.67). Overall the incidence rates seen in the cohort were similar to those seen nationally, with reported figures for England in 2012 (19) of 0.8 per 100,000 at ages 50-54, rising to 2.2 per 100,000 at 70-74, see Fig 5.1).

Table 6-1 Participant characteristics and incidence rates, vaginal cancer models

Vaginal cancer analysis – characteristics			
Number of participants (%)			
	All	1,300,042	(100.0)
	Vaginal cancer cases	170	(0.01)
Mean age at recruitment (SD)			
	All participants	56.1	(4.9)
	Vaginal cancer cases	56.3	(4.9)
Mean age at vaginal cancer registration (SD)			
	Squamous cell cancers	65.6	(6.7)
	Adenocarcinomas	63.5	(6.6)
	All	64.7	(6.3)
Participant characteristics at recruitment			
	Ever used oral contraceptive pill % (n)	58.6	(761,700)
	Current smoker % (n)	19.2	(249,182)
	Hysterectomy % (n)	24.4	(316,927)
	Tubal ligation % (n)	22.3	(289,625)
	Nulliparous % (n)	10.8	(140,630)
	Body mass index > 30 (obese) % (n)	17.0	(220,976)
	Current hormone therapy user % (n)	33.0	(428,539)
	Postmenopausal % (n)	86.3	(1,122,569)
	Most deprived quintile % (n)	19.7	(255,560)

Vaginal cancer incidence rates by time since recruitment (5-year groups) and by age (10-year groups)

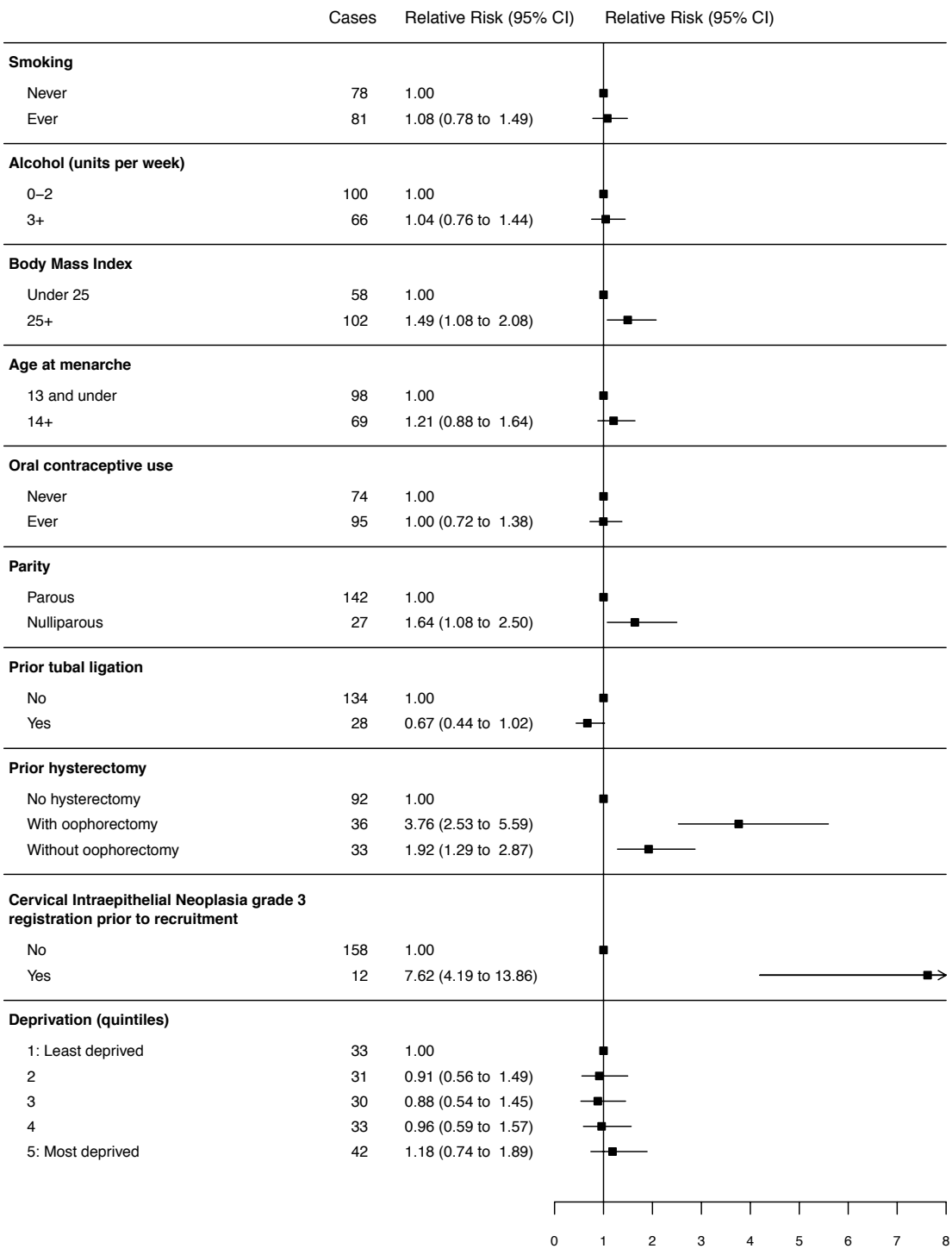
Time (years)	Cases	Rate / 100,000 (95% CI)	Person time (years)
<5	46	0.73 (0.54-0.97)	6,342,722
5-9	66	1.10 (0.87-1.40)	5,981,188
10-14	54	1.05 (0.81-1.37)	5,132,744
15+	4	0.82 (0.31-2.20)	485,480

Age group	Cases	Rate (95% CI)	Person-time (years)
<50	0	0	37.94
50-59	40	0.76 (0.56-1.04)	5,267,481
60-69	91	0.96 (0.78-1.18)	9,469,480
70+	39	1.22 (0.89-1.67)	3,205,135

In the main analysis, associations between various reproductive and lifestyle risk factors and incident vaginal cancer were examined in an adjusted multivariate model using age as the underlying time variable (Figure 6-1). The exposure with the strongest association with risk of vaginal cancer later in life was a history of high-grade screen-detected abnormality of the cervix, in the form of a registration of CIN 3 prior to recruitment. This was associated with a RR of vaginal cancer of 7.62 (95% 4.19-13.86, p<0.00001) compared to women without a history of CIN 3.

Hysterectomy with or without oophorectomy was associated with a 2-3 times increase in risk of vaginal cancer. Women who reported a hysterectomy with oophorectomy prior to recruitment had a RR of 3.76 (95% CI 2.53-5.59), and those reporting hysterectomy without oophorectomy had a RR of 1.92 (95% CI 1.29-2.87), p<0.00001. The overall risk associated with having a hysterectomy (irrespective of oophorectomy status) vs. no hysterectomy was 2.54 (95% CI 1.87-3.47, p<0.00001).

Women who were overweight or obese had around a 50% increase in risk of vaginal cancer (RR of 1.49, 95% CI 1.08-2.08, p = 0.02) compared with women whose BMI was less than 25. The increase in risk was similar for women in the overweight (BMI 25-29) and obese (BMI 30+) groups, RR 1.54 (95% CI 1.08-2.18) and RR 1.40 (95% CI 0.90-2.18) respectively. Women who reported never having given birth were also at an increased risk of vaginal cancer later in life; nulliparity was associated with a RR of 1.64 (95% CI 1.08-2.50, p = 0.03) compared with women who had given birth. Smoking, alcohol consumption, age at menarche, past oral contraceptive use, tubal ligation, and deprivation were not associated with risk of incident vaginal cancer in our study.



RRs adjusted for smoking, alcohol use, BMI, age at menarche, oral contraceptive use, parity, tubal ligation, hysterectomy/oophorectomy, CIN 3, and deprivation

Figure 6-1 Forest plot showing associations between vaginal cancer and various lifestyle and reproductive factors in an adjusted multivariate model.

When the analysis was restricted to women who reported being postmenopausal at recruitment, younger age at menopause was not significantly related to vaginal cancer risk in women with a natural menopause who had never used HT (Table 6-2).

When we considered any postmenopausal participant, use of menopausal hormone therapy also showed no significant association with subsequent risk of vaginal cancer (Table 6-2). This lack of effect persisted when we considered ever (past and current) vs. never use of HT (RR 1.04, 95% CI 0.74-1.47, p = 0.80, not shown in table).

Table 6-2 Association between age at menopause and use of menopausal hormone therapy and vaginal cancer risk in postmenopausal participants

Exposure	Cases/population at risk	RR	95% confidence interval	p-value
Age at menopause in women with natural menopause or oophorectomy who have never used hormone therapy				
50+	30/237,144	**1.00**	(reference)	
45-49	12/125,989	**0.75**	(0.38-1.48)	0.56
<45	8/49,480	**1.19**	(0.54-2.62)	
Use of menopausal hormone therapy at baseline, all postmenopausal participants				
Never	69/493,167	**1.00**	(reference)	
Past	18/192,727	**0.69**	(0.41-1.17)	0.13
Current: <5 years	26/174,251	**1.26**	(0.78-2.03)	
5 years +	40/242,043	**1.27**	(0.84-1.93)	

RRs adjusted for smoking, alcohol use, BMI, age at menarche, oral contraceptive use, parity, tubal ligation, hysterectomy/oophorectomy, CIN 3, and deprivation; HT analysis also adjusted for age at menopause

Participants who answered the first resurvey, which was sent out on average three years after recruitment, were asked about current or recent co-habitation (Table 6-3). 19% of respondents reported not currently living with a husband or partner, and those women had around a 60% increase in risk of vaginal cancer, RR 1.63 (95% CI 1.02-2.59, p = 0.05). A very small number of women (23,996, or around 3% of respondents) reported never having had a cervical smear test. There was no association between vaginal cancer risk and cervical smear attendance based on self-reported data (RR 0.41, 95% CI 0.10-1.73, p = 0.17).

Table 6-3 Associations between living with a husband or partner on average 3 years after recruitment, self-reported cervical cancer screening attendance, and vaginal cancer risk

Exposure	Cases/population at risk	RR	95% confidence interval	p-value
Married or living with a partner				
Yes	64/638,180	**1.00**	(reference)	0.05
No	28/153,773	**1.63**	(1.02-2.59)	
Ever attended for cervical cancer screening				
Yes	55/558,215	**1.00**	(reference)	0.17
No	2/23,996	**0.41**	(0.10-1.73)	

RRs adjusted for smoking, alcohol use, BMI, age at menarche, oral contraceptive use, parity, tubal ligation, hysterectomy/oophorectomy, CIN 3, and deprivation

Registered vaginal cancers were then classified by histological subtype, using the ICDO-3 morphology codes. Just over half of the tumours were squamous cell carcinomas (n = 89, 52%) (Table 6-4). Adenocarcinomas accounted for just under 20% of the cases, (n = 31, 8%). The remaining cases were other epithelial tumours (n = 17, 10%); sarcomas (n = 17, 10%); melanocytic tumours (n= 13, 8%) or unspecified neoplasms (n = 3, 2%).

Three cases of vaginal cancer in the cohort had clear cell morphology; this is typically considered to be associated with DES exposure in younger women. We looked at mean age at registration for the three clear cell carcinoma cases compared with all other morphologies. Mean age at cancer registration was substantially younger for cancers with clear cell morphology, with a mean age of 56.3 (SD 1.2) for clear cell carcinoma cases, and 65.1 (SD 6.2) for other morphologies.

Table 6-4 Vaginal cancer cases by histological subtype, classified by International Classification of Diseases for Oncology version 3 morphology code

ICDO3 morphology code	Histological subtype	Number (% of vaginal cancers)
	Epithelial tumours	
Squamous cell carcinoma (all variants)		
M8070/3	Squamous cell carcinoma, NOS	75 (44.1)
M8071/3	Squamous cell carcinoma, keratinizing, NOS	9 (5.3)
M8052/3, M8123/3, M8072/3	Papillary squamous cell carcinoma / Basaloid carcinoma / Squamous cell carcinoma, lg. cell, non-keratinizing	5 (3.0)
	Total squamous cell carcinomas	**89 (52%)**
Adenocarcinoma (all variants)		
M8140/3	Adenocarcinoma NOS	21 (12.4)
M8310/3	Clear cell adenocarcinoma, NOS	3 (1.9)
M8263/3, M8380/3 M8460/3,M8480/3 M8481/3	Adenocarcinoma in tubulovillous adenoma / Endometrioid carcinoma / Papillary serous cystadenocarcinoma / Mucinous adenocarcinoma / Mucin-producing adenocarcinoma	7 (4.1)
	Total adenocarcinomas	**31 (18%)**
Carcinoma not otherwise specified or other		
M8010/3	Carcinoma, NOS (Epithelial tumour, malignant)	7 (4.1)
M8012/3, M8020/3 M8021/3, M8033/3 M8041/3, M8050/3 M8246/3, M8560/3	Large cell carcinoma, NOS / Carcinoma, undifferentiated, NOS / Carcinoma, anaplastic, NOS /Pseudosarcomatous carcinoma / Small cell carcinoma, NOS /Papillary carcinoma, NOS / Neuroendocrine carcinoma / Adenosquamous carcinoma	10 (5.9)
	Total carcinoma NOS/other epithelial tumours	**17 (10%)**
	Non-epithelial tumours	
Melanocytic tumours		
M8720/3, M8721/3	Malignant melanoma NOS / Nodular melanoma	13 (8.1)
	Total melanocytic tumours	**13 (8%)**
Sarcomas		
M8800/3	Sarcoma NOS	2 (1.2)
M8801/3, 8890/3 M8933/3, M8950/3 M8980/3	Mixed Mullerian tumour / Leiomyosarcoma, NOS / Adenosarcoma / Spindle cell sarcoma / Carcinosarcoma, NOS	15 (0.9)
	Total sarcomatous tumours	**17 (10%)**
Other		
M8000/3	Neoplasm, malignant	3 (1.7)
	Total 'other' tumours	**3 (2%)**
	Total incident vaginal cancers (all morphologies)	**170 (100%)**

A competing hazards model was used to look at the relative risks of smoking, past oral contraceptive use, BMI, parity, and hysterectomy in squamous cell vaginal cancers vs. adenocarcinomas (Table 6-5). The model was mutually adjusted for the exposures examined, but CIN 3 examined, as there were no cases of adenocarcinoma in women with prior CIN 3. There was no significant heterogeneity in risk associated with these exposures, however the small number of cases severely limited our ability to detect any differences; our results for smoking in particular are suggestive of a difference.

Table 6-5 Associations between smoking, oral contraceptive use, body mass index, parity, hysterectomy and vaginal cancer by histological subtype

	Squamous cell cancer			Adenocarcinomas			Heterogeneity between tumour types
Exposure	**Cases**	**RR**	**95% CI**	**Cases**	**RR**	**95% CI**	**p**
Smoking							
Never	35/627,145	**1.00**	-	18/627,145	**1.00**	-	0.08
Ever	47/596,249	**1.39**	0.89-2.17	11/596,249	**0.65**	0.30-1.39	
Oral contraceptive pill use							
Never	37/523,823	**1.00**	-	16/523,823	**1.00**	-	0.15
Ever	52/761,699	**1.15**	0.74-1.78	14/761,699	**0.60**	0.29-1.27	
Body mass index							
<30	70/1,010,993	**1.00**	-	20/1,010,993	**1.00**	-	0.39
30+	15/220,976	**0.91**	0.52-1.60	7/220,976	**1.45**	0.61-3.46	
Parity							
Parous	74/1,157,186	**1.00**	-	27/1,157,186	**1.00**	-	0.39
Nulliparous	14/140,630	**1.72**	0.96-3.06	3/140,630	**0.98**	0.30-3.27	
Hysterectomy							
No	50/973,625	**1.00**	-	13/973,625	**1.00**	-	0.22
Yes	38/316,925	**2.33**	1.52-3.55	17/316,925	**3.90**	1.89-8.07	

RRs adjusted for smoking, alcohol use, BMI, oral contraceptive use, parity, tubal ligation, hysterectomy, CIN 3 and deprivation

6.5 Discussion

There were 170 vaginal cancer registrations in the cohort over an average of 14 years of follow-up. Just over half of the cancers registered were of squamous cell morphology (52%, n = 89), while the next commonest morphology was adenocarcinoma (18%, n= 31). Our figures are close to those that have been reported nationally, where around 60% of registered vaginal cancers are squamous cell carcinomas, and adenocarcinomas account for between 13-15% (16). A case series from the United States published in 2013 (21), which included 110 cases collected over 15 years (1990-2004), also had a broadly comparable distribution of tumour morphologies to those seen here, with 73% squamous cell carcinomas, 14% adenocarcinomas, and 13% with other tumour morphologies.

The mean age at vaginal cancer registration in our cohort was 64.7, which is similar to the ages reported in two large registry-based retrospective studies from the United States (118,119), which reported median ages of 68 and 65.7 years respectively for invasive vaginal cancer, with 5,430 and 2,149 cases. They found that women with squamous cell carcinoma had an older median age (70 years) than women with adenocarcinoma registrations (65 years). Age at vaginal cancer registration was similar in our cohort for squamous and adenocarcinomatous tumours.

It is notable that some of the older case-control studies have an over-representation of younger women in their vaginal cancer populations, with 40-50% of cases aged under 55 (25,26), which may in part explain why they reported associations with risk factors that were nearly identical to those seen for cervical cancer.

6.5.1 Past cervical intraepithelial neoplasia grade 3 (CIN 3)

The risk of anogenital cancer at other sites is known to be higher in women with a history of cervical cancer or precancerous change. High-risk strains of human papillomavirus (HrHPVs) are found in nearly 100% of cervical cancer tissue specimens (2,120,121), and HrHPVs are widely accepted to have a causal role in the aetiology of cervical, most anal and vaginal, and some vulval cancers (122). A Danish case-control study which examined HPV positivity in archival vaginal cancer tissue samples found that 89% of vaginal cancer cases , or 24/27 samples that were examined, were positive for HrHPV strains, mainly HPV 16 (91).

Women with prior cancer, including cervical cancer, were excluded from these analyses; in doing so it is hoped that the vaginal cancer cases identified are less likely to be extensions or unexcised cervical cancers. A CIN 3 registration prior to recruitment was associated with just over a 7 times greater risk of incident vaginal cancer in a fully adjusted model. Other groups have similarly found large increases in relative risk of vaginal cancer in women with a history of cervical disease; however the small numbers of cases in these studies mean that the actual estimates vary quite widely.

In their 2002 case-control study, Daling et al (26) found that 30% of vaginal cancer cases had a history of a prior anogenital tumour (nearly all of these were cervical), compared with 2% of controls (OR = 19.7, 95% CI 12.2-32.0). A Danish retrospective study (91), found an odds ratio (OR) of 5.37 (95% CI 2.79-10.3) for vaginal cancer in women with preinvasive or invasive cervical disease, in a multivariate model including sexual

behaviour, smoking and sociodemographic factors, and a weaker association with history of anogenital warts (OR 2.52, 95% CI 1.00-6.31).

A retrospective cohort study from Finland (62) also examined risk of subsequent vaginal cancer in 7564 women treated for CIN of any grade, and they found a standardised incidence ratio of 12.0 (95% CI 3.9-28.0), based on 5 cases of vaginal cancer, in a model stratified for age and sex, but otherwise unadjusted. Similarly, a Swedish study which examined risk of vaginal cancer after a diagnosis of CIN 3 found a 2-4x increase in risk, with increasing risk seen with age and more recent period of treatment (93). Finally, a recent retrospective Canadian registry-based study (123) identified 54,320 women with a diagnosis of CIN 2 or 3. After an average of 10 years of follow-up, they calculated a standardised incidence ratio (SIR) of 6.7 for vaginal cancer after CIN 2 or 3 (95% CI 3.0-12.8) compared with the expected population incidence; when they considered CIN 3 alone it rose to 8.5 (95% CI 4.3-15.2). These are similar to our estimate, and based on 47 cases.

It has been suggested that age at diagnosis may be affected by history of cervical disease. A Swedish retrospective cohort study (114) that looked at factors affecting the age at diagnosis in women with primary vaginal cancer found that women with a history of prior cervical dysplasia were significantly younger at diagnosis than those without such a history, with a mean age of 57.9 (95% CI 53.8-61.9) years in women with prior cervical dysplasia, compared with mean age of 70.8 (95% CI 69.4-71.8) in those without, p = 0.001. In our cohort, women with a history of CIN 3 had a mean age of 63.0 at vaginal cancer registration (SD 6.3), while those without such a history had similar ages, and were

on average 65.9 (SD 6.8) years old at registration, however cases that occurred prior to recruitment were excluded, so our mean age at diagnosis will consequently be older.

The relationship between CIN 3 and vaginal cancer is likely to be multifactorial. A number of vaginal cancers may arise directly from inadequately treated CIN 3 lesions, or incompletely excised cervical cancers (124); others will result from multifocal HrHPV infection. While it is clear that there is an increased relative risk of vaginal cancer in women with a history of CIN 3, the absolute risk is still very small. 12 of 170 cases of vaginal cancer in our cohort arose in women with a previous CIN 3 registration, meaning that 7% of vaginal cancer cases had a history of CIN 3, and around 1 in 1000 women with prior CIN 3 developed vaginal cancer. The majority of cases of vaginal cancer in our cohort occurred in women with no history of prior CIN 3 registration.

6.5.2 Hysterectomy

An association between hysterectomy and vaginal cancer has been suggested for some time (21,25,26,116,125,126), though not all studies have reported an association (117). It is not clear whether the association between hysterectomy and vaginal cancer is an independent one, or whether the increased risk seen is due to an association between cervical dysplasia and hysterectomy, with women diagnosed with high-grade cervical dysplasia more likely to have had a hysterectomy.

In a case-control study published in 2002, which included 37 women with squamous cell vaginal cancer, Daling *et al* (26) examined associations between hysterectomy and vaginal cancer, stratified by history of previous anogenital cancer. They found that while

hysterectomy in women who had not had a previous anogenital cancer was associated with an increased risk of vaginal cancer, OR 2.4 (95% CI 1.0-5.6), there was no significant relationship seen in between hysterectomy and vaginal cancer in women who did have a history of previous genital cancer. Women with prior cancer, including other anogenital cancers, were excluded from this analysis, so the relationship observed should not be due to residual tumour in this study.

The other retrospective study from the United States, by Brinton *et al* (25), which looked at risk factors for *in situ* and invasive vaginal cancers found an excess risk relating to hysterectomy; 44% of cases *vs* 18% of controls reported having had a hysterectomy one or more years prior to diagnosis of their vaginal disease. There was a significant interaction with age at hysterectomy, with surgical menopause prior to 40 years of age being associated with a RR of vaginal cancer of 6.73 (95% CI 1.4-32.2) compared with premenopausal women. When they adjusted this for previous abnormal pap smear, the risk was reduced to 5.3 (95% CI 1.5-19.0), but still significant. Most studies have not gathered data on indication for hysterectomy, which is essential for a full understanding of this relationship.

While there are many other indications for hysterectomy (including benign tumours such as uterine fibroids, or problems with heavy menstrual bleeding), hysterectomy was also historically offered as treatment for CIN, either because the patient had concurrent gynaecological problems, or as a definitive solution to dysplasia without the risk of cervical recurrence, which can occur with more conservative treatment methods such as ablation or excisional treatment (127). There is agreement in the literature that women

remain at risk of *in situ* and invasive vaginal lesions after hysterectomy for cervical dysplasia (92). The association is thought to be multifactorial: due in some cases to incomplete resection of CIN (either unclear surgical margins, or occult disease), or because of the multi-centric nature of some HrHPV lesions (114,126).

In a letter to the editor of the *Journal of Lower Genital Tract Disease*, Dr Ronald Jones of the National Women's Hospital in Auckland, New Zealand cautioned practitioners that he had "encountered a number of women with vaginal vault cancer who have previously had a hysterectomy (usually abdominal) for high-grade CIN…in some cases it was possible to confirm retrospectively incomplete excision of CIN at the hysterectomy vaginal margin. In these cases, it is likely that the cancer evolved from incompletely excised CIN 3/VAIN 3 buried in the vault at the time of hysterectomy"(128).

A retrospective case series from the United States (21) which looked at the characteristics of 110 women with vaginal cancer found that 73% had had a hysterectomy, and 22% of them had a history of cervical dysplasia. There was a long lag between hysterectomy and development of invasive vaginal cancer, with a median time to diagnosis of 26 years.

A Belgian group (127) who examined risk of subsequent vaginal neoplasia (VAIN 2+, or invasive cancer) amongst 125 women with hysterectomy for CIN 2, CIN 3, or early cervical cancer (Ia1) found that 2% of their participants developed vaginal cancer, while 7.4% developed vaginal intraepithelial neoplasia grade 2 or worse, with a mean interval of 45 months from hysterectomy to diagnosis.

There was clear evidence of a strong relationship between prior hysterectomy and subsequent vaginal cancer incidence, with a 2-3 times increase in risk. This was after adjustment for prior CIN 3 registration, but due to a lack of information the indication for hysterectomy could not be adjusted for.

These findings support the reported increase in risk of vaginal cancer in women who have had a hysterectomy, but information on indication for hysterectomy is crucial in order to further understand this relationship, and to clarify whether hysterectomy itself, which traumatises the vaginal epithelium at the time of removal of the hysterectomy specimen (when the vaginal 'vault' is created by surgical excision of the uterine cervix and a narrow adjacent cuff of tissue) is the mechanism by which the risk of vaginal cancer is increased. Alternatively, existing HrHPV disease may be to blame, either because it is incompletely excised and subsequently not looked for through screening; or because surgical damage to the vaginal epithelium, and subsequent burial of the traumatised skin at the edges of the vaginal vault creates a milieu that is favourable to reactivation or new HrHPV disease.

6.5.3 BMI

Women who were overweight or obese at recruitment had a significantly elevated risk of vaginal cancer. This is similar to the association observed for vulval cancer, and has not to our knowledge previously been reported for vaginal cancer. It is possible that the same mechanism underlies the increase in risk for both of these cancers; it is interesting to note that an analogous increase was not observed for anal cancer.

As there was no association seen between exogenous hormone use and vaginal cancer (either for oral contraceptive use or menopausal HT use), it seems less likely that adiposity affects the risk of vaginal cancer through its hormonal effects. While a potential for mechanical inflammation of the vulval skin related to adiposity seems possible, a similar process in the vagina is more difficult to envisage, though as discussed in chapter 2, the vaginal walls are generally apposed in the resting state, other than at its apex (in the region of the vaginal vault). The underlying mechanism for the relationship between vaginal cancer and adiposity is not clear, and merits further research.

6.5.4 Parity

Nulliparous women, who made up just 11% of participants in the study, had a greater risk of vaginal cancer later in life than those who reported ever having given birth, RR 1.64 (95% CI 1.08-2.50). A Swedish retrospective study which examined 341 cases of primary vaginal cancer found that nulliparity in their cohort (22%) was nearly double the population value of 12% (114). Daling *et al* (26) examined parity as a risk factor for squamous cell vaginal cancers in a small retrospective study, but did not find a significant association. Similarly, Brinton *et al* (25) found no significant association with parity in their study which considered both in situ and invasive vaginal lesions. However, they had small numbers: 41 cases, and only 3 women who reported never having been pregnant.

Women who reported never having given birth had an increased risk of vaginal cancer in this study. This is similar to what was seen for anal cancer in the cohort and may signify a common pathway or a common confounder (for example, sexual behaviour). Both vary markedly from the relationship seen between cervical cancer and parity, where there is a

strong increase in risk of cancer of the cervix associated with having given birth, which rises with number of births (129).

6.5.5 Co-habitation

Vaginal cancer has been found to be more common in separated and divorced women compared with married women in previous studies (114,130). Peters et al (1984) (131) found around a doubling of risk of vaginal cancer at any age in women who were separated or divorced. Similarly, a Danish case-control study reported around double the risk in separated or divorced women compared with married women (OR 2.18, 95% CI 1.02-4.68).

Around a 60% increase in risk of subsequent vaginal cancer was seen in women who reported not living with a husband or partner at follow-up. It is likely that most women in the cohort who reported not currently co-habiting are separated or divorced, and have been sexually active at some point (34). Marital instability may be associated with an increased number of sexual partners—either before or after the breakdown of the marriage. The increased risk of vaginal cancer seen in this group may be a reflection of different sexual behaviour, and an increased likelihood of HrHPV infection. The relationship between co-habitation and vaginal cancer observed here is similar to that seen earlier for anal cancer; in contrast, we saw no alteration in risk of vulval cancer by marital status, possibly because of its weaker relationship to HrHPV infection, particularly at older ages. The heterogeneity of effect observed by cancer site makes this relationship less likely to be related to health-seeking behaviour.

6.6 Conclusions

Vaginal cancer is the rarest of the three cancers examined in this thesis. With around 200 registrations annually in England, and 170 incident cases over an average of 14 years in the cohort, the power to detect associations with the factors examined here is somewhat limited for this cancer overall, but particularly by histological subtype. The difference in mean age at cancer registration for the women with clear cell tumours is interesting. We do not currently have any information on potential DES exposure, so are not able to comment further on any association with DES, however this merits further consideration.

Nonetheless, there was a significantly elevated relative risk of vaginal cancer associated with several of the exposures examined here, including prior registration of CIN 3, hysterectomy, obesity, not living with a husband or partner, and being nulliparous. These associations are similar to those seen in the preceding anal cancer analysis, and with the obvious exception of hysterectomy, are similar to those previously reported by other groups for cervical cancer, which is probably a reflection of the importance of HrHPV infection in the aetiology of vaginal cancer.

Chapter 7 Subsequent risk of anal, vulval and vaginal cancers in women in England with a history of screen-detected cervical abnormalities

7.1 Introduction

In the preceding chapters it has been shown that that anal, vulval and vaginal cancers have a strong association with past cervical intraepithelial neoplasia grade 3 (CIN 3). Women with a CIN 3 registration prior to recruitment had close to a 3-fold increase in risk of vulval cancer, a 4-fold increase in risk of anal cancer, and more than a 7-fold increase in risk of vaginal cancer compared with women who had not had a CIN 3 registration.

CIN 3 is the only grade of cervical preinvasive lesion that is captured by the UK Cancer Registry, but NHS Cervical Cancer Screening Programme records permit an examination of whether there is a similar association between past cervical smear (cytological) abnormalities and subsequent anal, vulval and vaginal cancer to that seen for CIN 3, and, if so, whether the association varies by degree of abnormality (low-grade versus high-grade smear results) or by cancer type.

In the UK we have a well-organised national call and recall-based cervical cancer screening programme (the National Health Service Cervical Screening Programme, or NHSCSP), which had its roots in earlier regional screening, but was established in 1988 as a centralised, computer-based system, inviting women for regular cervical cytology-based screening (132).

The NHSCSP uses exfoliative cervical cytology testing to detect abnormalities caused by high-risk strains of the human papillomavirus in cells from the uterine cervix. Previously known as 'Pap smears', because cervical cells were picked up with a wooden spatula and smeared onto a glass slide in a technique developed over a century ago by Dr George

Papanicoloau (133), in more recent years samples in the UK have been taken using a small broom or brush, and placed into liquid medium in a technique known as 'liquid-based cytology', or LBC.

Cervical cancer screening programmes exist in many developed nations internationally, and their aim is to detect women who are carrying preinvasive cervical neoplasia and are therefore at an increased risk of developing cervical cancer. Cervical cancer screening uses the known natural history of the disease, which has a relatively long preinvasive stage, and aims to detect and treat cervical abnormalities before they become a cancer.

Cervical intraepithelial neoplasia, or CIN, is a spectrum of preinvasive cellular abnormalities of the cervical epithelium. At its low-grade end are borderline and mild cervical abnormalities, previously termed 'mild dyskaryosis' when seen in cytology in the UK. Low-grade abnormalities represent a spectrum of disease which are confined to the lower third of the epithelium (133) and are histologically seen as HPV-change, or CIN 1 on cervical punch biopsy. Low-grade disease is generally managed conservatively, with observation and repeat cytology until resolution of the abnormality (termed 'regression'), or progression of the abnormality to high-grade disease (CIN 2 or 3) (134).

High-grade disease is the more severe end of the spectrum. High-grade cervical lesions include CIN grades 2 and 3, which are disease states where more severe cellular abnormalities affect up to two-thirds (CIN 2) or more (CIN 3) of the cervical epithelium. Because high-grade disease carries a significant risk of progression to invasion (135), women in the UK with a diagnosis of CIN 2 or 3 are offered treatment, usually in the colposcopy clinic. This generally involves excisional biopsy using diathermy (a large loop

excision of the transformation zone, or LLETZ), or in some centres a destructive treatment using laser, heat or cold coagulation. High grade CIN is often, but not exclusively related to high-grade smear results; in the UK a proportion of high grade lesions are found in women with low-grade smear abnormalities. Thus whilst a high-grade smear is highly suggestive of a high-grade lesion, a low-grade cytology result can also be associated with a high-grade cervical lesion.

In 2014 we obtained permission to link Million Women Study data to screening outcomes from the National Health Service Cervical Cancer Screening Programme records. Getting the screening data ready to analyse was a large undertaking, and one that was very much a collaborative effort between myself and several people (see further details in the methods section of this chapter, 7.2). The screening data have been available for analysis (in usable form) since mid-2015, so the analyses that follow are preliminary, principally to examine whether evidence of cervical abnormalities apart from a registration of CIN 3 are also associated with subsequent risk of anal, vulval and vaginal cancer.

We have examined the cervical screening records of Million Women Study participants, particularly seeking to answer the following questions:

1. Do women who have attended for cervical screening have a different risk of subsequent anal, vulval and vaginal cancer than women who have not attended?
2. In cervical screening attenders, is there an increased risk of anal, vulval or vaginal cancer associated with less severe abnormalities than CIN 3?

7.2 Methods:

As elsewhere, participants were excluded from the analysis if they had any invasive cancer registered prior to recruitment, other than non-melanoma skin cancer (ICD-10 C44). Because linked screening records were only available for participants recruited in England, women recruited in Scotland (n = 111,824) were excluded from the analysis.

The exposures examined in this analysis are related to previous cervical screen-detected abnormalities, either from NHSCSP data (consisting of invitations to screening and resulting smear results), or from Cancer Registry data, (consisting of a cancer registration of CIN 3, cervical carcinoma *in situ*, D06). First, outcomes for women with vs. without recorded screening attendance (ever) were compared. Associations between screen-detected abnormalities (in screening attenders) and subsequent non-cervical anogenital cancers were then examined.

In 1988, when organised screening began in the NHS, Million Women Study participants had a mean age of 46 (range = 35-80 years). So, while many women may have been having regular screening prior to this, they will not have had the invitation-based 3-yearly screening from age 25 that is currently advocated in the UK. Current practice in England involves primary cytology testing using liquid based cytology (LBC) every 3 years for women aged 25 to 49, and every 5 years for women aged 50 to 65 with previously normal smear tests.

Liquid-based cytology began to replace conventional pap smears from 2003 in the UK, though some centres adopted the new technology as late as 2008 (136); also, following a trial carried out in several large centres, smears showing 'borderline' or 'mild' cytological abnormalities now have reflex HrHPV testing performed in many regions, and women with a low- grade smear that is positive for HrHPV are sent directly for colposcopy. Prior to this, and for most of the period for which screening data is available for the cohort, women were sent to colposcopy after one moderate or severe smear test, one or two consecutive mild tests, or three consecutive borderline smears taken 6 months apart (137).

7.3 The screening data

In order to consider screen-detected abnormalities and CIN 3 registrations in the same model, we derived a variable representing a woman's worst screening result prior to recruitment, which included registered CIN 3. Screening history was abstracted and classified according whether or not women had ever attended for cervical screening, and if so, their worst recorded cytology result. Cervical smear results are coded on the NHAIS database as follows (Table 7.1):

Table 7-1 National Health Authority Information System cervical screening cytology codes

NHAIS 'test' code	Cytology result	'Worst' cytology
1	Inadequate	Low-grade
2	Negative	No cytological abnormality
3	Mild	Low-grade
4	Severe	High-grade
5	Invasive	High-grade
6	Glandular neoplasia (endocervical)	High-grade
7	Moderate	High-grade
8	Borderline	Low-grade
9	Borderline (endocervical)	Low-grade
0	Glandular neoplasia (non-cervical)	High-grade
B	Borderline (HPV tested)	Low-grade
M	Mild (HPV tested)	Low-grade
N	Negative (HPV tested)	No cytological abnormality

As mentioned previously, the preparation of the screening data was a large task which involved several people as well as myself. I was around a year into my DPhil when we obtained permission to link to the NHSCSP data. I worked on initial test runs of the data, when we performed a trial linkage with 10,000 participants. Anna Brown (CEU senior IT programmer and database manager) led this work, and was the person who liaised with the NHSCSP data team on our behalf. We decided which NHAIS variables we wished to capture, and had a look at preliminary descriptive statistics for the first 10,000 women.

Full linkage was then requested, and when those data arrived they went initially Dr Roger Blanks and Rupert Alison, who work as part of the Cancer Screening Programmes Research Group. They cleaned the data and created 'per woman' variables, allowing us to look at screening history by woman rather than by test. Data cleaning principally involved removal of a small number of invalid data values (e.g. tests that putatively occurred prior to 1965, at an age younger than 12, or at non-integer repeat intervals).

I then worked with Roger, Rupert, and my supervisors to create a screening hierarchy. I decided that because we were not initially going to look at cervical cancer as an outcome, we would not at this juncture include a measure of adequacy of screening in our variable (something that would need to be done if we were examining risk of the condition being screened for itself).

7.4 Creating the screening hierarchy

There were 6,598,784 test results for the nearly 1.2 million Million Women Study participants included in this analysis. Screening history was censored at recruitment, but women were not excluded from this analysis based on age (i.e. we did not exclude women who were over 64 in 1988), as we wished to include any screen-detected abnormalities prior to recruitment amongst women who had ever attended for any cervical screening. The first data grouping was based on whether or not women had ever attended for screening (see Figure 7.1).

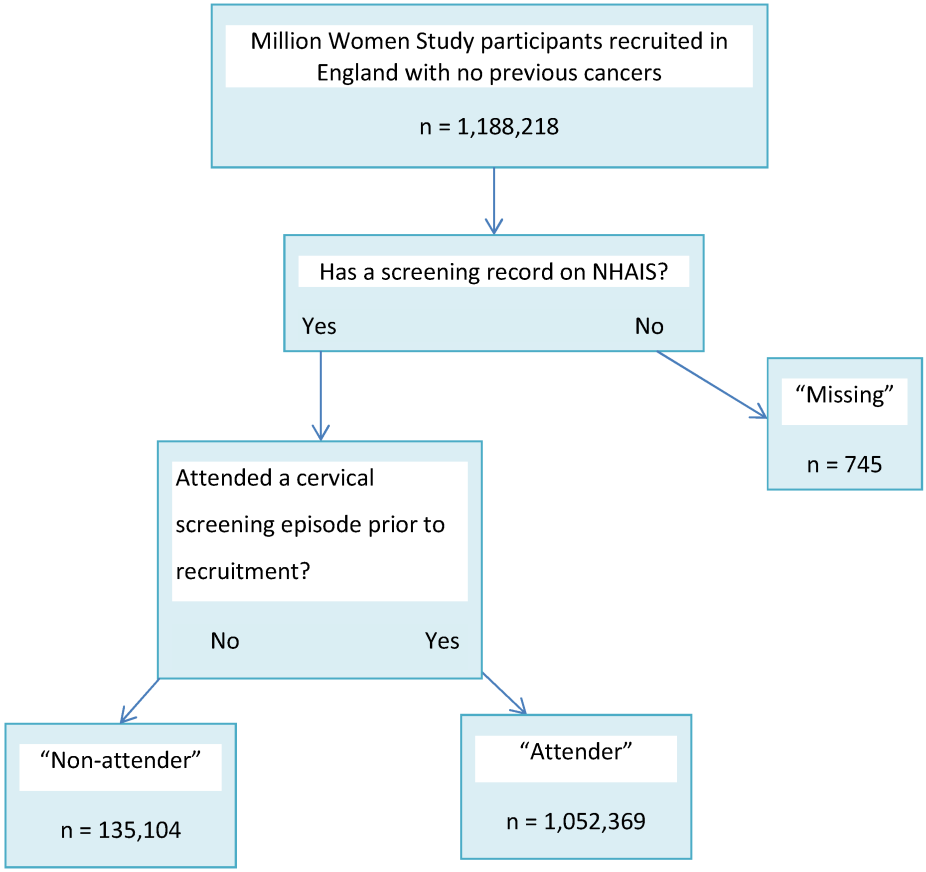


Figure 7-1 Attendance versus non-attendance for cervical cancer screening amongst Million Women Study participants recruited in England

After exclusion of women with no screening tests, Million women study participants had on average attended for 5.8 cervical screening tests prior to recruitment in the study. Because cervical screening records spanned a variable length of time and could have more than one severity of abnormality detected within one woman's screening record, we created a variable which grouped ever-screened women with a screening record (including women who had been invited but had never attended) by their most abnormal screening result prior to recruitment to the study (Figure 7.2).

Categories included 'no cytological abnormality'; 'low grade abnormality' (which captured borderline and mild dyskaryosis); 'high grade abnormality', which captured moderate and severe dyskaryosis (including glandular abnormalities of any grade and smears suggestive of malignancy); 'registered CIN 3' (a registration of CIN 3 in the ONS database) or 'non-attender' (did not attend for any screening), and 'missing' (not matched on the NHAIS database, i.e. no screening record). Women with any abnormality (low-grade or high-grade) who also had a CIN 3 registration were classed in the CIN 3 group.

Because screening history was limited to that which occurred prior to recruitment to the study, information on HPV testing was not captured. HPV testing as a routine part of the screening programme began in the past few years, and although not universal practice in the NHSCSP is routine in most of England. Screening history was censored at recruitment therefore a small number of women who attended for cervical screening after, but not before entry to the study, appear in the 'non-attender' group (n = 6,166, 0.5% of the cohort).

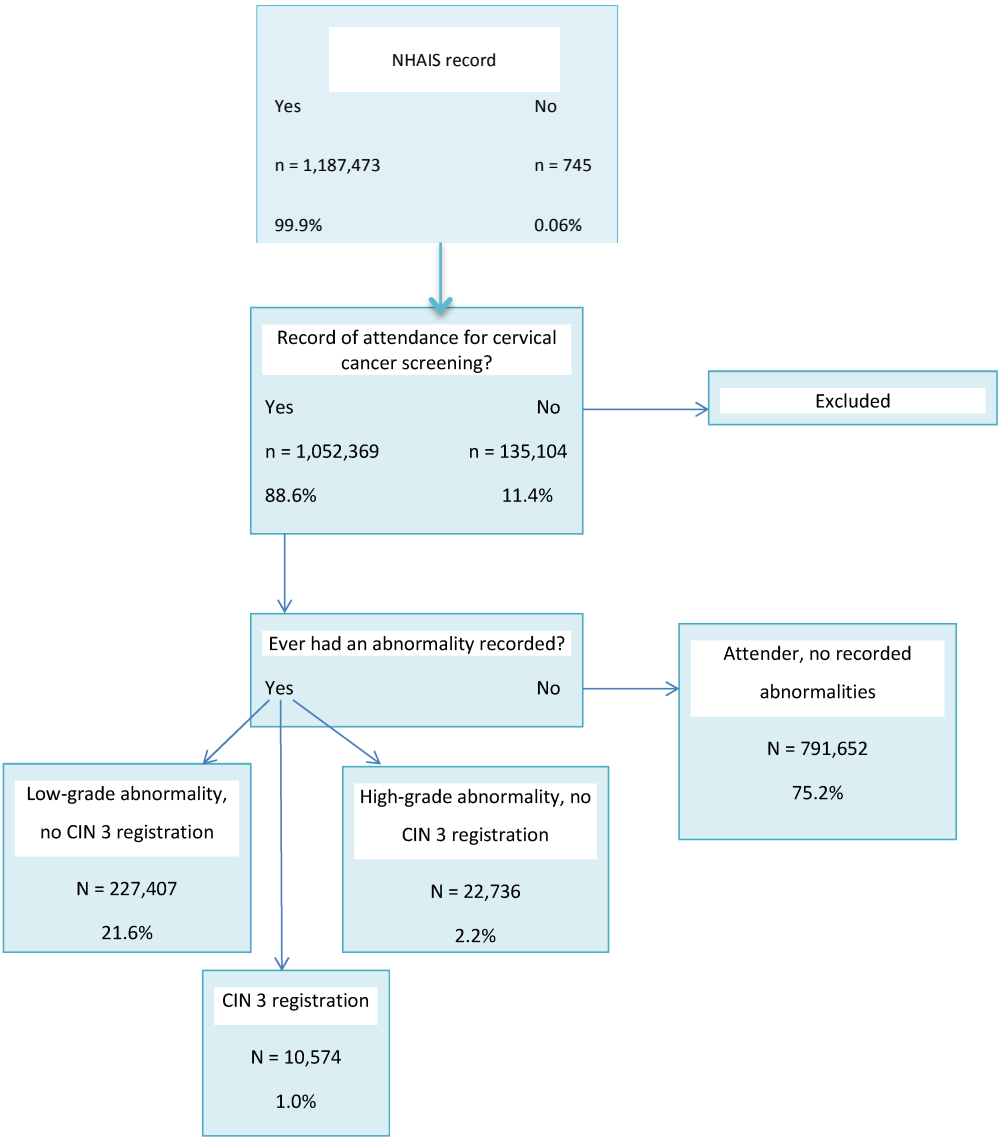


Figure 7-2 Screening hierarchy based on screen-detected cervical abnormalities prior to recruitment, using data from the National Health Service Cervical Screening Programme and the Cancer Registry.

Of the women with CIN 3 prior to recruitment, 2.3% were women who had no recorded attendances for cervical screening (n = 252); 24.2% had attended for screening but had no recorded abnormalities (n= 2,618); 24.4% had at worst a low grade abnormality (n= 2,646), and 49.0% had a prior high grade screen-detected abnormality (n = 5,308).

According to self-reported screening attendance, which was asked about on the first re-survey, only 3% of Million Women participants said that they had never had a cervical screening test. This is lower than the national figures cited by a recent British cross-sectional study, which found that 9% of women aged 40-74 reported not having had a cervical smear (155). 11 % of our participants did not have a record of a cervical smear on the NHAIS database, which is higher than we would expect, however as we have discussed a very high proportion of those women have had a hysterectomy, which may be why they have not had cervical screening.

7.4.1 Statistical analyses

Self-reported data from the recruitment and subsequent resurvey questionnaires were used to define adjustment variables, and analyses were adjusted for body mass index (<30/30+); past oral contraceptive pill use (never *vs*. ever); parity (parous *vs*. nulliparous); smoking (never, past, current); alcohol use (0-2 units/week, 2+ units/week); hysterectomy (no/yes, with oophorectomy/yes, without oophorectomy); and deprivation quintile (1 = least to 5= most deprived). The analysis was stratified by region of residence, consisting of 10 geographical areas corresponding to the cancer registry regions.

Cases of incident anogenital cancer were identified from the UK Cancer Registry as follows: “malignant neoplasm of anus”, ICD-10 C21; “malignant neoplasm of vulva”, ICD-10 C51; “malignant neoplasm of vagina”, ICD-10 C52.

A competing hazards model was used to examine the relationship between prior history of screen-detected cervical abnormalities and risk of subsequent anal, vulval and vaginal cancer. Hazard ratios were estimated with attained age as the underlying time variable, and are referred to as relative risks (RRs). Participants entered the analysis from recruitment or at the age of 50 for the small number (3%) who were younger than 50 at recruitment. Follow-up continued until the date of first of: cancer registration; death; emigration or other loss to follow-up; or 31 December 2013. The few women with missing data were included in a separate category for each adjustment variable and included in the analysis, but these data are not shown. Relative risks are reported with 95% confidence intervals. Analyses were performed in Stata 13 (1).

7.5 Results

Just under 1.2 million women recruited in England were included in this analysis, after exclusion of participants from Scotland for whom screening records were not available. Participants were followed on average for 14 years, yielding a total of 16,459 million person-years of follow-up (Table 7.2). During that time there were 513 anal cancers, 797 vulval cancers, and 160 vaginal cancers in the cohort.

The mean age at recruitment was 56.1 (SD 4.9). Nearly all participants had a screening record, with just 0.06% (n = 745) women who remained unmatched on NHAIS. Of the women with a record, 11% (n = 135,104) had no recorded attendances for a cervical screening test, and the rest, 89% (n = 1,052,369) had attended for screening at some point.

Table 7-2 Characteristics of participants at baseline and follow-up

Screen-detected cervical abnormalities: participant characteristics		
Number of participants (%)		
All	1,188,218	(100.0)
Anal cancer cases	513	(0.04)
Vulval cancer cases	797	(0.07)
Vaginal cancer cases	160	(0.01)
Mean age at recruitment (SD)		
All participants	56.1	(4.9)
Follow-up		
Woman-years (1000s)		16,459
Mean length of follow-up - years (SD)		13.9 (3.4)
Age at start of NHS Cervical Cancer Screening Programme (1988)		
Mean age, all participants – years (SD)	45.8 (5.0)	

First, associations between risk of subsequent non-cervical anogenital cancer and cervical screening attendance were compared, looking at risk of anal, vulval and vaginal cancer later in life in women who had or did not have any recorded cervical screening attendance (Table 7.3). As a group, Million Women Study participants (who were recruited via the NHS Breast Screening Programme) may be more likely to attend for cervical screening than other women nationally, and we wanted to see whether even in this population, non-attendance for cervical screening was associated with an increased risk of other anogenital cancers, either as a result of other high-risk behaviours, or possibly as a result of carriage of untreated HrHPV disease in the uterine cervix.

Table 7-3 Cervical screening history, characteristics at baseline by screening attendance, and worst recorded abnormality in women who attended for screening

Characteristics at recruitment	Attenders		Non-attenders	
Current or past smoker % (n)	45.6	(479,956)	45.9	(61,967)
Ever used oral contraceptive pill % (n)	59.8	(628,811)	47.8	(64,562)
Tubal ligation % (n)	21.2	(223,374)	20.0	(27,002)
Hysterectomy % (n)	17.0	(178,850)	85.0	(114,915)
Nulliparous % (n)	10.1	(106,369)	16.0	(21,502)
Ever hormone therapy user % (n)	48.7	(512,104)	58.9	(79,535)
Most deprived quintile % (n)	19.7	(207,255)	20.4	(27,551)
Mean age (SD)	55.9 (4.8)		58.0 (5.2)	
Worst abnormality recorded in women who attended	**%**		**n**	
No recorded abnormalities	75.2		(791,652)	
Low grade abnormality	21.6		(227,407)	
High grade abnormality	2.2		(22,736)	
Cervical intraepithelial neoplasia grade 3 registration	1.0		(10,574)	

Characteristics of Million Women Study participants who had and had not attended for cervical screening were overall very similar with a few exceptions (Table 7.3). Smoking history and deprivation were similar for both groups. However, women who had never had cervical screening were less likely to have used the oral contraceptive pill or have given birth--postnatal visits and contraceptive consultations would be healthcare episodes that might include an opportunistic smear-taking, so this difference is perhaps not unexpected.

The most heterogeneous characteristic was hysterectomy, with 85% of unscreened women reporting a hysterectomy prior to recruitment, and just 17% of screened women reporting having had one. Women who have had a hysterectomy but do not have a history of abnormal cervical smear results do not require further cervical screening (as in the majority of cases they no longer have a uterine cervix), and this is likely to be the main reason that such a high proportion of unscreened women had had a hysterectomy compared with those who had attended for screening. Similarly, unscreened women were more likely to have used HT (probably related to an increased use of HT in women who have had a hysterectomy).

There was no difference in risk of subsequent anal, vulval or vaginal cancer associated with non-attendance for cervical screening. Women who had no record of any cervical screening tests had a RR of 0.88 (95% CI 0.65-1.19) for anal cancer, RR 0.98 (95% CI 0.77-1.24) for vulval cancer, and RR 0.70 (95% CI 0.43-1.14) for vaginal cancer compared with those who had ever been screened (Table 7-4).

Table 7-4 Association between prior cervical screening attendance vs non-attendance and risk of subsequent anal, vulval or vaginal cancer amongst Million Women Study Participants with a screening record.

	Cases/population at risk			RR (95% CI)		
Exposure	**Anal**	**Vulval**	**Vaginal**	**Anal**	**Vulval**	**Vaginal**
Attender						
	447/1,052,174	689/1,051,932	136/1,0524,85	**1.00**	**1.00**	**1.00**
Non-attender						
	66/134,786	108/134,744	24/134,828	**0.88** (0.65-1.19)	**0.98** (0.77-1.24)	**0.70** (0.43-1.14)
				Test for heterogeneity p = 0.47		

Adjusted for smoking, BMI, oral contraceptive use, parity, hysterectomy & oophorectomy, tubal ligation, deprivation and stratified by recruitment region

We then looked at risk associated with severity of past abnormality amongst women who had a record of ever attending for screening. Most of the cohort (89%) had a record of some screening. The mean number of tests per woman after exclusion of unscreened women was 5.8. The majority of screening tests showed no abnormalities, and 75% of the cohort (791, 652) had attended for screening and never had an abnormality detected.

Around 1 in 5 screened women (21.5%, n = 227,407) had a history of low grade abnormality at some point prior to recruitment, and around 1 in 50 (2.2%, n = 22,736) had a history of high grade abnormality. Just 1% had a CIN 3 registration prior to recruitment (n = 10,574).

Table 7-5 Associations between past cervical screening attendance and detected abnormalities, and incident anal, vulval and vaginal cancers

	Cases			RR (95% CI)		
Exposure	**Anal**	**Vulval**	**Vaginal**	**Anal**	**Vulval**	**Vaginal**
Attender, no abnormalities						
	299	523	90	**1.00**	**1.00**	**1.00**
Low-grade abnormality						
	114	125	24	**1.47** (1.18-1.83)	**0.91** (0.75-1.11)	**1.07** (0.68-1.69)
High- grade abnormality						
	16	23	13	**1.97** (1.19-3.27)	**1.74** (1.14-2.65)	**5.42** (3.01-9.73)
CIN 3						
	18	18	9	**4.20** (2.60-6.80)	**2.92** (1.82-4.68)	**6.52** (3.25-13.10)
					Test for heterogeneity p = 0.001	

Adjusted for smoking, BMI, oral contraceptive use, parity, hysterectomy +/- oophorectomy, tubal ligation, deprivation, and stratified by recruitment region

Women who had attended for cervical screening and had a low-grade abnormality detected prior to recruitment had an increased risk of anal cancer, RR 1.47 (95% CI 1.18-1.83), compared to women who had attended but never had an abnormality recorded.

No significant alteration in risk was observed for vulval or vaginal cancer amongst women with a history of low grade abnormalities, compared with no abnormalities (RR 0.91, 95% CI 0.75-1.11 for vulval cancer; RR 1.07, 95% CI 0.68-1.69 for vaginal cancer). (Table 7.5)

In contrast, the relative risk of all three cancers was increased in women with a past high-grade abnormality. Women with a previous high-grade smear had doubling in risk of subsequent anal cancer (RR 1.97, 95% CI 1.19-3.27), a 70% increase in risk of vulval cancer (RR 1.74, 95% CI 1.14-2.65), and more than a 5-fold increase in risk of vaginal cancer (RR 5.42, 95% CI 3.01-9.73).

The greatest relative risk of all three cancers was seen in women with a past registration of CIN 3. Women with a CIN 3 registration prior to recruitment had a RR of subsequent anal cancer of 4.20 (95% CI 2.60-6.80), a relative risk of 2.92 (95% CI 1.82-4.68) for vulval cancer, and more than a 6-fold increase in risk of vaginal cancer (RR 6.52, 95% CI 3.25-13.10). In a competing hazards model the test for heterogeneity showed a significant difference in the risk associated with screening history between the three cancers, $p = 0.001$.

7.6 Discussion

Non-attendance for cervical screening was not associated with a different risk of subsequent anal, vulval or vaginal cancer, compared with women who had ever attended for cervical screening. Among women who had a record of ever attending for screening, those with past screen-detected cervical abnormalities had an increased risk of subsequent anal, vulval and vaginal cancer.

For those with at worst a low-grade abnormality, only anal cancer risk appears to be significantly elevated, with just under a 50% increase in risk; we did not observe a similar association between low-grade abnormalities and risk of vulval or vaginal cancer. Women with a history of high-grade cytology have an increased risk of all three cancers, with a doubling in risk of anal cancer, a 70% increase in risk of vulval cancer, and a five-fold increase in risk of vaginal cancer. A CIN 3 registration prior to recruitment was still associated with the highest relative risk of subsequent anal, vulval or vaginal cancer, which although higher, was not significantly different from the risk associated with a past high grade smear.

7.6.1 Cervical cancer screening attendance

Cervical screening is carried out to detect precancerous changes in the uterine cervix that can be treated before they become invasive. Although it is not designed to detect existing cancers, the screening programme in England has been reported to detect one invasive cervical cancer in every 2726 women it screens (136). Cervical cancer screening has also been reported to offer ongoing protection from cervical cancer after age 65 in women with adequate, negative screening histories (138). We have hypothesised that evidence of HrHPV disease in the genital tract detected during routine cervical screening may also act as a marker of risk for other anogenital cancers.

Women who attended for screening were quite different to those who did not. They were more likely to have used the oral contraceptive pill in the past, more likely to have given birth, less likely to have used HT, and far less likely to have had a hysterectomy. They were also slightly younger on average. Smoking, deprivation and history of sterilisation were broadly similar between the two groups.

When women were grouped according to whether or not they had a record of ever attending for cervical cancer screening, there was no difference in risk of subsequent anal, vulval or vaginal cancer between the two groups after adjustment for potential confounders. Women who had not attended for cervical screening had similar long-term risks of anal, vulval and vaginal cancer as women who had attended (irrespective of their smear results). So, unlike for cervical cancer, attending for screening itself does not appear to affect risk of subsequent other anogenital cancers.

7.6.2 Previous screen-detected abnormalities

Women with a record of attending for screening were then separately examined, in order to assess the association between screen-detected abnormalities of the cervix, and subsequent non-cervical anogenital cancer risk. A small increase in risk of anal cancer was associated with a past low-grade cervical abnormality, and a large increase in risk of all three cancers was seen for either cytological or histological evidence of high-grade disease, compared with women who had attended for screening but had never had an abnormality detected.

Screen-detected cervical abnormalities, particularly high-grade disease, are mostly attributable to a group of high risk HPVs, which have been classified by IARC as group 1 carcinogens in the cervix (139), the most common of which are HPV 16 & 18. A recent large meta-analysis reported that the association between screen-detected abnormalities and HPV 16 increased with grade of abnormality, with around 3% of negative smears positive for HPV 16, 20% of low-grade smears, 41% of high-grade smears, and 54% of CIN 3 cases (140). When they considered any strain of HrHPV, the same group found 13% of negative smears, 75% of low-grade, 85% of high-grade smears, and 92% of CIN 3 cases were positive.

A history of high-grade cervical abnormality has a strong correlation with HrHPV positivity, and it is widely accepted that persistent infection with HrHPV is a common aetiological precursor for most anal and vaginal, and some vulval cancers (141). A recent international study found HPV DNA in 84.9% of invasive cervical cancers, 90.0% of anal cancers , 74.3% of vaginal cancers, and 28.6% of vulval cancers (142).

HPV 16 and 18 are thought to be among the most important oncogenic strains for anal (143), vulval (15), and vaginal (112) cancers, as well as for cervical cancer (48,86,121). Past screen-detected cervical abnormalities are likely to be a good proxy marker of oncogenic HrHPV infection. Differences in the susceptibility of anal, vulval and vaginal epithelia to HPV infection may also affect the observed associations (57).

Other studies have examined the risk of subsequent non-cervical cancers in women diagnosed with high-grade dysplasia, but these have all been based on record-linkage with cancer registry data, and have not had information on the other exposures that have been examined here.

7.6.3 CIN 3

A recent large retrospective Canadian study (123) identified 54,320 women diagnosed with CIN 2 or 3 over a 25 year period, from 1980-2005. Gaudet *et al* found increased standardised incidence ratios (SIRs) of 6.7 for vaginal cancer (95% CI 3.0-12.8), and 2.9 for vulval cancer (95% CI 1.7-4.6), and a non-significant increase in risk of anal cancer, SIR 1.8 (95% CI 0.4-4.7). They had 10 years of median follow-up for women, and the mean age at diagnosis of CIN 2+ was age 35, so their cohort for the most part was younger than ours and is likely to accrue further cases of these age-related malignancies; at publication they had identified 96 vulval cancers, 47 vaginal cancers and only 20 anal cancers.

A study using historical data from New Zealand (124), which examined cervical cancer risk amongst women with untreated CIN 3 as a result of an unethical natural history study, found SIRs of 8.9 (95% CI 3.6-18.5) for vulval cancer, and 13.2 for anal cancer (95% CI 4.3-30.7).

These were based on 1063 women with an initial diagnosis of CIN 3, and a small number of subsequent vulval (n=7) and anal cancer cases (n=5). They did not have sufficient numbers to compare associations between non-cervical anogenital cancers and treated or untreated CIN 3 (which was the main analysis for cervical and vaginal cancers). They also looked at vaginal vault cancers, but in the context of untreated CIN 3 it is likely that many of the vaginal cancers were due to local spread of cervical disease.

A group from Finland (62) followed 7564 women treated for CIN 1-3 between 1974 and 2001, and followed to 2003, with a mean follow-up of 12 years. The mean age at treatment was 35 years of age. Similar to our study, they found an increased risk of vulval, vaginal and anal cancer in women treated for any grade of CIN, with a SIR of 5.7 for anal cancer (95% CI 1.2-17.0), 4.1 for vulval cancer (95% CI 1.5-8.9), and 12.0 (3.9-28.0) for vaginal cancer. Their estimates are higher than those we have observed, however the women they followed-up were for the most part younger than our participants, and for vulval cancer, probably more likely to have HPV-related lesions.

Another Scandinavian group, based in Stockholm (63), used the Swedish registry system to create a population cohort of 3,747,698 women aged 18-50 between 1968 and 2004. They linked their data to migration, cancer and death registries, identifying 125,292 women with a history of CIN 3 and comparing them with women without such a history. After adjustment for age, calendar period, socioeconomic status, and parity they found increased risks of anal, vulval and vaginal cancers in women with past CIN 3, with incidence rate ratios (IRRs) of 4.68 (95% CI 3.87-5.62) for anal cancer, 2.22 (95% CI 1.79-2.73) for vulval cancer, and 6.74 (95% CI 5.24-8.56) for vaginal cancer, which are very similar to our findings.

An older registry-based retrospective study from the UK (144) also found an increased risk of anal, vulval and vaginal cancer after CIN 3. They included 59,586 women with a CIN 3 registered between 1960 and 1999. They had a mean of 8 years of follow-up per woman, and the majority of their participants were aged under 50 at diagnosis of CIN 3 (91%). They found a raised standardised incidence ratio for anal cancer (SIR 5.9, 95% CI 3.7 to 8.8), vulval cancer (SIR 4.4, 95% CI 2.8 to 6.6), and vaginal cancer (SIR 18.5, 95% CI 13.0-25.5. While the risks of vulval and vaginal cancers were significantly increased in the first 14 years of follow-up, anal cancer risk was only significantly increased 10 or more years after the CIN 3 registration in their study.

Similar findings have also been reported from a large registry-based Swedish study (92), which examined risk of vaginal cancer in all women treated for CIN 3 between 1958-2002 (n = 132,493). They found an excess risk of vaginal cancer in women with a history of CIN 3, with a relative risk of 6.8 (95% CI 5.6-8.2). They did not consider anal or vulval cancers in their analysis. And finally, a Danish population-based case-control study (91) which investigated risk factors for vulval and vaginal cancers stratified by HPV status found a positive association between past cervical neoplasia (including preinvasive and invasive cases) and HPV positive (but not HPV negative) cases of both cancers, with an odds ratio of 4.91 (95% CI 2.34-10.29, $p< 0.001$) for vulval or vaginal squamous cell carcinoma after preinvasive or invasive cervical neoplasia.

7.6.4 Other screen detected abnormalities

While most research has concentrated on women with a diagnosis of CIN, particularly CIN 3, several retrospective studies have examined the relationship between cervical smear abnormalities and subsequent risk of anogenital cancer. Brinton *et al* (25) found a RR of 3.8 (95% CI 1.6-9.0) for vaginal cancer in women with a history of an abnormal pap smear. They also reported on risk factors associated with vulval cancer (100), where they found no significant alteration in risk of associated with history of abnormal pap smear (OR 1.41, 95% CI 0.5-3.6).

We were interested in screen-detected cervical abnormalities as a marker of HrHPV exposure and carriage. However, the possibility that a screen-detected abnormality at cervical screening may in fact arise from a non-cervical malignancy is relatively well-established, particularly in the case of glandular abnormalities (134), or squamous epithelial neoplasias of the vagina, which clinicians are urged to remember in women with high-grade cytological abnormalities and normal cervical findings.

A Finnish study (145) has estimated the subsequent incidence of other (non-cervical) gynaecological cancers in women with an abnormal cervical smear test, but who have had subsequent negative cervical histology. As a baseline group, they looked at women with a negative smear history, and as expected found no increase in risk of vulval or vaginal (or endometrial or ovarian) cancer in those women compared with general population values. When they looked at women with low-grade (Finnish class II smears), those with a history of a mildly abnormal smear and negative (normal) histology had a standardised incidence ratio of 1.4 (95% CI 0.9-2.0) for vulval cancer, and 2.7 (95% CI 1.7-4.1) for vaginal cancer.

When they considered those with high-grade abnormalities (class III-V, analogous to moderate, severe and malignant smears in the UK), they saw a large increase in risk of vulval and vaginal cancer, which was similar to what we have seen in our cohort, albeit with higher risk estimates of 5.8 (95% CI 2.3-12) for vulval cancer, and 16.4 (95% CI 7.1-32.0) for vaginal cancer. The reason for the magnitude of association they found compared to what we have seen is probably because they were specifically looking at cases where a smear abnormality was likely to originate in the genital tract, but not in the cervical epithelium.

Cervical smears originated as aspirated samples of pooled vaginal fluid 'smeared' onto glass slides, in which Dr George Papanicolaou incidentally observed cancer cells (133). As discussed above, smear-taking techniques have altered significantly over time, and cervical cancer screening programmes, including in the UK, now used liquid-based cytology where a brush is used to exfoliate cells from the surface of the cervix. Still, occasionally the cells acquired do not originate from the cervix.

In some instances, this is deliberate. There has been a considerable amount of research (and debate) surrounding the question of whether women who have had a hysterectomy with removal of the uterine cervix should continue to have vaginal cytology performed as part of the national cervical screening programme, in order to detect vaginal vault cancers.

Current NHSCSP guidelines (134) suggest that women with adequate negative cervical screening prior to hysterectomy who have no CIN in their hysterectomy specimen do not require vaginal vault cytology. Women who are not on 'routine recall', meaning that they either do not have an adequate screening history, or they have had an abnormality in the

past and have not completed their follow-up, and those who have had a hysterectomy for or with CIN, are offered varying numbers of follow-up vault cytology tests by the screening programme.

We did not exclude women with a hysterectomy from our analyses, so it is possible that a small number of the cervical screening tests in our model are actually vault smears, however, as we are interested in the association between past cytological abnormalities and subsequent non-cervical cancers, we did not feel that this was an issue.

7.7 Conclusion

In summary, we have seen in previous chapters that women with a history of high-grade cervical preinvasive neoplasia, or CIN 3 lesions of the uterine cervix, have a significantly increased relative risk of subsequent anal, vaginal, and to a lesser extent, vulval cancer, later in life. Like cervical cancer, the other anogenital cancers share varying degrees of association with high-risk strains of human papillomavirus, and it has been well-reported that women with anogenital cancer at one site are at greater risk of a subsequent cancer at another site.

Screen-detected cytological abnormalities of the uterine cervix are used to identify women at increased risk of cervical cancer. Well-organised national screening programmes like that in the UK have successfully used cervical cytology to identify women at increased risk, and by treating preinvasive precursors, have reduced the incidence and mortality of cervical cancer in screened women (137) . We now know that the highest risk of cervical cancer is seen in women with persistent HrHPV infection;

women who persistently carry HrHPV are at significant risk of developing invasive cervical cancer (121,146).

Given the strong relationship between CIN 3, which is the most severe grade of cervical precancer, and anal, vulval and vaginal cancer, we wished to examine whether cervical cytology results themselves showed an association with risk of subsequent non-cervical anogenital cancer, and if so, whether there were different associations seen by grade of abnormality or cancer type.

We found that attendance for cervical screening itself was not associated with a reduced risk of other anogenital cancers, in the way that it is for cervical cancer (where unscreened women have not had the chance to have pre-invasive disease treated in the same way that screen-attenders have). Women who had never attend had similar risks of anal, vulval and vaginal cancers as those who had attended for cervical screening.

A history of a low-grade cervical cytology result at any time prior to recruitment was associated with around a 50% increase in relative risk of anal cancer, but we did not observe any association with risk of vulval or vaginal cancer in the low-grade group. Women with a high-grade result (moderate, severe dyskaryosis or worse) had increased risks of all three cancers, with around a doubling in risk of anal cancer, a 70% increase in risk of vulval cancer, and around 5.5 times the risk of vaginal cancer after the age of 50. A registration of CIN 3 was still associated with the greatest increase in risk, with women at 4 times the risk of anal cancer, 3 times the risk of vulval cancer, and 6.5 times the risk of vaginal cancer after this grade of abnormality is detected.

Chapter 8 Conclusions

8.1 Summary of aims and findings

Anal, vulval and vaginal cancers are relatively rare malignancies that predominantly affect postmenopausal women. Although they share high-risk human papillomavirus (HrHPV) as an aetiological precursor (147), no previous large-scale prospective study has examined the exposures associated with an increased risk of these anogenital cancers in older women who are not, for the most part, members of known 'high-risk' groups (e.g. transplant recipients, or human immunodeficiency virus patients).

Using nationally held cancer registry data, as well as prospectively collected information on individual exposure to various reproductive and lifestyle factors, we sought to identify factors associated with an increased risk of anal, vulval and vaginal cancers in a large cohort of UK women, also examining the incidence of these cancers nationally and in our cohort. Finally, as all three cancers are known to be causally related to HrHPV in a variable proportion of cases, we assessed the association between previous screen-detected cervical abnormalities, and risk of anal, vulval and vaginal cancer, using NHSCSP data and ONS cancer registrations of CIN 3.

In chapter 2, publicly available data from the ONS were examined to assess anal, vulval, and vaginal cancer incidence in women in England in 2012. More than 85% of registered cases of these cancers occurred in women over the age of 50, contrasting markedly with the age-related incidence of cervical cancer, where more than 60% occur in women under 50, largely because of the effectiveness of the NHS Cervical Cancer Screening Programme. Cervical cancer remains the most common anogenital cancer until it is overtaken by vulval cancer around age 70.

Methods used were described in Chapter 3, and risk factors for anal cancer were reported in Chapter 4. Anal cancer is more common in women than in men internationally (47), and despite several well-known (and well-studied) high-risk groups, the majority of anal cancer registrations occur in women over 50 in the UK (7,47), but risk factors for anal cancer in older women have not previously been prospectively examined. There were 517 incident anal cancers registered in the cohort. CIN 3 registration prior to recruitment was found to be associated with the largest increase in risk of anal cancer, with an approximate quadrupling of risk of anal cancer in later life. Other factors associated with an increased risk were: ever smoking, past use of the oral contraceptive pill, nulliparity, tubal ligation, and not living with a husband or partner. The increased risk associated with smoking was seen in tumours with squamous cell morphology, but not adenocarcinomas; this histological heterogeneity of risk has previously been observed for cervical cancers (72).

When risk factors for vulval cancer were examined in chapter 5, nearly 70% of the 898 registered cases were found to be squamous cell carcinomas. A dual aetiology has been proposed for vulval cancer, where vulval cancers in younger women are more likely to be virally driven, and arise in a background of HrHPV-related VIN (vulval intraepithelial neoplasia), whereas those arising at older ages (which constitute the majority of cases) are more likely to be related to inflammatory vulval dermatoses such as lichen sclerosus. Most of the vulval squamous cell carcinomas were registered with squamous cell carcinoma 'NOS' (not otherwise specified) morphology, making it difficult to determine what proportion of the cancers in the cohort were likely to be related to HrHPV infection.

Nonetheless, some of the risk factors identified for incident vulval cancer were quite different from those seen in the analyses for anal cancer, and distinct from those commonly cited in the literature as being HrHPV-related. The strongest association was seen with a history of CIN 3 registration prior to recruitment; the associated increase in risk was smaller than that seen for anal cancer--a RR of 2.68 for vulval cancer, compared with a RR of 4.03 seen for anal cancer. Other factors significantly associated with an increased risk were adiposity, with increasing risk seen for women who were overweight or obese, and having a menopause prior to age 50. Parity, oral contraceptive use, smoking, and co-habitation were not related to vulval cancer risk in our cohort.

In chapter 6, risk factors for vaginal cancer, the rarest of the three cancers considered in this thesis, were examined. There were 170 primary vaginal cancers registered in the cohort over the study period, over on average 14 years of follow-up per woman.

As for anal and vulval cancer, a registration of CIN 3 prior to recruitment was the strongest predictor of risk of subsequent vaginal cancer in our study. Other exposures associated with an increase in risk of vaginal cancer were: hysterectomy, with or without oophorectomy, which was associated with a an approximate tripling of risk; adiposity, where women who were overweight or obese had around a 50% increase in risk of vaginal cancer, and nulliparity, which was associated with around a 60% increase in risk compared with women who had given birth. These associations were similar to those observed for anal cancer and are consistent with a strong relationship between HrHPV infection and vaginal cancer risk.

Finally, in chapter 7, NHSCSP screening data which only recently became available was used to assess whether history of a screen-detected abnormality of the uterine cervix was significantly associated with a subsequent non-cervical anogenital cancer.

There was no difference in risk of subsequent anal, vulval or vaginal cancer between women who had or had not ever attended for cervical cancer screening. Most women in the cohort (88%) had a record of ever attending for cervical screening, and 75% of those had never had a cytological abnormality detected. 25% of women had a history of some screen-detected abnormality, which for 22% was at worst low-grade, 2% high-grade, and 1% was a CIN 3 registration (an *in situ* cancer).

Having a history of a low-grade abnormality was associated with an increased risk of anal, but not vulval or vaginal cancer, compared with women who had been screened but had no recorded cytological abnormalities. Risks of anal, vulval and vaginal cancers were elevated in women with a prior high-grade abnormality, with a near doubling of risk of anal cancer, a 70% increase in risk of vulval cancer, and a five-fold increase in risk of vaginal cancer. CIN 3 was associated with the largest increases in relative risk of all three cancers, though this was not significantly greater than the increase in risk seen for high-grade smear.

8.2 Methodological considerations and limitations

8.2.1 The Million Women Study cohort

This thesis examines risk factors for anal, vulval, and vaginal cancers in a large cohort of UK women. These cancers are uncommon; anal, vulval and vaginal cancers combined had an annual total of fewer than 2000 cases registered in England in 2012 (n= 1,927). Of these, more than 85% occurred in women over the age of 50. This is the largest prospective cohort study to examine incident anogenital cancers in older women, and the availability of prospectively ascertained self-reported data on most exposures, and access to nationally-held linked cancer registry, mortality and screening data for most Million Women Study participants makes it a highly appropriate population in which to study these rare cancers.

There are, however, some limitations of this research. Million Women Study participants account for 1 in 4 women in their age cohort in regions that participated in the study, and are broadly representative of the approximately 70% of women aged 50-65 who attend for breast screening. Because women were recruited to the study via a screening programme, there is possibly some selection bias in our participants, and Million Women Study participants are likely to be representative of women who choose to attend for breast screening. Age-specific incidence rates of anal cancer were somewhat lower in the cohort than those observed nationally, while vulval and vaginal cancer rates were similar to the rates for England nationally in 2012. Some of this difference may be due to factors associated with breast screening attendance, and the under-representation of smokers in the cohort.

Women were invited to participate in the study by postal questionnaire, which was sent out with their routine breast screening invitation. Participants could only be enrolled in the study if they attended their index screening visit or posted in their questionnaire. Million Women Study participants were registered with a GP, as the NHSBSP relies on GP registrations to generate their participant lists. Participants were able to read English (or had someone to help them do so), and had sufficient literacy and numeracy to fill in a 4 page written questionnaire.

Despite these considerations, the very large numbers of participants, and the fact that the outcomes considered in this work are not cancers that are currently screened for means that the assessment of risk factors for non-cervical anogenital cancers are likely to be internally valid.

8.2.2 Exposure data

Detailed information was available for many exposures of interest, however there are some factors which would have been interesting to examine for which data were lacking. A key area to consider, given that the three cancers we examined are related to varying degrees to a sexually transmitted infection (HrHPV), would be information regarding age at first sexual intercourse, and number and gender of sexual partners. Using information that was collected from participants, proxy measures of some of these were examined, for example information on age at first birth in parous women, and age at first use of the oral contraceptive pill, were used as proxy measures of age at first sexual intercourse. However, because HrHPV is a sexually transmitted infection, sexual behaviour is likely to be both an important exposure, and a potential confounder, and it was not possible to adjust for it directly.

8.2.3 Screen-detected cervical abnormalities, and the NHSCSP data

The cervical screening programme data we used are nationally collected data on invitations and cervical smear abnormalities for over 1 million women in England, from whom we have also prospectively collected information on demographics, lifestyle and reproductive risk factors. Within their age group, our findings are likely to be generalizable for invited women nationally, although because they were recruited via the breast screening programme, our women are representative of women who attend for screening.

Participants in our study had a mean age of 46 at the inception of the NHSCSP. Few of our participants are likely to have been routinely screened from age 20-25, every three years to the age of 50, and every 5 years after that till age 65, which current NHSCSP guidelines recommend (134), and which would be crucial if we were considering the effectiveness of cervical screening in terms of cervical cancer prevention. We are not able to assess adequacy of lifetime screening in our cohort, as the organised call-recall system was not in place for our participants from age 25. As we are interested in cancer outcomes that are not the condition that is being screened for, we have instead used 'worst' abnormality in screened women, rather than assessing regularity or adequacy of screen-attendance.

Screen-detected cervical abnormalities represent non-invasive cellular abnormalities in the form of cervical intraepithelial neoplasia, which is graded from 1 to 3. CIN 1 is regarded as a benign, HPV-related abnormality that is confined to the lower third of the cervical epithelium, and is not currently routinely treated with excision. CIN 2 and 3, where the cellular abnormalities are more advanced and affect respectively up to the

middle or upper third of the cervical epithelium, are high-grade changes, and are routinely treated through excision or ablation (in selected cases). The diagnosis of CIN is the result of cervical biopsy, which is generally performed in the colposcopy clinic. Low-grade cytology results are often associated with disease found to be CIN 1 or less on histology, however a significant proportion of CIN 2+ cases arise from women identified as having low-grade cytology (156); indeed, in the Million Women Study cohort around half of the CIN 3 cases arose in women with no previous abnormalities or at worst a previous low-grade smear result. A high-grade cytology result is likely to correspond to CIN 2 or 3 in the majority of cases.

Although we have information on the cytological abnormality detected as part of the NHAIS data, and the administrative 'action' code for subsequent follow-up ('R', routine recall; 'S' suspend, generally meaning a referral to colposcopy; or 'C', cease, usually because of hysterectomy or when a woman reaches the upper age limit for screening), what actually happens to women as a result of their screen-detected abnormalities is not captured. While we can assume that women who have been suspended as a result of one or more cytological abnormalities have been referred to colposcopy, we don't know whether or not they attended, and if they did, what happened to them there, unless they subsequently had a CIN 3 registered.

A registration of CIN 3 means histologically confirmed cervical intraepithelial grade 3, the highest grade of cervical pre-cancer. A diagnosis of CIN 3 mandates treatment, so in general we can assume that women with a history of CIN 3 have abnormalities that will have been treated, generally by excisional treatment (large loop excision of the transformation zone, or LLETZ; cold knife cone biopsy; or hysterectomy).

8.2.4 Cancer registry data

Data incident cancer registrations that were used as outcomes in these analyses come from the NHS Central Registries. The UK Cancer Registry aims to capture data on all cancers diagnosed, and uses multi-source information, which was discussed in the methods chapter (Chapter 3). Notifications to the Cancer Registry mostly come from pathology laboratories, hospital clinics, cancer therapeutic centres, and death certificates.

Recent estimates of completeness of case ascertainment in the English cancer registries are quite high; a 2011 study estimated ascertainment of around 98-99% compared with cases identified from the Hospital Episode Statistics (HES) database for the period of 2001-2007 (148). There seems to be an improvement in estimates of ascertainment and completeness in more recent time, as older studies reported higher rates of discrepancy (149); this may be a reflection of a greater use of computerised records and multi-source information.

Most patients with cancer will interact with multiple contact points during their cancer 'journey': pathology laboratories, treatment centres, and for some, a death certificate. The rationale for multiple sources of notification is that fewer cases will be missed, but it means that cancer registries must have methods in place to ensure that one case is not registered multiple times, despite re-notification in for the same case from different sources (150). Death certificate only (DCO) registrations are considered to be a marker of completeness of registration, although it has been argued that this is inaccurate and an oversimplification (150). A recent National Cancer Information Network (NCIN) quality

and completeness summary for gynaecological cancers (16) reported that fewer than 4% of vulval and vaginal cancers were 'DCO' (death certificate only) registrations.

There is some evidence that cancer registry data completeness may decrease at older ages (151); this is reported to be a reduced reliability of stage and grade data (possibly due to differences in how cancers are investigated and treated in older patients). However, we have not looked at stage and grade in these analyses, so this is unlikely to affect the reliability of our estimates.

Cases of CIN (which were used as an exposure in most analyses) may not be as reliably reported, and it is possible that we have an underestimation of these cases (46); if there were a systematic under-reporting of cases in some regions, this could result in detection bias. CIN 3 is diagnosed either after a diagnostic punch biopsy, which results in a therapeutic excisional biopsy, or in a single diagnostic/therapeutic procedure done at the first visit to colposcopy. This may be the only interaction that women have relating to their CIN 3, apart from follow-up cervical cytology, which is done in primary care. Unlike invasive cancers, there are limited points at which CIN 3 diagnoses are likely to be notified to the cancer registry. We have no way of checking notification accuracy, however all analyses are stratified by region, which should limit the impact of any regional differences in completeness of CIN 3 notification.

Finally, we are not able to identify cases of CIN 2 in the cohort. CIN 2 is also 'high-grade' disease managed with excision biopsy, but CIN 2 cases are not captured by the cancer registry. While many of these will be women referred with high-grade cytological abnormalities (and therefore identified in the screening data), a proportion of CIN 2 cases will have been referred with low-grade cytology.

8.3 Future work

The cancers examined in this thesis are relatively rare, and as the cohort accrues further cases it may be possible to examine associations between the various exposures and vaginal cancer, as well as the histopathological subtypes of all three cancers, with more precision. It may also become possible to evaluate interactions between history of CIN 3/high grade smear, and other exposures and their influence on subsequent anogenital cancer risk (i.e. whether there are different associations for certain exposures amongst the subset of women with past high-grade disease).

Screen-detected cervical abnormalities, and their relationship with subsequent cancer risk in older women have been an area of research interest for other groups in the past few years. Several groups have reported that women remain at significant risk of cervical cancer after treatment of preinvasive disease, but none of these have had individual data on other risk factors (138,152–154). The work presented here could be extended to examine cervical cancer screening history and risk of cervical cancer at older ages. Identification of risk factors for on-going risk of cervical cancer after screen-detection of abnormalities may be possible with the data now available for Million Women Study participants. Such an analysis could also incorporate the use of a screening history variable that is updated through time.

8.4 Conclusions

Anal, vulval and vaginal cancers are rare malignancies that predominantly affect postmenopausal women. Although all three cancers share a relationship with high-risk strains of human papillomavirus infection, the risk factors associated with these cancers differ between cancer types, and in some cases show heterogeneity by histopathological subtype within the same cancer, suggesting likely differences in aetiology.

The associations observed in the analyses presented in this thesis are consistent with a close relationship between HrHPV and anal cancer. Several of the risk factors identified, such as smoking, past oral contraceptive use, marital status, and past CIN 3 are similar to those previously reported for cervical cancer. Significant associations were also observed with history of tubal ligation, and nulliparity.

Risk of vulval cancer was increased in women who were overweight or obese, those who had an earlier menopause, and women with a history of CIN 3; these relationships support the reported notion that cancers at older ages are in many cases may not be virally-driven, though there is still an increased relative risk in women with past high-grade cervical disease.

Factors associated with vaginal cancer showed some similarities to those associated with both anal and vulval cancer. Like vulval cancer, there was a significantly increased risk of vaginal cancer associated with overweight and obesity; and similar to anal cancer, women who had never given birth had an increased risk of vaginal cancer. In addition,

we saw a strong relationship with hysterectomy that was not seen for either of the other two cancers.

Also notable was the fact that although there was a very strong relationship with past CIN 3, smoking and oral contraceptive use which were significantly related to anal cancer risk and are known risk factors for cervical cancer, did not show a significant association with risk of vaginal cancer at older ages.

The future of cancer prevention for most cases of anal, vaginal, cervical and some vulval cancers may well lie in vaccination against HrHPV. It is hoped that as a consequence of the multivalent vaccines now in use, offering immunity to up to 7 strains of oncogenic HPV (and 2 low risk strains which commonly cause genital warts), HrHPV-related cancers will be a rare occurrence for the current generation of young women. Eradication of HrHPV infection amongst vaccinated cohorts is reported to be an achievable goal (140), with estimates of effective protection by the vaccine of 90%, 96%, 85% and 87% respectively for the HrHPV types associated with cervical, anal, vaginal and virally-driven vulval cancers if the nonavalent (9-valent) vaccine comes into use (142).

However, the unvaccinated cohort of women in the UK, and the millions of women in developing and middle-income countries who do not have access to the vaccine are and will remain at risk of these cancers, requiring on-going surveillance for HPV-related disease. Also, if most cases of vulval cancer at older ages (when its incidence peaks) are not virally-driven, the HPV vaccine may have little impact on rates of this cancer.

While attendance for cervical cancer screening itself did not appear to affect risk of subsequent non-cervical anogenital cancers, the risk of subsequent anal, vulval and vaginal cancers in cervical cancer screening-attenders was significantly associated with both high-grade cytology results and prior CIN 3 registration. Anal cancer risk was also significantly elevated in women with a past low-grade abnormality.

Screen-detected cervical abnormalities were not uncommon. 22% of the cohort had a cytological abnormality prior to recruitment, by far the majority of these were low-grade changes. Approximately 1 in 5 participants had a low-grade smear result, 1 in 50 had a high-grade smear result, and 1 in 100 had a registration of CIN 3 prior to recruitment.

While the relative risks of anal, vulval and vaginal cancers were elevated in women with a history of high-grade cervical disease, the absolute risk of these cancers associated with a past cervical abnormality, even a registration of CIN 3 (cervical carcinoma *in situ),* was small. Just over 4% of women with a past CIN 3 registration went on to develop a subsequent non-cervical anogenital cancer (n = 48), and around three-quarters of the cancer cases (74.8%) arose in screened women with no prior abnormality, or in women who had not been screened.

Clinicians who look after women with high-grade screen-detected cervical abnormalities should be aware that the relative risks of anal, vulval and vaginal cancers are elevated in these women. However, the rarity of these outcomes even in this higher-risk group is such that no practical recommendations for surveillance can be made without identification of more precise and robust markers of risk.

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Appendix A Million Women Study Documentation

A.1 Ethics approval

Original approval

MULTI-CENTRE RESEARCH ETHICS COMMITTEE
ANGLIA & OXFORD

c/o R & D Directorate
NHS Executive Anglia & Oxford
6-12 Capital Drive
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Professor Valerie Beral
Director
Imperial Cancer Research Fund
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30 October 1997

Dear Professor Beral

MREC Project Number MREC 97/01- "Million Women" Breast Cancer Study

The Anglia & Oxford MREC reviewed your application in October.

The members of the Committee agreed there was no objection on ethical grounds to your proposed study whose number is given at the head of this letter. I am, therefore, happy to give you our approval for a period of three years from the date of this letter. If you fail to start your research within three years it is necessary to reapply to this MREC. Approval is given subject to the following conditions.

Conditions of Approval

(a) you follow the protocol agreed and advise the MREC of any changes made. Any major changes to the protocol will require prior MREC approval.

(b) you complete the attached progress report and return it to the administrator of the MREC at the end of the first year after the start date of your project.

(c) you complete the final report form sent to you at the end of your project and return it to the administrator of the MREC.

(d) you notify any adverse events to this MREC, appropriate LRECs and your sponsor using the procedure set out in the Information for Researcher pack.

You will no doubt realise that whilst the MREC has given approval for your project on ethical grounds, it is still necessary for you to obtain approval, if you have not already done so, from the relevant Clinical Director and/or Chief Executives of Trusts (or DHAs) in which the work will be carried out.

It is also your responsibility to ensure any local researcher seeks approval of the relevant LREC before starting their research. Local researchers should only approach the appropriate LREC after MREC approval has been given. You should forward to the local researchers involved in the project a copy of your MREC application form, a copy of the protocol, this approval letter and attached response form and Annex D of the MREC application form for their completion. These documents will be required by the LREC when undertaking the local review of the project.

MREC Evaluation

During the first year after the establishment of the MREC process the Region would like to hear your views and experiences while using the new system. Please can you help us by completing the **Principal Researcher Evaluation Form** attached to this letter and returning to:

Ms Celia Richards, R & D Liaison Manager, NHS Executive Anglia & Oxford
6-12 Capital Drive, Linford Wood, Milton Keynes, MK14 6QP

Your help is also appreciated in ensuring any local researchers are sent the **Local Researcher Evaluation Form** also attached to this letter. Your views and comments are vital to ensure the process evolves and responds to the needs of multi-centre researchers and we look forward to receiving your comments.

Local Sites

While the MREC would like as much information as possible about local sites at the time you apply for approval it is understood that this is not always possible. You are asked, however, to send a completed copy of Annex C for each local site as soon as a researcher has been recruited. This is essential to enable the MREC to monitor the research it approves and to the smooth running of the evaluation.

Additional comments

The members of the MREC would also wish you to note:

(i) although there have been pilot studies attendance should continue to be monitored through out the study.

(ii) that effects on lead time of breast screening should be estimated simultaneously with effects on sensitivity.

With best wishes.

Yours sincerely

P H Archibald

pp **D Evered**
Chairman
Anglia & Oxford Multi-Centre Research Ethics Committee

A.2 Million Women Study protocol

THE MILLION WOMEN STUDY

a national survey of women invited for breast screening

PROTOCOL FOR THE STUDY

The principal aim of this research is to obtain reliable information on the risk of breast cancer associated with the use of hormone replacement therapy (HRT). Recent evidence suggests that the risk may be increased, but insufficient data are available in the entire world literature to describe that risk accurately or to determine whether HRT preparations containing oestrogen alone or oestrogen and progestogen have different effects. Large numbers of women, i.e. more than 5,000 with breast cancer, need to be studied to answer the questions reliably, and the NHSBSP offers a unique opportunity to do so. Indeed it would probably be impossible to carry out a study of the scale required anywhere else in the world. An additional consideration is that the age range of women who use HRT corresponds with the age when screening is offered, and a subsidiary question that would be answered by the proposed study is whether HRT use affects the sensitivity and specificity of mammography.

Summary of the study

The proposed study would involve sending a brief questionnaire to women when they are invited for screening. (A copy of the questionnaire is attached.) The questionnaire asks about factors known to affect breast cancer risk and about the use of hormone replacement therapy and other hormones. It also asks women for permission to obtain follow-up information about their health, and to provide personal details. Follow-up for cancers detected at mammography would be via the screening clinics and for other cancers and deaths via the NHS Central Registers. The risk of cancer and of death from various causes in women who had used HRT will then be compared to the risks in women who had not used HRT.

Results from pilot studies

Extensive piloting of the study has already been carried out on over 6000 women in West London and Oxford. These have demonstrated that the questionnaire is acceptable to women: a sample of women who were sent the questionnaire were interviewed by telephone and none expressed concern about any of the questions asked. By far the most common questions were about the effects of HRT, and many women said how pleased they were that this research was being done. Pilot studies have also shown that attendance rates did not differ significantly in women who were and were not sent a questionnaire. Furthermore, among the women who attended for screening, 78% completed the questionnaire, and 95% of these women gave permission for follow-up.

In conclusion, the results from pilot studies demonstrate that the enclosed questionnaire is acceptable and that sending it out to women does not alter attendance rates for screening. The response rate is high and women seem keen to take part in this type of research. Additionally, the prevalence of use of HRT is high, with 41% having used HRT at some time and 28% being current users of HRT.

Details of the study

Eligibility: All screening centres in the NHSBSP will be eligible to collaborate in this research and all women invited for screening will be eligible to take part. Participation is entirely voluntary.

Approach to clinics: Each breast screening centre will be approached and asked if it wishes to collaborate in this research. If so, the study team will discuss how best to arrange the sending out and collection of questionnaires and seek local ethical committee approval for the study.

The study is already underway in about 40 screening centres in England and the experience so far is that each centre has its own particular routine: an important principle of the study is that it should interfere as little as possible with the normal running of the screening programme. Therefore, there will be regular communication between the study team and staff at each participating centre to ensure that involvement in the study causes as little extra work as possible and that problems that arise are dealt with swiftly.

Approach to subjects: Women will be sent the attached questionnaire at the time they are invited for screening and asked to bring it with them when they attend for screening. A freephone number is provided if the women have any questions.

Follow-up: Once each year routine information about the breast cancers diagnosed at screening will be sought from each screening clinic. In addition, the study team will seek information about cancer incidence and deaths from the NHS Central Registries.

Logistics: Staff at the ICRF Cancer Epidemiology Unit, Oxford, will be responsible for the preparation and delivery of the questionnaires to the screening centres, for the collection of the questionnaires and for the follow-up. The screening clinics will be responsible for despatching the questionnaires to the women and for providing, once each year, routine details about the cancers diagnosed in study participants.

Numbers: At least 5,000 women with breast cancer are needed to detect a 25% difference in breast cancer risk in long term users of oestrogen alone compared with users of combinations of oestrogen and progestogen. In addition at least 500 interval cancers are needed in the first year after screening to see whether HRT use affects sensitivity of screening. To obtain such numbers about 1,000,000 women will need to be recruited.

Timetable: It is hoped that most centres that wish to participate will have begun sending questionnaires out by late 1997. Recruitment will last for about 3 years in each centre. The exact timing will be determined by the number of centres that collaborate.

Confidentiality and ethics: Local Ethical Committee approval will be sought separately for each screening centre. Participation in the study will be entirely voluntary. It will be made clear to women that they do not have to take part in the study if they do not want to and that their decision does not affect their management. All information provided will be stored in accordance with the regulations of the Data Protection Act (registration number with the Office of the Data Protection Registrar: K3039784). The data will be treated with utmost confidentiality and used only for medical research. Only the study team will have access to computerised data, via passwords. Any publication resulting from this work will not identify the individual women who took part.

Publication of results: This is a joint research project of the NHS Breast Screening Programme and the Imperial Cancer Research Fund (ICRF). Publications of the main results will be in the name of "The Million Women Study Group", and all collaborators will be named.

Million Women Study Co-ordinating Centre
ICRF Cancer Epidemiology Unit
Gibson Building
Radcliffe Infirmary
Oxford OX2 6HE
Tel: 01865 311933
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May 1997

A.3 Million Women Study Co-ordinating Centre Staff

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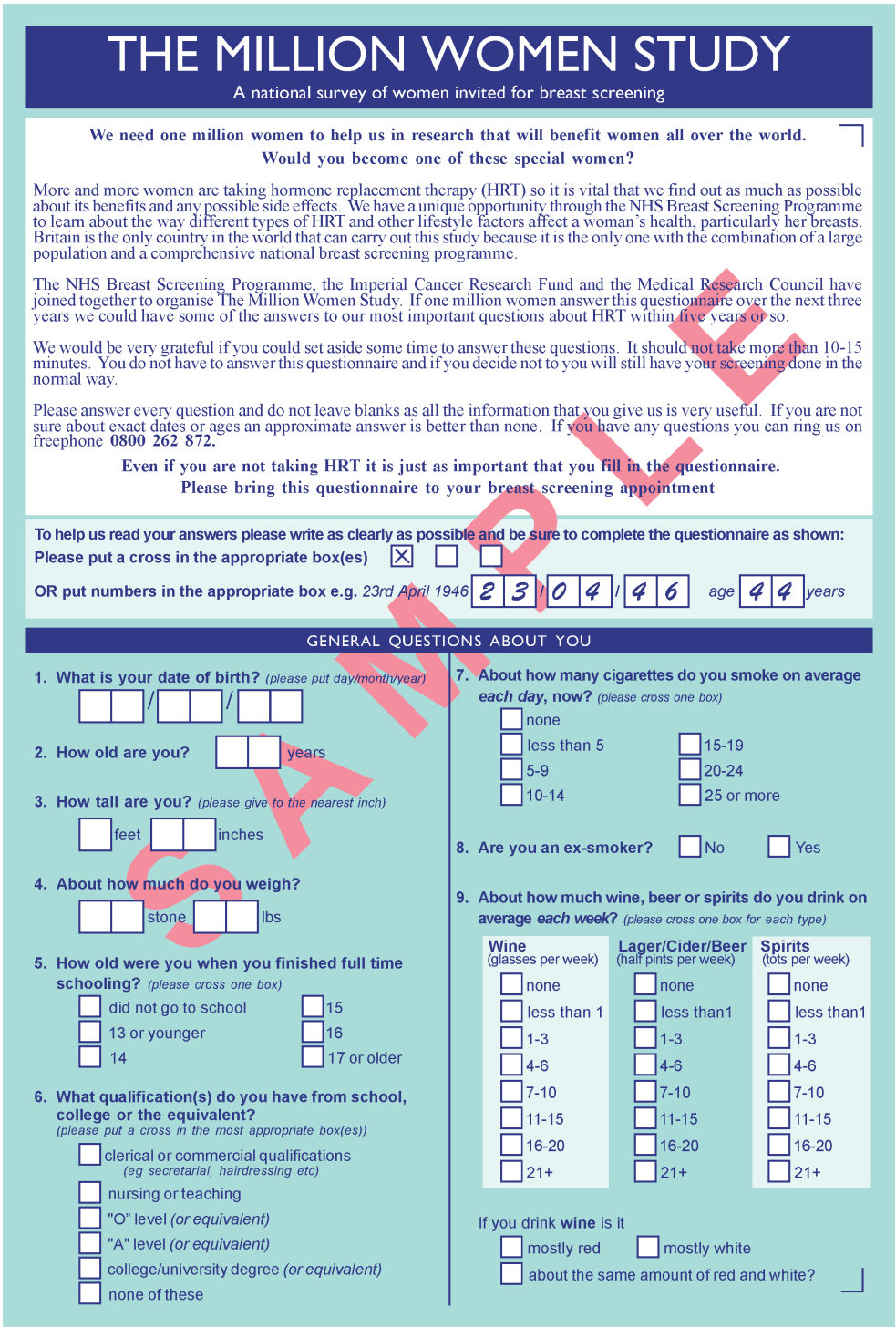
Million Women Study Advisory Committee: Emily Banks, Valerie Beral, Lucy Carpenter, Carol Dezateux, Jane Green, Julietta Patnick, Richard Peto, Cathie Sudlow.

Appendix B Million Women Study Questionnaires

B.1 The recruitment questionnaire



THE MILLION WOMEN STUDY

A national survey of women invited for breast screening

We need one million women to help us in research that will benefit women all over the world.
Would you become one of these special women?

More and more women are taking hormone replacement therapy (HRT) so it is vital that we find out as much as possible about its benefits and any possible side effects. We have a unique opportunity through the NHS Breast Screening Programme to learn about the way different types of HRT and other lifestyle factors affect a woman's health, particularly her breasts. Britain is the only country in the world that can carry out this study because it is the only one with the combination of a large population and a comprehensive national breast screening programme.

The NHS Breast Screening Programme, the Imperial Cancer Research Fund and the Medical Research Council have joined together to organise The Million Women Study. If one million women answer this questionnaire over the next three years we could have some of the answers to our most important questions about HRT within five years or so.

We would be very grateful if you could set aside some time to answer these questions. It should not take more than 10-15 minutes. You do not have to answer this questionnaire and if you decide not to you will still have your screening done in the normal way.

Please answer every question and do not leave blanks as all the information that you give us is very useful. If you are not sure about exact dates or ages an approximate answer is better than none. If you have any questions you can ring us on freephone **0800 262 872.**

Even if you are not taking HRT it is just as important that you fill in the questionnaire.
Please bring this questionnaire to your breast screening appointment

To help us read your answers please write as clearly as possible and be sure to complete the questionnaire as shown:

Please put a cross in the appropriate box(es) ☒ ☐ ☐

OR put numbers in the appropriate box e.g. *23rd April 1946* 2 3 / 0 4 / 4 6 *age* 4 4 *years*

GENERAL QUESTIONS ABOUT YOU

1. What is your date of birth? *(please put day/month/year)*

☐☐ / ☐☐ / ☐☐

2. How old are you? ☐☐ years

3. How tall are you? *(please give to the nearest inch)*

☐ feet ☐☐ inches

4. About how much do you weigh?

☐☐ stone ☐☐ lbs

5. How old were you when you finished full time schooling? *(please cross one box)*

- ☐ did not go to school
- ☐ 13 or younger
- ☐ 14
- ☐ 15
- ☐ 16
- ☐ 17 or older

6. What qualification(s) do you have from school, college or the equivalent?
(please put a cross in the most appropriate box(es))

- ☐ clerical or commercial qualifications *(eg secretarial, hairdressing etc)*
- ☐ nursing or teaching
- ☐ "O" level *(or equivalent)*
- ☐ "A" level *(or equivalent)*
- ☐ college/university degree *(or equivalent)*
- ☐ none of these

7. About how many cigarettes do you smoke on average *each day*, now? *(please cross one box)*

- ☐ none
- ☐ less than 5
- ☐ 5-9
- ☐ 10-14
- ☐ 15-19
- ☐ 20-24
- ☐ 25 or more

8. Are you an ex-smoker? ☐ No ☐ Yes

9. About how much wine, beer or spirits do you drink on average *each week*? *(please cross one box for each type)*

Wine (glasses per week)	**Lager/Cider/Beer** (half pints per week)	**Spirits** (tots per week)
☐ none	☐ none	☐ none
☐ less than 1	☐ less than1	☐ less than1
☐ 1-3	☐ 1-3	☐ 1-3
☐ 4-6	☐ 4-6	☐ 4-6
☐ 7-10	☐ 7-10	☐ 7-10
☐ 11-15	☐ 11-15	☐ 11-15
☐ 16-20	☐ 16-20	☐ 16-20
☐ 21+	☐ 21+	☐ 21+

If you drink **wine** is it

- ☐ mostly red
- ☐ mostly white
- ☐ about the same amount of red and white?

10. How often do you do *any* exercise?

- ☐ rarely/never
- ☐ less than once a week
- ☐ once a week
- ☐ 2-3 times a week
- ☐ 4-6 times a week
- ☐ every day

11. How often do you do *strenuous* exercise?

(that is, enough to cause sweating or a fast heart beat.)

- ☐ rarely/never
- ☐ less than once a week
- ☐ once a week
- ☐ 2-3 times a week
- ☐ 4-6 times a week
- ☐ every day

QUESTIONS ABOUT YOU AND YOUR FAMILY

12. Have you ever had any children? ☐ No ☐ Yes

- if No, please go on to question 15

13. How many children have you had? ☐☐

(please include stillbirths; it is not necessary to include miscarriages)

14. When was each child born, and for how many months did you breastfeed each child, if at all?

	DATE OF BIRTH (If you had twins or triplets please repeat the same date for each child) day / month / year	BREASTFEEDING (Months that you breastfed each child; put "0" if you did not breastfeed that child a "1" if you breastfed for month or less)
1st child	☐☐ / ☐☐ / ☐☐	☐☐ months
2nd child	☐☐ / ☐☐ / ☐☐	☐☐ months
3rd child	☐☐ / ☐☐ / ☐☐	☐☐ months
4th child	☐☐ / ☐☐ / ☐☐	☐☐ months
5th child	☐☐ / ☐☐ / ☐☐	☐☐ months
6th child	☐☐ / ☐☐ / ☐☐	☐☐ months
7th child	☐☐ / ☐☐ / ☐☐	☐☐ months
8th child	☐☐ / ☐☐ / ☐☐	☐☐ months
9th child	☐☐ / ☐☐ / ☐☐	☐☐ months
10th child	☐☐ / ☐☐ / ☐☐	☐☐ months

15. Have you ever been for breast screening before?

- ☐ No
- ☐ Yes- If **Yes**, about how many years ago was your last screen? ☐☐ years ago

16. Have you ever had a breast lump removed or any operations on your breast(s)?

- ☐ No
- ☐ Yes- If **Yes**, how old were you? ☐☐ years

(If you have had more than one operation please write your age at the first operation)

17. Have you ever had breast cancer diagnosed?

- ☐ No
- ☐ Yes- If **Yes**, how old were you when the cancer was first diagnosed? ☐☐ years

18. Has your mother ever had breast cancer diagnosed?

- ☐ No ☐ Don't know
- ☐ Yes- If **Yes**, how old was she when the cancer was first diagnosed? ☐☐ years

19. How many sisters do you have? ☐☐ sisters

(put"0" if you do not have any sisters, please include any sisters who have died)

20. Have any of your sisters ever had breast cancer diagnosed?

- ☐ No/No sisters ☐ Don't know
- ☐ Yes- If **Yes**, how old were they when the cancer was first diagnosed?

1st sister ☐☐ years **2nd sister** ☐☐ years

QUESTIONS ABOUT YOUR HEALTH

21. Have you ever had any other cancer?

☐ Yes ☐ No

Please describe

22. Have you EVER had:

(please cross "Yes" or "No" for each condition)

High blood pressure - when pregnant	☐ Yes	☐ No
High blood pressure - when not pregnant	☐ Yes	☐ No
Heart disease *(eg heart attack/angina)*	☐ Yes	☐ No
Stroke	☐ Yes	☐ No
Diabetes	☐ Yes	☐ No
High blood cholesterol	☐ Yes	☐ No
Blood clot *(thrombosis)*	☐ Yes	☐ No

23. Are you NOW being treated for:

High blood pressure *(hypertension)*	☐ Yes	☐ No
Heart disease	☐ Yes	☐ No
Diabetes	☐ Yes	☐ No
High blood cholesterol	☐ Yes	☐ No
Varicose veins	☐ Yes	☐ No
Clotting problems	☐ Yes	☐ No
Asthma	☐ Yes	☐ No
Rheumatoid arthritis	☐ Yes	☐ No
Osteoarthritis	☐ Yes	☐ No
Thyroid problems	☐ Yes	☐ No
Osteoporosis	☐ Yes	☐ No
Depression/Anxiety	☐ Yes	☐ No

24. Are you *NOW* being treated for any other *serious* illness?

☐ Yes ☐ No

Please describe this illness

Please describe the treatment

QUESTIONS ABOUT PAST OPERATIONS

25. Have you had a hysterectomy?

☐ No

☐ Yes- If **Yes**, how old were you? ☐☐ years

26. Have you had BOTH ovaries removed?

☐ No ☐ Not sure

☐ Yes- If **Yes**, how old were you? ☐☐ years

27. Have you been sterilised *(had your tubes tied)***?**

☐ No

☐ Yes- If **Yes**, how old were you? ☐☐ years

QUESTIONS ABOUT YOUR USE OF THE PILL

28. Have you ever used the pill *(oral contraceptive)*?

☐ Yes

☐ No ***- if No, please go to question 32***

29. About how old were you when you *first went on* the pill? ☐☐ years

30. About how old were you when you *last came off* the pill? ☐☐ years

31. For how many years *in total* did you take the pill? ☐☐ years

(Add together the years and months when you actually took the pill -do not count the years and months when you were not taking it. Please write "0" if you used the pill for less than a year in total)

QUESTIONS ABOUT YOUR USE OF HORMONE REPLACEMENT THERAPY (HRT)

32. Have you ever used hormone replacement therapy (HRT)? ☐ No ***- if No - please go to question 39***

☐ Yes

33. How old were you when you *first started* using HRT? ☐☐ years

34. Had your periods stopped before you started using HRT? *(Cross "Yes" if you had a hysterectomy before starting HRT)*

☐ No

☐ Yes **- if Yes**, how old were you when your periods stopped? ☐☐ years

35. For about how many years *in total* have you used HRT? ☐☐ years

(Add together the years and months when you used HRT - do not count the years and months when you were not using HRT. Please write "0" if you used HRT for less than a year in total)

36. Are you *now* using HRT?

☐ Yes

☐ No **- if No**, how old were you when you **last** used HRT? ☐☐ years

37. What is the name of the most RECENT HRT you have used?

☐ Prempak C 0.625mg	☐ Premarin 0.625mg
☐ Prempak C 1.25mg	☐ Premarin 1.25mg
☐ Tridestra	☐ Evorel 25mcg/50mcg
☐ Trisequens	☐ Evorel 75mcg/100mcg
☐ Trisequens Forte	☐ Progynova 1mg
☐ Cycloprogynova 1mg	☐ Progynova 2mg
☐ Cycloprogynova 2mg	☐ Estraderm 25mcg
☐ Estrapak	☐ Estraderm 50mcg
☐ Estracombi	☐ Estraderm 100mcg
☐ Climaval 1mg	☐ Zumenon 1mg
☐ Climaval 2mg	☐ Zumenon 2mg
☐ Premique Cycle	☐ Ethinyloestradiol
☐ Premique	☐ Micronor
☐ Nuvelle	☐ Provera
☐ Kliofem	☐ Duphaston
☐ Livial	

☐ Do not know ☐ Implants ☐ Oestrogel

Other *(please write here)*

38. For how many years in total did you use the most *recent* type of HRT? ☐☐ years

(Please write "0" if you used this recent HRT for less than a year in total)

QUESTIONS ABOUT YOUR PERIODS

39. About how old were you when your periods started? ☐☐ years

40. Have your periods NOW stopped?

*Cross "**Yes**"-if you are not having periods now, either because of your menopause, after a hysterectomy or after stopping HRT.*
*Cross "**No**"- if you are still having regular periods now, even if they are because you are taking HRT.*
*Cross "**Irregular**"-if your periods have been irregular and you think it might be because of the menopause.*

☐ **Yes**- If **Yes**, how old were you when they **stopped**? ☐☐ years

☐ **No**

☐ **Irregular**

FINAL SECTION

41. So that we can find out about your health in the future we may need to contact you again or look at your screening or medical records. We would be grateful if you gave us permission to contact you again or to use information from those records. **We guarantee that all information obtained will be treated with absolute confidentiality and used for medical research only.** Of course, you do not have to give permission. Your response to this request will not affect your screening or the treatment you receive in any way.

If you give permission, please sign here and print your name, address and other details in the section below. Please print in BLOCK CAPITALS as clearly as possible.

Signature: ☐ today's date: ☐☐ ☐☐ ☐☐

Surname: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Given name(s): ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

House number and street: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

District: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Town: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

County: ☐☐☐☐☐☐☐☐☐☐ Postcode: ☐☐☐☐☐☐☐☐

Surname at birth: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Town of birth: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

NHS (National Health Service)Number: ☐☐☐☐☐☐☐☐☐☐☐☐
(The number on your medical card)

Breast Screening Number: ☐☐☐☐☐☐☐☐☐☐☐☐
*(This is in the top left hand corner of your **screening invitation letter** and starts with the letters LGL)*

Please bring this questionnaire to your breast screening appointment

If you would like to post this questionnaire back to us, please send it to:
THE MILLION WOMEN STUDY CO-ORDINATING CENTRE
ICRF-CEU, GIBSON BUILDING,
RADCLIFFE INFIRMARY,
OXFORD OX2 6HE

FREEPHONE: 0800 262 872

Breast Screening Unit
Linton House
Thirlestaine Road
Cheltenham
GL53 7AS

LGL ☐

THANK YOU VERY MUCH FOR YOUR HELP

B.2 The first resurvey "9909" version

THE MILLION WOMEN STUDY

Confidential National Study of Women's Health

The Million Women Study is an important national study of women's health. A few years ago you received the first questionnaire with your invitation to the National Health Service Breast Screening Programme. Your help is needed again. Can you find time to complete this second questionnaire? Some of the questions may seem familiar and others are new, but all will provide vital up to date information for the study. Your answers are valuable and important - the enclosed leaflet explains how the study will benefit women and improve medical knowledge world-wide. We very much hope you are still willing to be one of the Million Women in the study.

We guarantee that all information provided will be treated with absolute confidentiality and used for medical research only. +

To help us read your answers please write as clearly as possible and complete the questionnaire as shown:

Please put a cross in the appropriate box(es) ☐ ☐

OR put numbers in the appropriate boxes

eg 28th October 1946 2 8 / 1 0 / 1 9 4 6

Any questions? Ring us on Freephone 0800 262 872

QUESTIONS ABOUT YOU AND YOUR HEALTH

Please answer every question as best you can as all the information that you give us is very useful. If you are not sure about exact dates or ages an approximate answer is better than none. Please use a black pen, if possible.

1. Have you had any of the following conditions diagnosed *for the first time in the last 5 years?*

Please cross "yes" if appropriate, and write when the condition was first diagnosed.

	Yes	If **Yes**, when was it first diagnosed? Month	Year
Heart disease *heart attack, angina etc*	☐		
Stroke	☐		
Diabetes	☐		
Blood clot in leg	☐		
Blood clot in lung or elsewhere	☐		
High blood cholesterol	☐		
High blood pressure *hypertension*	☐		
Asthma	☐		
Gallstones/gallbladder disease	☐		
Osteoporosis	☐		
Abnormal cervical smear test *Pap smear*	☐		
Breast cancer	☐		
Other cancer *Please describe the cancer below*	☐		

2. Have you had any broken/fractured bones, *in the last 5 years?*

☐ No ☐ Yes - ***if Yes***, when? Month ☐☐ Year ☐☐

If Yes, which bone(s) were broken? *(please cross)*

☐ hip ☐ ankle ☐ wrist/arm

☐ spine ☐ other- *please describe*_____

If Yes, how did the fracture(s) occur?

☐ after a fall ☐ in a car accident ☐ in another accident

☐ fracture found on X-ray without you knowing about it ☐ in some other way

3. Ha[illegible] you had any [illegible]er serious illness diagnosed [illegible] *first time, in the last 5 years?*

☐ N[illegible] ☐ Yes - ***if Yes***, when? Month ☐☐ Year ☐☐

If yes, please describe the illness

4. Have you had any major operations *in the last 5 years?*

☐ No ☐ Yes - ***if Yes***, when? Month ☐☐ Year ☐☐

If yes, please describe the operation(s)

5. Are you NOW being treated for:

	Yes	If yes, about how old were you when treatment began?
Heart disease *heart attack, angina etc*		
Diabetes	☐	☐☐
High blood pressure *hypertension*		
Asthma	☐	☐☐
Osteoarthritis	☐	☐☐
Osteoporosis	☐	☐☐
Any other serious illness or disability *(please describe below)*	☐	☐☐

6. How would you describe your health now? +

☐ excellent ☐ good ☐ fair ☐ poor

The Million Women Study is supported by the Imperial Cancer Research Fund, the Medical Research Council and the National Health Service Breast Screening Programme.

QUESTIONS ABOUT MEDICATIONS

7. Have you ever used H R T (hormone replacement therapy)? ☐ No - ***if No -*** *please go to question 12 .* ☐ Yes

8. How old were you when you *first started* using HRT? ☐☐ years old

9. Are you *now* using HRT?
☐ No - ***if No,*** when did you stop using HRT? ☐☐ Month ☐☐ Year
☐ Yes

10. For about how many years *in total* have you used HRT? ☐ years of use
(Add together all the years and months when you were using HRT. Please write "0" if you used HRT for less than a year in total)

11. Which HRT did you use MOST RECENTLY?

☐ Prempak C 0.625mg	☐ Premarin 0.625mg	
☐ Prempak C 1.25mg	☐ Premarin 1.25mg	
☐ Tridestra	☐ Evorel 25mcg/50mcg	
☐ Trisequens	☐ Evorel 75mcg/100mcg	
☐ Cycloprogynova 1mg	☐ Progynova 1mg	
☐ Cycloprogynova 2mg	☐ Progynova 2mg	
☐ Estrapak	☐ Estraderm 25mcg	
☐ Estracombi	☐ Estraderm 50mcg	
☐ Climaval 1mg	☐ Estraderm 100mcg	
☐ Climaval 2mg	☐ Zumenon 1mg	
☐ Premique Cycle	☐ Zumenon 2mg	
☐ Premique	☐ Ethinyloestradiol	
☐ Nuvelle	☐ Oestrogel	☐ Micronor
☐ Kliofem	☐ Implants	☐ Duphaston
☐ Livial	☐ Provera	☐ Do not know

Other (please write here)

12. Have you taken any medications (other than HRT) for most of the last 4 weeks? ☐ No ☐ Yes

If Yes, was it:

☐ thyroxine	☐ ibuprofen	☐ aspirin	
☐ tamoxifen	☐ bendrofluazide	☐ amlodipine *Istin*	☐ digoxin
☐ paracetamol	☐ propranolol	☐ atenolol	☐ warfarin
☐ prednisolone	☐ Losec/Zoton	☐ Prozac	☐ insulin
☐ co-proxamol *Distalgesic*	☐ amitriptyline *Tryptizol etc*	☐ sleeping pills	☐ lithium

Please give the name(s) of any other medication you have used for most of the last 4 weeks:

13. Do you regularly take any vitamins, minerals or supplements? ☐ No ☐ Yes - ***if Yes,*** do you take:

☐ multivitamins (with minerals)		☐ vitamin A
☐ multivitamins (without minerals)		☐ vitamin B (including B_6 B_{12})
☐ fish oil (including cod liver oil)		☐ vitamin C
☐ evening primrose oil	☐ garlic	☐ vitamin D
☐ iron	☐ zinc ☐ calcium	☐ vitamin E

☐☐☐☐☐☐☐☐☐☐☐☐
for office use only

QUESTIONS ABOUT YOUR DIET

We know it may be difficult for you to give exact answers to these questions about your diet. An approximate answer is very valuable for this study. So, please answer as best you can, thinking of a typical week.

14. Which types of meat do you eat about once a week or more often? *(you can cross more than one box)*

☐ beef	☐ bacon	☐ chicken/poultry
☐ lamb	☐ ham	☐ kidney
☐ pork	☐ sausages	☐ liver/pâté
☐ beefburger/hamburger		☐ never eat meat

15. Which types of fish do you eat about once a week or more often? *(you can cross more than one box)*

☐ tuna	☐ trout	☐ mackerel	☐ "fish & chips"
☐ salmon		☐ sardines	☐ other seafood *(prawns, scampi etc)*
☐ cod/haddock or other white fish			☐ never eat fish

16. About how many times each week do you eat:
(please count all meals and snacks. put '0' if eaten less than once a week)

meat	☐	number of times eaten each week *(remember meat in sandwiches)*
fish/ seafood	☐	number of times eaten each week
chips	☐	number of times eaten each week
potatoes (except chips)	☐	number of times eaten each week
pasta/ spaghetti	☐	number of times eaten each week
rice	☐	number of times eaten each week
cheese	☐	number of times eaten each week *(remember cheese in pizzas, quiches, cheese sauce, etc)*

17. About how many eggs do you eat each week?
eggs ☐ number of eggs eaten each week *(remember eggs in omelettes, quiches, cakes etc. put '0' if less than one)*

18. Which types of vegetables/salads (fresh, frozen or tinned) do you eat once a week or more often?
(you can cross more than one box)

☐ green peas	☐ tomatoes	☐ green beans
☐ broccoli	☐ onions	☐ baked beans
☐ cabbage	☐ garlic	☐ soya meat/tofu
☐ carrots	☐ swede	☐ chick peas/lentils
☐ courgettes	☐ spinach	☐ cauliflower
☐ beetroot	☐ sweetcorn	☐ green/red peppers
☐ leeks	☐ avocado	☐ brussels sprouts
☐ parsnip	☐ aubergine	☐ mushrooms
☐ lettuce	☐ celery	☐ cucumber

19. About how much do you eat each week of:
(put "0" if less than one)
cooked vegetables (except potatoes) ☐ number of heaped tablespoons each week
salad items/ raw vegetables ☐ number of heaped tablespoons each week *(please count lettuce, tomato etc in sandwiches)*

20. Which types of fruit do you eat once a week or more often, when in season? *(you can cross more than one box)*

☐ apples	☐ bananas	☐ oranges, satsumas, etc
☐ grapefruit	☐ pears	☐ stone fruit *(peaches, plums, nectarines etc)*

21. About how much fruit or fruit juice do you eat or drink each week? *(count 10 grapes, berries or raisins as one piece; put "0" if less than one a week)*

☐ number of pieces of **fresh fruit** eaten each week	☐ number of pieces of **dried fruit** eaten each week
☐ number of glasses of **fruit juice** each week	☐ number of tablespoons of **stewed or tinned fruit** eaten each week

MORE ABOUT YOUR DIET

22. About how many of the following do you eat: *(put "0" if none or less than one)*

slices/pieces of white bread	☐	slices each week
slices/pieces of brown/wholemeal bread *(also include granary, rye bread etc)*	☐	slices each week
crackers, crispbread etc *(ryvita, water biscuits etc)*	☐	number each week
crisps, hula hoops etc	☐	packets each week
sweet biscuits	☐	number each week
dairy desserts (yoghurts etc)	☐	number each week
cakes, puddings, pies, buns etc	☐	number each week
chocolate (in any food or drink)	☐	approx. number of pieces each week
boiled sweets, peppermints etc	☐	number each week
nuts (including peanut butter)	☐	tablespoons each week
gravy, cream/cheese sauces etc	☐	tablespoons each week
jams, marmalade	☐	tablespoons each week
breakfast type cereal	☐	bowls each week

If you eat breakfast cereal is it usually: *(please cross)*

☐ bran cereal *(allbran, branflakes etc)* ☐ muesli
☐ biscuit cereal *(weetabix, shreddies etc)* ☐ other *(cornflakes, rice crispies etc)*
☐ oat cereal *(porridge, ready brek etc)*

23. What type of spread do you use on bread, crispbreads etc, once a week or more often? *(you can cross more than one box)*

☐ butter ☐ margarine ☐ soft cheese
☐ low fat spread ☐ mayonnaise ☐ salad cream
☐ olive oil spread ☐ marmite etc ☐ rarely use spread

Do you spread it: ☐ thick? ☐ medium? ☐ thin? (please cross)

Do you add butter etc to: ☐ potatoes? ☐ other vegetables?

24. Which types of fats or oils do you use for cooking or salad dressing once a week or more often? *(you can cross more than one box)*

☐ butter ☐ soft (tub) margarine ☐ white flora
☐ olive oil ☐ hard (block) margarine ☐ lard/dripping
☐ corn oil ☐ sunflower oil ☐ mayonnaise
☐ soya oil ☐ other vegetable oil ☐ salad cream

Please put a cross in the box if you RARELY OR NEVER:

☐ use fats or oils for cooking ☐ use salad dressing/cream

25. Please put a cross in the box if you NEVER eat:

☐ beef ☐ pork/ham ☐ lamb ☐ dairy products
☐ kidney ☐ liver/pâté ☐ sugar ☐ wheat products
☐ salami ☐ sausages ☐ eggs ☐ beefburgers

26. What type of milk or cream do you drink or use once a week or more often? *(you can cross more than one box)*

☐ full cream milk ☐ single cream
☐ semi-skimmed milk ☐ double cream
☐ skimmed/fat free milk ☐ dairy ice cream
☐ soya milk ☐ never have milk/cream

27. Do you:

	never	some-times	usually	always
add milk to your tea?	☐	☐	☐	☐
add milk to your coffee?	☐	☐	☐	☐
add salt to your food?	☐	☐	☐	☐
remove fat from meat? *(cross "never" if vegetarian)*	☐	☐	☐	☐
eat breakfast?	☐	☐	☐	☐
eat an afternoon snack?	☐	☐	☐	☐
eat organic food?	☐	☐	☐	☐

28. Have you made any major changes to your diet in the last 5 years? ☐ No ☐ Yes-because of illness ☐ Yes-for some other reason

29. About how much alcohol do you drink each week?

☐ number of drinks of alcohol each week *(one drink = a glass of wine, half pint of lager, or tot of spirits; put "0" if you drink less than one drink each week)*

If you have more than one drink of alcohol each week:

is it usually with meals? ☐ No ☐ Yes ☐ it varies

on how many days each week do you usually drink? ☐ days each week

30. About how much do you drink EACH DAY of:

tea? *cups daily* ☐ milk, hot chocolate etc? *cups daily* ☐ fizzy/soft drink? *glasses daily* ☐
coffee? *cups daily* ☐ water? *glasses daily* ☐ fruit squash? *glasses daily* ☐

31. How many teaspoons of sugar do you add to tea, coffee, cereal, fruit etc EACH DAY? ☐ teaspoons of sugar each day

32. What size clothes do you wear now? *(you can cross more than one box if the size varies)*

Clothes	☐ 10 or less	☐ 12	☐ 14	☐ 16	☐ 18	☐ 20+
Bra	☐ 32	☐ 34	☐ 36	☐ 38	☐ 40	☐ 42+
Cup	☐ A/AA	☐ B	☐ C	☐ D	☐ DD/E +	

33. What is your: *(please put "0" if you do not know)*

waist measurement? ☐☐ inches hip measurement? ☐☐ inches

34. About how much do you weigh now? ☐ stone ☐ lbs *(Put "0" if you do not know)*

WHEN YOU WERE YOUNG

35. About how much did you weigh when you were born? ☐ lbs ☐ ozs *(Put "0" if you do not know)*

36. Were you breastfed when you were a baby?

☐ No ☐ Yes ☐ do not know

37. Did your parents smoke at around the time that you were born?

Mother ☐ No ☐ Yes ☐ do not know
Father ☐ No ☐ Yes ☐ do not know

38. Did your parents smoke at around the time that you were 10 years old?

Mother ☐ No ☐ Yes ☐ do not know
Father ☐ No ☐ Yes ☐ do not know

39. When you were about 10 years old, compared to average, would you describe yourself as *(please cross)*:

☐ thinner? ☐ plumper? ☐ about average?

40. What size clothes did you wear when you were about 20 years old? *(you can cross more than one box)*

☐ 8 or less ☐ 10 ☐ 12 ☐ 14 ☐ 16 ☐ 18 +

QUESTIONS ABOUT YOUR FAMILY AND LIFESTYLE

41. Is your mother still alive?

- ☐ Yes-please give her age now ☐☐ years old
- ☐ No-please give her age when she died ☐☐ years old
- ☐ Do not know

42. If your mother has died, what did she die from?

- ☐ heart disease *heart attack etc*
- ☐ stroke
- ☐ chest infection *pneumonia*
- ☐ "old age"
- ☐ breast cancer
- ☐ cancer of the womb
- ☐ cancer of ovary
- ☐ other/unknown_____

43. Has your mother or father ever suffered from:

mother	father		mother	father	
☐	☐	heart disease	☐	☐	breast cancer
☐	☐	stroke	☐	☐	bowel cancer
☐	☐	high blood pressure	☐	☐	lung cancer
☐	☐	diabetes		☐	prostate cancer
☐	☐	Alzheimer's disease	☐	☐	osteoporosis
☐	☐	Parkinson's disease	☐	☐	hip fracture
☐	☐	severe depression	☐	☐	severe arthritis

44. How tall is/was your mother? *(Put "0" if you do not know)* ☐ feet ☐ inches

How tall is/was your father? ☐ feet ☐ inches

45. Have you ever been a smoker?

- ☐ No - ***if No*** *- please go to question 49.*
- ☐ Yes

46. How old were you when you started smoking regularly? ☐☐ years old

47. Are you a smoker now?

- ☐ No - ***if No*** - how old were you when you stopped smoking? ☐☐ years old
- ☐ Yes -***if Yes*** - please write the tar & nicotine content of your usual brand of cigarettes:

(this is written on each packet of cigarettes) tar ☐ mg nicotine ☐ mg

48. About how many cigarettes do you/*did you* smoke on average *each day*? *(If you are an ex-smoker, how many did you smoke on average when you smoked?)*

☐☐ cigarettes per day

49. Have you had your menopause?

- ☐ No ☐ Not sure *(because periods irregular, taking HRT etc)*
- ☐ Yes- How old were you when you had your menopause? ☐☐ years old

50. Are you now in paid work?

☐ No ☐ Yes, full time ☐ Yes, part time

51. Are you currently married or living with a partner?

☐ No ☐ Yes- ***If Yes***- does your husband/partner smoke? ☐ No ☐ Yes

52. About how often do you use a mobile phone?

☐ Never ☐ less than once a day ☐ every day

For how long have you used one? *(put "0" if never or less than 1 year)* ☐ years

53. Do you belong to or participate in any of the following?

- ☐ religious group ☐ art/craft group ☐ bingo
- ☐ voluntary work ☐ music/singing group
- ☐ adult education ☐ sports club (swimming,golf etc)
- ☐ dancing group ☐ yoga,etc ☐ other group activity

54. How often do you feel:

	rarely/ never	some-times	usually	most of the time
happy	☐	☐	☐	☐
relaxed	☐	☐	☐	☐
in control	☐	☐	☐	☐
stressed	☐	☐	☐	☐
tired	☐	☐	☐	☐

55. Do you have a nap during the day?

☐ rarely/never ☐ sometimes ☐ usually

56. About how many hours sleep do you get in every 24 hours? ☐ hours sleep *(please include naps)*

57. To which ethnic group do you consider you belong?

- ☐ White ☐ Black - Caribbean, African etc.
- ☐ Asian ☐ Other -*please specify*_____

	Day	Month	Year
58. What is your date of birth?	☐☐	☐☐	☐☐
59. On what date did you fill in this form?	☐☐	☐☐	☐☐

60. In case we need to check on any details, it would be helpful if you would write your telephone number below.

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

STD code Telephone number

THANK YOU VERY MUCH FOR YOUR HELP

Please put your completed questionnaire in the pre-paid envelope and post it back to us

If your name /address has changed or is incorrect could you please cross this box ☐ & give the correct details below.

Surname: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Given name(s): ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

House number and street: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

District: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Town/County: ☐☐☐☐☐☐☐☐☐☐ Postcode: ☐☐☐☐☐☐☐☐

For office use only

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9909

PROFESSOR VALERIE BERAL, THE MILLION WOMEN STUDY, ICRF-CEU, GIBSON BUILDING, RADCLIFFE INFIRMARY, OXFORD OX2 6HE

FREEPHONE: 0800 262 872

B.3 The first resurvey "0012" version

THE MILLION WOMEN STUDY

Confidential National Study of Women's Health

The Million Women Study is an important national study of women's health. A few years ago you received the first questionnaire with your invitation to the National Health Service Breast Screening Programme. Your help is needed again. Can you find time to complete this second questionnaire? Some of the questions may seem familiar and others are new, but all will provide vital up to date information for the study. Your answers are valuable and important - the enclosed leaflet explains how the study will benefit women and improve medical knowledge world-wide. We very much hope you are still willing to be one of the Million Women in the study.

We guarantee that all information provided will be treated with absolute confidentiality and used for medical research only.

To help us read your answers please write as clearly as possible and complete the questionnaire as shown:

Please put a cross in the appropriate box(es) ☒ ☐

OR put numbers in the appropriate places

eg 54 *(age)* 0 2 0 8 2 0 0 0
2nd August 2000

Any questions? Ring us on Freephone 0800 262 872

QUESTIONS ABOUT YOU AND YOUR HEALTH

Please answer every question as best you can as all the information that you give us is very useful. If you are not sure about exact dates or ages an approximate answer is better than none. Please use a black pen, if possible.

1. In the last 5 years has a doctor told you that you have had any of the following conditions?

*If **YES** please cross the box ☒ and write your age when the condition was first diagnosed (eg. 57 (age))*

Yes			Yes			Yes	
☐ High blood pressure	____ (age)		☐ Asthma	____ (age)		☐ Gallstones/gall bladder problems	____ (age)
☐ High blood cholesterol	____ (age)		☐ Osteoporosis	____ (age)		☐ Blood clot in leg	____ (age)
☐ Diabetes	____ (age)		☐ Thyroid problem	____ (age)		☐ Blood clot elsewhere (please describe below)	____ (age)
☐ Heart problem (please describe below)	____ (age)		☐ Breast cancer	____ (age)		☐ Inflammatory bowel disease (please describe below)	____ (age)
☐ Stroke/TIA (please describe below)	____ (age)		☐ Other cancer (please describe below)	____ (age)		☐ Any other serious illness (please describe below)	____ (age)

Please give as many details as possible about any illness mentioned above

2. Are you NOW being treated for: *If YES please cross the box ☒ and write your age when the condition was first treated (eg. 60 (age))*

Yes			Yes			Yes	
☐ High blood pressure	____ (age)		☐ Asthma	____ (age)		☐ Osteoporosis	____ (age)
☐ Diabetes	____ (age)		☐ Rheumatoid arthritis	____ (age)		☐ Depression/anxiety	____ (age)
☐ Heart problem (please describe below)	____ (age)		☐ Osteoarthritis	____ (age)		☐ Other serious illness or disability (please describe below)	____ (age)

Please give further details of any serious illness or disability you are now being treated for

3. Have you had any broken/fractured bones in the last 5 years? *(please cross)* ☐ No ☐ Yes

If Yes, which bones were broken? ☐ wrist ☐ arm ☐ ankle ☐ hip ☐ spine ☐ other _____ (please describe)

If Yes, how did the fracture occur? ☐ after a fall ☐ in a car accident ☐ some other way _____ (please describe)

If Yes, when did it occur? ☐☐ month ☐☐☐☐ year *(give month/year of the most recent fracture, if you have had more than one)*

4. Have you had any major operations in the last 5 years? ☐ No ☐ Yes - ***If Yes***, when? month ☐☐ year ☐☐☐☐

*If **Yes**, please describe the operation and why it was done. (If you have had more than one operation please give the dates and details of each)*

5. When did you last go for breast screening? ☐☐ month ☐☐☐☐ year

6. Have you ever had a cervical smear test? ☐ No ☐ Yes - ***If Yes***, when was your last test? month ☐☐ year ☐☐☐☐

About how many cervical smear tests have you had in the last 10 years? ☐ number of tests

Were you told that any of the cervical smear tests (in the last 10 years) were abnormal? ☐ No ☐ Yes

7. How would you describe your health now? ☐ excellent ☐ good ☐ fair ☐ poor

QUESTIONS ABOUT MEDICATIONS

8. Have you ever used H R T (hormone replacement therapy)? ☐ No - ***if No*** - *please go to question 13*
☐ Yes

9. How old were you when you *first started* using HRT? ☐☐ years old

10. Are you *now* using HRT?
☐ No - ***if No,*** when did you stop using HRT? ☐☐ month ☐☐☐☐ year
☐ Yes

11. For about how many years *in total* have you used HRT? ☐☐ years of use
(Add together all the years and months when you were using HRT. Please write "0" if you used HRT for less than a year in total)

12. Which HRT did you use MOST RECENTLY?

☐ Prempak C 0.625mg	☐ Premarin 0.625mg	
☐ Prempak C 1.25mg	☐ Premarin 1.25mg	
☐ Tridestra	☐ Evorel 25mcg/50mcg	
☐ Trisequens	☐ Evorel 75mcg/100mcg	
☐ Cycloprogynova 1mg	☐ Progynova 1mg	
☐ Cycloprogynova 2mg	☐ Progynova 2mg	
☐ Estrapak	☐ Estraderm 25mcg	
☐ Estracombi	☐ Estraderm 50mcg	
☐ Climaval 1mg	☐ Estraderm 100mcg	
☐ Climaval 2mg	☐ Zumenon 1mg	
☐ Premique Cycle	☐ Zumenon 2mg	
☐ Premique	☐ Ethinyloestradiol	
☐ Nuvelle	☐ Oestrogel	☐ Micronor
☐ Kliofem	☐ Implants	☐ Duphaston
☐ Livial	☐ Provera	☐ Do not know

Other (please write here)

13. Have you taken any medications (other than HRT) for most of the last 4 weeks? ☐ No ☐ Yes

If Yes, was it:

☐ thyroxine	☐ ibuprofen	☐ aspirin	
☐ tamoxifen	☐ bendrofluazide	☐ amlodipine *Istin*	☐ digoxin
☐ paracetamol	☐ propranolol	☐ atenolol	☐ warfarin
☐ prednisolone	☐ Losec/Zoton	☐ Prozac	☐ insulin
☐ co-proxamol *Distalgesic*	☐ amitriptyline *Tryptizol etc*	☐ sleeping pills	☐ lithium

Please give the name(s) of any other medication you have used for most of the last 4 weeks:

14. Do you regularly take any vitamins, minerals or supplements? ☐ No ☐ Yes - ***if Yes,*** do you take:

☐ multivitamins (with minerals)		☐ vitamin A
☐ multivitamins (without minerals)		☐ vitamin B (including B_6, B_{12})
☐ fish oil (including cod liver oil)		☐ vitamin C
☐ evening primrose oil	☐ garlic	☐ vitamin D
☐ iron ☐ zinc	☐ calcium	☐ vitamin E

☐ other *(please describe)*_____

QUESTIONS ABOUT YOUR DIET

We know it may be difficult for you to give exact answers to these questions about your diet. An approximate answer is very valuable for this study. So, please answer as best you can, thinking of a typical week.

15. Which types of meat do you eat about once a week or more often? *(you can cross more than one box)*

☐ beef	☐ bacon	☐ chicken/poultry
☐ lamb	☐ ham	☐ kidney
☐ pork	☐ sausages	☐ liver/pâté
☐ beefburger/hamburger		☐ never eat meat

16. Which types of fish do you eat about once a week or more often? *(you can cross more than one box)*

☐ tuna	☐ sardines	☐ trout	☐ "fish & chips"
☐ salmon	☐ kippers/herring		☐ other seafood *(prawns, scampi etc)*
☐ cod/haddock or other white fish	☐ mackerel		☐ never eat fish

17. About how many times each week do you eat:
(please count all meals and snacks. put '0' if never eaten or eaten less than once a week)

meat	☐☐	number of times eaten each week *(remember meat in sandwiches)*
fish/ seafood	☐☐	number of times eaten each week
chips	☐☐	number of times eaten each week
potatoes (except chips)	☐☐	number of times eaten each week
pasta/ spaghetti	☐☐	number of times eaten each week
rice	☐☐	number of times eaten each week
cheese	☐☐	number of times eaten each week *(remember cheese in pizzas, quiches, cheese sauce, etc)*

18. About how many eggs do you eat each week?
eggs ☐☐ number of eggs eaten each week *(remember eggs in omelettes, quiches, cakes etc. put '0' if less than one)*

19. Which types of vegetables/salads (fresh, frozen or tinned) do you eat once a week or more often?
(you can cross more than one box)

☐ green peas	☐ tomatoes	☐ green beans
☐ broccoli	☐ onions	☐ baked beans
☐ cabbage	☐ garlic	☐ soya meat/tofu
☐ carrots	☐ swede	☐ chick peas/lentils
☐ courgettes	☐ spinach	☐ cauliflower
☐ beetroot	☐ sweetcorn	☐ green/red peppers
☐ leeks	☐ avocado	☐ brussels sprouts
☐ parsnip	☐ aubergine	☐ mushrooms
☐ lettuce	☐ celery	☐ cucumber

20. About how much do you eat each week of:
(put "0" if less than one)

cooked vegetables (except potatoes)	☐☐	number of heaped tablespoons each week
salad items/ raw vegetables	☐☐	number of heaped tablespoons each week *(please count lettuce, tomato etc in sandwiches)*

21. Which types of fruit do you eat once a week or more often, when in season? *(you can cross more than one box)*

☐ apples	☐ bananas	☐ oranges, satsumas, etc
☐ grapefruit	☐ pears	☐ stone fruit *(peaches, plums, nectarines etc)*

22. About how much fruit or fruit juice do you eat or drink each week? *(count 10 grapes, berries or raisins as one piece; put "0" if less than one a week)*

☐☐ number of pieces of **fresh fruit** eaten each week	☐☐ number of pieces of **dried fruit** eaten each week
☐☐ number of glasses of **fruit juice** each week	☐☐ number of tablespoons of **stewed or tinned fruit** eaten each week

MORE ABOUT YOUR DIET

23. About how many of the following do you eat: *(put "0" if none or less than one)*

slices/pieces of white bread	☐	slices each week
slices/pieces of brown/wholemeal bread *(also include granary, rye bread etc)*	☐	slices each week
crackers, crispbread etc *(ryvita, wafer biscuits etc)*	☐	number each week
sweet biscuits	☐	number each week
dairy desserts (yoghurts etc)	☐	number each week
cakes, puddings, pies, buns etc	☐	number each week
chocolate (in any food or drink)	☐	approx. number of pieces each week
nuts (including peanut butter)	☐	tablespoons each week
soup	☐	bowls/cups each week
gravy, cream/cheese sauces etc	☐	tablespoons each week
breakfast type cereal	☐	bowls each week

If you eat breakfast cereal is it usually: *(please cross)*

☐ bran cereal *(allbran, branflakes etc)* ☐ muesli
☐ biscuit cereal *(weetabix, shreddies etc)* ☐ other *(e.g. cornflakes, rice crispies etc)*
☐ oat cereal *(porridge, ready brek etc)*

24. Which type of spread do you use on bread, crispbreads etc, once a week or more often? *(you can cross more than one box)*

☐ butter ☐ margarine ☐ soft cheese
☐ low fat spread ☐ mayonnaise ☐ salad cream
☐ olive oil spread ☐ marmite etc ☐ rarely use spread

Do you spread it: ☐ thick? ☐ medium? ☐ thin? *(please cross)*

Do you add butter etc to: ☐ potatoes? ☐ other vegetables?

25. Which types of fats or oils do you use for cooking or salad dressing once a week or more often? *(you can cross more than one box)*

☐ butter ☐ soft (tub) margarine ☐ white flora
☐ olive oil ☐ hard (block) margarine ☐ lard/dripping
☐ corn oil ☐ sunflower oil ☐ mayonnaise
☐ soya oil ☐ other vegetable oil ☐ salad cream

Please put a cross in the box if you RARELY OR NEVER:

☐ use fats or oils for cooking ☐ use salad dressing/cream

26. Please put a cross in the box if you NEVER eat:

☐ beef ☐ pork/ham ☐ lamb ☐ dairy products
☐ kidney ☐ liver/pâté ☐ sugar ☐ wheat products
☐ salami ☐ sausages ☐ eggs ☐ beefburgers

27. Which type of milk or cream do you drink or use once a week or more often? *(you can cross more than one box)*

milk: ☐ full cream ☐ semi-skimmed ☐ skimmed ☐ soya
cream: ☐ single ☐ double ☐ other
other: ☐ dairy ice cream ☐ never have milk/cream

28. Do you:

	never	some-times	usually	always
add milk to your tea?	☐	☐	☐	☐
add milk to your coffee?	☐	☐	☐	☐
add salt to your food?	☐	☐	☐	☐
remove fat from meat? *(cross "never" if vegetarian)*	☐	☐	☐	☐
eat organic food?	☐	☐	☐	☐

29. Have you made any major changes to your diet in the last 5 years? ☐ No ☐ Yes-because of illness ☐ Yes-for some other reason

30. About how much alcohol do you drink each week?

☐ number of drinks of alcohol each week
one drink = a glass of wine, half pint of lager, or tot of spirits
(put "0" if you do not drink, or have less than one drink each week)

If you have more than one drink of alcohol each week:

is it usually with meals? ☐ No ☐ Yes ☐ it varies

on how many days each week do you usually drink? ☐ days each week

31. About how much do you drink EACH DAY of:

tea? ☐ *cups daily* milk? *(include hot chocolate etc)* ☐ *cups daily* fizzy/soft drink? ☐ *glasses daily*
coffee? ☐ *cups daily* water? ☐ *glasses daily* fruit squash? ☐ *glasses daily*

32. How many teaspoons of sugar do you add to tea, coffee, cereal, fruit etc EACH DAY? ☐ teaspoons of sugar each day

33. What size clothes do you wear now? *(you can cross more than one box if the size varies)*

Clothes	☐ 10 or less	☐ 12	☐ 14	☐ 16	☐ 18	☐ 20+
Bra	☐ 32	☐ 34	☐ 36	☐ 38	☐ 40	☐ 42+
Cup	☐ AA	☐ B	☐ C	☐ D	☐ DD/E +	

34. What is your: *(please put "0" if you do not know)*

waist measurement? ☐☐ inches hip measurement? ☐☐ inches

35. About how much do you weigh now? ☐ stone ☐ lbs *(Put "0" if you do not know)*

36. About how many hours each week do you spend doing:

housework? *(include cooking, cleaning etc)* ☐ hours per week

	summer	winter	
gardening?	☐	☐	hours per week
walking?	☐	☐	hours per week
cycling?	☐	☐	hours per week
any work or exercise causing sweating or a fast heartbeat?	☐	☐	hours per week

WHEN YOU WERE YOUNG

37. About how much did you weigh when you were born? ☐ lbs ☐ ozs *(Put "0" if you do not know)*

38. Were you breastfed when you were a baby?

☐ No ☐ Yes ☐ do not know

39. Did your parents smoke:

at around the time that you were born?

Mother ☐ No ☐ Yes ☐ do not know
Father ☐ No ☐ Yes ☐ do not know

at around the time that you were 10 years old?

Mother ☐ No ☐ Yes ☐ do not know
Father ☐ No ☐ Yes ☐ do not know

40. When you were about 10 years old, compared to average, would you describe yourself as *(please cross)*:

☐ thinner? ☐ plumper? ☐ about average?

41. What size clothes did you wear when you were about 20 years old? *(you can cross more than one box)*

☐ 8 or less ☐ 10 ☐ 12 ☐ 14 ☐ 16 ☐ 18 +

QUESTIONS ABOUT YOUR FAMILY AND LIFESTYLE

42. Is your mother still alive?

- ☐ Yes-please give her age now ☐☐ years old
- ☐ No-please give her age when she died ☐☐ years old
- ☐ Do not know

43. If your mother has died, what did she die from?

- ☐ heart disease *heart attack etc*
- ☐ stroke
- ☐ chest infection *pneumonia*
- ☐ "old age"
- ☐ breast cancer
- ☐ cancer of the womb
- ☐ cancer of ovary
- ☐ other/unknown_____

44. Has your mother or father ever suffered from:

mother	father		mother	father	
☐	☐	heart disease	☐	☐	breast cancer
☐	☐	stroke	☐	☐	bowel cancer
☐	☐	high blood pressure	☐	☐	lung cancer
☐	☐	diabetes		☐	prostate cancer
☐	☐	Alzheimer's disease	☐	☐	osteoporosis
☐	☐	Parkinson's disease	☐	☐	hip fracture
☐	☐	severe depression	☐	☐	severe arthritis

other*(mother)*_____ other*(father)*_____

45. How tall is/was your mother? *(Put "0" if you do not know)* ☐☐ feet ☐☐ inches

How tall is/was your father? ☐☐ feet ☐☐ inches

46. Have you ever been a smoker?

- ☐ No - ***if No*** - *please go to question 50*
- ☐ Yes

47. How old were you when you started smoking regularly? ☐☐ years old

48. Are you a smoker now?

- ☐ No - ***if No*** - how old were you when you stopped smoking? ☐☐ years old
- ☐ Yes -***if Yes*** - please write the tar & nicotine content of your usual brand of cigarettes:

(this is written on each packet of cigarettes) tar ☐☐☐ mg nicotine ☐☐☐ mg

49. About how many cigarettes do you/*did you* smoke on average *each day*? *(If you are an ex-smoker, how many did you smoke on average when you smoked?)*

☐☐ cigarettes per day

50. Have you had your menopause?

- ☐ No ☐ Not sure *(because periods irregular, taking HRT etc)*
- ☐ Yes- How old were you when you had your menopause? ☐☐ years old

51. Are you now in paid work?

- ☐ No ☐ Yes, full time ☐ Yes, part time

If Yes, does your work involve physical effort? ☐ No ☐ Yes

At work, do you mostly ☐ stand? ☐ sit? ☐ both

52. Are you currently married or living with a partner?

- ☐ No ☐ Yes- ***If Yes-*** does your husband/partner smoke? ☐ No ☐ Yes

53. About how often do you use a mobile phone?

- ☐ Never ☐ less than once a day ☐ every day

For how long have you used one? *(put "0" if never or less than 1 year)* ☐☐ years

54. Do you belong to or participate in any of the following?

- ☐ religious group ☐ art/craft group ☐ bingo
- ☐ voluntary work ☐ music/singing group
- ☐ adult education ☐ sports club (swimming,golf etc)
- ☐ dancing group ☐ yoga,etc ☐ other group activity

55. How often do you feel:

	rarely/ never	some-times	usually	most of the time
happy	☐	☐	☐	☐
relaxed	☐	☐	☐	☐
in control	☐	☐	☐	☐
stressed	☐	☐	☐	☐
tired	☐	☐	☐	☐

56. Do you have a nap during the day?

- ☐ rarely/never ☐ sometimes ☐ usually

57. About how many hours sleep do you get in every 24 hours? ☐☐ hours sleep *(please include naps)*

58. To which ethnic group do you consider you belong?

- ☐ White ☐ Black - Caribbean, African etc.
- ☐ Asian ☐ Other -*please specify*_____

59. What is your date of birth? day ☐☐ month ☐☐ year ☐☐☐☐

60. On what date did you fill in this form? ☐☐ ☐☐ ☐☐☐☐

61. In case we need to check on any details, it would be helpful if you would write your telephone number below.

☐☐☐☐☐☐☐☐☐☐☐☐☐☐

STD code Telephone number

THANK YOU VERY MUCH FOR YOUR HELP

Please put your completed questionnaire in the pre-paid envelope and post it back to us

If your name /address has changed or is incorrect could you please cross this box ☐ & give the correct details below.

Surname: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Given name(s): ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

House number and street: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

District: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Town/County: ☐☐☐☐☐☐☐☐☐☐ Postcode: ☐☐☐☐☐☐☐☐

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Appendix C Publications

C.1 Lifestyle and reproductive risk factors associated with anal cancer in women aged over 50 years, British Journal of Cancer

FULL PAPER

BJC

British Journal of Cancer (2015), 1–7 | doi: 10.1038/bjc.2015.89

Keywords: anal cancer; human papillomavirus; Million Women Study; epidemiology

Lifestyle and reproductive risk factors associated with anal cancer in women aged over 50 years

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Background: Anal cancer incidence increases with age and is higher in women than men. Risk factors in this group other than high-risk human papillomavirus infection are unclear.

Methods: In all, 1.3 million women were recruited in 1996–2001 and followed for incident anal cancer. Cox regression models were used to calculate relative risks (RRs) for anal cancer by various potential risk factors.

Results: Five hundred and seventeen incident anal cancers were registered over 13 years of follow-up. The largest RR was associated with a history of cervical intraepithelial neoplasia grade 3 (CIN 3; RR = 4.03, 95% CI 2.59–6.28). Other factors associated with significantly increased risks in multivariate analyses were: ever smoking (RR = 1.49, 1.24–1.80); previous use of oral contraceptives (RR = 1.51, 1.24–1.83); nulliparity (RR = 1.61, 1.24–2.07); tubal ligation (RR = 1.39, 1.13–1.70) and not living with a partner (RR = 1.82, 1.40–2.38). The association with smoking was significantly greater for squamous cell carcinoma than adenocarcinoma of the anus (RR 1.66 *vs* 0.89, *P* for heterogeneity = 0.04).

Conclusions: History of CIN 3, smoking, past oral contraceptive use, nulliparity, tubal ligation and not living with a partner are risk factors for anal cancer in women. There was a significant increase in risk associated with smoking for squamous cell anal cancers but not adenocarcinomas.

In the past 20 years there has been more than a doubling in incidence of female squamous cell anal cancer registrations in the UK, the rise being most marked in middle-aged and older women (Wilkinson *et al*, 2014). Incidence rates increase rapidly with age, and women in England have higher rates of anal cancer than men (see Figure 1, based on data from the Office of National Statistics, 2014). There are very few cases of anal cancer before age 40; in England in 2012 64% (665) of the 1043 registered anal cancer cases occurred in women and 88% (584) of the female cases were in women aged over 50 years.

High-risk human papillomavirus (HrHPV) infections, which cause the majority of cases of cervical cancer (Jacobs *et al*, 1999), are also responsible for between 80 and 90% of anal cancers (International Agency for Research on Cancer, 2005; Steinau *et al*, 2013). Similarly, HPV 16 and 18 appear to be the commonest HPV types associated with anal tumours (Steinau *et al*, 2013). Despite the evidence of a strong association between high-risk strains of the HPV virus and several cancers, including anal cancer, HrHPV oncogenesis is puzzling; the majority of sexually active women are exposed to HPV infections during their lifetimes (Baseman and Koutsky, 2005), but the anogenital cancers they cause remain relatively rare. Identification of other risk factors that have a role in HPV-related anogenital carcinogenesis is clearly important if we wish to identify individuals with an increased risk of developing an anal malignancy.

Several high-risk populations for anal cancer are already known, including human immunodeficiency virus (HIV)-positive individuals (Silverberg *et al*, 2012), organ transplant recipients (Madeleine *et al*, 2013), men who have sex with men (MSM) (Machalek *et al*, 2012), and women with a history of preinvasive or

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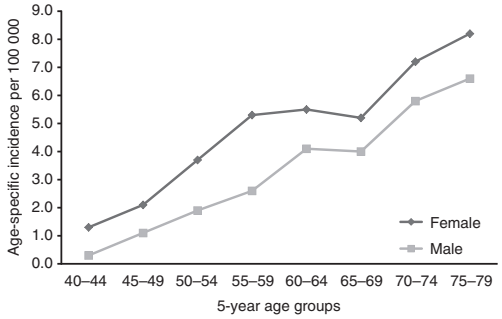

Figure 1. Age-specific rates of anal cancer per 100 000 population, England 2012 (ONS, 2014).

invasive cervical or vulval lesions (Ouhoummane *et al*, 2013). Most published research has focused on these groups, particularly HIV-positive MSM (Stanley *et al*, 2012). The literature on the role of anal HPV infection in non-HIV infected individuals has also mostly examined risk in men, with twice as many studies involving males than females (Giuliano *et al*, 2014). Although older women account for a large proportion of those developing anal cancer, they have not been the focus of previous large-scale epidemiological research.

Other than HrHPV infection, risk factors for anal cancer in women, particularly older women who have the highest incidence of anal cancer, are unclear.

Our aim is to examine reproductive, lifestyle, hormonal, and other risk factors for incident anal cancers in women aged over 50 years in a large cohort of UK women.

MATERIALS AND METHODS

The Million Women Study is a population-based prospective study, which recruited women via the National Health Service Breast Screening Programme from 1996 to 2001. The study was established to examine the association between menopausal hormone therapy and breast cancer, and to investigate associations between lifestyle, reproductive and hormonal risk factors, and other health outcomes (The Million Women Study Collaborative Group, 1999).

Women were invited to participate in the study via a postal questionnaire, which was distributed with their usual breast-screening invitation. Full details of the study design and methods are described in detail elsewhere (Beral and The Million Women Study Collaborative Group, 2003). About 1.3 million women returned a completed questionnaire. Participants gave written consent for use of their questionnaire data for research, and for ongoing linkage to nationally held registry and health data. The Study has Multi-Centre Research Ethics Committee approval (97/01).

The main analysis excluded participants if they had breast or any other invasive cancer registered prior to recruitment, with the exception of non-melanoma skin cancer (International Classification of Diseases, 10th revision; ICD-10 C44). Self-reported data from the recruitment and subsequent resurvey questionnaire were used to define most exposures; exposures were not updated. Deprivation index was calculated using post-code and 1991 census data (Townsend, 1987).

Million Women Study participants are flagged on the UK National Health Service Central Registers, which provide the study team with regularly updated information on incident cancers and deaths. Cases of incident anal cancer are those coded as C21 'malignant neoplasm of anus and anal canal' according to the (ICD-10) (World Health Organization, 2011). Cervical intra-epithelial neoplasia grade 3 (CIN 3) is routinely captured by the UK cancer registry as an *in situ* cancer: CIN 3 registrations (ICD-10 D06) that occurred prior to recruitment were also identified from the cancer registry data.

Risk factors with sufficient numbers of cases in exposed and unexposed women were also examined separately for the two most common histological subtypes of anal cancer: squamous cell carcinoma and adenocarcinoma. These were identified using ICDO-3 (ICD for Oncology version 3) morphology codes. Squamous tumours included the following ICDO-3 codes: M80810/3, M8070/3, M8071/3, M8072/3, M8083/3, M8094/3, M8123/3, and M8124/3. Adenocarcinomas included codes M8140/3, M8210/3, M8263/3, M8480/3, and M8481/3.

Statistical analyses. Cox proportional hazards models were used to examine the relationship between exposures and subsequent risk of anal cancer. Hazard ratios, described here as relative risks (RRs), were estimated with attained age as the underlying time variable. Participants entered the analysis from the date they reported the relevant exposure (which was the date they filled in the questionnaire, either at recruitment or subsequent resurvey). The small number of women who were under the age of 50 at recruitment (3%) entered the analysis at age 50. Women were followed to the earliest of date of first cancer registration, death, emigration, or 31 December 2012, the date of the most recent ONS follow-up.

Analyses were stratified by region of residence (10 geographical areas which correspond to the cancer registry regions: Oxford, East Anglia, South West, Thames, West Midlands, Northern & Yorkshire, Trent, North West (Mersey), North West (Manchester/Lancashire), Scotland) and adjusted where appropriate for previous registration of CIN 3 (yes/no); smoking (never *vs* ever); oral contraceptive pill use (never *vs* ever); parity (parous *vs* nulliparous); tubal ligation (no/yes); deprivation (quintile of Townsend Deprivation Index); age at menarche ($\leqslant$12, 13, 14+); alcohol use (0–2 units per week, 3–7 units per week, 8+ units per week); and body mass index ($<$25, 25–30, 30+). Where smoking and oral contraceptive pill use were examined as exposures, responses were categorised by amount smoked: smoking (never, past, current $<$15 cigarettes per day, current $>$15 cigarettes per day); and duration of use oral contraceptive pill use (none, $<$5 years, 5+ years).

Age at menopause (50+, 45–49, $<$45) and hormone therapy use (never/ever) were examined as exposures in postmenopausal women only; the association with age at menopause was further restricted to naturally postmenopausal women or those reporting a bilateral oophorectomy, who had never used hormone therapy. Apart from CIN 3 registration and deprivation quintile, these exposures were all based on self-reported questionnaire data collected from participants at baseline.

We also examined the impact of being married or living with a partner (yes/no), and of self-reported attendance for cervical cancer screening (ever/never) in fully adjusted models. These exposures were asked about on the first resurvey, which was sent out on average 3 years after recruitment.

The small number of women with missing data (fewer than 2% for each variable) were included in a separate category for each variable of interest and included in the analysis but these data are not shown. Relative risks are reported with 95% confidence intervals. Analyses were performed in Stata 13 (StataCorp, 2013).

We used a competing hazards model to examine the interaction between anal cancer histological subtype and past CIN 3, smoking, and oral contraceptive use.

RESULTS

In all, 1.3 million women were followed for 16.9 million person-years, giving an average of 13 years of follow-up per woman. During this time, 517 were registered with incident anal cancer. Table 1 shows participant characteristics at baseline. Mean age at recruitment was 56.1 (s.d. 4.9); 46% reported ever having smoked, with 19% currently smoking at recruitment; 59% reported ever using oral contraceptives, and 33% reported >5 years of use. Most women had given birth at least once, with 11% reporting being nulliparous.

About 1% (12 531) of the women in the cohort had a registration of CIN 3 prior to recruitment, and this was the strongest predictor of risk of anal cancer after the age of 50 (RR = 4.03, 95% CI 2.59–6.28, $P<0.0001$), after adjustment for smoking, alcohol use, BMI, age at menarche, ever use of the oral contraceptive pill, parity, tubal ligation, and deprivation (Figure 2).

Ever having smoked was associated with a 50% increase in risk of subsequent anal cancer, with a RR of 1.49 (95% CI 1.24–1.80; $P<0.0001$) compared with never smokers. Current smokers who reported smoking >15 cigarettes per day at recruitment had more than a doubling of risk (RR = 2.16, 1.65–2.83; $P<0.0001$) (Figure 2). No significant increase in risk was seen with alcohol use, adiposity, age at menarche, or deprivation.

Past use of the oral contraceptive pill was significantly associated with anal cancer risk (RR = 1.51, 1.24–1.83; $P<0.0001$) for ever *vs* never use. When stratified by years of use, women who reported 5 or more years of oral contraceptive use had a significantly increased risk of anal cancer, RR = 1.68 (95% CI 1.37–2.07; $P<0.0001$) (see Figure 2).

Overall, nulliparous women had a 60% greater risk of anal cancer when compared with parous women, with a RR of 1.61 (95% CI 1.24–2.07; $P<0.0001$). Women who reported ever having given birth had a lower risk of developing anal cancer, irrespective of the number of children they had, with a RR of 0.58 (95% CI 0.44–0.75; $P<0.0001$) for those reporting 1–2 children, and a RR of 0.69 (95% CI 0.52–0.91; $P<0.0001$) for women with 3+ children, compared with the nulliparous group.

Female sterilisation was also associated with an increased risk of anal cancer; women who reported having had a bilateral tubal ligation were significantly more likely to develop an anal cancer, with a RR of 1.39 (95% CI 1.13–1.70; $P<0.0001$). Neither age at menopause nor use of hormone therapy was significantly associated with anal cancer risk (Table 2).

Table 1. Participant characteristics at recruitment

Number of women	1 300 101	
Age in years—mean (s.d.)	**56.1**	**4.9**
	N	**%**
Body mass index >30	220 986	17.0
Ever used oral contraceptives	761 728	58.6
Ever smokers	596 284	45.9
Parous	1 157 243	89.0
Three or more children	427 331	32.9
History of tubal ligation	289 634	22.3
Hysterectomy	316 944	24.4
Postmenopausal, never HT user	424 872	32.7
Ever used hormone therapy	644 831	49.6
Most deprived quintile	255 619	19.7
Prior cervical intraepithelial neoplasia grade 3 registration	12 531	1.0

Abbreviation: HT = hormone therapy.

At resurvey, on average 3 years after recruitment, women were asked whether they were currently married or living with a partner. About a fifth (19%) reported not living with a husband or partner, and their risk of anal cancer was increased (RR of 1.82, 1.40–2.38; $P<0.0001$) (Table 3). Only 4% reported never having had a previous cervical screening (smear) test. The risk of anal cancer was not significantly different in women who had and had not been screened (Table 3).

We also compared associations for squamous cell cancers (SCC) *vs* adenocarcinomas of the anus (Table 4). The majority of the anal cancers registered in the cohort ($n=425$, 82%) were SCCs. Glandular tumours were less common, with 62 adenocarcinomas (12%) registered. The remaining 27 (5%) were rarer histological subtypes, including anal melanoma; this group had insufficient numbers to examine separately.

The RR of adenocarcinoma of the anus in women who reported any lifetime smoking was 0.89 (95% CI 0.51–1.54), which was significantly different (P for heterogeneity = 0.04) from the risk in women with squamous cell tumours (RR 1.66, 95% CI 1.35–2.05). In contrast, the association between past CIN 3 and oral contraceptive use were similar in the two subtypes. We were not able to examine all factors by histological subtype; the other exposures that showed a positive association with anal cancer (tubal ligation, parity, and not living with a husband or partner) had an insufficient number of cases of adenocarcinoma to permit valid subgroup analysis.

DISCUSSION

We examined lifestyle, reproductive, and other risk factors for incident anal cancer in a large prospective study of UK women aged over 50 years. Factors significantly associated with an increased risk of anal cancer were: prior high grade cervical disease (CIN 3); ever having smoked, with a doubling of risk in current smokers of >15 cigarettes a day; ever having used the oral contraceptive pill; nulliparity; a history of tubal ligation; and not currently living with a husband or partner. Among smokers, the risk of squamous cell anal carcinoma differed significantly from the risk of adenocarcinoma, with an elevated risk of squamous cell tumours but not of adenocarcinoma.

The strongest association with risk of anal cancer in our study was in the 1% of women who had a registration of CIN 3 before they were recruited. Women with HrHPV-associated neoplasia in one part of the anogenital tract are known to be more likely to have it elsewhere (Crawford *et al*, 2011). After cervical cancer, anal cancer has the closest association with HrHPV infection (Grulich *et al*, 2012), with an estimated 80–90% of anal cancers caused by oncogenic papillomaviruses (International Agency for Research on Cancer, 2005; Steinau *et al,* 2013; Stewart and Wild, 2014).

Anal cancer, and other HPV-related anogenital cancers are increased in women with a history of anogenital warts (Blomberg *et al,* 2012), cervical dysplasia (Kalliala *et al*, 2005; Edgren and Sparén, 2007), and previous anogenital malignancy at another site (Frisch *et al*, 1994; Jiménez *et al*, 2009; Saleem *et al*, 2011). In the United Kingdom, CIN 3 but not other grades of cervical dysplasia are registered, so we were not able to examine the risk associated with any other grade of CIN. There are no data on the completeness of CIN 3 registration. Cases are notified to the cancer registries by pathology laboratories, and it is possible that some cases may have been missed.

Though smoking has been found to be a risk factor for anal cancer in some other studies (Daling *et al* 1987, 1992, 2004; Frisch *et al*, 1999), not all have reported such an association (Ouhoummane *et al,* 2013). A recent Brazilian retrospective study reported a doubling of anal cancer risk in women associated with

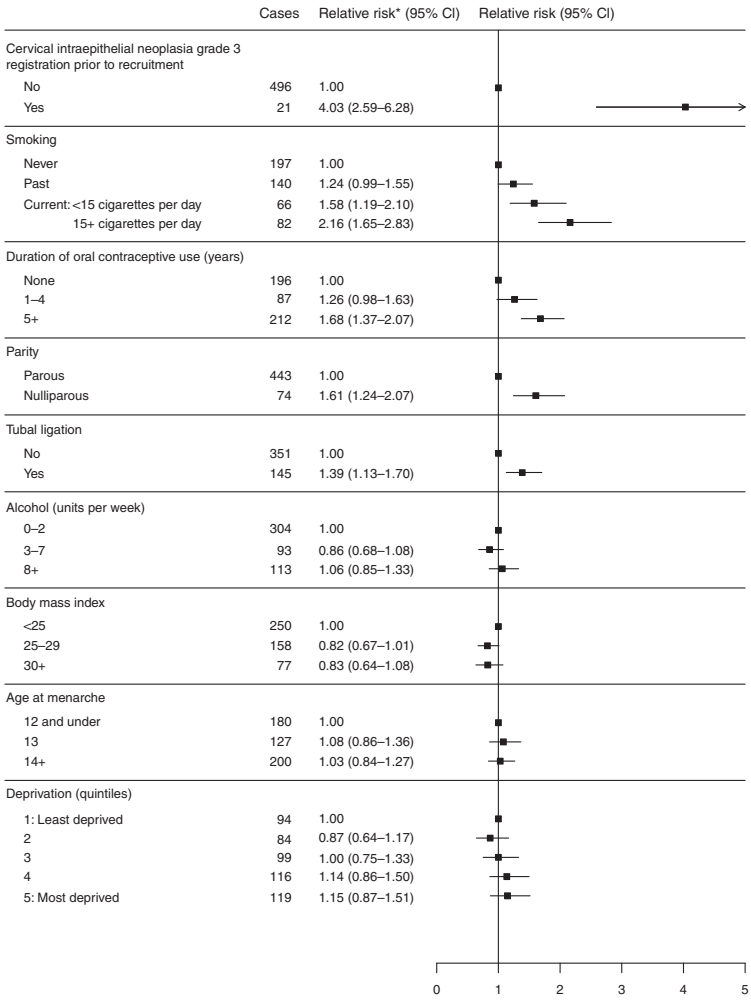

Figure 2. Association between anal cancer and various lifestyle and other factors. *Estimates of relative risk are adjusted by age, region of residence, and all other factors shown above, where appropriate.

Table 2. Association between anal cancer and risk factors associated with menopause

Exposure	Cases/population at risk	RR	95% confidence interval
Age at menopause in never users of hormone therapy in women with natural menopause or bilateral oophorectomy			
50+	80/237 157	1.00	—
45–49	52/126 008	1.18	0.83–1.68
<45	18/49 489	0.95	0.45–1.59
Use of hormone therapy at baseline, all postmenopausal participants			
Never	189/497 518	1.00	—
Ever	276/625 025	1.19	0.98–1.44

Abbreviation: RR = relative risk. Adjusted for smoking, alcohol use, BMI, OC use, age at menarche, parity, tubal ligation, previous CIN 3, deprivation and stratified by region.

smoking, similar to our findings (Moura *et al*, 2014). Another retrospective study from Denmark and Sweden (Frisch *et al*, 1999) with 417 anal cancer patients (324 women and 93 men) also found about a doubling of risk of anal cancer in female smokers. Laboratory studies have shown increased concentrations of nicotine and other metabolites in the cervical mucus of smokers

(Sasson *et al,* 1985; Hellberg *et al,* 1988; Prokopczyk *et al*, 1997), suggesting a potential biological pathway for the increase in risk seen in cervical cancer, which may also apply to anal epithelium. This could be through direct carcinogenesis with constituents of tobacco acting as carcinogens; indirectly through suppression of the immune response to HrHPV; or through some other mechanism.

We also found that the risk associated with smoking varies significantly by tumour histology, with an increase in anal SCCs, but not in adenocarcinomas. Similar heterogeneity has previously been reported for cervical cancer—smoking was significantly related to cervical SCCs but not adenocarcinomas (International Collaboration of Epidemiological Studies of Cervical Cancer, 2006). The heterogeneity in risk by histological subtype observed here for anal cancers, and previously seen in cervical cancers, suggests that anogenital cancers arising in squamous and glandular issues may have different biological pathways. It has been suggested that the majority of cases of anal adenocarcinoma actually represent downward spread from rectal tumours (International Agency for Research on Cancer, 2009). In this study, 8% of the adenocarcinoma cases (5/62) had ICDO-3 morphology codes that are usually associated with rectal (ICD-10 C18-20) and not anal cancers (ICD-10 C21).

Past use of oral contraceptives was a clear predictor of risk of subsequent anal cancer in our study, particularly for women with longer-term use of 5 or more years. This is a little-investigated association; we found only one previous study of anal cancer risk that mentioned oral contraceptive use, and no significant association was found (Frisch *et al*, 1999). Oral contraceptives have been shown to be associated with an increased risk of cervical cancer during use and for a period of time after cessation (International Collaboration of Epidemiological Studies of Cervical Cancer, 2007). It has been suggested that sex hormones may stimulate HrHPV gene expression or exert an effect on the immune microenvironment in the cervical epithelium (Delvenne *et al*, 2007), and it is possible that a similar effect could occur in anal epithelium.

The increased risk of anal cancer in women who reported a history of tubal ligation has not, to our knowledge, previously been reported. Tubal ligation is highly correlated with parity; over 97% of the women in our cohort who reported being sterilised had given birth to at least one child. Sterilised women had more children, with 49% reporting three or more children, compared with 33% of women in the cohort overall. Given that in our analysis the risk of anal cancer was decreased in parous women, the relationship between tubal ligation and increased risk was unexpected. Adjusting for parity, age at first birth, or number of children did not affect the association.

HPV infections are sexually transmitted. The risk of anal cancer and its precursor lesions have been shown to be closely related to sexual behaviour in both women and men, including number of partners and sexual practices (Holly *et al,* 2001; Tseng *et al* 2003; Grulich *et al*, 2012). A limitation of our study is that we did not collect information on sexual behaviour from our participants. We examined current marital status and co-habitation, and found that women who reported not currently being married or living with a partner had an increased risk of subsequent anal cancer.

Given that HPV is sexually transmitted, this might seem counter-intuitive; however, most women aged over 50 years who are not married or living with a partner are widowed, separated, or divorced (Floud *et al*, 2014). Being separated or divorced has been reported to be associated with an increased risk of anal cancer in both men and women; Peters and Mack (1983) found a doubling of risk for squamous cell anal cancers in separated or divorced women. Cervical cancer is also more common in separated and divorced than in currently married women (Beral, 1974), and it seems probable that differences in past sexual behaviour account for the findings related to marital status at older ages.

In a large cohort of over a million women with prospectively collected information on exposures, with an average 13 years of follow-up, we found that prior CIN 3, smoking, past use of the oral contraceptive pill, nulliparity, tubal ligation, and not currently living with a husband or partner were risk factors for incident anal cancer in women over 50 years of age. Anal cancer has the closest relationship to HrHPV after cervical cancer (Grulich *et al*, 2012), and it is possible that the risk factors we have identified are also associated with the risk of HPV acquisition, and/or a decreased clearance of existing infection, leading to the increased risk of anal cancer seen in association with certain exposures, or that some of these exposures themselves increase risk of anal cancer.

Table 3. Association between living with a partner and participation in cervical screening on incidence of anal cancer in respondents to resurvey 3 years after recruitment

Exposure	Cases/population at risk	RR	95% confidence interval
Married or living with a partner			
Yes	178/638 188	1.00	—
No	88/153 782	1.82	1.40–2.38
At least one prior cervical smear test			
Yes	177/558 237	1.00	—
No	7/23 997	0.84	0.39–1.81

Abbreviation: RR = relative risk. Adjusted for smoking, alcohol use, BMI, OC use, age at menarche, parity, tubal ligation, previous CIN 3, deprivation and stratified by region.

Table 4. Associations for anal cancer by tumour histology

	Squamous cell carcinoma			Adenocarcinoma			
Exposure	Cases/population at risk	RR	95% CI	Cases/population at risk	RR	95% CI	Heterogeneity by tumour type *P*-value
Past CIN 3 registration							
No	406/1 287 570	1.00	—	61/1 287 509	1.00	—	0.51
Yes	19/12 531	4.43	2.79–7.05	1/12 531	2.12	0.29–15.42	
Ever smoked							
Never	151/627 169	1.00	—	32/627 169	1.00	—	0.04
Ever	253/596 284	1.66	1.35–2.04	23/596 284	0.89	0.51–1.54	
Oral contraceptive use							
Never	138/523 851	1.00	—	24/523 851	1.00	—	0.74
Ever	282/761 728	1.52	1.23–1.89	38/761 728	1.68	0.98–2.88	

Abbreviations: CI = confidence interval; RR = relative risk. Adjusted for smoking, alcohol use, BMI, OC use, age at menarche, parity, tubal ligation, previous CIN 3, deprivation and stratified by region.

When we looked separately at the two main histological subtypes of anal cancer, we found a significant difference in risk associated with smoking for tumours with squamous cell morphology compared with adenocarcinomas. To our knowledge, this has not previously been reported in anal cancer; however, similar heterogeneity between squamous and adenocarcinomas has been reported for other cancers, including cancer of the cervix (International Collaboration of Epidemiological Studies of Cervical Cancer, 2006), and lung, where squamous cell tumours have been found to be more strongly associated with smoking (Houston *et al*, 2014).

Anal cancer, like the other anogenital cancers, has a strong relationship with high-risk strains of HPV. HrHPV infections are extremely common, but the cancers they cause are rare. We have identified several additional risk factors with strong associations to anal cancer risk in older women, particularly smoking and past oral contraceptive use.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

MILLION WOMEN STUDY COLLABORATORS

The NHS Breast Screening Centres which took part in the recruitment of participants were: Avon, Aylesbury, Barnsley, Basingstoke, Bedfordshire and Hertfordshire, Cambridge and Huntingdon, Chelmsford and Colchester, Chester, Cornwall, Crewe, Cumbria, Doncaster, Dorset, East Berkshire, East Cheshire, East Devon, East of Scotland, East Suffolk, East Sussex, Gateshead, Gloucestershire, Great Yarmouth, Hereford and Worcester, Kent, Kings Lynn, Leicestershire, Liverpool, Manchester, Milton Keynes, Newcastle, North Birmingham, North East Scotland, North Lancashire, North Middlesex, North Nottingham, North of Scotland, North Tees, North Yorkshire, Nottingham, Oxford, Portsmouth, Rotherham, Sheffield, Shropshire, Somerset, South Birmingham, South East Scotland, South East Staffordshire, South Derbyshire, South Essex, South Lancashire, South West Scotland, Surrey, Warrington Halton St Helens and Knowsley, Warwickshire Solihull and Coventry, West Berkshire, West Devon, West London, West Suffolk, West Sussex, Wiltshire, Winchester, Wirral, Wycombe.

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GYNECOLOGIC EPIDEMIOLOGY

Effects of obesity and hormone therapy on surgically-confirmed fibroids in postmenopausal women

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Abstract To examine the association between body mass index (BMI), use of menopausal hormone therapy (HT), and incidence of uterine fibroids in postmenopausal women, 610,604 postmenopausal women without prior hysterectomy or diagnosis of fibroids were followed as part of a large United Kingdom prospective cohort study. We used Cox regression models to calculate adjusted relative risks (RRs) of surgically-confirmed fibroids (defined as a hospital admission with uterine fibroids as a primary diagnosis with a related surgical procedure), in relation to BMI and use of HT. During an average of 11.4 years of follow-up, 3561 women were admitted to hospital with surgically-confirmed fibroids. Five-year incidence rates decreased with age, from 0.50 % (1 in 200 women) at age 50–54, to 0.11 % (1 in 1000 women) at age 75–79. The 5-year rate in postmenopausal women aged 50–54 was about a quarter that seen in premenopausal women of the same age (1 in 200 vs. 1 in 50). Compared with normal weight women, obese women had a RR of surgically-detected fibroids of 1.46 (95 % CI 1.33–1.59; $p < 0.0001$). HT use was associated with a RR of 2.33 (95 % CI 2.18–2.49; $p < 0.0001$) in ever versus never users. When we analysed HT use and BMI together, obese vs. normal weight never users had a RR of 2.00 (95 % CI 1.77–2.26): the highest risks were seen in women who were obese and had ever used HT, RR = 3.30 (95 % CI 2.88–3.79). Uterine fibroids continue to occur in postmenopausal women; obesity and hormone therapy use are important modifiable risk factors.

Keywords Uterine leiomyoma · Fibroids · Postmenopausal · BMI · HRT · Million Women Study

On behalf of The Million Women Study Collaborators.

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Introduction

Uterine fibroids are benign monoclonal smooth muscle tumours of the uterus [1], and are the most common pelvic tumour in women [2]. Asymptomatic in at least 50 % of cases [3], fibroids are nonetheless an important cause of morbidity and a common reason for surgery. A recent study estimated the annual cost of treating uterine fibroids in the United States at $34.4 billion dollars [4].

Prevalence rates ranging from 5 to 77 % [5] have been reported, reflecting differences in case definition and population studied. The highest estimates come from pathologic examination of hysterectomy specimens [6]. Fibroids are an indication for a large proportion of hysterectomies [7]; estimates based on pathological findings are likely to overestimate prevalence, and may preferentially identify women with specific symptoms, such as pain or bleeding [8]. Reported risk factors for uterine fibroids

include black ethnicity, family history, parity, and obesity [1, 2, 9–11].

Factors that increase exposure to oestrogen appear to increase the incidence of uterine fibroids [10]. Fibroids are not seen before puberty, occur most commonly in women of reproductive age, and are commonly said to regress after the menopause [12]. The reduction in size or resolution of fibroids in postmenopausal women [10–12] is thought to be due to the lower average levels of endogenous ovarian hormones after the menopause. However, in postmenopausal women average oestrogen levels have been found to increase with increasing body mass index (BMI) [13]. Use of exogenous hormones in the form of menopausal hormone therapy (HT) might also be expected to increase risk, although evidence for this has been relatively scarce [12].

The Million Women Study provides a unique opportunity to examine the relationship between adiposity, HT use and the incidence of uterine fibroids in postmenopausal women. This prospective cohort study collected detailed information at recruitment on participants' menopausal status, use of HT, and height and weight. There is virtually complete follow-up for cause-specific hospital admissions, incident cancers and death.

In order to clarify the public health impact of fibroids in postmenopausal women, we used a strict case definition, including only clinically relevant disease involving surgical detection and/or treatment. We aimed to determine whether postmenopausal women who were overweight or obese were more likely to have surgically-confirmed fibroids than normal weight women. We also investigated whether women who had ever used HT were at an increased risk of fibroids, and whether the use of HT modified the association with BMI.

We have used the term 'incidence rate' throughout the study, however surgically-confirmed fibroids in postmenopausal women represent a subset of true incidence; cases in our analysis are those in which uterine fibroids, or a co-morbid condition, have resulted in a surgical diagnosis of fibroids.

Materials and methods

The Million Women Study is a large prospective cohort study which recruited 1.3 million women, most aged 50–64 years, between 1996 and 2001 via the National Health Service (NHS) Breast Screening Programme. Full details of the study design and methods are described elsewhere [14]. Approximately one in two women in this age range in the recruitment areas agreed to participate in the study, or around one in four women in this age range in the entire United Kingdom. Participants gave written informed consent for use of their questionnaire data for research, and for ongoing linkage to nationally held registry and health data. The study has Multi-Centre Research Ethics Committee approval (MREC 97/01).

Information about personal characteristics including height and weight, reproductive history, medical history, family history, menopausal status, and HT use was collected at baseline. The recruitment and follow-up questionnaires can be viewed at www.millionwomenstudy.org.

Postmenopausal women were defined as those who reported having undergone a natural menopause or having had a bilateral oophorectomy prior to recruitment. Body mass index (kg/m^2) was calculated from self-reported weight and height at recruitment. This has been shown in Million Women Study participants to correlate closely with values based on measured variables [15]. Standard World Health Organization definitions [16] were used to categorise women with a BMI of <25 kg/m^2 as 'normal', 25–29.9 kg/m^2 as 'overweight', and 30 kg/m^2 or more as 'obese'. HT use was self-reported by participants at baseline. Reliability of reporting has been checked on a subset of participants and found to be excellent when compared with general practice prescription records [17].

All study participants are flagged on the NHS Central Registers, so that cancer registrations and deaths are routinely notified to study investigators. In addition, participants are linked using their name, date of birth, and NHS number (a unique personal identifier on all NHS health records) to NHS hospital admission databases, Hospital Episode Statistics (HES) in England and the Scottish Morbidity Records (SMR) in Scotland [18, 19]. The hospital records include day case and overnight stays to all NHS hospitals from April 1997 to March 31, 2011 (England); and from January 1981 to December 31, 2008 (Scotland). For each hospital admission, diagnoses are coded according to the International Classification of Diseases 10th revision (ICD-10) [20] and procedures coded according to the Office of Population Censuses Classification of Surgical Operations and Procedures (OPCS-4) [21].

The outcome of interest ('surgically-confirmed fibroids') is defined as a first hospital admission (including day-case admissions) after recruitment into the study with a primary diagnosis of uterine fibroids (ICD-10 D25) together with a related surgical procedure during the admission, limited to one (or more) of the following: dilation of cervix uteri and curettage of uterus ('D&C', OPCS-4 code Q10); diagnostic endoscopic examination of uterus (Q18); abdominal hysterectomy (Q074); vaginal hysterectomy (Q089); endoscopic resection of lesion of uterus (Q171); open myomectomy (Q092); open excision of lesion of uterus (Q093); vaginal excision of lesion of uterus (Q161); or diagnostic laparoscopy (T43).

Women who reported a natural menopause or bilateral oophorectomy at recruitment were included in the main analysis. Exclusion criteria included self-reported hysterectomy at baseline, hospital diagnosis of fibroids or record of hysterectomy prior to recruitment. Pre- or peri-menopausal women, those with indeterminate menopausal status, and those who began using HT prior to menopause were also excluded, as were those with missing information on height, weight, HT use or recruitment date.

We conducted an additional analysis looking at rates of surgically-confirmed fibroids in women who reported being pre-menopausal or peri-menopausal at recruitment, in order to compare them with postmenopausal women of the same age (50–54). These results are presented separately.

Statistical analysis

Each participant contributed person-years to the analysis from the date of recruitment until the first identified endpoint: date of hospital admission with surgically-confirmed fibroids, hysterectomy, date of death, emigration, or the end of the follow-up period. For women recruited in England follow-up was to 31 March 2011, and for women in Scotland to 31 December 2008. For the small proportion of women (5 %) recruited in England before 1 April 1997, person-years were calculated from this date. Earlier HES data, which was available in England from 1989, does not carry the individual's NHS number thus we were only able to link to records from 1997 onwards.

Multivariate Cox regression models, with attained age as the underlying time variable, were used to estimate the relative risk of surgically-confirmed fibroids associated with BMI and HT use. Analyses were stratified for recruitment region (10 geographic regions corresponding to the areas covered by the cancer registries), and adjusted for deprivation using Townsend Deprivation Index quintiles [7], smoking (never, past, current), oral contraceptive use (never/ever), parity (0/1/2/3+), BMI (<25, 25–29, 30+), and HT use (never vs. ever). Missing values were included in the analysis as a separate category for each adjustment variable. Analyses were performed using Stata version 13 (Statacorp, 2013).

Results

610,604 participants in the Million Women Study were postmenopausal, had not had a prior hysterectomy or recorded diagnosis of fibroids at recruitment, and provided information on their height, weight and HT use. 34 % were ever users of menopausal hormone therapy at baseline. 47 % were of normal weight, 36 % were overweight and 17 % were obese. Obese women were less likely to smoke, use HT, take regular vigorous exercise, or to come from the highest socioeconomic groups (Table 1).

During 7 million person-years of follow-up, on average 11.4 years per woman, 3561 postmenopausal participants were admitted to hospital with a primary diagnosis of fibroids and a related surgical intervention.

Overall, the 5-year incidence rate of surgically-confirmed fibroids in the cohort was 0.3 %, or around 1 in 300 participants. This rate fell with age (Fig. 1), from 0.50 admissions per 100 women (0.50 %, 95 % CI 0.45–0.0.55) at ages 50–54, to 0.11 (95 % CI 0.08–0.15) in women aged 75–79. Women who reported never using HT had 5-year incidence rates which increased from 0.1 % (around 1 in 1000) in normal weight women to 0.3 % (1 in 300) in obese women, a three-fold increase (Fig. 2). Higher rates were seen in all BMI groups in women who reported ever using HT, giving an overall rate of 0.4 %, (or 1 in 250) over 5 years, with a smaller increase in rate with increasing adiposity compared with that seen in never users of HT.

At age 50–54, premenopausal women had a 5-year incidence rate of surgically-confirmed fibroids of 1.94 % (95 % CI 1.85–2.02), which was about four times that seen in postmenopausal women of the same age (0.50 %, 95 % CI 0.45–0.55).

Just over half (n = 1810, 51 %) of the women with surgically-confirmed fibroids had an abdominal or vaginal hysterectomy during their admission. A small number had another open procedure: myomectomy/excision of uterine lesion (n = 30, 1 %), and 7 % had a diagnostic laparoscopy (n = 244). The remainder had less extensive procedures, most commonly hysteroscopy, dilatation and curettage, or vaginal or endoscopic resection of uterine lesion.

Ever-use of HT increased the risk of surgically-confirmed uterine fibroids (Table 2). Among women who reported ever using menopausal hormone therapy, the relative risk was 2.33 (95 % CI 2.18–2.49), compared with never users, adjusted for smoking, oral contraceptive use, parity and deprivation.

Body mass index also had a significant impact on risk (Table 3). Compared with women of normal weight, those who were overweight (BMI 25–29.9) had a RR of 1.15 (95 % CI 1.07–1.24), and those who were obese (BMI 30+) a RR of 1.46 (95 % CI 1.33–1.59), adjusted for smoking, oral contraceptive use, parity and deprivation.

While both HT use and BMI significantly increased risk of surgically-confirmed fibroids in postmenopausal women, when we looked at the interaction between the two risk factors, it was clear that BMI had a stronger effect in never-users of HT than in those who reported use of HT (Table 4). In never users of HT, there was a doubling of risk associated with obesity, RR 2.00 (95 % CI 1.77–2.26) compared with that seen in normal weight women. In

Table 1 Participant characteristics and follow-up, by body mass index (BMI)

Characteristic	All women	BMI		
		<25 kg/m^2 'Normal'	25.0–29.9 kg/m^2 'Overweight'	≥30 kg/m^2 'Obese'
Number of women (%)	610,604 (100.0)	287,208 (47.0)	217,546 (35.6)	105,850 (17.3)
Mean age at recruitment (SD)	57.8 (4.7)	57.6 (4.8)	58.0 (4.6)	57.9 (4.5)
Mean age at menarche (SD)	13.1 (1.6)	13.2 (1.6)	13.0 (1.6)	12.7 (1.6)
Past use of oral contraceptive %	52.2 (316,194)	54.5 (155,394)	50.9 (109,948)	48.5 (50,852)
Mean number of full term pregnancies (SD)	2.1 (1.3)	2.0 (1.2)	2.2 (1.3)	2.3 (1.4)
Nulliparous (n %)	12.3 (75,103)	13.6 (39,107)	11.0 (23,982)	11.4 (12,014)
Current smoker (n %)	20.4 (117,234)	22.8 (61,944)	19.1 (39,012)	16.5 (16,278)
Mean alcohol intake, units/week (SD)	3.9 (5.2)	4.3 (5.4)	3.8 (5.1)	2.8 (4.6)
Ever user of HT (n %)	34.0 (207,634)	36.3 (104,338)	33.5 (72,889)	28.7 (30,407)
Lowest quintile of socioeconomic status (n %)	26.3 (121,477)	23.6 (49,064)	26.5 (44,050)	33.0 (28,363)
Vigorous physical exercise at least once a week (n %)	38.6 (227,161)	44.1 (122,443)	36.9 (77,301)	27.0 (27,417)
Follow-up	All women	<25 kg/m^2 'Normal'	25.0–29.9 kg/m^2 'Overweight'	≥30 kg/m^2 'Obese'
Woman-years (1000s)	6974	3302	2484	1188
Mean length of follow-up (SD)	11.4 (2.5)	11.5 (2.5)	11.4 (2.5)	11.2 (2.6)
Number of women with surgically-confirmed fibroids	3561	1508	1273	780

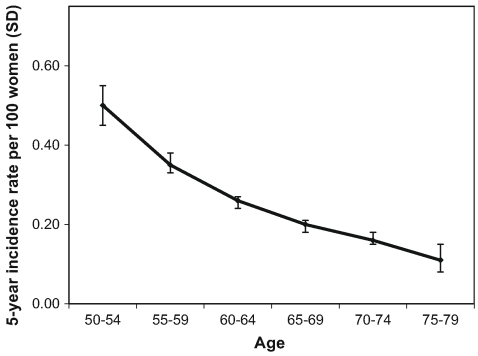

Fig. 1 Five-year incidence rates per 100 women of surgically-confirmed fibroids in postmenopausal women aged 50–79, by 5-year age group

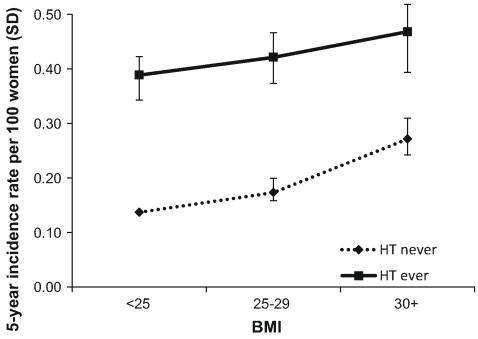

Fig. 2 Five-year incidence rates per 100 women of surgically-confirmed fibroids by body mass index and menopausal hormone therapy use

women who reported ever having used HT, the absolute risks overall were much higher, although the increase in relative risk associated with rising BMI was smaller. The additional increase in risk associated with adiposity was still present, but the magnitude of this effect was smaller in women who used HT.

The fully adjusted relative risk of fibroids in ever users of HT of normal BMI was 2.78 (95 % CI 2.50–3.08), which rose to 3.05 (95 % CI 2.73–3.41) in the overweight group, and further to 3.30 (95 % CI 2.86–3.79) in women who were obese (Table 4).

Discussion

We examined the relationship between adiposity, menopausal hormone therapy and the risk of surgically-confirmed uterine fibroids in postmenopausal women amongst

Table 2 Adjusted[a] relative risks of surgically-confirmed fibroids by menopausal hormone therapy (HT) use (ever vs. never)

HT use	Number of women	Number with fibroids	Adjusted RR (95 % CI)
Never	402,970	1607	1.00 (Reference group)
Ever	207,634	1954	2.33 (2.18–2.49)

[a] Adjusted for smoking, oral contraceptive use, parity and deprivation, stratified by region

Table 3 Adjusted[a] relative risks of surgically-confirmed fibroids by body mass index (BMI)

BMI	Number of women	Number with fibroids	Adjusted RR (95 % CI)
<25	287,208	1508	1.00 (Reference group)
25–29.9	217,546	1273	1.15 (1.07–1.24)
30+	105,850	780	1.46 (1.33–1.59)

[a] Adjusted for smoking, oral contraceptive use, parity and deprivation, stratified by region

Table 4 Adjusted[a] relative risks of surgically-confirmed fibroids by hormone therapy use and body mass index

Menopausal hormone therapy use	BMI	Number of women	Number with fibroids	Adjusted RR (95 % CI)
Never	<25	182,870	576	1.00 (Reference group)
	25–29.9	144,657	572	1.30 (1.16–1.46)
	30+	75,443	459	2.00 (1.77–2.26)
Ever	<25	103,338	932	2.78 (2.50–3.08)
	25–29.9	72,889	701	3.05 (2.73–3.41)
	30+	30,407	321	3.30 (2.86–3.79)

[a] Adjusted for smoking, oral contraceptive use, parity and deprivation, stratified by region

participants in a large UK prospective cohort study. Use of HT doubled the risk of incident surgically-confirmed uterine fibroids in postmenopausal women at any BMI. Adiposity alone had a lesser, but still significant effect, with a 46 % increase in risk of fibroids in obese women irrespective of history of HT use. When stratified by HT use, increasing adiposity had a much stronger effect in women who had never used HT, with a doubling of risk in the obese group. The effect of BMI was also present in ever users of HT, but they had much higher risks at any BMI, and the additional effect of increased adiposity was smaller.

Other groups have reported that uterine fibroid risk is increased in overweight and obese women, although published findings are inconsistent. The majority of studies show an increasing risk with increasing BMI [11, 22–24] but some have reported a J-shaped association [25–27], no association [28–30], or even a decreased risk in obese women [31]. Two other cohort studies, the Nurses' Health Study II [25] and the Black Women's Health Study [27] used surgically-defined cases, and both found strong associations between BMI and the risk of fibroids, which our findings support.

There has been comparatively little published on the effect of HT on uterine fibroid risk. Several small studies with short-term follow-up have been published, and results have been contradictory. For example, Yang et al. [32] followed 37 early postmenopausal HT users and 35 matched controls who did not receive HT. All participants had a solitary uterine fibroid at recruitment. Fibroids were measured by transvaginal ultrasound at baseline and then annually for 3 years. In the 3rd year there was a significant increase in the fibroid volume in the HT group, but not in the control group. The women treated with HT were also significantly more likely to have an increase in fibroid volume of >25 %, however numbers were small. A review by Parker [10] which included Yang's study, concluded that despite being hormone-sensitive tumours, for the majority of postmenopausal women with fibroids hormone therapy did not stimulate uterine or fibroid growth.

Two previous epidemiological studies that have examined the association between fibroids and HT use both reported increased risks. In a retrospective case–control study, Reed et al. [33] reported that use of combined oestrogen–progestagen HT was associated with an increased risk of fibroids, although the excess risk was largely confined to women who were not overweight (with BMI <24 kg/m^2). In the California Teachers Study [34] women taking HT had a higher risk of surgically treated fibroids than women who had never used HT. An increased risk

was seen both in women who used oestrogens alone (RR = 2.03, 95 % CI 1.17–3.52), and in those using combined HT (RR = 2.38, 95 % CI 1.66–3.41), similar to our overall findings.

Ovarian hormones are thought to play a key role in the aetiology of fibroids. Early menarche and obesity increase the incidence of fibroids, whereas high parity appears protective [35]. Fibroids are hormone-sensitive tumours, and so it is perhaps not surprising that use of HT doubles incidence rates in postmenopausal women. Adiposity is thought to affect the risk of fibroids in postmenopausal women because peripheral tissues, principally body fat, become the major source of circulating oestrogens (when women are not taking HT) [36].

Our results suggest that fibroids do continue to be an issue in postmenopausal women. Rates fall with age, and in women 50–54, the only age group which includes substantial numbers of both premenopausal and postmenopausal women, menopausal status affects incidence. At all ages, uterine fibroids occur more frequently in postmenopausal women who are obese, and those who use HT. Data in our study were collected prospectively, and we limited our analysis to women with a primary diagnosis of uterine fibroids during their hospital admission who also had a related operative procedure. Our aim was to identify clinically significant fibroids associated with a surgical intervention. A limitation of our study is that we were not able to assess whether having a raised BMI or using HT increased the risk of developing new fibroids in the postmenopausal period, or whether these factors decrease the likelihood of regression or encourage growth of pre-existing fibroids.

What is the public health relevance of our findings? Over half of the postmenopausal women with surgically-confirmed fibroids in our cohort underwent a hysterectomy or other major abdominal surgical procedure associated with their disease. Approximately 50,000 hysterectomies are performed annually in the UK [37]. At around £3282 per case [38], the 1810 hysterectomies in our study alone would have cost the NHS about £6 million. The cost in terms of quality of life, lost earnings, and morbidity to the women may have been even greater. Surgically-confirmed fibroids continue to occur in postmenopausal women, especially those who are obese or using menopausal hormone therapy. HT and BMI are potentially modifiable risk factors for uterine fibroids in postmenopausal women.

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Conflict of interest The authors report no conflict of interest.

Ethical standard Participants gave written informed consent for use of their questionnaire data for research, and for ongoing linkage to nationally held registry and health data. The Study has Multi-Centre Research Ethics Committee approval (MREC 97/01), and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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