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Predicting patients at risk of developing gastrointestinal symptoms after treatment with pelvic radiotherapy
a longitudinal observational cohort study

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PREDICTING PATIENTS AT RISK OF DEVELOPING GASTROINTESTINAL SYMPTOMS AFTER TREATMENT WITH PELVIC RADIOTHERAPY: A LONGITUDINAL OBSERVATIONAL COHORT STUDY

A thesis submitted for the degree of

Doctor of Philosophy

in

Nursing Research

Ann Cecile Georges Muls

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King's College London

Florence Nightingale Faculty of Nursing,

Midwifery & Palliative Care

“Prediction is very difficult, especially if it is about the future.”

Niels Bohr, Danish physicist (1885 - 1962)

Abstract

Background

Over 350,000 people are diagnosed with cancer in the UK each year of whom over 21,000 women are diagnosed with a new gynaecological malignancy. Pelvic radiotherapy, often used to treat gynaecological malignancies, can result in long-term changes in gastrointestinal function that impact severely on quality of life. There is no predictive model to stratify women at risk of gastrointestinal toxicity. Changes in the metabolic signatures of gut microbiome may be a viable way of identifying women at high risk of toxicity.

Methodology

The need for this study was supported by a prospective cohort describing the clinical setting of a specialist service assessing and managing people with long-term GI toxicity following cancer treatment. A cohort of 220 women who accessed the service is described and highlights the complexity of the gastrointestinal symptoms these women experience (chapter two). A systematic review was undertaken to identify studies examining the gut microbiome in women treated for gynaecological cancer. No studies included analysis of the gut microbiome metabolites and the update of the review (chapter three) highlighted the emerging importance of the vaginal as well as the intestinal microbiome. A prospective cohort study was then used to recruit 41 women newly diagnosed with a gynaecological malignancy who received pelvic radiotherapy as part of their treatment (chapter five). Demographic and treatment variables were collected at six different time points with several samples- saliva, urine, stool, rectal swab-

analysed using metabolic signature analysis to find a potential predictive framework. This included analysis by electronic sensing (e-nose) and the gold standard Gas Chromatography/ Ion Mass Spectrometry (CG-IMS). Particular attention was given to faecal calprotectin as a potential biomarker for long-term radiation induced gastrointestinal toxicity, using a generalised linear mixed model (GLMM). In addition, dietary intake, measured by 7- day food diaries, was analysed using a GLMM, to assess whether there were any changes in dietary intake over time. Gastrointestinal toxicity was measured using the modified Inflammatory Bowel Disease – Bowel subset- questionnaire (IBDQ-B) as primary outcome. Statistical analysis included descriptive statistics and predictive modeling statistics. To test the ability of the olfactory analysis to predict GI toxicity, the samples of the participants were grouped in three groups according to their IBDQ-B scores and again in according to their stool consistency as measured by the Bristol Stool Chart (BSC) and included area under the curve, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) analysis.

Results

Two years after completing radiotherapy, 24 women completed the study. The median IBDQ-B score at baseline was 68.5 (range: 38-70). The median score was at its lowest (worst) at the end of treatment (median 58, range 23-66) but this improved by two years after completion of radiotherapy. There was no relationship between the IBDQ-B scores and age, Body Mass Index, the presence of lymphovascular space invasion, chemotherapy and radiotherapy dose. Performance status, tumour type and surgery correlated with the IBDQ-B scores but not two years after radiotherapy. The evaluation of faecal calprotectin levels at

the end of treatment showed some evidence of correlation with IBDQ-B scores two years after radiotherapy (r= -0.45, p= 0.04) in this patient cohort but this effect was lost when when applying a bootstrap of 10.000 samples (p= 0.36). Overall, faecal calprotectin was a weak indicator of chronic radiation-induced GI toxicity (Pearson's R = -0.20, p= 0.03) as measured by the IBDQ-B score but this effect was also lost when accounting for the different timepoints (p= 0.64). The food diary analysis did not show any statistically significant changes in weight, BMI, dietary fat, fibre, protein or carbohydrate intake. The olfactory signatures of the metabolites of the samples showed that the e-nose analysis was more effective than analysis using GC-IMS in most instances and for all samples in predicting GI toxicity two years after treatment. Stool, urine and saliva samples could be used to distinguish the IBDQ-B score groups at the beginning of radiotherapy using the e-nose analysis ($p<0.05$). Stool samples, saliva and rectal swabs were all able to distinguish the three stool consistency groups at the beginning of treatment using the e-nose analysis ($p<0.05$).

Conclusion

Whilst this study had a small cohort, the results showed that is possible to use a variety of samples at the beginning of treatment to predict GI toxicity, based on IBDQ-B scores and stool consistency, two years after completion of radiotherapy using the e-nose analysis technique. This could be developed further for application in clinical practice and identify women at risk of long-term GI toxicity following treatment for gynaecological cancer.

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I dedicate this thesis to all of you!

Publications related to work conducted in this thesis

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Cirillo Sanchez, C., Czuber-Dochan, W., Cox, S., Murrells, T., Norton, C., & Muls, A. (2020). Dietary habits of women with gynecological cancer before, during and after treatment: a long-term prospective cohort study. *Nutrition & Cancer,* 11;1-11. doi: 10.1080/01635581.2020.1856386.

Muls, A., Cirillo Sanchez, C., Norton, C., & Czuber-Dochan, W. (2020). A systematic review: dietary habits of women with gynecological cancer before, during and after treatment. *Nutrition and Cancer,* 5:1-11. DOI: 10.1080/01635581.2020.1839518.

Muls, A., Taylor, A., Murrells, T., Norton, C., & Hart, A. (2021). Evaluating faecal calprotectin as a potential marker of long-term GI toxicity in women treated for gynaecological cancer with radiotherapy. *Journal of Cancer Science and Treatment,* 3(1): 201-209.

Glossary of abbreviations

16S rRNA	16S ribosomal Ribonucleic Acid
25[OH]D	25-hydroxycholecalciferol vitamin D
α	α- diversity (alpha diversity)
ACE	Angiotensin Converting Enzyme
ADL	Activities of Daily Living
AJCC	American Joint Committee on Cancer
AMPK	Adenosine Monophosphate-activated Protein Kinase
ANOVA	Analysis Of Variance
AUC	Area Under the Curve
β	β- diversity (beta-diversity)
BMI	Body Mass Index
BNF	British National Formulary
BSC	Bristol Stool Chart
BT	Brachytherapy
CASP	Critical Appraisal Skills Programme
CCR	Committee for Clinical Research
CI	Confidence Interval
CORDIS	Community Research and Development Information Service
CTCAE	Common Terminology Criteria for Adverse Events
CTGF	Connective Tissue Growth Factor
CRF	Case Report Form
CTC	Common Toxicity Criteria

CXT	Chemotherapy
DNA	Deoxyribonucleic Acid
EBRT	External Beam Radiotherapy
ECOG	European Cooperative Oncology Group
EPIC	Expanded Prostate Cancer Index Composite self-assessment questionnaire
FIBER	a randomised controlled trial investigating the role of low or high fibre diets in patients undergoing pelvic radiotherapy
FIGO	International Federation of Gynecology and Obstetrics
FOX4000	Electronic nose manufactured by Alpha MOS, France
FUP	Follow-up
G.A.S.	Gesellschaft für Analytische Sensorsysteme
GCMS	Gas Chromatography Mass Spectometry
GCSF	Granulocyte Stimulating Growth Factor
GC/ IMS	Gas Chromotography/ Ion Mobility Spectometry
GDPR	General Data Protection Regulation
GI	Gastrointestinal
GIANT	Gastrointestinal and Nutritional Team (Royal Marsden Hospital)
GOMS	Gut OncoMicrobiome Signatures
GLMM	General Linear Mixed Model
GORD	Gastro-oesophageal Reflux Disease
GP	General Practitioner
GSRS	Gastrointestinal Symptom Rating Score

Gy	Gray (unit of absorbed dose or radiotherapy)
HNA	Holistic Needs Assessment
HPV	Human Papilloma Virus
HPV-16	Human Papilloma Virus -16
IATA	International Airport Transport Association
IBDQ	Inflammatory Bowel Disease Questionnaire
IBDQ-B	IBDQ Bowel subset
IBDQ-E	IBDQ Emotional impact subset
IBDQ-SOC	IBDQ Social impact subset
IBDQ-SYS	IBDQ Systemic symptoms subset
IBDQ-T	IBDQ Total score
ICD-10	International Statistical Classification of Diseases and Related Health Problems version 10
IgA	Immunoglobulin A
IgB	Immunoglobulin B
IgG	Immunoglobulin G
IL	Interleukin
IV	Intravenous
J’	Pelou’s species evenness
kPa	Kilopascal
LDA	Linear Discriminant Analysis
LPS	Lipopolysaccharide
LREC	Local Research Ethics Co-ordinator
MDT	Multidisciplinary Team
MTA	Material Transfer Agreement

n	number (of participants)
N1mi	The presence of micrometastasis (greater than 0.2 mm and/or more than 200 cells, but none greater than 2.0 mm).
NA	Not Appliccable or Not possible to Annalyse
NCIN	National Cancer Intelligence Network
NHS	National Health Service
NICE	National institute for Health and Care and Excellence
NIHR	National institute for Health Research
NOS	Nitric Oxide Synthase
NS	Not (statistically) Significant
NSAID	Non-Steroidal Anti-Inflammatory Drugs
ORBIT	Optimising Radiotherapy Bowel Injury Therapy study
OTUs	Operational Taxonomic Units
PAI	Physical Activity Index
PCRRG	Patient and Carer Research Review Group
PD	Faith's Phylogenic Diversity
PEN-3	Portable Electronic Nose version 3
PORTEC-3	Randomised Trial of Radiation Therapy With or Without Chemotherapy for Endometrial Cancer- Phase 3
PPI	Proton Pump Inhibitor
PRDA	Pelvic Radiation Disease Association
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International database of prospectively registered systematic reviews in health and social care

PS	Performance Status
PTFE	Polytetrafluoroethylene (Teflon)
QoL	Quality of Life
rRNA	ribosonal RiboNucleic Acid
r (Pearson's R)	Pearson's Correlation Coefficient
RE	Radiation Enteropathy
ROC	Receiver Operator Curve
ROS	Reactive Oxygen Species
RT	Radiotherapy
RTOG	Radiation Therapy Oncology Group
SAP	Statistical Analysis Plan
SCFAs	Short Chain Fatty Acids
SD	Standard Deviation
SeHCAT	tauroselcholic [75 selenium] acid
SPSS	Statistical Package for Social Sciences
SSRI	Selective Seretonin Reuptake Inhibitors
T (ie. T0, T1, T2)	Time point
TGF	Tissue Growth Factor
Th	T helper cell
TJ	Tight Junction
TLRs	Toll Like Receptors
TMAO	Trimethylamine-N-oxide
TNF	Tumour Necrosis Factor
TReg	T Regulator cell
UK	United Kingdom

UN	United Nations
UPGMA	Unweighted Pair-Group Method using Arithmetic Averages
USA	United States of America
VAS	Visual Analogue Scale
VDR	Vitamin D Receptor
WHO	World Health Organization
VOC	Volatile Organic Compounds
WAR	Whole Abdominal Radiotherapy

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Figure 5.25 the effect of faecal calprotectin level at the end of treatment on the IBDQ-B scores one year post RT is (r= -0.17, p= 0.46) and two years post RT (r= -0.45, p= 0.036).

Figure 6.1 Poster of the Nose Opera production.

Figure 6.2 Student performance ticket.

Figure 6.3 The “nasoteque” (personal archive)

Figure 6.4 The noses of Soho (personal archive).

Introduction to the PhD

Having taken up the role of Nurse Consultant in gastrointestinal consequences of cancer and its treatment at the end of 2011, the next step to develop my skills to further research in this field was to embark on a doctoral study. The subject of my research was inspired by one of the presentations about the emerging research in the intestinal microbiome at the United European Gastroenterology Week in Stockholm a few weeks after starting this role. As my clinical experience grew, I was fascinated by the prospect of researching how the intestinal microbiome may impact cancer treatment outcomes and more specifically the development of long-term changes in gastrointestinal function afterwards.

Consequences of cancer and its treatment have gained importance during the last decade as cancer treatment outcomes and survival have continued to improve. The National Cancer Survivorship Initiative played a major role in demonstrating the relevance of consequences of cancer treatment within the National survivorship agenda. Long-term changes in bowel function after cancer treatment can affect many tumour groups and sometimes have a major impact on quality of life. Research in this field has mainly focused on patients treated with pelvic radiation which directly affects the intestinal tract but it is now recognised that any form of anti-cancer therapy may impact on gut function and many people receive multimodal treatments.

Only a handful of organisations in the UK offer services dealing with gastrointestinal consequences of cancer treatment and often people are referred after many years of having burdensome symptoms. Inspired by the patients with bowel problems we see in clinic, my main motivation has been to find potential indicators to predict who will go on to develop long-term changes in bowel function at an earlier stage in the treatment and recovery pathway and to reduce the time patients experience these symptoms before they seek appropriate help.

The intestinal microbiome hosts a wide variety of bacteria, protozoa, viruses, fungi and interacts with many intestinal functions. Research has shown that whilst the intestinal microbiome is relatively stable over time, specific disease states and treatments can alter the composition of the microbiome and thus its function. Therefore, the gut microbiome may also play a significant part in how intestinal function alters after cancer treatments.

Women treated for a gynaecological cancer make up a fifth of the service users who access our clinic and they often experience severe changes in bowel function which impacts on their quality of life and stops them participating in routine activities. This group of women has already received a high treatment burden as their treatment pathway is multimodal and complex and the additional impact of these long-term gastrointestinal consequences is significant.

This doctoral research study aims to research the possibility of using changes in the intestinal microbiome, combined with clinical data, to risk-stratify women treated for a gynaecological malignancy earlier in their treatment pathway so

that radiation induced GI toxicity can be managed sooner and reduce symptom burden. Changes in the intestinal microbiome may herald new treatment options for long-term bowel changes and lessen the chronic symptom burden.

This PhD thesis includes a background chapter presenting current understanding regarding gastrointestinal toxicity, survivorship issues in women treated for a gynaecological malignancy and the microbiome. The second chapter decribes the clinical setting that inspired this study and chapter three discusses a systematic review of studies analysing the gut and vaginal microbiome in women treated for a gynaecological malignancy. The methodology for the PREDICT study is outlined in chapter four. Study results are presented in chapter five followed by chapter six including discussion and implications of the findings. The reference section is followed by the appendices relating to this study.

Chapter 1

Background in current understanding regarding gastrointestinal toxicity and survivorship issues in women treated for a gynaecological malignancy.

This first chapter describes our current understanding regarding gastrointestinal toxicity and survivorship issues in women treated for a gynaecological malignancy and highlights current knowledge about the oral, gut and vaginal microbiome.

1.1 Gynaecological malignancies

1.1.1 Incidence

Over 350,000 people are newly diagnosed with cancer every year in the UK and there are yearly over 21,000 women diagnosed with a gynaecological malignancy in the UK (Cancer Research UK, 2020). Gynaecological cancers include cancer of the endometrium (or uterus), cervix, ovary, vagina and vulva. In the UK in 2017, there were about 9,500 new cases of endometrial cancer, nearly 7,450 women were newly diagnosed with ovarian cancer, around 3,150 new cases of cervical cancer; about 1,300 new diagnoses of vulvar cancer and 250 new cases of vaginal cancer (Cancer Research UK, 2020).

Cancer of the endometrium (or uterus)

Almost two-thirds of uterine cancers occur in women aged 55–75 with a peak in the rates for women in their early 70s. Obesity is a significant risk factor for adenocarcinoma of the endometrium, especially in post-menopausal women (Saso et al., 2011; Royal College of Obstetricians and Gynaecologists; 2012). Cases of endometrial adenocarcinoma in younger women are often associated with polycystic ovary syndrome and obesity. Endometrial hyperplasia is a pre-cancer change in the endometrium, which can progress to adenocarcinoma of the

endometrium. The use of tamoxifen to treat breast cancer and the decrease in hysterectomy procedures done for sterilisation or treatment of heavy menstrual bleeding are linked to an increase in endometrial cancer incidence (Jones et al., 2012; Temkin, Minasian, & Noone, 2016). In the UK, 77% of uterine cancers are endometrioid adenocarcinomas, 7% clear cell and serous carcinoma and 6% carcinosarcoma (National Cancer Intelligence Network, 2013).

Cancer of the cervix

Worldwide, cervical cancer is the most common gynaecological cancer, and the fourth most common cancer in women, accounting for nearly 7% of all female cancers (Arbyn et al., 2020). In the UK the incidence has reduced significantly since the introduction of routine screening (Castanon, Landy, & Sasieni, 2016). Cervical cancer has the highest incidence in women aged 30–35 years with more than 60% of cases affecting women under 50 years old. Risk factors for cervical cancer include human papillomavirus infection (HPV), smoking and socioeconomic status (Emmett, Gildea, Nordin, Hirschowitz, & Poole, 2017; Steinbeck et al., 2017; Brusselaers, Shrestha, Wijgert, & Verstraelen, 2019).

Cancer of the ovary

Ovarian cancer (including fallopian tube and primary peritoneal of gynaecological origin) is currently the fifth most common malignancy in women in the UK (Cancer Research UK, 2020). Over 80% of ovarian cancer cases are diagnosed in women over the age of 50. The majority of women (60%) present with advanced disease with little prospect of cure (London Cancer Alliance, 2014).

Cancer of the vulva

Carcinoma of the vulva is an uncommon tumour representing 4% of gynaecological malignancies, mostly affecting post-menopausal women (London Cancer Alliance, 2014; Sznurkowski, 2016).

Cancer of the vagina

Primary cancer of the vagina is rare and accounts for less than 2% of gynaecological cancers in the UK (London Cancer Alliance, 2014; Chang et al., 2016).

1.1.2 Classification and staging

Staging guidelines from the International Federation of Gynecology and Obstetrics (FIGO) and the American Joint Committee on Cancer (AJCC) are based on surgical findings: the depth of invasion of the tumour into the surrounding tissue and the grade of the tumour influence the recommendations for adjuvant postoperative therapy. The grade of the tumour depends on the amount of cancer cells in the specific cell types of the tissue layers and is determined by histopathology. Each of the gynaecological cancers have specific guidelines regarding staging (London Cancer Alliance, 2014). In this study, only women receiving treatment with curative intent were included.

1.1.3 Treatment modalities

Radiotherapy is used as a primary or adjuvant treatment for many women with gynaecological cancer with the exception of those with ovarian cancer where it is used more frequently in the palliative care setting. The close proximity to bowel and bladder means the treatment fields inevitably encompass some normal tissue (Taylor, Fernandes, Kumar, & Muls, 2016). This results in a range of potential acute and long-term radiation effects that can have significant impact on quality of life. With increasing numbers of cancer survivors, management of the consequences of treatment is a major clinical problem. Gastrointestinal (GI) symptoms are common and can have a major impact on daily activity (Abayomi, Kirwan, & Hackett, 2009; Ribas et al., 2020).

Cancer of the endometrium (or uterus)

Surgery is the main treatment for cancer of the endometrium with total hysterectomy and bilateral salpingo-oophorectomy. The aim of surgical treatment is to stage the cancer and achieve local clearance of the disease (Saso et al., 2011). Following surgery, the histopathology is reviewed in the specialist multidisciplinary team meeting (MDT) and recommendations for adjuvant treatment are based on these histopathological findings. Advanced disease may benefit from neo-adjuvant chemotherapy. Primary radiotherapy may be an option if surgery is not feasible due to extensive local disease or co-morbidities. Pelvic radiotherapy after surgery improves local control. In stage I disease Table 1.1), two-thirds of recurrences occur in the vaginal vault and vaginal vault brachytherapy alone significantly reduces the risk of central relapse. For

intermediate-risk stage I patients, there is a 15–25% risk of local relapse after surgery, which can be reduced to around 5% with post-operative radiotherapy although there is no survival advantage. For high-risk stage I patients, lymph node involvement is around 35% and overall 5-year survival is 55–60%. High-risk patients and those with locally advanced disease die of metastatic spread and the addition of concurrent and adjuvant chemotherapy (carboplatin and paclitaxel, 3-weekly for 6 cycles) has shown a significant improvement in survival in the PORTEC-3 trial (de Boer et al., 2019). Chemotherapy (carboplatin and paclitaxel, 3-weekly for 4 to 6 cycles) is usually given before radiotherapy but may be given afterwards for patients with definite residual disease. There is no evidence to support the use of adjuvant hormone therapy (Saso et al., 2011; Taylor et al., 2016).

11	**Stage grouping**	**FIGO Stage**	**Stage description endometrial cancer**
I	T1 N0 M0	I	The cancer is growing inside the uterus. It may also be growing into the glands of the cervix, but not into the supporting connective tissue of the cervix (T1). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IA	T1a N0 M0	IA	The cancer is in the endometrium (inner lining of the uterus) and may have grown less than halfway through the underlying muscle layer of the uterus (the myometrium) (T1a). It has not spread to nearby lymph nodes (N0) or distant sites (M0). *Low risk of recurrence:* Stage IA, Grade 1 (well differentiated) and Grade 2 (intermediate differentiated) endometrioid adenocarcinoma *Intermediate risk of recurrence:* Stage IA, Grade 3 (poorly differentiated) endometrioid adenocarcinoma *High risk of recurrence:* Stage IA, non-endometrioid adenocarcinoma (clear cell adenocarcinoma or serous adenocarcinoma)

Table 1.1 Summary of AJCC and FIGO staging guidelines for cancer of the endometrium.

AJCC Stage	Stage grouping	FIGO Stage	Stage description endometrial cancer (Cont.)
IB	T1b N0 M0	IB	The cancer has grown from the endometrium into the myometrium. It has grown more than halfway through the myometrium, but has not spread beyond the body of the uterus (T1b). It has not spread to nearby lymph nodes (N0) or distant sites (M0). *Intermediate risk of recurrence:* Stage IB, Grade 1 (well differentiated) and Grade 2 (intermediate differentiated) endometrioid adenocarcinoma *High risk of recurrence:* Stage IB, Grade 3 (poorly differentiated) endometrioid adenocarcinoma or Stage IB, non-endometrioid adenocarcinoma (clear cell adenocarcinoma or serous adenocarcinoma)
II	T2 N0 M0	II	The cancer has spread from the body of the uterus and is growing into the supporting connective tissue of the cervix (the cervical stroma). But it has not spread outside the uterus (T2). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
III	T3 N0 M0	III	The cancer has spread outside the uterus, but has not spread to the inner lining of the rectum or urinary bladder (T3). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IIIA	T3a N0 M0	IIIA	The cancer has spread to the outer surface of the and/or to the fallopian tubes or ovaries (the adnexa) (T3a). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IIIB	T3b N0 M0	IIIB	The cancer has spread to the vagina or to the tissues around the uterus (the parametrium) (T3b). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IIIC1	T1-T3 N1, N1mi or N1a M0	IIIC1	The cancer is growing in the body of the uterus. It may have spread to some nearby tissues, but is not growing into the inside of the bladder or rectum (T1 to T3). It has also spread to pelvic lymph nodes (N1, N1mi, or N1a), but not to lymph nodes around the aorta or distant sites (M0).
IIIC2	T1-T3 N2, N2mi or N2a M0	IIIC2	The cancer is growing in the body of the uterus. It may have spread to some nearby tissues, but is not growing into the inside of the bladder or rectum (T1 to T3). It has also spread to lymph nodes around the aorta (para-aortic lymph nodes) (N2, N2mi, or N2a), but not to distant sites (M0).

Table 1.1 (Cont.) Summary of AJCC and FIGO staging guidelines for cancer of the endometrium.

AJCC Stage	Stage grouping	FIGO Stage	Stage description endometrial cancer (Cont.)
IVA	T4 Any N M0		The cancer has spread to the inner lining of the rectum or urinary bladder (T4). It may or may not have spread to nearby lymph nodes (Any N), but has not spread to distant sites (M0).
IVB	Any T Any N M1	IVB	The cancer has spread to inguinal lymph nodes, the upper abdomen, the omentum, or to organs away from the uterus, such as the lungs, liver, or bones (M1). The cancer can be any size (Any T) and it might or might not have spread to other lymph nodes (Any N).

Table 1.1 (Cont.) Summary of AJCC and FIGO staging guidelines for cancer of the endometrium.

Cancer of the cervix

The optimal treatment of cervical cancer depends on the stage of disease (Table 1.2), tumour volume, lymph node status and the fitness of the patient. It can incorporate surgery, radiotherapy and chemotherapy. Ideally, a single radical modality should be used as there is no survival advantage but there is greater morbidity with a combined surgical and radiotherapy approach (London Cancer Alliance, 2014). Whereas surgery is preferable for early stage disease, radiotherapy is the treatment of choice when there is parametrial or nodal involvement which emphasises the need for optimal imaging to assess disease at presentation. External beam radiotherapy (EBRT) with concomitant chemotherapy (cisplatin, weekly for 5 cycles) followed by brachytherapy is the most common treatment modality (Emmett et al., 2017). Organs at risk involve the bladder, rectum, bowel and femoral heads. The kidneys and the spinal cord are involved when radiation to the para-aortic lymph nodes is necessary (London Cancer Alliance, 2014).

AJCC Stage	Stage grouping	FIGO Stage	Stage description cervical cancer
	I	IA	There is a very small amount of cancer on the surface of the cervix, and it can be seen only under a microscope. It has not spread to nearby lymph nodes or distant sites.
		IA1	The area of cancer can only be seen with a microscope and is less than 3 mm deep. It has not spread to nearby lymph nodes or distant sites.
		IA2	The area of cancer can only be seen with a microscope and is between 3 mm and 5 mm deep. It not has not spread to nearby lymph nodes or distant sites.
		IB	This includes stage I cancer that has spread deeper than 5 mm but is still limited to the cervix. It has not spread to nearby lymph nodes or distant sites.
		IB1	The cancer is deeper than 5 mm but not more than 2 cm in size. It has not spread to nearby lymph nodes or distant sites.
		IB2	The cancer is at least 2 cm in size but not larger than 4 cm. It has not spread to nearby lymph nodes or distant sites.
		IB3	The cancer is at least 4 cm in size and limited to the cervix. It has not spread to nearby lymph nodes or distant sites.
	II	IIA	The cancer has grown beyond the cervix and uterus but has not spread to the walls of the pelvis or the lower part of the vagina. It has not spread to nearby lymph nodes or distant sites.
		IIA1	The cancer is not larger than 4 cm. It not has not spread to nearby lymph nodes or distant sites.
		IIA2	The cancer is 4 cm or larger. It has not spread to nearby lymph nodes or distant sites.
		IIB	The cancer has grown beyond the cervix and uterus and has spread into the tissues next to the cervix (the parametria). It has not spread to nearby lymph nodes or distant sites.
	III	IIIA	The cancer has spread to the lower part of the vagina but not the walls of the pelvis. It has not spread to nearby lymph nodes or distant sites.
		IIIB	The cancer has grown into the walls of the pelvis and/or is blocking one or both ureters causing hydronephrosis. It has not spread to nearby lymph nodes or distant sites.
		IIIC	The cancer can be any size. Imaging tests or a biopsy show the cancer has spread to nearby pelvic lymph nodes (IIIC1) or para-aortic lymph nodes (IIIC2). It has not spread to distant sites.
	IV	IVA	The cancer has spread to the bladder or rectum or it is growing out of the pelvis.
		IVB	The cancer has spread to distant organs outside the pelvic area, such as distant lymph nodes, lungs or bones.

Table 1.2 Summary of AJCC and FIGO staging guidelines for cancer of the cervix.

Cancer of the ovary

The current standard of care for newly diagnosed ovarian cancer consists of a combination of cytoreductive surgery and platinum-based chemotherapy (Lin, Egorova, Franco, Prasad-Hayes, & Bickell, 2016). There remains a significant risk of recurrence and resistance to therapy. Systemic therapy options for relapsed ovarian cancer are guided by the platinum-free interval. The platinum-free interval is the time between the last treatment with platinum chemotherapy and evidence of disease progression and is an important marker in predicting the response to further treatment (Eng, Hanlon, Bradley, & Szender, 2015). Management of fallopian tube and primary peritoneal tumours is currently the same as for ovarian cancer. Whole-abdomen radiation (WAR) has been used to treat ovarian cancer, but its popularity has waned because of the favourable toxicity profiles of current chemotherapeutic regimens (Fields, McGuire, Lin, & Temkin, 2017). Treatment with additional radiotherapy boosts to the pelvis and para-aortic lymph nodes may be combined with WAR for total doses of 45-50 Gy to these regions. Unfortunately, small bowel obstruction several years following WAR was a common complication and so this is currently not recommended (London Cancer Alliance, 2014). Recently, clinical trials are examining the role of immunotherapy in women who no longer respond to platinum-based chemotherapy (Borella et al., 2020). Palliative radiotherapy is offered to patients who have focally symptomatic recurrences of ovarian cancer (Jiang, Balboni, Taylor, Liu, & Lee, 2018). The field arrangements and dose schedules are based on the site of recurrence and the patient's overall status (London Cancer Alliance, 2014). As women offered radiotherapy for cancer of the ovary have had multiple

previous systemic chemotherapeutic agents and receive radiotherapy for symptomatic relief, these women have not been included in the present study.

Cancer of the vulva

Some 90% are squamous cell carcinomas, while less common histological types are melanomas, adenocarcinomas, basal cell carcinomas, verrucous carcinomas, and sarcomas (London Cancer Alliance, 2014; Sznurkowski, 2016). The inguinal and femoral nodes are the primary sites of regional spread and involvement to pelvic nodes is considered as distant metastasis (Table 1.3). The presence of nodal metastasis greatly impacts on survival, with 5-year survival rates falling from 80% to 50% if the groin nodes are involved and to 10–15% if the iliac or pelvic nodes are involved. Around 30% of women may present with nodal involvement.

Surgery has been the mainstay of treatment for this malignancy. Radical vulvectomy combined with bilateral groin node dissection has been practised widely. Increasingly, care of such patients is individualised with a partial radical excision and unilateral node dissection; or with sentinel node dissection in selected patients (Sznurkowsky, 2016). Also, patients are increasingly being offered combined modality therapy with the use of radiotherapy or chemo-radiotherapy (cisplatin +/- 5-Fluorouracil +/- mitomycin, weekly). This seems to be particularly useful for the management of patients with more advanced disease, or where excision of midline structures is not feasible (London Cancer Alliance, 2014; Sznurkowski, 2016).

AJCC Stage	Stage grouping	FIGO Stage	Stage description vulvar cancer
IA	T1a N0 M0	IA	The cancer is in the vulva or perineum or both and has grown no more than 1 mm into underlying tissue (stroma) and is 2 cm or smaller (T1a). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IB	T1b N0 M0	IB	The cancer is in the vulva or the perineum or both and is either more than 2 cm or it has grown more than 1 mm (0.04 inches) into underlying tissue (T1b). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
II	T2 N0 M0	II	The cancer can be any size and is growing into the anus or the lower third of the vagina or urethra (T2). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IIIA	T1 or T2 N1 M0	IIIA	Cancer is in the vulva or perineum or both (T1) and may be growing into the anus, lower vagina, or lower urethra (T2). It has either spread to a single nearby lymph node with the area of cancer spread 5 mm or more **or** it has spread to 1 or 2 nearby lymph nodes with both areas of cancer spread less than 5 mm (N1). It has not spread to distant sites (M0).
IIIB	T1 or T2 N2a or N2b M0	IIIB	Cancer is in the vulva or perineum or both (T1) and may be growing into the anus, lower vagina, or lower urethra (T2). The cancer has spread either to 3 or more nearby lymph nodes, with all areas of cancer spread less than 5 mm (N2a); **or** the cancer has spread to 2 or more lymph nodes with each area of spread 5 mm or greater (N2b). It has not spread to distant sites (M0).
IIIC	T1 or T2 N2c M0	IIIC	Cancer is in the vulva or perineum or both (T1) and may be growing into the anus, lower vagina, or lower urethra (T2). The cancer has spread to nearby lymph nodes and has started growing through the outer covering of at least one of the lymph nodes (extracapsular spread; N2c). It has not spread to distant sites (M0).
IVA	T1 or T2 N3 M0	IVA	Cancer is in the vulva or perineum or both (T1) and may be growing into the anus, lower vagina, or lower urethra (T2). The cancer has spread to nearby lymph nodes and has become fixed to the underlying tissue or has caused an ulcer(s) to form on the lymph node(s) (N3). It has not spread to distant sites (M0).
	T1 or T2 Any N M0		The cancer has spread beyond nearby tissues to the bladder, rectum, pelvic bone, or upper part of the urethra or vagina (T3). It might or might not have spread to nearby lymph nodes (any N). It has not spread to distant sites (M0).
IVB	Any T Any N M1	IVB	The cancer has spread to distant lymph nodes (pelvic) or organs such as lung or bone (M1). The cancer can be any size and might or might not have spread to nearby organs (Any T). It might or might not have spread to nearby lymph nodes (Any N).

Table 1.3 Summary of AJCC and FIGO guidelines of staging for vulvar cancer.

Cancer of the vagina

Radiotherapy is the most widely used primary treatment modality for all stages of the disease. The role of surgery (partial/total radical vaginectomy) in the management of vaginal carcinoma is limited (Hacker, Eifel, & van der Velden, 2015; Chang et al., 2016). Stage I and II small lesions involving only the vault of the vagina with no or early parametrial involvement may be treated with radical vaginectomy or hysterectomy and pelvic and para-aortic node dissection (Table 1.4). For lesions that lie adjacent to the bladder or rectum, radiation therapy should be considered; however, some may require exenteration. Where the lower third of the vagina is involved or there are suspicious or positive groin nodes, then radical vulvectomy and groin node dissection may be considered. Vaginal reconstruction should be considered when vaginectomy is performed. In lesions involving the bladder or rectum, without distant spread or pelvic sidewall involvement, anterior, posterior or total exenteration may be considered (London Cancer Alliance, 2014). Exenterative surgery may also have a role in the treatment of radiation failures. Treatment with radiotherapy depends on the site of disease: lesions in the upper third of the vagina are treated as a stage IIA cervical cancer; treatment for lesions in the middle and lower third of the vagina may be treated with brachytherapy alone or wide local excision of the lesion in a stage I lesion less than 2cm in dimension with no lymphovascular space involvement and with a depth of invasion of less than 3mm. All other cases are treated with radical chemoradiation to whole vagina, pelvic (to common iliac region) and inguinal nodes delivering 45–50.4 Gy in 28 fractions with weekly cisplatin chemotherapy. Radiologically abnormal nodes should receive additional boost doses to 55–60 Gy. The total dose to the primary tumour should ultimately

be in excess of 65 Gy using interstitial or superficial brachytherapy or external beam radiotherapy to boost the primary site. Radiation is indicated post-surgery when poor prognostic features such as positive nodes are present or when there are close or involved margins (London Cancer Alliance, 2014; Hacker et al., 2015; Chang et al., 2016).

AJCC Stage	Stage grouping	FIGO Stage	Stage description vaginal cancer
IA	T1a N0 M0	I	The cancer is only in the vagina and is no larger than 2 cm (T1a). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IB	T1b N0 M0	I	The cancer is only in the vagina and is larger than 2.0 cm (T1b). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IIA	T2a N0 M0	II	The cancer has grown through the vaginal wall, but not as far as the pelvic wall and is no larger than 2.0 cm (T2a). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IIB	T2b N0 M0	II	The cancer has grown through the vaginal wall, but not as far as the pelvic wall and is larger than 2.0 cm (T2b). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
III	T1 to T3 N1 M0	III	The cancer can be any size and might be growing into the pelvic wall, and/or is growing into the lower 1/3 of the vagina, and/or has caused (T1 to T3). It has also spread to nearby lymph nodes in the pelvis or iguinal area (N1) but not to distant sites (M0).
	T3 N0 M0		The cancer is growing into the pelvic wall, and/or is growing into the lower 1/3 of the vagina, and/or has caused hydronephrosis (T3). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
IVA	T4 Any N M0	IVA	The cancer is growing into the bladder or rectum or is growing out of the pelvis (T4). It might or might not have spread to lymph nodes in the pelvis or inguinal area (Any N). It has not spread to distant sites (M0).
IVB	Any T Any N M1	IVB	The cancer has spread to distant organs such as the lungs, liver, or bones (M1). It can be any size and might or might not have grown into nearby structures or organs (Any T). It might or might not have spread to nearby lymph nodes (Any N).

Table 1.4 Summary of AJCC and FIGO staging guidelines for cancer of the vagina.

1.1.4 Survival

Long-term survival varies depending on the primary site of disease. Age and stage of presentation are also important factors (Cancer Research UK, 2020) (Table 1.5).

Cancer site	one year survival	5 years	10 years	Age/ stage of disease	Trend
Endometrium	90%	76%	72%	Highest survival if < 40y age + early stage	Improving
Cervix	81%	61%	51%	Highest survival if < 40y age	Improving
Ovary	72%	43%	35%	Highest survival if < 40y age + early stage	Improving
Vagina	82%	64%	53%	Highest survival if < 50y age	No change
Vulva	86%	67%	53%	Highest survival if < 50y age + early stage	No change

Table 1.5 Summary of survival statistics for women with a gynaecological malignancy in England data sourced from Cancer Research UK 2013-2017.

1.1.5 Survivorship issues following treatment

Half of people diagnosed with cancer in England and Wales survive their disease for ten years or more (Cancer Research UK, 2020). Cancer survival is improving and has doubled in the last 40 years. Advances in multimodal treatments offer better chances of survival but also impact on the body in multiple ways, not only during treatment but in the long-term. The long-term impact of cancer treatment is the focus of the National Survivorship Initiative for those living with and beyond cancer (Macmillan Cancer Support, 2013; National Cancer Survivorship Initiative, 2013).

The Royal Marsden Hospital GI and Nutrition Team (GIANT) service provides care for patients experiencing ongoing gastrointestinal (GI) symptoms after completion of their cancer treatment and improves continuity of long-term management of changes in GI function and quality of life within the context of the cancer survivorship agenda. The GIANT clinic has a multi-disciplinary set-up which includes consultant gastroenterologists, specialised dietitians, physiotherapists and nurses and has developed guidance on the practical management of gastrointestinal symptoms following cancer treatment (Andreyev et al., 2014).

A service evaluation spanning April 2013 to March 2016 showed that there were 4134 clinic appointment slots, 1158 (28%) new patients and 2686 (65%) follow-up patients were seen and 290 slots (7%) were left unused by patients not attending. During this period, 1117 cancer patients were discharged from the GIANT clinic. 66% were referred by in-house oncology teams, 21% from other centres and 12% by GPs. The original cancer was urological (39%), gynaecological (21%), colorectal (12%), upper GI (10%) haematological (5%) and other cancers (12%).

As women with a gynaecological malignancy account for a fifth of our service users, this sub-cohort is presented separately in Chapter two.

1.2 Treatment induced gastrointestinal toxicity

All treatment modalities potentially impact on bowel function acutely or long-term. Surgery for gynaecological cancer may be local, or extensive, resulting in pelvic exenteration and the formation of a stoma. This has a direct impact on bowel function. Chemotherapy also may alter GI function. Research has mainly focused on acute GI toxicity as diarrhoea and vomiting are the main treatment and dose limiting factors.

Studies in patients with locally advanced cervical cancer treated by radiotherapy alone or in combination with a cisplatin-based regimen reported that late toxicities were not increased when adding chemotherapy (Eifel et al., 2004; Maduro, Pras, Wilemse, & de Vries, 2003; Rose et al., 2007). These findings may be partially explained by the relatively short follow-up and an underestimation of long-term gastrointestinal effects particularly in cancer survivors. Long-term studies are needed to specifically estimate more accurately the late adverse effects of chemotherapeutic agents (Di Fiore & Van Cutsem, 2009).

Gastrointestinal toxicity is a common consequence during and following pelvic radiotherapy. Within days of starting a fractionated course of treatment, radiotherapy affects the rapidly proliferating mucosal epithelial cells. Loss of progenitor cells located in the intestinal crypts leads to insufficient replacement of the villus epithelium. Crypt cell death causes acute inflammatory changes that disrupt the mucosal barrier allowing penetration of bacterial products and other activating agents. Mucosal pathology with impaired bowel function peaks at 2 weeks into a fractionated course of radiotherapy and improves by the final week,

in contrast to the clinical picture (Stacey & Green, 2014). Pathophysiology of gastrointestinal function is complex as radiotherapy can affect multiple tissues and cellular functions at different levels in the GI tract (Hauer-Jensen, Denham, & Andreyev, 2014). Chronic changes may develop with prolonged cytokine activation, connective tissue fibrosis and endarteritis with neovascularisation.

Although the incidence of severe complications has reduced since the introduction of modern radiotherapy techniques, even low-moderate grade toxicity can significantly impact on quality of life (Gillespie, Goode, Hackett, & Andreyev, 2009; Roszak, Wareńczak-Florczak, Bratos, & Milecki, 2012; Campbell, Thomas, Hand, Lee, Taylor, & Donovan, 2019). In general, the incidence of physician scored moderate to severe GI toxicity following pelvic radiotherapy for gynaecological cancer is 5-15% whereas patient reported outcomes are consistently much higher (Taylor et al., 2016). A recent study showed that women with cancer of the endometrium and cervix reported GI symptoms up to nearly 20% more frequently than clinicians when using patient reported outcome measures (Yeung et al., 2020). In three studies including women treated with radiotherapy for gynaecological cancer, long-term bowel problems were reported in 28 to 80% women with the most common symptoms being faecal urgency, diarrhoea, faecal incontinence and rectal bleeding (Barraclough et al., 2012; Kuku, Fragkos, McCormack, & Forbes, 2013; Ribas et al., 2020). Bowel toxicity has historically not been investigated or managed actively but identification of the underlying physiological mechanism can guide treatment and significantly improve symptoms (Andreyev et al., 2014; Teo, Sebag-Montefiore, & Donnellan, 2015; White et al., 2020).

As indicated by the service evaluation in Chapter two, women treated for gynaecological malignancies often receive mutlimodal therapies. This makes this group of patients a complex cohort as it is difficult to ascertain which type of treatment has changed specific GI functions and what the cumulative affect is. In addition, acute GI symptoms such as mucositis, nausea, vomiting and diarrhoea caused by chemotherapeutic agents have been studied for many years, but studies reporting long-term changes in bowel function have only been conducted more recently.

1.3 The microbiome

1.3.1 The role of the microbiome in health and disease

The microbiome involves every organ, even those previously deemed sterile such as the lung (Shukla, Budden, Neal, & Hansbro, 2017). New research in this field is rapidly changing the way we think about medicine and healthcare as a result. Originally, specific microbiomes were mostly studied in isolation but interactions and feedback mechanisms between microbiomes are now emerging. The term microbiome refers to the whole microbial community that occupies a defined habitat and has distinct physicochemical properties (Young, 2017; Anwar et al., 2019). The microbiota are the types of organisms that are present in an environmental habitat, whether they are bacteria, viruses or eukaryotes (Marchesi et al., 2015). The most common way of studying the gut microbiome is 16S rRNA sequencing of stool samples or biopsies to identifies which microbiota are present, their abundance (how many) and their richness in diversity. In the

gut microbiome, a high diversity and high abundance is beneficial to gut health. Table 1.6 describes which terms are commonly used to describe biodiversity in the analysis of the intestinal microbiome in published papers. Methods to examine the metabolism of the microbiome and hence its function are now more frequently incorporated in studies. This section discusses the oral, gut and vaginal microbiome in health and explores how this may relate to women with a gynaecological malignancy. Chapter three further details a systematic review of studies examining the gut or vaginal microbiome in women treated with radiotherapy for gynecological cancer.

Term	Definition
Operational taxonomic Unit (OTU)	A taxonomic species within a sample
Alpha- diversity	The richness and evenness of the number of organisms within an individual or sample
Shannon index	The higher the value, the higher the diversity
Simpson index	The higher the value, the lower the diversity
Faith's pylogenetic diversity (PD)	the sum of the branch lengths of a phylogenetic tree connecting all species in a sample.
Pielou's species evenness (J')	The relationship between richness and evenness within a sample or between samples
Beta- diversity	The richness and evenness of the number of organisms between individuals or samples

Table 1.6 Commonly used terms describing biodiversity.

1.3.2 The oral microbiome

The microorganisms found in the human oral cavity have been referred to as the oral microflora, oral microbiota, or more recently as the oral microbiome. Most of the studies and samples studying the oral microbiome have been obtained from within the oral cavity. However, studies have shown that different oral structures and tissues are colonised by distinct microbial communities (Mager, Ximenez-Fyvie, Haffajee, & Socransky, 2003; Aas, Paster, Stokes, Olsen, & Dewhirst, 2005). Approximately 280 bacterial species from the oral cavity have been isolated in culture and formally named. It has been estimated that less than half of the bacterial species present in the oral cavity can be cultivated using anaerobic microbiological methods and that there are likely 500 to 700 common oral species (Dewhirst et al., 2010). The bacterial community of the mouth is dominated by the phyla *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes* and *Fusobacteria* which account for 96% of species detected (Dewhirst et al., 2010). Oral microorganisms adapt to changing environments within protective biofilms, which are the complex colonies of microorganisms that predominate on both hard and mucosal surfaces in the oral cavity (Flemmig & Beikler, 2011). Dental plaque is a multispecies biofilm that packs as layers onto tooth surfaces and can either entrap and prevent an existing oral pathogen from flourishing, or provide a refuge for a pathogen to hide from salivary flow and the host's immune system (Avila, Ojcius, & Yilmaz, 2009). Under healthy conditions, an ecological balance between microbe composition and activity keeps biofilms healthy and stable (Flemmig & Beikler, 2011). Saliva is crucial to the oral cavity because it plays a key role in maintaining homeostasis and defending from

disease (Nieuw Amerongen & Veeman, 2008; Pedersen & Belstrøm, 2019). While it helps maintain a climate that allows biofilms to flourish, saliva also detaches layers of plaque and contains numerous proteins, minerals, and antimicrobial enzymes that control biofilm build up and activity (Nieuw Amerongen & Veeman, 2008). Saliva also provides nutrients that protect tooth enamel and antibodies that defend the oral cavity and the rest of the body from infection (Nieuw Amerongen & Veeman, 2008; Filoche, Wong, & Sissons, 2010). Studies have shown both salivary flow and microbiome composition to be unique to an individual's oral cavity (Zarco, Vess, & Ginsburg, 2012). This may be of importance in women receiving chemotherapeutic agents such as cisplatin used in gynaeological malignancies which can affect the oral mucosa and result in nausea, vomiting, dry mouth or oral mucositis.

The oral microbiome in disease

The oral microbiome is not only implicated in diseases of the oral cavity (gingivitis, periodontitis, dental caries, and dental infections) but also acts as a reservoir for infection at other body sites. Oral bacteria can gain access to the bloodstream via the gingival crevice and untreated carious lesions and are a significant cause of infectious endocarditis, brain and liver abscesses (Kilian et al., 2016; Willis & Gabaldón, 2020) and play a role in artherosclerotic plaque formation in coronary heart disease (Chhibber-Goel et al., 2016; Belstrøm, 2020; Willis & Gabaldón, 2020). Oral bacteria have also been detected in the lungs of cystic fibrosis patients (Rivas Caldas et al., 2015). In addition, it has been suggested that the composition of the oral microbiome may be linked to diseases at other body sites either in a causative way or as a reflection of systemic changes

in the body and oral microbiomic profiles could be used as diagnostic biomarkers for other diseases (Ahn, Segers, & Hayes, 2012; Wade, 2013). The oral microbiome is implicated in the carcinogenesis of several cancers including oral (Zarco et al., 2012; Gholizadeh et al., 2016; Zhang, Lui, Zheng, & Zhang, 2020), gastric, oesophageal (Yu et al., 2014) and pancreatic cancer (Ertz-Archambault, Keim, & Von Hoff, 2016; Belstrøm, 2020; Willis & Gabaldón, 2020). The presence of *Candida* and *Porphyromonas gingivalis* has been identified as a biomarker for orodigestive tract cancer (Ahn et al., 2012). There is also some evidence to support associations between both oral fungal and viral organisms and cancer; the human papillomavirus 16 (HPV-16) infection is an established cause for the majority of oropharyngeal squamous cell carcinomas (Elrefaey, Massaro, Chiocca, Chiesa, & Ansarin, 2014). The salivary microbiome has been identified as a potential surrogate marker for monitoring health and disease (Yoshizawa et al., 2013).

The oral microbiome in women with gynaecological cancer

An early study analysed throat swabs from the oropharyngeal cavity, vaginal swabs, stool and urine samples using fungal culture in 31 women treated for cervical cancer before and during radiotherapy and showed that yeast counts – predominantly *Candida albicans* and *tropicalis* were increased at all sites for the majority of participants during the second week of therapy but returned to normal for all but 2 patients by the end of treatment (Talwar et al., 1992). Using conventional analysis methods, the study showed that radiation has the potential to disturb the equilibrium of yeast organisms even at distant sites. Currently, no

studies have considered sequencing fungal organisms when analysing the microbiome using newer techniques in women with gynaecological cancers.

1.3.3 The intestinal microbiome

The GI tract contributes to health in many ways and is not restricted to food processing and subsequent nutrient and fluid uptake alone (Bischoff, 2011). The gut communicates with bacteria that support digestion by their enzymatic activity, regulates major epithelial and immune functions, reports to the brain via the vagus nerve and hormones about energy uptake and affects mood and general well-being (Bischoff, 2011; Cani, 2018). Two functional entities are key to achieving and maintaining gut health: the GI microbiome and the gut barrier.

The intestinal microbiome is a complex ecosystem of all the bacteria, viruses, fungi, archaea, and eukaryotes that inhabit the human gastrointestinal tract (Ferranti, Dunbar, Dunlop, & Cowin, 2014; Thomas & Jobin, 2015). There are 10 times the number of microbial cells in the human gut compared to the whole human body, amounting to about 100 trillion microbes representing more than 1,000 different species and weighing approximately 2 kilogram (Ferranti et al., 2014; Salazar, Valdés-Varela, González, Gueimonde, & de los Reyes-Gavilán, 2017). Advances in identifying the composition of the intestinal microbiome have found 1,900 previously uncultured bacterial species confirming the vast diversity of the collective human intestinal microbiome (Almeida et al., 2019).

Development of the intestinal microbiome

Historically, the intestinal microbiome was thought to develop during birth and establish itself during the first few years of life. But several studies have shown that there are micro-organisms present in amniotic fluid, the foetal membrane, placenta, umbilical cord and meconium (Collado, Rautava, Aakko, Isolauri, & Salminen, 2016; Tanaka & Nakayama, 2017, Lim, Rodriguez, & Holtz, 2018). It was also thought that infants delivered vaginally have a microbiome akin to their mother's vaginal microbiome, dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* and children born via caecarian section have a microbiome resembling the skin microbiome, dominated by *Staphylococcus*, *Corynebacterium* and *Propionibacterium* (Dominguez-Bello et al., 2010). Some studies show that by 6 weeks of age, the microbial community structure and function in neonates significantly expanded and diversified and that there was no effect of the mode of delivery on the early microbiota beyond the immediate neonatal period (Chu et al., 2017; Stinson, Payne, & Keelan, 2018). However, a recent study found that children born by vaginal delivery have more *Bifidobacterium* species in their faecal samples compared to an abundance in *Klebsiella* species in babies born via caesarian section. In those delivered via caesarian section, there was a delay in acquiring *Bifidobacterium* species and those children developed more pediatric allergies and nosocomial infections within the first year of their life (Reyman, van Houten, Arp, Sanders, & Bogaert, 2019). There is not yet any evidence regarding how these early changes may impact on the intestinal microbiome later in life in both health and disease (Stinson et al., 2018).

Other factors affecting the development of the intestinal microbiome include whether a newborn is breast-fed or formula-fed and the timing of introducing solid food. Several studies have shown that breastfeeding increases *Bifidobacterium* and starting to eat solid food shifts the composition of the microbiome from being dominated by *Bifidobacterium* to being *Bacteroidetes* and *Firmicutes*-dominant (Tanaka & Nakayama, 2017). Previously, it was thought that the intestinal microbiome was fully established by the age of three but a recent study has shown it may still be under development in school going children whereby *Bifidobacterium* levels may gradually decrease until adulthood (Derrien, Alvarez, & de Vos, 2019). The species vary greatly between individuals but the individual microbiotic composition usually remains stable for 60% of the species making up the profile over time (Guarner & Malagelada, 2003, Floch, 2010). Although the human ageing process results in a gradual deterioration in physiological function, the intestinal microbiome does not age in the same way and remains relatively stable (Buford, 2017; Nagpal et al., 2018). Whilst there are some changes in the composition of the intestinal microbiome over the host's entire lifespan, it is not known how much these changes may be due to variables such as demographics, diet, genetics and gender or to the aging process and associated disease states as there are no studies examining the intestinal microbiome life-long (Tanaka & Nakayama, 2017; Santoro et al., 2018). Studies have shown that people co-habiting have a similar intestinal microbiome composition to each other and cat or dog-ownership correlates with a skin microbiome related to that of the cat or dog but does not affect the intestinal microbiome (Song et al., 2013; Hoffmann, Proctor, Surette, & Suchodolski, 2016; Torres et al., 2017; Dill-McFarland et al., 2019).

Functions of the intestinal microbiome

The intestinal microbiome is one factor responsible for integrity and function of the gastrointestinal tract. The microbiome plays a role in the defense from pathogenic microorganisms, the stimulation of immune responses, the control of metabolic and trophic function of the epithelial cells and synthesis of vitamins and nutrients, bile acid synthesis, conjugation and reabsorption (Gasbarrini et al., 2007; Mai & Draganov, 2009; Nicholson et al., 2012). Furthermore, it also promotes the development and maintenance of gut sensory and motor functions such as the intestinal propulsive activity (Gasbarrini et al., 2007).

The gut barrier refers to a functional entity of the intestinal lumen consisting of a mucous layer, epithelial layer and the lamina propria (König et al., 2016). Within the mucous layer, the thickness and composition of the mucous affects the host defense against infiltration of micro-organisms into the intestinal epithelium (Cornick, Tawiahy, & Chadee, 2015; Cani, 2018). This mucous layer is produced by the goblet cells which secrete mucins. Bacteria contain adhesins that bind to the mucins and help prevent the bacteria from moving from the intestinal lumen into the body (Cornick et al., 2015). Tight junction (TJ) proteins connect the intestinal epithelial cells and regulate the way substances move between the cells (paracellular permeability) (König et al., 2016). These tight junction proteins play an important role in infection through a complex mechanism of disruption and reorganisation of the tight junction proteins, increasing the epithelial permeability and enabling pathogens to enter into the body (König et al., 2016). The intestinal epithelial and immune cells also have toll-like receptors (TLRs) which, when interacting with a pathogen, trigger a host of immune responses by

producing cytokines and activating the T-cells to regulate the release of glucose and lipids providing energy to fight inflammation (Yiu, Dorweiler, & Woo, 2017; Cani, 2018). Bacteria and their metabolites play an inherent part in immunity through the proliferation and differentiation of T cells such as regulatory T (Treg) and helper T cells (Th) and the production of Immunoglobulin A (IgA) and G (IgG) by the B cells (Tanaka & Nakayama, 2017, Wells et al., 2017; Cani, 2018).

In addition to the complex interaction between the gut microbiota and the immune system, the metabolites produced by the microbiota such as folate, indoles, secondary bile acids, trimethylamine-N-oxide (TMAO), but also neurotransmitters (eg. serotonin, gamma amino butyric acid), and short-chain fatty acids (SCFAs) stimulate host metabolism from a distance (Thursby & Juge, 2016; Cani, 2018). The SCFAs (eg. butyrate, propionate and acetate) are recognised by G-protein-coupled receptors on every human cell membrane. Stimulation of these receptors triggers the secretion of intestinal peptides involved in glucose metabolism of food intake and encourages enteroendocrine cells to produce key hormones (Cani, 2018; Hills et al., 2019). Propionate also regulates immune cells to produce antimicrobial factors and, therefore, may act as an immune regulator, including a reduction of cancer cell proliferation. Distinct microbial metabolites may play different roles, ranging from the regulation of glucose levels to immunomodulatory effects, in host metabolism. These mechanisms remain a key area for further research (Cani, 2018).

The intestinal microbiome in disease

The link between gut microbiotic composition, and other diseases has also been recognised (Musso, Gambini, & Cassader, 2010; Garrett et al., 2010; Hart & Ng, 2011). People with inflammatory bowel disease have a less diverse intestinal microbiome than healthy people (Garrett et al., 2010; Hart and Ng, 2011; Glassner, Abraham, & Quigley, 2020). Equally, the intestinal microbiome of people with obesity and type 2 diabetes compared to healthy individuals has been studied extensively, especially within the context of bariatric surgery which alters the intestinal microbiome (Musso et al., 2010). However, a recent systematic review cautions that no consistent difference was found between lean and obese people; although associations have been suggested, there is a lack of evidence of causality (Castaner et al., 2018). Further investigation into obesity and changes in the intestinal microbiome may be of importance in women with a high body mass index (BMI) who receive treatment for a gynaecological cancer.

Many confounding factors influence the gastrointestinal microbiome (Debelius et al., 2016; Zhernakova et al., 2016; Quigley, 2017) such as diet (Pal, Banerjee, & Ghosh, 2012; Thursby & Juge, 2016; Singh et al., 2017; Castaner et al., 2018; Hills et al., 2019), alcohol (Engen, Green, Voigt, Forsyth, & Keshavarzian, 2015; Meroni, Longo, & Dongiovanni, 2019), smoking (Capurso, & Lahner, 2017; Savin, Kivity, Yonath, & Yehuda, 2018; Huang & Shi, 2019), exercise (Cerdá et al., 2016; Allen et al., 2018), the use of antibiotics (Van Eyk & van Schalkwyk, 2012; Iizumi, Battaglia, Ruiz, & Perez Peres, 2017; Willmann et al., 2019), other medications such as proton pump inhibitors and metformin (Imhann et al., 2017; Le Bastard

et al., 2018; Maier et al., 2018) and stool consistency (Vandeputte et al., 2016; Tigchelaar et al., 2016; Gilbert & Alvery, 2016).

Diet and the intestinal microbiome

Dietary intake varies enormously between individuals and is also dependent on culture and local custom. For instance, a Western diet contains more fat; a Mediteranean diet is rich in olive oil, grains, fruit, vegetables and seafood, but low in meat and dairy products; a Japanese diet features a lot of rice, soy and fish; a rural African diet contains mostly fibre; and Australian diet is rich in meat but also dietary fibre. Geographical differences are reflected in the composition of the intestinal microbiome (Senghor, Sokhna, Ruimy, & Lagier, 2018). Carbohydrates (sugars and starches) are the main carbon and energy source for the gut microbiota (Senghor et al., 2018). Dietary fibre is a key nutrient for a healthy microbiome and bulks up the stool in the gastrointestinal tract which dilutes toxins, reduces pressure in the colon, shortens transit time and increases defaecation frequency (Conlon & Bird, 2015; Singh et al., 2017). Fibres can also increase faecal mass to a lesser degree by stimulating fermentation, which leads to bacterial proliferation and increased biomass through the production of SCFAs (Conlon & Bird, 2015; Senghor et al., 2018; Valdes, Walter, Segal, & Spector 2018). Dietary protein intake such as eggs, meat and nuts, provides amino acids required to maintain health as it stimulates carbohydrate metabolism and is a major source of nitrogen which bacteria use in the fermentation process producing SCFAs (Conlon & Bird, 2015; Senghor et al., 2018; Hills et al., 2019). Several lipids with biological activity are produced by bacteria, including lipopolysaccharide (LPS), a component of the cell wall of gram negative bacteria

that can cause tissue inflammation; this is increased by high fat diets which also influence bile acid metabolism (Conlon & Bird, 2015; Senghor et al, 2018). It is thought that long-term dietary habits shape the intestinal microbiome and that dietary changes can modify this, however, the long-term effects are yet to be determined (Senghor et al., 2018; Valdes et al., 2018).

In women treated for gynaecological cancer, dietary habits before, during or after treatment have only been described in three studies to date (Muls, Cirillo Sanches, Norton, & Czuber-Dochan, 2020) (Appendix 1) and none have been done in relation to the gut microbiome.

The use of probiotics has no effect on the intestinal microbiome in healthy people (Mayor, 2016; Singh et al., 2017) but its effect has been demonstrated in preventing and treating recurrent *Clostridium difficile* infections (Trick et al., 2018). Many studies have researched the potential of probiotics in reducing acute diarrhoea in patients receiving radiotherapy but results have been equivocal (Ciorba, Hallemeier, Stenson, & Parikh, 2015; Liu, Li, Shu, & Zhan, 2017).

Vitamin D is important for bone health but is also known to have immunomodulatory characteristics and deficiency impacts on the gut barrier attributing to translocation of endotoxins and inflammation (Luthold, Fernandes, Franco-de-Moraes, Folchetti, & Ferreira, 2017). The main source of vitamin D is exposure to sunlight but up to a fifth comes from dietary intake. In the UK, people of all age groups are reported to have a low vitamin D status, as indicated

by serum 25-hydroxyvitamin D (25[OH]D) concentrations below 25 nmol/L (Public Health England and Food Standards Agency, 2014). A recent study showed that vitamin D deficiency is linked to gastrointestinal symptoms such as bloating, diarrhoea, constipation, abdominal pain and that short-term supplementation of vitamin D in combination with Vitamin B can resolve those symptoms (Gominak, 2016). A study including 17 patients with ulcerative colitis (n= 9 inactive disease, n= 8 active disease) and 8 healthy volunteers showed that there was no difference in the diversity of the microbiome before supplementation with vitamin D between the groups. After 8 weeks of supplementation, there was still no difference in diversity but there was an increase in *Clostridium colinae* and Enterobacteriacae species across all groups and *Ruminococcus gnavus* was marginally decreased acros the participants. In patients with active disease, inflammation markers in the gut were improved following vitamin D supplementation (Garg et al., 2018). Although there is evidence to suggest the intestinal microbiome is responsive to vitamin D supplementation, the mechanisms remain unclear as highlighted in a systematic review including 14 studies with human participants (Waterhouse et al., 2018). There is also clinical evidence to support that adequate vitamin D levels or supplementation in those with low levels reduces the risk of developing gynaecological cancer, but the molecular pathways remain poorly understood (Deuster, Jeschke, Ye, Mahner, & Czogalla, 2017).

Caffeine intake through coffee or tea can stimulate colonic activity in some but not all people (Boekema, Samsom, van Berge Henegouwen, & Smout, 1999; Papakonstantinou et al., 2016). Studies have shown that coffee and tea can

increase *Bifidobacterium* in the intestinal microbiome (Jaquet, Rochat, Moulin, Cavin, & Bibiloni, 2009; Gurwara et al., 2019; Bond & Derbyshire, 2019).

Alcohol consumption, depending on the type of alcoholic beverage, modulates the gastric environment by breaking down the gastric barrier, changing gastric acid secretion and increasing the risk of *Helicobacter pylori* infection (Capurso, & Lahner, 2017). Several studies have shown that the impact of alcohol on the intestinal microbiome results in dysbiosis of the luminal microbiota with an increase in *Proteobacteria*, a decrease in *Bacteroidetes* and increased permeability of the epithelial gut barrier leading to translocation into the blood stream (Engen et al., 2015; Thursby & Juge, 2016; Capurso, & Lahner, 2017; Meroni et al., 2019). However, the consumption of red wine which contains polyphenols, has a beneficial effect by increasing the amount of *Enterococcus*, *Bacteriodetes, Bifidobacterium* (Engen et al., 2015; Capurso, & Lahner, 2017) and *Faecalobacterium Prausnitzii* (Zhernakova et al., 2016). In addition, red wine polyphenols inhibit the growth of *Clostridium* which is also linked to the development of colonic carcinoma (Guarner & Malagelada, 2003; Engen et al., 2015; Le Roy et al., 2019).

The impact of smoking on the intestinal microbiome

Cigarettes contain an enormous amount of chemical compounds which affect different organs and recent studies suggest that smoking may alter the periodontal, oesophageal, gastric and respiratory microbiomes (Capurso, & Lahner, 2017; Savin et al., 2018; Huang & Shi, 2019). The exact mechanisms of how smoking alters the intestinal microbiome have not been established (Parkes,

Whelan, & Lindsay, 2014; Savin et al., 2018) but cigarette smoking may influence the microbiome via changes to immune homeostasis, biofilm formation, oxygen tension, or through direct contact with microbes within it (Huang & Shi, 2019). Changes in gastric acidity due to smoking may explain a higher rate of *Helicobacter pylori* eradication failure and may further contribute to alterations in the intestinal microbiome (Capurso, & Lahner, 2017). An increased risk of *Clostridium difficile* infection has also been observed in smokers (Capurso, & Lahner, 2017; Savin et al., 2018). Current smokers show a higher relative abundance of *Bacteroidetes* and a lower relative abundance of *Firmicutes* (Parkes et al., 2014; Lee et al., 2018). In the small and large intestine, smoking cessation induced increased diversity in the microbiome with an increase of *Firmicutes* and *Acinetobacteria* and a decrease in *Bacteroidetes* and *Proteobacteria* (Conlon & Bird, 2015; Capurso, & Lahner, 2017; Savin et al., 2018). A recent study comparing cigarette smoking to using electronic cigarettes (vaping) showed that cigarette smokers had significantly different bacterial profiles when compared to non-smokers and electronic cigarette users both in stool and oral swabs (Stewart et al., 2018). The most significant associations were found in the gut, with a higher relative abundance of *Prevotella* ($p=0.006$) and lowered *Bacteroides* ($p= 0.036$) in tobacco smokers. The alpha-diversity was also significantly reduced ($p= 0.009$) in stool samples collected from cigarette smokers compared to non-smokers indicating a lower diversity (Stewart et al., 2018).

Physical activity and the intestinal microbiome

Guidelines for physical activity in the UK recommend that adults should aim to be active daily. Over a week, activity should add up to at least 150 minutes (2½ hours) of moderate intensity activity in bouts of 10 minutes such as walking up stairs, carrying groceries, yoga or for instance by doing a brisk walk, cycling, ball room dancing for 30 minutes on at least 5 days a week (Department of Health UK, 2011). Whilst there are no guidelines specifically for people with cancer, the same recommendations are given (Cancer Research UK, 2015). Several studies have looked into the effect exercise has on the intestinal microbiome of lean and obese people (Allen et al., 2018) and found that whilst beta-diversity in the intestinal microbiome (the diversity between species in different people) was different between lean and obese people at baseline, this difference disappeared after a 6 week exercise programme of three 30 to 60 minute exercise sessions per week. However, no difference in alpha-diversity (the diversity in species within an individual) was found at any time. Most other studies have been done in animal models with the exception of studies examining the intestinal microbiome in elite athletes (Clarke et al., 2014; Petersen et al., 2017) which does not reflect physical activity levels of women undergoing treatment for a gynaecological malignancy.

The impact of medications on the intestinal microbiome

The most obvious medications to alter the intestinal microbiome are antibiotics. Whilst women with gynaecological cancer may be treated with antibiotics for a number of reasons, European guidelines advocate the use of prophylactic antibiotics to prevent or reduce the risk of subsequent infections in women

receiving a hysterectomy for malignant indications (Negoi et al., 2016). Surgery is the first intervention in women diagnosed with cancer of the endometrium and those with cervical cancer depending on staging (Saso et al., 2011; London Cancer Alliance, 2014). In addition, indwelling urinary catheters increase the risk of developing urinary tract infections post-surgery (Crosby-Nwaobi & Faithfull, 2011). Changes in the intestinal microbiome due to antibiotics can remain after long periods of time from months to years but the precise impact and duration until recovery of the intestinal microbiome following the use of antibiotics may be different for each individual depending to the variation in the intestinal microbiome before treatment (Van Eyk & van Schalkwyk, 2012; Macfarlane, 2014; Abeles et al., 2016; Francino, 2016; Thursby & Juge, 2016). There are different patterns in changes in the intestinal microbiome manifested by the class of antibiotic, dose and duration of treatment (Iizumi et al., 2017; Willmann et al., 2019).

Many other commonly used medications such as proton pump inhibotors (PPIs), anti-depressants (ie. selective serotonin inhibitors (SSRIs), statins, anti-diabetic medications, non-steroidal anti-inflammatories (NSAIDs) and opioids have been shown to have an impact on the intestinal microbiome (Le Bastard et al., 2018; Maier et al., 2018). For most, the exact mechanisms of action remain elusive. Considering that many people are on several long-term medications when also being diagnosed and treated for cancer, this requires further understanding and consideration.

Proton Pump Inhibitors (PPIs) are the most prescribed medication world-wide (Jackson et al. 2015; Imhann et al., 2017; Le Bastard et al., 2018; Shi et al., 2019). The use of PPIs for the treatment of gastro-oesophageal reflux disease (GORD), functional dyspepsia and prevention of gastric ulceration is associated with an increased risk of infection with *Clostridium difficile, Campylobacter, Salmonella* and *Shigella* (Jackson et al., 2015; Imhann et al., 2017; Le Bastard et al., 2018; Takagi et al., 2018). PPIs reduce gastric acid and raise pH hence making the gastric barrier more prone to become a growing ground for pathogenic microbial species which would otherwise not survive (Imhann et al., 2017; Le Bastard et al., 2018). The use of PPIs has been associated with an increased risk of cardiovascular disease which may indicate that PPIs could also affect bile acid and lipid metabolism (Imhann et al., 2017). It has been indicated that there is a significantly lower diversity in the microbiota of PPI users compared to those not using the drug (Jackson et al., 2015; Shi et al., 2019). However, a study in Japan analysing the intestinal microbiome of 36 PPI users and 36 healthy volunteers did not show any statistically significant differences in the composition of the intestinal microbiome when comparing the two groups and controlling for age and gender (Takagi et al., 2018). However, the study did not describe the use of any other medications by the participants.

Metformin remains one of the main treatments used in type 2 diabetes mellitus (Imhann et al., 2017; Pollak, 2017; Wu et al., 2017; Le Bastard et al., 2018). Although the mechanisms of how metformin influences the intestinal microbiome remain unclear, the use of metformin has been shown to decrease the abundance of *Intestinibacter* and increases butyrate production. This in turn, impacts on

glucose and carbohydrate metabolism and may include changes in bile acid metabolism, enteric hormones, reduced oxidative phosphorylation which results in AMP- activated protein kinase (AMPK) reducing energy consumption (Pollak, 2017; Wu et al., 2017). Furthermore, AMPK activation is implied in T cell memory and hence immune function (Pollak, 2017; Wu et al., 2017; Le Bastard et al., 2018).

The impact of intestinal transit time and stool consistency on the intestinal microbiome

Transit time within the intestine affects nutrient and fluid absorption and determines the clearance of the microbial population during defaecation (Lewis & Heaton, 1997; Gilbert & Alvery, 2016). Investigating the intestinal microbiome in 53 healthy women, a negative correlation was found in the richness of the species between stool consistency types; harder stool consistency (type 1-3) was associated with a richer microbiome, looser stool with less diversity (Vandeputte et al., 2016). A larger study with 1126 participants did however not confirm the association between stool consistency and richness of species (Tigchelaar et al., 2016). Another study showed that long colonic transit was associated with increased microbial α-diversity but not with stool consistency (Müller et al., 2020).

The intestinal oncobiome

The interplay between the human microbiome and cancer development has been coined the oncobiome although its significance is currently unclear (Sekirov, Russell, Antunes, & Finlay, 2010; Schwabe & Jobin, 2013; Thomas & Jobin, 2015;

Astudillo-de la Vega, Alonso-Luna, Ali-Pérez, López-Camarillo, & Ruiz-Garcia, 2019). The intestinal microbiome may also affect cancer by promoting oestrogen metabolism to increase the generation of estradiol, thereby promoting oestrogen-driven malignancies such as endometrial cancer (Berger, 2014). In addition, the metabolistic abilities of the gut microbiota can influence drug metabolism such as chemotherapeutic agents (cisplatin) used to treat gynaecological malignancies (Roy & Trinchieri, 2017). Furthermore, the intestinal microbiome may undergo significant changes during treatment with chemotherapy (Zwielehner et al., 2011; Alexander, Kohoutova, & Powell, 2019) and pelvic radiation (Ferreira, Muls, Dearnaley, & Andreyev, 2014; Touchefeu et al., 2014; Chase et al., 2015; Alexander et al., 2019). Two studies showed that the intestinal microbiome has a high diversity in healthy people and a low diversity in people with diarrhoea after pelvic radiotherapy (Manichanh et al., 2008; Floch, 2010). Currently, a large European study aims to identify and validate core or cancer-specific Gut OncoMicrobiome Signatures (GOMS) associated with cancer occurrence, prognosis, response to, or progression on, therapy or adverse effects (CORDIS, 2020).

Whilst the focus of analysing the gut microbiota in general remains mainly on the bacterial species, recent studies also recognise the need to explore the role of viruses and fungi (Cadwell, 2015; Lynch & Pedersen, 2016; Young, 2017; Cani, 2018). A further limitation of our current understanding includes the fact that most of the studies have been conducted in developed countries so there may be a bias towards the 'Western' microbiome.

1.3.4 The vaginal microbiome

A healthy vaginal microbiome is typically populated by aerobic members of the Firmicutes phylum, dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jensenii* (Ravel et al., 2011). In contrast to the intestinal microbiome, a healthy vaginal microbiome is low in diversity. Alterations in the vaginal microbiome lead to symptomatic conditions such as bacterial vaginosis with a reduction of vaginal lactobacilli and an overgrowth of other anaerobic bacteria which are normally not present (Chase, Goulder, Zenhousen, Monk, & Herbst-Kralovetz, 2015; van de Wijgert et al., 2015; Kovachev, 2020). Altered communities of micro-organisms in the vagina are not only implicated in septic postpartum and neonatal infections but also in pelvic inflammatory disease (Taylor, Darville, & Haggerty, 2013), increased HIV acquisition and onward transmission (Hayes, Watson-Jones, Celum, van de Wijgert, & Wasserheit, 2010). A recent systematic review concluded that women with less *Lactobacillus iners* had a higher prevalence of HPV, dysplasia and cancer compared to those with more *Lactobacillus crispatus* (Norenhag et al., 2020).

Research in the emerging field of the microbiome is currently biased towards the intestinal microbiome and only a little is known about interactions with the vaginal microbiome and the oral microbiome. Moreover, the intestinal virome has only recently started to be researched (Garmaeva et al., 2019; Shkoporov et al., 2019). This may be important in women with gynaecological cancer where HPV is a causal factor in the development of invasive cervical carcinoma and cancer treatment can induce immunosuppression.

The vaginal microbiome in women with gynaecological cancer

Several studies have examined the vaginal microbiome of women at different stages of gynaecological malignancy compared to that of women without cancer (Lee, So, Piyathilake, & Kim, 2013; Oh et al., 2015; Mitra et al., 2015; Audirac-Chalifour et al., 2016; Piyathilake et al., 2016; Kyrgiou, Mitra, & Moscicki, 2017). However, no studies reported long-term follow-up data to determine the role changes have in the vaginal microbiome in progression from pre-cancerous lesions to cancer diagnosis; only a few studies to date have examined changes in the vaginal or cervical microbiome in women undergoing treatment for gynaecological cancer (Colbert et al., 2018; Bai, Jhaney, Daniel, & Bruner, 2019; Tsementzi et al., 2020). These studies have been described in detail in Chapter three. Studies remain mostly focused on sequencing the microbiome, but its clinical relevance for using these techniques in isolation may be limited. Inclusion of metabonomic analysis techniques offers additional avenues to develop risk stratification pathways and targeted treatment options for those experiencing long-term gastrointestinal toxicity following cancer treatment.

1.4 Metabolite production of gut microbiota

1.4.1 Volatile organic compounds

Normal gut microbiota produce gases from their metabolites (Volatile Organic Compounds or VOCs) that can be detected and analysed. VOCs are organic chemicals which derive from biological samples such as breath, faeces, saliva, sweat or urine and are gas phase biomarkers which reflect the metabolic condition of a person (Shirasu & Touhara, 2011; Arasaradnam, Covington, Harmston, & Nwokolo, 2014; Schmidt & Podmore, 2015).

The detection of specific chemical compounds that reflect changes in the metabolism of the gut microbiota which are involved in colonic fermentation processes enables the diagnosis of specific conditions. Sulphur containing compounds such as hydrogen sulphide, dimethyl disulphide, methyl disulphide and dimethyl trisulphide have been found to be the main odiroferous compounds of faecal gases for the diagnosis of gastrointestinal disease (Probert et al., 2009). Differences in the VOC profiles in urine and stool samples of people with ulcerative colitis, Crohn's disease and healthy people can be separated (Arasaradnam et al., 2014; Tiele, Wicaksono, Kansara, Arasaradnam, & Covington, 2020).

1.4.2 Techniques for analysis of Volatile Organic Compounds (VOCs)

There are a number of different analytical approaches to analyse volatile organic compounds (VOCs). At present, the gold standard is considered gas chromatography/mass spectrometry (GCMS), which combines a GC front-end for pre-separation followed by a MS detector. Though highly successful, these techniques have a number of limitations, in particular, the equipment is expensive and specialised laboratory facilities and highly trained staff are required to use the equipment and analysis programs. Therefore, these techniques are unlikely to be a viable option in day-to-day clinical practice (Arasaradnam et al., 2014; Wilson, 2018).There are many alternative techniques, however, two methods that have found extensive use for clinical applications include the electronic nose (e-nose) and Gas Chromatography- Ion Mobility Spectrometry (GC-IMS).

The electronic nose is an instrument that replicates the biological olfactory system, by analysing samples as a whole, rather than identifying specific chemicals, as with GCMS. These instruments are formed from an array of discrete sensors combined with a pattern recognition approach and were invented in the 1980s (Persaud & Dodd, 1982). The air above the sample (called the headspace) is drawn into the e-nose and passed over this array of chemical sensors. As each sensor in the array is different, its interaction with the odour is unique. When the response of all the sensors are brought together it creates a signature, which is identified by a pattern recognition algorithm. The size of the array varies, but all commercial instruments vary between 6 and 32 sensors (Covington, Wedlake,

Andreyev, & Ouaret, 2012). The PEN III airsense e-nose uses 18 sensors (Figure 1.1).

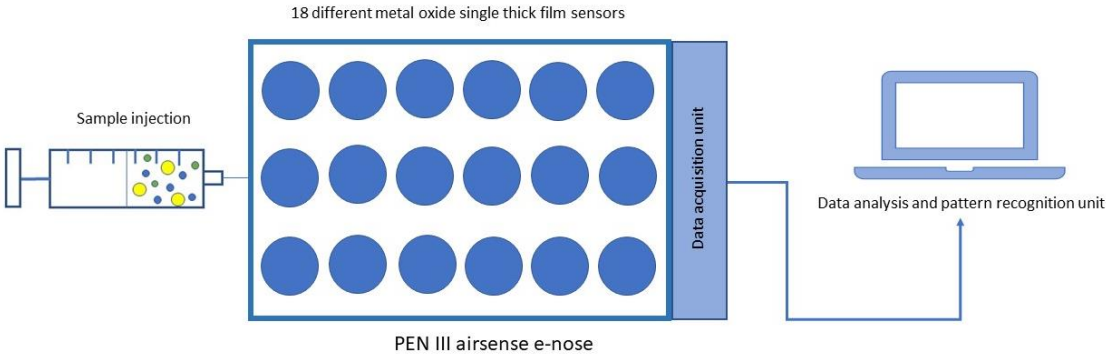


Figure 1.1 PEN III e-nose analysis.

Gas Chromatography- Ion Mobility Spectrometry (GC-IMS) is a newer technology capable of separating gas phase ions at atmospheric pressure and using air as the carrier gas. In GC-IMS (Figure 1.2), molecules are first separated by a gas chromatograph, which is formed of a long capillary column that separates molecules depending on their interaction with a thin coating on the inside of the column. This results in molecules eluding from the column one after the other. These molecules are then ionised using a radioactive source and then pushed along a drift tube by a high electric field. Against the drift of the ions, a buffer gas flows, where interactions between the ions and the gas flow slow the ions down. Thus, the time taken to exit the drift tube is based on the interaction of the ion with the electric field and the number of collisions to the buffer gas. GC-IMS systems are incredible sensitive (more than GCMS) and are also used extensively for security applications such as airports (Covington et al., 2012; Schmidt & Podmore, 2015; Vautz, Seifert, Liedtke, & Hein 2016; Gallegos, Arce, Jordano, Arce, & Medina, 2017).

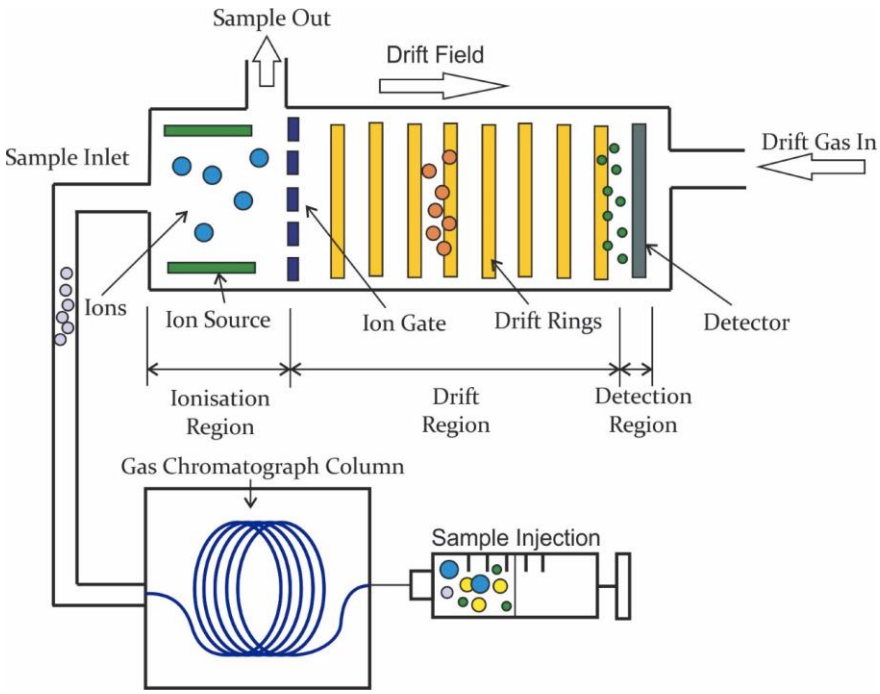


Figure 1.2 Gas Chromatography- Ion Mobility Spectometry (GC-IMS) analysis courtesy of Professor James Covington, Warwick University.

Focusing on gastrointestinal disorders, these techniques have been used successfully to identify consistent changes in the VOCs produced by patients with inflammatory bowel disease (Arasaradnam et al., 2014, Tiele et al., 2020), bile acid malabsorption (Covington et al., 2013; Sagar, Cree, Covington, & Arasaradnam, 2015), colorectal cancer (Arasaradnam et al., 2014), coeliac disease (Rouvroye et al., 2019), *Clostridium difficile* infection (Patel, Fowler, Sizer, & Walton, 2019) and differences can be identified compared to healthy individuals (Covington et al., 2012; Arasaradnam et al., 2014; Chan, 2018; Wilson, 2018).

A retrospective blinded pilot study using similar techniques to analyse stool samples collected before the start of radiotherapy and after completion of treatment from 23 patients treated for prostate cancer identified two completely

distinct groups from the samples taken before treatment: patients who would develop severe toxicity during treatment and those who would not (Covington et al., 2012). The presence of hydrogen sulphate was identified in patients with severe GI toxicity after treatment with pelvic radiation from sample taken before the start of radiotherapy and indicates that it could be possible to determine the risk of GI toxicity following radiotherapy (Arasaradnam et al., 2014). Whilst VOC analysis techniques have been used in many disease states as highlighted above, no other studies have applied this analysis technique to predict long-term GI toxicity following radiotherapy.

1.5 Risk stratification of patients at risk: implications for clinical practice

The risk factors for developing radiation toxicity are multi-factorial being both patient and treatment dependent. Treatment related risk factors include total cumulative dose, dose per fraction, treated volume, relative contribution from radiation modality (external beam or brachytherapy) and concomitant drugs, particularly chemotherapy. Tumours vary in terms of histology, anatomical location and genotype. Patient related factors include underlying co-morbidities (e.g. inflammatory bowel disease, connective tissue disorders), low body mass index, smoking, genomic make-up and age. Prior surgery increases the risk of bowel toxicity due to adhesions causing immobile bowel loops, while alteration of the vascular supply can induce hypoxia that then increases radio-resistance. Aside from a few genetic syndromes wherein hypersensitivity to radiation is well documented, such as ataxia-telangiectasia, an immunodeficiency disease, and

Nijmegen Breakage syndrome, a condition characterised by short stature syndrome, microcephaly, recurrent respiratory tract infection and increased risk of cancer, specific predictive biomarkers for radiation sensitivity have not yet been identified (Pollard & Gatti, 2009). However, several recent studies have demonstrated some promise with immunological markers (Sprung, Forrester, Siva, & Martin, 2015). The early tissue response is driven by a complex immune-mediated process involving interactions with the reactive oxygen species (ROS) and nitric oxide molecules (NOS) produced by the destructive effect of ionising radiation on double stranded DNA and hydrolysis of water. This results in secretion of damage associated molecular patterns involving pro-inflammatory cytokines and growth factors including interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF)-α and tumour growth factor (TGF)-β (Kim, Jenrow, & Brown, 2014). These factors modulate several intracellular signaling pathways and provide potential targets for mitigating radiation toxicity (Wedlake et al., 2013).

Chronic inflammatory and immune-modulated processes also drive long-term effects. Radiation-induced fibrosis in any tissue can cause significant clinical impact. TGF-β has been demonstrated as pivotal in up-regulation of myofibroblasts and indeed its own activation is reported to be radiation dose-dependent (Wynn & Ramalingam, 2015). Genetic studies have concluded that patients with higher numbers of TGF-β single nuclear polymorphisms may have a higher risk of radiation-induced fibrosis (Reuther et al., 2012).

Whilst these risk factors are taken into consideration when planning and treating patients, there is currently no model to predict what risk of developing long-term

bowel changes women have when treated with radiotherapy for gynaecological cancers. Risk stratification based on a combination of the factors above and new insights from the microbiome interactions may yield new approaches for early intervention and prevention of radiation induced GI toxicity.

1.6 Thesis statement and research hypotheses

1.6.1 Thesis statement

By analysing the metabolomic signatures of the intestinal microbiome and clinical data we may be able to risk-stratify women at risk of developing long-term gastrointestinal toxicity following treatment for gynaecological cancer so that radiation induced GI toxicity can be managed sooner and reduce symptom burden. Changes in the intestinal microbiome may herald new treatment options for long-term bowel changes and lessen the chronic symptom burden.

1.6.2 Research hypotheses

The following hypotheses have been formulated to provide a framework for this research:

1. The signatures created by the electronic sensing technique will show there is a difference in the emitted volatiles and gas profiles between cancer patients treated with pelvic radiotherapy who develop GI symptoms and those who do not. These signature profiles can be used to predict risk of GI toxicity.

2. Analysis of patient characteristics will elicit differences between cancer patients treated with pelvic radiotherapy who develop GI symptoms and those who do not.

1.7 Conclusion

This chapter has provided a background to current understanding regarding gastrointestinal toxicity and survivorship issues in women treated for a gynaecological malignancy and described current knowledge about the microbiome. Long-term GI toxicity in women treated with radiotherapy for gynaecological cancer can occur in over 40% of patients and can have a great impact on quality of life. A healthy gut microbiome is diverse in terms of the different species present, but each individual has a unique gut microbiotic composition. Many factors can influence the gut microbiome composition including diet, smoking, physical exercise, medications and specific disease states. A pilot study showed that VOC signatures from the gut microbiome can be used to detect changes in the signatures of those with and without long-term radiation induced GI toxicity from before the start of treatment.

Chapter two details the clinical context which forms the basis for this research and highlights the clinical importance of this research for women treated for gynaecological cancer experiencing long-term changes in gastrointestinal function following their treatment. Chapter three explores the current literature on how radiotherapy changes the gut microbiome in women treated for gynaecological cancer.

Chapter 2

The clinical setting: a longitudinal cohort of women treated for gynaecological cancer attending the GI and Nutrition Team Service.

This chapter describes the clinical context of the Royal Marsden Hospital GI and Nutrition Team service (GIANTs) which forms the basis for this research and highlights the clinical importance of this research for women treated for gynaecological cancer experiencing long-term changes in gastrointestinal function following their treatment. It describes a prospective cohort of 220 women who were treated for gynaecological cancer and accessed the service with long-term GI toxicity following cancer treatment.

2.1 Demographic and clinical characteristics

Between April 2013 and March 2016, 1117 patients were discharged from the GIANT service. Of those, 235 (21%) were women treated for gynaecological cancers. Fifteen women did not wish to have any further assessment or investigations and were excluded from this cohort leaving 220 reported here.

Women were referred from within the organisation (n= 94, 43%), from other centres (n= 77, 35%) or by their GP 22% (n=48). One person (<1%) referred herself to the service. The majority of women had been treated for cancer of the cervix (n= 109, 50%) and endometrium (n= 62, 28%), followed by ovarian (n= 33, 15%), vaginal or vulvar (n= 16, 7%) cancer. The median age was 57 years (range: 24-83). The median time between cancer diagnosis and referral to service was 4 years and 10 months (range: 6 months - 47.5 years). 10% of the women had been seen previously and were re-referred. Several women received multimodal therapies (Table 2.1).

Cancer treatment	Cervical cancer n (%)	Endometrial cancer n (%)	Ovarian cancer n (%)	Vaginal/ vulvarcancer n (%)	Total n (%)
Chemotherapy alone	11	1	8	2	22 (10%)
Radiotherapy alone	36	13	1	2	52 (24%)
Surgery alone	10	16	11	2	39 (18%)
CXT + RT (no surgery)	24	1	0	3	28 (13%)
CXT + surgery (no RT)	0	0	8	3	11 (5%)
RT + surgery (no CXT)	12	18	0	1	31 (14%)
CXT + RT + surgery	16	13	5	3	37 (17%)
TOTAL	109 (50%)	62 (28%)	33 (15%)	16 (7%)	220 (100%)

Table 2.1 Cancer treatment regimens per cancer diagnosis group (n= 220) (CXT, chemotherapy; RT, radiotherapy) (Muls et al., 2020)

2.2 Assessment of GI toxicity and impact on quality of life

At each clinic visit, people attending self-reported the presence and severity of their symptoms using a modified Gastrointestinal Symptom Rating Scale (GSRS). The GSRS (Appendix 2) has been previously validated for use in those with gastro-oesophageal reflux disease, peptic ulcer disease, those who have undergone GI surgery (e.g. pancreatectomy), coeliac disease, chronic intestinal pseudo-obstruction, chronic non-specific abdominal complaints and irritable bowel syndrome (Dimenäs et al., 1993; Revicki, Wood, Wiklund, & Crawley, 1998; Svedlund, Sjödin, & Dotevall, 1998; Lohiniemi, Mäki, Kaukinen, Laippala, & Collin, 2000; Van den Heuvel-Janssen et al. 2006; Iwarzon, Gardulf, & Lindberg, 2009; Rashid & Velanovich, 2012). Patients also indicated the frequency of bowel movements and the consistency of their stool using a Bristol Stool Chart (Lewis & Heaton, 1997). In addition, they scored their perceived Quality of Life (QoL) and the impact of their GI symptoms on a Visual Analogue Scale (VAS) with 0 indicating the worst possible QoL and 10 equating the best possible QoL.

A holistic needs assessment (HNA) was also offered to all patients attending our service. The HNA (Appendix 3) is part of the cancer recovery package and comprises of a questionnaire covering an array of potential areas which may be a concern for anyone living with and beyond cancer (NHS England, 2016). Practical, emotional, spiritual, family issues can be identified as a concern to discuss with a qualified health care professional alongside 22 physical concerns and any other medical condition.

2.3 Results

The most prevalent gastrointestinal symptoms at assessment rated as severe included increased frequency of defaecation (88%), diarrhoea (36%), urgency (31%) and incontinence (21%). In addition, many women also reported fatigue (87%), urinary problems (53%) and sexual concerns (38%) (Figure 2.1). In this cohort, 71% of these women completed a holistic needs assessment. The distress thermometer (part of the HNA) showed a high level of anxiety (62%) (Figure 2.2) and indicated that women scored a median of 5 on a scale from 0 ("I am not distressed") to 10 ("I am extremely distressed"). Thirty-five percent of women scored more than 7, justifying a psychological support service referral.

A median of 8 investigations (range: 1-14) were requested for this cohort using our algorithm version 10 (Andreyev et al., 2016). These included a blood screen (n = 213; 97%), gastroscopy (n = 178; 80%) with duodenal aspirate (n = 168; 76%), glucose hydrogen methane breath test (n = 177; 80%), SeHCAT scan (n = 161; 73%), stool sample for faecal elastase (n = 152; 69%), flexible sigmoidoscopy (n = 114; 52%), colonoscopy (n = 56; 25%) and abdominal X-ray (n = 35; 16%).

These investigations revealed a median of four factors (range: 1-9) possibly contributing to symptoms with 68% of women having more than three. The most common diagnoses were vitamin D deficiency (n= 133, 60%), small intestinal bacterial overgrowth (SIBO) (n= 118, 54%), and bile acid malabsorption (n= 104, 52%).

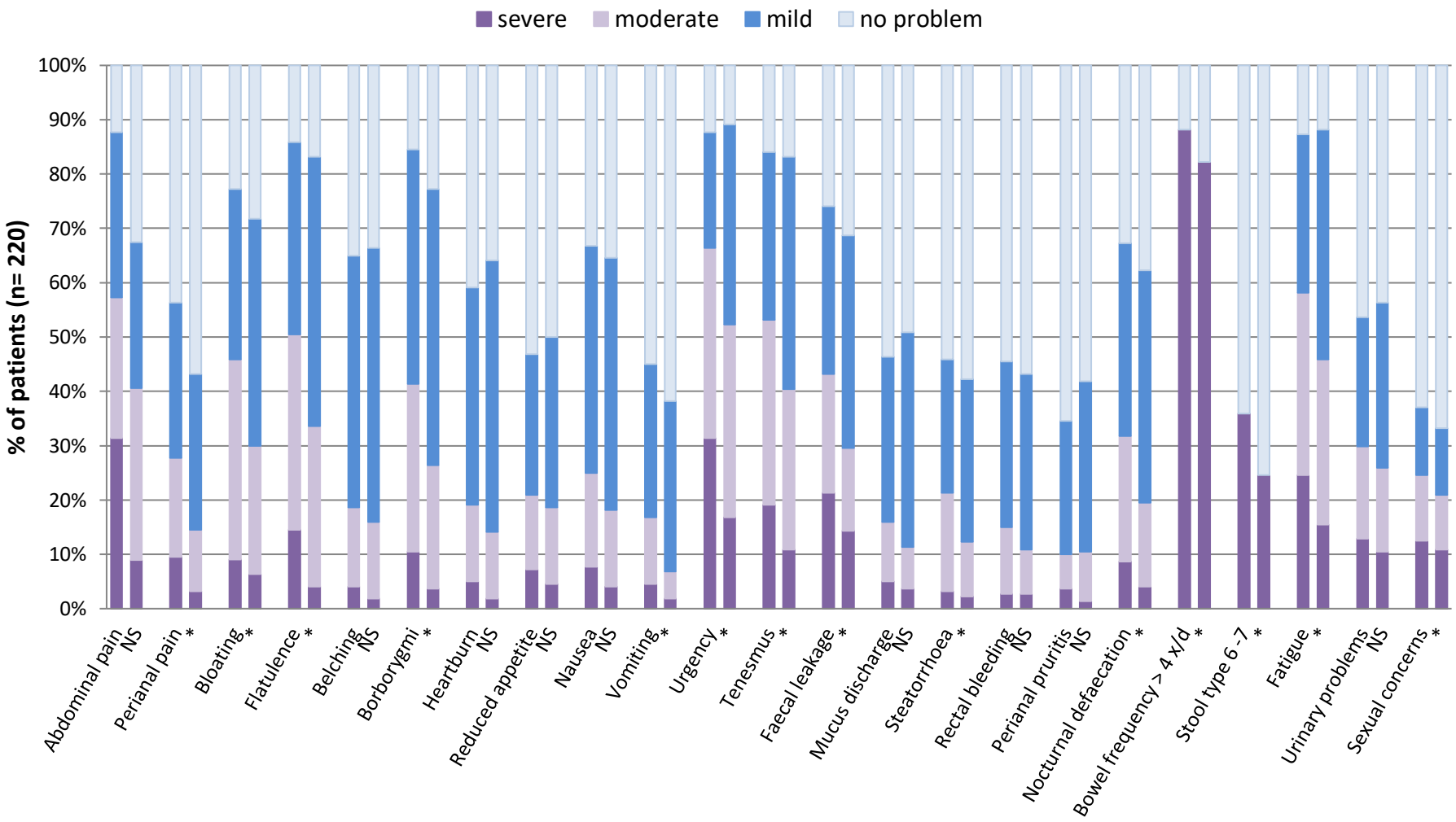


Figure 2.1. Paired symptom scores (n= 220) at baseline and discharge; left bar = baseline assessment, right bar = discharge assessment. (NS= not significant, * = p< 0.05) (Muls et al., 2020)

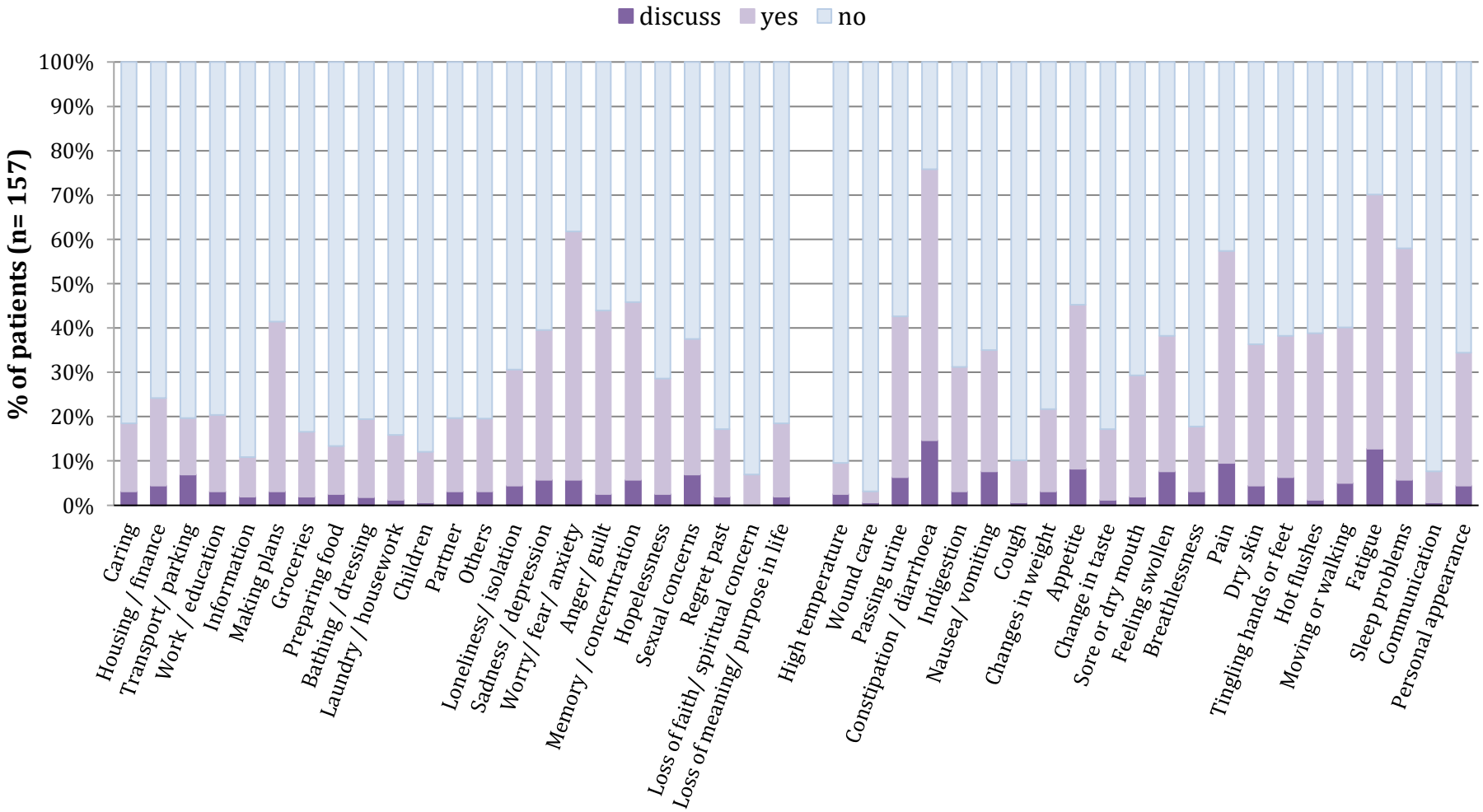


Figure 2.2. Concerns highlighted by women treated for gynaecological malignancy on the holistic needs assessment (n= 157) (Muls et al., 2020)

Gastritis was found in a third of women (n= 68, 31%), vitamin B12 deficiency in 30% (n= 65), weak pelvic floor musculature in 21% (n= 36), telangiectasia on the rectal wall in 15% (n= 33), trace element deficiency in 14% (n= 14), and a new bowel polyp in 11% (n= 24). Less common diagnoses included: hiatus hernia (10%), faecal loading (confirmed by abdominal Xray) (8%), oesophagitis (7%), iron deficiency (7%), pancreatic insufficiency (7%), thyroid problems (5%), gastro-oesophageal reflux disease (4%), haemorrhoids (3%), rectal ulcer (2%), a new GI cancer (2%) and anal fissure (1%) (Table 2.2).

The median number of consultations was 4 (range: 1-17) with 90% requiring more than one visit. After treating contributing GI pathologies, symptom burden was reduced at discharge with 12 gastrointestinal symptoms improving with statistical significance (Table 2.3) and general quality of life improved from a median of 4 at first assessment to a median of 6 at discharge ($p < 0.001$).

Diagnosis	Prevalence, n (%)
Vitamin D deficiency	133 (60%)
Small intestinal bacterial overgrowth	118 (54%)
Bile acid malabsorption	104 (47%)
Gastritis	68 (31%)
Vitamin B12 deficiency	65 (30%)
Weak pelvic floor musculature on rectal exam	36 (21%)
Telangiectasia on the rectal wall	33 (15%)
Trace element deficiency	31 (14%)
New GI polyp	24 (11%)
Hiatus hernia	22 (10%)
Faecal loading (confirmed on abdominal Xray)	18 (8%)
Dietary fibre excess on fibre quiz	16 (7%)
Oesophagitis	16 (7%)
Iron deficiency	16 (7%)
Pancreatic insufficiency	16 (7%)
Duodenitis	13 (6%)
Thyroid problems	11 (5%)
Diverticular disease	9 (4%)
Gastro-oesophageal reflux disease	8 (4%)
Haemorrhoids	7 (3%)
Inflammatory bowel disease	4 (2%)
Rectal ulcer	4 (2%)
New GI cancer	5 (2%)
Anal fissure/ anal sphincter defect	3 (1%)

Table 2.2 Prevalence of new gastrointestinal or nutritional diagnoses identified following assessment in clinic (n= 220) (Muls et al., 2020)

Symptoms	Baseline (n=220)	Discharge (n=220)	p-value
	n (%)	n (%)	
Perianal pain			
no problem	96 (44)	125 (57)	
mild	63 (29)	63 (29)	<0.001
moderate	40 (18)	25 (11)	
severe	21 (10)	7 (3)	
Bloating			
no problem	50 (23)	62 (28)	
mild	69 (31)	92 (42)	<0.001
moderate	81 (37)	52 (24)	
severe	20 (9)	14 (6)	
Flatulence			
no problem	31 (14)	37 (17)	
mild	78 (35)	109(50)	<0.001
moderate	79 (36)	65 (30)	
severe	32 (15)	9 (4)	
Borborygmi			
no problem	34 (15)	50 (23)	
mild	95 (43)	112 (51)	<0.001
moderate	68 (31)	50 (23)	
severe	23 (10)	8 (4)	
Vomiting			
no problem	121 (55)	136 (62)	
mild	62 (28)	69 (31)	0.002
moderate	27 (12)	11 (5)	
severe	10 (5)	4(2)	
Urgency			
no problem	27 (12)	24 (11)	
mild	47 (21)	81 (37)	< 0.001
moderate	77 (35)	78 (35)	
severe	69 (31)	37 (17)	
Tenesmus			
no problem	35 (16)	37 (17)	0.003
mild	68 (31)	94 (43)	
moderate	75 (34)	65 (30)	
severe	42 (19)	24 (10)	
Faecal incontinence			
no problem	50 (23)	60 (28)	
mild	59 (27)	85 (39)	< 0.001
moderate	58 (26)	45 (20)	
severe	53 (24)	30 (14)	
Steatorrhoea			
no problem	119 (54)	127 (58)	
mild	54 (25)	66 (30)	0.045
moderate	40 (18)	22 (10)	
severe	7 (3)	5 (2)	

Table 2.3 Symptoms which improved at a statistically significant level ($p < 0.05$). (Muls et al., 2020)

Symptoms (Cont.)	Baseline (n=220)	Discharge (n=220)	p-value
	n (%)	n (%)	
Nocturnal defaecation			
no problem	72 (33)	83 (38)	
mild	78 (35)	94 (43)	<0.001
moderate	51 (23)	34 (15)	
severe	19 (9)	9 (4)	
Bowel frequency > 4x/day			
No	26 (12)	39 (18)	0.009
Yes	194 (88)	181 (82)	
Diarrhoea (type 6 or 7)			
No	142 (65)	165 (75)	0.013
Yes	78 (35)	55 (25)	
Fatigue			
no problem	28 (13)	26 (12)	
mild	64 (29)	93 (42)	0.001
moderate	74 (34)	67 (30)	
severe	54 (26)	34 (15)	
Sexual concerns			
no problem	137 (62)	147 (67)	
mild	28 (13)	27 (12)	0.004
moderate	27 (12)	22 (10)	
severe	28 (13)	24 (11)	

Table 2.3 Cont. (Muls et al., 2020)

2.4 Clinical importance

At the time of this study, women were referred to a specialist team at the earliest one year after completion of radiotherapy due to the current follow-up pathway but in reality, the median time between cancer diagnosis and referral to our specialist service for women treated for a gynaecological malignancy was 4.8 years (range: 0.5 - 47.5).

The ability to stratify those at risk would lead to proactive identification and more intensive follow-up for those at risk with early intervention to manage GI toxicity and reduce or prevent GI symptom burden.

These findings were presented as a poster presentation at the British Society of Gastroenterology Annual meeting in 2018 (Appendix 4) and published with a proposed tailored investigational algorithm for women treated for gynaecological cancer with long-term gastrointestinal consequences (Muls et al., 2020) (Appendix 5).

2.5 Conclusion

This chapter has described the clinical context which forms the basis for this research and highlights the clinical importance of this research for women treated for gynaecological cancer experiencing long-term changes in gastrointestinal function following their treatment. It showed there was a high GI symptom burden and that QoL improved following appropriate management of underlying treatment induced GI pathology.

Chapter three will discuss a systematic review of the literature regarding current understanding of the intestinal and vaginal microbiome in women receiving treatment for gynaecological cancer.

Chapter 3

Literature review of studies exploring the gut and vaginal microbiome in women treated for a gynaecological malignancy.

This chapter describes a systematic literature review undertaken to support evidence for this research study regarding current understanding of the intestinal and vaginal microbiome in women undergoing treatment for gynaecological cancer. The original systematic review was published in September 2017 (Appendix 6) and updated in July 2020.

3.1 Introduction

A systematic literature review was conducted to identify current studies exploring the intestinal and vaginal microbiome in women treated for a gynaecological malignancy. This systematic review used the PRISMA guidelines to ensure transparent and complete reporting (Appendix 7) (Liberati et al., 2009). The review protocol was registered on the International PROSPERO review database (Muls, Norton, & Hart, 2016).

3.2 Aim

The main aim of the systematic literature review was to explore current understanding of the impact of cancer treatment (surgery, chemotherapy, radiotherapy) on the gut and vaginal microbiome in women with a gynaecological malignancy.

3.3 Methods applied in conducting the review

3.3.1 Search strategy

Suitable studies for this review were identified by the primary author (AM) searching the following databases to ensure all potential sources of published evidence were covered: Embase <1974 to 2016 August 16>, Global Health <1973 to 2016 Week 31>, HMIC Health Management Information Consortium <1979 to July 2016>, Journals@Ovid Full Text <August 16, 2016>, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to August, 2016>Ovid Medline database. In addition, the PubMed database was searched. Both the PROSPERO database and Cochrane Library were searched for existing similar systematic reviews, with none found. The search was repeated on the 08th of July 2020 with the time limit 2016- current (see appendix 8).

To ensure that the search was comprehensive and any relevant research had not been missed or wrongly excluded from the electronic database search, searches on general search engines, and hand searches of the reference lists of included papers were conducted. Contact was also be made with the authors of the papers when further information was required.

The main search terms included: gynaecological cancer, gynaecological malignancy, gut microbiota, intestinal microbiota, microbiome, chemotherapy, radiotherapy. Figure 3.1 shows the details of the search conducted.

3.3.2 Inclusion and exclusion criteria

The inclusion criteria were defined using the following components:

Patient population (P): women treated for a gynaecological malignancy.

The exposure of interest (I): cancer treatment: surgery, chemotherapy or radiotherapy.

The comparator (C): before and after treatment for gynaecological cancer (ie, surgery, chemotherapy or radiotherapy).

The outcome (O): the change in the gut or vaginal microbiome following treatment for gynaecological cancer.

The study designs (S) of interest: randomised controlled trials (RCTs), prospective observational cohort studies and retrospective studies.

Animal studies were excluded. Case reports or studies including single subjects relevant to this review, expert opinion that included narrative literature reviews and consensus statements were excluded. Due to recent advances in the delivery of treatment (specifically radiotherapy) and the development of new techniques to analyse the microbiome, studies using older techniques such as conventional cultures were excluded to ensure the relevance to current practice in this review.

As a systematic review protocol assessing the impact of probiotics for the prevention or treatment of chemotherapy or radiotherapy related diarrhoea in cancer patients (Wei et al., 2018) has been published by the Cochrane

collaboration, studies including the use of pre- or probiotics as an intervention were also excluded.

3.3.3 Management of review process

The primary researcher (AM) independently conducted an initial screening of titles based on the eligibility criteria. The abstracts for the titles that could not be excluded were obtained and independently examined against the inclusion criteria by the primary reviewer (AM). The full papers for the abstracts that could not be excluded based on the eligibility criteria, were obtained and independently examined against the inclusion criteria by the primary reviewer (AM). Any concerns were discussed with the review team (AH, CN), and a majority decision reached. The list of included studies was discussed with the review team. For the update, AM conducted the search, screened and reviewed suitable papers for inclusion. Full papers of multiple reports of a study were collated to ensure that each study, as opposed to each paper, was the unit of interest in the review. No studies were excluded due to a lack of information where a decision about inclusion or exclusion could not be reached. The primary reviewer contacted the corresponding authors of two papers and one abstract for more information but none responded with additional files. One informed the reviewer that a full paper was in preparation for publication and this paper was published in April 2020 (Tsementzi et al., 2020). Any disagreements were discussed in accordance with the protocol. The study selection process was piloted in a sample of retrieved papers to ensure that the

inclusion and exclusion criteria could be interpreted reliably and consistently, and that it appropriately classified the studies.

The primary reviewer (AM) extracted the data from studies that meet the inclusion criteria onto a data extraction form designed and piloted for this review. Extracted information for quantitative studies included, but was not limited to, method, participants, intervention, and outcomes. As it was expected that the majority of studies would consist of observational cohort studies, the papers were critically appraised using the Critical Appraisal Skills Programme study checklist for observational cohort studies (Critical Appraisal Skills Programme (CASP, 2014; Table 2). The final results of the original review were also reviewed by the collaborators (AT, SL and JA) prior to submission for publication (Muls, Andrevey, Lalondrelle, Taylor, Norton, & Hart, 2017) (Appendix 9).

Study selection

In addition to the three papers identified in the original systematic review, eight new papers were included in the update.

Study assessment

As all studies were prospective cohort studies, these are primary reports within the classification category B (A: randomised controlled trials, B: primary reports; C: non-randomised controlled/ crossover trials, case control studies, D: non-controlled trials, case reports, observational studies) (Handu et al., 2016). All studies received a CASP quality score out of a maximum of 12 and a grading

of the level of evidence: grade I: strong evidence, grade II: fair evidence, grade III: limited or weak evidence, grade IV: expert opinion only or grade V: grade not assignable (Handu et al., 2016).

3.3.4 Suitability of data for meta-analysis

Due to the small number of studies found in the original review meta-analysis was not performed as a part of this systematic review and thus a narrative review is presented.

3.3.5 Summary of results of the original systematic review

Three original included studies (Table 3.1 and 3.2) were identified using sequencing of changes in the gut microbiome in women treated with radiotherapy for a gynaecological malignancy. All were prospective cohort studies and described a total of 23 women with gynaecological cancer.

All three studies included patients treated with radiotherapy. The radiotherapy dosage ranged from 43.0 to 54.0 Gy across the studies but did not provide specific information about radiotherapy fields, the use of boosts or additional brachytherapy, making comparison difficult. One study included 7 women who received concomittent chemoradiation (Nam, Kim, Seo, Kang, & Bae, 2013). Whilst all studies acknowledge that radiotherapy may result in toxicity, Nam et al. (2013) did not assess any grade of gastrointestinal toxicity in the participants. In the two other studies, the Common Toxicity Criteria version 0.2

(Manichanh et al., 2008) and the Common Terminology Criteria for Adverse Events version 0.3 (Wang et al., 2015) were used to assess bowel function. Where stool consistency was assessed, 8 women (57%) developed grade 2 or 3 diarrhoea during radiotherapy.

The outcomes of the three studies suggested that there is a correlation between changes in the intestinal microbiome and receiving radiotherapy. All studies yielded similar results and showed a decrease in abundance and diversity of the intestinal bacterial species. One study identified a decrease in *Firmicutes* by 10% ($p=0.09$) and an increase in *Fusobacteria* by 3% ($p=0.05$) and unclassified bacteria by 9.9% ($p=0.04$) during radiotherapy (Nam et al., 2013). An increase in unspecified bacterial species was seen in those who developed diarrhoea but patients without diarrhoea maintained their bacterial profiles (Manichanh, 2008; Wang et al., 2015). More specifically, prior to radiotherapy, compared to patients who did not develop diarrhoea, those who did had an increased abundance of *Bacteroides, Dialister, Veillonella* ($p<0.01$), and a decreased abundance of *Clostridium XI* and *XVIII, Faecalibacterium, Oscillibacter, Parabacteroides, Prevotella* and unclassified bacteria ($p<0.05$) (Wang et al., 2015).

Reference	Type of study	Number of patients with gynaecological tumour types	Number of healthy volunteers as control	Surgery	Chemotherapy type defined	Radiotherapy dose (Gy)	Analysis technique microbiome	Type of sample	Diarrhoea defined	No of patients reporting diarrhoea	Before- after design	Number of follow-up time points	Latest time point after radiotherapy	Antibiotic use as exclusion ?	Steroid use as exclusion?	Immunotherapy as exclusion?
Manichanh et al., 2008	Prospective cohort	6 cervix (n=1) uterus (n=1) endometrium (n=4)	4	?	N/A	43-54	16S rRNA sequencing	Stool	Yes CTC v0.2	4	Yes	4	2 weeks	Yes	Yes	Yes
Nam et al., 2013	Prospective cohort	9 cervix (n=7) endometrium (n=2)	6	?	Yes	50.4	16S rRNA sequencing	Stool	No	-	Yes	4	1-3 months	Yes	Yes	Yes
Wang et al., 2015	Prospective cohort	8 cervix (n=8)	4	?	Excl	44-50	16S rRNA sequencing	Stool	Yes CTCAE v0.3	4	Yes	2	3-5 weeks	Yes	Yes	Yes

Table 3.1 Original studies describing changes in the gut or vaginal microbiome in women treated with radiotherapy for a gynaecological malignancy (Muls et al., 2017)

Author / year Study design	Class of evidence	CASP score	Quality grade	Population studied and sample size	Primary outcome measures	Conclusions
Manichanh et al., 2008 Prospective cohort	B	6/11	III Limited/ weak	Setting: Spain Sample: • 10 cancer patients • 5 healthy volunteers • Mixed tumour groups: Gynaecological cancer (n=6) + lower GI cancer (n=4)	Outcome measures: Stability and diversity of faecal microbiota before and after RT using similarity index and cluster analysis Comparison with healthy individuals Sub-analysis of those with and without diarrhoea GI toxicity: CTC v 0.2	Mixed tumour type + mixed gender cohort, use of different radiotherapy fields, unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period. Results do not include any missing data or drop-out rates which may reflect selective reporting.
	Main findings	Patients without diarrhoea maintained their microbiotic profile for 60% during RT Healthy controls and patients with no diarrhoea maintained their bacterial profiles with a similarity near 60% over the 7-wk time course. Patients with diarrhoea showed a significantly modified bacterial profile ($p < 0.05$) after the beginning of the radiotherapy (49% of similarity, SD = 17), at the end of the radiotherapy (29% similarity, SD = 17), and 2 wk after (35% similarity, SD = 15) with the detection of *actinobacteria* + increase in *firmicutes* (bacilli). Patients that develop diarrhoea clustered separately from those that did not develop diarrhoea using the Pearson's correlation and the unweighted pair-group method using arithmetic averages (UPGMA).					
Nam et al., 2013 Prospective cohort	B	7/11	III Limited/ weak	Setting: South Korea Sample: • 9 cancer patients • 6 healthy volunteers • single tumour group: gynaecological cancer (n=9)	Outcome measures: Stability and diversity of faecal microbiota before and after RT using ANOVA for difference in OTUs and principal coordinates analysis plots Comparison with healthy individuals GI toxicity: not formally measured, discussion section mentions 8/9 patients developed diarrhoea	Single tumour type in cohort. Use of chemotherapy in 7/9 patients. No information of eligibility, selection of participants, recruitment period. No formal measurement of GI toxicity but discussion informs 8/9 patients developed a degree of diarrhoea. Results do not include any missing data or drop-out rates which may reflect selective reporting.
	Main findings	Compared to T0, family Eubacteriaceae in each sample at T2, and T3 were significantly decreased ($p < 0.032$). *Fusobacteriaceae* significantly increased at T2 and *Streptococcaceae* significantly increased at T1 compared to T0. Family level taxon *Veillonellaceae, Enterococcaceae, Lactobacillales* bacterium and Butyrate-producing bacterium at T0, T1, and T2 were not different from each other, but the T0 and T3 samples showed significant differences ($p=0.050$). Overall richness in bacterial species decreased: - decrease in *firmicutes* by 10% ($p= 0.09$), decrease in *bacteriodes* during RT but increase after 3 months (no p-value). Increase in *fusobacteria* by 3% ($p=0.05$).					

Wang et al., 2015 Prospective cohort	B	7/11	III Limited/ weak	Setting: China Sample: • 11 cancer patients • 4 healthy volunteers • Mixed tumour groups: gynaecological cancer (n=8) + GI cancer (n=3)	Outcome measures: Stability and diversity of faecal microbiota before and after RT using difference in OTUs, Shannon Index, Good's coverage, Chao1 richness estimator. Comparison with healthy individuals Sub-analysis of those with and without diarrhoea GI toxicity: CTCAE v 0.3	Mixed tumour type + mixed gender cohort, use of different radiotherapy fields, unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period. 9 patients were excluded from final analysis for various reasons which may reflect selective reporting.
	Main findings	Patients with diarrhoea had lower microbial diversity ($p < 0.01$) Prior to radiotherapy, compared to patients who did not develop diarrhoea, those who did had increased abundance of *Bacteroides, Dialister, Veillonella*, and decreased abundance of *Clostridium XI* and *XVIII, Faecalibacterium, Oscillibacter, Parabacteroides, Prevotella* and unclassified (Genus: others)				

Table 3.2 Appraisal of studies included in the original review using the CASP tool. (Muls et al., 2017).

3.3.6 Results of the updated review

Figure 3.1 shows the flow diagram for the updated search. Since 2016, there were five publications involving analysis of the gut microbiome in women treated for gynaecological cancer including three abstracts related to different aspects of a single study as no full paper was yet available. There were three full papers examining the vaginal microbiome. In total, 182 women with gynaecological cancer are described in these recent study reports (Table 3.3).

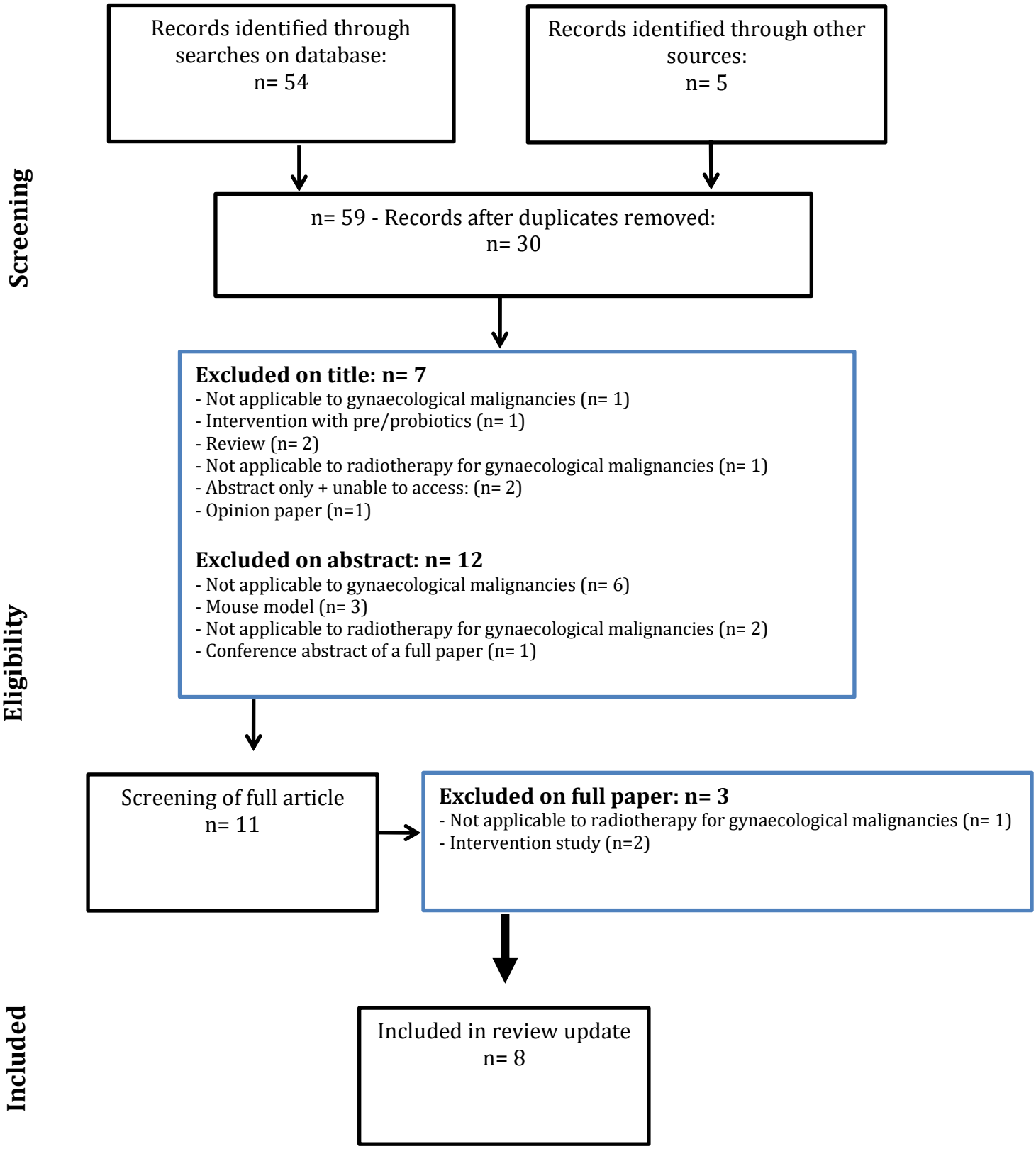


Figure 3.1 Flow diagram of excluded and included studies.

Several studies have examined the vaginal microbiome of women at different stages of malignancy compared to that of women without cancer (Lee et al., 2013; Oh et al., 2015; Mitra et al., 2015; Audirac-Chalifour et al., 2016; Piyathilake et al., 2016). Bai, Jhaney, Daniel, & Bruner (2019) have published the first full paper reporting long-term sequential follow-up data to determine the role of changes in the vaginal microbiome in progression from pre-cancerous lesions to cancer diagnosis. 16S rRNA sequencing of vaginal swabs, taken prior to treatment and up to one year after completion of treatment, showed that *Lactobacillus* was depleted in the 19 American women receiving treatment for gynaecological cancers compared to the healthy population. In addition, microbial diversity was higher compared to what would be expected in a healthy vaginal microbiome (Table 3.3). Compared to those before treatment, women with gynaecologic cancer showed a higher alpha diversity (richness in species within a sample of the same individual) in the Shannon's index ($p=0.01$) and number of observed OTUs (species) ($p=0.001$) after radiotherapy. Caucasian women with gynaecologic cancer showed higher trends in alpha diversity than African American women (Shannon's index: $p=0.02$; observed OTUs: $p=0.02$). The only differential taxa abundance related to race was *Brucellaceae* at the family level (Bai et al., 2019). Women after radiotherapy had higher abundances of the taxa *Mobiluncus, Atopobium,* and *Prevotella,* but lower abundances of *Lactobacillus, Gardnerella,* and *Peptostreptococcus* than before treatment (Table 3.4).

The same group (Tsementzi et al., 2020) reported on 65 women with gynaecolofical malignancy and 69 healthy volunteers with a sub-analysis of 25

women with repeated samples of the vaginal microbiome before and after radiotherapy. 16S rRNA sequencing of the vaginal sampls showed a higher α-diversity in samples from cancer patients compared to samples from healthy volunteers with a depletion in *Lactobacillus*, *Bifidobacteria* and *Shuttleworthia*. Furthermore, 15 phylogroups not normally present in the vaginal microbiome of healthy women: *Prevotella, Porphyromonas, Peptoniphyllus, Fusobacterium, Anaerococcus, Dialister, Sneathia, Mycoplasma, Ezakiella, trichomonas, Moryella, Parvimonas, Peptostreptococcus, Leptotrichiaceae* and *Mobiluncus* (LDA > 3). Samples after radiotherapy showed a higher α-diversity than samples before radiotherapy. Differences in β-diversity could not be fully explained by major contributing clinical factors.

Delgado Medrano et al. (2017) collected rectal and vaginal samples before and during radiotherapy in 20 American women, but none after treatment (Table 3.3). 16S rRNA sequencing of the rectal samples showed a reduction in the diversity of the intestinal microbiome during treatment and this decrease was associated with higher gastrointestinal toxicity. The results of the vaginal samples and changes in the vaginal microbiome were reported separately in 30 women regarding their response to chemoradiation. Using 16S rRNA sequencing, early response was associated with lower quantity of species within the vaginal microbiome and an increased abundance of *Corynebacteriales* ($R = 0.90$; $p < 0.0001$), *Actinomycetales* ($R = 0.83$; $p < 0.0001$) and *Bifidobacteriales* ($R = 0.82$; $p < 0.0001$). The study indicates that an increased diversity of the vaginal microbiome is negatively associated with

outcome, supporting previous clinical studies in non-cancer settings (Colbert et al., 2018).

Two of the studies focused on the difference in gut microbiome between women with cervical cancer and healthy controls and the participants did not yet seem to have received any treatment with radiotherapy. In analyses independently adjusted for age and race, *Prevotella, Porphyromonas*, and *Dialister* were significantly enriched in samples of women with cervical cancer, while *Blautia, Alistipes* and members of the *Lachnospiracea* family were preferentially more abundant in controls ($p < 0.05$, LDA score > 3) (Sims et al., 2019). The other study identified different microbiota: *Proteobacteria, Parabacteroides, Escherichia, Shigella*, and *Roseburia* were all significantly more abundant in the faecal samples from the patients with cervical cancer and significantly less abundant in the faecal samples from healthy controls ($p < 0.05$, LDA score > 4) (Wang et al., 2019) (Table 3.4).

The studies focused on acute gastrointestinal toxicity as the longest follow-up time point to collect stool was 14 months post radiotherapy. Long-term changes in bowel function may manifest many months to years following treatment (Morris & Haboubi, 2015). Future research with more longitudinal follow-up is warranted as exemplified in the newer studies conducted by Bai et al. (2019) and Tsementzi et al. (2020). This review will be updated again in July 2021 to prepare for submission as an update of the original systematic review paper, reflecting advances within this field during the last 5 years.

Reference	Type of study	Number of patients with gynaecological tumour types	Number of healthy volunteers as control	Surgery	Chemotherapy type defined	Radiotherapy dose (Gy)	Analysis technique microbiome	Type of sample	Diarrhoea defined	No of patients reporting diarrhoea	Before- after design	Number of follow-up time points	Latest time point after radiotherapy	Antibiotic use as exclusion ?	Steroid use as exclusion?	Immunotherapy as exclusion?
Colbert et al., 2017 (abstract)	Prospective cohort	20 cervix (n=20)	0	?	No	?	16S rRNA sequencing	Stool	No	N/A	Yes	3	Last week of RT	?	?	?
Delgado Medrano et al., 2017 (abstract)	Prospective cohort	20 cervix (n=20)	0	?	No	?	16S rRNA sequencing	Stool	Yes EPIC	?	Yes	3	Last week of RT	?	?	?
Colbert et al., 2018 (abstract)	Prospective cohort	30 cervix (n=30)	0	?	No	?	16S rRNA sequencing	Cervical sample	No	N/A	Yes	3	Last week of RT	?	?	?
Wang et al., 2019	Prospective cohort	18 cervix (n=18)	0	no	No	50.4	16S rRNA sequencing	Stool	Yes RTOG grading system	10	Yes	2	1 day after treatment	Yes	Yes	Yes
Bai et al., 2019	Prospective cohort	19 endometrium (n=10) cervix (n=8) vulva (n=1)	0	Yes	No	Mean 52.21	16S rRNA sequencing	Vaginal swab	No	N/A	Yes	4	14 months post treatment	Yes	Yes	?
Sims et al., 2019	Prospective cohort	42 cervix (n=42)	46	?	No	?	16S rRNA sequencing	Rectal swab Stool	No	N/A	No	N/A	N/A	Yes	?	?
Wang et al., 2019	Prospective cohort	8 cervix (n=8)	5	?	No	?	16S rRNA Sequencing	Stool	No	N/A	No	N/A	N/A	yes	?	?
Tsementzi et al., 2020	Prospective cohort	25 endometrium (n=15) cervix (n=10)	69	Yes	Yes	45-50.4 Gy	16S rRNA Sequencing	Vaginal swab	No	N/A	Yes	2	2-4 months post RT	Yes	Yes	yes

Table 3.3 Additional studies describing changes in the gut or vaginal microbiome in women treated with radiotherapy for a gynaecological malignancy.

Author / year Study design	Class of evidence	CASP score	Quality grade	Population studied and sample size	Primary outcome measures / results	Conclusions
Colbert et al., 2017 Prospective cohort	C	4/11	IV Very weak	Setting: USA Sample: • Single cohort: ca cervix (n= 20) • No healthy volunteers	Outcome measures: OTUs, response to treatment subgroup analysis, rectal + cervical microbiota No GI toxicity Results: Diversity of the microbiome in rectal samples decreased throughout the course of treatment. 3 OTUs mapping to (genus/genera) were significantly more abundant in cervical samples from responding patients over non-responding patients ($p < 0.0001$): two *Tenericutes* and one *Firmicute*. 5 OTUs were significantly more abundant in rectal samples from non-responding patients: three *Bacteroides* ($p=0.03$ to 0.005) and two *Firmicutes* ($p=0.05$).	Abstract report only Limited information on radiotherapy fields, unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period
Delgado Medrano et al., 2017 Prospective cohort	C	5/11	IV Very weak	Setting: USA Sample: Single cohort: ca cervix (n=20) No healthy volunteers	Outcome measures: OTUs, rectal samples, GI Toxicity: EPIC Results: Increased Expanded Prostate cancer Index Composite bowel assessment (EPIC toxicity score) (Appendix 10) was inversely associated with time and directly associated with increased Shannon diversity index. On multivariate linear regression, increased Shannon diversity index was independently associated with increased EPIC toxicity score as was time ($p<0.0001$). No individual OTUs significantly associated with increased toxicity.	Abstract report only Limited information on radiotherapy fields, unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period
Colbert et al., 2018 Prospective cohort	C	4/11	Very weak	Setting: USA Sample: • Single cohort: ca cervix (n= 30) • No healthy volunteers	Outcome measures: OTUs, response to treatment subgroup analysis, cervical microbiotaNo GI toxicity Results: Early response to chemoradiation was associated with lower quantity of observed OTUs of vaginal microbiota (25.0 [SD 12.68]) ($p=0.03$) and increased abundance of *Corynebacteriales* ($R = 0.90$; $p < 0.0001$), *Actinomycetales* ($R = 0.83$; $p < 0.0001$) and *Bifidobacteriales* ($R = 0.82$; $p < 0.0001$). Increased diversity of the vaginal microbiome is negatively associated with response to treatment.	Abstract report only Limited information on radiotherapy fields, unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period.

Table 3.4 Appraisal of studies included in the updated review using the CASP tool.

Author / year Study design	Class of evidence	CASP score	Quality grade	Population studied and sample size	Primary outcome measures / results	Conclusions
Wang et al., 2019 Prospective cohort	B	8/11	III Limited/ weak	Setting: China Sample: • 18 cancer patients • no healthy volunteers • single tumour type: ca cervix (n=18)	Outcome measures: Stability and diversity of faecal microbiota before and after RT using difference in OTUs, Simpson Index, Shannon Index. Sub-analysis of those with and without radiation enteropathy per grade GI toxicity: RTOG grading system Results: Dysbiosis was observed among patients with RE, which was characterised by significantly reduced α-diversity ($p< 0.006$) but increased β-diversity ($p< 0.004$) with a relative higher abundance of Proteobacteria and Gammaproteobacteria and lower abundance of Bacteroides. Coprococcus was clearly enriched ($p= 0.034$) prior to radiotherapy in patients who later developed radiation enteritis (RE). Meta-analysis further revealed unique grade- related microbial features, such as more abundant Virgibacillus and Alcanivorax in patients with mild enteritis. Additionally, using bacterial-epithelial co-cultures, RE patient-derived microbiota induced epithelial inflammation and barrier dysfunction, enhanced TNF-α and IL-1β expression compared with control microbiota.	Very cohesive cohort in terms of clinical characteristics and treatment Very strict exclusion criteria used No mention of drop-out rate No mention of missed data
Bai et al., 2019 Prospective cohort	B	7/11	III Limited/ weak	Sample: USA • Sample: 19 cancer patients • no healthy volunteers • single tumour group: gynaecological cancer (n=19) but mixed histology types	Outcome measures: Stability and diversity of faecal microbiota before and after RT using difference in OTUs, Shannon Index, Good's coverage, Chao1 richness estimator. No measurement of GI toxicity Results: vaginal microbiome changes during and after pelvic radiotherapy: Compared to those pre-RT, women with gynecologic cancer post-RT showed a higher alpha diversity in Shannon's index ($p=0.01$), observed OTUs ($p=0.001$), Faith's PD ($p=0.001$), and Pielou's species evenness ($p=0.04$). Race was significantly associated with alpha diversity metrics (Shannon's index: $p=0.02$; observed OTUs: $p=0.02$; Faith's PD: $p=0.02$; Pielou's species evenness: $p=0.03$) Women post-RT had higher abundances of the taxa *Mobiluncus, Atopobium,* and *Prevotella,* but lower abundances of *Lactobacillus, Gardnerella,* and *Peptostreptococcus* than pre-RT The only differential taxa abundance related to race was *Brucellaceae* at the family level.	Single tumour type (gynaecological cancer) Unable to assess whether cohort is representative as no information of eligibility, selection of participants. Baseline samples: n=17 but n=19 participants reported ? 2 did not provide baseline samples? Missing data not accounted for But this is the first cohort study looking at the vaginal microbiome.

Table 3.4 (Cont.)

Author / year Study design	Class of evidence	CASP score	Quality grade	Population studied and sample size	Primary outcome measures / results	Conclusions
Sims et al., 2019 Prospective cohort	B	6/11	III Limited/ weak	Setting: USA Sample: • 42 cancer patients • 46 healthy volunteers • single tumour type: ca cervix (n=42)	Outcome measures: Stability and diversity of faecal microbiota before RT between healthy controls and women with ca cervix using difference in OTUs, α- diversity and β-diversity. Results: *Prevotella, Porphyromonas*, and *Dialister* were significantly enriched in samples in women with cervical cancer. In analyses independently adjusted for age and race, *Prevotella, Porphyromonas*, and *Dialister* were significantly enriched in samples from women with cervical cancer, while *Blautia, Alistipes* and members of the *Lachnospiracea* family were preferentially more abundant in controls ($p < 0.05$, LDA score > 3)	One time point only Strict exclusion criteria used No mention of drop-out rate No mention of missed data Different sampling methods: Stool sample for patients, rectal swabs for health controls.
Wang et al., 2019 Prospective cohort	C	5/11	IV very weak	Setting: China Sample: • 8 cancer patients • 5 healthy volunteers single tumour type: ca cervix (n=8)	Outcome measures: Stability and diversity of faecal microbiota before RT between healthy controls and women with ca cervix using difference in OTUs. Results: *Proteobacteria, Parabacteroides, Escherichia_Shigells*, and *Roseburia* were all significantly more abundant in the faecal samples from the patients with cervical cancer and conversely, significantly less abundant in the faecal samples from healthy controls ($p < 0.05$, LDA score > 4)	One time point only Strict exclusion criteria used No mention of drop-out rate No mention of missed data
Tsementzi et al., 2020	B	9/11	II Fair evidence	Setting: USA Sample: • 65 cancer patients • 69 healthy volunteers ** Subcohort of pre-post RT analysis mixed tumour type: ca endometrium (n= 36) ca cervix (n=29)	Outcome measures: Stability and diversity of vaginal microbiota before RT between healthy controls and women with gynae cancer + between women before and after RT using difference in OTUs, α- diversity and β-diversity. Results: *Sneathia, Prevotella, Fusobacterium, Porphyromonas, Anaerococcus, Dialister* were all more abundant in women with cancer compared to healthy controls ($p < 0.05$, LDA > 3) with a depletion of *Lactobacillus*, *Bifidobacteria* and *Shuttleworthia*. This was enhanced in women with cancer post RT.	Limited number of patients with repeated samples but this is a landmark study. Very clear description of methods, inclusion and exclusion criteria.

Table 3.4 (Cont.)

3.3.7 Discussion of the evidence

This review highlights the increasing interest of charting changes in the gut or vaginal microbiomes in women with gynaecological malignancies prior to, during and following treatment. Whilst the gut microbiome in healthy people is diverse in species and has a high abundance of *Bacteroidites*, *Bifidobacterium* and *Lactobacillus*, a healthy vaginal microbiome is typically populated by aerobic members of the Firmicutes phylum, dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jensenii* and is limited in diversity (Ravel et al., 2011).

Alterations in these microbiomes are, to date, not well understood. But these recent vaginal microbiome studies show similar results regarding a higher α-diversity and specific OTUs that occur in women with gynaecological cancer. In all studies the rationale for conducting the study was clearly outlined based on the acknowledgment that treatment with radiotherapy can result in serious complications, leading to interruption of treatment and that the underlying cause remains unknown. A broad range of supporting literature including mainly opinion papers and reviews is used in the background sections of the papers and the studies represent feasibility work in this field to strengthen the rationale for future research. This is especially relevant in view of the emerging studies evaluating the use of probiotics to reduce acute gastrointestinal toxicity (Delia et al., 2007; Giralt et al., 2008; Chitapanarux et al., 2010; Kumagai, Rahman, & Smith 2018). Results of probiotics so far have varied in success rates and this may be due to current gap in understanding of the impact of

gynaecological malignancies and multimodal therapies on the gut and vaginal microbiome. These recent studies have provided basic building blocks to our understanding and ability to offer treatment options targeted to the individual patient.

Limitations of the studies

As in the studies included in original systematic review, these studies were single-centred studies, no information about the clinical activity of the oncology units regarding how many patients are annually treated, how many patients would have been eligible, how eligible patients were selected or the time scale for recruitment was provided. This may have indicated some selection bias. The researchers do not specify what the guidelines to stop recruitment were. This raises concerns about equity for potential study participants and whether this was openly discussed with the candidates concerned. Information about catchment areas and number of patients treated could have provided additional insight into whether the sample population is representative for the wider population. Some of the studies used healthy volunteers as controls but none described the selection methods or demographic information.

None of the study reports described missing data or drop-out rates. This increases the risk of selecting only those participants who were able to provide all data for inclusion in the final analysis and may have resulted in reporting bias. Interpretation of the results may also become difficult if it is not known how many possible participants were identified but not included in the studies.

The discussion sections address a number of key areas; the researchers acknowledge the limitations of 16S rRNA sequencing as not all bacterial species can be adequately identified and support future studies with larger cohorts.

There are several factors contributing to bias. The inclusion of different tumour types may complicate interpretation of the results as individual patient outcomes could not be separated for analysis. Little is known about the selection procedure for recruiting, the small number of participants included and the completeness of the data impacting on the external validity and thus generalisability of the study outcomes. In addition, several countries were involved in conducting the studies which may indicate diversity of lifestyle factors, predominantly diet and health care provision.

Several of the studies specifically described excluding patients who had been treated with antibiotics for at least one month prior to commencing radiotherapy. In addition, six of these studies excluded the use of corticosteroids and five immune-suppressants. Whilst the long-term impact of the use of antibiotics resulting in sustained reductions in gut microbiota diversity has been acknowledged (Van Eyk & van Schalkwyk, 2012; Macfarlane, 2014; Francino, 2016; Thursby & Juge, 2016), excluding those patients from future studies does not reflect oncological management in the clinical setting and this should be considered in future studies. The studies lacked information about other known confounding factors such as diet, body mass index, other medications affecting the gut microbiome or gastrointestinal function,

treatment with previous surgery or chemotherapy, co-morbid factors such as diabetes or treatment induced nausea and vomiting.

Research in the emerging field of the microbiome is currently biased towards the gut microbiome and only little is known about interactions between the vaginal microbiome and the intestinal microbiome. Moreover, the viromes continue to be neglected and this may be important in women with gynaecological cancer where HPV is a causal factor in the development of invasive cervical carcinoma as well as treatment-induced immunosuppression.

The studies to date also remain mostly focused on sequencing the microbiome but the clinical relevance for using these techniques in isolation may be limited. In our original review, we identified one older prospective study applying electronic sensing to analyse vaginal swabs in 68 women with a clinical diagnosis of bacterial vaginosis and found a positive predictive value of the test was 61.5% (Chandiok et al., 1997). However, no studies were found using VOC analysis to describe the vaginal microbiome in women with gynaecological cancer nor how treatment such as surgery, chemotherapy and pelvic radiotherapy may impact on the vaginal microbiome of these women. Inclusion of metabonomic analysis techniques offers additional avenues to develop risk stratification pathways and targeted treatment options for those experiencing long-term gastrointestinal toxicity following cancer treatment.

Limitations of the review

This review was limited to prospective cohort studies assessing the gut and vaginal microbiome of women treated for a gynaecological malignancy. No animal studies were included. In view of recent advances in novel, more targeted radiotherapy techniques, and technology available to analyse the microbiome the findings of older studies need to be interpreted with caution. All studies used current sequencing methods but did not include other metabonomic analysis techniques. Whilst it is still not common to register review protocols, registration of this review on the International PROSPERO database reduced the risk of multiple reviews addressing the same question, limited publication bias and provided transparency in updating the review and preparing this for publication in the future (Liberati et al., 2009).

Implications for future research

The limited but growing evidence to date implies that larger studies including both the vaginal and gut microbiome in women treated for a gynaecological malignancy are warranted to explore the impact of cancer treatments on the microbiome and its relation to developing long-term gastrointestinal toxicity.

3.4 Conclusion of the review

The outcomes of these studies support that there is a correlation between changes in the intestinal microbiome and receiving radiotherapy. All studies yielded similar results. All studies showed a decrease in abundance and diversity of the intestinal bacterial species. Two full papers and one abstract

focused on changes in the vaginal microbiome and these are the first study reports to date to describe that there is an increase in abundance and diversity in the vaginal microbiome of women treated with radiotherapy for a gynaecological cancer.

Further characterisation of potential differences or changes in the vaginal and gastrointestinal microbiome and intestinal function before and after treatment- especially pelvic radiotherapy- could improve understanding of why up to 80% of patients develop bowel symptoms during and after radiotherapy and why others remain symptom-free. This may lead to new avenues to stratify those at risk and explore personalised treatment options and prevention of gastrointestinal toxicity following cancer treatment.

Chapter 4

Research methods

The cohort analysis of women treated for gynaecological malignancy who accessed a specialist gastroenterology service to assess, investigate and manage their long-term changes in gastrointestinal function, as described in Chapter two, shows there is a clinical need to establish possible clinical indicators to identify people who will develop long-term changes in gastrointestinal function earlier as women waited on average nearly 5 years before seeking appropriate help (Muls et al., 2020). In addition, there is a lack of studies examining the role of the gut microbiome in the development of long-term gastrointestinal toxicity following treatment for cancer. The systematic review described in Chapter three evidenced the sparsity of robust evidence of the understanding of how the vaginal or gut microbiome change in women undergoing treatment for gynaecological malignancies and what factors may be predictors for long-term gastrointestinal toxicity. However, the updated review revealed a growing interest in this field.

This chapter describes the methods used in the design and conduct of a longitudinal observational cohort study titled: 'Predicting patients at risk of developing gastrointestinal symptoms after treatment with pelvic radiotherapy', abbreviated to 'the PREDICT study'. It provides a rationale for the selection of the methods, including a critical discussion of the literature in the area, as well as a detailed description of the methods used. The methods include the assessment of GI toxicity, dietary intake, microbiome metabolites in samples of stool, urine, saliva and rectal swabs and the use the metabolomics signatures to predict long-term GI toxicity following treatment with radiotherapy in women with gynaecological cancer.

4.1 Study design

A prospective observational cohort study design was employed to study women under the care of participating consultants undergoing radiotherapy for a new gynaecological malignancy at Royal Marsden NHS Foundation Trust in Sutton and Chelsea. The rationale for employing a longitudinal cohort study design was based on the lack of literature evidencing studies which include long-term GI toxicity beyond a few months after completion of treatment (Maduro et al., 2003; Berveling et al., 2011). The need for a study encompassing time points up to two years after completion of treatment reflects the clinical journey of people developing long-term changes in bowel function and allows for a better understanding of which factors may play a role in this process (Muls et al., 2020).

4.1.1 Aims

This doctoral research study aimed to explore the possibility of using changes in the gut microbiome metabolites, combined with clinical data to risk-stratify women treated for a gynaecological malignancy earlier in their treatment pathway so that those likely to develop long-term gastrointestinal are identified proactively and more intensively followed-up with early intervention to manage GI toxicity. Changes in the gut microbiome may herald new treatment options for long-term bowel changes and lessen the chronic symptom burden.

The study applied the electronic sensing technique and Gas Chromotography – Ion Mobility Spectometry (GC-IMS) to analyse the metabolites of gut microbiota in samples from a carefully characterised cohort of patients to ascertain the potential for predicting in advance the likely development of GI symptoms from pelvic radiotherapy in correlation to demographic and clinical features.

4.1.2 Objectives

The primary objective for this study was:

To identify potential predictive markers of chronic radiation induced GI toxicity in women treated for gynaecological cancer using the metabolomic signatures of the microbiota in stool, urine, saliva and rectal swabs.

Secondary objectives were:

- To identify the optimal time point to predict chronic GI toxicity.
- To determine which of the samples (rectal swab, stool sample, urine sample, saliva sample) will best predict chronic GI toxicity.

4.1.3 Hypotheses

4.1.3.1 Primary hypothesis

Primary hypothesis:

The null hypothesis is that there is no difference in the signatures created by the electronic sensing technique and GC-IMS in the emitted volatiles and gas

profiles of the gut microbiota between cancer patients treated with pelvic radiotherapy who develop gastrointestinal symptoms and those who do not.

The alternative hypothesis is that the signatures created by the electronic sensing technique and GC-IMS will show there is a difference in the volatiles and gas profiles emitted by the gut microbiota between cancer patients treated with pelvic radiotherapy who develop GI symptoms and those who do not. These signature profiles can be used to predict risk of GI toxicity.

4.1.3.2 Secondary hypothesis

Analysis of patient and clinical characteristics will elicit indicators of differences between cancer patients treated with pelvic radiotherapy who develop GI toxicity and those who do not.

4.2 Setting

This study was hosted by the Royal Marsden NHS Foundation Trust at Chelsea and Sutton which is a tertiary cancer centre in London. Participants were recruited from both sites between November 2015 and November 2017. Follow-up concluded in November 2019.

4.2.1 Protocol and ethical approval

Ethical approval was obtained from National Research Ethics Committee in August 2015 (Appendix 11). The study protocol was also approved by the Royal Marsden NHS Foundation Trust's Research and Development Committee and assigned protocol reference CCR 4201 (Appendix 12). Two further amendments were submitted, the first in January 2016 to broaden inclusion criteria so that private patients could also be recruited and the second in August 2017 to allow a possible extension of the recruitment period from November 2017 to April 2018 if needed.

4.2.2 Time points for data collection

Time points for data and sample collection coincided with existing oncology treatment or follow-up appointments to minimise the need for additional visits to the hospital. Oncology follow-up for women treated for gynaecological cancer is weekly during treatment, one month after completion of radiotherapy, then three-monthly for the first year, four-monthly in the second year and every six months during year 3 to 5 following treatment. Women are discharged from oncology follow-up 5 years after treatment. In this study, there were 6 time points for most participants, but patients due to receive a course of chemotherapy prior to starting radiotherapy, had an extra time point after chemotherapy and before the start of radiotherapy (Figure 4.1).

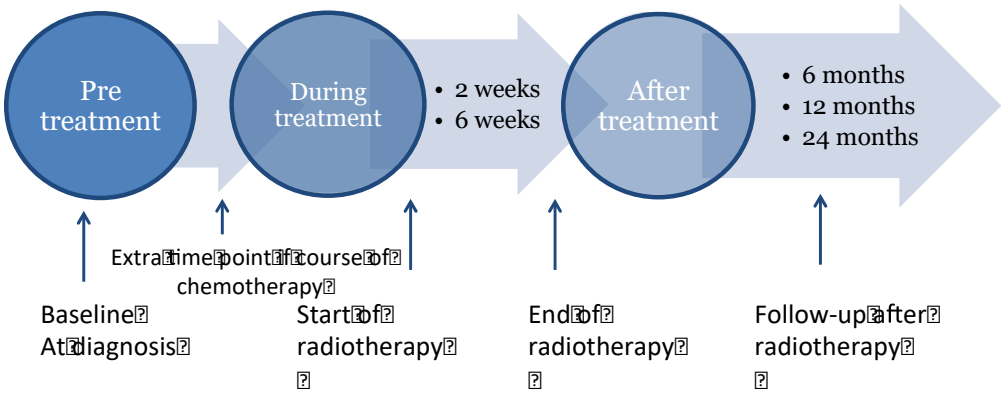


Figure 4.1 Data collection time points.

4.3 Participants

Patients eligible for the study were women newly diagnosed with a gynaecological cancer due to be treated with radical, adjuvant or neo-adjuvant radiotherapy. Due to the proximity of healthy tissues in the GI tract, all these women are at risk of developing long-term GI toxicity (Taylor et al., 2016).

4.3.1 Inclusion and exclusion criteria

Women aged 18 years or above were eligible to participate in the study. In theory, young adults aged 16 or above were also able to consent. Young adults were being treated in a different department but would have been referred on to the gynaecology oncology team. However, no young adults under the age of 18 years were diagnosed with a gynaecological malignancy during the recruitment period. Potential participants had to be able to provide informed consent.

Patients were excluded if:

- they were pregnant at the time of diagnosis as this would delay the start of treatment.
- they had been diagnosed with recurrent disease as they would have been treated with pelvic radiotherapy previously.
- they had received treatment for another cancer in the pelvis within the last 5 years.
- they were on studies with conflicting end-points such as dietary interventions which might alter the gut microbiome.
- they were due to have their post-treatment follow-up elsewhere in the country or abroad as they would not be returning for routine oncology follow-up appointments.

4.3.2 Screening of eligible participants

The clinic lists of the two medical gynaecological oncologists were screened on a weekly basis in addition to their joint clinic lists with the surgical team. A total of 13 clinic lists per week were screened. The gynaecology cancer multidisciplinary meeting was also attended to pick up late referrals to the teams. The exclusion criteria were applied to identify suitable participants and clinics were attended to approach the women who were eligible to participate. If they wanted to hear about the study, the aim of the study was explained to them in detail and they were given a patient information sheet (Appendix 13) confirming what was involved. If the participant wanted to consider

participating in the study, they agreed to be contacted by phone to establish whether they had read the information and were interested in participating.

4.3.3 Consent

At the next planned visit to hospital either for a radiotherapy planning scan, further staging or a clinic appointment, the women were met by the researcher to discuss their participation again and sign a consent form if they wished to participate (Appendix 14) hence recruiting them to the study. The consent form was printed on triple carbon copy paper of which one copy was given to the participant. The other copies were filed with the case report forms and uploaded in the electronic patient record. The participant was assigned a unique identifier on the electronic patient record system and registered as recruited for the study. Having signed the consent, a date was set with the participant depending on their next hospital visit, prior to the start of treatment to collect baseline samples, questionnaires and clinical data. At each meeting, an estimated date for the next contact and data collection point was agreed.

4.3.4 Withdrawal from the study

At each time point during the study, the participant was asked if they wanted to continue to contribute and if they did, it was agreed they were contacted by phone prior to the next meeting to confirm this and set up a date and time for

the next data and sample collection. If the participant indicated they wanted to withdraw from the study, they were invited to sign the withdrawal paperwork (Appendix 15). As with the consent form, a copy was kept with the case report forms and the reason for withdrawal was noted both on the paperwork and electronic database.

4.4 Variables

4.4.1 Data governance

At each time point several clinical parameters were collected. The data were entered on a paper case report form (CRF) (Appendix 16) and at a later date entered in a dedicated database specific to the study by an independent data manager. Throughout the study, participant data were handled in accordance with the requirements of the Data Protection Act (1998) and in accordance with the Data Protection Act 2018 which is the UK's implementation of the General Data Protection Regulation (GDPR). As laboratory analysis of samples was conducted at Warwick University, a Material Transfer Agreement between the Royal Marsden NHS Foundation Trust and Warwick University was in place (Appendix 17). All data were treated as strictly confidential and held in a secure location, in line with the Royal Marsden NHS Foundation Trust data protection and confidentiality arrangements.

4.4.2 Baseline only variables

At baseline the following characteristics were collected from the relevant sections of the electronic patient record and confirmed with the participant:

- age: years
- cancer tumour site: as described in the diagnosis section
- histology: as described on the histological diagnosis section
- the presence of lymphovascular space invasion: as described on the histology section
- surgery: as described on the surgery report
- chemotherapy: as described in the e-prescribing records of anti-cancer drugs
- radiotherapy: Gy and number of fractions as described in the radiotherapy planning section
- marital status: as described in the patient detail section
- number of children and mode of delivery: as described in the pre-operative assessment notes
- smoking status and alcohol intake: as described in the pre-operative assessment notes
- type of diet: as described by the participant: includes meat, pesceterian, vegetarian, vegan, lactose free diet, gluten free diet, other.

4.4.3 Baseline and follow-up variables

At all time points the data below were collected:

- weight: kg, measured on calibrated electronic hospital scales (Marsden, The Weighing Company ®: DP2400V5BMI Indicator)

- height: cm, measured on calibrated hospital stadiometer

- Body Mass Index (BMI): calculated using the online NHS BMI calculator: https://www.nhs.uk/live-well/healthy-weight/bmi-calculator/

- co-morbidities: as described in the electronic patient record and listed by the participant. Co-morbidities were entered on the CRF as coded in the international classification of diseases version 10 (World Health Organisation, 2016).

https://www.who.int/classifications/icd/icdonlineversions/en/

- medications: participants described which medications they were taken and if any medications had been stopped or started at each time point. Generic names as listed in the British National Formulary (Joint Formulary Committee, 2015) were used on the CRF. https://bnf.nice.org.uk/

- performance status: the participant described their level of activity to the researcher who scored it using the ECOG performance Status and Physical Activity Index (Table 4.1 and 4.2). The scoring systems are slightly different and require specific definitions to be applied. Two different performance status assessments were used by the researcher to achieve greater precision in the scoring as cancer patients tend to overestimate their own activity level (Oken et al., 1982; Cust et al., 2008, Health Improvement Directorate, 2009; Smith et al., 2019).

Variables also included GI toxicity, dietary intake, faecal calprotectin, rectal swabs, stool, urine and saliva samples. The case report form (Appendix 16) noted which questionnaires were completed, the score of the questionnaires, which samples were collected, the date they were stored and results of any other relevant blood tests and faecal calprotectin. The completed questionnaires were added to the case report forms. At each time point, it was noted whether the participant experienced GI symptoms warranting a referral to a gastroenterology service and if so, what new GI diagnoses had been made. There was also an open text box to include any other relevant information the participant wished to share. Table 4.3 shows all collection time points for samples and questionnaires.

GRADE	**ECOG PERFORMANCE STATUS**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self care; totally confined to bed or chair
5	Dead

Table 4.1 ECOG Performance Status Developed by the Eastern Cooperative Oncology Group (Oken et al., 1982).

PHYSICAL ACTIVITY INDEX (PAI) Score Patients can be classified into four categories:	
Inactive	Sedentary job and no physical exercise or cycling
Moderately inactive	Sedentary job and some but < 1 hour physical exercise and / or cycling per week OR Standing job and no physical exercise or cycling
Moderately active	Sedentary job and 1-2.9 hours physical exercise and / or cycling per week OR Standing job and some but < 1 hour physical exercise and / or cycling per week OR Physical job and no physical exercise or cycling
Active	Sedentary job and ≥ 3 hours physical exercise and / or cycling per week OR Standing job and 1-2.9 hours physical exercise and / or cycling per week OR Physical job and some but < 1 hour physical exercise and / or cycling per week OR Heavy manual job

Table 4.2 The Physical Activity Index (Cust et al., 2008).

	Baseline before treatment	***If having chemotherapy, before radiotherapy***	***2 weeks after start of radiotherapy***	***End of radiotherapy***	***6 months after completion of radiotherapy***	***12 months after completion of radiotherapy***	***24 months after completion of radiotherapy***
Patient characteristics	***X***	Updated if incomplete or changed	Updated if incomplete or changed	Updated if incomplete or changed	Updated if incomplete or changed	Updated if incomplete or changed	Updated if incomplete or changed
Treatment characteristics	***X***	Updated if incomplete or changed	Updated if incomplete or changed				
Saliva sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Urine sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Rectal swab (optional)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Stool sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Faecal calprotectin	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Blood sample (translational)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Blood sample (fibrotic markers)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Inflammatory Bowel Disease Questionnaire (IBDQ)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
St. Mark's incontinence score	***X***	***X***	***X***	***X***	***X***	***X***	***X***
GI section of the Common Terminology Criteria for Adverse Events (CTCAE) (version 3.0) score	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Bristol Stool Chart	***X***	***X***	***X***	***X***	***X***	***X***	***X***
7-day food diary	***X***	***X***	***(optional)***	***X***	***X***	***X***	***X***

Table 4.3 PREDICT study sample and questionnaire collection time points.

4.5 Gastrointestinal toxicity

4.5.1 Modified Inflammatory Bowel Disease Questionnaire (IBDQ) (Appendix 18)

The IBDQ is a quality of life (QoL) tool which groups 32 questions into 4 dimensions: bowel, systemic, social, and emotional (Pallis, Mouzas, & Vlachonikolis, 1999). The top score is 224 for good bowel function and quality of life. Higher scores indicate a better QoL. The original IBDQ, validated for patients with inflammatory bowel disease, has a good internal consistency, with a Cronbach α of 0.85 for the bowel-related quality of life items (Pallis et al., 1999). The modified version of the IBDQ was developed for oncology patients (Olopade et al., 2005) and has been used in several studies such as the ORBIT study and the FIBRE study (Andreyev et al., 2013; Wedlake et al., 2017). This modified IBDQ has been used in this study but will be referred to as the 'IBDQ' from here on.

The use of the IBDQ in this study was the result of a multi-professional discussion and based on previous research experience indicating it a feasible tool to use in oncology patients for assessing GI toxicity. However, during data cleaning, it became apparent that there is a problem regarding interpreting and scoring two questions of the emotional subscale (question 21 and question 32). These questions are worded positively, where all other questions are worded negatively and the score (1- 7) needs to be reversed. The researcher (AM) had not been aware of this, nor was this described in the original paper (Olopade et al., 2005). This was discussed at length with the researcher's supervisors and

the senior author of the original paper was contacted to confirm the extent of the problem. As a result, the answers provided by the participants on the IBDQ forms were reviewed to gain an estimate of how many participants may have misinterpreted the way they answered these two questions and a sensitivity analysis was performed between the original and adjusted scores as the emotional function sub-scale impacts on the total IBDQ score and hence may misrepresent both the emotional impact and impact on total quality of life of the IBDQ bowel subset.

4.5.2 IBDQ Bowel subset (IBDQ-B) (Appendix 19)

Ten of the 32 questions in the IBDQ make up the bowel subset questionnaire score which can range from 10 (poorest bowel function) to 70 (best bowel function). A fall in score of 10 points between baseline and one year after treatment was indicative of late GI symptoms in patients treated with radiotherapy ($p= 0.049$) when using IBDQ-B scores. The correct prediction of true positives or sensitivity has been found to be 60% and the correct prediction of true negatives or specificity was 65% (Wedlake et al., 2010). However a cut-off score for determining 'good' versus 'bad' bowel function has not been clearly defined in the literature and a difference of 6 points has been used in several other studies to indicate a clinically meaningful difference (Andreyev et al., 2013; Wedlake et al., 2017). In the ORBIT study, 2 groups were identified to indicate high symptom burden with an IBDQ-B score of < 60, with 60-70 indicating low symptom burden (Andreyev et al., 2013).

In view of the lack of evidence supporting a difference of 6 points, a conservative difference of 10 points has been used in this study with those with a reduction of 10 or more points in the IBDQ-B score between baseline and the fourth (one year) and final (two year) time point used to determine who developed long-term gastrointestinal symptoms.

For the analysis of the metabolic signatures in the samples, the following three IBDQ-B groups were defined:

Group A: women with a drop of ≥ 10 points in their IBDQ-B score at the end of treatment compared to baseline and did not improve two years after radiotherapy compared to baseline.

Group B: women with a drop of ≥ 10 points in their IBDQ-B score at the end of treatment compared to baseline but then improved.

Group C: women who did not have a change in their IBDQ-B scores between baseline and 2 years after radiotherapy.

4.5.3 St-Mark's Incontinence Score (Appendix 20)

The St. Mark's Incontinence Score assesses the severity of faecal incontinence and contains items about the type (gas, fluid, solid) and frequency of incontinence, and addresses social impact: the need to wear a pad or plug, the use of constipating medication and the presence of urgency. The total score on the St. Mark's score ranges from 0 (complete continence) to 24 (complete incontinence) (Vaizey, Carapeti, Cahill, & Kamm, 1999). Previous studies showed that the St. Mark's Incontinence Score is a more sensitive tool to assess

incontinence than other currently available incontinence scales (Khalid et al., 2006; Olopade et al., 2005) and it also correlates well with patients' perceptions of their symptom burden (Maeda, Pares, Norton, Vaizey, & Kamm, 2008).

4.5.4 The Common Terminology Criteria for Adverse Events (CTCAE) (Appendix 21)

The Common Terminology Criteria for Adverse Events (CTCAE) (Trotti et al., 2003), is a standardised classification used in assessing side effects of cancer therapy developed by the National Cancer Institute in the USA. Currently version 4.0 has been released but in practice version 3.0 remains in use on the Royal Marsden Electronic Patient Record and trial management system. Most US and UK drug trials base their observations on this system which has a range of grades from 1 to 5. Specific conditions and symptoms may have values or descriptive comment for each level, but the general guideline is: 1 – Mild, 2 – Moderate, 3 – Severe, 4 - Life threatening, 5 – Death (Trotti et al., 2003). Each of the symptoms have a description of what change denotes a specific level of severity (see Table 4.4 for an example). Whilst this is commonly completed by researchers in clinical trials, in this study, participants completed the grading themselves. The CTCAE version 0.3 is used as a standard measure of toxicity in clinical trials within oncology and a toxicity of grade 3 and 4 informs the need to withdraw treatment and admit the patient to hospital. Grades 1 and 2 diarrhoea on the CTCAE are considered to represent mild to moderate symptoms in oncology whereas gastroenterology guidelines using Truelove and Witt's

severity index which describe an increase of stools of 4 times a day as mild and up to 6 times a day as severe diarrhoea and would indicate a reason to admit a patient with inflammatory bowel disease to hospital (NICE, 2019).

Adverse event	**Grade**				
	1	**2**	**3**	**4**	**5**
Diarrhoea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 – 6 stools per day over baseline; IV fluids indicated	Increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; hospitalisation; severe increase in ostomy output compared to baseline; interfering with activities of daily living	Life-threatening consequences (e.g., haemodynamic collapse)	Death

Table 4.4 Example of CTCAE version 3.0 scoring for the severity of diarrhoea.

GI symptoms of interest were: abdominal pain, bloating, constipation, diarrhoea, faecal incontinence, flatulence, frequency, rectal bleeding, rectal pain, tenesmus, urgency and 'other' symptom (Appendix 20: CTCAE version 3.0). There is no difference in the definition of GI symptom between version 3.0 and version 4.0 (Muls et al., 2017).

4.5.5 Bristol Stool Chart (BSC) (Appendix 22)

The Bristol Stool Chart categorises the types of stool a person passes into 7 categories (Lewis & Heaton 1997). It is a helpful tool to assess stool consistency in combination with other tools assessing GI symptoms (Heaton, 2009) and is widely used in clinical practice (Gee, Andreyev, & Muls, 2018). Participants completed the Bristol Stool Chart. For the analysis of the metabolic profiles of the samples, the following BSC groups were defined:

Group E: women who reported type 1 and 2 stool consistency but no diarrhoea (type 6 or 7) two years after radiotherapy (constipation).

Group D: women who reported type 3-5 stool consistency but no constipation or diarrhoea two years after radiotherapy (normal stool).

Group F: women who reported type 6 or 7 stool consistency but no constipation (type 1 or 2) two years after radiotherapy (diarrhoea).

4.6 Dietary intake assessment (Appendix 23)

As diet is presumed to significantly influence the gut microbiome (Pal et al., 2012; Conlon & Bird, 2015; Thursby & Juge, 2016; Castaner et al., 2018; Senghor et al., 2018; Valdes et al., 2018; Hills et al., 2019) and many cancer patients change their diet during treatment as they become aware of diet and cancer links (Pal et al., 2012), a seven-day food diary which is routinely used in clinical practice at the Royal Marsden NHS Foundation Trust (Appendix 23) was used to provide information about what an individual's dietary intake consists of as this can be analysed in a systematic manner (Shim, Oh, & Kim, 2014;

Naska, Lagiou, & Lagiou, 2017). This method was chosen above a food frequency questionnaire or 24hr diet recall as it reduces memory bias, is independent of interviewer skills and is less resource heavy (Shim et al., 2014). Repeated assessments are most useful to measure changes over time (Shim et al., 2014). Food diaries were completed at baseline, at the end of treatment and all follow-up time points. The paper food diaries were stored with the case report form and completed questionnaires.

The information recorded in the food diaries by the participants was entered into the Nutritics programme by a Master's degree student at King's College London. Nutritics is a software programme used by healthcare and sports professionals that allows the input of ingredients and meals and assists on dietary analysis (Nutritics, 2019). Each time point was entered into Nutritics as a different participant with specific codes per time point to enable the input of weight and height for every time point and track weight and BMI over time. The data were exported into Excel and analysed using SPSS. The selection of 22 dietary parameters was informed by the studies described in a systematic literature review undertaken in collaboration with the Master's student (Muls et al., 2020) and extensive discussion with this MSc supervision team.

4.7 Biomarkers of gastrointestinal toxicity

4.7.1 Faecal calprotectin

Faecal calprotectin is a biomarker of inflammation in the GI tract. It is commonly used to determine whether a patient has an acute episode or flare of Crohn's Disease or ulcerative colitis (Alibrahim, Aljasser, & Sahl, 2015; Walsham & Sherwood, 2016). Neutrophils are part of the white blood cells in blood and help heal damaged tissue and resolve inflammation. Neutrophils are made in the bone marrow by granulocyte stimulating growth factor (GCSF) and stored in the blood circulation. Under healthy circumstances, the intestinal epithelium acts as a physical and immunological barrier that prevents direct contact between luminal microbiota and intestinal mucosa and is free of neutrophils. When epithelium is injured, neutrophils can be recruited to the site of infection as phagocytes and producers of anti-bacterial metabolites such as hydrogen peroxide (Hidalgo, Chilvers, Summers, & Koenderman, 2019; Wéra, O., Lancellotti, P., & Oury, 2016). Within this process, neutrophils also produce a protein called calprotectin (Bjarnason, 2017). Neutrophils are eliminated by macrophages and the metabolites and proteins can be found in stool (Bratton & Henson, 2011).

A stool sample is tested for faecal calprotectin and if negative, has a value equal or less than 15 microgram per kilogram (mg/kg) stool. The test is positive with a faecal calprotectin level of more than 60 mg/kg stool which leaves a grey zone for interpretation between those values. In general, a cut off of 50 mg/kg stool has been agreed for patients with inflammatory bowel disease (NICE, 2013).

For a cut-off of 50 mg/kg, the sensitivity of faecal calprotectin to correctly identify individuals with inflammatory bowel disease in adults lies between 82% and 98% (Tibble, Sigthorsson, Foster, Forgacs, & Bjarnason, 2002; Von Roon et al., 2007; Gisbert & McNicholl, 2009; Van Rheenen, Van de Vijver, & Fidler, 2010; Pavlidis, Chedgy, & Tibble, 2013; Burri et al., 2014). The ability to correctly exclude inflammatory bowel disease or specificity reported in the same studies ranges from 68% to 96%.

Suspected inflammatory bowel disease is mainly diagnosed by general practitioners in primary care where when requesting a faecal calprotectin stool sample analysis 0.4% is false negative and 5.6% is false positive (NICE, 2013). Patients are then referred to a gastroenterologist where diagnosis is confirmed with colonoscopy and biopsies.

Several studies in patients treated with radiotherapy for pelvic cancer showed a correlation between raised faecal calprotectin levels and GI inflammation during treatment and longitudinal studies, measuring faecal calprotectin a year and 40 months post treatment also showed elevated levels in those with chronic GI toxicity compared to levels below 60 mg/kg in those without chronic GI toxicity (Wedlake et al., 2008; Varela et al., 2009; Hille et al., 2017). For this study, the cut-off point was set at 100 mg/kg to indicate those with treatment induced inflammation and to minimise the risk of false positive correlation with IBDQ-B scores. Stool samples for faecal calprotectin were collected at each time point. The samples were then frozen by the biochemistry department and sent

in batches to a laboratory in Sheffield University for analysis which handles samples for the Royal Marsden NHS Foundation Trust. Results were accessed on the Electronic Patient Record and reported on the case report form.

Evaluation of faecal calprotectin as a potential biomarker for long-term radiation-induced GI toxicity is presented in Chapter five. Associations overall, and across time-points are described using the Pearson correlation coefficient. A general linear mixed model (GLMM) for repeated measures (nested within participants), a population-average model, was used to test the relationship between IBDQ-B and faecal calprotectin scores overall across time. The dependent variable was the IBDQ-B score and the independent variable the faecal calprotectin level. The fitted model accounts for serial correlation across time points within women. Random effects were omitted due to lack of variability that prevented the model from converging. A time point and faecal calprotectin interaction was added to the model to determine whether this association varied across time. The model residuals were skewed to the left. To address this, faecal calprotectin, which was skewed to the right, was natural log transformed and IBDQ-B scores, which were skewed to the left, was natural log transformed after taking the IBDQ-B score for each patient from 71 (i.e. one more than the highest possible IBDQ-B score). The model was refitted using these transformed variables and the residuals were normally distributed. Pearson's correlation between faecal calprotectin level at the end of treatment and IBDQ-B scores at one year and two years post RT were calculated.

4.7.2 Metabolite production of the gut microbiome

Gastrointestinal microbiota produce gases from their metabolites (Volatile Organic Compounds or VOCs) that can be detected and analysed from biological samples such as breath, faeces, saliva, sweat or urine and are gas phase biomarkers which reflect the metabolic condition of a person (Shirasu & Touhara, 2011; Arasaradnam et al., 2014; Schmidt & Podmore, 2015; Chen, Wang, Kuo, & Tsai, 2019).

A stool sample provides a specimen in which gastrointestinal microbiota are present directly from which the gases can be analysed. A less onerous way of collecting gastrointestinal microbiota may be through a rectal swab which reduces the burden of providing a stool sample, especially when experiencing GI toxicity during and after treatment.

Urine contains metabolic breakdown products from a wide range of foods, drinks, drugs, environmental contaminants, endogenous waste metabolites and bacterial by-products and is easy to obtain from patients in appropriate volumes (Bouatra et al., 2013). Urine, therefore, could also contain metabolites relating to the gut microbiota and GI toxicity (Covington et al., 2012; Arasaradnam et al., 2014).

As discussed in chapter one, it has been suggested that the composition of the oral microbiome in saliva may be linked to diseases at other body sites either in a causative way or as a reflection of systemic changes in the body and oral

microbiomic profiles could be used as diagnostic biomarkers for other diseases (Ahn et al., 2012; Wade, 2013).

The following samples were requested from participants at all time points: stool, urine, rectal swabs and saliva. Participants were given specific instruction on how to collect these samples (Appendix 24). They were asked to collect the specimens as close to their appointment as possible.

Three rectal swabs were taken by the participants themselves using the Copan Italia Sterilin SPA ® culture swab transport system suitable for both aerobe and anaerobe bacteria. The tip of the swab was cut with sterile scissors by the researcher, stored into a Thermo Scientific 1.8ml NUNC tube, labelled and frozen at -80 C. Rectal swabs have been shown to be a reliable proxy for analysing the gut microbiome compared to stool samples in children and in adults including those treated for cancer (Bassis et al., 2017; Reyman, van Houten, Arp, Sanders, & Bogaert, 2019, Biehl et al., 2019). As patients receiving pelvic radiotherapy can develop diarrhoea during treatment, the rectal swabs were included to check whether this would be a viable alternative to providing stool samples within this cohort.

Urine, stool and saliva samples were collected in Sarstedt 80/50 yellow 70ml sterile containers. The urine sample was aliquoted into 5 smaller sterile Fisherscientific cryogenic 4.5ml vials and labelled. The stool sample was also aliquoted into 5 Sarstedt faecal sample pots with screw cap and spoon and

labelled. The saliva sample was aliquoted in 3 Thermo Scientific 1.8ml NUNC tubes and labelled.

The labels included the study allocation number, initials of participant, time point, date of collection and sample type (Figure 4.2).

Figure 4.2 Examples of sample labeling.

All samples were frozen at -80 °C and logged in the FreezerPro sample database. Sample storage was organised for all samples per time point and per participant number in appropriate cryogenic boxes.

When samples needed to be moved to a larger storage facility, this was done according to the International Airport Transport Association guidelines (International Airport Transport Association, 2018) on dry ice and in suitable containers labelled appropriately (Figure 4.3).

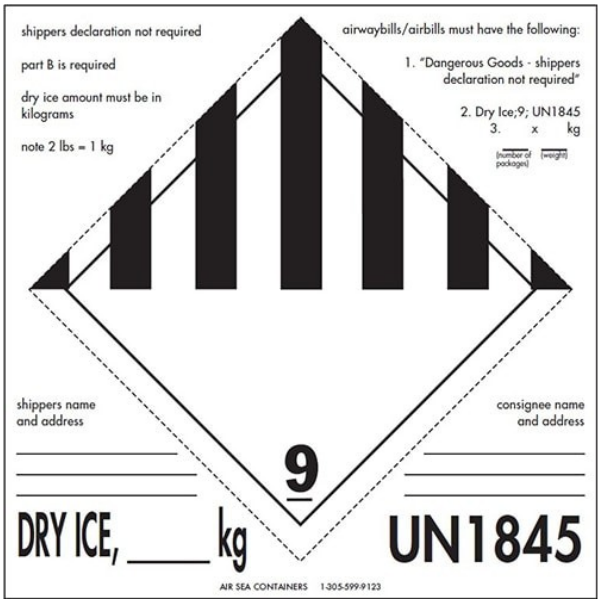


Figure 4.3 United Nations 1845 label for transportation of samples on dry ice.

City Sprint was the courier used to transport samples from the Royal Marsden NHS Foundation Trust to the laboratory at the University of Warwick. Samples were stored on dry ice and the box was labelled in line with the dangerous goods regulations (International Airport Transport Association, 2018). A summary of the samples was included in a sealed envelope.

At the University of Warwick, samples were stored in the freezer facility at -20 °C prior to analysis. At analysis samples were defrosted in a 4 °C fridge over night before analysis to minimise chemical deterioration. All samples were prepared to undergo VOC analysis by two methods: the electronic nose and GC-IMS.

Of the stool samples, a sub-sample of 0.5 g was weighed using a calibrated scale (A and D, Japan) into a glass vial of 20 mL for GC-IMS and electronic nose. Of each urine sample the required sample amount was taken using a calibrated

Gilson micro-pipet and pipetted in a glass vial which was then sealed with a PTFE crimp cap (Thames Restek, UK). For GC-IMS 5mL of sample was pipetted into a 20mL glass vial (Thames Restek, UK) required 3mL of sample in a 10mL sample vial. Of the saliva samples, 0.5ml was taken and pipetted into the glass vial of the appropriate size and sealed with a crimp cap. The rectal swabs were inserted in a glass vial and crimp capped.

Analysis with the electronic nose

The samples were tested by commercial electronic nose instruments – specifically a PEN-3 (Airsense Analytical, Germany) and an FOX4000 (AlphaMOS, France) analysis. Samples were treated the same, with being agitated and heated to 37-40 °C for 10 min before 2.5 ml of the sample headspace was injected into the electronic nose. Previous work has shown body temperature to be the optimal temperature to reduce loss of chemical components within the sample (Covington et al., 2015).

Samples were loaded onto the loading tray in the auto-sampler where up to 42 samples can be loaded at once. The auto-sampler picks up one vial at a time with a sensor lever and moves it into the analysis component of the device injecting a needle into the head space of the sample to analyse the chemical composition of the gas emitted by the sample.

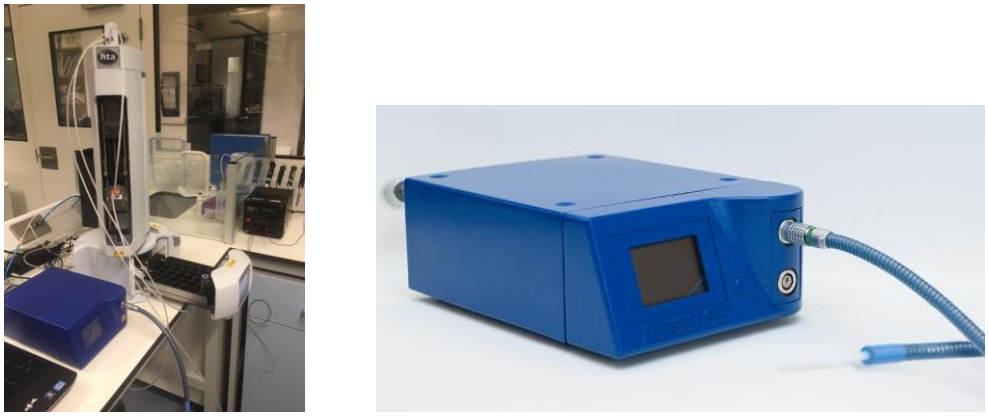


Figure 4.4: The Air sense PEN 3 device and autosampler.

The raw sensor output from the e-nose is a 2D Spreadsheet with sensors along one axis and time in the other. The table is formed of sensor resistance values taken throughout the measurement process. From these features were extracted, in this case, the maximum resistance divided by the baseline, the maximum resistance minus the baseline and the area. These are features commonly used with electronic noses. These features were then run on a statistical analysis program written in the R Statistics Package. This used a 10-fold cross validation approached, where the data was split into a 10% training set and a 90% test set. The models were trained to the training set and applied to the test set. This was repeated 10 times until all the samples have been test samples. Five different models were used (Gaussian Process, NeuralNet, RandomForest, SparseLogisticRegression and SupportVectorMachine) and from the resultant probabilities, statistical values, including area under the curve, sensitivity, specificity, positive predictive value and negative predictive value were calculated. A probability (p-value) was calculated based on the probabilities generated by the statistical models. The statistical analysis of the electronic nose data was undertaken by the team at Warwick University.

Analysis with GC-IMS

The GC-IMS analysis was done using the G.A.S. FlavourSpec machine (Dortmund, Germany) (Figure 4.5) in accordance with settings described by Bosch et al. (2020). Samples were loaded into the auto-sampler which can load 32 samples at a time. A 15m, SE-54 capillary column was used (CS Chromatographie, Germany). Samples were heated and agitated at 40°C for ten minutes before 0.5mL of the headspace was injected directly into the GC-IMS. The GC analysis was performed at 40 °C using nitrogen 99.99% as the carrier gas (3.5 bar) and the IMS was performed at 45 °C, also using nitrogen. Flow rates were set at 20 ml/min (34.175kPa) for the GC and 150 ml/min (0.364kPa) for the IMS and the analysis time lasted 8 minutes.

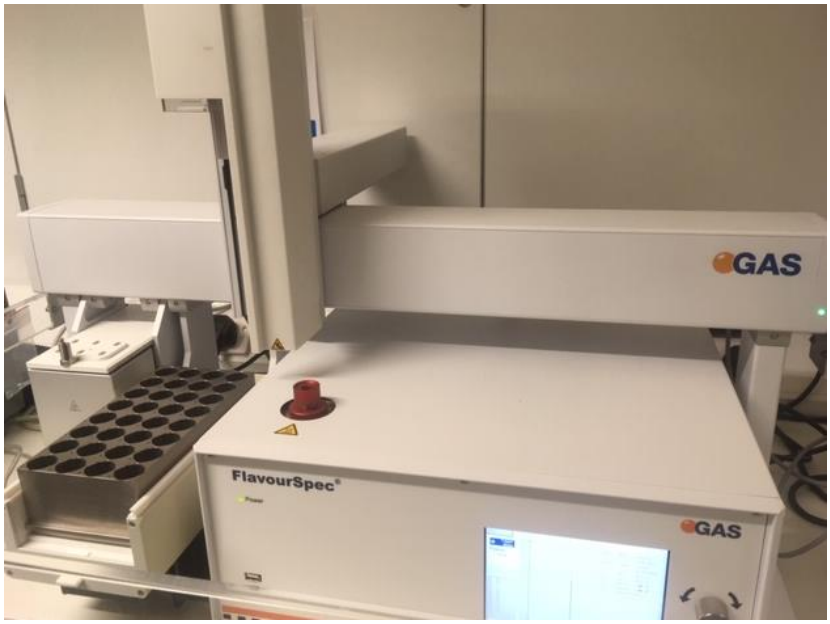


Figure 4.5 The G.A.S. FlavourSpec machine.

GC-IMS generates much higher dimensional datasets than produced by the electronic nose. As the chemical information is located in one area of the output, this area is cropped to reduce the overall dimensionality. The same crop values were used for all files. Then a small threshold was applied to remove the background (thus background values become zero in the output dataset). As with the e-nose data a 10-fold cross-validation was used, splitting the data into 10% training set and 90% test set. Features were selected in the training set using a rank-sum test, with the features with the 20 lowest p-values used to create the model. Thus, the feature selection and training were independent of the test samples. As with the e-nose data, different classifiers were applied to the data and statistical parameters calculated. The GC-IMS statistical analysis was undertaken by the team at Warwick University.

4.8 Bias

Sampling bias may be an issue in this study as the recruited population and the population who declined participation may not similar with regards to, age, tumour stage, performance status or other demographic variables. A systematic error caused by non-random sampling could be important. Therefore, differences in baseline characteristics between those recruited, those who provided data at more than one time point and those who completed the study will be reported.

In addition, this was a single centre study in a tertiary oncology centre which may limit the generalisability to women treated for gynaecological malignancies in general hospitals as, even though treatment protocols are defined nationally, there may be variances in individualised treatment plans.

Response bias, where the participant provides incomplete or false information, needs to be acknowledged with regards to the food diaries for assessing nutritional intake. In addition, observation bias may be an issue as respondents may change their intake during the diary period. Correlating BMI with total calorie intake will provide a measure of the likelihood of participants under or over reporting their dietary intake.

The attempt to avoid unnecessary hospital visits for the participants as oncology follow-up appointment did not always match the exact study time points, especially at the one and two year time points after treatment may also lead to some bias as this resulted in a time margin of a few months either side of the actual time point.

Samples were stored for different lengths of time before being sent to the University of Warwick for analysis and this may incur bias in the laboratory analysis as samples may degrade over time (Esfahani et al., 2016). As the original stool and urine samples were split into 5 separate containers per time point to allow enough material for the different VOC analysis and because there is little consensus in the literature describing sample deterioration when stored

at -80 °C, a second aliquot of the stool and urine samples were sent to the laboratory exactly one year later to assess the impact of different storage times.

4.9 Study sample size

This observational cohort study was not powered to a specific end point but the following assumptions were made to estimate the number of patients feasible to recruit.

In preparation of the study protocol the number of women referred to the organisation during 2013 were requested from our information department: 918 patients were referred with a gynaecological cancer (cervix, ovary, endometrium, vagina, and uterus). Of the 780 patients that were treated and alive at one year, 29 (4%) received pelvic radiotherapy alone (3 after surgery) and a further 100 (13%) received brachytherapy in combination with pelvic radiotherapy (Unpublished data). On paper, these 129 patients (17%) met the eligibility criteria for this study.

The recruitment rate for studies within our team has been consistent between 30 and 40%. This suggested a potential annual recruitment of 55 patients (or 5 per month), 110 patients over two years. However, the data did not differentiate how many women were treated for recurrent disease, how many were overseas patients or how many might be unable or unwilling to consent.

4.10 Patient and public involvement

At the Royal Marsden NHS Foundation Trust all research proposals are reviewed by patient, carer and GP representatives, especially trained in guiding research priorities and ensuring the viewpoints of these groups are incorporated in the outline of the proposal. In addition, the patient and carer research review group (PCRRG) reviews all information available for patients and carers. On the Royal Marsden Hospital website (www.royalmarsden.nhs.uk/pages/home.aspx) there is a section for patients and carers to propose clear research priorities. This subject was identified as a particular interest by patients who have experienced the devastating effect of long-term changes in their bowel function and GI symptoms after completing their cancer treatment.

The impetus for this study originated from a combination of sources including patients and carers. The author has been involved in extensive discussion with members of the Pelvic Radiation Disease Association (PRDA) and Jo's Trust about patient priorities. Both are charities that provide support for people living with and beyond cancer and they identified that a key topic is better information pre-treatment as to the risks of developing GI consequences of cancer treatments. In addition, the Chairman of PRDA wrote a letter of support explaining his association's viewpoint (Appendix 25). They found this study highly appealing as the clinical relevance is enormous, the study is relatively simple and, if effective, could yield many benefits for the management of cancer

patients at all stages in the disease and treatment trajectory, from diagnosis onwards.

In addition, the study was well received at the Royal Marsden NHS Foundation Trust's Patient Carer Research Group meeting on the 23rd of April 2013 where a power point presentation and lay summary were presented. Participants discussed the relevance of the study and more specifically its importance for direct patient care and service development within a broader context of cancer survivorship.

Following completion of the data collection and analysis, the main research findings will be disseminated to members of the public on the PRDA website.

4.11 Statistical analysis methods

A Statistical Analysis Plan (SAP) was written by the researcher (AM) and approved by the study Chief Investigator (Appendix 26). Statistical analysis was conducted in partnership with the study statistician at the Royal Marsden NHS Foundation Trust and the health statistician at King's College London using SPSS version 24 for the statistical analysis. Statistical analysis of the metabolonic signatures of the samples was conducted under the guidance of Professor James Covington, using a bespoke statistical analysis programme in the R Statistics Package at the University of Warwick.

After data input into a secure database by an independent data manager, 100% of primary data were cleaned by the researcher (AM) and the database was updated by the data manager prior to its release. Once imported into SPSS, all data were checked for normality.

Analysis of study recruitment data included the number of patients screened, number of patients excluded with reasons, number of patients missed, number of patients who declined participation with reasons and the number of participants who withdrew or died during the study were presented using the CONSORT diagram (chapter five).

Participants are described with respect to age, cancer tumour site, histology, the presence of lymphovascular space invasion, surgery, chemotherapy, radiotherapy (Gy and number of fractions), marital status, number of children, mode of delivery, smoking status, alcohol intake, type of diet, weight at baseline, BMI, performance status, co-morbidities and use of medications both for all who provided baseline data, those who provided data at more than one time point and those who completed the study.

Statistical significance was set at $p < 0.05$ and tests were applied 2-sided. Categorical data will be summarised by numbers and percentages. Continuous data are summarised by median and range and associations between the primary outcome (IBDQ-B score) and other variables of interest are presented using Pearson's correlation coefficients, ANOVA or independent t-test results.

The incidence of new onset GI symptoms after pelvic radiotherapy is reported using descriptive statistics. The relationship between the incidence of new symptoms, radiotherapy regimen and other potential confounding factors (e.g. age, Body Mass Index, performance status) and patient characteristics using the change in Inflammatory Bowel Disease Questionnaire (IBDQ) score and IBDQ-B subset score, change in Vaizey incontinence score, change in the GI section of the Common Terminology Criteria for Adverse Events (CTCAE) (version 4.0) score and change in Bristol Stool Chart parameters (stool frequency, type) are reported using non-parametric analysis.

Changes in the IBDQ-B scores in between time points are described as well as its value in predicting GI toxicity one and two years after treatment. Comparisons were from baseline to 6 months after treatment with the final two time points using a Generalised Linear Mixed Model (GLMM) and correlation analysis. This approach is able to fit a model in the presence of missing data under the assumption that the data are missing-at-random (Seltman, 2018).

A generalised linear mixed model (GLMM) was also the approach selected for the analysis of the dietary intake. GLMMs allow for a variety of correlation patterns over time to be incorporated into the model.

4.12 Conclusion

This chapter has described the key methods used in the design and conduct of this longitudinal observational cohort study and provides a rationale for the

selection of the methods, including a critical discussion of the literature in the area, as well as a detailed description of the actual methods used. The outcomes of the analysis and clinical findings are presented in chapter five.

Chapter 5

Results

This chapter reports the findings of the PREDICT study using the STROBE checklist of items to include in reports of cohort studies (von Elm et al., 2008) (Appendix 26).

5.1 Study performance

5.1.1 Recruitment

From 01 November 2015 until 31 October 2017, 945 women were screened from the clinic and MDT lists of the gynaecology oncology clinics at the Royal Marsden Hospital Chelsea and Sutton sites. 310 (33%) were excluded because they did not have a gynaecological malignancy but were referred to one of the medical oncologists also treating people with skin cancer. Of the remaining 635 (77%) with gynaeological cancer, 524 (83%) were excluded mainly because they had a previous pelvic cancer (n= 166), received radiotherapy < 45Gy for symptom control or palliative reasons rather than with curative intent (n= 99), were not due to be treated with radiotherapy (n= 58), were not a UK resident (n= 36), would receive treatment elsewhere (n= 35) or had communication problems (n= 20). Other reasons are highlighted in Figure 5.1.

A total of 111 (17% of those with gynaecological cancer) women were eligible to participate in the study. Some of these women (n= 27, 24%) were missed due to being added late onto a clinic list with no opportunity to approach them for the study prior to the start of treatment. 43 (39%) declined participation in the study. Reasons included not being interested in study participation (n=12, 28%), feeling unable to provide the samples requested (n=7, 16%), feeling too

distressed by their cancer diagnosis and impending treatment (n= 8, 19%) or feeling unable to participate due to personal circumstances (n= 1). Fifteen women (35%) did not offer a reason.

Forty-one women (37% of those eligible) agreed to participate in the study, consented and were assigned a study participation identifier. Thirty-six women (88% of those consenting) provided baseline samples, completed questionnaires and a food diary and allowed clinical data to be collected. However, six women (16%) did not provide further data at the second time point (10 days into radiotherapy). A total of 24 participants completed the study at the last time point, two years after completion of treatment. Therefore, data of 24 participants has been included in the analysis.

As only two women had an additional time point after receiving chemotherapy before radiotherapy, this time point was omitted as it would not be able to pick up any chemotherapy related toxicity in such small number of participants.

5.1.2 Withdrawn participants

Five of the 41 recruited women (12%) withdrew before providing baseline samples and questionnaires and another six provided baseline data but then decided to discontinue (Table 5.1). The main reasons these women gave was that they felt unable or were too unwell to provide the samples (n= 8) or that they felt too burdened by the prospect of their treatment to contribute (n= 3). Withdrawal throughout the study was 3 (8%) before the third time point (at the

end of radiotherapy), 3 (8%) before the fourth time point (6 months following completion of radiotherapy), none before the fifth time point (one year after completion of radiotherapy) or the final time point (two years after finishing radiotherapy). All participants who indicated they no longer wished to continue with the study signed a study withdrawal form (Appendix 14) indicating that they consented to the data already collected being included in the final analysis. Appendix 28 shows the timing schedule for the data collection points throughout the study.

Time point	**N (%)**
Recruited	41 (100%)
Provided baseline data	36 (87%)
Provided data at more than 1 time point	30 (71%)
Provided data at the end of RT	28 (68%)
Provided data at 6 months post RT	23 (56%)
Provided data at 1 year after RT	24 (59%)
Provided data at 2 years after RT	24 (59%)

Table 5.1 participant numbers (%) who completed data collection throughout the PREDICT study.

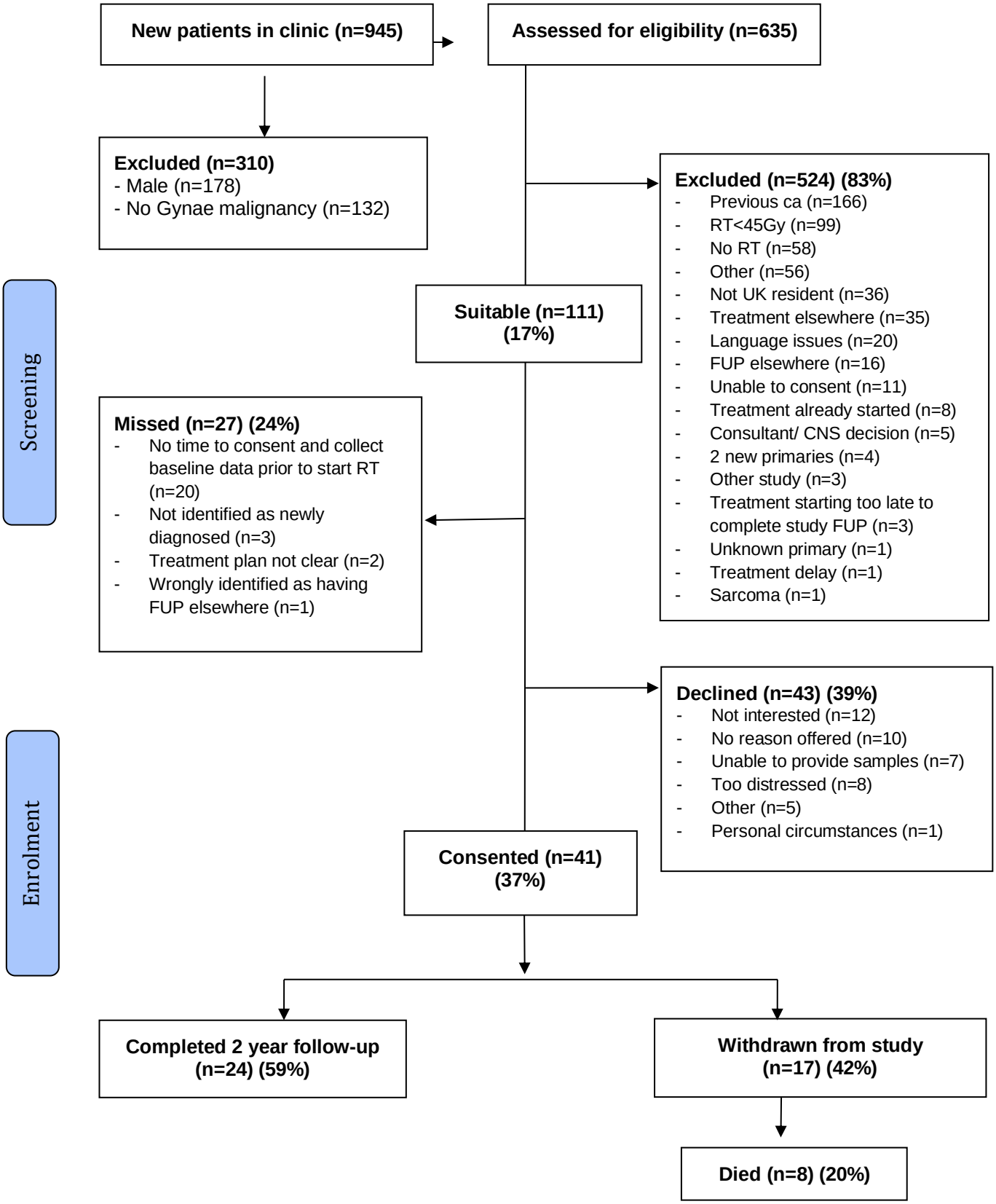


Figure 5.1 Screening and recruitment diagram PREDICT Study

5.2 Data analysis

5.2.1 Participant clinical and demographic characteristics

As the primary end point was long-term gastroenterological toxicity, data from participants who completed the final time point (two years after completion of radiotherapy) were included in the final analysis. Women who withdrew were more likely to have lymphovascular space invasion (n= 9, 53% vs. n= 11, 46%), less likely to consume alcohol (n= 7, 41% vs. n= 17, 71%) and were less likely to be nullipara than those who completed the study. (n= 4, 24% vs. n= 11, 46%) (Table 5.2).

Of the 24 women included, the median (range) age of the cohort was 54 (27-78) years. The most common ethnicity was White British (n= 17, 71%). There was only one women of Black African ethnicity and two were of Asian descent. The remaining women were White European origin (n=4, 17%). The most prevalent gynaecological cancer diagnosis was endometrioid adenocarcinoma of the endometrium (n= 13, 43%). Nearly half of the participants (n= 11, 46%) had lymphovascular space invasion which means there are malignant cells within the blood vessels or lymph system and which carries an increased risk of metastatic disease. Nearly 80% (n= 19) had received surgery prior to their radiotherapy and 75% (n= 17) were also treated with chemotherapy. All women received external beam radiotherapy (EBRT) (median dose: 45 Gy, range: 45- 58 Gy) and brachytherapy (BT) (median dose: 8 Gy, range: 6- 20 Gy).

Characteristic	All recruited (n= 41)	Withdrew before or at baseline (n=11)	Withdrew before or at 6 months (n= 6)	Completed 2 year time point (n= 24)
Tumour site: n (%)				
Endometrium	22 (54)	6 (55)	3 (50)	13 (54)
Cervix	16 (39)	5 (45)	2 (33)	9 (38)
Vagina	2 (5)	-	1 (17)	1 (4)
Vulva	1 (2)	-	-	1 (4)
Histology: n (%)				
Adenocarcinoma	8 (20)	4 (36)	2 (33)	2 (8)
Adenosquamous carcinoma	1 (2)	-	-	1 (4)
Carcinosarcoma	1 (2)	-	-	1 (4)
Clear cell carcinoma	1 (2)	1 (9)	-	0 (0)
Endometrioid adenocarcinoma	14 (34)	3 (27)	1 (17)	10 (42)
Mucinous carcinoma	1 (2)	-	-	1 (4)
Serous carcinoma	3 (6)	-	1 (17)	2 (8)
Squamous cell carcinoma	12 (29)	3 (27)	2 (33)	7 (29)
Lymphovascular space invasion: n (%)				
Yes	20 (49)	7 (64)	2 (33)	11 (46)
No	21 (51)	4 (36)	4 (67)	13 (54)
Surgery: n (%)				
Yes	29 (71)	6 (55)	4 (67)	19 (79)
No	12 (29)	5 (45)	2 (33)	5 (21)
Concomitant chemotherapy: n (%)				
Cisplatin	21 (51)	4 (36)	4 (67)	13 (54)
Carboplatin	3 (7)	3 (27)	-	-
Carboplatin + Placlitaxel	8 (20)	3 (27)	1 (17)	4 (17)
None	9 (22)	1 (9)	1 (17)	7 (29)
Radiotherapy: n (%)				
EBRT	41 (100)	11 (100)	6 (100)	24 (100)
Brachytherapy	38 (93)	8 (73)	6 (100)	24 (100)
Radiotherapy dose (Gy)				
EBRT: median (range)	45 (45- 58)	50.4(45- 56)	47.5 (45- 56)	45 (45- 58)
Brachytherapy: median (range)	18 (4- 22.5)	16.5 (8- 23)	16.5 (4- 20)	8 (6- 20)
Radiotherapy dose (fractions)				
EBRT: median (range)	25 (24- 29)	28 (24-29)	25 (25- 28)	25 (24- 28)
Brachytherapy: median (range)	2 (1- 4)	3 (2- 5)	3 (1- 4)	3 (2- 4)

Table 5.2 Baseline clinical characteristics of41 recruited participants

The majority of participants were married (n= 16, 67%) and half (n= 12, 50%) had one (n= 5, 21%) or more (n= 7, 29%) children. Most women (n= 9, 75%) had given birth vaginally as opposed to birth via caesarian section (n=2, 16%). More than half (n= 14, 55%) never smoked and four women were current smokers (17%). Nearly a third of women did not drink any alcohol (n= 7, 29%).

For those who did, the median of alcoholic units per week was 5 (range: 2-28). Most women included meat in their diet (n= 20, 80%). Median weight at baseline was 70 kg (range: 62-131 kg) and median BMI was 27 (range: 17-48).

Characteristic	All recruited (n= 41)	Withdrew before or at baseline (n=11)	Withdrew before or at 6 months (n= 6)	Completed 2 year time point (n= 24)
Age: years				
Median (range)	57 (26- 84)	57 (32- 82)	61.5 (36-75)	54 (27- 78)
Ethnicity: n (%)				
African, Black	3 (7)	2 (18)	-	1 (4)
Asian	6 (15)	3 (27)	1 (17)	2 (8)
White, British	26 (63)	5 (45)	4 (66)	17 (71)
White, Irish	1 (2)	1 (9)	-	-
White, Other	5 (12)	-	1 (17)	4 (17)
Marital status: n (%)				
Married	26 (63)	6 (55)	4 (67)	16 (67)
Single	8 (20)	3 (27)	-	5 (21)
Co-habiting	3 (6)	1 (9)	-	2 (8)
Widowed	3 (6)	1 (9)	1 (17)	1 (4)
Divorced	1 (2)	0 (0)	1 (17)	0 (0)
Children: n (%)				
Nullipara	15 (37)	3 (27)	1 (17)	11 (46)
Unipara	7 (17)	2 (18)	-	5 (21)
Multipara	19 (46)	6 (55)	5 (83)	8 (33)
Mode of delivery: n (%)	26 (100)	8 (100)	5 (100)	13 (100)
Normal delivery	20 (86)	6 (75)	3 (60)	11 (85)
C-section	3 (12)	1 (12)	-	2 (16)
Both	3 (12)	1 (12)	2 (40)	-
Smoking status: n (%)				
Current smoker	5 (12)	1 (9)	-	4 (17)
Ex-smoker	10 (24)	3 (27)	2 (33)	5 (21)
Never smoked	26 (63)	7 (64)	4 (67)	15 (62)
Number of cigarettes per day				
Median (range)	1 (1- 60)	20	0	1 (1- 60)
Alcohol intake: n (%)				
Yes	24 (59)	4 (36)	3 (50)	17 (71)
No	17 (41)	7 (64)	3 (50)	7 (29)
Number of alcoholic units per week				
Median (range)	9 (2- 28)	14.5 (12- 21)	20 (14- 28)	5 (2-28)
Diet: n (%)				
Includes meat	36 (88)	10 (91)	6 (100)	20 (84)
Vegetarian	3 (6)	1 (9)	-	2 (8)
Pescetarian	2 (4)	-	-	2 (8)
Weight at baseline: kg				
Median (range)	71 (62-145)	66 (52-145)	78 (60-113)	70 (62-131)
BMI at baseline				
Median (range)	27 (17- 54)	26.3 (20- 54)	32.3 (24- 42)	27.1 (17- 48)

Table 5.3 Demographic characteristics of 41 recruited participants.

5.2.2 Primary end-point

5.2.2.1 IBDQ-B scores

The IBDQ bowel subset score ranged from 10 (poorest bowel function) to 70 (best bowel function). The median IBDQ-B score, for the 24 participants who completed the study, at baseline, was 68.5 (range: 38-70). The median at the end of treatment was at its lowest with a median score of 58 (range: 23-66) but this improved six months, 1 year and two years after radiotherapy (Table 5.4). For each question, participants can indicate the occurrence of a symptom from 'not at all' to 'more than ever before'. The categories 'moderate increase in frequency' to 'more than ever before' are considered to reflect clinically significant symptom burden. Nine women (37.5%) did not have a change in their IBDQ-B scores between baseline and two years after radiotherapy. Nine women (37.5%) did have a drop of ≥ 10 points in their IBDQ-B scores as defined in Chapter three (section 4.5.2) at the end of treatment compared to baseline but recovered two years after completion of radiotherapy and six women (25%) had a drop of ≥ 10 points in their IBDQ-B scores at the end of treatment compared to baseline but did not recover two years after completion of radiotherapy. These three groups were used to analyse differences in the metabolic signatures from stool, urine, saliva samples and rectal swabs (see section 5.2.2.2).

	Baseline	Start of RT	End of RT	6 months post RT	12 months post RT	24 months post RT
Complete scores	n= 24	n= 24	n= 23	n= 22	n= 24	n= 24
IBDQ-B Median Range	68.5 (38- 70)	62 (34- 70)	58 (23-66)	65.5 (38- 70)	65 (46-70)	63 (31- 70)

Table 5.4 The IBDQ-B scores per time point (median, range)

The IBDQ-B answers per question showed that most of the participants (n= 19, 79%) had a normal bowel frequency before the start of treatment (Figure 5.2). This reduced to only 8% at the end of radiotherapy (n= 2). The number of women indicating normal bowel frequency increased 6 months and one year post radiotherapy but dropped again two years after radiotherapy.

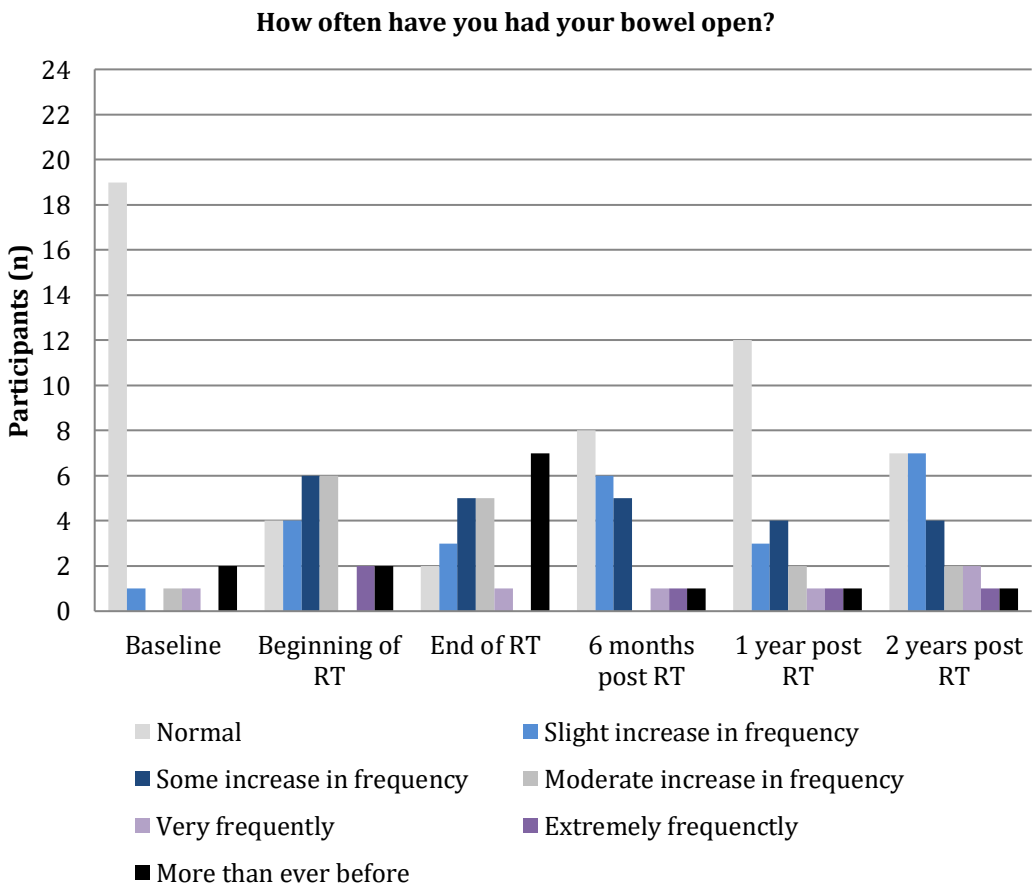


Figure 5.2 IBDQ-B question bowel frequency.

The IBDQ-B question relating to stool consistency (Figure 5.3) showed that most women had normal stool consistency at baseline (n= 18, 75%). This reduced to only one woman reporting she had not experienced loose bowel movements at the end of treatment with more than half continuing to have loose stool (n= 14, 58%) two years after the completion of radiotherapy.

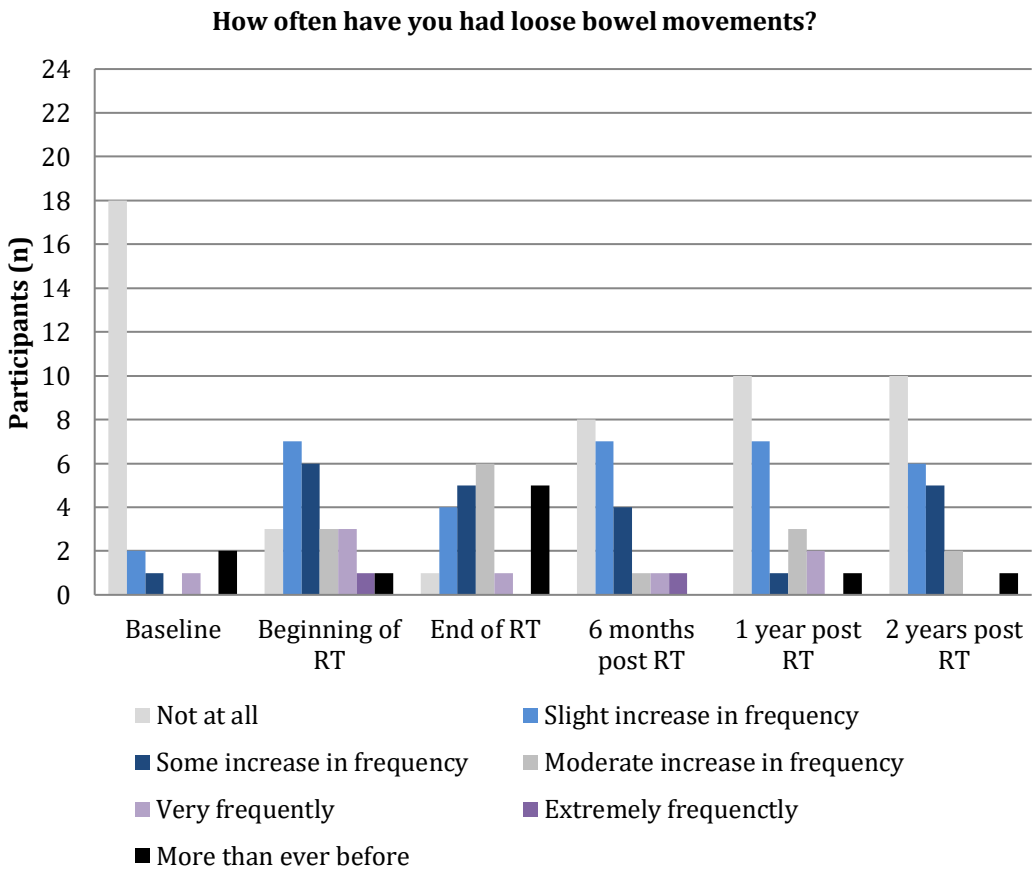


Figure 5.3 IBDQ-B question stool consistency.

Some degree of peri-anal pain was experienced at the end of treatment in half of the cohort (n= 12, 50%) with pain generally lower at other time points (Figure 5.4).

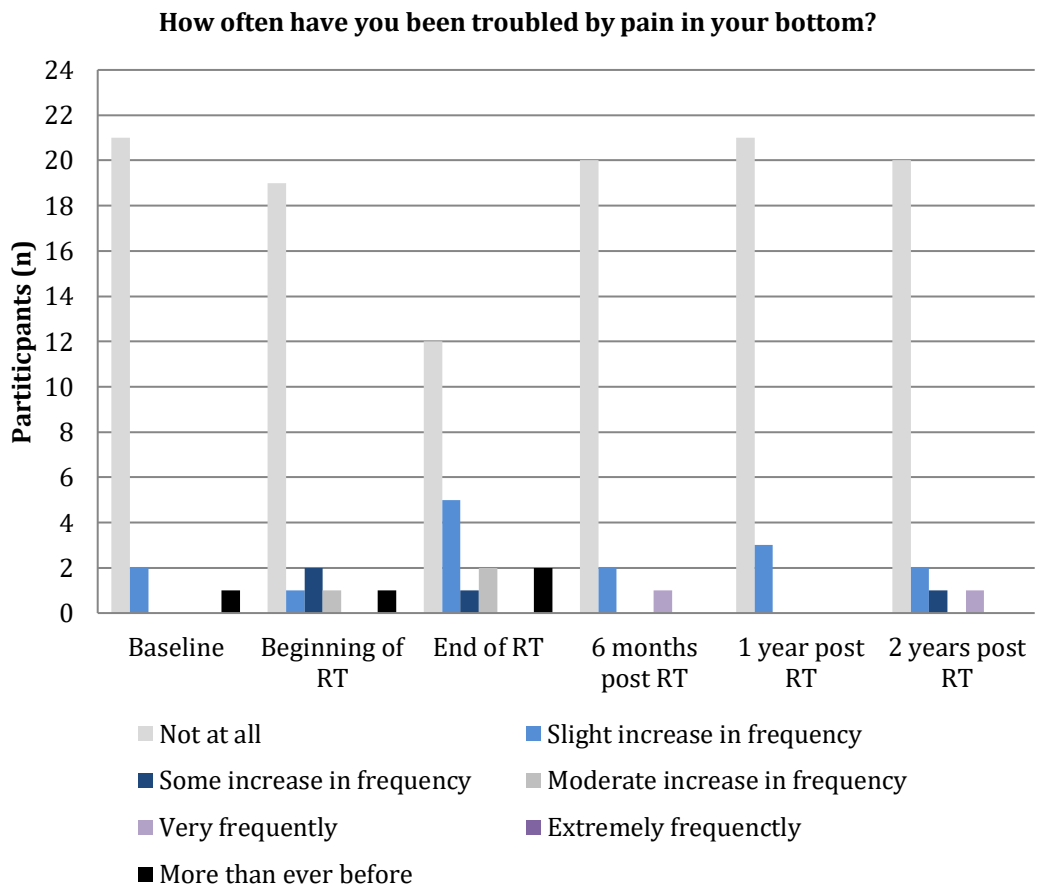


Figure 5.4 IBDQ-B question perianal pain.

Abdominal cramps (Figure 5.5) were reported by 71% (n= 17) of the women at the end of treatment. One woman did not complete her questionnaire at the end of treatment (Table 5.4). Abdominal cramps continued to be reported by 46% two years after completion of treatment (n= 11).

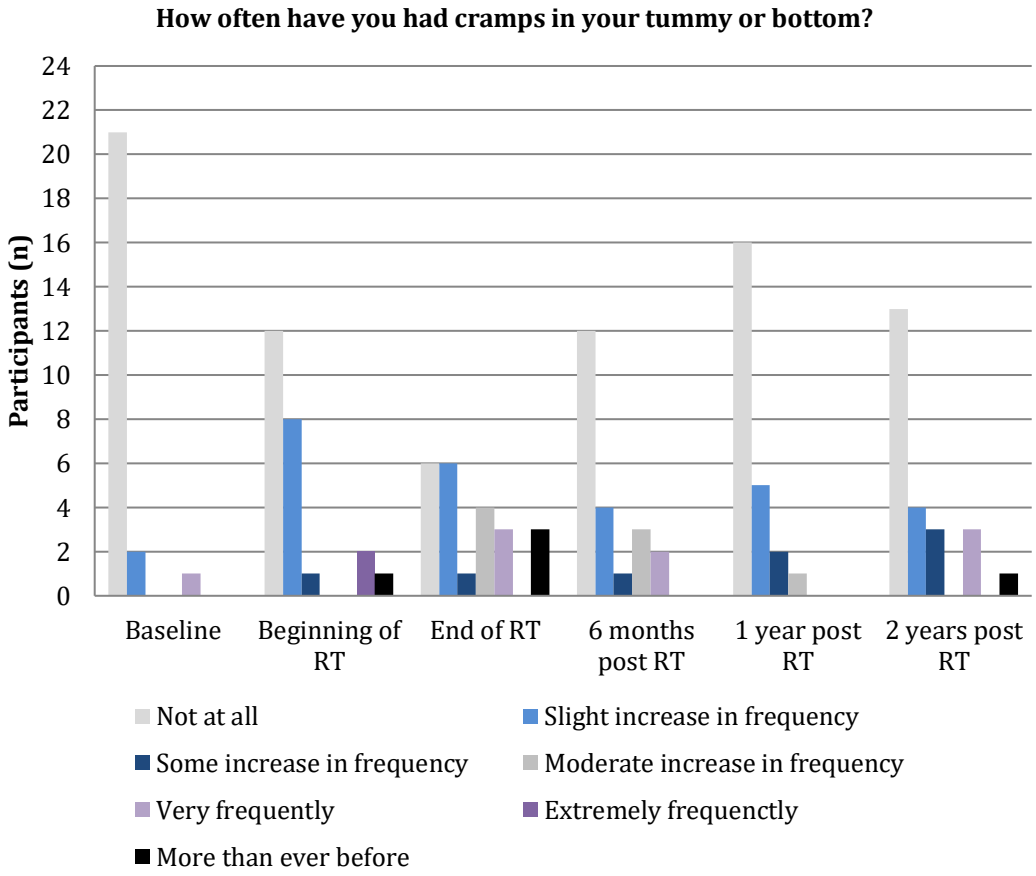


Figure 5.5 IBDQ-B question abdominal cramps.

Flatulence was reported as not a problem or a slight increase in frequency by most women (Figure 5.6). The same two women reported severe excessive flatulence at six months post radiotherapy and two years after completion of radiotherapy.

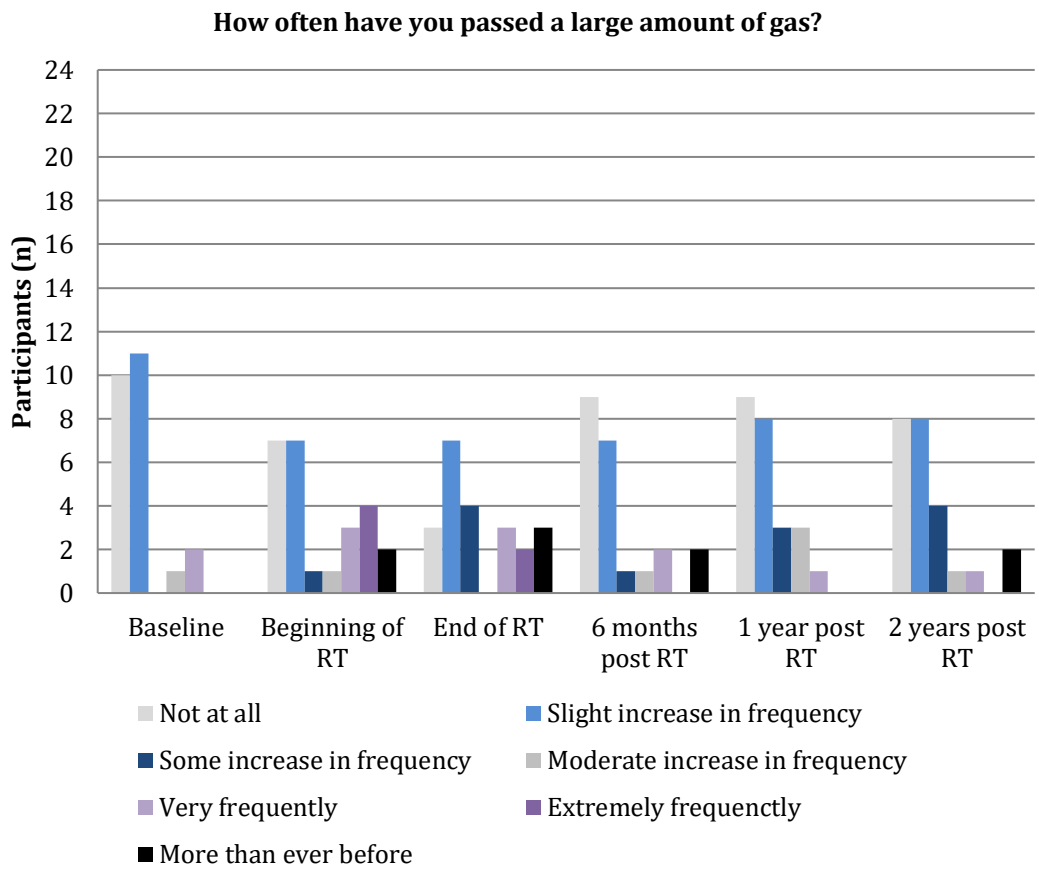


Figure 5.6 IBDQ-B question flatulence.

Bloating was also not often reported as an issue with a moderate to a more than ever before increase in frequency (Figure 5.7).

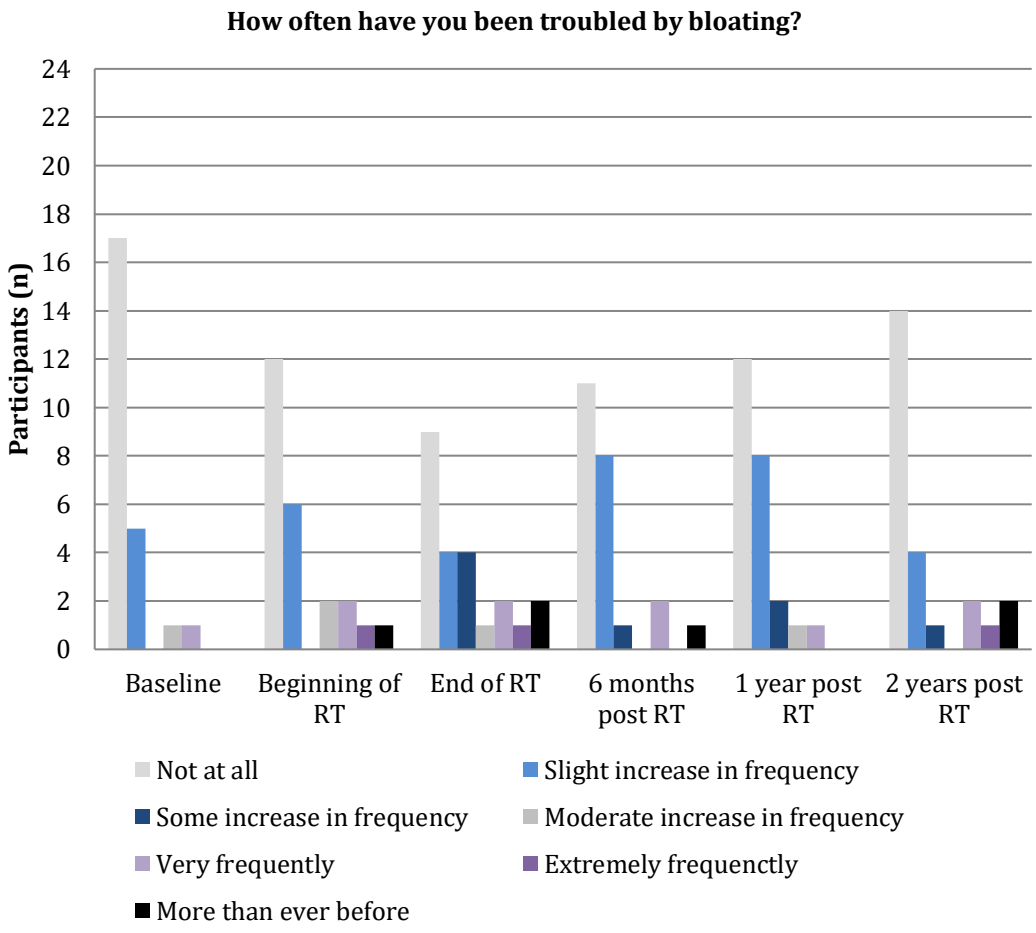


Figure 5.7 IBDQ-B question bloating.

Rectal bleeding was experienced infrequently, including two years after radiotherapy (Figure 5.8).

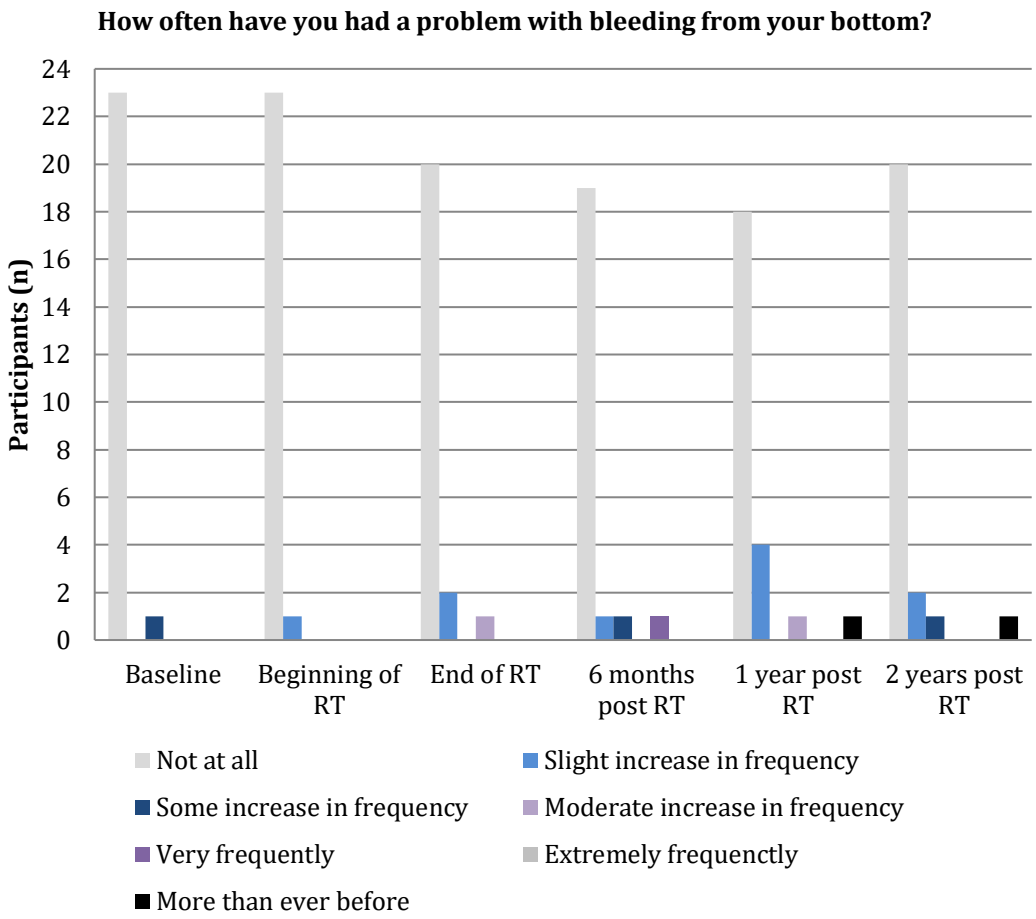


Figure 5.8 IBDQ-B question rectal bleeding.

Tenesmus occurred either 'not at all' or with a 'slight increase' for most women across at all time points (Figure 5.9) and affected women least often one year post radiotherapy (n= 6, 25%).

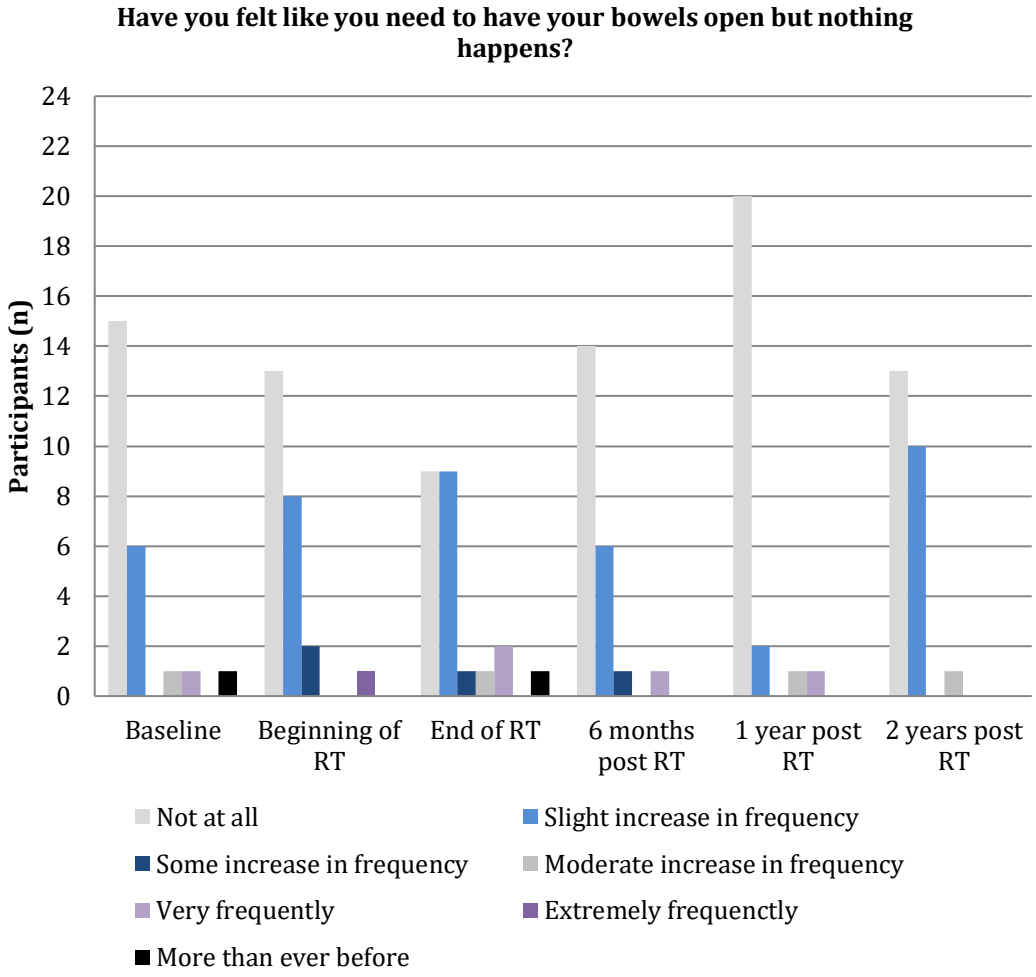


Figure 5.9 IBDQ-B question tenesmus.

Increased frequency of faecal incontinence (Figure 5.10) occurred more often in women at the end of treatment and two years after completion of radiotherapy (n= 9, 38%) compared to other time points. Whilst the majority reported this as only a slight increase, the impact of experiencing faecal incontinence on quality of life is not refected in these results.

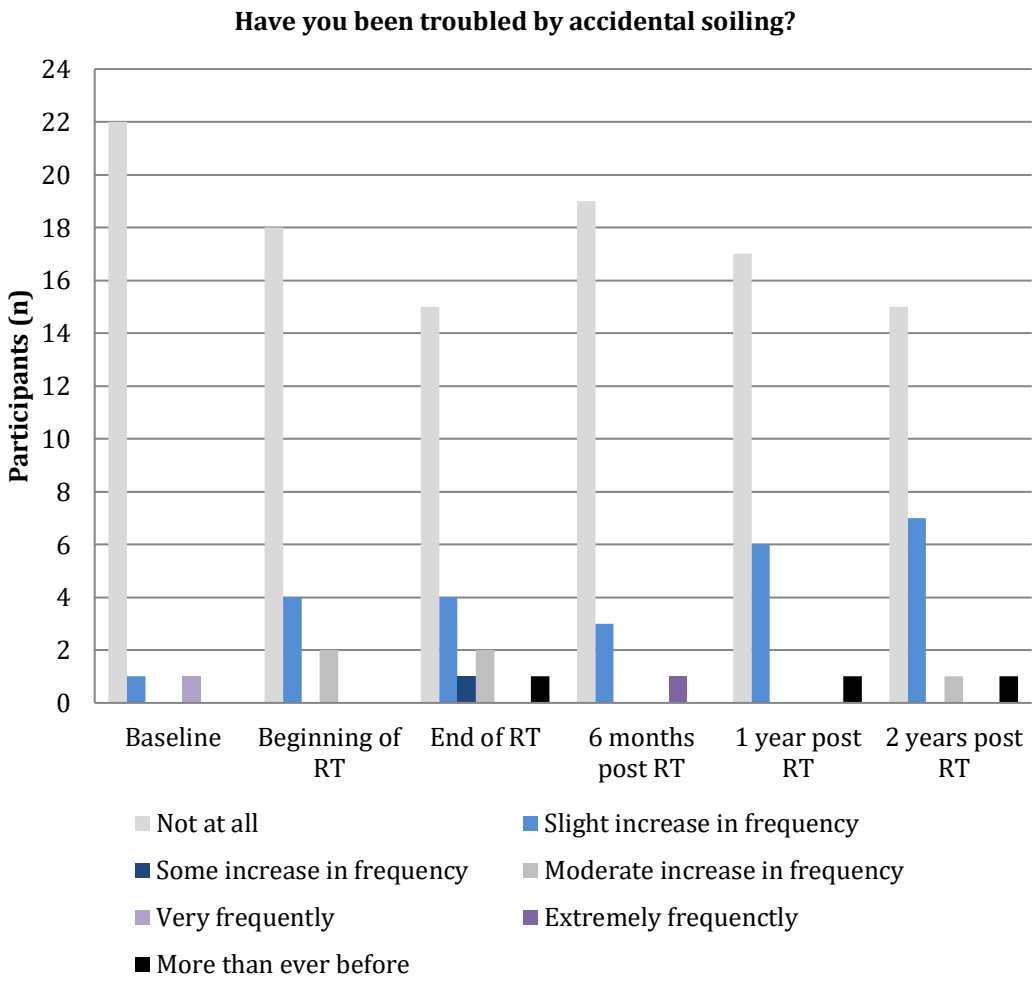


Figure 5.10 IBDQ-B question faecal incontinence.

Women felt most disgusted about their bowel problems at end of treatment (7, 29%) (Figure 5.11).

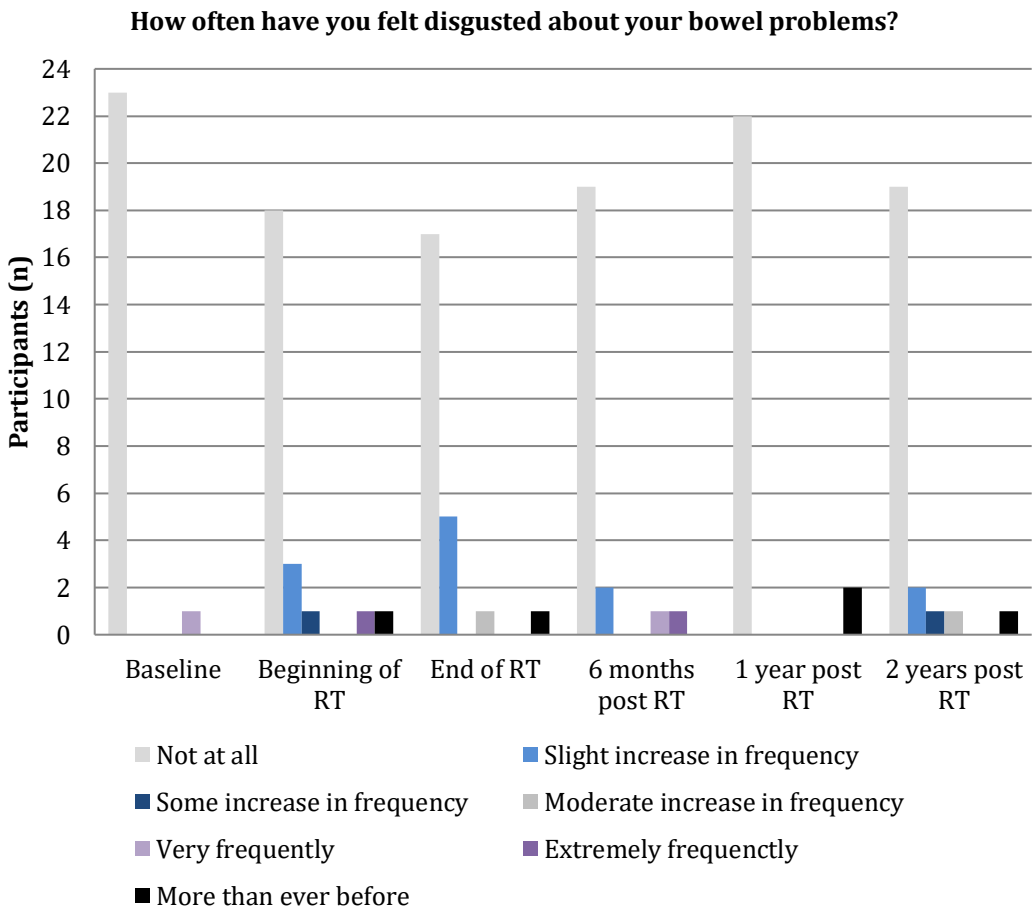


Figure 5.11 IBDQ-B question feeling disgusted.

5.2.2.2 Relationship of IBDQ-B scores with other variables

The relationship between the following clinical variables and the IBDQ-B scores over time was explored: age, BMI, ECOG performance status, tumour type, the presence of lymphovascular space invasion, surgery, chemotherapy and dose of EBRT and brachytherapy.

In the Generalised Linear Mixed Model (GLMM) with age as the independent variable, the age * time interaction was not statistically significant (F= 0.13 [numerator df =5, denominator df=40.58], p=0.98) suggesting that if there was an effect of age on IBDQ score it did not vary across time. None of the time specific correlations between IBDQ score and age were statistically significant (Table 5.6). The overall correlation with age was weak (r= 0.10, p= 0.24). The fixed effect beta coefficient for age was 0.06 (95% CI -0.05 to 0.18, p= 0.29) and was also not statistically significant. Therefore, age does not appear to influence the IBDQ-B scores in this cohort.

Table 5.5 shows the BMI categories for all time points. The majority of women (n= 16, 77%), were overweight or obese, two years after radiotherapy. In the GLMM with BMI as the independent variable the BMI * time interaction was not statistically significant (F= 0.72 [numerator df =5, denominator df=43.96], p= 0.62) suggesting that if there was an effect of BMI on IBDQ scores it did not vary across time. The fixed effect beta coefficient for BMI was 0.23 (95% CI 0.04 to 0.43, p= 0.018) and was statistically significant. Whilst, BMI appears to influence the IBDQ-B scores within this cohort and this remains consistent

across time, the overall correlation with BMI was weak (r= 0.17, p= 0.044) and none of the time specific correlations between IBDQ scores and BMI were statistically significant (Table 5.6).

BMI n (%)	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
< 18.5 Underweight	1 (4)	1 (4)	1 (4)	1 (4)	1 (4)	1 (4)
18.5-24.9 Healthy	6 (25)	6 (25)	6 (25)	7 (29)	9 (37.5)	7 (29)
25-29.9 Overweight	7 (29)	7 (29)	8 (33)	4 (17)	3 (12.5)	8 (33)
30-39.9 Obese	9 (37.5)	9 (37.5)	8 (33)	9 (37.5)	8 (33)	6 (25)
< 40 Morbidly obese	1 (4)	1 (4)	1 (4)	1 (4)	2 (8)	2 (8)

Table 5.5 BMI categories per time point

The GLMM with EBRT dose as the independent variable showed that the EBRT dose * time interaction was not statistically significant (F= 0.63 [numerator df =5, denominator df=40.29], p= 0.68) suggesting that if there was an effect of EBRT dose on IBDQ scores it did not vary across time. The fixed effect beta coefficient for EBRT was 0.14 (95% CI -0.21 to 0.48, p= 0.43) and was not statistically significant. None of the time specific correlations between IBDQ scores and EBRT dose were statistically significant (Table 5.6).

Equally, in the GLMM with brachytherapy dose as the independent variable the BT dose * time interaction was not statistically significant (F= 0.48 [numerator df =5, denominator df=41.40], p= 0.79) suggesting that if there was an effect of

BT dose on IBDQ scores it did not vary across time. The fixed effect beta coefficient for BT dose was -0.06 (95% CI -0.40 to 0.27, p= 0.72) and was not statistically significant. None of the time specific correlations between IBDQ scores and BT dose were statistically significant (Table 5.6).

IBDQ-B correlation with	Pearson's correlation *r*	p- value
Age		
Overall	0.10	p= 0.24
Baseline	0.03	p= 0.90
Beginning of RT	0.21	p= 0.33
End of RT	0.11	p= 0.62
6 months post RT	0.04	p= 0.87
1 year post RT	0.13	p= 0.54
2 years post RT	0.52	p= 0.81
BMI		
Overall	0.17	p= 0.044
Baseline	-0.13	p= 0.55
Beginning of RT	0.13	p= 0.56
End of RT	0.31	p= 0.15
6 months post RT	0.30	p= 0.19
1 year post RT	0.36	p= 0.086
2 years post RT	0.20	p= 0.36
EBRT dose		
Overall	0.09	p= 0.30
Baseline	-0.01	p= 0.96
Beginning of RT	0.35	p= 0.10
End of RT	0.17	p= 0.44
6 months post RT	-0.08	p= 0.73
1 year post RT	0.07	p= 0.75
2 years post RT	-0.03	p= 0.90
Brachytherapy dose		
Overall	0.01	p= 0.87
Baseline	-0.23	p= 0.29
Beginning of RT	0.10	p= 0.63
End of RT	0.18	p= 0.43
6 months post RT	-0.14	p= 0.54
1 year post RT	-0.04	p= 0.85
2 years post RT	0.05	p= 0.82

Table 5.6 Pearson correlation coefficients for age, BMI, ECOG scores, EBRT and brachytherapy dose.

The GLMM with lymphovascular space invasion as the independent variable showed that the LVSI * time interaction was not statistically significant (F= 1.12 [numerator df =5, denominator df=41.24], p= 0.37) suggesting that if there was an effect of LVSI on IBDQ scores it did not vary across time. The fixed effect beta coefficient for LVSI was 1.66 (95% CI -1.26 to 4.58, p= 0.26) and was not statistically significant. Using an independent t-test, none of the time specific correlations between IBDQ scores and LVSI were statistically significant (Table 5.7).

The GLMM with surgery as the independent variable showed that the surgery * time interaction was not statistically significant (F= 0.90 [numerator df =5, denominator df=38.72], p= 0.49) suggesting that if there was an effect of having had surgery prior to radiotherapy on IBDQ scores it did not vary across time. The fixed effect beta coefficient for surgery was 3.75 (95% CI 0.23 to 7.28, p= 0.26) and was not statistically significant. Using an independent t-test showed that having had surgery prior to radiotherapy resulted in a mean IBDQ-B score of 64 compared to a mean of 59 for those who did not have surgery one year after radiotherapy (p<0.001) (Table 5.7).

In the GLMM with chemotherapy as the independent variable the chemotherapy * time interaction was not statistically significant (F= 0.17 [numerator df =5, denominator df=40.39], p= 0.97) suggesting that if there was an effect of chemotherapy on IBDQ scores it did not vary across time. The fixed effect beta coefficient for chemotherapy was -1.74 (95% CI -3.91 to 0.42, p= 0.12) and was not statistically significant. Using an independent t-test, none

of the time specific correlations between IBDQ scores and chemotherapy were statistically significant (Table 5.7).

IBDQ-B score	Yes	No	p-value
LVSI mean (SD)			
Baseline	67.00 (5.44)	63.46 (9.36)	p= 0.095
Beginning of RT	56.73 (11.43)	60.15 (9.16)	p= 0.48
End of RT	52.36 (13.43)	54.33 (12.23)	p= 0.37
6 months post RT	64.90 (7.09)	59.17 (10.06)	p= 0.13
1 year post RT	62.18 (7.08)	63.38 (6.78)	p= 0.91
2 years post RT	63.73 (6.18)	58.23 (10.39)	p= 0.14
Surgery (Y/N)			
Baseline	65.42 (7.99)	63.80 (8.08)	p= 0.46
Beginning of RT	58.95 (9.74)	57.20 (12.87)	p= 0.58
End of RT	53.00 (12.31)	55.25 (15.56)	p= 0.65
6 months post RT	64.12 (6.23)	53.80 (12.13)	p= 0.08
1 year post RT	63.95 (4.78)	58.60 (11.59)	p< 0.001*
2 years post RT	60.79 (9.21)	60.60 (9.10)	p= 0.62
Chemotherapy mean (SD)			
Baseline	64.29 (9.11)	67.00 (3.11)	p= 0.055
Beginning of RT	56.76 (11.36)	63.00 (4.62)	p= 0.061
End of RT	51.44 (13.68)	57.86 (8.80)	p= 0.16
6 months post RT	60.33 (10.03)	64.86 (6.41)	p= 0.18
1 year post RT	61.65 (7.33)	65.71 (4.46)	p= 0.17
2 years post RT	59.53 (9.76)	63.71 (6.40)	p= 0.36

Table 5.7 Independent t-test of mean (SD) IBDQ-B scores per time point according to the presence of LVSI, previous surgery, treatment with chemotherapy.

As there were only two women with carcinoma of the vulva and vagina, these were excluded as the comparison groups were too small. Women with cancer of the endometrium (n= 13) were compared to women with cancer of the cervix (n= 9) using an independent t-test. In the GLMM with tumour type as the independent variable the tumour type * time interaction was not statistically significant (F= 0.44 [numerator df =5, denominator df=37.47], p= 0.81) suggesting that if there was an effect of tumour type on IBDQ scores it

did not vary across time. The fixed effect beta coefficient for tumour type was -5.51 (95% CI -8.56 to -2.45, p= 0.001) and was statistically significant. All of the time specific correlations between IBDQ scores and tumour type using an independent t-test were statistically significant with the exception of the last time point, two years after radiotherapy (Table 5.8). Women with carcinoma of the cervix had lower IBDQ-B scores than women with carcinoma of the endometrium indicating their bowel function was worse but the impact of tumour type on difference in mean IBDQ-B scores was no longer detectable two years after completion of radiotherapy.

Tumour type	**Endometrium**	**Cervix**	**p- value**
IBDQ-B score: mean, SD			
Baseline	67.54 (2.57)	60.78 (11.58)	P< 0.001*
Beginning of RT	61.08 (5.17)	54.44 (14.66)	P< 0.001*
End of RT	56,54 (8.82)	49.88 (17.64)	p= 0.001*
6 months post RT	63.77 (7.70)	57.14 (11.61)	p= 0.003*
1 year post RT	65.00 (4.67)	59.22 (8.61)	p= 0.045*
2 years post RT	60.46 (11.08)	60.67 (6.75)	p= 0.37

Table 5.8 Independent t- test for tumour type per time point.

Physical activity was measured at each time point using the ECOG performance status (Appendix 15). Even at baseline, 42% (n= 10) of the participants did not consider themselves fully active. This increased to 83% (n= 20) by the end of radiotherapy. Two thirds of women (n= 16, 67%) reported restricted physical activity or were able to undertake self-care activities but no strenuous activity two years after completing radiotherapy (Figure 5.12).

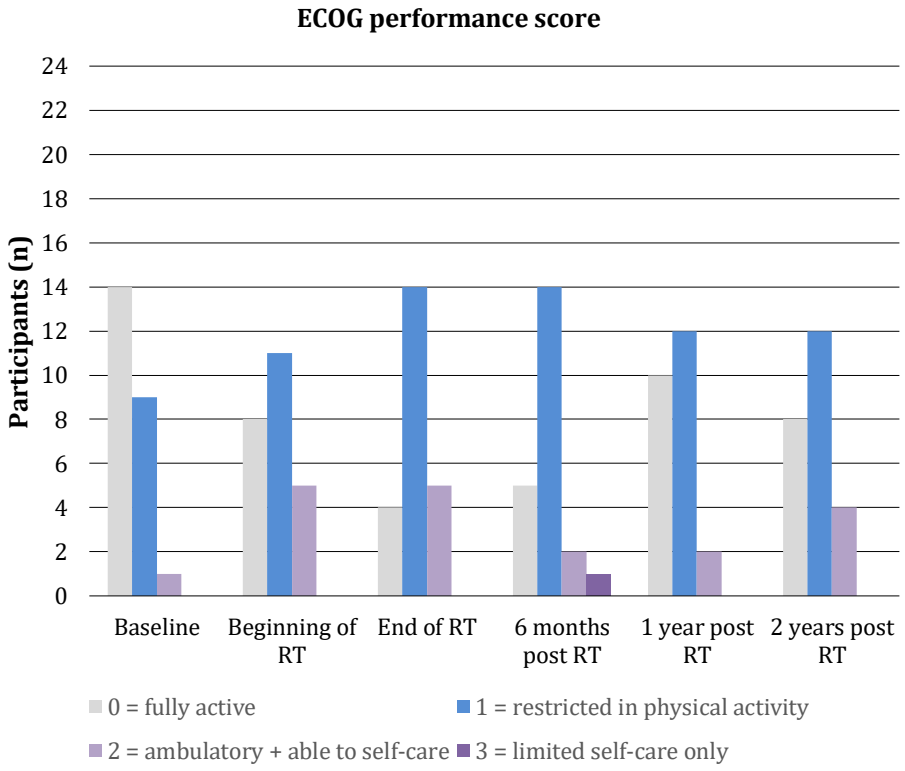


Figure 5.12 The ECOG Performance Status scores per time point.

In the GLMM with ECOG performance status as the independent variable the ECOG performance status * time interaction was not statistically significant (F= 1.30 [numerator df =5, denominator df=39.18], p= 0.28) suggesting that if there was an effect of ECOG performance status on IBDQ scores it did not vary across time. The fixed effect beta coefficient for ECOG performance status was

-3.15 (95% CI -5.31 to -0.99, p< 0.001) and was statistically significant. Therefore, performance status appears to influence the IBDQ-B scores within this cohort irrespective of time although time-specific comparisons using ANOVA found statistically significant differences between the performance status groups at baseline and the beginning of treatment but not at any later time points (Table 5.9).

ECOG performance status	**0**	**1**	**2-3**	**p-value**
IBDQ-B score: mean, SD				
Baseline	66.57 (5.45)	65.78 (5.73)	38.00 (-)	p< 0.001*
Beginning of RT	66.00 (2.67)	55.45 (10.20)	53.60 (12.46)	p= 0.03*
End of RT	56.75 (10.24)	53.50 (11.53)	50.40 (18.30)	p= 0.77
6 months post RT	66.80 (3.11)	60.64 (8.29)	58.67 (17.90)	p= 0.37
1 year post RT	63.40 (6.82)	61.92 (7.23)	65.50 (6.36)	p= 0.76
2 years post RT	61.88 (6.03)	59.92 (11.12)	61.00 (8.60)	p= 0.90

Table 5.9 ANOVA test for ECOG Performance score per time point (0 = fully active, 1= restricted in physical strenuous activity, 2= ambulatory and capable of self-care, 3= capable of limited self-care).

5.2.2.3 VOC olfactory signatures

The e-nose creates a heat map of the gasses found within the samples. Figure 5.13 below provides some examples of such outputs.

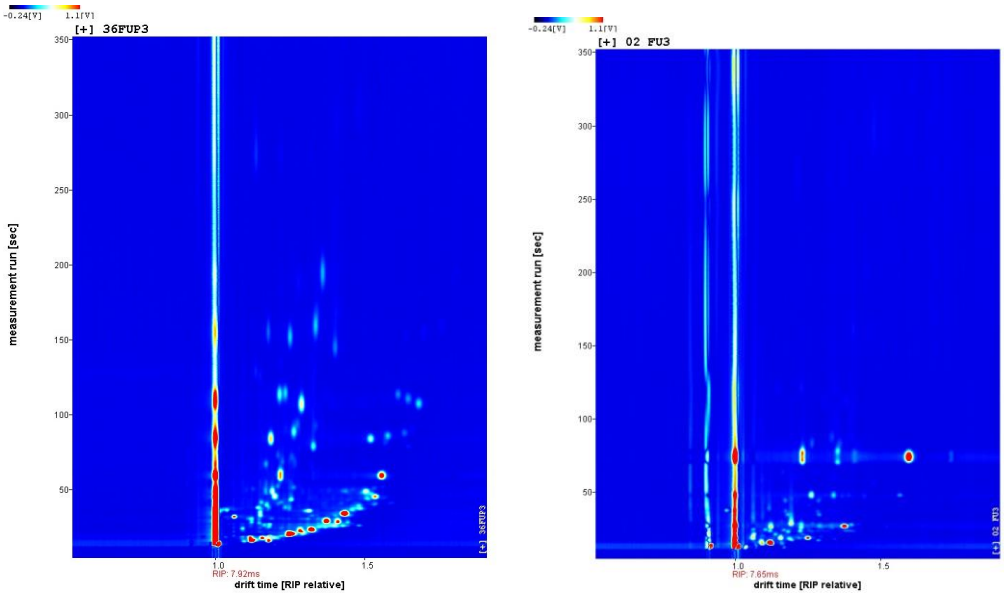


Figure 5.13 Analysis heat map with e-nose of stool (Left) and urine (Right). The coloured areas depict the VOCs in the sample.

5.2.2.4 VOC analysis per IBDQ-B scores

For the analysis of the IBDQ-B and the olfactory signatures, the participants were categorised in 3 groups (Table 5.10). These definitions are used throughout this chapter.

Group A	Women with a drop of ≥ 10 points in their IBDQ-B score at the end of treatment compared to baseline and did not improve two years after radiotherapy compared to baseline	n= 6 (25%)
Group B	Women with a drop of ≥ 10 points in their IBDQ-B score at the end of treatment compared to baseline but then improved	n= 9 (37.5%)
Group C	Women who did not have a change in their IBDQ-B scores between baseline and 2 years after radiotherapy	n= 9 (37.5%)

Table 5.10 Definitions of groups A-C as per IBDQ-B scores.

Analysis of the VOC signatures with the e-nose showed Group B can be clearly separated in stool samples at baseline from groups A and C. There is some overlap between group A and C which may be due to some women having no change in their IBDQ-B scores but having already quite a low IBDQ-B score at baseline. However, two women in group C did not have a baseline stool sample with sufficient material for analysis (Figure 5.14).

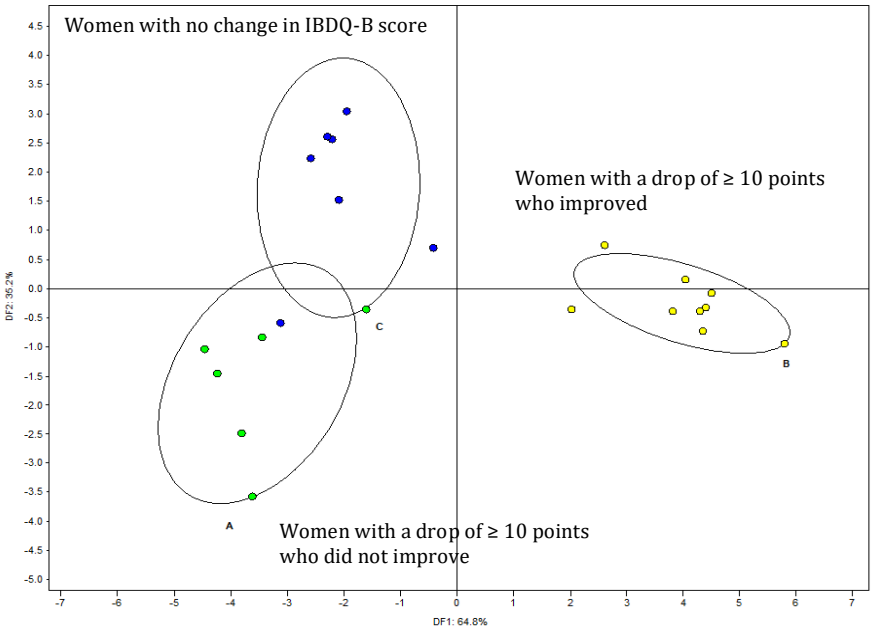


Figure 5.14 VOC signatures of stool per IBDQ-B group at baseline (A, women with a drop of ≥ 10 points who did not improve; B, women with a drop of ≥ 10 points who improved; C, women with no change in IBDQ-B scores)

Sensitivity and specificity were calculated and compared between groups A, B and C. The analysis shows that analysis by the e-nose technique compared to GC-IMS provides more consistent results in separating the groups regarding differences in IBDQ-B scores. Stool, urine and saliva samples at the beginning of treatment, within 10 days of starting radiotherapy, (purple section in Table 5.11) can predict GI toxicity two years after completion of radiotherapy. Further

details of the area under the curve (AUC), PPV and NPV show how efficient each method is for detecting the different groups per sample type.

Analysis technique	Sample type	Group	Baseline	Beginning of RT	End of RT	6 months post RT	1 year post RT	2 years post RT
e-nose	Stool	A vs C						
		B vs C						
	Urine	A vs C						
		B vs C						
	Saliva	A vs C						
		B vs C						
	Rectal swab	A vs C						
		B vs C						
GC-IMS	Stool	A vs C						
		B vs C						
	Urine	A vs C						
		B vs C						
	Saliva	A vs C						
		B vs C						
	Rectal swab	A vs C						
		B vs C						

Table 5.11 Comparison of the ability to distinguish between IBDQ-B groups of the e-nose and GC-IMS analysis (blue= statistically significant, purple= statistically significant for all groups, white= not statistically significant).

5.2.2.3.1 Stool sample analysis

The stool sample analysis for groups A and group C shows that analysis of the VOCs by the e-nose is most effective in distinguishing between those two groups at the end of treatment (Table 5.12). The area under the curve is the largest at the end of radiotherapy (AUC= 0.92) with a sensitivity of 67% and a specificity of 83%. The positive predictive value for the e-nose analysis at the end of radiotherapy was 80% which means that performing this analysis at this time point increases the likelihood to set apart someone who has no change in IBDQ-B scores and someone who continues to have a drop of ≥ 10 points two years after treatment for both groups by more than 40 to 55%. A negative predictive value of 71% means there is quite a high risk of misclassification in doing the test at the end of radiotherapy.

e-nose Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.77 (0.53-0.97)	0.86 (0.65-1)	0.92 (0.75-1)	0.79 (0.50-1)	0.83 (0.58-1)	0.87 (0.68-1)
Sensitivity (95% CI)	1 (1-1)	0.83 (0.50-1)	0.67 (0.33-1)	0.83 (0.50-1)	1 (1-1)	0.83 (0.50-1)
Specificity (95% CI)	0.43 (0.13-0.75)	0.57 (0.25-0.88)	0.83 (0.50-1)	0.75 (0.50-1)	0.25 (0.50-1)	0.89 (0.70-1)
PPV	1	0.80	0.80	0.71	1	0.83
NPV	0.23	0.63	0.71	0.86	0.46	0.89
p-value	0.125	0.035*	0.015*	0.081	0.065	0.022*
GC-IMS Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.75 (0.40-1)	Unable	0.55 (0.22-0.88)	0.80 (0.53-1)	Unable	0.88 (0.67-1)
Sensitivity (95% CI)	0.67 (0.33-1)	To	0.50 (0.17-0.86)	0.80 (0.50-1)	To	0.60 (0.20-1)
Specificity (95% CI)	0.75 (0.33-1)	process	0.72 (0.60-1)	0.83 (0.56-1)	Process	0.88 (0.67-1)
PPV	0.80		0.67	0.83		0.75
NPV	0.60		0.75	0.80		0.78
p-value	0.937		0.836	0.216		0.030*

Table 5.12 E-nose and GC-IMS analysis of group A and C.

The stool sample analysis for group B and group C shows that analysis of the VOCs by the e-nose is again more effective in distinguishing between those two groups at all time points except 6 months after radiotherapy compared to the same analysis by the GC-IMS (Table 5.13). The area under the curve is the largest at baseline (before radiotherapy) (AUC= 0.89) with a sensitivity of 89% and a specificity of 86%. The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 87% which means that performing this analysis at this time point increases the likelihood to separate a person who had an improvement in IBDQ-B score from someone who did not have any change in IBDQ-B scores two years after radiotherapy by at least 50%. A negative predictive value of 86% means that it would not be possible to differentiate between those with no change in IBDQ-B scores and those who improved two years after radiotherapy in nearly 15%.

e-nose Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.89 (0.70-1)	0.89 (0.71-1)	0.88 (0.69-1)	0.82 (0.57-1)	0.83 (0.60-1)	0.83 (0.63-0.99)
Sensitivity (95% CI)	0.89 (0.67-1)	0.45 (0.14-0.75)	0.13 (1-0.33)	0.86 (0.60-1)	0.63 (0.33-0.89)	0.67 (0.39-1)
Specificity (95% CI)	0.86 (0.60-1)	1 (1-1)	1 (1-1)	0.57 (0.25-0.88)	0.75 (0.50-1)	0.78 (0.55-1)
PPV	0.87	0.58	0.46	0.80	0.71	0.70
NPV	0.86	1	1	0.67	0.67	0.75
p-value	0.011*	0.011*	0.020*	0.053	0.028*	0.022*
GC-IMS Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.56 (0.27-0.83)	0.78 (0.50-1)	0.90 (0.69-1)	0.80 (0.64-1)	unable	0.79 (0.58-0.98)
Sensitivity (95% CI)	0.40 (0.25-0.88)	0.50 (0.17-0.86)	0.86 (0.60-1)	0.80 (0.50-1)	to	0.78 (0.55-1)
Specificity (95% CI)	0.50 (0.17-0.83)	0.80 (0.50-1)	0.57 (0.25-0.88)	0.80 (0.50-1)	process	0.63 (0.33-0.89)
PPV	0.50	0.57	0.67	0.80		0.70
NPV	0.57	0.75	0.80	0.80		0.71
p-value	0.731	0.126	0.011*	0.056		0.049*

Table 5.13 E-nose and GC-IMS analysis for group B and C.

5.2.2.3.2 Urine sample analysis

The urine sample analysis for group A and group C shows that analysis of the VOCs by the e-nose is more effective in distinguishing between those two groups at baseline and the beginning of treatment with compared to the same analysis by the GC-IMS (Table 5.14). The area under the curve is the largest before radiotherapy (AUC= 0.90) with a sensitivity of 100% and a specificity of 78%. The positive predictive value for the e-nose analysis at baseline was 75% which means that performing this analysis at this time point increases the likelihood of being able to tell apart someone who has no change in IBDQ-B scores two years after radiotherapy from those continue to have a drop of ≥ 10 points from baseline by (50%).

e-nose Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.89 (0.67-1)	0.90 (0.71-1)	0.78 (0.51-1)	0.90 (0.27-1)	0.88 (0.70-1)	0.82 (0.58-1)
Sensitivity (95% CI)	1 (1-1)	1 (1-1)	0.5 (0.00-1)	1 (1-1)	1 (1-1)	1 (1-1)
Specificity (95% CI)	0.78 (0.50-1)	0.38 (0.11-0.67)	0.75 (0.45-1)	0.63 (0.33-0.89)	0.46 (0.17-0.73)	0.33 (0.10-0.60)
PPV	0.75	1	0.50	1	1	1
NPV	1	0.50	0.75	0.63	0.55	0.46
p-value	0.016*	0.019*	0.154	0.019*	0.018*	0.062
GC-IMS Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.83 (0.63-1)	0.67 (0.40-0.92)	0.73 (0.48-0.94)	0.64 (0.33-0.92)	0.61 (0.30-0.89)	0.51 (0.24-0.75)
Sensitivity (95% CI)	0.50 (0.14-0.83)	0.67 (0.33-1)	1 (1-1)	0.20 (0.00-0.50)	0.67 (0.33-1)	0.83 (0.50-1)
Specificity (95% CI)	0.89 (0.70-1)	0.38 (0.11-0.67)	0.11 (0.00-0.33)	1 (1-1)	0.44 (0.17-0.71)	0.33 (0.10-0.63)
PPV	0.75	0.60	1	1	0.67	0.75
NPV	0.73	0.44	0.38	0.67	0.44	0.45
p-value	0.039*	0.345	0.182	0.435	0.517	1.00

Table 5.14 E-nose and GC-IMS analysis for group A and C.

The urine sample analysis for group B and group C shows that analysis of the VOCs by the e-nose is then again more effective in distinguishing between those two groups at the beginning of treatment with compared to the same analysis by the GC-IMS (Table 5.15). The area under the curve is the largest at the beginning of radiotherapy (AUC= 0.84) with a sensitivity of 45% and a specificity of 88%. The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 86% which means that performing this analysis at this time point increases the likelihood that it is possible to separate those who had no change in IBDQ-B scores versus those who did not improve IBDQ-B scores two years after radiotherapy by more than 50%. The negative predictive value of 77% means that in nearly 25% doing this analysis would not be able to differentiate who is at risk of not improving their bowel function two years after radiotherapy.

e-nose Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.82 (0.50-1)	0.84 (0.65-1)	0.67 (0.40-0.89)	0.80 (0.56-1)	0.84 (0.67-0.97)	0.81 (0.60-0.97)
Sensitivity (95% CI)	0.83 (0.50-1)	0.75 (0.50-1)	0.67 (0.40-0.91)	0.71 (0.40-1)	0.56 (0.27-0.73)	0.63 (0.33-0.89)
Specificity (95% CI)	0.11 (0.30-1)	0.88 (0.67-1)	0.75 (0.50-1)	0.63 (0.33-0.89)	0.89 (0.70-1)	0.78 (0.50-1)
PPV	0.50	0.86	0.67	0.63	0.67	0.71
NPV	0.62	0.77	0.75	0.71	0,73	0.7
p-value	0.052	0.021*	0.268	0.054	0.017*	0.039*
GC-IMS Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.65 (0.39-0.89)	0.71 (0.41-0.96)	0.57 (0.34-0.81)	0.72 (0.44-1)	0.85 (0.65-1)	0.61 (0.36-0.83)
Sensitivity (95% CI)	0.83 (0.50-1)	0.83 (0.50-1)	0.44 (0.17-0.72)	0.71 (0.40-1)	0.89 (0.69-1)	0.33 (0.10-0.40)
Specificity (95% CI)	0.33 (0.10-0.62)	0.50 (0.20-0.78)	0.56 (0.25-0.83)	0.88 (0.67-1)	0.78 (0.50-1)	0.56 (0.25-0.83)
PPV	0.75	0.80	0.50	0.83	0.80	0.45
NPV	0.45	0.56	0.50	0.78	0.88	0.53
p-value	0.377	0.228	0.626	0.152	0.013*	0.453

Table 5.15 E-nose and GC-IMS analysis for group B and C.

5.2.2.3.3 Saliva sample analysis

The saliva sample analysis for group A and group C shows that analysis of the VOCs by the e-nose is more effective in distinguishing between those two groups at the beginning of treatment with compared to the same analysis by the GC-IMS. The area under the curve is 0.90 with a sensitivity of 100% and a specificity of 50% (Table 5.16). The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 100% which means that performing this analysis at this time point increases the likelihood that we can correctly separate the two groups. With a negative predictive value of 60% but there is quite a high risk of wrongly categorising the groups.

e-nose Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.93 (0.76-1)	0.90 (0.73-1)	0.89 (0.67-1)	0.75 (0.47-1)	0.90 (0.71-1)	0.80 (0.52-1)
Sensitivity (95% CI)	1 (1-1)	1 (1-1)	1 (1-1)	0.67 (0.33-1)	1 (1-1)	1 (1-1)
Specificity (95% CI)	0.25 (0.00-0.50)	0.5 (0.20-0.80)	1 (1-1)	0.75 (0.50-1)	1 (1-1)	0.25 (1-0.50)
PPV	1	1	NA	0.67	NA	1
NPV	0.45	0.6	0.58	0.75	0.43	0.46
p-value	0.284	0.013*	0.030*	0.142	0.013*	0.093
GC-IMS Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.62 (0.34-0.88)	0.80 (0.56-1)	0.73 (0.43-0.67)	0.94 (0.82-1)	0.64 (0.36-0.90)	0.81 (0.53-1)
Sensitivity (95% CI)	1 (1-1)	0.83 (0.56-1)	1 (1-1)	1 (1-1)	1 (1-1)	0.80 (0.50-1)
Specificity (95% CI)	0.33 (0.11-0.60)	0.57 (0.25-0.88)	0.38 (0.11-0.67)	0.75 (0.50-1)	0.25 (0.00-0.50)	0.43 (0.13-0.78)
PPV	1	0.80	1	1	1	0.75
NPV	0.45	0.63	0.50	0.75	0.45	0.50
p-value	0.505	0.073	0.222	0.005*	0.435	0.073

Table 5.16 E-nose and GC-IMS analysis for group A and C.

The saliva sample analysis for group B and group C shows that analysis of the VOCs by the e-nose is more effective in distinguishing between those two groups at the beginning and end of treatment compared to the same analysis by GC-IMS (Table 5.17). The area under the curve is the largest at the end of radiotherapy (AUC= 0.88) with a sensitivity of 100% and a specificity of 50%. The positive predictive value for the e-nose analysis at the end of radiotherapy of 64% means that performing this analysis at this time point this analysis of a saliva sample is able to separate women at risk of developing those with a drop of ≥10 points in their IBDQ-B score at the end of treatment compared to baseline but then improved and those who did not have a change in their IBDQ-B score two years post radiotherapy since baseline. A negative predictive value of 100% for the e-nose analysis means that there is little risk at misclassification of the two groups.

e-nose Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.75 (0.48-0.95)	0.82 (0.57-1)	0.88 (0.68-1)	0.68 (0.42-0.91)	0.89 (0.70-1)	0.74 (0.49-0.96)
Sensitivity (95% CI)	0.57 (0.25-0.88)	0.85 (0.60-1)	1 (1-1)	0.57 (0.25-0.86)	0.88 (0.67-1)	0.67 (0.38-0.91)
Specificity (95% CI)	0.623 (0.33-0.89)	0.25 (0.00-0.50)	0.5 (0.20-0.80)	0.63 (0.33-0.89)	0.63 (0.33-0.89)	0.63 (0.33-0.89)
PPV	0.63	0.67	0.64	0.63	0.70	0.67
NPV	0.57	0.50	1	0.57	0.83	0.63
p-value	0.121	0.040*	0.014*	0.281	0.007*	0.112
GC-IMS Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.73 (0.49-0.93)	0.60 (0.26-0.87)	0.75 (0.50-0.95)	0.88 (0.69-1)	0.79 (0.36-0.95)	0.76 (0.53-0.96)
Sensitivity (95% CI)	0.67 (0.38-0.90)	0.50 (0.17-0.83)	0.86 (0.60-1)	0.71 (0.40-1)	0.63 (0.33-0.89)	0.67 (0.38-0.91)
Specificity (95% CI)	0.67 (0.38-0.91)	0.71 (0.40-1)	0.75 (0.50-1)	0.75 (0.50-1)	0.63 (0.33-0.90)	0.63 (0.33-0.90)
PPV	0.67	0.62	0.86	0.75	0.63	0.63
NPV	0.67	0.60	0.75	0.71	0.63	0.67
p-value	0.112	0.628	0.121	0.014*	0.050*	0.083

Table 5.17 E-nose and GC-IMS analysis for group B and C.

4.2.2.3.4 Rectal swab analysis

The rectal swab sample analysis for group A and group C shows that analysis of the VOCs by the e-nose is also more effective in distinguishing between those two groups at the end of treatment with compared to the same analysis by the GC-IMS (Table 5.18). The area under the curve is 0.88 with a sensitivity of 100% and a specificity of 100%. However, due to small sample sizes, caution is required to assign any value to its use in clinical practice.

e-nose Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	Unable	0.87 (0.60-1)	0.88 (0.63-1)	0.90 (0.70-1)	0.82 (0.54-1)	0.88 (0.66-1)
Sensitivity (95% CI)	To	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-1)
Specificity (95% CI)	Process	0.17 (0.00-0.50)	1 (1-1)	0.25 (0.00-0.50)	0.14 (0.00-0.60)	0.25 (0.00-0.50)
PPV		0.50	NA	1	1	1
NPV		1	0.33	0.46	0.4	0.4
p-value		0.052	0.048*	0.019*	0.109	0.048*
GC-IMS Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.68 (0.33-1)	Unable	0.67 (0.30-0.98)	0.95 (0.83-1)	Unable	0.71 (0.43-1)
Sensitivity (95% CI)	0.25 (0.00-0.67)	To	1 (1-1)	1 (1-1)	To	0.75 (0.33-1)
Specificity (95% CI)	0.71 (0.40-1)	Process	0.25 (0.00-0.50)	0.50 (0.18-0.80)	Process	0.57 (0.25-0.88)
PPV	0.33		1	1		0.80
NPV	0.63		0.40	0.56		0.50
p-value	0.412		0.368	0.006*		0.315

Table 5.18 E-nose and GC-IMS analysis for group A and C.

The rectal swab sample analysis by the e-nose for group B and group C is most effective at baseline or 6 months after radiotherapy (Table 5.19).

e-nose Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.83 (0.63-1)	0.83 (0.61-1)	0.68 (0.43-0.90)	0.82 (0.61-1)	0.84 (0.64-1)	0.72 (0.47-0.93)
Sensitivity (95% CI)	0.63 (0.33-0.89)	0.57 (0.25-0.88)	0.67 (0.40-0.91)	0.86 (0.60-1)	0.75 (0.44-1)	0.63 (0.33-0.89)
Specificity (95% CI)	0.67 (0.33-1)	0.83 (0.50-1)	0.75 (0.50-1)	0.50 (0.20-0.80)	0.71 (0.40-1)	0.50 (0.20-0.80)
PPV	0.57	0.63	0.67	0.80	0.75	0.57
NPV	0.29	0.80	0.75	0.60	0.71	0.56
p-value	0.043*	0.051	0.229	0.040*	0.029*	0.161
GC-IMS Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.64 (0.36-0.89)	0.69 (0.40-0.94)	0.71 (0.46-0.94)	0.58 (0.28-0.88)	0.75 (0.50-0.95)	0.56 (0.31-0.82)
Sensitivity (95% CI)	0.50 (0.20-0.80)	0.63 (0.33-0.89)	0.71 (0.43-1)	0.67 (0.33-1)	0.50 (0.20-0.80)	0.56 (0.27-0.83)
Specificity (95% CI)	0.57 (0.25-0.86)	0.75 (0.33-1)	0.50 (0.20-0.80)	0.75 (0.44-1)	0.86 (0.60-1)	0.63 (0.33-0.89)
PPV	0.50	0.83	0.67	0.67	0.60	0.63
NPV	0.57	0.50	0.56	0.75	0.80	0.56
p-value	0.397	0.368	0.189	0.662	0.121	0.700

Table 5.19 E-nose and GC-IMS analysis for group B and C.

In summary, the analysis by the e-nose technique provides more consistent results as shown in Table 5.11 in separating the groups regarding differences in IBDQ-B scores. All groups can be separated on stool, urine and saliva samples at the beginning of radiotherapy.

5.2.2.5 VOC analysis per stool consistency (Bristol Stool Chart) groups

For the analysis of stool consistency (Bristol Stool Chart) and the olfactory signatures, the participants were also categorised in 3 groups (Table 5.20). Those definitions have been used throughout the presentation of analysis results in this chapter. Further details of the Bristol Stool Chart data are presented in section 5.2.3.4.

Group D	Women who reported type 1 and 2 stool consistency but no diarrhoea (type 6 or 7) 2 years after radiotherapy (constipation)	n= 8 (33%)
Group E	Women who reported type 3-5 stool consistency but no constipation or diarrhoea two years after radiotherapy (normal stool)	n= 8 (33%)
Group F	Women who reported type 6 or 7 stool consistency but no constipation (type 1 or 2) two years after radiotherapy (diarrhoea)	n= 8 (33%)

Table 5.20 Definitions of groups D-F as per Bristol Stool Chart.

Distinguishing patients per stool consistency type is more effective using the e-nose analysis than the GC-IMS analysis (purple section Table 5.21). Stool, saliva and rectal swab samples can be used at the beginning of treatment.

Analysis technique	Sample type	Group	Baseline	Beginning of RT	End of RT	6 months post RT	1 year post RT	2 years post RT
e-nose	Stool	D vs E						
		E vs F						
	Urine	D vs E						
		E vs F						
	Saliva	D vs E						
		E vs F						
	Rectal swab	D vs E						
		E vs F						
GC-IMS	Stool	D vs E						
		E vs F						
	Urine	D vs E						
		E vs F						
	Saliva	D vs E						
		E vs F						
	Rectal swab	D vs E						
		E vs F						

Table 5.21 Comparison of the ability to distinguish between BSC groups of the e-nose and GC-IMS analysis (blue= statistically significant, purple= statistically significant for all groups, white= not statistically significant).

5.2.2.5.1 Stool sample analysis

The stool sample analysis for group D and group E shows that analysis of the VOCs by the e-nose is more effective in distinguishing between those two groups at all time points except 6 months after treatment compared to the same analysis by the GC-IMS (Table 5.22). The area under the curve is the largest at the beginning of radiotherapy (AUC= 1) with a sensitivity of 81% and a specificity of 100%. The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 81% which means that performing this analysis at this time point increases the likelihood to differentiate between someone who develops constipation and someone with normal stool consistency two years after radiotherapy by half (48%). The strength of the test at the beginning of radiotherapy is a NPV of 100%.

e-nose Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.98 (0.92-1)	1 (1-1)	0.93 (0.78-1)	0.77 (0.50-1)	0.84 (0.64-1)	0.94 (0.81-1)
Sensitivity (95% CI)	0.63 (0.33-0.88)	0.81 (0.40-1)	0.57 (0.25-0.87)	0.71 (0.40-1)	0.85 (0.60-1)	0.88 (0.67-1)
Specificity (95% CI)	1 (1-1)	1 (1-1)	1 (1-1)	0.71 (0.40-1)	0.62 (0.33-0.88)	1 (1-1)
PPV	0.70	0.81	0.67	0.75	0.83	1
NPV	1	1	1	0.83	0.66	0.89
p-value	<0.001*	<0.001*	0.008*	0.097	0.029*	0.002*
GC-IMS Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.76 (0.49-1)	unable	0.55 (0.24-0.86)	Unable	Unable	0.58 (0.29-0.85)
Sensitivity (95% CI)	0.86 (0.60-10)	To	0.5 (0.17-0.88)	To	To	0.71 (0.40-1)
Specificity (95% CI)	0.40 (0.00-0.80)	process	0.57 (0.25-0.88)	Process	Process	0.49 (0.13-0.75)
PPV	0.67		0.57			0.60
NPV	0.67		0.50			0.56
p-value	0.149		0.836			0.620

Table 5.22 E-nose and GC-IMS analysis for group D and E.

The stool sample analysis for group E and group F shows that analysis of the VOCs by the e-nose is again more effective in distinguishing between those two groups at the beginning of treatment with compared to the same analysis by the GC-IMS (Table 5.23). The area under the curve is the largest at the beginning of radiotherapy (AUC= 0.92) with a sensitivity of 85% and a specificity of 87%. The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 86% which means that performing this analysis at this time point increases the likelihood to separate a person who develops diarrhoea from someone who maintains normal stool consistency two years after radiotherapy by 53%. A negative predictive value of 85% means that it would not be possible to differentiate between those developing chronic diarrhoea or constipation two years after radiotherapy in 15%.

e-nose Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.80 (0.55-1)	0.92 (0.78-1)	0.86 (0.63-1)	0.67 (0.35-0.98)	0.83 (0.62-1)	0.63 (0.38-0.87)
Sensitivity (95% CI)	0.71 (0.40-1)	0.85 (0.60-1)	0.83 (0.50-1)	0.67 (0.33-1)	0.36 (0.11-0.66)	0.88 (0.67-1)
Specificity (95% CI)	0.71 (0.40-1)	0.87 (0.66-1)	0.57 (0.25-0.88)	0.71 (0.40-1)	1 (1-1)	0.5 (0.18-0.80)
PPV	0.71	0.86	0.63	0.67	0.55	0.64
NPV	0.71	0.85	0.80	0.71	1	0.80
p-value	0.073	0.040*	0.040*	0.366	0.043*	0.442
GC-IMS Stool	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.64 (0.36-0.91)	0.63 (0.29-0.94)	0.60 (0.32-0.88)	0.85 (0.58-1)	Unable	0.91 (0.73-1)
Sensitivity (95% CI)	0.80 (0.50-1)	0.75 (0.33-1)	0.71 (0.40-1)	0.80 (0.50-1)	To	0.86 (0.60-1)
Specificity (95% CI)	0.71 (0.40-1)	0.71 (0.40-1)	0.29 (0.00-0.60)	1 (1-1)	Process	0.88 (0.67-1)
PPV	0.67	0.83	0.50	1		0.88
NPV	0.83	0.60	0.50	0.86		0.88
p-value	0.432	0.527	0.535	0.052		0.006*

Table 5.23 E-nose and GC-IMS analysis for group E and F.

5.2.2.5.2 Urine sample analysis

Urine sample analysis for group D and group E (Table 5.24) shows that analysis of the VOCs by the e-nose is more effective in distinguishing between the two groups than the GC-IMS analysis at the end of radiotherapy (AUC= 0.91) with a sensitivity of 83% and a specificity of 66%. The positive predictive value for the e-nose analysis at the end of radiotherapy was 80% which means that performing this analysis at this time point using a urine sample increases the likelihood to differentiate between someone who develops constipation and a person who will have normal stool consistency two years after radiotherapy by nearly half (49%). The negative predictive value at the end of radiotherapy means that in nearly a third (29%) it may not be possible to separate who is at risk of developing chronic constipation two years after radiotherapy.

e-nose Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC	0.82 (0.60-1)	0.79 (0.54-1)	0.91 (0.75-1)	0.92 (0.78-1)	0.84 (0.65-1)	0.78 (0.56-0.97)
Sensitivity (95% CI)	1 (1-1)	0.71 (0.40-1)	0.83 (0.50-1)	0.83 (0.57-1)	0.63 (0.22-0.89)	0.75 (0.50-1)
Specificity (95% CI)	0.5 (0.20-0.80)	0.57 (0.25-0.85)	0.66 (0.33-1)	0.71 (0.55-1)	0.75 (0.50-1)	0.75 (0.44-1)
PPV	1	0.66	0.80	0.83	0.71	0.75
NPV	0.60	0.62	0.71	0.71	0.67	0.75
p-value	0.043	0.073	0.015*	0.008*	0.021*	0.065
GC-IMS Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC	0.63 (0.33-0.92)	0.7 (0.36-1)	0.84 (0.60-1)	0.74 (0.43-1)	0.77 (0.54-0.96)	0.72 (0.47-0.93)
Sensitivity (95% CI)	0.67 (0.33-1)	1 (1-1)	0.71 (0.40-1)	0.83 (0.50-1)	0.63 (0.33-0.89)	0.63 (0.33-0.89)
Specificity (95% CI)	0.75 (0.45-1)	0.20 (0.00-0.50)	0.88 (0.67-1)	0.49 (0.15-0.75)	0.75 (0.45-1)	0.75 (0.45-1)
PPV	0.67	1	0.83	0.75	0.67	0.67
NPV	0.75	0.56	0.78	0.56	0.71	0.71
p-value	0.491	0.310	0.029*	0.181	0.083	0.161

Table 5.24 E-nose and GC-IMS analysis for group D and E.

Urine sample analysis for group E and group F shows that analysis of the VOCs by the e-nose is again more effective in distinguishing between those two groups at the beginning of treatment with compared to the same analysis by the GC-IMS (Table 5.25). The area under the curve is the largest at the beginning of radiotherapy (AUC= 0.81) with a sensitivity of 71% and a specificity of 57%. The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 66% which means that performing this analysis at this time point increases the likelihood that it is possible to separate those who develop diarrhoea two years after radiotherapy from those who maintain normal stool consistency by 33%. The negative predictive value of 62% means that in nearly 40% doing this analysis would be able to differentiate who is at risk of developing chronic diarrhoea two years after radiotherapy.

e-nose Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.70 (0.43-0.93)	0.81 (0.68-1)	0.83 (0.61-1)	0.71 (0.44-0.94)	0.83 (0.62-1)	0.75 (0.48-0.98)
Sensitivity (95% CI)	0.75 (0.50-1)	0.71 (0.40-1)	0.83 (0.50-1)	0.71 (0.40-1)	0.87 (0.66-1)	0.62 (0.33-0.88)
Specificity (95% CI)	0.71 (0.40-1)	0.57 (0.25-0.85)	0.57 (0.25-0.87)	0.71 (0.40-1)	0.62 (0.33-0.88)	0.67 (0.33-1)
PPV	0.75	0.66	0.80	0.71	0.83	0.57
NPV	0.71	0.62	0.62	0.71	0.7	0.71
p-value	0.232	0.017*	0.051	0.209	0.028*	0.142
GC-IMS Urine	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.59 (0.30-0.86)	0.52 (0.23-0.85)	0.77 (0.52-0.96)	0.88 (0.67-1)	0.82 (0.60-1)	0.79 (0.55-0.99)
Sensitivity (95% CI)	0.75 (0.50-1)	0.67 (0.33-1)	0.75 (0.50-1)	0.71 (0.40-1)	0.88 (0.67-1)	0.29 (0.00-0.60)
Specificity (95% CI)	0.43 (0.13-0.75)	0.71 (0.40-1)	0.75 (0.50-1)	0.57 (0.25-0.88)	0.75 (0.50-1)	0.88 (0.67-1)
PPV	0.60	0.67	0.75	0.63	0.78	0.67
NPV	0.60	0.71	0.75	0.67	0.86	0.58
p-value	0.613	0.945	0.083	0.017*	0.028*	0.072

Table 5.25 E-nose and GC-IMS analysis for group E and F.

5.2.2.5.3 Saliva sample analysis

The saliva sample analysis of the VOCs by the e-nose is more effective in distinguishing between those all three stool consistency groups at the beginning of radiotherapy than the GC-IMS analysis (Table 5.26). The saliva sample analysis for group D and group E has an AUC of 0.93 with a sensitivity of 100% and a specificity of 88%. The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 100% which means that performing this analysis at this time point using a saliva sample can identify all women who will develop chronic constipation from those with normal stool consistency two years after radiotherapy. The negative predictive value at the beginning of radiotherapy is 88% carries a risk of not being able to distinguish between the two groups in 12% of cases.

e-nose Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.95 (0.83-1)	0.93 (0.80-1)	0.90 (1-0.73)	0.69 (0.40-0.94)	0.74 (0.49-0.94)	0.72 (0.50-0.94)
Sensitivity (95% CI)	0.71 (0.43-1)	1 (1-1)	1 (1-1)	0.33 (0.00-0.67)	0.5 (0.16-0.86)	0.71 (0.40-1)
Specificity (95% CI)	0.83 (0.50-1)	0.88 (0.67-1)	0.50 (0.20-0.80)	0.86 (0.60-1)	0.63 (0.33-0.88)	0.62 (0.33-0.88)
PPV	0.83	1	1	0.67	0.50	0.71
NPV	0.71	0.88	0.56	0.60	0.63	0.62
p-value	0.005*	0.004*	0.019*	0.295	0.142	0.152
GC-IMS Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.58 (0.31-0.83)	0.67 (0.42-0.88)	0.60 (0.26-0.93)	0.60 (0.33-085)	0.79 (0.50-1)	0.71 (0.44-0.92)
Sensitivity (95% CI)	0.63 (0.33-0.89)	0.57 (0.25-0.88)	0.80 (0.50-1)	0.67 (0.33-1)	0.83 (0.50-1)	0.71 (0.40-1)
Specificity (95% CI)	0.63 (0.33-0.89)	0.71 (0.40-1)	0.13 (0.00-0.33)	0.50 (0.20-0.80)	0.13 (0.00-0.33)	0.63 (0.33-0.90)
PPV	0.63	0.63	0.50	0.67	0.50	0.71
NPV	0.63	0.67	0.36	0.50	0.42	0.63
p-value	0.645	0.359	0.724	0.573	0.081	0.189

Table 5.26 E-nose and GC-IMS analysis for group D and E.

The saliva sample analysis for group E and group shows that analysis of the VOCs by the GC-IMS is more effective in distinguishing between those two groups at the beginning of treatment with compared to the same analysis by the e-nose (Table 5.27). The area under the curve is the largest at the beginning of radiotherapy (AUC= 1) with a sensitivity of 100% and a specificity of 100%. The positive predictive value for the GC-IMS analysis at the beginning of radiotherapy was 100% compared to 62% with the e-nose which means that performing this analysis at this time point this analysis of a saliva sample would separate all women at risk of developing diarrhoea from those with normal stool consistency two years after treatment with radiotherapy. In addition, a negative predictive value of 100% for the GC-IMS analysis (compared to 83% for the e-nose analysis) means that there is limited risk of false negative results.

e-nose Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.69 (0.38-1)	0.89 (0.68-1)	0.75 (0.52-0.95)	0.82 (0.60-1)	0.88 (0.67-1)	0.82 (0.59-1)
Sensitivity (95% CI)	1 (1-1)	0.62 (0.33-0.88)	0.62 (0.34-0.89)	0.62 (0.33-0.88)	0.63 (0.33-0.88)	0.68 (0.33-0.88)
Specificity (95% CI)	0.58 (0.25-0.87)	0.62 (0.33-0.88)	0.85 (0.60-1)	1 (1-1)	1 (1-1)	0.86 (0.60-1)
PPV	1	0.62	0.77	0.70	1	0.66
NPV	0.66	0.83	0.83	1	0.73	0.82
p-value	0.295	0.013*	0.121	0.040*	0.010*	0.040*
GC-IMS Saliva	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.74 (0.46-0.98)	1 (1-1)	0.63 (0.34-0.86)	0.63 (0.34-0.88)	0.69 (0.42-0.91)	0.66 (0.33-0.89)
Sensitivity (95% CI)	0.86 (0.60-1)	1 (1-1)	0.50 (0.20-0.80)	0.50 (0.20-0.80)	0.50 (0.20-0.80)	0.63 (0.33-0.89)
Specificity (95% CI)	0.43 (0.13-0.75)	1 (1-1)	0.57 (0.25-0.88)	0.71 (0.40-1)	0.71 (0.40-1)	0.57 (0.22-0.88)
PPV	0.75	1	0.50	0.56	0.56	0.57
NPV	0.60	1	0.63	0.67	0.67	0.63
p-value	0.165	0.003*	0.463	0.463	0.232	0.336

Table 5.27 E-nose and GC-IMS analysis for group E and F.

4.2.2.5.4 Rectal swab analysis

Rectal swab analysis for group D and group E shows that analysis of the VOCs by the e-nose is effective in distinguishing between those two groups the beginning of treatment compared to the same analysis by the GC-IMS (Table 5.28). The area under the curve is 0.89 with a sensitivity of 57% and a specificity of 100%. The positive predictive value for the e-nose analysis at the beginning of radiotherapy was 57% so while performing this analysis at this time point only increases the likelihood to distinguish someone develops constipation from someone who has normal stool consistency two years after radiotherapy by 24%. The negative predictive value of 100% means that there would be little risk of wrongly categorising the groups.

e-nose Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.77 (0.43-1)	0.89 (0.66-1)	0.94 (0.81-1)	0.93 (0.79-1)	0.74 (0.44-1)	0.69 (0.39-0.95)
Sensitivity (95% CI)	0.60 (0.20-1)	0.57 (0.25-0.85)	1 (1-1)	0.71 (0.4-1)	0.71 (0.4-1)	0.67 (0.33-1)
Specificity (95% CI)	0.83 (0.56-1)	1 (1-1)	0.85 (0.62-1)	0.83 (0.50-1)	0.80 (0.20-1)	0.71 (0.40-1)
PPV	0.75	0.57	1	0.71	0.71	0.67
NPV	0.71	1	0.94	0.83	0.60	0.71
p-value	0.177	0.042*	0.009*	0.008*	0.202	0.295
GC-IMS Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.83 (0.58-1)	unable	0.75 (0.48-0.99)	0.58 (0.26-0.88)	0.54 (0.22-0.85)	0.96 (0.85-1)
Sensitivity (95% CI)	0.58 (0.60-1)	To	0.67 (0.33-1)	0.33 (0.00-0.67)	0.43 (0.13-0.75)	0.86 (0.60-1)
Specificity (95% CI)	0.50 (0.17-0.83)	process	0.81 (0.50-1)	0.50 (0.17-0.83)	1 (1-1)	0.86 (0.60-1)
PPV	0.75		0.71	0.40	0.56	0.86
NPV	0.67		0.80	0.43	1	0.86
p-value	0.051		0.180	0.699	0.876	0.002

Table 5.28 E-nose and GC-IMS analysis for group D and E.

The rectal swab sample analysis for group E and group F is almost identical and most effective 6 months after radiotherapy for both analysis methods (Table 5.29). However, e-nose analysis can distinguish between those two groups both before the start of treatment or at the beginning of treatment a sensitivity and PPV of 100% at both time points. The specificity is better at baseline (71%) with a negative predictive value of 75% which means that at that time point the test can separate those developing chronic diarrhoea two years after radiotherapy from those with normal stool consistency but in 25% the test may be false negative compared to 43% at the beginning of treatment with radiotherapy.

e-nose Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.86 (0.64-1)	0.89 (0.67-1)	0.78 (0.53-1)	0.91 (0.71-1)	0.80 (0.53-1)	0.90 (0.70-1)
Sensitivity (95% CI)	1 (1-1)	1 (1-1)	0.83 (0.50-1)	1 (1-1)	0.60 (0.20-1)	0.86 (0.60-1)
Specificity (95% CI)	0.71 (0.42-1)	0.57 (0.25-0.85)	0.43 (0.22-0.75)	0.71 (0.43-1)	1 (1-1)	0.86 (0.60-1)
PPV	1	1	0.75	0.75	1	0.86
NPV	0.75	0.57	0.66	1	0.78	0.86
p-value	0.035*	0.042*	0.101	0.014*	0.106	0.011*
GC-IMS Rectal swabs	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
AUC (95% CI)	0.64 (0.36-0.92)	0.73 (0.41-1)	0.66 (0.36-0.91)	0.91 (0.73-1)	0.53 (0.23-0.86)	0.78 (0.52-0.98)
Sensitivity (95% CI)	0.5 (0.14-0.83)	1 (1-1)	0.83 (0.57-1)	1 (1-1)	0.40 (0.00-0.80)	0.71 (0.40-1)
Specificity (95% CI)	0.57 (0.25-0.88)	0.13 (0.00-0.33)	0.29 (0.00-0.60)	0.57 (0.25-0.88)	0.67 (0.33-1)	0.86 (0.60-1)
PPV	0.50	1	0.67	1	0.50	0.83
NPV	0.57	0.36	0.50	0.67	0.57	0.75
p-value	0.445	0.214	0.366	0.014*	0.931	0.097

Table 5.29 E-nose and GC-IMS analysis for group E and F.

In summary, analysis by the e-nose technique provides more consistent results in separating the groups based on stool consistency regarding differences in IBDQ-B scores. All groups can be separated on a rectal swab, stool or saliva sample at the beginning of treatment. No sample type is consistent at all time points.

5.2.3 Secondary end points

For all other bowel symptom questionnaires, the completeness was the same as for the IBDQ-B score (Table 5.4 section 5.2.2.1).

5.2.3.1 Total IBDQ scores, IBDQ-Emotional subset, IBDQ-Social subset and IBDQ-Systemic subset

The total IBDQ score (IBDQ-T) contains three further subsets apart from the bowel subset (IBDQ-B): IBDQ-Emotional (IBDQ-E), IBDQ-Social (IBDQ-SOC) and IBDQ-Systemic (IBDQ-SYS). Table 5.30 shows that the median emotional sub-scores remained stable throughout the study but there was a wide range. The lowest IBDQ-E score possible is 12, the highest score possible is 84. The impact of the problem with the phrasing of two of the questions and the reverse scoring was assessed by a sensitivity analysis comparing the original IBDQ-E scores the IBDQ-E scores without the two questions (Appendix 29). The correlation between those two versions of IBDQ-E was high ($r= 0.93$, $p< 0.001$).

The social impact as measured by the median IBDQ-SOS scores also remained stable across the time points. The lowest IBDQ-SOS score possible is 7, the highest is 35. The median score for systemic symptoms (IBDQ-SYS) was the lowest at the end of treatment with a score of 27 out of a maximum IBDQ-SYS score of 35. These sub-scores together with the IBDQ-B make up the total IBDQ-B (IBDQ-T) score which denotes the impact of bowel symptoms on overall quality of life. The minimum score is 32, the maximum score is 224. As there were concerns that the reverse question in the IBDQ-E subset may impact on the IBDQ-T score (chapter three, section 4.5.1), a sensitivity analysis was performed comparing the original IBDQ-T scores and the IBDQ-T scores without those two questions (Appendix 29). The correlation between those two versions was high ($r= 0.97$, $p< 0.001$).

	Baseline	Start of RT	End of RT	6 months post RT	12 months post RT	24 months post RT
IBDQ-E Median Range	74 (41-84)	70 (25-84)	68 (34-84)	72 (44-84)	75 (52-84)	72 (36-84)
IBDQ-SOC Median Range	35 (19-35)	35 (17-35)	32 (11-35)	35 (23-35)	35 (22-25)	35 (17-35)
IBDQ-SYS Median Range	33 (17-35)	30 (18-35)	27 (9-35)	30 (19-35)	32 (22-35)	30 (14-35)
IBDQ-T Median Range	209 (157-224)	194 (121-224)	188 (80-217)	199 (124-224)	200 (145-224)	200 (98-221)

Table 5.30 IBDQ subsets and IBDQ-T scores (median, range)

5.2.3.2 St Mark's Incontinence scores

The St Mark's Incontinence score (Appendix 19) was completed by the participants at each time point (Table 5.31). Out of a maximum score of 24 (complete incontinence) the median baseline score was 0 (complete continence) (range: 0-20). The score deteriorated the most at the end of radiotherapy (median 5.5, range: 0-21), improved 6 months and 1 year after radiotherapy but deteriorated again two years after the end of radiotherapy to a median of 4 (range: 0-15).

St Mark's score	Baseline	Start of RT	End of RT	6 months post RT	1 year post RT	2 years post RT
Median Range	0 (0-20)	4 (0-24)	5.5 (0-21)	1 (0-11)	2 (0-10)	4 (0-15)

Table 5.31 St Mark's Incontinence scores across time points (median, range)

Most women indicated that they had not been incontinent for solid stool (Figure 5.15).

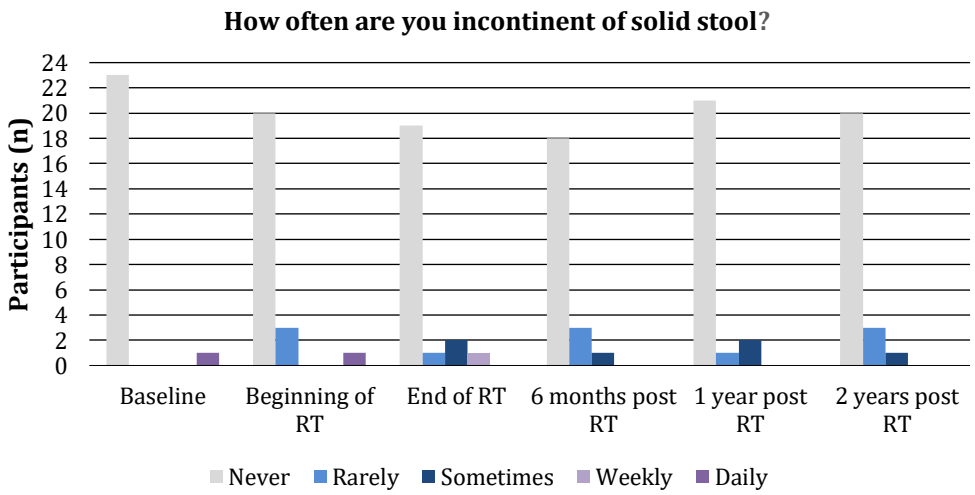


Figure 5.15 St Mark's incontinence for solid stool.

Several women indicated they had experienced some incontinence with diarrhoea (Figure 5.16). One woman reported incontinence at all time points.

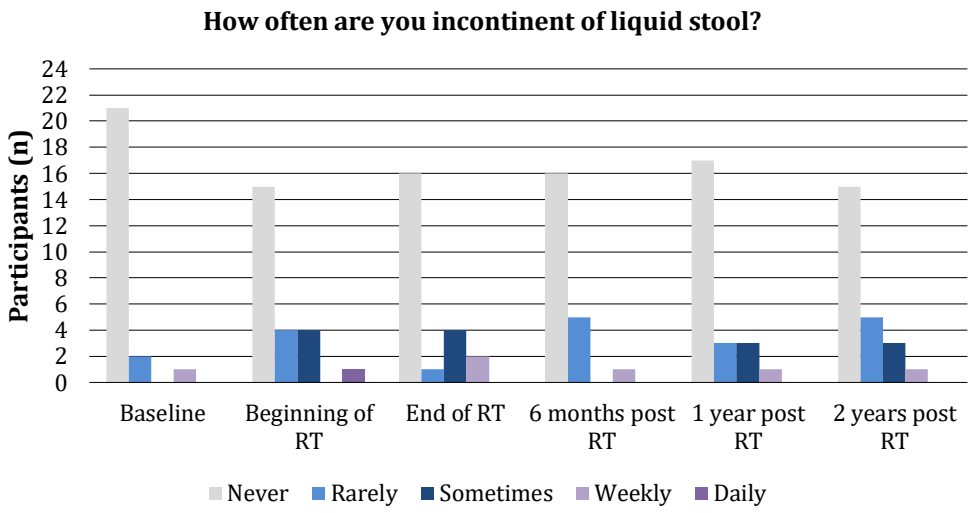


Figure 5.16 St Mark's incontinence for diarrhoea.

Incontinence for gas was more frequently reported on the St Mark's incontinence questionnaire than incontinence for stool (Figure 5.17).

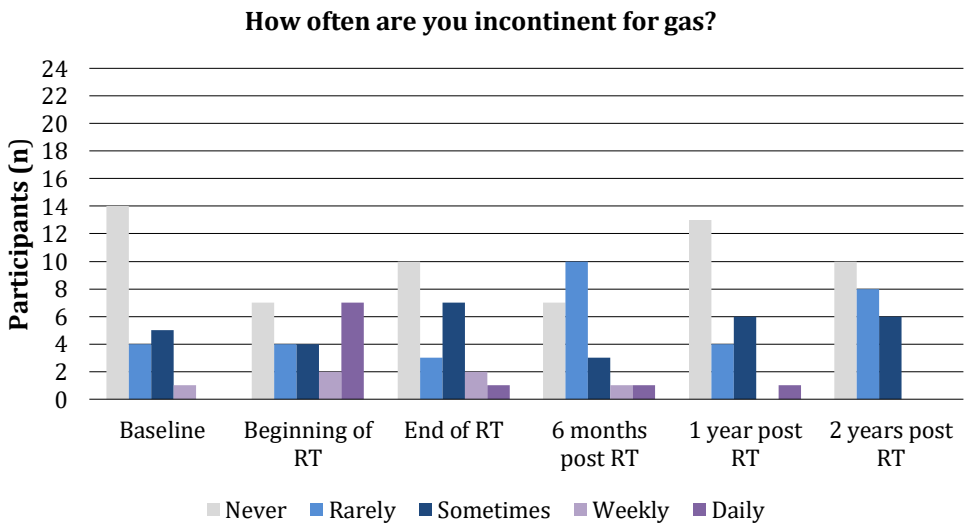


Figure 5.17 St Mark's incontinence for gas.

A minority of women felt their problems with bowel incontinence had an impact on their lifestyle but this was reported by 40% (n=10) two years after radiotherapy (Figure 5.18).

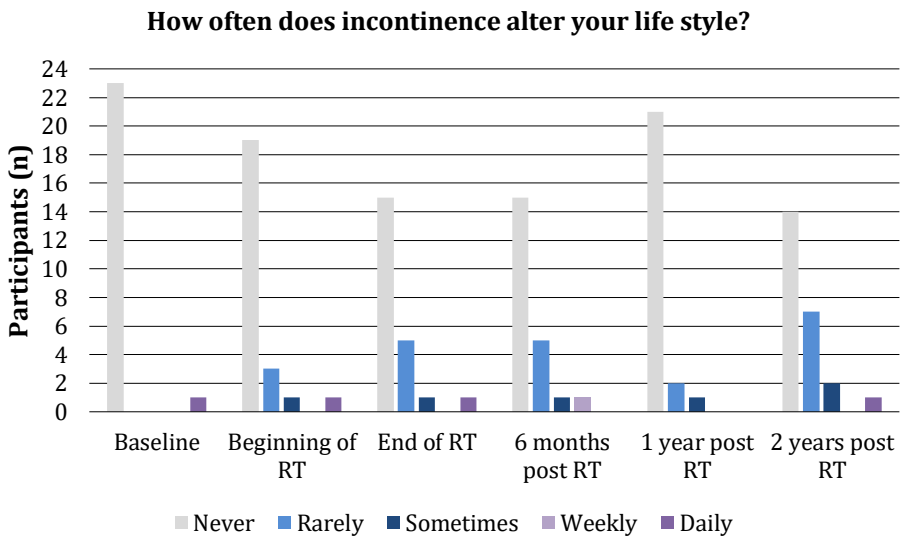


Figure 5.18 St Mark's impact on lifestyle.

At the end of radiotherapy, 25% of women (n= 6) had to wear a pad due to accidental incontinence (Figure 5.19). This may be due to pre-existing incontinence problems or additional bladder incontinence which is comon in women treated for gynaecological cancer and might imact further on lifestyle and quality of life.

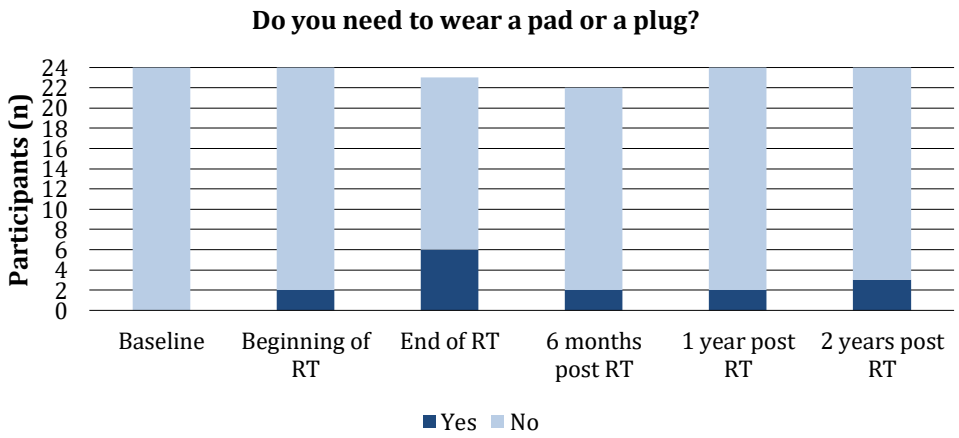


Figure 5.19 St Mark's need to wear protection.

The use of anti-diarrhoeal medication was the highest during radiotherapy and some women continued to need loperamide two years after completion of radiotherapy (Figure 5.20).

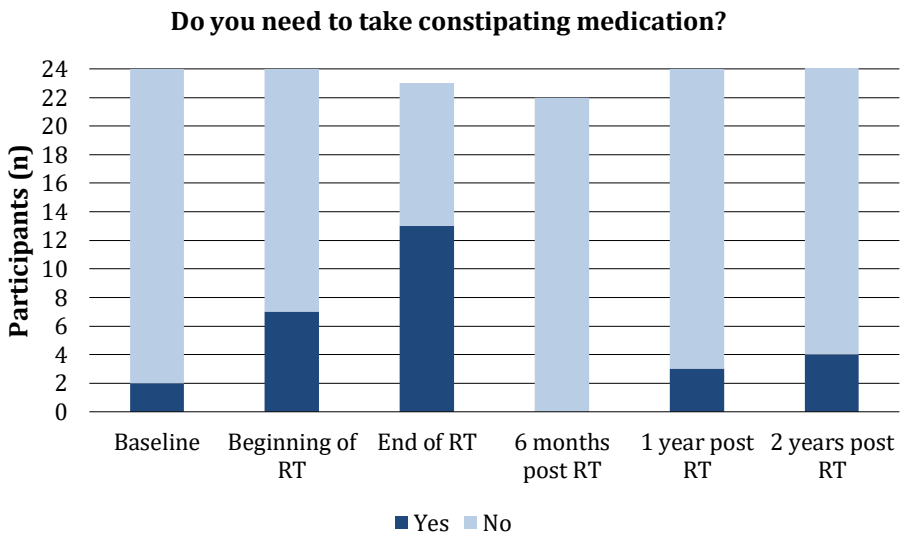


Figure 5.20 St Mark's use of anti-diarrhoeal medication.

At the end of radiotherapy, more than half of women (n= 13) reported difficulty in postponing to open their bowels for 15 minutes (Figure 5.21). Ten women (42%) reported they could not defer for 15 minutes two years after radiotherapy.

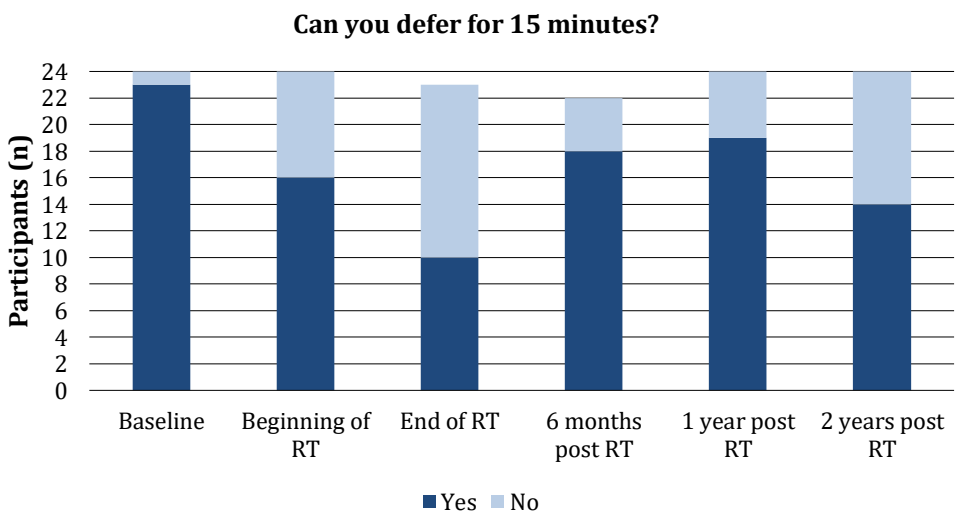


Figure 5.21 St Mark's ability to defer defaecation.

The St Mark's scores show more detailed information than the IBDQ-B as it differentiates types of incontinence. In the GLMM with the St Mark's score as the independent variable the St Mark's score * time interaction was not statistically significant (F= 0.49 [numerator df =5, denominator df=35.71], p= 0.78) suggesting that if there was an effect of the St Mark's score on IBDQ scores it did not vary across time. The fixed effect beta coefficient for St Mark's score was -1.27 (95% CI -1.56 to -0.98, p< 0.001) and was statistically significant. All time-point specific correlations between IBDQ score and St Mark's score were negative, ranging from -0.45 to -0.71 and were all statistically significant (Table 5.32). The overall Pearson correlation across all time points was -0.62.

St Mark's Incontinence score	Pearson's R	p-value
Overall	-0.62	p< 0.001*
Baseline	-0.69	p< 0.001*
Beginning of RT	-0.63	p= 0.001*
End of RT	-0.71	p< 0.001*
6 months post RT	-0.54	p= 0.010*
1 year post RT	-0.53	p= 0.008*
2 years post RT	-0.45	p= 0.028*

Table 5.32 Pearson's correlation between IBDQ-B and St Mark's scores.

5.2.3.3 Common Terminology Criteria for Adverse Events

Participants were asked to complete a CTCAE questionnaire (Appendix 20) at each time point. None of the participants indicated any grade 4 severity for their symptoms. The most frequent gastrointestinal symptoms all occurred at the end of treatment. Two years after completion of radiotherapy, the only grade 3 symptom noted was abdominal pain limiting self-care and interfering with activities of daily life (Figure 5.22).

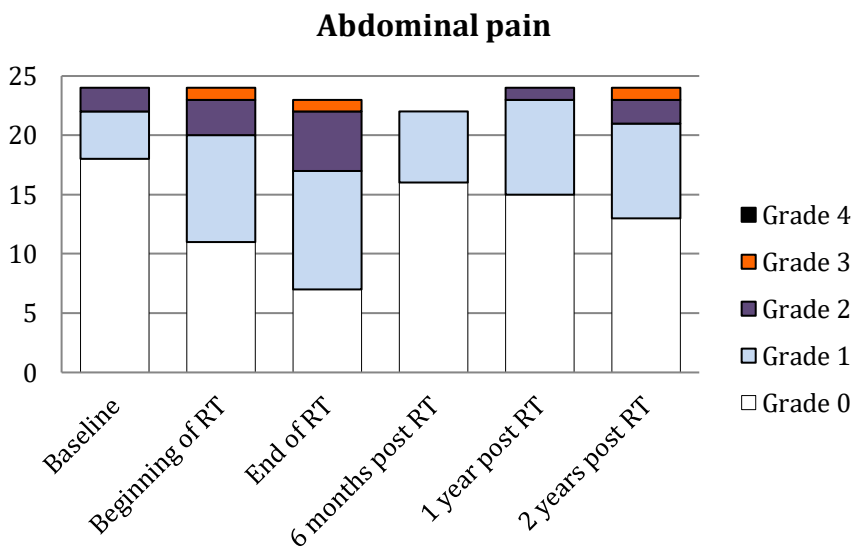
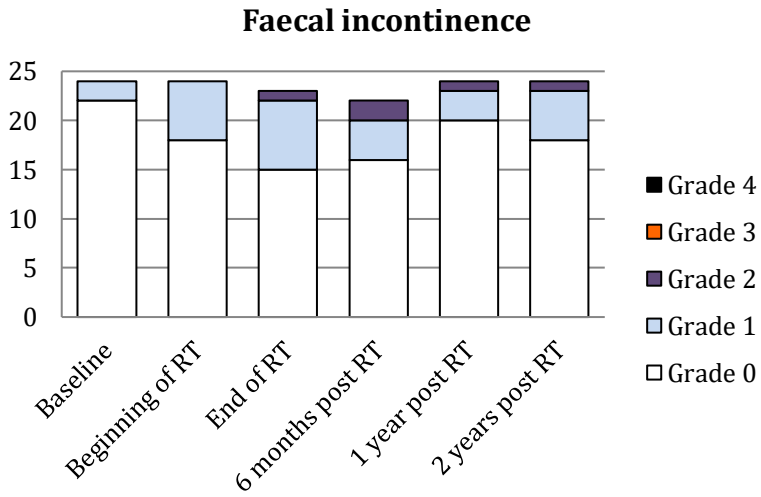
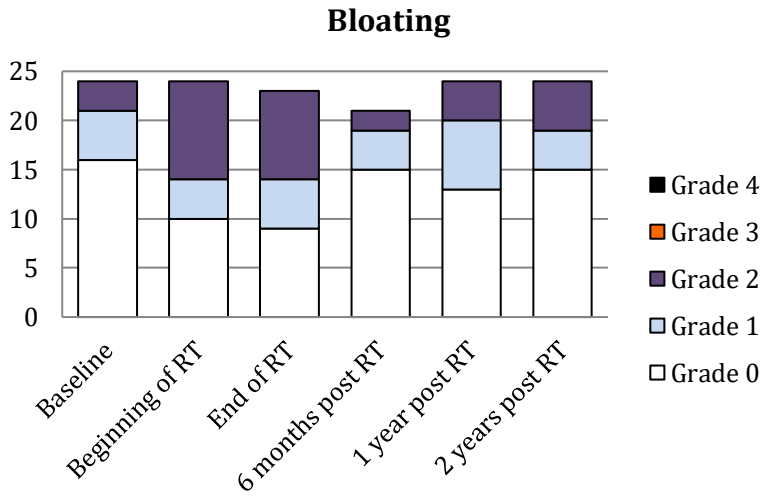
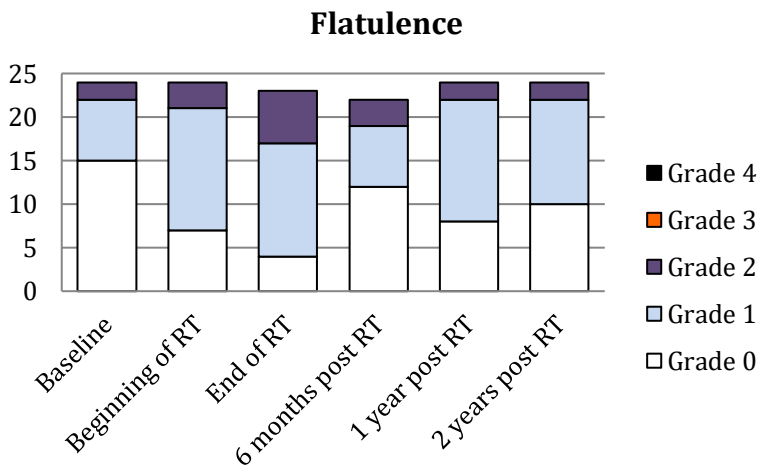


Figure 5.22 CTCAE version 3.0 Abdominal pain (n).







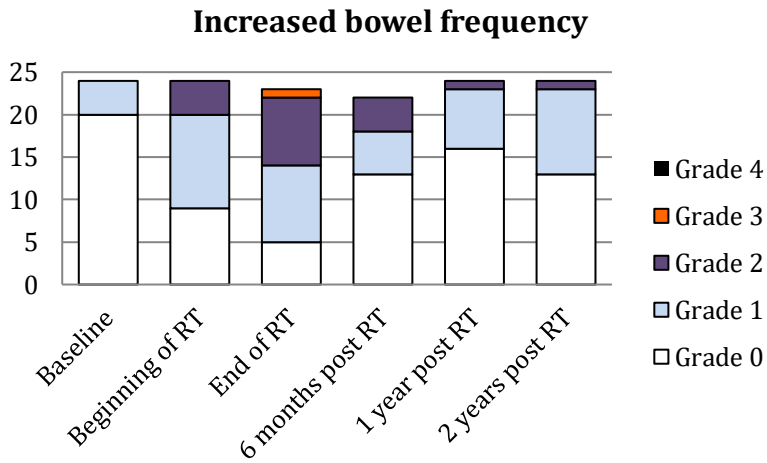
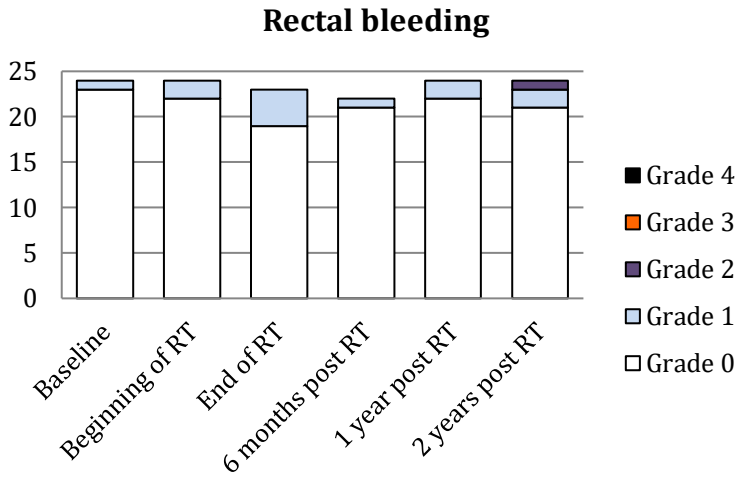
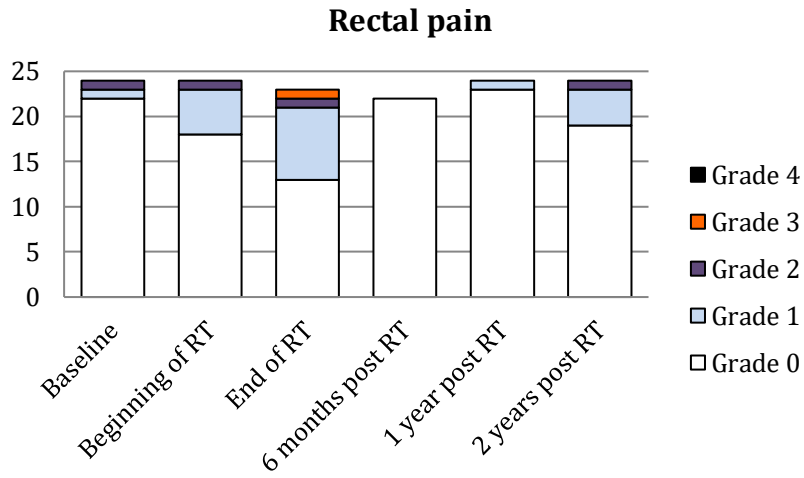
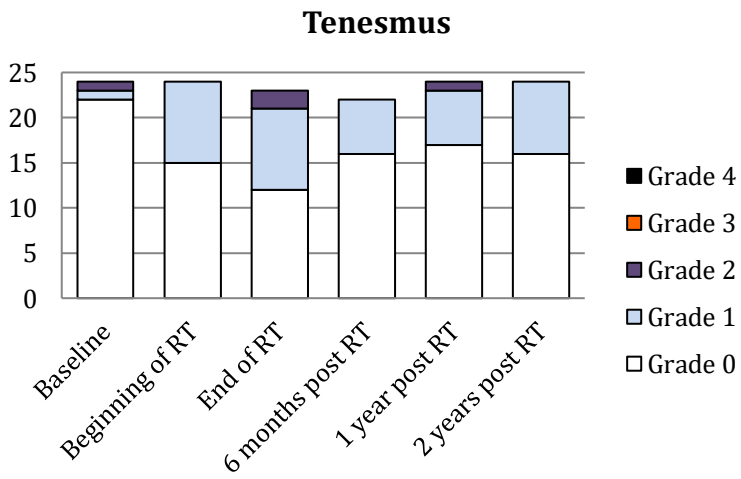


Figure 5.22 (Cont.). CTCAE version 3.0 faecal incontinence, bloating, flatulence and increased bowel frequency (n).







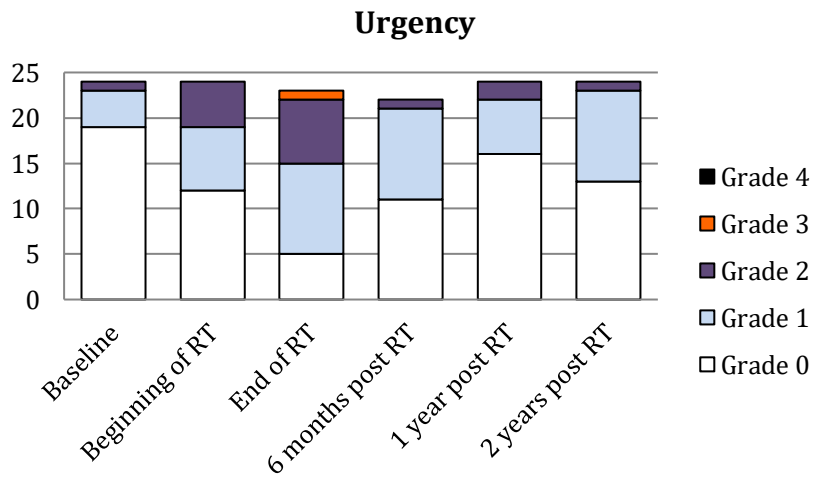


Figure 5.22 (Cont..) CTCAE version 3.0 rectal bleeding, rectal pain, tenesmus and urgency (n).

5.2.3.4 Bristol Stool Chart data

Participants also completed a Bristol Stool Chart (BSC) (Appendix 21) at all time points to indicate their stool consistency throughout the study. Consistency types 1 and 2 indicate constipation, types 3-5 normal formed stool and types 6 and 7 represent diarrhoea. Most women highlighted variable stool consistency as they reported they passed several types of stool consistency at each time point. Figure 5.23 shows distribution per stool consistency type and time point. As radiotherapy often results in acute gastrointestinal toxicity, diarrhoea is an expected clinical outcome, it is not surprising up to two thirds of women experienced type 6 or 7 stool consistency at the end of treatment (Table 5.33). In addition, several were prescribed anti-diarrhoeal medication (loperamide) by their oncology team. At the start of treatment and at the end of radiotherapy, over 40% of women were on loperamide, 4 women (17%) at both time points during treatment. One of those women was still on loperamide 6 months after treatment. Even though one year after treatment, nearly half of the participants (46%) reported type 1 or 2 stool consistency, only one woman was on a regular laxative and three were on a laxative two years after treatment (Table 5.33). Whilst no participants used laxatives or loperamide at the same time at any time in the study, some women reported both constipation (BSC stool type 1 or 2) and diarrhoea (BSC stool type 6 or 7). This may indicate constipation with overflow diarrhoea, constipation due to the use of anti-diarrhoeal medication or loose stool as a result of laxative use.

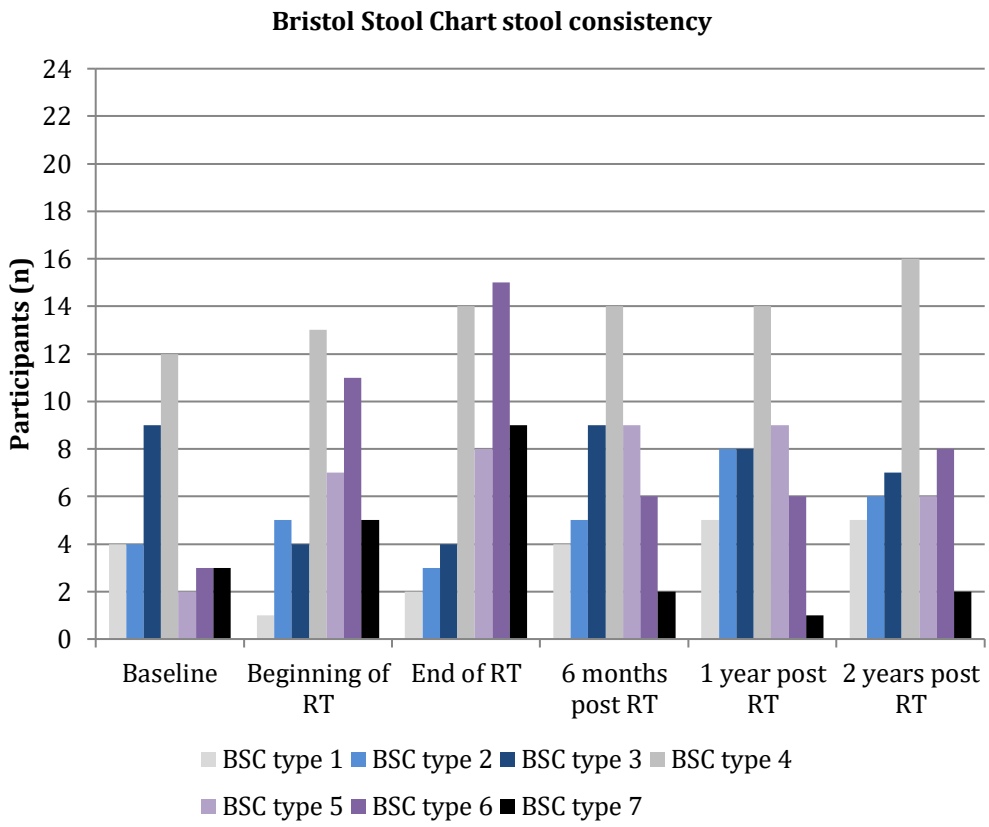


Figure 5.23 BSC stool consistency per type and time point (%).

Stool type	Baseline	Start of RT	End of RT	6 months post RT	12 months post RT	24 months post RT
BSC type 6 or 7 n (%)	3 (13%)	12 (50%)	16 (67%)	6 (25%)	6 (25%)	8 (33%)
On loperamide n (%)	0	10 (42%)	10 (42%)	1 (4%)	1 (4%)	1 (4%)
BSC type 1 or 2 n (%)	6 (25%)	7 (29%)	4 (17%)	6 (25%)	11 (46%)	8 (33%)
On laxative n (%)	2 (8%)	4 (17%)	0	0	1 (4%)	3 (13%)
BSC type 1 or 2 and type 6 or 7 n (%)	0	5 (21%)	3 (13%)	2 (8%)	3 (13%)	0

Table 5.33 BSC stool consistency and use of loperamide or laxatives across time points (n, %).

Use of other medications

In addition to laxative and loperamide usage, the use of other medications was also recorded and collected on the CRF throughout the study. This did not show any particular patterns. The median was a use of no regular medications (range 0-3) at the beginning of treatment. This increased to a median of one medication (range: 0-7) one year after completion of radiotherapy and reduced back to the baseline median and range two years after radiotherapy. The most commonly used medication used was analgesia (paracetamol or an NSAID). In addition, many women received antibiotics, mainly for infections of the urinary tract. A full list of medications and supplements used can be found in Appendix 30.

5.2.3.5 Seven-day food diary analysis

Of the 24 participants, the food diaries of 15 women were analysed in 2019 in collaboration with a Master in Science Degree student from King's College London as those had completed the two year time point at that time. The results of this analysis have been submitted for publication in Nutrition and Cancer (Cirillo Sanchez et al., 2020) (Appendix 27). There were six missing diaries out of a possible 75, leaving 69 for analysis.

The results showed that there was a median weight decrease of 1 kg at the end of treatment compared to baseline (Table 5.34). Weight increased 6 months after completion of treatment but decreased by nearly 2 kg below

baseline weight by 1 and 2 year after radiotherapy, but this was not statistically significant (p= 0.59). At all time points, the median BMI was above the UK recommendation for a healthy BMI (18.1 to 24.9). BMI decreased by 1.2 kg/m2 at the end of radiotherapy and was at its lowest one year following treatment (median: 27.4, range: 23.9 - 33). Two years after completion of pelvic radiotherapy, BMI was 0.4 kg/m2 below baseline BMI (p= 0.58). From baseline, daily caloric intake decreased by a median of 140 kcal one year after radiotherapy and increased above baseline to nearly 1500 kcal/ day two years after completion of treatment, but these changes were not statistically significant (p= 0.89).

Changes in macronutrient intake

Baseline intakes of fat, carbohydrate and fibre intake were below the UK daily recommendation of 78 g/day, 267 g/day and 30 g/day respectively, but protein intake was adequate above 45 g/day (Table 5.35a). Fat intake increased by a median of 1 g/day two years after completion of radiotherapy (53.18 g, IQR 38.68- 68.87 g) from baseline (52.06 g, IQR 42.71- 78.34 g). Carbohydrate intake reduced by a median of 10 g/day from baseline (175.65 g, IQR 143.88- 201.08 g), two years after completion of radiotherapy (164.68 g, IQR 147.16- 194.49 g). None of these changes were statistically significant. Median daily intake of fibre fluctuated across the time points (range: 14- 18 g/ day) but remained well below the recommended 30 g/day. Whilst initially the changes in fibre intake were statistically significant, this was no longer the case when adjusted for multiple testing.

Time point	Baseline	End of treatment	6 months post RT	1 year post RT	2 years post RT	p-value
Weight (kg)	73.00 (67.95, 84.90)	72.00 (61.34, 85.45)	74.35 (61.73, 86.70)	71.10 (63.50, 83.00)	71.10 (56.68, 82.90)	p= 0.59
BMI (kg/m^2)	29.10 (26.15, 31.70)	27.90 (24.65, 31.63)	28.30 (24.93, 31.68)	27.40 (23.90, 33.00)	28.70 (23.75, 33.45)	p= 0.58
Energy (kcal)	1466.87 (1216.66, 1846.80)	1455.93 (1077.42, 1824.85)	1429.31 (1212.50, 1860.03)	1306.53 (1241.77, 1643.83)	1498.39 (1134.87, 1806.18)	p= 0.89

Table 5.34 Weight, BMI, energy and nutrient intake per time point, median (25th percentile, 75th percentile).

* statistically significant level type I error at 5% level under multiple testing requires p <0.023.

Macronutrients and fibre	Baseline	End of treatment	6 months post RT	1 year post RT	2 years post RT	p-value
Fat (g)	52.06 (42.71, 78.34)	60.89 (38.02, 73.00)	53.19 (42.65, 75.69)	52.19 (41.75, 58.95)	53.18 (38.68, 68.87)	p= 0.73
Saturated Fat (g/d)	18.94 (13.73, 25.98)	22.01 (15.08, 26.01)	19.10 (16.22, 26.21)	16.90 (12.54, 21.15)	18.46 (13.23, 27.60)	p= 0.24
Omega-3 (Total) (g/d)	0.94 (0.51, 1.48)	0.87 (0.52, 1.45)	0.48 (0.25, 1.26)	0.75 (0.56, 1.18)	0.66 (0.37, 1.35)	p= 0.39
Omega-6 (Total) (g/d)	3.26 (2.73, 4.68)	3.03 (1.53, 4.24)	2.66 (1.67, 3.43)	2.78 (2.05, 3.43)	2.44 (2.19, 3.23)	p= 0.24
Carbohydrate (g/d)	175.65 (143.88, 201.08)	156.44 (131.36, 191.33)	156.18 (127.58, 194.72)	147.24 (128.52, 155.53)	164.68 (147.16, 194.49)	p= 0.41
Free Sugars (g/d)	14.80 (12.17, 25.27)	15.95 (12.23, 19.82)	10.46 (8.95, 24.58)	9.68 (7.85, 22.11)	19.93 (5.84, 24.38)	p= 0.36
Protein (g/d)	70.72 (58.23, 90.75)	63.62 (49.68, 73.87)	72.50 (58.09, 84.47)	69.42 (57.13, 75.55)	66.55 (55.70, 88.68)	p= 0.092
Protein intake per kg body weight (g/kg/d)	0.96 (0.85, 1.06)	0.88 (0.81, 0.86)	0.97 (0.94, 0.97)	0.98 (0.90, 0.91)	0.94 (0.98, 1.07)	-
Fibre (g/d)	16.51 (13.90, 22.70)	13.82 (13.11, 16.62)	15.63 (11.19, 20.00)	17.75 (12.69, 19.21)	16.42 (10.96, 20.60)	p= 0.004*

Table 5.35a Macronutrient and fibre intake per time point, median (25th percentile, 75th percentile).

Changes in micronutrient intake

Micronutrient intake across all time points is shown in Table 5.35b. None of the changes in daily intake across time points of the micronutrients examined were statistically significant either before or after adjusting for multiple testing. At baseline, the median iron intake was 8.72 mg/day (IQR 6.90- 11.25 mg); this reduced slightly to a median of 7.33 mg/day (IQR 6.15- 10.76 mg) at the end of the study. Only one woman was taking ferrous sulphate at baseline, but none took iron supplements during or after treatment. Baseline Vitamin D intake through diet or supplements was a median of 3.43 µg/day (IQR 2.09- 4.63 µg). At that time, three women were taking a vitamin D supplement. All participants stopped their vitamin and herbal supplements during treatment. Intake through diet was at its lowest 6 months after radiotherapy (1.85 µg, IQR 1.02- 5.61 µg) and increased slightly to a median of 3.62 µg/day (IQR 1.67- 4.51 µg) two years after the end of treatment. At that time, only one woman was still taking a Vitamin D supplement. In contrast, at all time points, median vitamin B12 intake was higher than the recommended dose of 1.5 µg/day. One woman was taking a vitamin B12 supplement at baseline but discontinued this and one other participant was taking an over the counter multivitamin two years after treatment. Baseline intake was a median of 3.95 µg/day (IQR 3.43- 5.11 µg) and increased by an additional 1 µg/day at the end of the study (4.90 µg, IQR 3.40- 5.64 µg). Dietary folate intake remained below the recommended 200 µg/day throughout the study.

Micronutrients	Baseline	End of treatment	6 months post RT	1 year post RT	2 years post RT	p-value
Calcium (Ca) (mg/d)	575.15 (468.37, 725.98)	637.84 (379.97, 745.77)	565.20 (391.37, 735.46)	558.99 (477.92, 731.04)	522.94 (430.75, 669.38)	p= 0.87
Iron (Fe) (mg/d)	8.72 (6.90, 11.25)	6.78 (6.37, 9.11)	9.03 (5.30, 11.81)	7.23 (6.36, 9.57)	7.33 (6.15, 10.76)	p= 0.45
Magnesium (Mg) (mg/d)	195.08 (174.09, 259.03)	198.36 (161.01, 226.15)	234.79 (156.30, 267.64)	190.96 (165.99, 270.48)	191.47 (156.08, 260.11)	p= 0.82
Phosphorus (P) (mg/d)	896.64 (747.22, 1153.16)	811.72 (732.76, 1090.03)	857.79 (711.53, 1057.52)	813.61 (721.98, 1061.20)	879.96 (681.49, 938.27)	p= 0.58
Sodium (Na) (mg/d)	1476.30 (1322.86, 2336.40)	1430.07 (1084.51, 1843.45)	1544.10 (1374.04, 1957.18)	1585.73 (1230.80, 1815.68)	1476.90 (1293.03, 2100.93)	p= 0.86
Vitamin B_{12} (Cobalamin) (µg/d)	3.95 (3.43, 5.11)	4.95 (2.99, 6.26)	4.75 (3.49, 6.30)	4.04 (3.03, 5.47)	4.90 (3.40, 5.64)	p= 0.68
Vitamin C (mg/d)	69.07 (60.41, 93.41)	68.75 (36.56, 110.78)	61.84 (43.29, 105.45)	73.78 (57.32, 126.38)	55.29 (33.66, 108.71)	p= 0.76
Vitamin D (µg/d)	3.43 (2.09, 4.63)	2.22 (1.90, 3.41)	1.85 (1.02, 5.61)	2.86 (2.15, 4.02)	3.62 (1.67, 4.51)	p= 0.65
Folate (B9) Natural (µg/d)	159.32 (142.19, 264.02)	152.03 (133.47, 199.40)	168.60 (103.87, 202.39)	177.38 (132.33, 204.28)	167.93 (141.34, 188.13)	p= 0.13

Table 5.35b Micronutrient intake per time point, median (25th percentile, 75th percentile).

Other	Baseline	End of treatment	6 months post RT	1 year post RT	2 years post RT	p-value
Alcohol (g/d)	3.11 (1.99, 6.94)	5.16 (3.93, 5.35)	3.82 (2.26, 7.64)	5.69 (1.91, 8.45)	5.53 (3.25, 7.83)	p= 0.96
Caffeine (mg/d)	68.89 (47.83, 187.69)	39.62 (10.78, 114.49)	100.26 (32.36, 226.46)	122.95 (60.79, 231.49)	84.40 (59.04, 172.93)	p= 0.023*

Table 5.35c Alcohol and caffeine intake per time point, median (25th percentile, 75th percentile).

Alcohol and caffeine intake

There was an increase in alcohol intake across the five time points in those who drank alcohol; 40% of all participants did not consume any alcohol (Table 5.35c). Median baseline caffeine intake was (68.89 mg, IQR 47.83- 187.69 mg). Caffeine intake decreased at the end of treatment (39.62 mg, IQR 10.78- 114.49 mg), peaked one year after completion of treatment (122.95 mg, IQR 60.79- 231.49 mg) and decreased again a year later (84.40 mg, IQR 59-04- 172.93 mg) (p= 0.02).

GI toxicity

GI toxicity as defined by the change in IBDQ-B scores in section 5.2.2.4 (Table 5.9) in this sub-cohort of 15 women, was worst at the end of treatment for all groups and was most noticeable in the group who had a deterioration of ≥ 10 points in IBDQ-B scores at the end of treatment and six months after radiotherapy but then improved one year and two years after radiotherapy (Table 5.36). Due to the small number of participants in the groups and due to not having found any significant changes in dietary intake over time, no further statistical analysis was attempted to correlate GI toxicity with dietary intake.

IBDQ-B Median (range)	**Baseline**	**Beginning of RT**	**End of RT**	**6 months post RT**	**1 year post RT**	**2 years post RT**
Group A n= 4	70 (68-70)	63.5 (62-68)	56.5 (52-58)	65.5 (62-70)	67 (59-69)	56 (48-63)
Group B n= 5	68 (56-69)	58 56-66)	54 (41-61)	57 (38-67)	65 (46-67)	65 (62-70)
Group C n= 6	68.5 (54-69)	65.5 (57-70)	64 (62-65)	67.5 (54-70)	67 (65-68)	67 (54-69)
Total n= 15	69 (54-70)	64 (56-70)	60.5 (41-65)	66 (38-70)	67 (46-69)	64 (48-70)

Table 5.36 The IBDQ-B scores per time point of the 15 women whose food diaries were analysed (median, range).

A detailed discussion was included in the paper submitted for publication (Cirillo Sanchez et al., 2020) (Appendix 27) and the results showed that there were no significant changes in the dietary habits of the included participants over time. This means that it is likely that the development of symptoms relating to gastrointestinal toxicity is due to other factors rather than dietary changes and also that it is unlikely that dietary intake is the cause of any changes in the microbiome.

5.2.3.6 Evaluation of faecal calprotectin as potential biomarker for long-term GI toxicity

Faecal calprotectin levels of the 24 participants were assessed as a potential biomarker for long-term GI toxicity as measured by the IBDQ-B scores. The median baseline IBDQ-B score was 68.5 (maximum score 70, range: 38- 70). The median baseline faecal calprotectin level was 37 mcg/g which is within the normal limit. There was one outlier with known Crohn's disease and faecal calprotectin level greater than 2000 mcg/g at baseline and at all other time points who was excluded from the final analysis. Without this participant, the median baseline faecal calprotectin level was 35 mcg/g and 2 women (9%) had a faecal calprotectin level of > 100 mcg/g and median baseline IBDQ-B score was 68 (range: 51- 70) with 3 women (13%) having an IBDQ-B score below 60. At the end of treatment the median IBDQ-B score had dropped to 57.5 (range: 23- 66) and more than half of the participants (n= 12, 52%) had an IBDQ-B score less than 60 and the median faecal calprotectin level increased to 73.5 mcg/g (range: 0-373) with 8 women (45%) having a level above 100 mcg/g. Two years after radiotherapy the median faecal calprotectin decreased to 14 mcg/g and 4 women (17%) had a faecal calprotectin level above 100 mcg/g; the median IBDQ-B score was 63.5 (range: 31- 70) and 6 women (26%) had an IBDQ-B score below 60 (Table 5.37).

Time points		Baseline	FUP 1 Start of RT	FUP 2 End of RT	FUP 3 6 months post RT	FUP 4 12 months post RT	FUP 5 24 months post RT
IBDQ-B score (10-70)	Median (range)	68.5 (38- 70)	62.5 (34- 70)	57.5 (23- 66)	64.5 (38- 70)	65.0 (46- 70)	63.5 (31- 70)
IBDQ-B score < 60	N (%)	3 (13%)	8 (35%)	12 (52%)	6 (26%)	5 (22%)	6 (26%)
IBDQ-B score > 60		20 (87%)	15 (65%)	11 (48%)	17 (74%)	18 (78%)	17 (48%)
Faecal calprotectin levels (0-500)	Median (range)	35 (7-229)	43 (0-403)	73.5 (0-373)	44 (5- 298)	27 (5- 187)	14 (1- 234)
Faecal calprotectin < 100 mcg/g	N (%)	21 (91%)	20 (87%)	15 (65%)	19 (83%)	20 (87%)	19 (83%)
Faecal calprotectin > 100 mcg/g		2 (9%)	3 (13%)	8 (45%)	4 (17%)	3 (13%)	4 (17%)

Table 5.37 IBDQ-B scores and faecal calprotectin levels (n= 23) (Muls et al., in preparation for submission).

Of the 8 women with a faecal calprotectin level above 100 mcg/g at the end of treatment, three women had an IBDQ-B score below 60 one year post RT and two had an IBDQ-B score below 60 two years after radiotherapy. Only one of those participants had a high IBDQ-B score (66/70) at baseline, a raised faecal calprotectin level at the end of treatment (373 mcg/g) and IBDQ-B scores of 55/70 (one year post RT) and 31/70 (two years post RT) (Figure 5.24).

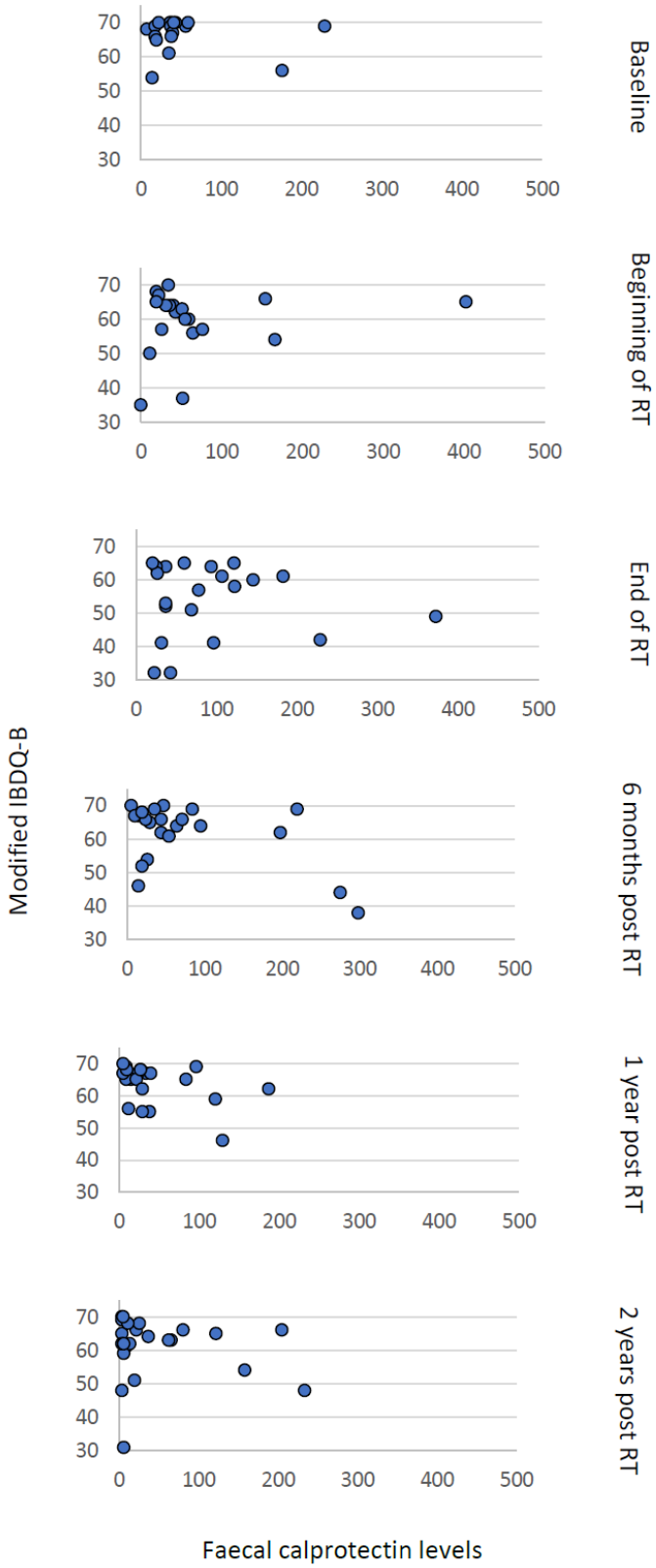


Figure 5.24 Scatterplot Faecal calprotectin level (mcg/ g) and IBDQ-B scores by time point. (Muls et al., in preparation for submission).

In the GLMM with faecal calprotectin as the independent variable the faecal calprotectin * time interaction was not statistically significant (F= 0.69 [numerator df =5, denominator df=43.45], p=0.64) suggesting that if there was an effect of faecal calprotectin on IBDQ scores it did not vary across time. The fixed effect beta coefficient for faecal calprotectin was -0.02 overall (95% CI -0.04 to -0.01, p= 0.030) and was statistically significant. Correlations between faecal calprotectin and IBDQ-B scores were negative except at the beginning of RT (Pre-RT -0.15, Beginning of RT 0.14, End of RT -0.11, 6 months post RT -0.40, one year post RT -0.18, two years post RT -0.35).

When looking at the correlation between between faecal calprotectin levels at the end of treatment and IBDQ-B scores 1 year post RT, the Pearson correlation was -0.17 (p=0.46) and 2 years post RT, the Pearson's correlation was -0.45 (p= 0.04) (Figure 5.25). More so, when applying the bootstrap of 10.000 samples to address issues of non-normality, the statistical significance was lost 2 years post RT (p= 0.36).

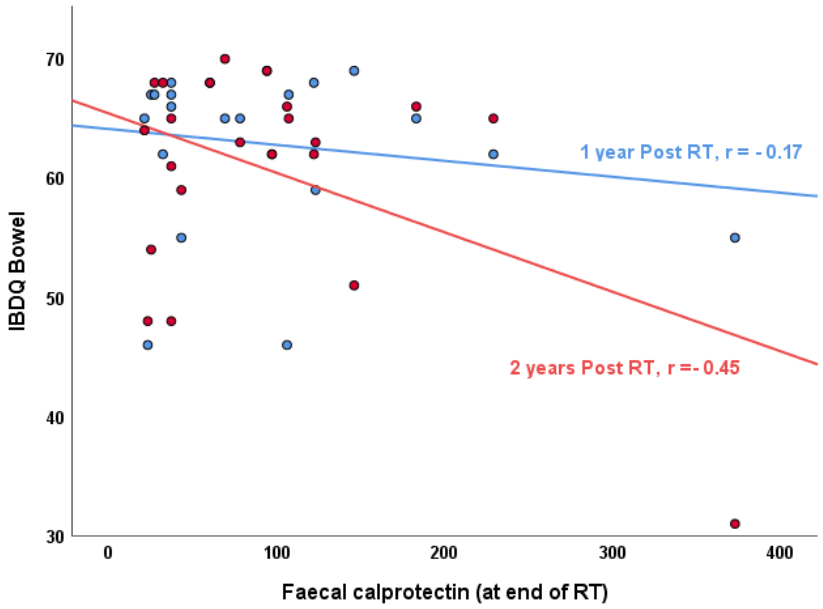


Figure 5.25 the effect of faecal calprotectin level at the end of treatment on the IBDQ-B scores 1 year post RT is (r= -0.17, p= 0.46) and 2 years post RT (r= -0.45, p= 0.036) (Muls et al., in preparation for submission).

These results suggest that faecal calprotectin provides some evidence of correlation with IBDQ-B scores in this patient cohort but is a weak indicator of chronic radiation-induced GI toxicity as measured by the IBDQ-B score. Raised faecal calprotectin levels in the acute phase of radiotherapy do not explain long-term deterioration in IBDQ-B scores one or two years after treatment.

Research time commitment

In total there were 277 face to face contacts during the study including the initial contact to explain the study, signing consent and withdrawal forms. Each contact lasted between 30 minutes and 1 hour. In between contacts, participants were telephoned to arrange the next data collection contact and to remind them again of the meeting. For each time point a sample collection pack was sent out to the participant. Preparing the sample collection receptacles for the participants with labels and instructions took 15 minutes per pack. A total of 330 telephone contacts were made. Each telephone contact lasted between 5 and 10 minutes. Providing the sample storage receptacles with labels took 20 minutes. Sample processing and storage took between 2 and 3.5 hours per sample collection. Annotation on the Electronic Patient Record and CRF took about 20-30 minutes and upgrading sample logs about the same. In total, each time point per participant took about 7 hours to complete. An estimated total of 1950 working hours was spent on preparation for meeting participants, travel time between sites, data and sample collection. This equates to 260 working days of 7.5 hours or one full time work year.

5.3 Conclusion

In summary, this chapter described the findings of the PREDICT study. Whilst there were no obvious statistically significant correlations found with clinical or treatment related variables, dietary food intake or faecal calprotectin levels, women with cervical cancer had lower IBDQ-B scores at all time points compared to women with carcinoma of the endometrium at all time points including one year after radiotherapy ($p < 0.05$) but this difference was no longer statistically significant two years after completion of radiotherapy ($p=0.37$). The analysis of the metabolites in the gut microbiome showed it is possible to predict long-term GI toxicity from a variety of samples taken earlier in the treatment pathway. Stool, urine and saliva samples could be used at the beginning of radiotherapy to predict GI toxicity two years after cancer treatment using the e-nose based on changes in IBDQ-B scores. This may be a useful tool in clinical practice to risk stratify women at risk of developing long-term GI toxicity and offering specialist GI assessment earlier in their follow-up after treatment rather than waiting until women have become distressed by their GI function not improving spontaneously.

The strengths and limitations of the methodology used in this study, generalisability, interpretation of the results and a personal reflection is discussed in chapter six.

Chapter 6

Discussion

This final chapter contains the key findings, limitations and interpretation of the PREDICT study and considers implications for future research and clinical practice. A personal reflection closes the chapter.

6.1 Summary of key findings

In order to assess potential predictive indicators of long-term radiation-induced GI toxicity it is important to collect detailed data on the development of GI symptoms throughout the treatment and follow-up pathway. This is the first prospective cohort study to systematically record GI symptoms and examine clinical variables alongside metabolic signatures of the gut microbiome in women treated for a gynaecological malignancy from before treatment until two years after completion of radiotherapy.

6.1.1 Hypothesis

The primary hypothesis is that the signatures created by the electronic sensing technique and GC-IMS show there is a difference in the volatiles and gas profiles emitted by the gut microbiota between cancer patients treated with pelvic radiotherapy who develop GI symptoms and those who do not. These signature profiles can be used to predict risk of GI toxicity. This hypothesis appears to be supported by the e-nose analysis for stool, urine and saliva samples at the beginning of radiotherapy based on the IBDQ-B groups. Thus, this hypothesis

can be accepted with caution due to the small number of participants in the study.

The secondary hypothesis that patient and treatment characteristics are different between cancer patients treated with pelvic radiotherapy who develop GI toxicity and those who do not can only partially be accepted. No statistically significant correlations were found between IBDQ-B scores and age, lymphovascular space invasion, chemotherapy or dose of radiotherapy. BMI, tumour type, surgery and European Cooperative Oncology Group Performance Status were correlated with IBDQ-B scores, but this was not sustained two years after radiotherapy.

Although associations have been suggested between high BMI and changes in the gut microbiome, Castaner et al. (2018) caution that no consistent difference was found between the microbiomes of lean and obese people in 11 studies including adults in their systematic review. No direct link has been described between BMI and long-term radiation induced GI toxicity. The results suggest that whilst a better performance status, and thus being more physically active before and at the beginning of treatment, is correlated with better GI function as measured by the IBDQ score, the individual time-specific correlations suggest this might not be sustained over time.

Women with cancer of the cervix had statistically significantly lower IBDQ-B scores and thus worse bowel function than women with cancer of the

endometrium from baseline throughout treatment and including one year after radiotherapy but this difference was not present two years after radiotherapy. It may be that women who receive primary radiotherapy for cervical cancer have a larger primary cancer and thus more disease bulk impacting on GI function compared to women with endometrial cancer who receive adjuvant radiotherapy following surgery.

Equally, among the treatment related variables such as chemotherapy and dose of radiotherapy, only having had surgery was correlated with long-term GI toxicity as measured with the IBDQ-B score one year after radiotherapy but this effect was not present at any other time points.

In this study, 25% (n= 6) of the participants experienced long-term GI toxicity, defined as a drop of ≥ 10 points on the IBDQ-B score, two years after radiotherapy compared to baseline. No change in IBDQ-B scores was reported by 37.5% (n= 9) of women two years from baseline and 37.5% (n= 9) experienced acute GI toxicity at the end of treatment compared to baseline but their IBDQ-B scores recovered two years after radiotherapy. This is lower to long-term radiotherapy induced GI toxicity figures reported in other studies, and may be due to many of those studies using a retrospective cohort design without baseline data about gastrointestinal function (Gillespie et al., 2009; Kuku et al., 2015; Ribas et al., 2020).

Analysis of stool, urine, saliva samples and rectal swabs showed that the e-nose is more effective at distinguishing based on the IBDQ-B score groups. The e-nose may be more sensitive and specific than GC-IMS as it has 18 sensors that analyse samples as a whole as opposed to the GC-IMS which detects specific metabolites.

Stool, urine and saliva could be used at the beginning of treatment with radiotherapy to predict GI toxicity two years after radiotherapy. Women may find it easier to provide a urine sample than a stool or saliva sample in the clinical setting.

Based on stool consistency, the outcomes of the analysis with the e-nose were more consistent than GC-IMS analysis overall. Women who reported constipation two years after treatment could be distinguished from women who reported normal stool consistency and those who reported diarrhoea two years after radiotherapy using stool, saliva and rectal swabs from the beginning of radiotherapy. However, urine was not the best sample of choice based on the BSC group analysis using the e-nose. Why this was the case is not clear.

Caution in interpreting these results is needed due to the very small numbers in the groups but these results give an indication that this is a viable way of assessing risk of long-term GI toxicity in clinical practice.

In the pilot study involving men treated for prostate cancer, higher levels of hydrogen sulphide were identified in men with long-term radiation induced GI toxicity compared to men with no GI toxicity following radiotherapy (Covington et al. 2012). High levels of hydrogen sulpide are associated with gut inflammation but lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium* can reduce inflammation in the intestinal tract (Dordevića, D., Jančíkováa, S., Vítězováb,. M, & Kushkevych, I., 2020). Due to the COVID-19 epidemic, the laboratory facilities at Warwick University were closed and further analysis to ascertain which metabolites were involved in the signatures or which metabolites changed over time was not possible. This analysis may be able to continue next year when laboratories are able to accept non-urgent, non-COVID related work.

6.1.2 Systematic review of the intestinal and vaginal microbiome and clinical cohort paper

The systematic review describing studies that examined the gut or vaginal microbiome in women treated for gynaecological malignancies and the clinical cohort paper showed that even though the number of participants in the present study was less than expected, it is relevant to clinical practice as there is a lack of studies including prospective cohorts of women treated for gynaecological cancer and there is a great clinical unmet need in understanding long-term gastrointestinal toxicity following cancer treatment in order to improve clinical management. The update emphasises the growing interest within this field and the relevance of the vaginal microbiome. In the future,

interactions between different microbiomes (such as the link found between the gut microbiome and brain function) may also become more important but this remains under-researched at present.

6.1.3 Systematic review of dietary habits and food diary analysis

The systematic review of literature describing dietary intake of women with a gynaecological cancer before, during or after treatment highlighted a lack of studies and showed that our understanding of dietary habits of this cohort remains limited. This was surprising considering the growing number of studies currently using dietary interventions to mitigate GI toxicity and promote healthy lifestyle changes to prevent recurrent disease. Analysis of the food diaries of 15 of the women participating in this study did not show any changes over time. Within the full cohort (n=24), most women (n= 16, 77%) maintained a median BMI of > 24.9 kg/m^2, categorising them as overweight (n= 8, 33%), obese (n= 6, 25%) or morbidly obese (n= 2, 8%). This is similar to findings in a recent randomised clinical trial including 35 women with gynaecological cancer where BMI also remained > 24.9 kg/m^2, one year after radiotherapy (White et al., 2020). It is known that obesity carries a risk of disease recurrence in gynaecological cancer and it seems women are not benefitting from the teachable moment to change their diet to manage their weight (Royal College of Obstetricians and Gynaecologists, 2012). GI toxicity in this sub-cohort had a similar pattern to the IBDQ-B scores of the total participants but due to the small number of women in each of the IBDQ-B

subgroups, it was not feasible to undertake further statistical analysis to correlate GI toxicity with dietary intake.

6.1.4 Evaluating faecal calprotectin as a potential biomarker for long-term GI toxicity

The evaluation of faecal calprotectin as a potential biomarker for long-term GI toxicity showed, in line with previous studies (Wedlake et al., 2008; Varela et al., 2009; Hille et al., 2017), that faecal calprotectin levels rise at the end of treatment and IBDQ-B scores worsen. There was only a weak correlation (Pearson's R: -0.20) between the IBDQ-B scores and faecal calprotectin levels (p= 0.03).

The pathophysiology of GI injury involves dynamic interactions among the intestinal vasculature, epithelial stem cells and stromal elements including resident and infiltrating macrophages and mast cells. Microvascular injury plays a significant and early role in the development of the acute phase of the GI injury. Vascular ischemia contributes to secondary enterocyte depletion, mucosal barrier breach, bacterial translocation, and structural damage of the intestine and resulting in fibrosis (Yarnold & Vozenin Brotons, 2010; Kim et al., 2014). The increase in faecal calprotectin levels seen at the end of radiotherapy are likely a result of acute inflammatory changes. However, with a Pearson's R of -0.20, in clinical practice, faecal calprotectin is a weak indicator for symptoms of chronic radiation-induced toxicity related to fibrosis 2 years post radiotherapy and faecal calprotectin testing in clinical practice has a limited

role in determining radiation-induced toxicity as early faecal calprotectin levels do not predict symptoms of long-term GI toxicity. Long-term, mildly raised faecal calprotectin levels could be due to small intestinal bacterial overgrowth or bile acid malabsorption which are present in women treated for gynaecological cancer with radiotherapy up in about 50% (Muls et al., in preparation for submission) and patients warrant further investigation into the potential contributing GI pathology.

6.2 Limitations

6.2.1 Recruitment

Recruitment across the two hospital sites was much more time consuming than anticipated and nearly 25% of eligible participants were missed due a lack of time to complete the consent process prior to the commencement of treatment. Over the 2 years of screening and recruiting, 111 women were eligible to participate. This was less than the expected 130 eligible participants a year. The most noticeable factor was that there were fewer potential participants identified in screening than anticipated from earlier data. A number of methods were used to attempt to improve recruitment of participants such as allowing recruitment of private patients and an extension of the recruitment period. The first resulted in an additional three women recruited (two completed the study). The extension of the recruitment period was not utilised as it was clear the original recruitment target would not be met and this would extend the

follow-up data collection and hamper the analysis of samples within the scheduled time-frame to complete this PhD.

Recruitment rates in clinical trials within oncology are known to be low (Walters et al., 2019) and researchers generally overestimate the number of participants available for a trial (Bertram et al., 2017). Muench's third law states that the number of cases promised in any clinical study must be divided by a factor of at least 10 (Bearman et al., 1979). In addition, this was not a clinical trial but a prospective cohort study whereby participants did not have any direct benefit from participating. In total, nearly 40% of eligible participants were recruited into the study and all of the women who completed the study were very enthusiastic and generous in their contribution to the study showing the study was feasible, if very time consuming, to undertake.

6.2.2 Withdrawal

Of note was the under-representation of non-White ethnicity within the study, especially among the participants who completed the time point two years after finishing radiotherapy. Proportionally, the withdrawal rate among Black African and Asian participants was not dissimilar from women with a White ethnicity. Participants withdrew mainly due to feeling unable or too unwell to provide samples or the burden of anti-cancer treatment. Nine women (22% of those recruited) had died by the end of the study and eight of those had withdrawn quite early on in the study. Perhaps these women were less well

and thus less able to participate in a study. Whilst this is the nature of clinical practice in an oncology setting it is something to be considered for recruiting into future studies.

6.2.3 GI toxicity assessment

As there is no gold standard to diagnose long-term GI toxicity following cancer treatment and no clear biological markers, GI toxicity was assessed by assessing symptoms relating to changes in GI function through a variety of GI symptom questionnaires. The IBDQ-B subscale was used as the primary end point. Previous studies have used a reduction of 6 points or more as a cut-off (Andreyev et al., 2013; Wedlake et al., 2017). However as only a fall of -10 points in the IBDQ-B score had shown a predictive value (ROC AUC 0.61, p= 0.049) (Wedlake et al., 2010) and there is a lack of any other studies defining a cut-off value, a reduction of 10 points or more was used as an indication of deteriorating GI function in this study. This may have resulted in a more strict application of the definition of GI toxicity than other studies have used.

The Bristol Stool Chart, St Mark's Incontinence score and CTCAE were also completed by the participants. The St Mark's Incontinence score correlated strongly with the IBDQ-B scores at all time points and this is likely because there are overlapping elements of the IBDQ-B score. The CTCAE score is usually completed by health care professionals but in this study, participants completed this themselves. More recently a PRO-CTCAE specific to be completed by

patients has been developed (National Cancer Institute, 2020; Yeung et al., 2020). This may be a more participant friendly way of assessing GI toxicity in future studies as the symptoms can be specifically adjusted to the study.

In line with other studies, diarrhoea, urgency and faecal incontinence were the more frequent reported GI symptoms at the end of treatment and long-term (Barraclough et al., 2012; Kuku et al., 2013; Ribas et al., 2020; White et al., 2020).

6.2.4 Feasibility

The 24 women who completed the study, provided their samples and symptom questionnaires with a high completion rate showing the study was feasible even though there was no direct benefit for the participants. As data collection in this study was so time-consuming, a reduced number of samples and questionnaires to be completed would lessen the burden both on the participant and the researcher.

6.3.3 Statistical analysis

Numbers in this study were small and this has implications for the statistical analysis and its interpretation. So as to not over-test from a statistical perspective, other demographic and clinical variables were presented using descriptive statistics only.

To check correlations between the IBDQ-B scores and clinical and treatment related variables, a GLMM was used to allow for data that does not meet certain statistical criteria expected of data with larger samples. GLMMs enabled examination of the impact of continuous variables within and between different time points and the primary outcome measure of the IBDQ-B scores but this method does not allow similar analysis for categorical variables. Under the guidance of Mr Murrells, an independent t-test was used to analyse nominal variables and ANOVA for the categorical variables. Acknowledgement of the small number of participants is required when interpreting the p-values (Ahmrein, Greenland & McShane, 2019).

The biological sample analysis was done by the team at Warwick University as this required bespoke statistical programming and data handling. Bias may have resulted in a deterioration of the samples as they were frozen at -80°C for different lengths of time. Comparison of urine and stool samples that had been stored for one year longer than the original batch had been planned to check for stability of results, but this analysis was not possible due to the COVID-19 laboratory restrictions.

An additional sensitivity analysis was undertaken to ascertain the impact of the reverse scoring for questions 21 and 32 of the IBDQ-E sub-questionnaire as participants may have misinterpreted how to answer those two questions (section 5.2.3.1). Paired sample correlations showed a strong correlation

between the IBDQ-E scores with and without those questions. Similarly, the total IBDQ score was not affected.

6.3 Interpretation of the results

Whilst it is known that women treated for gynaecological cancer are at risk of developing osteoporosis and bone fractures (Lee, Park, Lee, & Yong, 2020), most studies have identified this risk through examining large retrospective study cohorts and these risks are taken into account in routine oncology follow-up. Similarly, it is reported that many women treated for gynaecological cancer develop unintended long-term toxicities on many levels including gastrointestinal toxicity (Barraclough et al., 2012; Campbell et al., 2019; White et al., 2020) and it is recognised that long-term gastrointestinal toxicity is a potential outcome for many tumour groups (Teo et al., 2015; Gegechkori, Haines, & Lin, 2017). No specific predicitive factors have been identified in this study to determine the risk of long-term GI toxicity in women treated for gynaecological cancer. Finding little correlation between IBDQ-B scores and clinical and demographic variables may be due to the small number of participants in this study but may also indicate that long-term GI toxicity following cancer treatment is not only due to the treatment exposures.

As evidenced in the systematic review (chapter three), research only recently has focused on the gut microbiome of women treated for gynaecological cancer (Manichanh et al., 2008; Nam et al., 2013; Wang et al., 2015; Colbert et al., 2017;

Delgado-Medrano et al., 2017; Wang et al., 2019; Sims et al., 2019; Wang et al., 2019). Whilst results are variable in terms of specific species identified, the general finding is that, overall, the gut microbiome is less diverse in individuals when they develop GI toxicity with some bacterial species being present in higher abundance than normal and some no longer present. As studies to date identified different species involved, the composition of the gut microbiome before the start of radiotherapy could become a potentially important predictor for long-term GI toxicity following cancer treatment. This study has shown that the signatures of gut microbiome metabolites are also a candidate for predicting long-term GI toxicity following radiotherapy.

Results from the VOC analysis using the e-nose technique varied for the different sample types but it was possible to distinguish the IBDQ-B groups using stool, urine and saliva samples at the beginning of treatment to predict GI toxicity two years after radiotherapy using the e-nose analysis. Based on the stool consistency (BSC) groups, urine was not a good sample to use. It is not clear why different sample types yielded different results at different time points. One possible explanation may be that the different sample types represent different microbiomes and currently our understanding of this remains limited. However, this provides several options in clinical practice to identify women who are likely to develop long-term GI toxicity following radiotherapy. For instance a stool or urine sample could be sent off at the beginning of treatment so that personalised information can be provided

regarding the likelihood that long-term GI toxicity will develop and a plan can be made to assess and investigate that woman as soon as symptoms occur.

Because several studies have suggested faecal calprotectin as a biological marker of long-term GI toxicity following radiotherapy (Wedlake et al., 2008; Varela et al., 2009; Hille et al., 2017), special attention was given to evaluate this potential. As expected, an inverse relationship between IBDQ-B scores and faecal calprotectin levels was detected in stool samples: high IBDQ-B scores (good bowel function) correlated with low faecal calprotectin levels (no inflammation present) and vice versa. However, visual inspection of the scatterplot of faecal calprotectin levels and IBDQ-B scores at each time point did not show a clear cut-off level where faecal calprotectin correlates more strongly with IBDQ-B scores. Even though some correlation was found between faecal calprotectin level at the end of treatment and IBDQ-B scores one year post RT ($r= -0.17$, $p=0.46$) and two years post RT ($r= -0.45$, $p= 0.04$) the statistical significance at two years post radiotherapy was lost when using a bootstrap to approximate a larger sample. Moreover, in addition to the woman who was excluded due to persistently high faecal calprotectin levels due to known Crohn's disease, only one woman had a raised faecal calprotectin level at the end two years following treatment with radiotherapy despite 25% of women experiencing long-term radiotherapy induced GI toxicity. Raised faecal calprotectin levels above 100mcg/g during treatment may not be a good marker for chronic radiation-induced GI toxicity. The small sample size of this study limits the conclusions that can be made but indicated that raised faecal

calprotectin levels in the acute phase of radiotherapy do not fully explain long-term deterioration in IBDQ-B scores one or two years after treatment. Faecal calprotectin testing in patients with long-term radiotherapy-induced GI toxicity has limited value in clinical practice if no acute inflammatory pathology is suspected.

6.4 Future research implications

The study included a number of blood samples for translational research and clear plans have been set out to continue the work using these additional samples.

6.4.1 Analysis of fibrotic markers in blood samples

Previous studies have shown that cytokines such as CTGF, TGFβ1, IL-6 are involved in the development of long-term fibrosis (Yarnold and Vozenin Brotons, 2010; Wynn et al., 2012; Westbury et al., 2014). Blood samples were taken at each time point of the women participating in this study, processed and stored. Professor Abraham (Professor of cell and molecular biology, Royal Free Hospital London) has a special interest in radiation induced fibrosis and a Material Transfer Agreement (MTA) has been drawn up to analyse those samples as soon as laboratory facilities are able to undertake non-urgent work.

6.4.2 Sequencing of the microbiome

In addition, all stool, urine, saliva samples and rectal swabs were allocated in duplicate aliquots to enable further sequencing of the microbiomes. Professor Marchesi (University College London) has in principle agreed to collaborate in this work.

New research questions that have arisen include the role of the vaginal microbiome and which changes occur throughout and following cancer treatment and whether there is a link between changes in the gut microbiome and the vaginal microbiome.

6.5 Generalisability and implications for clinical practice

This study captured data from a small cohort of women treated for gynaecological cancer in a single centre. This reduces the ability to generalise the findings to a wider population or a different setting but it does build a basis for further clinical research in this area.

The processing time of the samples to prepare them for storage was considerable. In addition, analysis of the samples required a specialised laboratory. In theory, the e-nose technology can be used in clinical practice but the statistical analysis of the output generates a vast amount of data and is complex to analyse. The turn-over time for results could make its application in clinical practice more challenging. On the other hand, the results showed that

women who had acute GI toxicity at the end of treatment but recovered long-term, could be distinguished from those who had no change in their bowel function and those who developed on-going GI toxicity from the beginning of radiotherapy. Using this analysis in clinical practice would enable us to identify those women who would benefit earlier assessment and intervention from samples taken before or shortly after their treatment started. If the result of this analysis was available by the end of their treatment, women could receive individual counselling on how to monitor their GI symptoms and seek appropriate advice. Alternatively, patients could be asked earlier to identify those who need further assessment through active case finding in routine clinical practice.

In the near future, manipulation of the intestinal microbiome to reduce GI symptom burden is no longer fiction. The first case report of five women with radiation induced GI toxicity following treatment for gynaecological cancer who received a faecal transplant was published this year (Ding et al., 2020). This is promising as there were no safety concerns reported and three out of the five women reported an improvement in their GI symptoms lasting three to six months which was also confirmed by an improvement in visual features at colonoscopy (Ding et al., 2020). Other options could include altering the gut microbiome before the start of treatment to prevent or reduce long-term GI toxicity by using probiotics or faecal transplants, or taking faecal samples from before treatment to transplant back to the individual if they develop GI toxicity later on.

6.6 Funding

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In addition, the Friends of the Royal Marsden provided financial support for the purchase of an autosampler device to enable faster analysis of the samples.

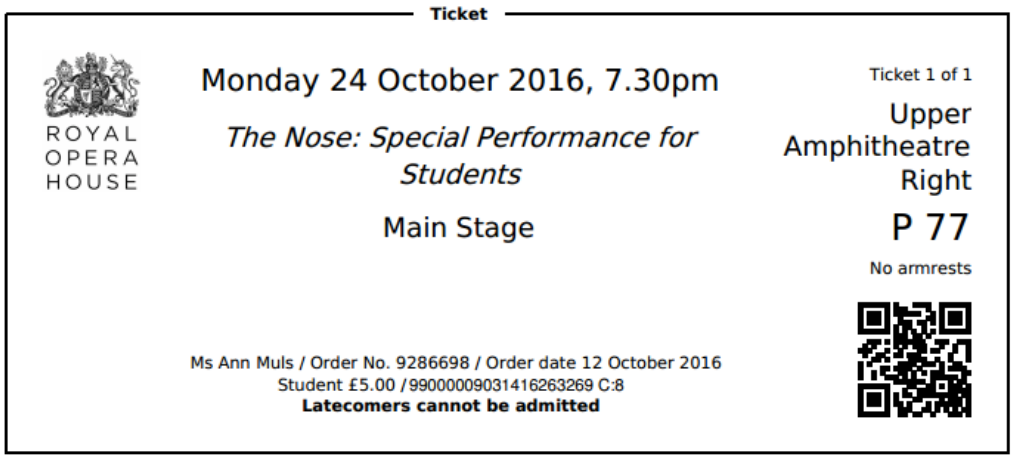
6.7 Personal reflection

Whilst I am immensely grateful for all the support and encouragement I have received throughout this PhD journey, there have also been big challenges. What this has taught me is that by discussing these challenges, eventually, a solution always emerges and I have also learnt that I need to accept what I cannot change myself. There is always a silver lining somewhere, even if you can't see it yet.

I am incredibly indebted to Professor Ailsa Hart and Professor Christine Norton for guiding me through this journey and I could not have done this work without their support. In addition, many people behind the scene highlight that no researcher works in isolation.

What I have also enjoyed immensely was reading around and exploring the subject of the 'nose'. Of course, the most famous story featuring a nose is Pinocchio (Collodi, 1881). Having read Nikolai Gogol's short story "The nose" (Gogol, 1836) about what happens to a man when he wakes up without his nose, I later on went to see the opera production of that same story with music by Dmitri Shostakovich at the Royal Opera House which was both hilariously funny and cleverly done.

Figure 6.1 Poster of the Nose Opera production.


Ticket

ROYAL OPERA HOUSE

Monday 24 October 2016, 7.30pm

The Nose: Special Performance for Students

Main Stage

Ticket 1 of 1

Upper Amphitheatre Right

P 77

No armrests

Ms Ann Muls / Order No. 9286698 / Order date 12 October 2016
Student £5.00 /99000009031416263269 C:8
Latecomers cannot be admitted

Figure 6.2 My student performance ticket.

One of the most wonderful exhibitions was the one I stumbled on in Copenhagen's NY Carlsberg Glyptoteket where there is a "nasoteque". It is a small display of marble noses that have been cut off from Roman statues.

Figure 6.3 The "nasoteque" (personal archive).

In addition, London's walk around Soho to find the seven noses and one "ghost" nose hidden on various facades of buildings was one I repeated with many members of my family and friends.

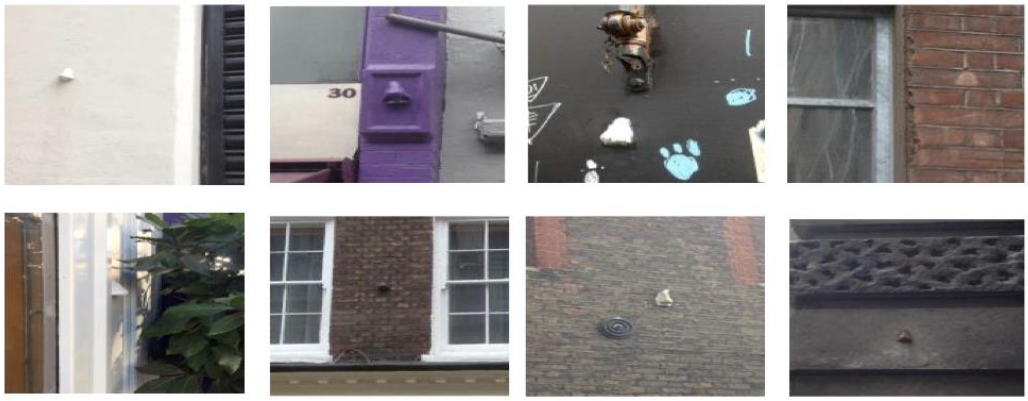


Figure 6.4 The noses of Soho (personal archive).

The Camel's nose, a book by Knut Schmidt-Nielsen (1998), describes the memoires of a "Curious Scientist" in his quest to understand why camels can go without drinking for a long time when faced with high desert temperatures (it turns out they do not sweat through their skin and are able to reabsorb water from the air they exhale via their nose).

Representation of the nose in literature and art is beautifully done in the "Nose Book" by De Rijke et al. (2000) and provided much opportunity to ponder about the importance of this specific organ.

Other skills that I gained during my PhD research were laboratory skills. Preparing samples for freezing and storage, using centrifuges and pipetting. It felt very Crime Scene Investigation-like and I really liked this part of the study.

I also enjoyed the opportunity to support the MSc student from King's as she chose the food diary analysis as her Master's degree thesis project and learning from the other academic supervisors as the student progressed.

I never experienced the "change in pace" that many clinicians expect when they move from a full-time clinical post to undertaking research at doctoral level. This was mainly because my PhD programme was part-time and I remained clinically engaged in our service for two days a week. This also meant I struggled to meet increasing clinical demands set by others but also myself. Near the end, I learnt better how to manage my time and expectations.

One of the unexpected things I discovered was that Brian Eno (yes, the musician and producer) and Peter Schmidt, in 1975, designed a set of 100 cards with "Oblique Strategies" to help artists or anyone who needed to boost their inspiration when faced with a problem (Marshall & Loydell, 2017). Being a huge Brian Eno fan, I was so surprised I had never come across this and it was only when I read a feature mentioning the set of cards during lockdown, I discovered it. I promptly ordered a set and used it when I had a dilemma to solve. You spread out the 100 cards and then pick one.

The occasions, I was curious as to how it would help, my dilemmas were all PhD related, especially during the analysis and interpretation stage.

I got the following answers:

"Always first steps" → go back to your baseline question.

"Do something boring" → start sorting the tables, figures and references.

"Allow and easement" → don't expect too much, it is ok not to get everything.

"Use an old idea" → go back to basics.

"Trust in the you of now" → believe that your intention was good and solid.

"Take some advice" → discuss with supervisors.

The very last one, a few weeks before submission of this PhD, very aptly stated "Tidy up" (!).

I think these cards could be used more often in health care to aide collaboration, problem solution, clinical supervision and reflective practice both in clinical practice and health research.

On a different note, the situation that made me laugh the most (and still does when I think of it) was the fact that at my very last stool sample collection, on opening the container, the sample exploded. I knew that if the container is overfilled and the bacteria have some time to produce gas, it increases the pressure within the container, but I in my whole career and throughout the study collecting hundreds of stool samples, this had never happened. I could not believe it happened with the last sample and it really was as if a monster within the sample escaped…

Despite often feeling this PhD was a horrible beast, I also saw that is was a magical creature that has taught me a lot about collaboration, support, steady work pace and determination.

About my future? I would like to continue a clinical academic research pathway. Anything is possible… Who (k)nose?

References

Aas, J., Paster, B., Stokes, L., Olsen, I., & Dewhirst, F. (2005). Defining the normal bacterial flora of the oral cavity. *J. Clin. Microbiol., 43*(11),5721-5732.

Abayomi, J., Kirwan, J., & Hackett, A. (2009). The prevalence of chronic radiation enteritis following radiotherapy for cervical or endometrial cancer and its impact on quality of life. *Eur J Oncol Nurs, 13*(4), 262-267.

Abeles, S., Jones, M., Santiago-Rodriguez, T., Ly, M., Klitgord, N., Yooseph, S., … & Pride, D. (2016). Microbial diversity in individuals and their household contacts following typical antibiotic courses. *Microbiome, 4*(1), 39-51.

Ahmrein, V., Greenland, S., & McShane, B. (2019). Retire statistical significance. *Nature, 567*, 305-307.

Ahn, J., Segers, S., & Hayes, R. (2012). Periodontal disease, Porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality. *Carcinogenesis, 33*(5), 1055-1058.

Alexander, J., Kohoutova, D., & Powell, N. (2019). Science in Focus: The Microbiome and Cancer Therapy. *Clinical Oncology, 31*(1), 1-4.

Alibrahim, B., Aljasser, M. & Salh, B. (2015). Fecal calprotectin use in inflammatory bowel disease and beyond: A mini-review. *Can J Gastroenterol Hepatol, 29*(3), 157–163.

Allen, J., Mailing, L., Niemro, G., Moore, R., Cook, M., White, B., … & Woods, J. (2018). Exercise alters gut microbiota composition and function in lean and obese humans. *Medicine & Science in Sports & Exercise, 50*(4), 747-757.

Almeida, A., Mitchell, A., Boland, M., Forster, S., Gloor, G., Tarkowska, A., … & Finn, R. (2019). A new genomic blueprint of the human gut microbiota. *Nature, 568*, 499–504.

Andreyev, H.J.N., Benton, B., Lalji, A., Norton, C., Mohammed, K., Gage, H., … & Lindsay, J. (2013). Algorithm-based management of patients with gastrointestinal symptoms in patients after pelvic radiation treatment (ORBIT): a randomised controlled trial. *Lancet, 382*, 2084–2092.

Andreyev, H.J.N., Muls, A., Norton, C., Ralph, C., Watson, L., … & Lindsay, J. (2014). Guidance: The practical management of the gastrointestinal symptoms of pelvic radiation disease. *Frontline Gastroenterol, 6*, 53-72.

Anwar, H., Ifran, S., Hussain, G., Faisal, M., Muzaffar, H., Mustafa, I., … & Ullah, M. (2019). Chapter 10: Gut Microbiome: A new organ system in the body. In Bastidas, G., & Kamboh, A. (Eds). *Parasitology and Microbiology Research.* (1st ed., pp. 217-238). IntechOpen, London. Retrieved April, 04, 2020 from https://www.intechopen.com/books/parasitology-and-microbiology-research/gut-microbiome-a-new-organ-system-in-body

Arasaradnam, R., McFarlane, M., Ryan-Fisher, C., Westenbrink, E., Hodges, P., Thomas, M., … & Covington, J. (2014). Detection of Colorectal Cancer (CRC) by Urinary Volatile Organic Compound Analysis. *PLoS One*, *9*(9), e108750.

Arasaradnam, R., Ouaret, N., Joseph, M., Nwokolo, C., Wedlake, L., Andreyev, J., … & Covington, J. (2011). Radiation-induced gut damage: identifying 'at risk' patients with an 'electronic nose' (e-nose). *Gut, 60*(Suppl 1), A108-A109.

Arasaradnam, R., Covington, J., Harmston, C. & Nwokolo, C. (2014). Review article: next generation diagnostic modalities in gastroenterology - gas phase volatile compound biomarker detection. *Aliment Pharmacol Ther, 39*(8), 780-789.

Arbyn, M., Weiderpass, E., Bruni, L., de Sanjosé, S., Saraiya, M., Ferlay, J., & Bray, F. (2020). Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *The Lancet Global Health*, *8*(2), e191–e203.

Astudillo-de la Vega, H., Alonso-Luna, O., Ali-Pérez, J., López-Camarillo, C. & Ruiz-Garcia, E. (2019). Oncobiome at the Forefront of a Novel Molecular Mechanism to Understand the Microbiome and Cancer. In Ruiz-Garcia, E., Astudillo-de la Vega, H. (Eds.), *Translational Research and Onco-Omics Applications in the Era of Cancer Personal Genomics. Advances in Experimental Medicine and Biology* (Vol. 1168., pp. 147-156.) Springer, Cham.

Audriac-Chalfour, A., Torres-Poveda, K., Bahena-Román, M., Téllez-Sosa, J., Martínez-Barnetche, J., Cortina-Ceballos, B., … & Madrid-Marina, V. (2016). Cervical microbiome and cytokine profile at various stages of cervical cancer: a pilot study. *PLoS One, 11*(4), e0153274.

Avila, M., Ojcius, D., & Yilmaz, Ö. (2009). The oral microbiota: Living with a permanent guest. *DNA Cell Biol, 28*(8), 405–411.

Bai, J., Jhaney, I., Daniel, G., & Bruner, D. (2019). Pilot Study of Vaginal Microbiome Using QIIME 2™ in Women With Gynecologic Cancer Before and After Radiation Therapy. *Oncology Nursing Forum, 46*(2): E48–E59.

Bai, J., Patel, P., Shelton, J., Corwin, E., Daniel, G., Mulle, J., … & Bruner, D. (2018). Vaginal Microbiome Profiles in Women with Gynecologic Cancers Treated with Radiation Therapy. *The Southern Nursing Research Society,* conference abstract. Retrieved August, 22, 2018 from: https://www.researchgate.net/publication/324101202_Vaginal_Microbiome_Profiles_in_Women_with_Gynecologic_Cancers_Treated_with_Radiation_Therapy.

Barraclough, L., Routledge, J., Farnell, D., Burns, M., Swindell, R., Livsey, J., & Davidson, S. (2012). Prospective analysis of patient-reported late toxicity following pelvic radiotherapy for gynaecological cancer. *Radiotherapy and Oncology, 103*, 327-332.

Bassis, C., Moore, N., Lolans, K., Seekatz, A., Weinstein, A., Young, V., & Hayden, M. for the CDC Prevention Epicenters Program. (2017). Comparison of stool versus rectal swab samples and storage conditions on bacterial community profiles. *BMC Microbiology, 17*(1), 78- 85.

Bearman, J., Loewenson, R., & Gullen, W. (1974). Muench's postulates, laws and corollaries, or biometrician's views on clinical studies (Biometric Note 4). *Bethesda (MD): Office of Biometry and Epidemiology, National Eye Institute, National Institutes of Health.*

Belstrøm, D. (2020). The salivary microbiota in health and disease. *Journal of Oral Microbiology, 12*(1), 1723975.

Berger, N. (2014). Obesity and cancer pathogenesis. *Annals of the New York Academy of Sciences, 1311*(1), 57-76.

Bertram, W., Moore, A., Wylde, V., & Gooberman-Hill, R. (2019). Optimising recruitment into trials using an internal pilot. *Trials, 20*, article number 207.

Berveling, M., Langendijk, J., Beukema, J., Maurits, M., Reuners, A. & Pras, E. (2011). Health-related quality of life and late morbidity in concurrent chemoradiation and radiotherapy alone in patients with locally advanced cervical carcinoma. *J Gynecol Oncol, 22*(3), 152–160.

Biehl, L., Garzetti, D., Farowski, F., Ring, D., Koeppel, M., Rohde, H., … & Vehreschild, M. (2019). Usability of rectal swabs for microbiome sampling in a cohort study of hematological and oncological patients. *PLoS One, 14*(4), e0215428.

Bischoff, S. (2011). Gut health: a new objective in medicine? *BMC Medicine, 9*, arcticle number 24.

Bjarnason, I. (2017). The Use of Fecal Calprotectin in Inflammatory Bowel Disease. *Gastroenterology & Hepatology, 13*(1), 53- 56.

Boekema, P., Samsom, S., van Berge Henegouwen, G., & Smout, A. (1999). Coffee and Gastrointestinal Function: Facts and Fiction: A Review, *Scandinavian Journal of Gastroenterology, 34*(230), 35-39.

Bond, T., & Derbyshire, E. (2019). Tea Compounds and the Intestinal microbiome: Findings from Trials and Mechanistic Studies. *Nutrients, 11*(10), 2364- 2376.

Borella, F., Ghisoni, E., Giannone, G., Cosma, S., Benedetto, C., Valabrega, G. & Katsaros, D. (2020). Immune Checkpoint Inhibitors in Epithelial Ovarian Cancer: An Overview on Efficacy and Future Perspectives. *Diagnostics, 10*(3), 146-163.

Bosch, S., Bot, R., Wicaksono, A., Savelkoul, E., van der Hulst, R., Kuijvenhoven, J., … & de Boer, N. (2020). Early detection and follow-up of colorectal neoplasia based on faecal volatile organic compounds. *Colorectal disease: the official journal of the Association of Coloproctology of Great Britain and Ireland*, 10.1111/codi.15009. Advance online publication. https://doi.org/10.1111/codi.15009.

Bouatra, S., Aziat, F., Mandal, R., Guo, A., Wilson, M., Knox, C., … & Wishart, D. (2013). The human urine metabolome. *PLoS One, 8*(9), e73076.

Bratton, D. & Henson, P. (2011). Neutrophil Clearance: when the party's over, cleanup begins. *Trends Immunol, 32*(8), 350–357.

Brusselaers, N., Shrestha, S., Wijgert, J., & Verstraelen, H. (2019). Vaginal dysbiosis, and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *American Journal of Obstetrics and Gynecology, 221*, 9-18.

Buford, T. (2017). (Dis)Trust your gut: the intestinal microbiome in age-related inflammation, health, and disease. *Microbiome, 5*(1), 80-91.

Burri, E., Manz, M., Schroeder, P., Froehlich, F., Rossi, L., Beglinger, C., & Lehmann, F. (2014). Diagnostic yield of endoscopy in patients with abdominal

complaints: incremental value of faecal calprotectin on guidelines of appropriateness. *BMC gastroenterology, 14,* 57.

Cadwell, K. (2015). The virome in host health and disease. *Immunity, 42*(5), 805-813.

Campbell, G., Thomas, T., Hand, L., Lee, Y., Taylor, S., & Donovan, H. (2019). Caring for survivors of gynecologic cancer: Assessment and management of long-term and late effects. *Semin Oncol Nurs, 35*, 192-201.

Cancer Research UK. (2020). Cancer statistics- new cases. Retrieved May, 27, 2020 from http://www.cancerresearchuk.org/health-professional/cancer-statistics.

Cancer Research UK. (2015). Exercise guidelines for cancer patients. Retrieved September, 12, 2018 from https://www.cancerresearchuk.org/about-cancer/coping/physically/exercise-guidelines.

Cani, P. (2018). Human intestinal microbiome: hopes, threats and promises. *Gut, 67*, 1716-1725.

Capurso, G., & Lahner, E. (2017). The interaction between smoking, alcohol and the gut microbiome. *Best practice & research. Clinical gastroenterology*, *31*(5), 579–588.

Castaner, O., Goday, A., Park, Y. M., Lee, S. H., Magkos, F., Shiow, S., & Schröder, H. (2018). The Gut Microbiome Profile in Obesity: A Systematic Review. *International Journal of Endocrinology*, *2018*, article number 4095789.

Castanon, A., Landy, R., & Sasieni, P. (2017). By how much could screening by human papillomavirus testing reduce cervical cancer incidence in England*? J Med Screen, 24*(2), 110–112

Cerdá, B., Pérez, M., Pérez-Santiago, J., Tomero-Aguillera, J., González-Soltero, R., & Larrosa, M. (2016). Gut microbiota modification: another piece in the puzzle of the benefits of physical exercise in health? *Frontiers in Physiology, 7*, article number 51.

Chan, A. (2018). Application of Electronic-Nose Technologies and VOC-Biomarkers for the Noninvasive Early Diagnosis of Gastrointestinal Diseases. *Sensors, 18*: 2613-2642.

Chandiok, S., Crawley, B., Oppenheim, B., Chadwick, P., Higgins, S. & Persaud, K. (1997). Screening for bacterial vaginosis: a novel application of artificial nose technology. *J Clin Pathol, 50*, 790–795.

Chang, J., Jang, W., Kim, Y., Kim, J., Kim, Y., Kim, Y., … Kim, H. (2016). Definitive treatment of primary vaginal cancer with radiotherapy: multi- institutional retrospective study of the Korean Radiation Oncology Group (KROG 12-09). *J Gynecol Oncol, 27*(2): e17.

Chase, D., Goulder, A., Zenhausen, F., Monk, B., & Herbst-Kralovetz, M. (2015). Review: The vaginal and gastrointestinal microbiomes in gynaecologic cancers: A review of applications in etiology, symptoms and treatment. *Gynecologic Oncology, 138*, 190-200.

Chen, M., Wang, S-Y., K, C-H., & Tsai, I-L. (2019). Metabolome analysis for investigating host-gut microbiota interactions. *Journal of the Formosan Medical Association, 118*, S10-S22.

Chhibber-Goel, J., Singhal, V., Bhowmik, D., Vivek, R., Parakh, N., Bhargava, B., & Sharma, A. (2016). Linkages between oral commensal bacteria and atherosclerotic plaques in coronary artery disease patients. *npj Biofilms and Microbiomes, 2*: article number: 7.

Chitapanarux, I., Chitapanarux, T., Traisathit, P., Kudumpee, S., Tharavichitkul, E., & Lorvidhaya, V. (2010). Randomized controlled trial of live lactobacillus acidophilus plus bifidobacterium bifidum in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. *Radiat Oncol, 5,* 31.

Chu, D., Ma, J., Prince, A., Antony, K., Seferovic, M., & Aagaard, K. (2017). Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nature Medicine, 23*(3), 314-328.

Ciorba, M., Hallemeier, C., Stenson, W., & Parikh, P. (2015). Probiotics to prevent gastrointestinal toxicity from cancer therapy: An interpretive review and call to action. *Curr Opin Support Palliat Care, 9*(2), 157–162.

Cirillo Sanchez, C., Czuber-Dochan, W., Cox, S., Murrells, T., Norton, C., & Muls, A. (2020). Dietary habits of women with gynecological cancer before, during and after treatment: a long-term prospective cohort study. *Nutrition & Cancer,* submitted for peer review.

Clarke, S., Murphy, E., O'Sullivan, O., Lucey, A., Humphreys, M., Hogan, A., … & Cotter, P. (2014). Exercise and associated dietary extremes impact on gut microbial diversity. *Gut, 63*, 1913–1920.

Colbert, L., Delgado Medrano, A., Previs, R., Eifel, P., Jhingran, A., Ramondetta, L., … & Klopp, A. (2018). Association of changes in vaginal microbiome with oligoclonal T-cell expansion and early response to chemoradiation for cervical cancer. *Journal of Clinical Oncology, 36*(5), suppl 8-8.

Colbert, L., Previs, R., Delgado Medrano, A., Eifel, P., Jhingran, A., Ramondetta, L., … & Klopp, A. (2017). Rectal Microbiome Diversity Predicts Disease Response at Completion of Radiation Therapy for Squamous Cell Carcinoma of the Cervix. *Oral Scientific Sessions, 99*(2S): S51.

Collado, M., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep., 6*, article number 23129.

Community Research and Development Information Service (CORDIS). (2020). Gut OncoMicrobiome Signatures (GOMS) associated with cancer incidence, prognosis and prediction of treatment response. *European Commission CORDIS EU research results.* Retrieved April, 17, 2020 from https://cordis.europa.eu/project/id/825410

Conlon, M. & Bird, A. (2015). The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. *Nutrients, 7*, 17-44.

Cornick, S., Tawiahy, A., & Chadee, K. (2015). Roles and regulation of the mucus barrier in the gut. *Tissue Barriers, 3*:1-2, e982426.

Covington, J., Wedlake, L., Andreyev, H.J.N., & Ouaret, N. (2012). The detection of patients at risk of gastrointestinal toxicity during pelvic radiotherapy, by electronic nose : a pilot study. *Sensors, 12*, 1-9.

Covington, J., Westenbrink, E., Ouaret, N., Harbord, R., Bailey, C., O'Connell, N., … & Arasaradnam, R. (2013). Application of a novel tool for diagnosing bile acid diarrhoea. *Sensors (Basel, Switzerland)*, *13*(9), 11899–11912.

Covington, J., van der Schee, M., Edge, A., Boyle, B., Savage, R., & Arasaradnam, R. (2015). The application of FAIMS gas analysis in medical diagnostics. *The Analyst, 140*(20), 6775-6781.

Critical Appraisal Skills Programme (CASP). (2014). *Study checklist for observational cohort studies. Critical Appraisal Skills Programme (CASP)*, Oxford Retrieved October, 16, 2016 from http://media.wix.com/ugd/dded87_e37a4ab637fe46a0869f9f977dacf134.pdf .

Crosby-Nwaobi, R., & Faithfull, S. (2011). High risk of urinary tract infections in post-operative gynaecology patients: a retrospective case analysis. *Eur J Cancer Care, 20*(6), 825-831.

Cust, A., Smith, B., Chau, J., van der Ploeg, H., Friedenreich, C., Armstrong, B., & Bauman, A. (2008). Validity and repeatability of the EPIC physical activity questionnaire: a validation study using accelerometers as an objective measure. *International Journal of Behavioral Nutrition and Physical Activity, 5*, 33.

Data Protection Act. (1998). Retrieved May 20, 2018 from http://www.legislation.gov.uk/ukpga/1998/29

Data Protection Act. (2018). Retrieved March 30, 2020 from http://www.legislation.gov.uk/ukpga/2018/12/contents/enacted

de Boer, S., Powell, M., Mileshkin, L., Katsaros, D., Bessette, P., Haie-Meder, C., … & Creutzberg, C. on behalf of the PORTEC Study Group (2019). Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial. *Lancet Oncol, 20*(9), 1273-1285.

De Rijke, V., Østermark-Johansen, L., & Thomas, H. (2000). *Nose book: representations of the nose in literature and the arts.* (1st ed.). Middlesex University Press.

Debelius, J., Song, S., Vasquez-Baeza, Y., Xu, Z., Gonzalez, A., & Knight, R. (2016). Tiny microbes, enormous impacts: what matters in intestinal microbiome studies? *Genome Biology, 17*, 217-229.

Delgado Medrano, A., Colbert, L., Hutchinson, D., Ajami, N., Petrosino, J., Mikkelson, M., … & Klopp, A. (2017). Increased Diversity of the Intestinal Microbiome Predicts for Lower Rates of Patient Reported Acute Gastrointestinal Toxicity in Women Receiving Radiation Therapy for Cervical Cancer. *International Journal of radiation Oncology Biology Physics, 99*(25), S111, 241.

Delia, P., Sansotta, G., Donato, V., Frosina, P., Messina, G., De Renzis, C., & Famularo, G. (2007). Use of probiotics for prevention of radiation-induced diarrhea. *World J Gastroenterol, 13*(6): 912–915.

Department of Health UK. (2011). *Physical activity guidelines for adults (Factsheet 4 and 5).* Retrieved September 12, 2018 from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/213740/dh_128145.pdf.

Derrien, M., Alvarez, A., & de Vos, W. (2019). The Gut Microbiota in the First Decade of Life. *Trends in Microbiology, 27*(12), 997-1010.

Deuster, E., Jeschke, U., Ye, Y., Mahner, S., & Czogalla, B. (2017). Vitamin D and VDR in Gynecological Cancers- A Systematic Review. *Int J Mol Sci., 18*(11), article number 2328.

Dewhirst, F., Chen, T., Izard, J., Paster, B., Tanner, A., Yu, W-H., … & Wade, W. (2010). The human oral microbiome. *J. Bacteriol., 192*(19), 5002-5017.

Di Fiore, F., & Van Cutsem, E. (2009). Acute and long-term gastrointestinal consequences of chemotherapy. *Best Practice & Research Clinical Gastroenterology, 23*: 113–124.

Dill-McFarland, K., Tang, Z., Kemis, J., Kerby, R., Chen, G., Palloni, A., … & Herd, P. (2019). Close social relationships correlate with human gut microbiota composition. *Sci Rep, 9*, 703.

Dimenäs, E., Glise, H., Hallerbäck, B., Hernqvist, H., Svedlund, J., & Wiklund, I. (1993). Quality of life in patients with upper gastrointestinal symptoms. An improved evaluation of treatment regimens? *Scand J Gastroenterol, 28*, 681-687.

Dominguez-Bello, M., Costello, E., Contreras, M., Magris, M., Hidalgo, G., Fierere, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA., 107*(26): 11971-11975.

Ding, X., Li, Q., Li, P., Chen, X., Xiang, L., Bi, L., … & Zhang, F. (2020). Fecal microbiota transplantation: a promising treatment for radiation enteritis? *Radiotherapy and Oncology, 143*, 12-18.

Dordevića, D., Jančíkováa, S., Vítězováb,. M, & Kushkevych, I. (2020). Hydrogen sulfide toxicity in the gut environment: Meta-analysis of sulfate-reducing and lactic acid bacteria in inflammatory processes. *Journal of Advanced Research*, In press. Retrieved 09 September 2020 from https://doi.org/10.1016/j.jare.2020.03.003.

Eifel, P., Winter, K., Morris. M., Levenback, C., Grigsby, P., Cooper, J., … & Muth, D. (2004). Pelvic radiation with concurrent chemotherapy versus pelvic and para-aortic radiation for high-risk cervical cancer: an update of Radiation Therapy Oncology Group Trial (RTOG) 90-01. *Journal of Clinical Oncology, 22*(5), 872-880.

Elrefaey, S., Massaro, M., Chiocca, S., Chiesa, F., & Ansarin, M. (2014). HPV in oropharyngeal cancer: the basics to know in clinical practice. *Acta Otorhinolaryngol Ital., 34*(5), 299–309.

Emmett, M., Gildea, C., Nordin, A., Hirschowitz, L., & Poole, J. (2017). Variations in Treatment of Cervical Cancer According to Tumor Morphology V Population-Based Cohort Analysis of English National Cancer Registration Data. *Int J Gynecol Cancer, 27*, 138-146.

Eng, K., Hanlon, B., Bradley, W., & Szender, J. (2015). Prognostic factors modifying the treatment-free interval in recurrent ovarian cancer. *Gynecol Oncol., 139*(2), 228–235.

Engen, P., Green, S., Voigt, R., Forsyth, C., & Keshavarzian, A. (2015). Alcohol effects on the composition of intestinal microbiota. *The Gastrointestinal Microbiome, 37*(2), 223-236.

Ertz-Archambault, N., Keim, P., & Von Hoff, D. (2017). Microbiome and pancreatic cancer: A comprehensive topic review of literature. *World J Gastroenterol., 23*(10), 1899–1908.

Esfahani, S., Sagar, N., Kyrou, I., Modziak, E., O'Connell, N., Nwokolo, C., … & Covington, J. (2016). Variation in Gas and Volatile Compound Emissions from Human Urine as It Ages, Measured by an Electronic Nose. *Biosensors*, *6*, article number 4.

Ferranti, E., Dunbar, S., Dunlop, A., & Corwin, E. (2014). 20 Things you Didn't Know About the Human intestinal microbiome. *J Cardiovasc Nurs., 29*(6), 479–481.

Ferreira, M., Muls, A., Dearnaley, D., & Andreyev, H.J.N. (2014). Microbiome and radiation-induced bowel toxicity: lessons from inflammatory bowel disease for the radiation oncologist. *Lancet Oncol, 15*(3), e139-47.

Fields, E., McGuire, W., Lin, L., & Temkin, S. (2017). Radiation Treatment in Women with Ovarian Cancer: Past, Present, and Future. *Front. Oncol., 7*, article number 177.

Filoche, S., Wong, L., & Sissons, C. (2010). Oral biofilms: emerging concepts in microbial ecology. *J Dent Res, 89*, 8–18.

Flemmig, T., & Beikler, T. (2011). Control of oral biofilms. *Periodontol 2000, 55*, 9–15.

Floch, M. (2010). Fecal Bacteriotherapy, Fecal Transplant, and the Microbiome. *Journal of Clinical Gastroenterology, 44*(8), 529-530.

Francino, M. (2016). Antibiotics and the human intestinal microbiome: dysbiosis and accumulation of resistances. *Frontiers in Microbiology, 6*, article number 1543.

Gallegos, J., Arce, C., Jordano, R., Arce, L., & Medina, L. (2017). Target identification of volatile metabolites to allow the differentiation of lactic acid

bacteria by gas chromatography-ion mobility spectrometry. *Food Chemistry, 220*: 362-370.

Garg, M., Hendy, P., Ding, J., Shaw, S., Hold, G., & Hart, A. (2018). The effect of vitamin D on intestinal inflammation and faecal microbiota in patients with ulcerative colitis. *Journal of Crohn's and colitis, 2018*, 963-972.

Garmaeva, S., Sinha, T., Kurilshikov, A., Fu, J., Wijmenga, C., & Zhernakova, A. (2019). Studying the gut virome in the metagenomic era: challenges and perspectives. *BMC Biol, 17*, article number 84.

Garrett, W., Gallini, C., Yatsunenko, T., Michaud, M., DuBois, A., Delaney, M., … & Glimcher, L. (2010). Enterobacteriaceae act in concert with the intestinal microbiome to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe, 8*(3), 292-300.

Gasbarrini, A., Lauritano, A., Gabrielli, M., Scarpellini, E., Lupascu, A., Ojetti, V., Gasbarrini, G. (2007). Small Intestinal Bacterial Overgrowth: Diagnosis and Treatment. *Dig Dis, 25*, 237-240.

Gee, C., Andreyev, J., & Muls, A. (2018). Developing advanced clinical practice skills in gastrointestinal consequences of cancer treatment. *British Journal of Nursing, 27*(5), 237-247.

Gegechkori, N., Haines, L., & Lin, J. J. (2017). Long-Term and Latent Side Effects of Specific Cancer Types. *The Medical clinics of North America, 101*(6), 1053-1073.

Gerstner, G., Kucera, H., Weghaupt, K., & Rotter, M. (1982). Endometrial bacteriology in patients with endometrial cancer before and after primary intracavity irradiation using IR-192 and an afterloading technique. *Archives of Gynecology*, *231*, 299-306.

Gholizadeh, P., Eslami, H., Yousefi, M., Asgharzadeh, M., Aghazadeh, M., & Kafil, H. (2016). Role of oral microbiome on oral cancers, a review. *Biomedicine & Pharmacotherapy, 84*, 552-558.

Gilbert, J., & Alverdy, J. (2016). Stool consistency as a major confounding factor affecting microbiotic composition: an ignored variable? *Gut, 65*(1), 1-2.

Gillespie, C., Goode, C., Hackett, C, & Andreyev, H.J.N. (2007). The clinical needs of patients with chronic gastrointestinal symptoms after pelvic radiotherapy. *Aliment Pharmacol Ther, 26*, 555-563.

Giralt, J., Regadera, J., Verges, R., Romero, J., de la Fuente, I., Biete, A., … & Guarner, F. (2008). Effects of probiotic Lactobacillus casei DN-114 001 in prevention of radiation-induced diarrhea: results from multicenter, randomized, placebo-controlled nutritional trial. *Int J Radiat Oncol Biol Phys, 71*, 1213–1219.

Gisbert, J., & McNicholl, A. (2009). Questions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease. *Dig Liver Dis., 41*(1), 56–66.

Glassner, K., Abraham, B., & Quigley, E. (2020). The microbiome and inflammatory bowel disease. *The Journal of Allergy and Clinical Immunity, 145* (1), 16-27.

Gogol, N. (1836). *The nose.* (Published Feb 2015). Little Black Classics # 46. Penguin Classics.

Gominak, S. (2016). Vitamin D deficiency changes the intestinal microbiome rediucing B vitamin production in the gut. The resulting lack of pantothenic acid adversely affects the immune system, producing a "pro-inflammatory" state associated with atherosclerosis and autoimmunity. *Medical Hypotheses, 94*, 103-107.

Gordon, A., Martens, M., LaPread, Y., & Faro, S. (1989). Response of lower genital tract flora to external pelvic irradiation. *Gynecologic Oncology, 35*, 233-235.

Guarner, F., & Malagelada, J. (2003). Gut flora in health and disease. *The Lancet*, *360*, 512-519.

Gurwara, S., Dai, A., Ajami, N., El-Serag, H., Graham, D., & Jiao, L. (2019). Caffeine consumption and the colonic mucose-associated gut microbiota. *American College of Gastroenterology Annual Scientific Meeting Abstracts 2019*, Program no. P1916.

Hacker, N., Eifel, P., & van der Velden, J. (2015). Cancer of the vagina. *International Journal of Gynecology and Obstetrics, 131*, S84–S87.

Handu, D., Moloney, L., Wolfram, T., Ziegler, P., Acosta, A., & Steiber, A. (2016). Academy of Nutrition and Dietetics Methodology for Conducting Systematic Reviews for the Evidence Analysis Library. *Journal of the Academy of Nutrition and Dietetics, 116*(2), 311-318.

Hart, A., & Ng, S. (2011). *Inflammatory Bowel Disease: An Evidence-Based Practical Guide.* (1st ed.) T.F.M. Publishing Limited, Nr Shrewsbury, UK.

Hauer-Jensen, M., Denham, J., & Andreyev, H.J.N. (2014). Radiation enteropathy--pathogenesis, treatment and prevention. *Nat Rev Gastroenterol Hepatol*, *11*(8), 470-479.

Hayes, R., Watson-Jones, D., Celum, C., van de Wijgert, J., & Wasserheit, J. (2010). Treatment of sexually transmitted infections for HIV prevention: end of the road or new beginning? *AIDS, 24*, S15–S26.

Health Improvement Directorate. (2009). *General Practice Physical Activity Questionnaire: A screening tool to assess adult physical activity levels, within primary care.* Retrieved September, 12, 2018 from http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/@ps/documents/digitalasset/dh_112134.pdf

Heaton, K. (2009). Response to Constipation in Adults, BMJ, 338, b831.

Henderson, P., Anderson, N., & Wilson, D. (2014). The diagnostic accuracy of fecal calprotectin during the investigation of suspected pediatric inflammatory

bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol., 109*, 637–645.

Hidalgo, A., Chilvers, E., Summers, C., & Koenderman, L. (2019). New Advances in Neutrophil Immunity Feature Review The Neutrophil Life Cycle. *Trends in Immunology, 40*(7), 584- 597.

Hille, A., Schmidt-Giese, E., Hermann, R. M., Herrmann, M. K., Rave-Fränk, M., Schirmer, M., … & Ramadori, G. (2008). A prospective study of faecal calprotectin and lactoferrin in the monitoring of acute radiation proctitis in prostate cancer treatment. *Scandinavian journal of gastroenterology*, *43*(1), 52–58.

Hills, R., Pontefract, B., Mishcon, H., Black, C., Sutton, S., & Theberge, C. (2019). Gut Microbiome: Profound Implications for Diet and Disease. *Nutrients*, *11*(7), article number 1613.

Hoffmann, A., Proctor, L., Surette, M., & Suchodolski, J. (2016). The Microbiome: The Trillions of Microorganisms That Maintain Health and Cause Disease in Humans and Companion Animals. *Veterinary Pathology, 53*(1), 10-21.

Huang, C., & Shi, G. (2019). Smoking and microbiome in oral, airway, gut and some systemic diseases. *J Transl Med, 17*, article number 225.

Iizumi, T., Battaglia, T., Ruiz, V., & Perez Peres, G. (2017). Intestinal microbiome and antibiotics. *Archives of Medical Research, 48*, 727-734.

Imhann, F., Vila, A., Bonder, M., Lopez Manosalva, A., Koonen, D., Fu, J., Wijmenga, C., … & Weersma, R. (2017). The influence of proton pump inhibitors and other commonly used medication on the gut microbiota. *Gut Microbes, 8*(4), 351-358.

International Airport transport Association. (2018). *Dangerous Goods Regulations.* (59th ed). IATA. Retrieved May, 26, 2019, from https://www.iata.org/publications/store/Pages/dgr-print-manuals.aspx

Iwarzon, M., Gardulf, A., & Lindberg, G. (2009). Functional status, health- related quality of life and symptom severity in patients with chronic intestinal pseudo-obstruction and enteric dysmotility. *Scand J Gastroenterol, 44*, 700-707.

Jackson, M., Goodrich, J., Maxan, M., Freedberg, D., Abrams, J., Poole, A., … & Steves, C. (2015). Proton pump inhibitors alter the composition of the gut microbiota. *Gut, 0*, 1-8.

Jaquet, M., Rochat, I., Moulin, J., Cavin, C., & Bibiloni, R. (2009). Impact of coffee consumption on the gut microbiota: A human volunteer study. *International Journal of Food Microbiology*, *130*, 117- 121.

Jiang, G., Balboni, T., Taylor, A., Liu, J., & Lee, L. (2018). Palliative radiation therapy for recurrent ovarian cancer: Efficacy and predictors of clinical response. *International Journal of Gynecological Cancer, 28*(1), 43-50.

Jones, E., van Leeuwen, F., Hoogendoorn, W., Mourits, M., Hollema, H., Boven, H., … & Swerdlow, A. (2012). Endometrial cancer survival after breast cancer in

relation to tamoxifen treatment: Pooled results from three countries. *Breast Cancer Res., 14*(3), R91.

Joint Formulary Committee. (2015). British National Formulary (online) London: BMJ Group and Pharmaceutical Press. Retrieved 2015-2020 from https://bnf.nice.org.uk/

Khalid, U., McGough, C., Hackett, C., Blake, P., Harrington, K., Khoo, V., … & Andreyev, H.J.N. (2006). A modified Inflammatory Bowel Disease Questionnaire and the St. Mark's Incontinence Questionnaire are more sensitive measures of acute gastrointestinal toxicity during pelvic radiotherapy than RTOG grading. *Int J Radiat Oncol Biol Phys., 64*(5), 1432-1441.

Kilian, M., Chapple, I., Hannig, M., Marsh, P., Meuric, V., Pedersen, A., … & Zaura, E. (2016). The oral microbiome – an update for oral healthcare professionals. *British Dental Journal, 221*, 657– 666.

Kim, J., Jenrow, K., & Brown, S. (2014). Mechanisms of radiation-induced normal tissue toxicity and implications for future clinical trials. *Radiat Oncol J., 32*(3), 103–115.

König, J., Wells, J., Cani, P., García-Ródenas, C., MacDonald, T., Mercenier, A., … & Brummer, R. (2016). Human Intestinal Barrier Function in Health and Disease. *Clinical and Translational Gastroenterology, 7*, e196.

Kovachev, S. (2020). Cervical cancer and vaginal microbiota changes. *Arch Microbiol., 202*(2), 323-327.

Kuku, S., Fragkos, C., McCormack, M., & Forbes, A. (2013). Radiation-induced bowel injury: the impact of radiotherapy on survivorship after treatment for gynaecological cancers. *Br J Cancer, 109*(6), 1504-1512.

Kumagai, T., Rahman, F., & Smith, A. (2018). The Microbiome and Radiation Induced-Bowel Injury: Evidence for Potential Mechanistic Role in Disease Pathogenesis. *Nutrients, 10*(10), E1405.

Kyrgiou, M., Mitra, A., & Moscicki, A. (2017). Does the vaginal microbiota play a role in the development of cervical cancer? *Transl Res*, *179*, 168-182.

Le bastard, Q., Al-Ghalith, G., Grégoire, M., Chapelet, G., Javaudin, F., Dailly, E., … & Montassier, E. (2018). Systematic review: human gut dysbiosis induced by non-antibiotic prescription medications. *Aliment Pharmacol Ther., 47*, 332-345.

Le Roy, C., Wells, P., Si, J., Raes, J., Bell, J., & Spector, T. (2019). Red Wine Consumption Associated With Increased Gut Microbiota α-Diversity in 3 Independent Cohorts. *Gastroenterology, 158*, 270-272.

Lee, J., Park, C., Lee, E., Yong, I. (2020). Effect of gynecological cancer and its treatment on bone mineral density and the risk of osteoporosis and osteoporotic fracture. *Obstet Gynecol Sci, 64*(4), 470-479.

Lee, J., So, K., Piyathilake, C., & Kim, M. (2013). Mild Obesity, Physical Activity, Calorie Intake, and the Risks of Cervical Intraepithelial Neoplasia and Cervical Cancer. *PLoS One, 8*(6), e66555.

Lee, S., Yun, Y., Kim, S., Lee, E., Chang, Y., Ryu, S., … & Lee, J. (2018). Association between cigarette smoking status and composition of the gut microbiota: population-based cross-sectional study. *Journal of Clinical Medicine, 7*, 282-295.

Lewis, S., & Heaton, K. (1997). Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol, 32*, 920-924.

Liberati, A., Altman, D., Tetzlaff, J., Mulrow, C., Gøtzsche, P., Ioannidis, J., … & Moher, D. (2009). The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. *PLoS Med, 6*(7), e1000100.

Lim, E., Rodriguez, C., & Holtz, L. (2018). Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. *Microbiome, 6*, 87.

Lin, J., Egorova, N., Franco, R., Prasad-Hayes, M., & Bickell, N. (2016). Ovarian Cancer Treatment and Survival Trends Among Women Older Than 65 Years of Age in the United States, 1995–2008. *Obstet Gynecol, 127*, 81–89.

Liu, M., Li, S., Shu, Y., & Zhan, H. (2017). Probiotics for prevention of radiation-induced diarrhea: A meta-analysis of randomized controlled trials. *PLoS One, 12*(6), e0178870.

Lohiniemi, S., Mäki, M., Kaukinen, K., Laippala, P., & Collin, P. (2000). Gastrointestinal symptoms rating scale in coeliac disease patients on wheat starch-based gluten-free diets. *Scand J Gastroenterol, 35*, 947-949.

London Cancer Alliance. (2014). *Gynaecological Cancer Clinical Guidelines.* Retrieved May, 26, 2019, from http://rmpartners.cancervanguard.nhs.uk/pathways-and-protocols/.

Luthold, R., Fernandes, G., Franco-de-Moraes, A., Folchetti, L., & Ferreira, S. (2017). Gut microbiota interactions with the immunomodulatory role of vitamin D in normal individuals. *Metabolism Clinical and Experimental, 69*, 76-86.

Lynch, S., & Pedersen, O. (2016). The Human Intestinal Microbiome in Health and Disease. *N Engl J Med, 375*, 2369-2379.

Macfarlane, S. (2014). Antibiotic treatments and microbes in the gut. *Environ. Microbiol, 16*(4), 919–924.

Macmillan Cancer Support. (2013). *Throwing light on cancer and its consequences.* Retrieved July, 20, 2017, from https://www.macmillan.org.uk/.../throwinglightontheconsequencesofcanceranditstreat...

Maduro, J., Pras, E., Willemse, P., & de Vries, E. (2003). Acute and long-term toxicity following radiotherapy alone or in combination with chemotherapy for locally advanced cervical cancer. *Cancer Treat Rev, 29*, 471–488.

Maeda, Y., Pares, D., Norton, C., Vaizey, C., & Kamm, M. (2008). Does the St. Mark's incontinence score reflect patients' perceptions? A review of 390 patients. *Diseases of the Colon & Rectum, 51*, 436-442.

Mager, D., Ximenez-Fyvie, L., Haffajee, A., & Socransky, S. (2003). Distribution of selected bacterial species on intraoral surfaces. *J. Clin. Periodontol., 30*, 644-654.

Mai, V., Draganov, P. (2009). Recent advances and remaining gaps in our knowledge of associations between gut microbiota and human health. *World J Gastroenterol, 15*(1), 81-85.

Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E. E., … & Typas, A. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, *555*(7698), 623-628.

Manichanh, C., Varela, E., Martinez, C., Antolin, M., Llopis, M., Doré, J., … & Malagelada, J. (2008). The gut microbiome predispose to the pathophysiology of acute postradiotherapy diarrhea. *Am J Gastroenterol, 103*, 1754-1761.

Marchesi, J., Adams, D., Fava, F., Hermes, G., Hirshfield, G., Hold, G., … & Hart, A. (2015). The gut microbiota and host health: a new clinical fonrtier. *Gut BMJ, 65*, 330-339.

Marshall, K., & Loydell, R. (2017). Thinking inside the box: Brian Eno, music, movement and light. *Journal of Visual Art Practice, 16*(2), 104-118.

Mayor, S. (2016). Probiotics have no effect on gut microbiota in healthy people, review suggests. *BMJ, 353*, i2617.

Meroni, M., Longo, M., & Dongiovanni, P. (2019). Alcohol or Gut Microbiota: Who Is the Guilty? *Int J Mol Sci., 20*(18), E4568.

Mitra, A., MacIntyre, D., Lee, Y., Smith, A., Marchesi, J., Holmes, E., … & Kyrgiou, M. (2015). The vaginal microbiome of women with cervical intraepithelial neoplasia. *International Journal of Gynecological Cancer, 25*(9 suppl 1), 976-977.

Morris, K., & Haboubi, N. (2015). Pelvic radiation therapy: Between delight and disaster. *World journal of gastrointestinal surgery*, *7*(11), 279–288.

Müller, M., Hermes, G., Canfora, E., Smidt, H., Masclee, A., Zoetendal, E., & Blaak, E. (2020). Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit. *American Journal of Physiology Gastrointestinal and Liver Physiology, 318*(2), G361-G369.

Muls, A., Andrevey, H.J.N., Lalondrelle, S., Taylor, A., Norton, C., & Hart, A. (2017). Systematic Review: The Impact of Cancer Treatment on the Gut and Vaginal Microbiome in Women With a Gynecological Malignancy. *International Journal of Gynecological Cancer, 27*(7), 1550–1559.

Muls, A., Cirillo Sanchez, C., Norton, C., & Czuber-Dochan, W. (2020). A systematic review: dietary habits of women with gynecological cancer before, during and after treatment. *Nutrition and Cancer,* 5:1-11. DOI: 10.1080/01635581.2020.1839518.

Muls, A., Norton, C., & Hart, A. (2016). Systematic Review Protocol: The Impact of Cancer Treatment on the Gut and Vaginal Microbiome in Women With a Gynecological Malignancy. (PROSPERO 2016:CRD42016047121 Available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016047121).

Muls, A., Taylor, A., Lalondrelle, S., Kabir, M., Norton, C., Hart, A., & Andreyev, H.J.N. (2020). A proposed tailored investigational algorithm for women treated for gynaecological cancer with long-term GI consequences. *Journal of Supportive Care in Cancer, 2020*. https://doi.org/10.1007/s00520-020-05309-z.

Muls, A., Taylor, A., Murrells, T., Norton, C., & Hart, A. (2021). Evaluating faecal calprotectin as a potential marker of long-term GI toxicity in women treated for gynaecological cancer with radiotherapy. *Journal of Cancer Science and Treatment,* 3(1): 201-209.

Musso, G., Gambino, R., & Cassader, M. (2010). Obesity, diabetes, and intestinal microbiome: the hygiene hypothesis expanded? *Diabetes Care, 33*(10), 2277-2284.

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., … & Yadav, H. (2018). Intestinal microbiome and aging: physiological and mechanistic insights. *Nutrition and Healthy Aging, 4*, 267-285.

Nam, Y., Kim, H., Seo, G., Kang, S., & Bae, J. (2013). Impact of pelvic radiotherapy on intestinal microbiome of gynecological cancer patients revealed by massive pyrosequencing. *PLoS One, 8*, e82659.

Naska, A., Lagiou, A., & Lagiou, P. (2017). Dietary assessment methods in epidemiological research: current state of the art and future prospects. *F1000Research 2017, 6*(F1000 Faculty Rev), 926.

National Cancer Institute. (2020). Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE™). Retrieved May, 14, 2020 from https://healthcaredelivery.cancer.gov/pro-ctcae/

National Cancer Intelligence Network. (2013). *Outline of Uterine Cancer in the United Kingdom: Incidence, Mortality and Survival.* Retrieved July, 20, 2017 from www.ncin.org.uk/view?rid=2398

National Cancer Survivorship Initiative. (2013). *Living with and beyond cancer: taking action to improve outcomes.* National Cancer Survivorship Initiative, London. Retrieved July, 20, 2017 from https://www.gov.uk/government/publications/living-with-and-beyond-cancer-taking-action-to-improve-outcomes

National Health Service England. (2016). *Implementing the Cancer Taskforce Recommendations: Commissioning person centred care for people affected by cancer.* NHS, England. Retrieved July, 20, 2017 from https://www.england.nhs.uk/wp-content/uploads/2016/04/cancer-guid-v1.pdf

National Institute for Health and Care Excellence. (2013). *Diagnostics guidance [DG11]: Faecal calprotectin diagnostic tests for inflammatory diseases of the bowel.* Retrieved July, 22, 2018 from www.nice.org.uk/guidance/dg11

National Institute for Health and Care Excellence. (2019). *NICE Guidance [NG130]: Ulcerative colitis: management.* Retrieved 25 February 2021 from https://www.nice.org.uk/guidance/ng130/chapter/Recommendations

Negoi, I., Beuran, M., Paun, S., Moldoveanu, A., Negoim R., Bocicor, J., … & Hostiuc, M. (2016). Antibiotic prophylaxis in gynecological surgery. A literature review. *Gineco.eu, 12*(45), 142-145.

Nicholson, J., Holmes, E., Kinross, J., Darzi, A., Takats, Z., & Lindon, J. (2012). Metabolic phenotyping in clinical and surgical environments. *Nature, 491*, 384-392.

Nieuw Amerongen, A., & Veeman, E. (2008). Saliva – the defender of the oral cavity. *Oral Dis, 8*, 12–22.

Norenhag, J., Du, J., Olovsson, M., Verstraelen, H., Ensgstrand, L., & Brusselaers, N. (2020). The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis. *BJOG, 127*, 171–180.

Nutritics©. (2019). *About Nutritics*©. Retrieved November, 05, 2019 from https://en-gb.nutritics.com/p/home.

Oh, H., Kim, B., Seo, S., Kong, J., Lee, J-K., Park, S-Y., … & Kim, M. (2015). The association of uterine cervical microbiota with an increased risk for cervical intraepithelial neoplasia in Korea. *Clin Microbiol Infect, 21*, 674.e1-674.e9.

Oken, M., Creech, R., Tormey, D., Horton, D., Davis, E., McFadden, E., & Carbone, P. (1982). Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol., 5*, 649-655.

Olopade, F., Norman, A., Blake, P., Dearnaley, D., Harrington, K., Khoo, V., … & Andreyev, H.J.N. (2005). The inflammatory bowel disease questionnaire and the

Vaizey incontinence questionnaire are useful to identify gastrointestinal toxicity after pelvic radiotherapy. *British Journal of Cancer, 92*(9), 1663-1670.

Pal, D., Banerjee, S., & Ghosh, A. (2012). Dietary-induced cancer prevention: An expanding research arena of emerging diet related to healthcare system. *J Adv Pharm Technol Res., 3*(1), 16–24.

Pallis, A., Mouzas, I., & Vlachonikolis, I. (2004). The Inflammatory Bowel Disease Questionnaire: a review of its national validation studies. *Inflamm Bowel Dis., 10*(3), 261-269.

Papakonstantinou, E., Kechribari, I., Sotirakoglou, K., Tarantilis, P., Gourdomichali, T., Michas, G., … & Zampelas, A. (2016). Acute effects of coffee consumption on self-reported gastrointestinal symptoms, blood pressure and stress indices in healthy individuals. *Nutrition Journal, 15*, 26-37.

Parkes, G., Whelan, K., & Lindsay, J. (2014). Smoking in inflammatory bowel disease: Impact on disease course and insights into the aetiology of its effect. *Journal of Crohn's and Colitis, 8*, 717-725.

Patel, M., Fowler, D., Sizer, J., & Walton, C. (2019). Faecal volatile biomarkers of Clostridium difficile infection. *PLOS One, 14*(4), e0215256.

Pavlidis, P., Chedgy, F., & Tibble, J. (2013). Diagnostic accuracy and clinical application of faecal calprotectin in adult patients presenting with gastrointestinal symptoms in primary care. *Scandinavian Journal of Gastroenterology, 48*(9), 1048-1054.

Pedersen, L., & Belstrøm, D. (2019). The role of natural salivary defences in maintaining a healthy oral microbiota. *J Dent., 80*(Suppl 1), S3–S12.

Persaud, K., & Dodd, G. (1982). Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose. *Nature, 299*, 352-355.

Petersen, L., Bautista, E., Nguyen, H., Hanson, B., Chen, L., Lek, S., … & Weinstock, G. (2017). Community characteristics of the intestinal microbiomes of competitive cyclists. *Microbiome, 5*(1), 98-111.

Piyathilake, C., Ollberding, N., Kumar, R., Macaluso, M., Alvarez, R., & Morrow, C. (2016). Cervical Microbiota Associated with Higher Grade Cervical Intraepithelial Neoplasia in Women Infected with High-Risk Human Papillomaviruses. *Cancer Prev Res (Phila), 9*(5), 357-366.

Pollard, J., & Gatti, R. (2009). Clinical radiation sensitivity with DNA repair disorders: an overview. *Int J Radiat Oncol Biol Phys., 74*(5), 1323-1331.

Pollak, M. (2017). The effects of metformin on gut microbiota and the immune system as research frontiers. *Diabetologia, 60*, 1662-1667.

Probert, C., Ahmed, I., Khalid, T., Johnson, E., Smith, S., & Ratcliffe, N. (2009). Volatile organic compounds as diagnostic biomarkers in gastrointestinal and liver diseases. *J Gastrointestin Liver Dis., 18*(3), 337-343.

Public Health England and Food Standards Agency. (2014). *National Diet and Nutrition Survey Results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009 – 2011/2012).* Retrieved August, 7, 2019 from

https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012.

Quigley, E. (2017). Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet? *Nature Reviews. Gastroenterology & Hepatology, 14*(5), 315-320.

Rashid, L., & Velanovich, V. (2012). Symptomatic change and gastrointestinal quality of life after pancreatectomy. *HPB, 14*, 9–13.

Ravel, J., Gajera, P., Abdob, Z., Schneiderc, G., Koeniga, S., McCulle, S., … & Forney, L. (2011). Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA, 108*(Suppl 1), 4680-4687.

Reuther, S., Szymczak, S., Raabe, A., Borgmann, K., Ziegler, A., Petersen, C., … & Hoeller, U. (2015). Association between SNPs in defined functional pathways and risk of early or late toxicity as well as individual radiosensitivity. *Strahlenther Onkol, 191*(1), 59-66.

Revicki, D., Wood, M., Wiklund, I., & Crawley, J. (1998). Reliability and validity of the Gastrointestinal Symptom Rating Scale in patients with gastroesophageal reflux disease. *Qual Life Res, 7*, 75-83.

Reyman, M., van Houten, M., Arp, K., Sanders, E., & Bogaert, D. (2019). Rectal swabs are a reliable proxy for faecal samples in infant gut microbiota research based on 16S-rRNA sequencing. *Scientific Reports, 9*, 16072-16080.

Ribas, Y., Bonet, M., Torres, L., Núñez, M., Jovell-Fernández, E., Aranda, E., & Andreyev, H.J.N. (2020). Bowel dysfunction in survivors of gynaecologic malignancies. [published online ahead of print, 2020 Mar 14]. *Support Care Cancer*. 2020;10.1007/s00520-020-05402-3.

Rivas Caldas, R., Le Gall, F., Revert, K., Rault, G., Virmaux, M., Gouriou, S., … & Boisramé, S. (2015). Pseudomonas aeruginosa and periodontal pathogens in the oral cavity and lungs of cystic fibrosis patients: a case-control study. *J Clin Microbiol, 53*, 1898-1907.

Rose, P., Ali, S., Watkins, E., Tate Thigpen, J., Deppe, G., Clarke-Pearson, D., & Insalaco, S. (2007). Long-term follow-up of a randomized trial comparing concurrent single agent cisplatin, cisplatin-based combination chemotherapy, or hydroxyurea during pelvic irradiation for locally advanced cervical cancer: a Gynecologic Oncology Group Study. *J Clin Oncol, 25*, 2804-2810.

Roszak, A., Wareńczak-Florczak, Z., Bratos, K., & Milecki, P. (2012). Incidence of radiation toxicity in cervical cancer and endometrial cancer patients treated with radiotherapy alone versus adjuvant radiotherapy. *Rep Pract Oncol Radiother., 17*(6), 332–338.

Rouvroye, M., Wicaksono, A., Bosch, S., Savelkoul, E., Covington, J., Beaumont, H., … & de Boer, N. (2019). Faecal Scent as a Novel Non-Invasive Biomarker to Discriminate between Coeliac Disease and Refractory Coeliac Disease: A Proof of Principle Study. *Biosensors, 9*(2), article number 69.

Roy, S., & Trinchieri, G. (2017). Microbiota: a key orchestrator of cancer therapy. *Nature Reviews, 17*, 271-285.

Royal College of Obstetricians and Gynaecologists. (2012). Endometrial cancer in obese women. Scientific Impact Paper No 32. Retrieved April 04 2018 from https://www.rcog.org.uk/globalassets/documents/guidelines/scientific-impact-papers/sip_32.pdf

Sagar, N., Cree, I., Covington, J., & Arasaradnam, R. (2015). The Interplay of the Gut Microbiome, Bile Acids, and Volatile Organic Compounds. *Gastroenterology Research and Practice, 2015*, article number 39858.

Salazar, N., Valdés-Varela, L., González, S., Gueimonde, M., & de los Reyes-Gavilán, C. (2017). Nutrition and the intestinal microbiome in the elderly. *Gut Microbes, 8*(2), 82-97.

Santoro, A., Ostan, R., Candela, M., Biagi, E., Brigidi, P., … & Franceschi., C. (2018). Gut microbiota changes in the extreme decades of human life: a focus on centenarians. *Cell. Mol. Life Sci., 75*, 129-148.

Saso, S., Chatterjee, J., Georgiou, E., Ditri, A., Smith, R., & Ghaem-Maghami, S. (2011). Clinical Review: Endometrial cancer. *BMJ, 342*, d3954.

Savin, Z., Kivity, S., Yonath, H., & Yehuda, S. (2018). Smoking and the intestinal microbiome. *Archives of Microbiology, 200*, 677-684.

Schmidt, K., & Podmore, I. (2015). Current Challenges in Volatile Organic Compounds Analysis as Potential Biomarkers of Cancer. *Journal of Biomarkers, 2015*, article number 981458.

Schmidt-Nielsen K. (1998). *The camel's nose.* (1st ed.). Washington D.C.: Island Press/ Shearwater Books.

Schwabe, R., & Jobin, C. (2013). The microbiome and cancer. *Nat Rev Cancer, 13*(11), 800-812.

Sekirov, I., Russell, S., Antunes, C., & Finlay, B. (2010). Gut microbiota in health and disease. *Physiol Rev, 90*, 859-904.

Seltman, H. (2012). Chapter 15: Mixed Models. In Seltman, H. (Ed). *Experimental Design and Analysis*, (pp. 357-378). Pennsylvania: Carnegie Mellon University. Retrieved February, 13, 2020 from http://www.stat.cmu.edu/~hseltman/309/Book/Book.pdf.

Senghor, B., Sokhna, C., Ruimy, R., & Lagier, J. (2018). Gut microbiota diversity according to dietary habits and geographical provenance. *Human Microbiome Journal, 7*, 1-9.

Shi, Y., Cai, S., Tian, Y., Zhao, H., Zhang, Y., Chen, J., … & Yang Y. (2019). Effects of Proton Pump Inhibitors on the Gastrointestinal Microbiota in Gastroesophageal Reflux Disease. *Genomics Proteomics Bioinformatics, 17*, 52–63.

Shim, J., Oh, K., & Kim, H. (2014). Dietary assessment methods in epidemiologic studies. *Epidemiol Health, 36*, e2014009.

Shirasu, M., & Touhara, K. (2011). The scent of disease: volatile organic compounds of the human body related to disease and disorder. *Biochem, 150*(3), 257-266.

Shkoporov, A., Clooney, A., Sutton, T., Ryan, F., Daly, K., Nolan, J., … & Hill, C. (2019). The Human Gut Virome Is Highly Diverse, Stable, and Individual Specific. *Cell Host & Microbe, 26*(4), 527-541.

Shukla, S., Budden, K., Neal, R., & Hansbro, P. (2017). Microbiome effects on immunity, health and disease in the lung. *Clin Transl Immunology, 6*(3), e133.

Sims, T., Colbert, L., Zheng, J., Delgado Medrano, A., Hoffman, K., Ramondetta, L., … & Klopp, A. (2019). Gut microbial diversity and genus-level differences identified in cervical cancer patients versus healthy controls. *Gynecol Oncol., 155*(2), 237-244.

Singh, R., Chang, W., Yan, D., Lee, K., Ucmak, D., Wong, K., … & Liao, W. (2017). Influence of diet on the intestinal microbiome and implications for human health. *J Transl Med, 15*(1), article number 73.

Smith, L., Lee, J., Mun, J., Pakpahan, R., Imm, K., Izadi, S., … & Yang, L. (2019). Levels and patterns of self-reported and objectively-measured free-living physical activity among prostate cancer survivors: A prospective cohort study. *Cancer, 125*(5), 798-806.

Song, S., Lauber, C., Costello, E., Lozupone, C., Humphrey, G., Berg-Lyons, D., … & Knight, R. (2013). Cohabiting family members share microbiota with one another and with their dogs. *eLife, 2*, e00458.

Sprung, C., Forrester, H., Siva, S., & Martin, O. (2015). Immunological markers that predict radiation toxicity. *Cancer Letters, 368*, 191-197.

Stacey, R., & Green, J. (2014). Radiation-induced small bowel disease: latest developments and clinical guidance. *Ther Adv Chronic Dis, 5*(1), 15-29.

Steinbeck, G., Sjöberg, F., Skokic, V., Bull, C., Wilderäng, U., Alevronta, E., … & Jörnsten, R. (2017). Late radiation-induced bowel syndromes, tobacco smoking, age at treatment and time since treatment – gynecological cancer survivors. *Acta Oncologica, 56*(5), 682-691.

Stewart, C., Auchtung, T., Ajami, N., Velasquez, K., Smith, D., De La Garza, R., … & Petrosino, J. (2018). Effects of tobacco smoke and electronic cigarette vapor exposure on the oral and gut microbiota in humans: a pilot study. *PeerJ, 6*, e4693.

Stinson, L., Payne, M., & Keelan, J. (2018). A Critical Review of the Bacterial Baptism Hypothesis and the Impact of Cesarean Delivery on the Infant Microbiome. *Front. Med., 5*, article number 135.

Svedlund, J., Sjödin, I., & Dotevall, G. (1988). GSRS – a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syn- drome and peptic ulcer disease. *Dig Dis Sci, 33*,129–134.

Sznurkowski, J. (2016). Vulvar cancer: initial management and systematic review of literature on currently applied treatment approaches. *European Journal of Cancer Care, 25*, 638–646.

Takagi, T., Naito, Y., Inoue, R., Kashiwagi, S., Uchiyama, K., Mizushima, K., … & Itoh, Y. (2018). The influence of long-term use of proton pump inhibitors on the gut microniota: an age-sex matched case control study. *J Clin Biochem Nutr., 62*(1), 100-105.

Talwar, P., Chakrabarti, A., Patel, F., Ghoshal, S., Chander, J., & Gupta, B. (1992). Yeasts at different mucosal sites in patients with carcinoma cervix before and following radiotherapy. *J Hyg Epidemiol Microbiol Immunol., 36*(3), 311-316.

Tanaka, M., & Nakayama, J. (2017). Development of the gut microbiota in infancy and its impact on health in later life. *Allergology International., 66*, 515-522.

Taylor, A., Fernandes, A., Kumar, P., & Muls, A. (2016). Chapter 178: Acute and chronic toxicity of pelvic radiotherapy. In Ayhan, A., Reed, N., Gultekin, M., & Dursun, P. (Eds.) Textbook of Gynaecologocal Oncology. (3rd ed, pp.1425-1436). European Society of Gynaecological Oncology, Güneş Publishing, Ankarra. Retrieved August, 22, 2018 from http://ebooks.esgo.org/textbook3.2/mobile/index.html#p=2

Taylor, B., Darville, T., & Haggerty, C. (2013). Does bacterial vaginosis cause pelvic inflammatory disease? *Sex Transm Dis, 40*, 117-122.

Temkin, S., Minasian, L., & Noone, A. (2016). The End of the Hysterectomy Epidemic and Endometrial Cancer Incidence: What Are the Unintended Consequences of Declining Hysterectomy Rates? *Front Oncol., 6*, article number 89.

Teo, M., Sebag-Montefiore, D., & Donnellan, C. (2015). Prevention and management of radiation-induced late gastrointestinal toxicity. *Clinical Oncology, 27*, 656-667.

Thomas, R., & Jobin, C. (2015). The Microbiome and Cancer: Is the 'Oncobiome' Mirage Real? *Trends in Cancer, 1*(1), 24-35.

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal, 474*, 1823-1836.

Tibble, J., Sigthorsson, G., Foster, R., Forgacs, I., & Bjarnason, I. (2002). Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterol., 123*, 450-460.

Tiele, A., Wicaksono, A., Kansara, J., Arasaradnam, R., & Covington, J. (2020). Breath analysis using e-nose technology to diagnose inflammatory bowel disease- a pilot study. *Biosensors, 9*, article number 55.

Tigchelaar, E., Bonder, M., Jankipersadsing, S., Fu, J., Wijmenga, C., & Zhernakova, A. (2016). Gut microbiota composition associated with stool consistency. *Gut, 65*(3), 540-542.

Torres, S., Clayton, J., Danzeisen, J., Ward, T., Huang, H., Knights, D., & Johnson, T. (2017). Diverse bacterial communities exist on canine skin and are impacted by cohabitation and time. *PeerJ, 5*, e3075.

Touchefeu, Y., Montassier, E., Nieman, K., Gastinne, T., Potel, G., Bruley des Varannes, S., … & de La Cochetiere, M. (2014). Systematic review: the role of the

gut microbiome in chemotherapy- or radiation-induced gastrointestinal mucositis - current evidence and potential clinical applications. *Alimentary Pharmacology & Therapeutics, 40*(5), 409-421.

Trick, W., Sokalski, S., Johnson, S., Bunnell, K., Levato, J., Ray, M., & Weinstein, R. (2018). Effectiveness of Probiotic for Primary Prevention of Clostridium difficile Infection: A Single-Center Before-and-After Quality Improvement Intervention at a Tertiary-Care Medical Center. *Infect Control Hosp Epidemiol., 39*(7), 765-770.

Trotti, A., Colevas, A., Setser, A., Rusch, V., Jaques, D., Budach, V., … & Rubin, P. (2003). CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol, 13*, 176-181.

Tsementzi, D., Pena-Gonzalez, A., Bai, J., Hu, Y., Patel, P., Shelton, J., … & Bruner, D. (2020). Comparison of vaginal microbiota in gynecologic cancer patients pre- and post-radiation therapy and healthy women. *Cancer Medicine, 9*, 3714-3724.

Vaizey, C., Carapeti, C., Cahill, J., & Kamm, M. (1999). Prospective comparison of faecal incontinence grading systems. *Gut, 44*, 77–80.

Valdes, A., Walter, J., Segal, E., & Spector, T. (2018). Role of the gut microbiota in nutrition and health. *BMJ, 361*, j2179.

Van de Wijgert, J., Borgdorff, H., Verhelst, R., Crucitti, T., Francis, S., Verstraelen, H., … & Jerpsers, V. (2014). The Vaginal Microbiota: What Have We Learned after a Decade of Molecular Characterization? *PLoS One, 9*(8), e105998.

Van den Heuvel-Janssen, H., Borghouts, J., Muris, J., Koes, B., Bouter, L., & Knottnerus, J. (2006). Chronic non-specific abdominal complaints in general practice: a prospective study on management, patient health status and course of complaints. *BMC FamPract, 7*, 1-8.

Van Eyk, N., & van Schalkwyk, J. (2012). Antibiotic prophylaxis in gynaecologic procedures. *J Obstet Gynaecol Can, 34*(4), 382-391.

Van Rheenen, P., Van de Vijver, A., & Fidler, V. (2010). Faecal calprotectin for screening for patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ, 341*, c3369.

Vandeputte, D., Falony, G., Vieira-Silva, S., Tito, R., Joosens, M., & Raes, J. (2016). Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut, 65*, 57-62.

Varela, E., Antolin, M., Guarner, F., Verges, R., Giralt, J., & Malagelada, J. (2009). Faecal DNA and calprotectin as biomarkers of acute intestinal toxicity in patients undergoing pelvic radiotherapy. *Aliment Pharmacol Ther, 30*, 175-185.

Vautz, W., Seifert, L., Liedtke, S., & Hein, D. (2014). GC/IMS and GC/MS analysis of pre-concentrated medical and biological samples. GC/IMS and GC/MS analysis of pre-concentrated medical and biological samples. *Int. J. Ion Mobil. Spec., 17*, 25-33.

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., Vandenbroucke, J. P., & STROBE Initiative (2008). The Strengthening the Reporting of

Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Journal of clinical epidemiology, 61*(4), 344-349.

von Roon, A., Karamountzos, L., Purkayastha, S., Reese, G., Darzi, A., Teare, J., … & Tekkis, P. (2007). Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol, 102*, 803-813

Wade, G. (2013). The oral microbiome in health and disease. *Pharmacol Res., 69*(1), 137-143.

Walsham, N., & Sherwood, R. (2016). Fecal calprotectin in inflammatory bowel disease. *Clin Exp Gastroenterol., 9*, 21-29.

Walters, S., Bonacho dos Anjos Henriques-Cadby, I., Bortolami, O., Flight, L., Hind, D., Jacques, R., … & Julious, S. (2017). Recruitment and retention of participants in randomised controlled trials: a review of trials funded and published by the United Kingdom Health Technology Assessment Programme. *BMJ Open, 7*, e015276.

Wang, A., Ling, Z., Yang, Z., Kiela, P., Wang, T., Wang, C., … & Wang, J. (2015). Gut Microbial Dysbiosis May Predict Diarrhea and Fatigue in Patients Undergoing Pelvic Cancer Radiotherapy: A Pilot Study. *PLoS One, 10*(5), e0126312.

Wang, Z., Wang, Q., Wang, X., Zhu, L., Chen, J., Zhang, B., … & Yuan, Z. (2019). Gut microbial dysbiosis is associated with development and progression of radiation enteritis during pelvic radiotherapy. *J Cell Mol Med., 23*(5), 3747–3756.

Wang, Z., Wang, X., Zhao, J., Gong, L., Zhang, Y., Wang, X., & Yuan, Z. (2019). Altered diversity and composition of the gut microbiome in patients with cervical cancer. *AMB Expr, 9*, 40-49.

Waterhouse, M., Hope, B., Krause, L., Morrison, M., Protani, M., Zakrzewski, M., & Neale, R. (2018). Vitamin D and the intestinal microbiome: a systematic review of in vivo studies. *European Journal of Nutrition, 58*, 2895–2910.

Wedlake, L., Korsa, K., Bhaja, A., Karen, T., David, A., & Andreyev, H.J.N. (2013). Letter in Response to Article: A Pilot Study Examining Circulating Interleukin-6 and Transforming Growth Factor-β Levels During Pelvic Radiation Therapy. *American Journal of Clinical Oncology, 36*(1), 102-103.

Wedlake, L., McGough, C., Hackett, C., Thomas, K., Blakes, P., Harrington, K., … & Andreyev, H.J.N. (2008). Can biological markers act as non-invasive, sensitive indicators of radiation-induced effects in the gastrointestinal mucosa? *Aliment Pharmacol Ther., 27*, 980–987.

Wedlake, L., Shaw, C., McNair, H., Lalji, A., Mohammed, K., Klopper, T., … & Andreyev, H.J.N. (2017). Randomized controlled trial of dietary fibre for the prevention of radiation-induced gastrointestinal toxicity during pelvic radiotherapy. *Am J Clin Nutr., 106*(3), 849-857.

Wedlake, L., Thomas, K., Lalji, A., Blake, P., Khoo, V., Tait, D., & Andreyev, H.J.N. (2010). Predicting late effects of pelvic radiotherapy: is there a better approach? *Int. J. Radiation Oncology Biol. Phys., 78*(4), 1163-1170.

Wei, D., Heus, P., van de Wetering, F., van Tienhoven, G., Verleye, L., & Scholten, R. (2018). Probiotics for the prevention or treatment of chemotherapy- or radiotherapy-related diarrhoea in people with cancer. *Cochrane Database of Systematic Reviews, 8*, article number CD008831.

Wells, J., Brummer, R., Derrien, M., MacDonald, T., Troost, F., Cani, P., … & Garcia-Rodenas, C. (2017). Homeostasis of the gut barrier and potential biomarkers. *Am J Physiol Gastrointest Liver Physiol, 312*, G171– G193.

Wéra, O., Lancellotti, P., & Oury, C. (2016). The Dual Role of Neutrophils in Inflammatory Bowel Diseases. *Journal of Clinical Medicine, 5*, 118-132.

Westbury, C., Haviland, J., Davies, S., Gothard, L., Abdi, B., Sydenham, M., … & Yarnold, J. (2014). Cytokine levels as biomarkers of radiation fibrosis in patients treated with breast radiotherapy. *Radiat Oncol.*, 30(9), article number 103.

White, K., Henson, C., Hann, M., Eden, M., Burden, S., Lal, S., & McLaughlin, J. (2020). Randomised clinical trial of a gastrointestinal care bundle to reduce symptoms in patients with pelvic cancer undergoing chemoradiotherapy. *BMJ Open Gastroenterology, 7*, e000432.

Willis, J., & Gabaldón, T. (2020). The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems. *Microorganisms, 8*, 308- 336.

Willmann, M., Vehreschild, M., Biehl, L., Vogel, W., Dörfel, D., Hamprecht, A., … & Peter, S. (2019). Distinct impact of antibiotics on the gut microbiome and resistome: a longitudinal multicenter cohort study. *BMC Biol, 17*, article number 76.

Wilson, A. (2018). Applications of Electronic-Nose Technologies for Noninvasive Early Detection of Plant, Animal and Human Diseases. *Chemosensors., 6*(4), article number 45.

World Health organization (WHO). (2016). *International Classification of Diseases (version 10).* Retrieved July-August, 2019 from https://www.who.int/classifications/icd/icdonlineversions/en/

Wu, H., Esteve, E., Tremaroli, V., Khan, M., Caesar, R., Mannerås-Holm, L., … & Bäckhed, F. (2017). Metformin alters the intestinal microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effect of the drug. *Nature Medicine, 23*(7), 850-858.

Wynn, T., & Ramalingam, T. (2012). Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med, 18*(7), 1028-1040.

Yarnold, J., & Vozenin Brotons, M. (2010). Pathogenetic mechanisms in radiation fibrosis. *Radiotherapy and Oncology, 97*, 149-161.

Yeung, A., Pugh, S., Klopp, A., Gil, K., Wenzel, L., Westin, S., … & Kachnic, L. (2020). Improvement in patient-reported outcomes with intensity-modulated radiotherapy (RT) compared with standard RT: a report from the NRG Oncology RTOG 1203 study. *J Clin Oncol., 38*(15), 1685-1692.

Yiu, J., Dorweiler, B., & Woo, C. (2017). Interaction between gut microbiota and toll-like receptor: from immunity to metabolism. *J Mol Med, 95*, 13-20.

Yoshizawa, J., Schafer, C., Schafer, J., Farrell, J., Paster, B., & Wong, D. (2013). Salivary biomarkers: toward future clinical and diagnostic utilities. *Clinical microbiology reviews, 26*(4), 781–791.

Young, V. (2017). The role of the microbiome in human health and disease: an introduction for clinicians. *BMJ, 356*, j831.

Yu, G., Gail, M. H., Shi, J., Klepac-Ceraj, V., Paster, B., Dye, B., … & Abnet, C. (2014). Association between upper digestive tract microbiota and cancer-predisposing states in the esophagus and stomach. *Cancer Epidemiol Biomarkers Prev., 23*(5), 735-741.

Zarco, M., Vess, T., & Ginsburg, G. (2012). The oral microbiome in health and disease and the potential impact on personalized dental medicine. *Oral Diseases, 18*, 109-120.

Zhang, L., Liu, Y., Zheng, H., & Zhang, C. (2020). The Oral Microbiota May Have Influence on Oral Cancer. *Front. Cell. Infect. Microbiol., 9*, article number 476.

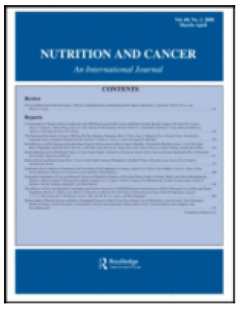
Zhernakova, A., Kurilshikov, A., Bonder, M., Tigchelaar, E., Schirmer, M., Vatanen, T., … & Fu, J. (2016). Population-based metagenomics analysis reveals markers for intestinal microbiome composition and diversity. *Science*, *352*(6285); 565-569.

Zwielehner, J., Lassl, C., Hippe, B., Pointner, A., Switzeny, O., Remely, M., … & Halsberger, A. (2011). Changes in Human Fecal Microbiota Due to Chemotherapy Analyzed by TaqMan-PCR, 454 Sequencing and PCR-DGGE Fingerprinting. *PLoS One, 6*(12), e28654.

Appendices

Appendix 1. Systematic review: dietary habits of women treated for gynaecological cancer before, during and after treatment.

Nutrition and Cancer: An International Journal



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A systematic review: dietary habits of women with gynecological cancer before, during and after treatment.

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Abstract

Introduction:

Cancer treatment can affect eating habits and some patients treated with pelvic radiotherapy develop chronic changes in their bowel function which may impact on dietary intake. This systematic review summarizes current research on dietary habits before, during and after treatment in women with a gynecological malignancy.

Methods:

MEDLINE, EMBASE, CINAHL and PubMed databases were searched until August 1, 2019, with no language restrictions. PRISMA guidelines for systematic reviews were used to ensure transparent and complete reporting (Liberati et al., 2009). Quantitative studies exploring dietary habits before, during or after treatment for gynecological cancer were included.

Results:

Three prospective cohort studies (98 participants) were included. All patients were treated with radiotherapy; some also had surgery and chemotherapy. Studies reported similar changes in weight, BMI and energy intake and found that patients lose weight during treatment, but this was not long-lasting. Long-term results suggest that fat and protein intake increase significantly ($p < 0.01$). There was no correlation of symptoms with any nutritional parameters.

Conclusion:

Evidence from three studies suggests further studies including long-term assessment of dietary intake in women treated for a gynecological malignancy are warranted to explore the impact of cancer treatments on dietary intake and its relation to developing long-term gastrointestinal toxicity.

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Introduction

There is increasing evidence that undergoing cancer treatment and living with cancer can affect the eating habits of patients (1-3).

Obesity is a significant risk factor for adenocarcinoma of the endometrium, especially in post-menopausal women (4). Prevalence figures for women in the general population in the United Kingdom show that 60% is overweight with a body mass index (BMI) between 25 and 29.9 kg/m^2 (31%), or obese with a BMI of > 30 kg/m^2 (29%). Moreover, 37% of women aged 45 to 54 years are overweight or obese (5). A rise in BMI of 4.78 points increases the risk of being diagnosed with or dying from endometrial cancer (odds ratio of 1.41) (6). In addition, excessive weight has an impact on cancer recurrence and second cancer development (7). There is not enough evidence to give specific dietary advice for cancer survivors but there is some evidence that reduction of dietary fat after breast cancer treatment could increase recurrence-free survival (8).

Gynecological cancers include cancer of the endometrium (or uterus), cervix, ovary, vagina and vulva. Worldwide, the incidence of gynecological cancer was more than 1.3 million in 2018 (9). In the UK in 2016, there were 21,000 new cases of gynecological malignancy (10). Many women treated for gynecological cancer with radiotherapy develop long-term changes in gastrointestinal function which can impact on what they eat; for example, some cut out specific food groups such as gluten or reduce the amount of fiber to reduce symptoms like bloating or diarrhea (11-14). Treatment for gynecological cancer can include surgery, chemotherapy and radiotherapy and all can impact on bowel function acutely or long-term. The risk factors for developing radiation toxicity are both treatment and patient dependent. Treatment related risk factors include total cumulative dose, dose per fraction, treated volume, treatment modality (external beam or brachytherapy) and concomitant drugs, particularly chemotherapy. Patient related factors include underlying co-morbidities (e.g. inflammatory bowel disease or connective tissue disorders), low body mass index, smoking, genomic make-up and age. Prior surgery increases the risk of bowel toxicity due to adhesions causing immobile bowel loops,

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while alteration of the vascular supply can induce hypoxia that then increases radio-resistance (15-16).

Surgery for gynecological cancer may be local, or extensive, resulting in pelvic exenteration and the formation of a stoma. This has a direct impact on bowel function. Chemotherapy also may alter gastrointestinal function. Research has mainly been focused on acute gastrointestinal toxicity as diarrhea and vomiting are the main treatment and dose limiting factors (16).

Although the incidence of severe complications has been reduced since the introduction of modern radiotherapy techniques, even low-moderate grade gastrointestinal toxicity such as increased bowel function, excessive wind, variable stool consistency, having to rush to the toilet or fecal leakage can significantly impact on quality of life (1, 11-12, 17).

Interventional studies have explored dietary changes such as fiber, fat or lactose modification during treatment to prevent or reduce long-term gastrointestinal toxicity following cancer treatment (12, 18). Studies have also examined the role of the gut microbiome in the development of both acute and long-term gastrointestinal toxicity and how changes in diet or the use of probiotics could improve symptom burden (19-22).

As half of people diagnosed with cancer in England and Wales survive their disease for ten years or more (10), the importance of managing negative gastrointestinal consequences increases.

Several studies have explored the dietary habits of people suffering from various types of cancer including head and neck, breast, testicular or prostate cancer. Due to the nature of the disease process and local impact of treatment in people treated for head and neck cancer, studies in this cohort have shown severe weight loss and the need for on-going nutritional support (23-25). Avery et al. (26) and Fassier et al. (27) found that men treated for prostate cancer maintained a stable daily caloric intake one year after treatment with radiotherapy compared to baseline. Studies including women treated for breast cancer showed a reduction in caloric energy intake of 90 to 100 kcal 6 months after treatment ($p = 0.01$) (2, 27) and 140 kcal

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two years after treatment ($p < 0.01$) (28). However, weight increased in both studies (2, 28) and BMI increased (2).

This systematic review aims to summarize the findings of studies where the dietary intake of women with a gynecological malignancy was evaluated before, during or after treatment in order to assess whether there are long-term changes in dietary intake.

Methods

This systematic review used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparent and complete reporting (http://links.lww.com/IGC/A485) (29). The Booth et al. (30) and Gough et al. (31) guidelines for systematic reviews were followed to ensure a systematic approach and rigor.

Data sources

Relevant studies were identified by two authors (CCS and AM) searching the following databases: MEDLINE, EMBASE 1974 to 2019 August 1, Journals@Ovid Full Text August 1, 2019, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to 2019 August 1, Ovid Medline database, CINAHL (Cumulative Index to Nursing and Allied Health Literature) and PubMed until August 01. Searching the PROSPERO database and Cochrane Library did not identify any existing similar systematic reviews. Additional resources on the PubMed database, such as "find similar search" and reference lists, were used to identify relevant studies. Specific exclusion criteria for the studies found through all these searches are described in Fig 1.

The main search terms included gyn(a)ecological cancer "AND" "OR" gyn(a)ecological malignancy "AND" "OR" cervical cancer "AND" "OR" endometrial cancer "AND" "OR" uterine cancer "AND" "OR" diet "AND" "OR" dietary intake "AND" "OR" dietary habit(s) "AND" "OR" food diaries "AND" "OR" nutrition "AND"

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"OR" nutritional intake "AND" "OR" radiotherapy "AND" "OR" pelvic radiotherapy "AND" "OR" chemoradiation "AND" "OR" treatment.

To ensure that the search was comprehensive, and studies had not been missed or wrongly excluded, general search engines (Google, Yahoo), and reference lists of included papers were checked.

Study eligibility

The inclusion criteria for studies were determined by following the components in the PEO(S) framework: the patient population (P) – women with gynecological cancer (womb/uterine/endometrial, ovarian, vaginal, vulval or cervical) before, during or after treatment; the exposure (E) – treatment for gynecological cancer (surgery, radiotherapy or chemoradiotherapy); the outcome of the study (O) – changes in the patients' dietary habits before, during or after treatment for gynecological cancer; and the study design (S) – prospective, observational, cohort studies using any quantitative food record data collection tools (30).

Studies that included adult participants with gynecological cancer, but did not report data for this group separately were excluded, as were studies that did not use any kind of dietary record to collect data and interventional studies altering dietary intake. In addition, studies that included patients who had undergone treatment for gynecological cancer in the past and were cancer survivors were excluded as there were no baseline data on dietary intake before and during treatment. No publication year restrictions were applied.

Study selection and quality assessment

The relevance of studies was assessed based on title and abstract. Two reviewers (CCS and AM) independently appraised the quality of the papers included using the Critical Appraisal Skills Programme (CASP) checklist specific to cohort studies (32) and discussed any difference to achieve consensus. A quality score was given out of 12, based on the number of sections and subsections that could be answered with

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'yes, no, or can't tell' in the CASP checklist. 'Yes' answers received a score of one and 'no' and 'can't tell' answers scored zero.

Results

A total of 3182 titles were found (Fig 1). The majority (n= 2968) were excluded on title. A further 142 papers were excluded after reading the abstract. 68 papers were excluded on reading the full text.

Four papers were included from three different studies (33-36). Bruning et al. (1985) referred to some of their dietetic analysis data in a separate publication (34). As it involved the same cohort, the information was amalgamated with the original paper. Table 1 summarizes the extracted data from each of the three studies and the CASP quality score.

Study characteristics

The three included studies (33, 35-36) reported on 98 women diagnosed with gynecological cancer: cervical (n=68), endometrial (n=29) and vaginal (n=1). Two of the studies (33, 35) had a mixed cohort (endometrial, cervical and vaginal); and the other only included women with cervical cancer (36). They were all longitudinal prospective cohort studies and had a repeated measures design, reporting dietary habits of women with gynecological cancer undergoing treatment, as well as having other objectives and additional data collection tools, including blood samples, other types of food intake questionnaires or CT scans (Table 1). All studies reported data before treatment started. All patients included in the three studies had radiotherapy. As all studies were prospective cohort studies, these are primary reports within the classification category B (A: randomized controlled trials, C: non-randomized controlled/ crossover trials, case control studies, D: non-controlled trials, case reports, observational studies) (37). All studies received a CASP quality score of 7 out of a maximum of 12 and a limited or weak level of evidence (Grade III, grade I: strong evidence- grade V: grade not assignable) (37).

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Changes in weight and Body Mass Index (BMI)

Bruning et al. (33) found a non-significant decrease in weight during 4 weeks of treatment with radiotherapy but this recovered back to baseline weight or more 5 months after treatment. Pía de la Maza et al. (35) found weight also decreased slightly by the end of treatment (p= 0.03) and it also increased by a median of 4.2 kg over baseline weight two years after treatment (p= 0.03). Sanchez et al. (36) found a median weight loss of 7.4 kg by the end of treatment compared to pre-treatment (p< 0.001) but there was no long-term follow-up data to determine later weight changes.

Overall, at all time points, in all studies, the median BMI was above the World Health organization (WHO) recommendation for a healthy BMI (which ranges between 18.1 and 24.9) (39). Hulshof et al. (34) described a median increase from baseline of 0.3 points in BMI, 3 months after treatment. Mapping the weight decrease found by the end of treatment, there was a slight decrease in BMI of 0.3 points (p= 0.02) but this increased two years after treatment by a median of 1.2 points (p< 0.01) (35). Sanchez et al. (36) also noted a reduction in BMI by the end of treatment of 3.7 points (p= 0.001) but did not present long-term data.

These changes in weight and BMI across the studies seem to indicate that patients lose weight during treatment, but this may not be long-lasting.

Changes in energy intake

Daily caloric intake (Table 2) decreased by a median of 125 kcal, 470 kcal and 670 kcal respectively from baseline by the end of treatment (33, 35-36). However, this trend was not sustained as participants increased their daily calorie intake from baseline by an average of 500 kcal a day 3 months post treatment in the study by Bruning et al. (33) and this was also found by Pía de la Maza et al. (35) reporting an increase from baseline of 280 kcal a day (p= 0.01) two years after treatment.

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Changes in macronutrient intake

All three studies showed a reduction in daily intake of fat, carbohydrates, protein and fiber compared to baseline by the end of treatment with statistical significance ($p < 0.01$) stated in two studies (35-36). In one study, the reduction in daily fat intake by the end of treatment was not sustained and increased again 2 years after treatment ($p < 0.01$) (35). However, daily intake of all other macronutrients increased 2 years after treatment compared to baseline (35). This was consistent with the results reported by Hulshof et al. (34) ($p < 0.05$). Long-term, results seem to suggest that fat and protein intake increase significantly compared to baseline ($p < 0.01$) and carbohydrate intake remains stable (34-35). This is in line with the finding that kcal intake and BMI also increased over time.

Changes in micronutrient intake

Sanchez et al. (36) did not assess micronutrient intake. No comparison between the micronutrient results of the two other studies could be drawn as they measured different micronutrients. However, Bruning et al. (33) noted that a mean of 11.6 mg daily iron intake by women treated for gynecological cancer indicated their intake of iron through diet was 15% below the Dutch recommended allowance. Pía de la Maza et al. (35) found a reduction of intake of vitamin B12 through diet by the end of treatment but a statistically significant increase two years after completion of treatment ($p = 0.01$) compared to baseline.

Additional findings

Pía de la Maza et al. (35) assessed gastrointestinal toxicity but did not use any formal gastrointestinal symptom assessment tool. Nine women (60%) were reported to experience substantial gastrointestinal symptoms 2 years after radiotherapy. Symptoms described were bloating, rectal bleeding and tenesmus. The outcomes suggested a decreased gastrointestinal transit time measured by a lactose breath test and a lower intestinal permeability measured by urinary excretion of ingested lactulose, mannitol and sucrose but there was no correlation with any nutritional parameters (35).

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Discussion

No studies were identified assessing dietary intake longitudinally of women with gynecological cancer undergoing surgery or chemotherapy only. Three studies described the dietary habits of women with gynecological cancer before, during or after treatment with radiotherapy. The outcomes of the studies indicate that there are changes in weight, BMI and dietary intake across time.

Each study was completed in a different country, with different food customs, nutritional habits and healthy dietary requirements, hence, the results may not be generalizable to other countries. Each study considered as reference the dietary requirements and BMI recommended for their country.

The study by Bruning et al. (33) reported that researchers measured volumes and weights from household items that the patients would be using to report their food intake. However, there is great variability in sizes of household measure receptacles in general households, for instance there can be a variation of 8-12% in a 1-teaspoon serving (39).

Two of the included studies, carried out short follow-up periods: just after the end of treatment (36) and 5 months after completion of radiotherapy (33). Pía de la Maza (35) included follow-up data two years after completion of treatment. Studies by Bruning et al. (33) and Pía de la Maza (35) discussed confounding factors, such as hormone replacement therapy, the use of steroids, alcohol intake and changes in appetite, but did not control for these in their statistical analysis.

There was a trend of on-going weight gain with an increase of BMI in a cohort of patients who already tend to be overweight before the start of their gynecological cancer treatment (33-36). The BMI of gynecological cancer survivors remained within the overweight or obese category for more than half of the women (14, 27, 40). Table 1 showed that all participants in the three studies had been treated with radiotherapy (33-36). However, the radiotherapy dose in older study by Bruning et al. (33) was 40Gy; in the more recent studies the dose was higher (40-45Gy) with the inclusion of brachytherapy (35-36). In addition, the participants in Sanchez et al. (36) received chemotherapy. These changes in treatment delivery may explain

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why Bruning et al. (33) only detected limited changes in weight loss at the end of treatment compared to the two other studies.

Nutritional status and quality of life improved in a mixed cohort of women with gynecological cancer 6 months after treatment compared to baseline but did not link this to BMI (41). This is similar to findings from studies including women treated for breast cancer (2, 28, 42) and men treated for prostate cancer (26). Deeper insight into the psychological mechanisms relating to weight gain is an area for future research as this may impact on long-term recurrence and survival (8, 27, 43-44).

Several studies involving women treated for gynecological malignancy have been identified which aimed to reduce BMI (45-48). Other studies examined modification of fiber, fat or lactose to prevent or reduce long-term gastrointestinal toxicity following cancer treatment (12, 18) or influence the development of both acute and long-term gastrointestinal toxicity by changes in diet or the use of probiotics (19-22). However, none included data relating to dietary habits prior to treatment or how treatment may have impacted dietary intake.

Strengths and limitations of this literature review

This is the first systematic review to summarize studies on the dietary intake of women with a gynecological malignancy before, during and after cancer treatment. A rigorous approach was employed to identify, analyze and assess the papers included.

The searches carried out for this systematic literature review were not restricted by publication year, resulting in the inclusion of older studies. In recent decades, there have been advances made to radiotherapy and chemotherapy treatment regimens, which should be considered when interpreting results from older studies. No meta-analysis was performed as only three studies were identified.

The dissimilar study designs, cohorts and data collection methods of the studies made comparison impossible and so, insight into this topic remains unclear. All studies included clearly stated aims and the issues they would be investigating.

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Evidence from previous literature was used to build a solid rationale in all studies, highlighting the gaps in knowledge and the need for these studies.

Food intake data collection methods varied and all have strengths and limitations. Heterogeneity of the measures used prevented direct comparison between the studies. Bruning et al. (33) collected 2-day food diaries and the other studies used dietary recall (35-36). Both 24h or 48h dietary recall or food frequency questionnaires are less labor intensive to analyze than food diaries, but sources of error include the participants' inability to fully and accurately recall their intakes and the risk of providing subjective and socially acceptable data. Furthermore, the ability of the food composition databases applied to convert the reported food consumption to energy and nutrient intake, may limit the validity of the generated information (49-50). An alternative form of real-time recording is food diaries (with or without weighing of foods consumed) and the duplicate portion method. When using food diaries, individuals are asked to record every food or beverage they consume in real time; in food diaries with weighing, individuals are also required to record the actual quantities consumed. This method is labor intensive, both for the participant and for the researcher, costly and generates complex data (49-50). Parameters such as macro- or micronutrients in blood can provide unbiased information but are affected by metabolism and disease state and do not reflect absorption from dietary intake (49).

Due to the limited number of studies identified, the small number of participants in each study and different geographical settings, the generalizability of the results, the evidence presented by these studies are limited or weak (Grade III).

Considerations for future studies

Only three studies found eligible and analyzed in this literature review highlights the gap in the literature exploring the dietary habits of women with gynecological cancer before, during and after treatment. This gap may lead to new insights to explore personalized treatment options and prevention of the gastrointestinal consequences of cancer treatments.

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Considering the growing amount of interventional studies that were found and the increasing number of studies examining the gut microbiome and its relation to long-term gastrointestinal toxicity following cancer treatment, it is surprising that dietary habits within this cohort have not been described in more studies. In addition, a growing number of current studies try to manipulate the gut microbiome to prevent or ameliorate gastrointestinal toxicity following cancer treatment.

The main findings of this review identified changes in weight, BMI and dietary intake across time. Further longitudinal research is required in order to fully comprehend the dietary habits of this cohort and how diet could contribute to the reduction of gastrointestinal toxicity following treatment and improvement of cancer treatment outcomes and overall wellbeing.

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References

1. Abayomi J, Kirwan J and Hackett A. The prevalence of chronic radiation enteritis following radiotherapy for cervical or endometrial cancer and its impact on quality of life. *Eur J Oncol Nurs.* **13**, 262-267, 2009.

2. Dias Custódio I, Da Costa Marinho E, Araujo Gontijo C, Silva Pereira T, Paiva C et al. Impact of Chemotherapy on Diet and Nutritional Status of Women with Breast Cancer: A Prospective Study. *PLoS One* **11(6)**, e0157113, 2016.

3. de Vries Y, van den Berg M, de Vries J, Boesveldt S, de Kruif J et al. Differences in dietary intake during chemotherapy in breast cancer patients compared to women without cancer. *Support Care Cancer.* **25(8),** 2581-2591, 2017.

4. Saso S, Chatterjee J, Georgiou E, Ditri A, Smith R et al. Clinical Review: Endometrial cancer. *BMJ* **342**, d3954, 2011.

5. Health and Social Care Information Centre. NHS Digital. Statistics on Obesity, Physical Activity and Diet, England, 2019. Accessed February 2020 via https://digital.nhs.uk/data-and-information/publications/statistical/statistics-on-obesity-physical-activity-and diet/statistics-on-obesity-physical-activity-and-diet-england-2019/part-3-adult-obesity#health-risks-associated-with-obesity-overweight-and-waist-circumference

6. Gharahkhani P, Ong JS, An J, Law H, Whiteman D et al. Effect of increased body mass index on risk of diagnosis or death from cancer. *British Journal of Cancer* **120**, 565–570, 2019.

7. Demark-Wahnefried W, Platz E, Ligibel J, Blair C, Courneya K et al. The Role of Obesity in Cancer Survival and Recurrence. *Cancer Epidemiol Biomarkers Prev* **21(8)**, 1244- 1259, 2012.

8. Jochems S, Van Osch F, Bryan R, Wesselius A, van Schooten F et al. Impact of dietary patterns and the main food groups on mortality and recurrence in cancer survivors: a systematic review of current epidemiological literature. *BMJ Open* **8**, e014530, 2017.

9. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **68(6)**, 394-424, 2018.

For Peer Review Only

10. Cancer Research UK. Women's cancers (gynaecological cancer). Cancer Research UK. 2017. [Viewed February 2020]. Available from: https://www.cancerresearchuk.org/about-cancer/womens-cancer

11. McGough C, Baldwin C, Frost G and Andrejev HJN. Role of nutritional intervention in patients treated with radiotherapy for pelvic malignancy. *British Journal of Cancer* **90**, 2278–2287, 2004.

12. Henson C, Burden S, Davidson S and Lal S. Nutritional interventions for reducing gastrointestinal toxicity in adults undergoing radical pelvic radiotherapy. *Cochrane Database of Systematic Reviews* **11**, Art. No.: CD009896, 2013.

13. van de Wetering F, Verleye L, Andreyev HJN, Maher J, Vlayen J et al. Non-surgical interventions for late rectal problems (proctopathy) of radiotherapy in people who have received radiotherapy to the pelvis. *Cochrane Database of Systematic Reviews* **4**, Art. No.: CD003455, 2016.

14. Lind H, Waldenstrom AC, Dunberger G, al-Abany M, Alevronta E et al. Late symptoms in long-term gynaecological cancer survivors after radiation therapy: a population-based cohort study. *Br J Cancer* **105**, 737–745, 2011.

15. Kuku S, Fragkos C, McCormack M and Forbes A. Radiation-induced bowel injury: the impact of radiotherapy on survivorship after treatment for gynaecological cancers. *Br J Cancer* **109(6)**, 1504-1512, 2013.

16. Taylor A, Fernandes A, Kumar P and Muls A. Chapter 179. Pelvic Irradiation: Acute and Late Toxicity. 1425- 1436. *European Society of Gynaecological Oncology. Textbook of gynaecological oncology.* Third Edition. 2016.

17. Gillespie C, Goode C, Hackett C and Andreyev J. The clinical needs of patients with chronic gastrointestinal symptoms after pelvic radiotherapy. *Aliment Pharmacol Ther,* **26**, 555-563, 2007.

18. Wedlake L, Shaw C, McNair H, Lalji A, Mohammed K et al. Randomized controlled trial of dietary fibre for the prevention of radiation-induced gastrointestinal toxicity during pelvic radiotherapy. *Am J Clin Nutr.* **106(3)**, 849-857, 2017.

For Peer Review Only

19. Touchefeu Y, Montassier E, Nieman K, Gastinne T, Potel G et al. Systematic review: the role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis - current evidence and potential clinical applications. *Aliment Pharmacol Ther.* **40(5)**, 409-421, 2014.

20. Ciorba M, Hallemeier C, Stenson W and Parikh P. Probiotics to prevent gastrointestinal toxicity from cancer therapy: An interpretive review and call to action. *Curr Opin Support Palliat Care.* **9(2)**, 157–162, 2015.

21. Kumagai T, Rahman F and Smith AM. The Microbiome and Radiation Induced-Bowel Injury: Evidence for Potential Mechanistic Role in Disease Pathogenesis. *Nutrients* **10(10)**, pii: E1405, 2018.

22. Ferreira M, Andreyev HJN, Mohammed K, Truelove L, Gowan S et al. Microbiota- and Radiotherapy-Induced Gastrointestinal Side-Effects (MARS) Study: A Large Pilot Study of the Microbiome in Acute and Late-Radiation Enteropathy. *Translational Cancer Mechanisms and Therapy.* 2019. published online first.

23. Farhangfar A, Makarewicz M, Ghosh S, Jha N, Scrimger R et al. Nutrition impact symptoms in a population cohort of head and neck cancer patients: Multivariate regression analysis of symptoms on oral intake, weight loss and survival. *Oral Oncology* **50**, 877–883, 2014.

24. Arribas L, Hurtós L, Taberna M, Peiró I, Vilajosana E et al. Nutritional changes in patients with locally advanced head and neck cancer during treatment. *Oral Oncology* **71**, 67–74, 2017.

25. Nejatinamini S, Kubrak C, Álvarez-Camacho M, Baracos V Ghosh S et al. Head and neck cancer patients do not meet recommended intakes of micronutrients without consuming fortified products. *Nutrition and Cancer* **70 (3)**, 474-482. 2018.

26. Avery K, Donovan J, Gilbert R, Davis M, Emmett P et al. Men with prostate cancer make positive dietary changes following diagnosis and treatment. *Cancer causes & control* **24(6)**, 1119-1128, 2013.

27. Fassier P, Zelek L, Lécuyer L, Bachmann P, Touillaud M et al. Modifications in dietary and alcohol intakes between before and after cancer diagnosis: Results from the prospective population based NutriNet-Sante cohort. *International Journal of Cancer* **141**, 457-470, 2017.

For Peer Review Only

28. Wayne S, Lopez S, Butler L, Baumgartner K, Baumgartner R et al. Changes in dietary intake after diagnosis of breast cancer. *Journal of the American Dietetic Association* **104(10)**, 1561- 1568, 2004.

29. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche P et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol.* **6**, e1-e34, 2009.

30. Booth A, Papaioannou D and Sutton A. *Systematic Approaches to a Successful Literature Review*. London: SAGE. 2012.

31. Gough D, Oliver S and Thomas J. *An Introduction to Systematic Reviews*. London: SAGE. 2012.

32. Critical Appraisal Skills Programme (CASP). Study checklist for observational cohort studies. Critical Appraisal Skills Programme Oxford 2018. Accessed September 2019 via https://casp-uk.net/wp-content/uploads/2018/01/CASP-Cohort-Study-Checklist_2018.pdf

33. Bruning P, Egger R, Gooskens A, Hermus R, Hulshof K et al. Dietary Intake, Nutritional Status and Well-being of Cancer Patients: a Prospective Study. *European Journal of Cancer and Clinical Oncology* **21(12)**, 1449-1459, 1985.

34. Hulshof K, Gooskens A, Wedel M, Bruning P. Food intake in three groups of cancer patients. A prospective study during cancer treatment. *Hum Nutr Appl Nutr.* **41(1)**, 23-37, 1987.

35. Pía de la Maza M, Agudelo G, Yudin T, Gattas V, Barrera G et al. Long-Term Nutritional and Digestive Consequences of Pelvic Radiation. *Journal of the American College of Nutrition* **23(2)**, 102-107, 2004.

36. Sanchez M, Castro-Eguiluz D, Luvian-Morales J, Jimenez-Lima R, Aguilar-Ponce J et al. Deterioration of nutritional status of patients with locally advanced cervical cancer during treatment with concomitant chemoradiotherapy. *Journal of Human Nutrition and Dietetics* **32**, 480-491, 2019.

For Peer Review Only

37. Handu D, Moloney L, Wolfram T, Ziegler P, Acosta A et al. Academy of nutrition and dietetics methodology for conducting systematic reviews for the evidence analysis library. *J Acad Nutr Diet* **116**, 311-318, 2016.

38. World Health Organisation. Global health Observatory Data. Mean Body Mass Index (BMI). https://www.who.int/gho/ncd/risk_factors/bmi_text/en/ Accessed 26 March 2020.

39. Wansink B and van Ittersum K. Spoons systematically bias dosing of liquid medicine. *Annals of Internal Medicine* **152(1)**, 66-67, 2010.

40. Von Gruenigen V, Waggoner S, Frasure H, Cavanagh M, Janata J et al. Lifestyle challenges in endometrial cancer survivorship. *Obstet Gynecol* **117**, 93–100, 2011.

41. Ryan M, White K, Roydhouse J and Fethney J. A description of the nutritional status and quality of life of Australian gynaecological cancer patients over time. European *Journal of Oncology Nursing* **16(5)**, 453-459, 2012.

42. Ceccatto V, Faria Di Pietro P, Nogueira Previdelli A, Vieira F, Cesa Schiavon C et al. Brazilian Healthy Eating Index Revised (BHEI-R) of women before and during adjuvant treatment for breast cancer. *Nutr Hosp.* **30(5)**, 1101-1109, 2014.

43. Salani R. Survivorship planning in gynecologic cancer patients. *Gynecologic Oncology* **130**, 389–397, 2013.

44. Morris K and Haboubi N. Pelvic radiation therapy: Between delight and disaster. *World J Gastrointest Surg.* **7(11)**, 279-88, 2015.

45. Nock N, Dimitropoulos A, Rao S, Flask C, Schluchter M et al. Rationale and design of REWARD (revving-up exercise forsustained weight loss by altering neurological reward and drive): a randomized trial in obese endometrial cancer survivors. *Contemp Clin Trials* **39(2)**, 236-245, 2014.

46. Kanera I, Willems R, Bolman C, Mesters I, Verboon P et al. Long-term effects of a web-based cancer aftercare intervention on moderate physical activity and vegetable consumption among early cancer survivors: a randomized controlled trial. *The International Journal of Behavioral Nutrition and Physical Activity* **14(1)**, Article number: 19, 2017.

For Peer Review Only

47. Cohen CW, Fontaine KR, Arend RC, Alvarez RD, Leath CA III et al. A Ketogenic Diet Reduces Central Obesity andSerum Insulin in Women with Ovarian or Endometrial Cancer. *J Nutr.* **148(8)**, 1253-1260, 2018.

48. Flores-Cisneros L, Castro-Eguiluz D, Reyes-Barretero DY, Jaimes E, Cano-Blanco C et al. Effects of Dietary Components During and After Concomitant Chemoradiotherapy, Radiotherapy, or Sequential Chemoradiotherapy to the Abdominopelvic Area. *Rev Invest Clin.* **70(3)**, 126-129, 2018.

49. Shim J-S, Oh K and Chang Kim H. Dietary assessment methods in epidemiologic studies. *Epidemiology and Health* **36**, 8pp, 2014.

50. Naska A, Lagiou A and Lagiou P. Dietary assessment methods in epidemiological research: current state of the art and future prospects [version 1; referees: 3 approved] *F1000Research* **6**(F1000 Faculty Rev), 926, 2017.

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Figure 1: PRISMA flow diagram of studies identified from the literature searches

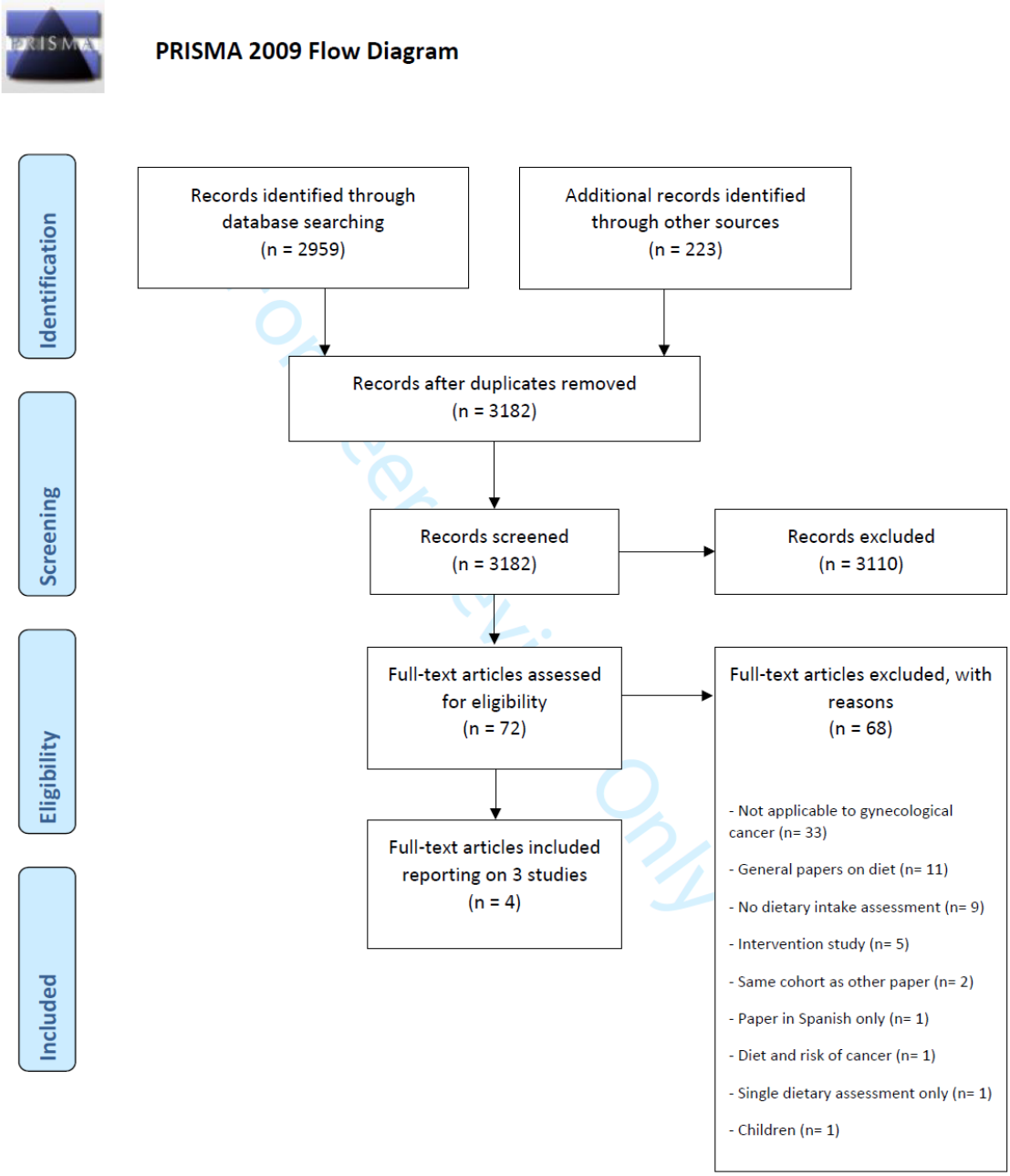

Table 1. Studies assessing dietary intake of women with a gynecological malignancy evaluated before, during and after treatment

Author and country	Study design	Number of participants Cancer diagnosis	Treatment	Main objective(s)	Data collection tool(s)	Number of data collection time points Duration of follow-up	Main limitations	CASP quality score (max score: 12)
Bruning et al. (1985) Holland **Hulshof et al. (1987)** Holland	Prospective cohort, repeated measures	28 Mixed: endometrial (n=25) cervical (n=3)	**Surgery** (n= 28, 100%) **Radiotherapy dose (Gy)** 40 Gy (n= 28, 100%) **Chemotherapy** None	To evaluate changes in dietary intake, nutritional status, body composition and well-being of cancer patients during and after radiotherapy	- Dietary history and cross-check for the baseline - 48-hour dietary records - Anthropometric measurements - Blood and urine samples - Checklist of physical and psychological complaints - Performance scales - Final interview	9 - Baseline - During treatment: 7 - End of treatment (interview) 5 months after the end of treatment	- total added number of participants (n=104) differs from total number mentioned in the text (n=108) - no mention of reason behind dropout of 24 people - short follow up time (~ 5 months after treatment) - many results are described as 'significant' but often no statistical significance is reported in the tables - no comparison with similar studies in discussion - old study: no comparison with current radiotherapy treatment possible	7/12
Key findings	Most patients met the Dutch recommended dietary allowances. Baseline diet was composed of 44% carbohydrates, 39% fat, 15% protein and 2% alcohol. Daily energy intake increased between weeks 5 and 19 with an average of 500 kcal and only 11% of participants had an energy intake lower than 2/3 of the Dutch recommended dietary allowances on two occasions during the 5 months. There was a slight decrease in weight during treatment, but this returned to were baseline values or increased by week 13 (no p-value stated) 17% of participants indicated they had paid more attention than usual to their dietary habits. Women with gynecological cancer had an iron intake 15% lower, and a retinol intake 25% lower than 2/3 of the Dutch recommended dietary allowances. There was no statistically significant change in dietary habits 5 months after treatment.							

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Author and country	Study design	Number of participants Cancer diagnosis	Treatment	Main objective(s)	Data collection tool(s)	Number of data collection time points Duration of follow-up	Main limitations	CASP quality score (max score: 12)
Pía de la Maza et al. **(2004)** Chile	Prospective cohort, repeated measures	15 Mixed: Cervical (n=10) Endometrial (n=4) Vaginal (n=1)	**Surgery** (n= 7, 47%) **Radiotherapy dose (Gy)** 45-50 Gy (n= 15, 100%) + brachytherapy 10-15 Gy (n= 14, 93%) **Chemotherapy** None	To study long-term variations in nutritional status of women who were previously treated with radiotherapy for gynecological cancer	- 24-hour dietary recall - Blood test - Anthropometric measurements - double beam X ray absorptiometry	3 - baseline - week 5 of radiotherapy - 2 years after end of treatment 2 years after treatment	- participants recruited from previous study - no description of people who declined participation in original cohort - 24h diaries recall can incur bias - no clinical demographics table	7/12
Key findings	Statistically significant increase in weight, BMI, hip and waist circumference after 2 years (p= 0.03). Energy intake increased statistically significantly 2 years after completion of treatment by an average of 280 kcal (p= 0.01). Fat, protein and vitamin B12 intake also increased significantly (p = 0.01). Micronutrient intake remained below the recommended amount. Fiber and carbohydrate intake did not change over time. Lost lean tissue during treatment did not recover after two years from the end of treatment, despite higher protein intake. Energy expenditure from physical activity reduced significantly from baseline and persisted at the 2-year time-point (p <0.01).							

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Author and country	Study design	Number of participants Cancer diagnosis	Treatment	Main objective(s)	Data collection tool(s)	Number of data collection time points Duration of follow-up	Main limitations	CASP quality score (max score: 12)
Sanchez et al. (2019) Mexico	Prospective cohort, repeated measures	55 Cervical (n=55)	**Surgery** Not stated **Radiotherapy dose (Gy)** 50 Gy (n= 55, 100%) + brachytherapy 24 Gy (n= 55, 100%) **Chemotherapy** (n= 55, 100%)	To evaluate nutritional status and change in body composition before and during treatment. - body composition changes on tumor recurrence and overall survival	Anthropometric measurements - Waist and hip circumference - 24-hour recall questionnaires - Food frequency questionnaire - Patient-generated subjective global assessment tool - Blood sample - CT imaging	3 Baseline - during treatment - end of treatment No follow-up after completion of treatment 2 years after treatment	- limited information on data collection process - 24h diaries recall can incur bias - some important confounding factors were not considered, such as use of steroids, hormone replacement therapy and alcohol intake - no follow-up after treatment, short term follow-up (~11 weeks)	7/12
Key findings	Baseline diet was composed of 62% carbohydrates, 24% fat, 15% protein and 24% fiber. Statistically significant decrease in weight and BMI by the end of treatment (p< 0.001). Energy intake statistically significantly decreased by an average of 670 kcal (p= 0.001) Fat and fiber intake decreased during treatment (p= 0.001) but fat reduction was no longer statistically significant by the end of treatment. Carbohydrate and protein intake did not change. 30% of participants became malnourished and lost muscle tissue. Loss of >10% of muscle tissue has a higher risk of tumor recurrence (hazard ratio = 2.957; p= 0.006).							

Table 2. Macronutrient intake across the studies.

	Bruning et al., 1985 **Holland** As described in Hulshof et al., 1987			**Pía de la Maza et al., 2004** **Chile**			**Sanchez et al., 2019** **Mexico**		
Median (range)	BL	EOT	Long-term	BL	EOT	Long-term	BL	EOT	Long-term
Fat **g/ day**	94 (57- 131)	79 (40- 118)	105 (71- 139)	55 (26- 84)	34 (18- 50)	81 (38- 124.5)	37 (27- 47)	17 (6- 28)	-
Carbohydrate **g/ day**	203 (142- 264)	192 (136- 248)	228 (153- 303)	323.5 (209- 438)	265 (134- 396)	316 (162- 470)	220 (160- 280)	140 (85- 195)	-
Protein **g/ day**	69 (53- 85)	59 (42- 76)	71 (54- 88)	70 (37.5- 69)	51 (30- 73)	88 (46- 129)	61 (46- 76)	35 (20- 45)	-
Fibre **g/day**	21 g/ d (14- 28)	16 g/d (9- 23)	23 g/d (15- 31)	5.6 g/d (2.6- 8.6)	4.2 g/d (1- 7.4)	5.7 g/d (2.8- 8.6)	24 g/d	12.7 g/d	-
Daily energy intake kcal/d	1864	1739	2210	2054	1580	2336	1491	813	-
BMI kg/m²	26.3 (23.1- 28.9)	-	26.6 (23.8- 28.8)	27.3 (22.5- 32.1)	27.0 (22.2- 29.8)	28.5 (22.7- 34.3)	27.6 (19.6-36.3)	24.9 (17.1- 33.9)	-

Key: BL: baseline, EOT: end of treatment, Long-term: follow-up after completion of treatment.

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Appendix 2. The modified Gastro-intestinal Symptom Rating Scale.

1. Please rate your symptoms during the last month by placing a tick in the box that best describes each symptom

	Never	Occasional	Frequently affecting your life	Causes majo changes in your life
Experienced change in smell				
Experienced change in taste				
Bad breath/halitosis				
Difficulty swallowing liquids				
Difficulty swallowing solids				
Belching or burping				
Heartburn or acid regurgitation				
Feeling full after small amount of food				
Reduced appetite				
Hiccups				
Nausea/feeling sick				
Vomiting/being sick/retching				
Abdominal cramps/trapped wind				
Upper abdominal pain/discomfort				
Lower abdominal pain/discomfort				
Pain around your bottom				
Abdominal bloating/distension				
Excessive passing of wind from your bottom				
Stomach/abdominal gurgling				
Need to rush to open bowels				
Feeling that you have not emptied your bowel properly				

Please turn over page

Leakage/ soiling or lack of control of the bowel				
Mucus in the stool				
Greasy or oily stool				
Bleeding from your bottom				
Itchiness around the bottom				
Woken from sleep to have bowels open				
Tiredness/lethargy				
Problems passing urine				
Sexual concerns				

2. Please indicate how you would score your quality of life at present on the scale below

0 1 2 3 4 5 6 7 8 9 10

Very low — Perfect

3. How much do your bowel symptoms affect your quality of life?

0 1 2 3 4 5 6 7 8 9 10

Not at all — All the time

4. Currently, how often do you open your bowels? (please tick as many boxes as required)

	What is the least often you have your bowels open?	What is the most you have your bowels open
Less than once a week	☐	☐
Once every 4-7 days	☐	☐
Once every 2-4 days	☐	☐
Once a day	☐	☐
2-3 times a day	☐	☐
4-6 times a day	☐	☐
7 or more times a day	☐	☐

5. Please tick the box(es) which best describe(s) the stool you pass:

Bristol Stool Chart

Type 1	Separate hard lumps, like nuts (hard to pass)	☐
Type 2	Sausage-shaped but lumpy	☐
Type 3	Like a sausage but with cracks on its surface	☐
Type 4	Like a sausage or snake, smooth and soft	☐
Type 5	Soft blobs with clear-cut edges (passed easily)	☐
Type 6	Fluffy pieces with ragged edges, a mushy stool	☐
Type 7	Watery, no solid pieces. **Entirely Liquid**	☐

Appendix 3. The Holistic Needs Assessment (London Cancer Alliance)

LONDON CANCER ALLIANCE West and South LCA

LONDON CANCER NORTH AND EAST

London Holistic Needs Assessment

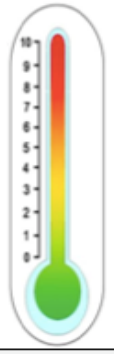
For each item below, please select **yes** or **no** if they have been a concern for you during the last week, including today. Please also select **discuss** if you wish to speak about it with your health professional.

Choose not to complete the assessment today by selecting this box ☐

Date:	Click here to enter text.
Name:	Click here to enter text.
Hospital/NHS number:	Click here to enter text.

Please **select the number** that best describes the overall level of distress you have been feeling during the last week, including today:

10	☐	**Extreme distress**
9	☐	
8	☐	
7	☐	
6	☐	
5	☐	
4	☐	
3	☐	
2	☐	
1	☐	
0	☐	**No distress**



For health professional use	
Date of diagnosis:	Click here to enter text.
Diagnosis:	Click here to enter text.
Pathway point:	Click here to enter text.

Practical concerns	**Yes**	**No**	**Discuss**
Caring responsibilities	☐	☐	☐
Housing or finances	☐	☐	☐
Transport or parking	☐	☐	☐
Work or education	☐	☐	☐
Information needs	☐	☐	☐
Difficulty making plans	☐	☐	☐
Grocery shopping	☐	☐	☐
Preparing food	☐	☐	☐
Bathing or dressing	☐	☐	☐
Laundry/housework	☐	☐	☐
Family concerns			
Relationship with children	☐	☐	☐
Relationship with partner	☐	☐	☐
Relationship with others	☐	☐	☐
Emotional concerns			
Loneliness or isolation	☐	☐	☐
Sadness or depression	☐	☐	☐
Worry, fear or anxiety	☐	☐	☐
Anger, frustration or guilt	☐	☐	☐
Memory or concentration	☐	☐	☐
Hopelessness	☐	☐	☐
Sexual concerns	☐	☐	☐
Spiritual concerns			
Regret about the past	☐	☐	☐
Loss of faith or other spiritual concern	☐	☐	☐
Loss of meaning or purpose in life	☐	☐	☐

Physical concerns	**Yes**	**No**	**Discuss**
High temperature	☐	☐	☐
Wound care	☐	☐	☐
Passing urine	☐	☐	☐
Constipation or diarrhoea	☐	☐	☐
Indigestion	☐	☐	☐
Nausea and/or vomiting	☐	☐	☐
Cough	☐	☐	☐
Changes in weight	☐	☐	☐
Eating or appetite	☐	☐	☐
Changes in taste	☐	☐	☐
Sore or dry mouth	☐	☐	☐
Feeling swollen	☐	☐	☐
Breathlessness	☐	☐	☐
Pain	☐	☐	☐
Dry, itchy or sore skin	☐	☐	☐
Tingling in hands or feet	☐	☐	☐
Hot flushes	☐	☐	☐
Moving around/walking	☐	☐	☐
Fatigue	☐	☐	☐
Sleep problems	☐	☐	☐
Communication	☐	☐	☐
Personal appearance	☐	☐	☐
Other medical condition	☐	☐	☐

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Presenting problems and outcomes of women with gynaecological cancer attending a specialist clinic for GI symptoms

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Introduction

Worldwide 1,470,900 women are diagnosed yearly with a gynaecological malignancy; 21,000 in the UK. Some women develop long-term changes in bowel function following treatment severely impacting on quality of life.

Methods

A service evaluation collecting prospective data was approved by the organisation's R&D department. Intestinal symptoms were measured using a modified Gastrointestinal Symptom Rating Scale and impact on quality of life (QoL) assessed by a visual analogue score (0= worst QoL, 10 = best QoL). The McNemar Chi-square and Wilcoxon signed rank tests were used to analyse changes in symptom burden between initial assessment and discharge from the service.

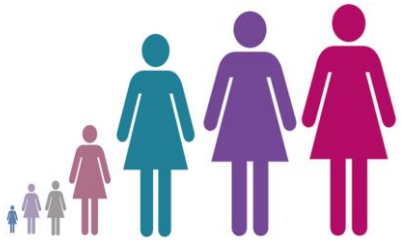
Results

From April 2013 to March 2016, 235 women treated for gynaecological cancers attended the clinic, representing a fifth of those using the service. Fifteen declined further intervention and were excluded, leaving 220 for analysis. Women had been treated for cancer of the cervix (50%), endometrium (28%), ovary (15%), vagina or vulva (7%). Most women received multi-modal therapies (Figure 1): chemoradiation (29%), surgery + radiotherapy (28%), surgery + chemoradiation (23%), surgery + chemotherapy (10%). Four women (2%) also received hormone therapy. Median age was 57 years (range: 24-83). The median time between cancer diagnosis and referral to the service was 4 years and 10 months (range: 6m - 47.5y). Most troublesome symptoms at assessment were bowel frequency of 4 or more times a day (88%), type 6 or 7 stool consistency (36%), urgency to defaecate (31%), faecal incontinence (21%) and fatigue (25%). Some women also reported urinary problems (17%) and sexual concerns (12%).

Following a published algorithm, a median of 8 investigations were requested (range: 1-14): blood screen (97%), gastroscopy (75%), glucose hydrogen methane breath test (77%), SeHCAT scan (71%), faecal elastase (69%), flexible sigmoidoscopy (52%), colonoscopy (25%) and abdominal Xray (18%). A median of four factors contributing to symptoms were found (range: 1-9) and 68% of women had more than three factors. The most common diagnoses were vitamin D deficiency (60%), treated with replacement; small intestinal bacterial overgrowth (54%), treated with antibiotics and bile acid malabsorption (52%), treated with bile acid sequestrants and low fat diet. The median number of consultations was 4 (range: 1-17). Median quality of life improved from 4 at first assessment to 6 at discharge ($p<0.001$); the reduction in urgency, incontinence, tenesmus, frequency, diarrhoea and fatigue was statistically significant ($p< 0.05$).

Figure 1. Cancer treatment modalities

- Chemotherapy alone (1%)
- Surgery alone (4%)
- Radiotherapy alone (5%)
- Surgery + chemotherapy (10%)
- Chemoradiation + surgery (23%)
- Surgery + radiotherapy (28%)
- Chemoradiation (29%)



Conclusion

Bowel symptoms after cancer treatment affect many women. In this uncontrolled series we have demonstrated that they can be discharged with symptom improvement using a systematic investigational and treatment approach. Earlier referral to specialist services is recommended.

Acknowledgements

NHS

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Appendix 5. A proposed tailored investigational algorithm for women treated for gynaecological cancer with long-term gastrointestinal consequences.

Supportive Care in Cancer
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ORIGINAL ARTICLE

A proposed tailored investigational algorithm for women treated for gynaecological cancer with long-term gastrointestinal consequences

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Abstract

Background and aim Long-term changes in gastrointestinal function impacting quality of life after treatment for cancer are common. Peer reviewed guidance to investigate and manage GI dysfunction following cancer treatment has been published. This study reviewed gastrointestinal symptoms of women previously treated for gynaecological cancer and considered whether suggested algorithms could be amended to optimise management for this cohort.

Methods Demographic and clinical data recorded for patients attending a specialist consequences of cancer treatment gastroenterology service prospectively are reported using median and range. The Wilcoxon signed rank test analysed changes in symptoms between initial assessment to discharge from the service.

Results Between April 2013 and March 2016, 220 women, with a median age of 57 years (range 24–83 years), treated for gynaecological cancer (cervical (50%)), endometrial (28%), ovarian (15%), vaginal or vulval (7%) attended. Twelve gastrointestinal symptoms were statistically significantly reduced by time of discharge from the specialist gastroenterology clinic including bowel frequency ≥4/day (88%), type 6 or 7 stool consistency (36%), urgency (31%) and incontinence (21%). General quality of life improved from a median score of 4 at first assessment to a median of 6 at discharge ($p < 0.001$). A median of four (range, 1–9) diagnoses were made.

Conclusion Women with gastrointestinal symptoms after cancer treatment benefit from a systematic management approach. After excluding disease recurrence, a proposed investigational algorithm and the oncology team includes FBC, U&Es, LFTs, thyroid function test, vitamin B_{12}, vitamin D, a hydrogen methane breath test and a SeHCAT scan. If rectal bleeding is present, iron studies, flexible sigmoidoscopy or colonoscopy should be performed. Patients with normal investigations or symptoms not responding to treatment require gastroenterology input.

Keywords Cancer · Gynaecological malignancy · Chemotherapy · Radiotherapy · Gastrointestinal · Side effects · Toxicity · Quality of life · Bile acid malabsorption · Small intestinal bacterial overgrowth · Endoscopy · Diarrhoea · Abdominal pain · Bloating · Incontinence · Urgency · Late effects · Consequences of treatment

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Background

Cancer survival has doubled in the last 40 years. Half of people diagnosed with cancer in England and Wales survive for 10 years or more [1]. Advances in multi-modal treatments offer better chances of survival but impact on the body in multiple ways, not only during treatment but long-term. The long-term impact of cancer treatment was the focus of the National Survivorship Initiative and has been adopted in the NHS England cancer plan [2, 3].

The Royal Marsden Gastrointestinal and Nutrition Team (GIANT) service, a clinic established specifically to care for

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patients experiencing ongoing gastrointestinal symptoms after cancer treatment, is multidisciplinary with input from a consultant gastroenterologist, specialist nurses and dietitians.

The team developed a systematic, checklist-based approach to assess and investigate these long-term gastrointestinal symptoms, tested in the ORBIT study [4]. This showed that patient outcomes when managed by a specialist nurse were not inferior to those managed by a consultant gastroenterologist. Subsequent evaluation of this clinical service suggested that the cost of managing gastrointestinal consequences of cancer treatment across tumour groups averages £1563 per patient [5].

The algorithm used to manage patients' abnormal symptoms was developed through 10 peer-reviewed versions [6] without regard to the primary cancer site treated. Since different treatment pathways are used for each type of cancer—including type of surgery, chemotherapy regimens, radiotherapy volumes and doses—the predominant causes for long-term gastrointestinal symptoms may vary. This may allow for a tailored algorithm in some patient groups.

Annually, 21,000 UK women are newly diagnosed with a gynaecological malignancy [7]. Following treatment for gynaecological cancer, data are inadequate to assess how many women experience permanent changes to gastrointestinal function which impact on quality of life, activity levels or return to work; however, several studies have highlighted this as a serious concern [8–11].

The aims of this study were to evaluate the symptoms of women previously treated for gynaecological cancer, assessed in a specialist gastroenterology clinic and to review whether the established algorithm could be amended to optimise management in this cohort by the gynaecology team. The study hypothesis was that there would be an improvement in symptom severity at discharge from the gastroenterology clinic compared with baseline.

Methods

This was an observational study with data collected prospectively on all users of the GIANT service. Following institutional review board approval for service evaluation (SE36), demographic and clinical data were collected on a clinical research form and entered onto database. Data included all requested investigations, resulting gastrointestinal diagnoses and treatments as dictated by the algorithm for patients who completed their episode of care from the clinic.

At each clinic visit, patients self-reported the presence and severity of their symptoms using a modified Gastrointestinal Symptom Rating Scale (GSRS). Symptom severity is categorised as 'causes major changes in your life', 'frequently affecting your life', 'occasional' or 'never'. The first two categories indicate severe and moderate symptoms. The GSRS has been validated for use in a wide variety of gastrointestinal disorders [12–15]. Patients indicated the frequency of bowel movements and stool consistency using a Bristol stool chart with 1 = hard stool and 7 = liquid stool [16]. In addition, they scored their perceived quality of life (QoL) on a visual analogue scale (VAS) with 0 indicating the worst possible QoL and 10 equating the best possible QoL and the impact of their gastrointestinal symptoms on QoL with 0 indicating no impact and 10 the worst impact possible.

A holistic needs assessment (HNA) was also offered to all patients at first assessment. The HNA comprises a questionnaire covering areas which may concern anyone living with and beyond cancer [2]. Practical, emotional and spiritual family issues can be identified to discuss with a qualified health care professional or highlighted as a concern without discussion, alongside physical concerns. As part of the assessment, the distress thermometer —a screening tool for assessing psychological distress in people affected by cancer—is a requirement of the National Institute of Clinical Excellence guidelines for supportive and palliative care [17]. Patients scored on a scale from 0 ('I am not distressed') to 10 ('I am extremely distressed'). A score of more than 7 justifies a psychological support service referral in a tertiary cancer centre [18].

The Wilcoxon signed rank test was used to analyse changes in symptom burden between initial assessment and the point of discharge from the service. This statistical analysis test allows for comparison of ordinal data (symptom severity) between 2 related groups.

Results

Between April 2013 and March 2016, there were 1158 new patients and 2686 follow-up appointments in the GIANT clinic. Two hundred thirty-five women (21%) had received treatment for gynaecological malignancy. Fifteen women who declined investigations and further management were excluded, leaving 220 for analysis. Referral to the clinic came from within the organisation (n = 94; 43%), other hospitals (n = 77; 35%), general practitioners (n = 48; 22%) and self-referral (n = 1; < 1%).

The primary cancer site was cervix (n = 109; 50%), endometrium (n = 62; 28%), ovary (n = 33; 15%) and vagina or vulva (n = 16; 7%). The median age was 57 years (range, 24–83). Several women received multi-modal therapies for their malignancy (Table 1). Nearly 70% (n = 151) received radiotherapy (including brachytherapy), 54% (n = 118) had surgery and 45% (n = 98) chemotherapy. Seventeen percent (n = 37) received all three therapies. Treatment characteristics per tumour group are presented in Table 1. The median time between cancer diagnosis and referral was 4 years and 10 months (range, 6 months–47.5 years). Ten percent of women had been seen previously and were re-referred. In patients seen for the

Table 1 Cancer treatment regimens per cancer diagnosis group ($n = 220$)

	Cervical cancer, *n* (%)	Endometrial cancer, *n* (%)	Ovarian cancer, *n* (%)	Vaginal/vulval cancer, *n* (%)	Total, *n* (%)
Cancer treatment					
Chemotherapy alone	11	1	8	2	22 (10%)
Radiotherapy alone	36	13	1	2	52 (24%)
Surgery alone	10	16	11	2	39 (18%)
CXT + RT (no surgery)	24	1	0	3	28 (13%)
CXT + surgery (no RT)	0	0	8	3	11 (5%)
RT + surgery (no CXT)	12	18	0	1	31 (14%)
CXT + RT + surgery	16	13	5	3	37 (17%)
Total	109 (50%)	62 (28%)	33 (15%)	16 (7%)	220 (100%)
Additional cancer treatment information per cancer diagnosis group ($n = 220$)					
EBRT w/o brachytherapy	37	29	5	4	71 (32%)
Brachytherapy w/o EBRT	3	3	0	1	7 (3%)
EBRT + brachytherapy	48	13	4	4	69 (31%)
RT total	88 (40%)	45 (20%)	9 (4%)	9 (4%)	151 (69%)
Chemotherapy total	51 (23%)	15 (7%)	21 (10)	11 (5%)	98 (45%)
Surgery total	38 (17%)	47 (21%)	24 (11%)	9 (4%)	118 (54%)
Hormone replacement therapy	1	4	4	0	9 (4%)
Other treatment	0	0	0	0	0
Colostomy	1	0	0	1	2 (1%)

first time, the median number of consultations was 4 (range, 1–17) with 38% requiring more than four. Those who were re-referred had a median of 4 consultations (range, 1–7) with 30% needing more than four.

The most prevalent gastrointestinal symptoms at assessment rated as severe included increased frequency of defaecation (88%), diarrhoea (36%), urgency (31%) and incontinence (21%). In addition, many women also reported fatigue (87%), urinary problems (53%) and sexual concerns (38%) (Fig. 1).

A median of 8 (range, 1–14) investigations were requested for this cohort using our algorithm version 10 [6]. These included blood screen ($n = 213$; 97%), gastroscopy ($n = 178$; 80%) with duodenal aspirate ($n = 168$; 76%), glucose hydrogen methane breath test ($n = 177$; 80%), SeHCAT scan ($n = 161$; 73%), stool sample for faecal elastase ($n = 152$; 69%), flexible sigmoidoscopy ($n = 114$; 52%), colonoscopy ($n = 56$; 25%) and abdominal X-ray ($n = 35$; 16%).

These investigations revealed a median of four abnormal findings (range, 1–9) with 68% of women ($n = 150$) having more than three. Blood screens showed that vitamin D deficiency is common in this group (60%). The vitamin B_{12} test was below the normal range for 30% of this cohort ($n = 65$). Thyroid function tests indicated abnormal values for 5% ($n = 11$) (Table 2).

Following the algorithm, in the presence of upper GI symptoms, a gastroscopy with duodenal aspirate was often ordered to test for small intestinal bacterial overgrowth (SIBO) in addition to upper gastrointestinal pathology. In this cohort, more than 75% ($n = 168$) of women had a duodenal aspirate. Of those, 60% ($n = 101$) did not report any growth. Of the 40% ($n = 67$) that did, half of the cases ($n = 35$) had sensitivities reported resulting in specific antibiotic treatment which in 15% ($n = 5$) differed from the recommendation of the algorithm. One hundred seventy-seven women had a glucose hydrogen methane breath test which was positive for both hydrogen and methane in 24% ($n = 43$), for methane alone in 12% ($n = 22$) and for hydrogen alone in 8% ($n = 14$). Nearly a third of women ($n = 33$) with persistent symptoms required multiple treatments with antibiotics (range, 2–9) and two women required long-term, rotating antibiotics.

Bile acid malabsorption was a common diagnosis in this group of women ($n = 104$; 47%); 73% ($n = 161$) had a SeHCAT scan which was positive in 65% ($n = 104$). The algorithm suggests that a SeHCAT should be requested for anyone reporting type 6 or 7 stool consistency, urgency or steatorrhoea [6]. For those women who reported urgency without diarrhoea, 60% ($n = 54$) had a positive SeHCAT scan.

Faecal loading complicated by overflow diarrhoea was diagnosed following an abdominal X-ray in 8% of the cohort ($n = 18$).

A stool sample for faecal elastase, sent in 69% ($n = 152$), confirmed pancreatic insufficiency in 7% ($n = 16$) requiring treatment with enzyme replacement and

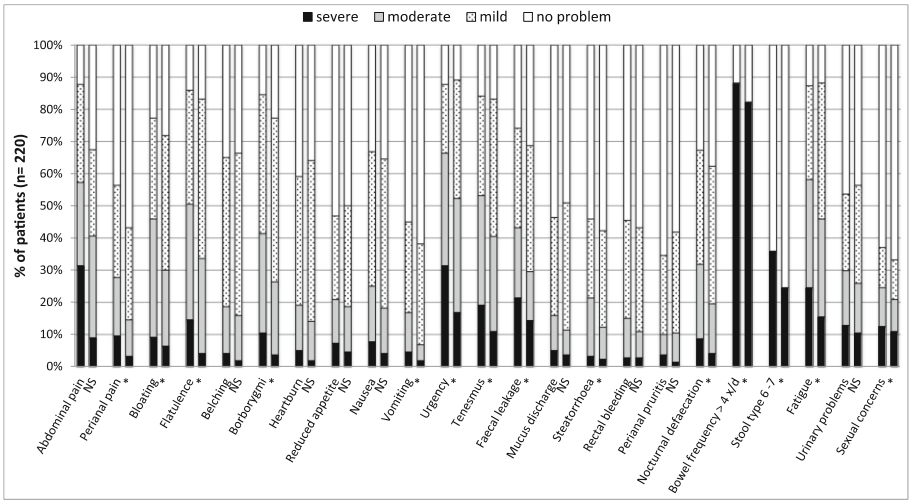

Fig. 1 Paired symptom scores ($n = 220$) at baseline and discharge; left bar, baseline assessment; right bar, discharge assessment (NS, not significant, $*p < 0.05$)

sometimes specialist dietetic advice. A coeliac screen was requested in 84% of the women ($n = 185$) but did not identify any women with the condition.

Table 2 Prevalence of new gastrointestinal or nutritional diagnoses ($n = 220$)

Diagnosis	Prevalence, n (%)
Vitamin D deficiency	133 (60%)
Small intestinal bacterial overgrowth	118 (54%)
Bile acid malabsorption	104 (47%)
Gastritis	68 (31%)
Vitamin B_{12} deficiency	65 (30%)
Weak pelvic floor musculature on rectal exam	36 (21%)
Telangiectasia on the rectal wall	33 (15%)
Trace element deficiency	31 (14%)
New GI polyp	24 (11%)
Hiatus hernia	22 (10%)
Faecal loading (confirmed on abdominal X-ray)	18 (8%)
Dietary fibre excess on fibre quiz	16 (7%)
Oesophagitis	16 (7%)
Iron deficiency	16 (7%)
Pancreatic insufficiency	16 (7%)
Duodenitis	13 (6%)
Thyroid problems	11 (5%)
Diverticular disease	9 (4%)
Gastro-oesophageal reflux disease	8 (4%)
Haemorrhoids	7 (3%)
Inflammatory bowel disease	4 (2%)
Rectal ulcer	4 (2%)
New GI cancer	5 (2%)
Anal fissure/anal sphincter defect	3 (1%)

The holistic needs assessment was completed by 71% ($n = 157$) (Fig. 2). The main concerns for women after treatment for gynaecological cancer were fatigue (70%), worry, fear and anxiety (62%), sleeping problems (58%) and pain (57%). Women scored a median of 5 (range, 0–10) on the distress thermometer and 35% ($n = 59$) scored 7 or more.

Twelve gastrointestinal symptoms were statistically significantly reduced by time of discharge from the specialist gastroenterology clinic (Table 3) ($p < 0.05$). The general quality of life score improved from a median of 4 at first assessment to a median of 6 at discharge ($p < 0.001$). The impact of residual or recurrent gastrointestinal symptoms on QoL did not improve with a median of 7 out of a maximum score of 10 ($p > 0.05$).

Discussion

This prospective cohort study shows that a specialist gastroenterology service can improve the outcomes for women living with and beyond cancer after treatment for gynaecological malignancy by reducing gastrointestinal symptom burden and improving quality of life.

The findings of this study are consistent with other studies [19] which show that the main concerns for women on holistic needs assessment are largely unchanged from their concerns at the end of treatment and in 1 in 3 justify a psychological

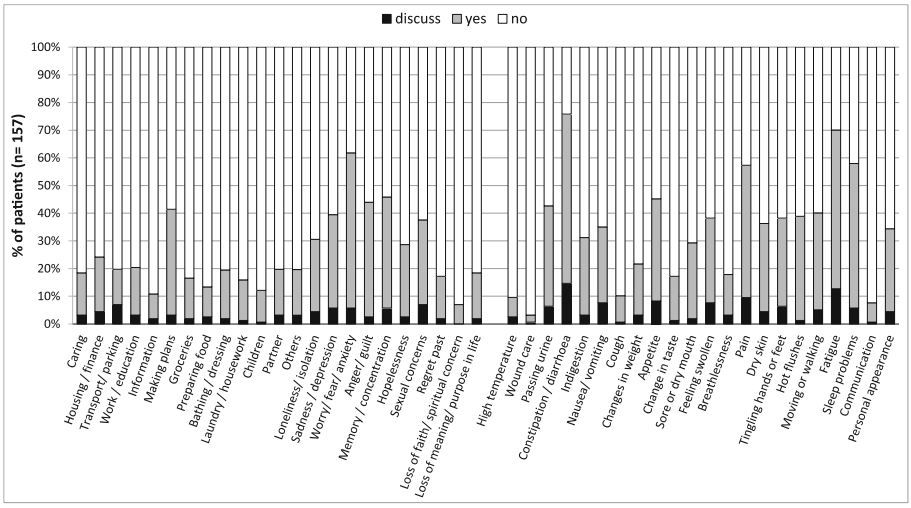

Fig. 2 Concerns highlighted by women treated for gynaecological malignancy on the holistic needs assessment ($n = 157$)

support service referral because of worry, fear and anxiety, fatigue and sleeping problems.

The relatively high prevalence of symptoms still present at discharge may partly be related to the fact that whilst specific treatments help improve symptoms, as yet there are no proven treatments to tackle the underlying pathological changes which cause pelvic radiation disease [20]. Despite this, there remains scope for further improvement. Support delivered by a specialist multidisciplinary team may have a beneficial psychological effect in itself. Furthermore, the importance to provide person-centred care and use patient recorded outcome measures (PROMS) is highlighted by the suggestion that even though general QoL improves from baseline, the impact of residual or recurring gastrointestinal symptoms on QoL remains the same at discharge. This may be due to heightened awareness of the causes contributing to these symptoms and need for ongoing, long-term management strategies including demanding lifestyle and dietary changes.

Whilst the reduction in severity reached statistical significance in 12 out of 20 gastrointestinal symptoms, the average time from diagnosis of cancer to referral to a specialist gastroenterology service was nearly 5 years. There is a need for earlier identification of patients requiring intervention. One way to do this is by developing assessment measures such as the ALERT-B questionnaire validated for use in oncology follow-up clinics in patients with prostate cancer who may benefit from further assessment [21]. After excluding disease progression, the next logical step would be for the oncology team to initiate first-line investigations to identify treatable symptoms. To this end, this study suggests an investigational algorithm tailored to symptomatic gynaecological oncology patients which any team should not find challenging. If symptoms remain unresolved or these tests are negative, a referral can be made to specialist gastroenterology services (Table 4).

Within this cohort, several tumour sub-groups were represented with different treatment regimens. Whilst nearly 70% received radiotherapy to the pelvic area, nearly 50% also received other treatments, making it difficult to establish which treatment modality contributes to gastrointestinal symptom burden. The heterogeneity of the patients included in the study regarding tumour and treatment types could limit the applicability of the algorithm to each different cancer type, treatment and stage. Further testing is needed to provide evidence of its use in clinical practice and its merit in reducing symptom burden for these patients.

In this study, two-thirds of women were deficient in vitamin D. Clinical evidence supports that adequate vitamin D levels or supplementation in those with low levels reduces the risk of developing gynaecological malignancies although the molecular pathways remain poorly understood [22]. Vitamin D is essential to aide the absorption of calcium and maintain bone health [23] and after pelvic radiotherapy patients are at increased risk of bone fractures. Whilst in the general population supplementation of vitamin D does not

Table 3 Symptoms which improved at a statistically significant level ($p < 0.05$), $n = 220$

Symptoms	Baseline ($n = 220$)	Discharge ($n = 220$)	p value
	n (%)	n (%)	
Perianal pain			
No problem	96 (44)	125 (57)	
Mild	63 (29)	63 (29)	< 0.001
Moderate	40 (18)	25 (11)	
Severe	21 (10)	7 (3)	
Bloating			
No problem	50 (23)	62 (28)	
Mild	69 (31)	92 (42)	< 0.001
Moderate	81 (37)	52 (24)	
Severe	20 (9)	14 (6)	
Flatulence			
No problem	31 (14)	37 (17)	
Mild	78 (35)	109(50)	< 0.001
Moderate	79 (36)	65 (30)	
Severe	32 (15)	9 (4)	
Borborygmi			
No problem	34 (15)	50 (23)	
Mild	95 (43)	112 (51)	< 0.001
Moderate	68 (31)	50 (23)	
Severe	23 (10)	8 (4)	
Vomiting			
No problem	121 (55)	136 (62)	
Mild	62 (28)	69 (31)	0.002
Moderate	27 (12)	11 (5)	
Severe	10 (5)	4(2)	
Urgency			
No problem	27 (12)	24 (11)	
Mild	47 (21)	81 (37)	< 0.001
Moderate	77 (35)	78 (35)	
Severe	69 (31)	37 (17)	
Tenesmus			
No problem	35 (16)	37 (17)	0.003
Mild	68 (31)	94 (43)	
Moderate	75 (34)	65 (30)	
Severe	42 (19)	24 (10)	
Faecal incontinence			
No problem	50 (23)	60 (28)	
Mild	59 (27)	85 (39)	< 0.001
Moderate	58 (26)	45 (20)	
Severe	53 (24)	30 (14)	
Steatorrhoea			
No problem	119 (54)	127 (58)	
Mild	54 (25)	66 (30)	0.045
Moderate	40 (18)	22 (10)	
Severe	7 (3)	5 (2)	
Nocturnal defaecation			
No problem	72 (33)	83 (38)	
Mild	78 (35)	94 (43)	< 0.001
Moderate	51 (23)	34 (15)	
Severe	19 (9)	9 (4)	
Bowel frequency > 4x/day			
No	26 (12)	39 (18)	0.009
Yes	194 (88)	181 (82)	
Diarrhoea (type 6 or 7)			
No	142 (65)	165 (75)	0.013
Yes	78 (35)	55 (25)	
Fatigue			
No problem	28 (13)	26 (12)	
Mild	64 (29)	93 (42)	0.001
Moderate	74 (34)	67 (30)	
Severe	54 (26)	34 (15)	
Sexual concerns			
No problem	137 (62)	147 (67)	
Mild	28 (13)	27 (12)	0.004
Moderate	27 (12)	22 (10)	
Severe	28 (13)	24 (11)	

improve bone health [24], this may be different in a cancer population and people with bile acid malabsorption.

Vitamin B_{12} deficiency is a potent cause for tiredness and is linked to memory problems, anaemia and breathlessness. Levels are often low in those with bile acid malabsorption and SIBO [25–27]. Up to 23% of women treated for gynaecological cancer were previously reported to have decreased vitamin B_{12} levels [28] and in this cohort, it was detected in 30%.

Interpretation of all currently available investigations to diagnose SIBO is problematic [29] and it is difficult to ascertain how effective treatment for SIBO is without systematic re-testing. A breath test should remain a first-line test in this patient cohort. In view of antibiotic stewardship [30], a duodenal aspirate may be helpful in people with recurrent SIBO or persistent symptoms.

Bile acid diarrhoea has an estimated prevalence in the general population of 1% and is frequently misdiagnosed as IBS [31, 32]. Bile acid malabsorption was diagnosed in 47% in our cohort. Some of these may have had primary bile acid diarrhoea and unrecognized pre-treatment, which exacerbated during treatment but for many, new onset symptoms were likely secondary to the effect of chemo-radiotherapy on the absorptive capacity for bile of the terminal ileum. Our finding is in keeping with other studies. Bile acid malabsorption is often thought as causing chronic watery stool. We have previously shown that a wide spectrum of symptoms improves when bile acid malabsorption is adequately treated [33]. It is therefore particularly interesting that 40% of patients in whom we made this diagnosis only had urgency and did not report loose stool. In some, opioids or anti-diarrhoeal medication may have masked their diarrhoea; this highlights that urgency in this patient group may be a marker for bile acid malabsorption.

Constipation is common in up to 60% of cancer patients and can be due to several causes following treatment: small intestinal bacterial overgrowth, reduced dietary fibre intake, medication, anorectal pain, anal fissure and co-morbidities such as diabetes [34]. Although bowel obstruction occurs more often in women treated for ovarian cancer, it is important to differentiate this from severe faecal loading through imaging [35]. In this cohort, abdominal X-ray had a 50% yield for identifying faecal loading which was thought to predispose to abdominal pain or overflow diarrhoea.

In this group, a stool sample for faecal elastase, which costs about £39, has a 7% yield for diagnosing pancreatic insufficiency. Thyroid function problems were detected in only 5% of patients but are routinely included in our blood screen which was done in 97% of the cohort. A coeliac screen is also routinely included, especially as people often cut gluten out of their diet. The cost of a

Table 4 Investigation algorithm tailored for women treated for gynaecological cancer with ongoing GI problems

Investigational algorithm tailored for gynaecological cancer	Original investigational algorithm (Frontline Gastroenterology, 2015)
First-line investigations (oncology clinic)	**First-line investigations**
Blood screen: FBC, U&Es, LFTs Vitamin D Vitamin B_{12} Glucose hydrogen methane breath	Blood screen: FBC, U&Es, LFTs, CRP, ESR Vitamin D Vitamin B_{12} Iron studies
SeHCAT scan If rectal bleeding is present: iron studies Flexible sigmoidoscopy or colonoscopy (avoid biopsies of irradiated tissue)	Thyroid function Coeliac screen Serum magnesium
If no rectal bleeding but anaemic: iron studies Flexible sigmoidoscopy or colonoscopy (avoid biopsies of irradiated tissue)	Stool sample for microscopy, culture and *Clostridium difficile*
Second-line investigations (gastroenterology team)	Stool sample for faecal elastase Glucose hydrogen methane breath test Gastroscopy with duodenal aspirate
Stool sample for faecal elastase	Carbohydrate challenge
Coeliac screen	SeHCAT scan
Thyroid function screen	Flexible sigmoidoscopy/colonoscopy (avoid biopsies of irradiated tissue)
Abdominal X-ray	**Second-line investigations**
Third-line investigations (gastroenterology team)	Colonoscopy with biopsies
Carbohydrate challenge	**Third-line investigations**
Gut hormone blood test, urinary 5-HIAA	Gut hormone challenge
Colonoscopy with biopsies	Urinary 5-HIAA
CT chest abdomen and pelvis/CT colonography to exclude structural GI pathology	CT chest abdomen and pelvis

coeliac screen is about 25–£40 with on-costs (personal communication). As we did not identify any new onset of coeliac disease in this cohort, and the low yield of faecal elastase and coeliac screening, it may be reasonable not to include them in the oncology clinic; however, this may delay diagnosis, affect clinic capacity and potentially increase costs as patients may require additional appointments before treatment has been optimised.

The most common and severe symptoms in this cohort were urgency and type 6 or 7 stool consistency. A new tiered approach to investigation (Table 2) in this cohort may facilitate the management of symptoms as it can be difficult to know which intervention has resulted in improvement or failed to have an impact.

In this prospective cohort, we were unable to correlate gastrointestinal symptoms to disease stage, treatment modalities, radiotherapy volumes or doses but this is important to include in further studies. Differences in disease stage could have influenced symptom incidence, severity and outcome. Pelvic radiotherapy is used to treat gynaecological malignancies in 30% of women. Moderate to severe long-term gastrointestinal symptoms are reported by clinicians in 5–15% of patients treated [36] and up to 50% when PROMs are used [37, 38]. Extrapolating the number of patients treated for gynaecological malignancies with pelvic radiotherapy, this would mean up to 3225 women need access to this kind of service in the UK where 21,500 gynaecological cancer diagnoses are made yearly [7].

Conclusion

Significant improvement in gastrointestinal symptoms for women with long-term bowel problems following treatment for gynaecological cancer can be achieved. The most common functional deficits are bile acid malabsorption, small intestinal bacterial overgrowth and vitamin D deficiency. The proposed investigational algorithm tailored to this cohort will need to be tested further in clinical practice to ascertain its clinical value. There is a need for earlier intervention and further research for predicting and treating bowel toxicity which correlates GI symptom burden with radiotherapy volumes and doses.

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Compliance with ethical standards

Conflict of interest Ms. Muls reports grants from the National Institute for Health Research, during the conduct of the study. Dr. Taylor has nothing to disclose. Dr. Lalondrelle reports personal fees from Sanofi Genzyme, grants from MSD, personal fees from Roche, grants and personal fees from Elekta, outside the submitted work. Mr. Mohammed has nothing to disclose. Professor Norton reports personal fees from Takeda, personal fees from Tillotts Pharma, personal fees from Ferring, outside the submitted work. Professor Hart has served as a consultant, advisory board member or speaker for AbbVie, Atlantic, Bristol-Myers Squibb, Celltrion, Falk, Ferring, Janssen, MSD, Napp Pharmaceuticals, Pfizer, Pharmacosmos, Shire and Takeda. She also serves on the Global Steering Committee for Genentech. Professor Andreyev reports non-financial support from GE Healthcare and manufacturers of SeHCAT, outside the submitted work.

The corresponding author has full control over the primary data.

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References

1. Vale C, Tierney J, Davidson S, Drinkwater KJ, Symonds P (2010) Substantial improvement in UK cervical cancer survival with chemoradiotherapy: results of a Royal College of Radiologists' audit. Clin Oncol (R Coll Radiol) 22:590–601. https://doi.org/10.1016/j.clon.2010.06.002
2. NHS England (2016) Guidance: implementing the Cancer Taskforce recommendations: commissioning person centred care for people affected by cancer. NHS England 2016: 17pp. Accessed August 2019 via https://www.england.nhs.uk/wp-content/uploads/2016/04/cancer-guid-v1.pdf
3. Department of Health. Living with and beyond cancer: taking action to improve outcomes. Department of Health 2013: 135pp. Accessed August 2019 via https://www.gov.uk/government/publications/living-with-and-beyond-cancer-taking-action-to-improve-outcomes
4. Andreyev HJN, Benton BE, Lalji A et al (2013) Algorithm-based management of patients with gastrointestinal symptoms in patients after pelvic radiation treatment (ORBIT): a randomised controlled trial. Lancet 382:2084–2092
5. Muls A, Lalji A, Marshall C, Butler L, Shaw C, Vyoral S, Mohammed K, Andreyev HJ (2016) The holistic management of consequences of cancer treatment by a gastrointestinal and nutrition team: a financially viable approach to an enormous problem? Clin Med 16(3):240–246
6. Andreyev HJN, Muls AC, Norton C, Ralph C, Watson L, Shaw C, Lindsay JO (2015) Guidance: the practical management of the gastrointestinal symptoms of pelvic radiation disease. Frontline Gastroenterol 6(1):53–72
7. Cancer Research UK (2019) Cancer statistics- new cases. Accessed May 2019 via: http://www.cancerresearchuk.org/health-professional/cancer-statistics
8. Gillespie C, Goode C, Hackett C, Andreyev HJ (2007) The clinical needs of patients with chronic gastrointestinal symptoms after pelvic radiotherapy. Aliment Pharmacol Ther 26:555–563. https://doi.org/10.1111/j.1365-2036.2007.03405.x
9. Lind H, Waldenstrom AC, Dunberger G et al (2011) Late symptoms in long-term gynaecological cancer survivors after radiation therapy: a population-based cohort study. Br J Cancer 105:737–745. https://doi.org/10.1038/bjc.2011.315
10. Salani R (2013) Survivorship planning in gynecologic cancer patients. Gynecol Oncol 130:389–397. https://doi.org/10.1016/j.ygyno.2013.05.022
11. Barnes D, Rivera R, Gibson S, Craig C, Cragun J, Monk B, Chase D (2018) The utility of patient reported data in a gynecologic oncology clinic. Gynecol Oncol Res Pract 5:4. https://doi.org/10.1186/s40661-018-0062-4
12. Revicki DA, Wood M, Wiklund I, Crawley J (1998) Reliability and validity of the gastrointestinal symptom rating scale in patients with gastroesophageal reflux disease. Qual Life Res 7:75–83
13. Van den Heuvel-Janssen HA, Borghouts JA, Muris JW et al (2006) Chronic non-specific abdominal complaints in general practice: a prospective study on management, patient health status and course of complaints. BMC FamPract 7:1–8
14. Iwarzon M, Gardulf A, Lindberg G (2009) Functional status, health- related quality of life and symptom severity in patients with chronic intestinal pseudo-obstruction and enteric dysmotility. Scand J Gastroenterol 44:700–707
15. Rashid L, Velanovich V (2012) Symptomatic change and gastrointestinal quality of life after pancreatectomy. HPB 14:9–13
16. Lewis SJ, Heaton KW (1997) Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol 32:920–924
17. Stewart-Knight K, Parry R, Abey A et al (2012) Does the distress thermometer work? A systematic review of the evidence for its use and validity. Support Palliat Care 2(Suppl_1):A30. https://doi.org/10.1136/bmjspcare-2012-000196.87
18. Sandsund C, Towers R, Thomas K et al (2017) Support Palliat Care: 1–14. https://doi.org/10.1136/bmjspcare-2016-001207
19. London Cancer Alliance. E-HNA data analysis: What are people's most commonly reported concerns? 1st April 2014–31 March 2015. Presentation data 2015
20. Hauer-Jensen M, Denham JW, Andreyev HJ (2014) Radiation enteropathy–pathogenesis, treatment and prevention. Nat Rev Gastroenterol Hepatol 11(8):470–479
21. Farnell D, Staffurth J, Sivell S et al (2019) The ALERT-B questionnaire provides a clinically validated questionnaire for the detection of gastroenterological late effects after radiotherapy for prostate cancer. Submitted for publication to Radiotherapy and Oncology
22. Deuster E, Jeschke U, Ye Y, Mahner S, Czogalla B (2017) Vitamin D and VDR in gynecological cancers—a systematic review. Int J

Mol Sci 18(11):23, 12pp–28. https://doi.org/10.3390/ijms18112328
23. Hill T, Aspray T (2017) The role of vitamin D in maintaining bone health in older people. Ther Adv Musculoskelet Dis 9(4):89–95. https://doi.org/10.1177/1759720X17692502
24. Bolland M, Grey A, Avenell A (2018) Effects of vitamin D supplementation on musculoskeletal health: a systematic review, meta-analysis, and trial sequential analysis. Lancet Diabetes Endocrinol 6:847–858. https://doi.org/10.1016/S2213-8587(18)30265-1
25. Camilleri M (2014) Advances in understanding of bile acid diarrhea. Expert Rev Gastroenterol Hepatol 8(1):49–61. https://doi.org/10.1586/17474124.2014.851599
26. Ghoshal U, Shukla R, Ghoshal U (2017) Small intestinal bacterial overgrowth and irritable bowel syndrome: a bridge between functional organic dichotomy. Gut and Liver 11(2):196–208. https://doi.org/10.5009/gnl16126
27. Green R (2017) Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood 129:2603–2611. https://doi.org/10.1182/blood-2016-10-569186
28. Snijders-Keilholz A, Griggoen G, Davelaar J, Trimbos J, Leer J (1993) Vitamin B12 malabsorption after irradiation for gynaecoligical tumours. Anticancer Res 13(5):1877–1881
29. Grace E, Shaw C, Whelan K, Andreyev J (2013) Review article: small intestinal bacterial overgrowth–prevalence clinical features, current and developing diagnostic tests, and treatment. Aliment Pharmacol Ther 38(7):674–688
30. National Institute of Clinical Excellence (2015) Antimicrobial stewardship: systems and processes for effective antimicrobial medicine use: NICE guideline. NICE 2015; 25pp. Accessed August 2019 via www.nice.org.uk/guidance/ng15
31. Wedlake L, A'hern R, Russel D et al (2009) Systematic review: the prevalence of idiopathic bile acid malabsorption as diagnosed by SeHCAT scanning in patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 30:707–717
32. Walters JR, Pattni SS (2010) Managing bile acid diarrhoea. Ther Adv Gastroenterol 3(6):349–357. https://doi.org/10.1177/1756283X10377126
33. Gupta A, Muls A, Lalji A, Thomas K, Watson L, Shaw C, Andreyev HJ (2015) Outcomes from treating bile acid malabsorption using a multidisciplinary approach. Support Care Cancer 23(10):2881–2890. https://doi.org/10.1007/s00520-015-2653-5
34. Wickham R (2017) Managing constipation in adults with cancer. J Adv Pract Oncol 8:149–161. https://doi.org/10.6004/jadpro.2017.8.2.3
35. Kolomainen D, Riley J, Wood J, Barton D (2017) Surgical management of bowel obstruction in gynaecological cancer. Obstet Gynaecol 19:63–70. https://doi.org/10.1111/tog.12341
36. Taylor A, Fernandes A, Kumar P et al (2016) Acute and chronic toxicity of pelvic radiotherapy. In: Textbook of gynaecological oncology, 3rd edn. The European Soiaty of Gynaecological Oncology. Chapter 138. 12pp
37. Donovan K, Boyington A, Judson P, Wyman JF (2014) Bladder and bowel symptoms in cervical and endometrial cancer survivors. Psychooncology. 23(6):672–678. https://doi.org/10.1002/pon.3461
38. Pfaendler K, Wenzel L, Mechanic et al (2015) Cervical cancer survivorship: long-term quality of life and social support. Clin Ther 37(1):39–48. https://doi.org/10.1016/j.clinthera.2014.11.013

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Systematic review: the impact of cancer treatment on the gut and vaginal microbiome in women with a gynaecological malignancy

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Abstract

Background and aim—Worldwide 1,470,900 women are diagnosed yearly with a gynaecological malignancy; 21,000 in the UK. Some patients treated with pelvic radiotherapy develop chronic changes in their bowel function. This systematic review summarises current research on the impact of cancer treatment on the gut and vaginal microbiome in women with a gynaecological malignancy.

Methods—The PRISMA guidelines for systematic reviews were used to ensure transparent and complete reporting. Quantitative studies exploring the gut or vaginal microbiome in this patient cohort were included. Animal studies were excluded. There were no language restrictions.

Results—No studies examined the possible effects of surgery or chemotherapy for gynaecological cancers on the gut or vaginal microbiome.

Three prospective cohort studies were identified using sequencing of changes in the gut microbiome reporting on a total of 23 women with treated for gynaecological cancer. All studies included patients treated with radiotherapy with a dosage ranging from 43.0 to 54.0 Gy. Two studies assessed gastrointestinal toxicity formally; 8 women (57%) developed grade 2 or 3 diarrhoea during radiotherapy. The outcomes suggest a correlation between changes in the intestinal microbiome and receiving radiotherapy and showed a decrease in abundance and diversity of the intestinal bacterial species. Prior to radiotherapy, those who developed diarrhoea had an increased abundance of Bacteroides, Dialister, Veillonella ($p<0.01$), and a decreased abundance of Clostridium XI and XVIII, Faecalibacterium, Oscillibacter, Parabacteroides, Prevotella and unclassified bacteria ($p<0.05$).

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Ethical/Legal Considerations

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Conclusion—The limited evidence to date implies that larger studies including both the vaginal and gut microbiome in women treated for a gynaecological malignancy are warranted to explore the impact of cancer treatments on the microbiome and its relation to developing long-term GI toxicity. This may lead to new avenues to stratify those at risk and explore personalised treatment options and prevention of gastrointestinal consequences of cancer treatments.

Keywords

gut microbiome; vaginal microbiome; gynaecology; malignancy; cancer; cancer treatment; gastrointestinal toxicity

Introduction

Over 14 million people worldwide are diagnosed with a new cancer annually.1 Yearly, 1,470,900 women are diagnosed with gynaecological malignancy, 21,000 in the UK.

Treatment for gynaecological cancers includes surgery, radiotherapy and chemotherapy. Radiation delivered by external beam radiotherapy or brachytherapy frequently causes acute gastrointestinal toxicity and up to half of all patients experience chronic change in bowel function. Few data are available with regard to long-term gastrointestinal toxicity following surgery and chemotherapy.2

Gastrointestinal symptoms following cancer treatment may include diarrhoea, abdominal pain, bowel frequency, faecal incontinence, borborygmi, tenesmus and flatulence.3 These symptoms cause fatigue, affect well-being, relationships and socio-economic status. The impact of these symptoms on quality of life and daily activities is often not assessed or addressed.

While progress has been made in defining optimal management of chronic changes in bowel function after cancer treatment3, the ability to predict and risk-stratify in advance those who might develop serious problems as a result of treatment would herald a major advance in outcomes for cancer survivors.

Mechanisms by which gastrointestinal symptoms occur after cancer treatment are starting to be understood and personal parameters which change the risk of side-effects for individuals, identified. Body mass index (BMI), concomitant chemotherapy, use of a statin or ACE inhibitor, diabetes mellitus, connective tissue disorders or HIV disease all alter risk of long-term consequences.4

The gut microbiome, however, may be the key to understanding gastrointestinal toxicity during and following cancer treatment.5 The bacteria within the gastrointestinal tract contribute to health, mood and general well-being by regulating major epithelial and immune functions and feedback to the brain via the vagus nerve and hormone secretion particularly about energy uptake.6–7

In health, microbiomes in the gut and vagina are separated from the host by a multi-level barrier supported by immune cells neutralising pathogens. Failure of this barrier either in gut or vaginal epithelia can cause low-grade chronic inflammation leading to cardiovascular

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disease, inflammatory bowel disease and cancer.7–9 Cancer itself can cause inflammation leading to dysbiosis, creating a positive feedback loop promoting disease.8,10

The gastrointestinal microbiome is an ecosystem of up to 1,000 bacterial species in any one individual.11 In health, 90% of the total gastrointestinal microbiome is populated by five major bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Fusobacteria.9 The composition within individuals remains stable for 60% of species over time.12–13

The gut microbiome has a high diversity in healthy people. There is a clear link between gut microbiotic composition and pathological states, eg inflammatory bowel disease5,14–15 The composition of the gut microbiome is less diverse in obese people who have a higher proportion of Firmicutes and fewer Bacteriodes.16–17. There is reduced diversity in people with diarrhoea after pelvic radiotherapy.13,18

A healthy vaginal microbiome is typically populated by aerobic members of the Firmicutes phylum, dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jensenii*.19 Alterations in the vaginal microbiome lead to conditions such as bacterial vaginosis where vaginal lactobacilli are reduced and replaced by anaerobic bacteria.20

Research into the vaginal and cervical microbiome was initially promoted by the desire to prevent and treat radiation fever which was believed to be due to ascending infection by pathogenic vaginal bacteria and could result in delays delivering radiotherapy, dose reduction and poorer treatment outcomes. 21–29 The notion that radiotherapy sterilised the cervix was dispelled by studies analysing aerobic and anaerobic cultures.21–29 These studies however, differed in their conclusions as to the role of the genital tract microbiome in oncogenesis, whether in the presence of gynaecological malignancy it differed from that in health and the significance of changing bacterial composition during and after treatment.

Febrile morbidity in women receiving pelvic radiotherapy is no longer a concern. Prophylactic use of antibiotics before gynaecological procedures, makes risk of infection following biopsies or surgical intervention less common.30 However, even short courses of antibiotics results in reduction in gut microbiota diversity lasting six months to four years.31 The use of antibiotics and its implications for maintaining human health and resilience to disease need to be acknowledged.32

Recent efforts have focused on how the vaginal microbiome impacts on gynaecological malignancy development. Women with vaginal dysbiosis have a higher risk of developing cervical pre-neoplastic changes over time (OR 2.00; 95% CI 1.31-3.05) however, women with *Candidiasis* do not have (OR 0.42; 95% CI 0.11-1.70).33,34–38 Disturbance of the vaginal flora increases the risk of acquiring oncogenic Human Papillomavirus (HPV)33,38 which plays a causal role in the development of precancerous cervical intra-epithelial neoplasia and invasive cervical cancer.34,38 Although HPV infection is common in sexually-active women and may be indicative of sexual behaviour predisposing to HPV, most infections are transient. Only a small proportion of infected women develop clinically

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significant pre-invasive lesions or invasive malignant disease. HIV and Human Papilloma Virus (HPV) status is now routinely tested in women with cervical cancer.9,39

The gut microbiome produces estradiol, which may promote oestrogen-driven malignancies such as endometrial cancer.40

The intestinal and vaginal microbiome also plays a significant role in response to oncological treatments and long-term toxicity from those treatments.41–42 The number of people living with and beyond cancer has tripled over the last decade and reducing long term toxicity is a priority.43

Analysing the gastrointestinal and genital tract microbiome

Older methods relied on bacterial culture using gram staining, gas-liquid chromatography or biochemical tests. These approaches are limited by the inability to culture all bacterial species and do not detect changes in bacterial functions.

Modern research uses next generation sequencing methodologies (DNA, rDNA, rRNA) and metagenomics to analyse the DNA of the entire microbial community and to understand how radiotherapy possibly induces changes in microbial composition. However, the virome is rarely included15,44 and these techniques are complex, expensive and mostly do not measure changes in microbial function which may be as important as changes in composition.

Alternative options for analysing the gut microbiome include examining gases produced by the metabolites of the gut microorganisms, Volatile Organic Compounds (VOCs). VOCs are organic chemicals which derive from biological samples such as breath, faeces, sweat, urine or vaginal fluid and are gas phase biomarkers reflecting individuals' metabolic state.45–47 The gold standard for analysing VOCs is gas chromatography/mass spectrometry (GCMS). However, this technique is expensive and specialised and is not a viable option for day-to-day clinical practice.47

Two alternative techniques can analyse VOCs to draw a unique olfactory signature: electronic sensing (e-nose) or High Field Asymmetric Ion Mobility Spectrometry (FAIMS). These detect specific chemical compounds indicating changes in gut microbiome metabolism involved in fermentation processes.47

This review aims to summarise existing research using modern analysis methodologies on the impact of cancer treatment (surgery, chemotherapy, radiotherapy) on the gut and vaginal microbiome in women with a gynaecological malignancy.

Methods

Literature identification

This systematic review used the PRISMA guidelines to ensure transparent and complete reporting (Appendix 1).48 The review protocol was registered on the International

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PROSPERO review database: PROSPERO 2016:CRD42016047121 http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016047121.

Search strategy (figure 1)

Relevant studies were identified by one author (ACM) searching the following databases: Embase 1974 to 2016 August 16, Global Health 1973 to 2016 Week 31, HMIC Health Management Information Consortium 1979 to July 2016, Journals@Ovid Full Text August 16, 2016, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present, Ovid Medline database. The PROSPERO database and Cochrane Library were searched for existing similar systematic reviews.

To ensure that the search was comprehensive and studies had not been missed or wrongly excluded, the unpublished and grey literature, general search engines, and reference lists of included papers were checked. Contact was made with the authors of the papers when further information was required. A second reviewer (CN) independently analysed the papers.

The main search terms included: gynaecological cancer, gynaecological malignancy, gynecological cancer, gynecological malignancy, cervix cancer, endometrial cancer, uterine cancer, gut microbiome, intestinal microbiome, vaginal microbiome, vaginal swabs, surgery, chemotherapy, radiotherapy, pelvic radiotherapy, chemoradiation, sequencing, VOC analysis. Appendix 2 presents an example of the full electronic search strategy for the OVID database.

Types of studies included

Quantitative studies exploring the gut or vaginal microbiome in women treated for a gynaecological malignancy were included. There were no language restrictions. Animal studies, case reports or studies including single subjects, expert opinions and consensus statements were excluded. Due to recent advances in treatment (specifically radiotherapy) and new techniques to analyse the microbiome, studies with older conventional culturing techniques were excluded.

As a systematic review protocol assessing the impact of probiotics for the prevention or treatment of chemotherapy or radiotherapy related diarrhoea in cancer patients has been published by the Cochrane collaboration49, studies including the use of pre- or probiotics as an intervention were excluded.

Inclusion criteria were defined using the following components: Patient population (P): women treated for a gynaecological malignancy, exposure of interest (I): cancer treatment: surgery, chemotherapy or radiotherapy, comparator (C): before and after treatment for gynaecological cancer, outcome (O): the change in the gut or vaginal microbiome following treatment for gynaecological cancer and the study designs (S) of interest: randomised controlled trials (RCTs), prospective observational cohort studies and retrospective studies.

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Results

No studies were identified examining the possible effects of surgery for gynaecological cancers on the gut microbiome or charting changes in the gut microbiome of women treated with chemotherapy alone. One study included 7 women who received concomitant chemoradiation.53 Analysis of gut microbiotic profiles showed that the number of operational and taxonomic units (OTUs) decreased as well as the richness in bacterial species. However, the impact of chemotherapy alone on the gut microbiome remains unclear.

No studies have examined changes in the vaginal microbiome during treatment for gynaecological malignancies and how this may affect long-term toxicity.

No studies were identified using VOC analysis methods examining the metabolites of gut or vaginal microbiome involving women treated for gynaecological tumours.

Five studies and two abstracts were identified using sequencing to analyse the gut microbiome during treatment with pelvic radiotherapy. One study was excluded50 as it described a single case of a women treated for ovarian cancer in a cohort of 19 patients receiving chemotherapy for a range of malignancies. Both abstracts were excluded as one related to a full publication (table 1) and one described the cohort as ‘cancer patients treated with pelvic radiotherapy’. Contact with the authors revealed this study only included men treated for prostate cancer.51

The remaining studies were included and critically appraised using the Critical Appraisal Skills Programme study checklist for observational cohort studies (Table 1 and 2).52 They reported on a total of 23 women with gynaecological cancer: endometrial cancer (n=7), cervical cancer (n=16). One study included only women with a gynaecological cancer.53 The two other studies also included patients receiving treatment for gastrointestinal cancers. 18,54 It was not possible to identify outcomes for the women with gynaecological cancer separately.

Outcomes of included studies

All three studies were prospective cohort studies and included patients treated with radiotherapy in a dose of 43.0 to 54.0 Gy but did not provide specific information about radiotherapy fields, the use of boosts or brachytherapy, making comparisons difficult. Whilst all studies acknowledge that radiotherapy may result in toxicity, one study did not report gastrointestinal toxicity.53 In the other studies, the Common Toxicity Criteria (CTC) version 2.018 and the Common terminology Criteria for Adverse Events (CTCAE) version 3.054 assessed bowel function. Where stool consistency was reported, 8 women (57%) developed grade 2 or 3 diarrhoea during radiotherapy. One study also assessed levels of fatigue using the Multidimensional Fatigue Inventory-20 general fatigue score and added measurement of biochemical markers of inflammation from blood samples.54

These studies suggest a correlation between changes in the intestinal microbiome and radiotherapy. All studies showed decrease in abundance and diversity of the intestinal bacterial species. One study identified 10% decrease in firmicutes (p=0.09), 3% increase in

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fusobacteria (p=0.05) and 9.9% in unclassified bacteria (p=0.04).53 An increase in unspecified bacterial species was seen in those with diarrhoea but patients without diarrhoea maintained their bacterial profiles.18,54 Before radiotherapy, those who developed diarrhoea compared to those who did not, had increased abundance of Bacteroides, Dialister, Veillonella (p<0.01), and a decrease in Clostridium XI and XVIII, Faecalibacterium, Oscillibacter, Parabacteroides, Prevotella and unclassified bacteria (p<0.05).54

Level of evidence is classified as B.55 The quality of the studies had CASP scores of 6/11 and 7/11 (Table 2).18,53–54

Discussion

This review highlights the lack of studies mapping changes in the gut or vaginal microbiomes in women with gynaecological malignancies before, during and following treatment.

In all identified studies the rationale for the study was clearly outlined, namely that radiotherapy can result in serious toxicity and the underlying cause remains unknown. Mainly opinion papers and reviews are used in the background sections of these papers. These studies are essentially feasibility studies which strengthen the rationale for further research. This is especially relevant in view of the emerging studies evaluating probiotics to reduce gastrointestinal toxicity.56–58 These report varying success rates perhaps due to the gap in understanding the impact of multi-modal therapies for gynaecological malignancies on the microbiome. This missing information prevents us offering treatment options targeted to the individual patient.

Two studies included multi-modal cancer therapies which draws attention to the complexity in determining how different treatment interact patho-physiologically with the microbiome in gynaecological malignancy. Whilst receiving chemotherapy was an exclusion criterion for two studies18,54, another53 included seven women receiving a variety of chemotherapeutic agents.

Only two studies assessed acute gastrointestinal toxicity formally, measuring diarrhoea. There was no difference in assessing diarrhoea between the tools used.59 As the last follow-up time-point was two months after radiotherapy, longer term gastrointestinal toxicity which manifests months or years following treatment3 was not assessed and future research needs to incorporate much longer follow-up.

Limitations of the studies

These were single-centred studies. No information regarding how many patients were treated in each centre, how many patients were eligible, how eligible patients were selected or the time scale for recruitment was provided. This may have introduced selection bias. The researchers did not specify guidelines used to stop recruitment. This raises concerns about equity for potential study participants. Information about catchment areas and number of patients treated could have provided additional insight into whether the sample population is

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representative for the wider population. All studies used healthy volunteers as controls but none described the selection methods or demographic information.

In all studies, no missing data were reported although one study mentions a drop-out rate of 45%. Reporting bias may potentially result if participants only included if they were able to provide all data for inclusion of the final analysis. The inclusion of different tumour types and mixed gender cohorts complicates interpretation of the results as individual patient outcomes could not be separated for analysis. In addition, all three studies were set in different countries and lacked information about other possible confounding factors such as diet, body mass index, medications affecting the gut microbiome or gastrointestinal function, treatment with previous surgery or chemotherapy and co-morbid factors.

The discussion sections addressed a number of key areas; the researchers acknowledge the limitations of 16S rRNA sequencing as not all bacterial species can be adequately identified and support future studies with larger cohorts. Two studies excluded patients treated with antibiotics before radiotherapy whilst all studies excluded those receiving antibiotics during treatment or the use of corticosteroids and immune-suppressants. Whilst the long-term impact of the use of antibiotics resulting in sustained reductions in gut microbial diversity has been acknowledged31, excluding those patients does not reflect clinical oncological management.

Research in the emerging field of the microbiome is currently biased towards the gut microbiome and little is known about interactions with the vaginal microbiome. The viromes and fungal populations have been neglected and may be important. Several studies have examined the vaginal microbiome of women at different stages of malignancy compared to that of women without cancer34–48,60–61 However, no studies reported long-term sequential follow-up data to determine the role of changes in the vaginal microbiome during progression from pre-cancerous lesions to cancer.

The studies to date mostly employ sequencing and did not include metabonomic analysis techniques. The clinical relevance of sequencing used in isolation may be limited. One older prospective study applying electronic sensing to analyse vaginal swabs with a clinical diagnosis of bacterial vaginosis, found the positive predictive value of the test was 61.5%.45 No studies were found using VOC analysis to describe the vaginal microbiome in women with gynaecological cancer nor how treatment such as surgery, chemotherapy and pelvic radiotherapy may impact on the vaginal microbiome of these women. Inclusion of metabonomic analysis techniques offers additional avenues to develop risk stratification pathways and targeted treatment options.

Limitations of the review

This review was limited to prospective cohort studies assessing the gut and vaginal microbiome of women treated for a gynaecological malignancy. No animal studies were included. In view of recent advances in novel, more targeted radiotherapy techniques, and technology available to analyse the microbiome the findings of older studies need to be interpreted cautiously. Due to the small number of studies found and the heterogeneity in study subjects in terms of treatment modalities and reporting methods, meta-analysis was

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not performed. Registration of this review on the International PROSPERO database reduced the risk of multiple reviews addressing the same question, limited publication bias and provided transparency for updating the review in the future.48

Implications for future research

The limited evidence implies that the role of the vaginal and gut microbiome in women treated for a gynaecological malignancy are relevant and require further study.

Conclusion

The outcomes of these studies support the hypothesis that radiotherapy changes the intestinal microbiome in patients with a decrease in abundance and diversity of the intestinal bacterial species.

Further characterisation of differences and changes in the genital and gastrointestinal microbiome and intestinal function before and after treatment could improve understanding of why some patients develop more severe toxicity and why others remain symptom-free. This may lead to new avenues to stratify those at risk and explore personalised treatment options and prevention of consequences of cancer treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Cancer Research UK. Cancer statistics- new cases. 2015. Accessed via: http://www.cancerresearchuk.org/health-professional/cancer-statistics January 2017
2. Andreyev HJN, Davidson SE, Gillespie C, Allum WH, Swarbrick E. Practice guidance on the management of acute and chronic gastrointestinal problems arising as a result of treatment for cancer. Gut. 2012; 61:179–92. [PubMed: 22057051]
3. Andreyev HJN, Muls A, Norton C, Ralph C, Watson L, Shaw C, Lindsay J. Guidance: The practical management of the gastrointestinal symptoms of pelvic radiation disease. Frontline Gastroenterol. 2014; 0:1–20. DOI: 10.1136/flgastro-2014-100468
4. Taylor, A.; Fernandes, A.; Kumar, P.; Muls, A. Textbook of the European Gynae Oncology Society. 2016. Acute and chronic toxicity of pelvic radiotherapy. Chapter 138. 12pp
5. Ferreira M, Muls A, Dearnaley D, Andreyev HJN. Microbiome and radiation-induced bowel toxicity: lessons from inflammatory bowel disease for the radiation oncologist. Lancet Oncol. 2014; 15(3):e139–47. [PubMed: 24599929]
6. Bischoff S. Gut health: a new objective in medicine? BMC Medicine. 2011; 9:24–38. DOI: 10.1186/1741-7015-9-24 [PubMed: 21401922]
7. Lynch S, Pedersen O. The Human Intestinal Microbiome in Health and Disease. N Engl J Med. 2016; 375:2369–2379. DOI: 10.1056/NEJMra1600266 [PubMed: 27974040]

Europe PMC Funders Author Manuscripts

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8. Schwabe R, Jobin C. The microbiome and cancer. Nat Rev Cancer. 2013; 13(11):800–812. DOI: 10.1038/ncr3610 [PubMed: 24132111]
9. Chase D, Goulder A, Zenhausen F, Monk B, Herbst-Kralovetz M. Review: The vaginal and gastrointestinal microbiomes in gynaecologic cancers: A review of applications in etiology, symptoms and treatment. Gynecologic Oncology. 2015; 138:190–200. DOI: 10.1016/j.ygyno.2015.04.036
10. Garrett W, Gallini C, Yatsunenko T, Michaud M, DuBois A, Delaney M, Punit S, Karlsson M, Bry L, Glickman J, Gordon J, et al. Enterobacteriaceae act in concert with the gut microbiome to induce spontaneous and maternally transmitted colitis. Cell Host Microbe. 2010; 8(3):292–300. DOI: 10.1016/j.chom.2010.08.004 [PubMed: 20833380]
11. Sekirov I, Russell S, Antunes C, Finlay B. Gut microbiota in health and disease. Physiol Rev. 2010; 90:859–904. DOI: 10.1152/physrev.00045.2009 [PubMed: 20664075]
12. Guarner F, Malagelada J. Gut flora in health and disease. The Lancet. 2003; 360:512–519.
13. Floch M. Fecal Bacteriotherapy, Fecal Transplant, and the Microbiome. Journal of Clinical Gastroenterology. 2010; 44(8):529–30.
14. Hart, AL.; Ng, SC. Inflammatory Bowel Disease. T.F.M. Publications; 2011. ISBN: 9781903378823
15. Marchesi J, Adams D, Fava F, Hermes G, Hirshfield G, Hold G, Quraishi M, Kinross J, Smidt H, Tuohy K, Thomas L, et al. The gut microbiota and host health: a new clinical fonrtier. Gut BMJ. 2015; 65:330–339.
16. DiBaise J, Zhang H, Crowell M, Krajmalnik-Brown R, Decker G, Rittmann B. Gut microbiome and its possible relationship with obesity. Mayo Clin Proc. 2008; 83(4):460–469. DOI: 10.4065/83.4.460 [PubMed: 18380992]
17. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiome: the hygiene hypothesis expanded? Diabetes Care. 2010; 33(10):2277–2284. DOI: 10.2337/dc10-0556 [PubMed: 20876708]
18. Manichanh C, Varela E, Martinez C, et al. The gut microbiome predispose to the pathophysiology of acute postradiotherapy diarrhea. Am J Gastroenterol. 2008; 103:1754–61. [PubMed: 18564125]
19. Ravel J, Gajera P, Abdob Z, Schneiderc GM, Koeniga S, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA. 2011; 108(Suppl 1):4680–4687. DOI: 10.1073/pnas.1002611107
20. Van de Wijgert J, Borgdorff H, Verhelst R, Crucitti T, Francis S, Verstraelen H, et al. The Vaginal Microbiota: What Have We Learned after a Decade of Molecular Characterization? PLoS ONE. 2014; 9(8):e105998.doi: 10.1371/journal.pone.0105998 [PubMed: 25148517]
21. Mead P. Cervical- vaginal flora of women with invasive cervical cancer. Obstet Gynecol. 1978; 52(5):601–604.
22. Blythe J. Cervical bacterial flora in patients with gynecological malignancies. Am J Obstet Gynecol. 1978; 131(4):438–445. [PubMed: 352153]
23. Thadepalli H, Savage E, Rao B. Anaerobic bacteria associated with cervical neoplasma. Gynecologic Oncology. 1982; 14:307–312. [PubMed: 7152363]
24. Gerstner G, Kucera H, Weghaupt K, Rotter M. Endometrial bacteriology in patients with endometrial cancer before and after primary intracavity irradiation using IR-192 and an afterloading technique. Archives of Gynecology. 1982; 231:299–306.
25. Choo Y, Seto W, Ma H. Cervical- vaginal bacterial flora in patients with cervical carcinoma treated with irradiation and febrile morbidity during intracavity radium therapy. Aus NZ J Obstet Gynaec. 1984; 24:34–38.
26. Gilstrap L III, Gibbs R, Michel T, Hauth J. Genital aerobic bacterial flora of women receiving radiotherapy for gynecologic malignancy. Gynecologic Oncology. 1986; 23:35–39. [PubMed: 3943749]
27. Gordon A, Martens M, LaPread Y, Faro S. Response of lower genital tract flora to external pelvic irradiation. Gynecologic Oncology. 1989; 35:233–235. [PubMed: 2807016]
28. Guijon F, Paraskevas M, Rand F, Heywood E, Brunham R, McNicol P. Vaginal microbial flora as a cofactor in the pathogenesis if uterine cervical intraepithelial neoplasma. Int J Gynecol Obstet. 1992; 37:185–191.

Europe PMC Funders Author Manuscripts

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29. Mikamo H, Sato Y, Hayasaki Y, Kawazoe K, Izumi K, Ito K, Yamada Y, Tamaya T. Intravaginal bacterial flora in patients with uterine cervical cancer. High incidence of detection of Gernerella vaginalis. J Infect Chemother. 1999; 5:82–85. [PubMed: 11810495]
30. Van Eyk N, van Schalkwyk J. Antibiotic prophylaxis in gynaecologic procedures. J Obstet Gynaecol Can. 2012; 34(4):382–391. [PubMed: 22472341]
31. Macfarlane S. Antibiotic treatments and microbes in the gut. Environ Microbiol. 2014; 16(4):919–924. [PubMed: 24471523]
32. Abeles S, Jones M, Santiago-Rodriguez T, Ly M, Klitgord N, Yooseph S, Nelson K, Pride D. Microbial diversity in individuals and their household contacts following typical antibiotic courses. Microbiome. 2016; 4(1):39.doi: 10.1186/s40168-016-0187-9
33. Engberts M, Verbruggen B, Boon M, van Haaften M, Heintz A. Candida and dysbacteriosis: a cytologic, population-based study of 100,605 asymptomatic women concerning cervical carcinogenesis. Cancer Cytopathology. 2007; 111(5):269–274. [PubMed: 17823916]
34. Oh H, Kim B, Seo S, Kong J, et al. The association of uterine cervical microbiota with an increased risk for cervical intraepithelial neoplasia in Korea. Clin Microbiol Infect. 2015; 21:674.e1–674.e9. DOI: 10.1016/j.cmi.2015.02.026
35. Mitra, A.; MacIntyre, D.; Lee, Y.; Smith, A.; Marchesi, J.; Holmes, E.; Nicholson, J.; Bennett, P.; Kyrgiou, M. The vaginal microbiome of women with cervical intraepithelial neoplasia. ESGO; International Journal of Gynecological Cancer. Conference: 19th International Meeting of the European Society of Gynaecological Oncology; 2015. p. 976-977.
36. Kwasniewski W, Kotanski J, Barkzynski B, Warchol W, et al. A metagenomicapproch to characteriztion of the cervix microbiome during HPV dependent carcinogenesis. International Journal of Gynecological Cancer. 2015; 25(9 suppl 1):848.
37. Audriac-Chalfour A, Torres-Poveda K, Bahena-Román M, Téllez-Sosa J, et al. Cervical microbiome and cytokine profile at various stages of cervical cancer: a pilot study. PLoS ONE. 2016; 11(4):e0153274.doi: 10.1371/journal.pone.0153274
38. Piyathilake C, Ollberding N, Kumar R, Macaluso M, Alvarez R, Morrow C. Cervical Microbiota Associated with Higher Grade Cervical Intraepithelial Neoplasia in Women Infected with High-Risk Human Papillomaviruses. Cancer Prev Res (Phila). 2016; 9(5):357–366. DOI: 10.1158/1940-6207.CAPR-15-0350
39. Gillet E, Meys J, Verstraelen H, Verhelst R, De Sutter P, Temmerman M, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. PLoS One. 2012; 7:e45201.doi: 10.1371/journal.pone.0045201 [PubMed: 23056195]
40. Berger N. Obesity and cancer pathogenesis. Annals of the New York Academy of Sciences. 2014; 1311(1):57–76. [PubMed: 24725147]
41. Touchefeu Y, Montassier E, Nieman K, Gastinne T, Potel G, Bruley des Varannes S, Le Vacon F, de La Cochetiere M. Systematic review: the role of the gut microbiome in chemotherapy- or radiation-induced gastrointestinal mucositis - current evidence and potential clinical applications. Alimentary Pharmacology & Therapeutics. 2014; 40(5):409–421. DOI: 10.1111/apt.12878
42. Kyrgiou M, Mitra A, Moscicki A. Does the vaginal microbiota play a role in the development of cervical cancer? Transl Res. 2016; pii: S1931-5244(16)30109-8. doi: 10.1016/j.trsl.2016.07.004
43. Maher J, McConnell H. New pathways of care for cancer survivors: adding the numbers. British Journal of Cancer. 2011; 105:S5–10. [PubMed: 22048033]
44. Cadwell K. The virome in host health and disease. Immunity. 2015; 42(5):805–13. DOI: 10.1016/j.immuni.2015.05.003 [PubMed: 25992857]
45. Chandiok S, Crawley BA, Oppenheim BA, Chadwick PR, Higgins S, Persaud KC. Screening for bacterial vaginosis: a novel application of artificial nose technology. J Clin Pathol. 1997; 50:790–795. DOI: 10.1136/jcp.50.9.790 [PubMed: 9389983]
46. Shirasu M, Touhara K. The scent of disease: volatile organic compounds of the human body related to disease and disorder. Biochem. 2011; 150(3):257–266. DOI: 10.1093/jb/mvr090
47. Arasaradnam RP, Covington JA, Harmston C, Nwokolo CU. Review article: next generation diagnostic modalities in gastroenterology - gas phase volatile compound biomarker detection. Aliment Pharmacol Ther. 2014; 39(8):780–9. DOI: 10.1111/apt.12657 [PubMed: 24612215]

Europe PMC Funders Author Manuscripts

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48. Liberati A, Altman D, Tetzlaff J, Mulrow C, et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. PLoS Med. 2009; 6(7):e1000100.doi: 10.1371/journal.pmed.1000100 [PubMed: 19621070]
49. van de Wetering F, Heus P, Verleye L, van Tienhoven G, et al. Probiotics for the prevention or treatment of chemotherapy or radiotherapy related diarrhoea in cancer patients. Editorial Group: Cochrane Gynaecological, Neuro-oncology and Orphan Cancer Group. 2013; doi: 10.1002/14651858.CD008831.pub2
50. Zwielehner J, Lassl C, Hippe B, Pointner A, Switzeny OJ, Remely M, et al. Changes in Human Fecal Microbiota Due to Chemotherapy Analyzed by TaqMan-PCR, 454 Sequencing and PCR-DGGE Fingerprinting. PLoS ONE. 2011; 6(12):e28654.doi: 10.1371/journal.pone.0028654 [PubMed: 22194876]
51. Covington J, Wedlake L, Andreyev HJN, Ouaret N. The detection of patients at risk of gastrointestinal toxicity during pelvic radiotherapy, by electronic nose and FAIMS: a pilot study. Sensors. 2012; 12:1–9.
52. Critical Appraisal Skills Programme (CASP). Study checklist for observational cohort studies. Critical Appraisal Skills Programme (CASP); Oxford: 2014. Accessed via October 2016 http://media.wix.com/ugd/dded87_e37a4ab637fe46a0869f9f977dacf134.pdf
53. Nam YD, Kim HJ, Seo JG, Kang SW, Bae JW. Impact of pelvic radiotherapy on gut microbiome of gynecological cancer patients revealed by massive pyrosequencing. PLoS ONE. 2013; 8:e82659. [PubMed: 24367534]
54. Wang A, Ling Z, Yang Z, Kiela PR, Wang T, Wang C, et al. Gut Microbial Dysbiosis May Predict Diarrhea and Fatigue in Patients Undergoing Pelvic Cancer Radiotherapy: A Pilot Study. PLoS ONE. 2015; 10(5):e0126312.doi: 10.1371/journal.pone.0126312 [PubMed: 25955845]
55. Handu D, Moloney L, Wolfram T, Ziegler P, Acosta A, Steiber A. Academy of Nutrition and Dietetics Methodology for Conducting Systematic Reviews for the Evidence Analysis Library. Journal of the Academy of Nutrition and Dietetics. 2016; 116(2):311–318. DOI: 10.1016/j.jand.2015.11.008 [PubMed: 26822985]
56. Delia P, Sansotta G, Donato V, Frosina P, Messina G, De Renzis C, et al. Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol. 2007; 13:912–915. [PubMed: 17352022]
57. Giralt J, Regadera J, Verges R, Romero J, de la Fuente I, Biete A, et al. Effects of probiotic Lactobacillus casei DN-114 001 in prevention of radiation-induced diarrhea: results from multicenter, randomized, placebo-controlled nutritional trial. Int J Radiat Oncol Biol Phys. 2008; 71:1213–1219. [PubMed: 18243569]
58. Chitapanarux I, Chitapanarux T, Traisathit P, Kudumpee S, Tharavichitkul E, Lorvidhaya V. Randomized controlled trial of live lactobacillus acidophilus plus bifidobacterium bifidum in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. Radiat Oncol. 2010; 5:31.
59. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, Langer C, Murphy B, Cumberlin R, Coleman CN, Rubin P. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol. 2003; 13:176–181. [PubMed: 12903007]
60. Lemen J, Bruner D. E-55. Characterising the vaginal microbiome of gynaecologic cancer patients: an intergrative review. Oncology Nursing Forum. 2016; 43(2):61–62.
61. Verstraelen H. The association between vaginal microbiome dysbiosis with HPV acquisition, HPV persistence, and cervical intraepithelial neoplasia: a systematic review. progress PROSPERO. 2016 CRD42016035620.

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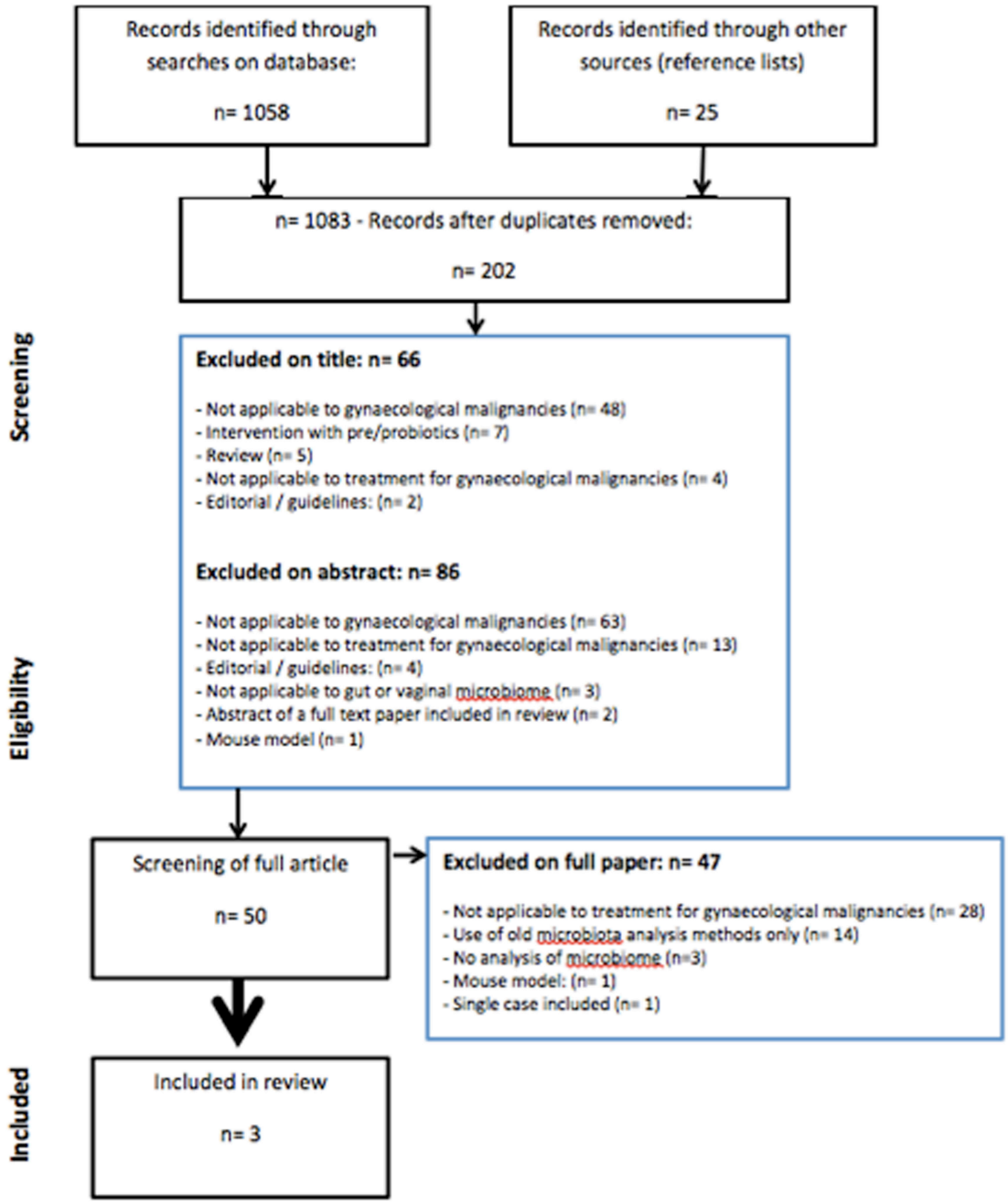

Figure 1.
Flow chart literature search

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Table 1

Studies using pyrosequencing of changes in the gut microbiome in women treated with radiotherapy for a gynaecological malignancy

Reference	Type of study	Number of patients with gynaecological tumour types	Number of healthy volunteers as control	Surgery	Chemotherapy type defined	Radiotherapy dose (Gy)	Analysis technique microbiome	Type of sample	Diarrhoea defined	No of patients reporting diarrhoea	Before- after design	Number of follow-up time points	Latest time point after radiotherapy	Antibiotic use as exclusion ?	Steroid use as exclusion?	Immunotherapy as exclusion?
Manichanh et al., 2008	Prospective cohort	6 cervix (n=1) uterus (n=1) endometrium (n=4)	4	?	N/A	43-54	16S rRNA sequencing	Stool	Yes CTC v0.2	4	Yes	4	2 weeks	Yes	Yes	Yes
	Main findings	Patients without diarrhoea maintained their microbiotic profile for 60% during RT Healthy controls and patients with no diarrhoea maintained their bacterial profiles with a similarity near 60% over the 7-wk time course. Patients with diarrhoea showed a significantly modified bacterial profile ($P < 0.05$) after the beginning of the radiotherapy (49% of similarity, SD = 17), at the end of the radiotherapy (29% similarity, SD = 17), and 2 wk after (35% similarity, SD = 15) with the detection of actinobacteria + increase in fermicutes (bacilli). Patients that develop diarrhoea clustered separately from those that did not develop diarrhoea using the Pearson's correlation and the unweighted pair-group method using arithmetic averages (UPGMA).														
Nam et al., 2013	Prospective cohort	9 cervix (n=7) endometrium (n=2)	6	?	Yes	50.4	16S rRNA sequencing	Stool	No	-	Yes	4	1-3 months	Yes	Yes	Yes
	Main findings	Compared to T0, family Eubacteriaceae in each sample at T2, and T3 were significantly decreased ($P < 0.032$). Fusobacteriaceae significantly increased at T2 and Streptococcaceae significantly increased at T1 compared to T0. Family level taxon Veillonellaceae, Enterococcaceae, Lactobacillales bacterium and Butyrate- producing bacterium at T0, T1, and T2 were not different from each other, but the T0 and T3 samples showed significant differences ($P = 0.050$). Overall richness in bacterial species decreased - decrease in firmicutes by 10% ($p= 0.09$) - increase in fusobacteria by 3% ($p=0.05$) - decrease in bacteriodes during RT but increase after 3 months (no p-value)														
Wang et al., 2015	Prospective cohort	8 cervix (n=8)	4	?	Excl	44-50	16S rRNA sequencing	Stool	Yes CTCAE v0.3	4	Yes	2	3-5 weeks	Yes	Yes	Yes
	Main findings	Patients with diarrhoea had lower microbial diversity ($p< 0.01$) Prior to radiotherapy, compared to patients who did not develop diarrhoea, those who did had increased abundance of Bacteroides, Dialister, Veillonella, and decreased abundance of Clostridium XI and XVIII, Faecalibacterium, Oscillibacter, Parabacteroides, Prevotella and unclassified (Genus: others)														

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Table 2

Appraisal of studies included using the CASP tool

Author / year	Study design	Class of evidence	CASP score	Quality grade	Population studied and sample size	Primary outcome measures / results	Author's conclusions
Manichanh et al., 2008	Prospective cohort	B	6/11	III Limited/ weak	Setting: Spain Sample: • 10 cancer patients • 5 healthy volunteers • Mixed tumour groups: Gynaecological cancer (n=6) + lower GI cancer (n=4)	Outcome measures: Stability and diversity of faecal microbiota before and after RT using similarity index and cluster analysis Comparison with healthy individuals Sub-analysis of those with and without diarrhoea GI toxicity: CTC v 0.2 Results: Patients that develop diarrhoea clustered separately from those that did not develop diarrhoea using the Pearson's correlation and the un-weighted pair-group method using arithmetic averages (UPGMA).	Mixed tumour type + mixed gender cohort, use of different radiotherapy fields, unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period. Results do not include any missing data or drop-out rates which may reflect selective reporting.
Nam et al., 2013	Prospective cohort	B	7/11	III Limited/ weak	Setting: South Korea Sample: • 9 cancer patients • 6 healthy volunteers • single tumour group: gynaecological cancer (n=9)	Outcome measures: Stability and diversity of faecal microbiota before and after RT using ANOVA for difference in OTUs and principal coordinates analysis plots Comparison with healthy individuals GI toxicity: not formally measured, discussion section mentions 8/9 patients developed diarrhoea Results: Overall richness in bacterial species decreased: decrease in firmicutes by 10% (p= 0.09), increase in	Single tumour type in cohort. Use of chemotherapy in 7/9 patients. Unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period. No formal measurement of GI toxicity but discussion informs 8/9 patients developed a degree of diarrhoea. Results do not include any missing data or drop-out rates which may reflect selective reporting.

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Author / year	Study design	Class of evidence	CASP score	Quality grade	Population studied and sample size	Primary outcome measures / results	Author's conclusions
						fusobacteria by 3% (p=0.05), decrease in bacteriodes during radiotherapy but increase after 3 months (no p-value)	
Wang et al., 2015	Prospective cohort	B	7/11	III Limited/ weak	Setting: China Sample: • 11 cancer patients • 4 healthy volunteers • Mixed tumour groups: Gynaecological cancer (n=8) + lower GI cancer (n=3)	Outcome measures: Stability and diversity of faecal microbiota before and after RT using difference in OTUs, Shannon Index, Good's coverage, Chao1 richness estimator. Comparison with healthy individuals Sub-analysis of those with and without diarrhoea GI toxicity: CTCAE v 0.3 Results: Patients with diarrhoea had lower microbial diversity (p< 0.01)	Mixed tumour type + mixed gender cohort, use of different radiotherapy fields, unable to assess whether cohort is representative as no information of eligibility, selection of participants, recruitment period. 9 patients were excluded from final analysis for various reasons which may reflect selective reporting.

Appendix 7. PRISMA Statement - Checklist of items to include when reporting a systematic review

Section/topic	*#*	*Checklist item*	*Reported on page # of review*
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	

Section/topic	*#*	*Checklist item*	*Reported on page # of review*
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see Item 12).	

Section/topic	*#*	*Checklist item*	*Reported on page # of review*
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

Appendix 8: Search strategy and example of results

```
-----Original Message-----
From: Ovid_Online@ovid.com <Ovid_Online@ovid.com>
Sent: 08 July 2020 15:44
To: Muls, Ann <ann.muls@kcl.ac.uk>
Subject: Ovid Results

Ovid Technologies, Inc. Email Service
-------------------------------
Database: Embase <1974 to 2020 Week 27>, Embase Classic <1947 to
1973>, Ovid MEDLINE(R) and Epub Ahead of Print, In-Process &
Other Non-Indexed Citations and Daily <1946 to July 07, 2020>,
Global Health <1973 to 2020 Week 26>, HMIC Health Management
Information Consortium <1979 to May 2020>, Journals@Ovid Full
Text <July 06, 2020>, APA PsycInfo <1806 to June Week 5 2020>
Search Strategy:
--------------------------------------------------------------------
---------------
1     gynaecological cancer.m_titl. (1481)
2     limit 1 to yr="2016 -Current" (391)
3     gynaecological malignancy.mp. [mp=ti, ab, hw, tn, ot, dm,
      mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy, bt, id,
      cc, tx, sh, ct, tc, tm, mh] (1471)
4     limit 3 to yr="2016 -Current" (420)
5     gynecological cancer.mp. [mp=ti, ab, hw, tn, ot, dm, mf,
      dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy, bt, id, cc,
      tx, sh, ct, tc, tm, mh] (8439)
6     limit 5 to yr="2016 -Current" (3222)
7     gynecological malignancy.mp. [mp=ti, ab, hw, tn, ot, dm,
      mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy, bt, id,
      cc, tx, sh, ct, tc, tm, mh] (2866)
8     limit 7 to yr="2016 -Current" (1093)
9     gut microbiota.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw,
      fx, dq, nm, kf, ox, px, rx, an, ui, sy, bt, id, cc, tx, sh,
      ct, tc, tm, mh] (65192)
10    limit 9 to yr="2016 -Current" (46250)
11    intestinal microbiota.mp. [mp=ti, ab, hw, tn, ot, dm, mf,
      dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy, bt, id, cc,
      tx, sh, ct, tc, tm, mh] (28340)
12    limit 11 to yr="2016 -Current" (15163)
13    microbiome.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx,
      dq, nm, kf, ox, px, rx, an, ui, sy, bt, id, cc, tx, sh, ct,
      tc, tm, mh] (99294)
14    limit 13 to yr="2016 -Current" (76732)
15    chemotherapy.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw,
      fx, dq, nm, kf, ox, px, rx, an, ui, sy, bt, id, cc, tx, sh,
      ct, tc, tm, mh] (1855551)
16    limit 15 to yr="2016 -Current" (466000)
17    radiotherapy.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw,
      fx, dq, nm, kf, ox, px, rx, an, ui, sy, bt, id, cc, tx, sh,
      ct, tc, tm, mh] (1089870)
18    limit 17 to yr="2016 -Current" (253892)
19    2 and 10 and 16 (1)
20    4 and 10 and 16 (0)
21    6 and 10 and 16 (4)
22    8 and 10 and 16 (4)
```

23 2 and 12 and 16 (0)
24 4 and 12 and 16 (0)
25 6 and 12 and 16 (1)
26 8 and 12 and 16 (1)
27 2 and 14 and 16 (1)
28 4 and 14 and 16 (1)
29 6 and 14 and 16 (10)
30 8 and 14 and 16 (5)
31 2 and 10 and 18 (0)
32 4 and 10 and 18 (0)
33 6 and 10 and 18 (3)
34 8 and 10 and 18 (3)
35 2 and 12 and 18 (0)
36 4 and 12 and 18 (0)
37 6 and 12 and 18 (1)
38 8 and 12 and 18 (1)
39 2 and 14 and 18 (0)
40 4 and 14 and 18 (3)
41 6 and 14 and 18 (10)
42 8 and 14 and 18 (5)

****
Search for: 6 and 14 and 18: Results: 10

1. Comparison of vaginal microbiota in gynecologic cancer patients pre- and post-radiation therapy and healthy women.
Tsementzi D., Pena-Gonzalez A., Bai J., Hu Y.-J., Patel P., Shelton J., Dolan M., Arluck J., Khanna N., Conrad L., Scott I., Eng T.Y., Konstantinidis K.T., Bruner D.W.
Embase Cancer Medicine. 9 (11) (pp 3714-3724), 2020. Date of Publication: 01 Jun 2020.
[Article]
AN: 2004533974
Publisher: Blackwell Publishing Ltd
Other Index Terms: adult; age; Anaerococcus; Article; Bifidobacterium; *cancer radiotherapy; cohort analysis; controlled study; dysbiosis; female; *female genital tract cancer / *radiotherapy / *surgery; Fusobacterium; human; human tissue; Lactobacillus; major clinical study; middle aged; nonhuman; Peptoniphilus; Peptostreptococcus; population abundance; Prevotella; priority journal; RNA sequence; *vagina flora; vagina pH; vaginitis.
Year of Publication: 2020

2. Comparison of Vaginal Microbiota in Gynecologic Cancer Patients Pre- and Post-Radiation Therapy and Healthy Women.
Bruner D.W., Tsementzi D., Gonzales A.P., Bai J., Hu Y.J., Liu T., Patel P.R., Shelton J.W., Dolan M., Arluck J., Khanna N., Corwin E., Mulle J.G., Konstantinidis K.
Embase International Journal of Radiation Oncology Biology Physics. Conference: The American Society for Radiation Oncology 61st Annual Meeting. United States. 105 (1 Supplement) (pp S229), 2019. Date of Publication: 1 September 2019.
[Conference Abstract]
AN: 2002634209
Conference End Date: 20190918
Publisher: Elsevier Inc.

Other Index Terms: Actinomyces; Adonis; adult; Bacillus; Bacteroides ovatus; *cancer patient; Clostridium; cohort analysis; controlled study; Corynebacterium; ethnicity; Faecalibacterium prausnitzii; female; *female genital tract cancer; genus; human; human tissue; infectious agent; library; major clinical study; Methylobacterium; microbiome; nonhuman; quality control; radiotherapy; rank sum test; reproducibility; Ruminococcus; Streptococcus anginosus; *vagina flora; vaginal injury.
Year of Publication: 2019

3. The impact of cancer treatment on the gut and vaginal microbiome in women with a gynecological malignancy.
Muls A., Andreyev J., Lalondrelle S., Taylor A., Norton C., Hart A.
Embase International Journal of Gynecological Cancer. 27 (7) (pp 1550-1559), 2017. Date of Publication: 2017.
[Article]
AN: 621682093
Publisher: Lippincott Williams and Wilkins (E-mail: kathiest.clai@apta.org)
Other Index Terms: Bacteroides; *cancer chemotherapy; *cancer radiotherapy; *cancer surgery; chemoradiotherapy; Clostridium; diarrhea; endometrium cancer / drug therapy / radiotherapy / surgery; Faecalibacterium; female; *female genital tract cancer / *drug therapy / *radiotherapy / *surgery; female genital tract cancer / drug therapy; gastrointestinal toxicity; human; *intestine flora; meta analysis; Parabacteroides; Prevotella; priority journal; systematic review; uterine cervix cancer / drug therapy / radiotherapy / surgery; *vagina flora; Veillonella.
Year of Publication: 2017

4. Systematic Review: The Impact of Cancer Treatment on the Gut and Vaginal Microbiome in Women With a Gynecological Malignancy.
[Review] Muls A; Andreyev J; Lalondrelle S; Taylor A; Norton C; Hart A.
Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily International Journal of Gynecological Cancer. 27(7):1550-1559, 2017 09.
[Journal Article. Meta-Analysis. Review. Systematic Review]
[2017/06/08 06:00]
UI: 28590950
Year of Publication: 2017

5. Anticancer effect of bacteria on cervical cancer: Molecular aspects and therapeutic implications.
Pourmollaei, Soraya a,b; Barzegari, Abolfazl c; Farshbaf-Khalili, Azizeh d; Nouri, Mohammad e,f; Fattahi, Amir e,g,h,*; Shahnazi, Mahnaz a,**; Dittrich, Ralf h
Journals@Ovid Full Text Life Sciences. 246:117413, April 1, 2020.
[Review] [Review article]
AN: 00076800-202004010-00015.

6. Risk factors for bloodstream infections in gynecological cancer.
Franza, Laura 1, 2; Costantini, Barbara 3; Corrado, Giacomo 3; Spanu, Teresa 4; Covino, Marcello 1; Ojetti, Veronica 1;

Quagliozzi, Lorena 3; Biscione, Antonella 3; Taccari, Francesco 2; Fagotti, Anna 5; Scambia, Giovanni 5; Tamburrini, Enrica 2
Journals@Ovid Full Text International Journal of Gynecological Cancer. 30(2):245-251, February 2020.
[Article] [Original research]
AN: 00009577-202002000-00015.

7. Posters.
Journals@Ovid Full Text Female Pelvic Medicine & Reconstructive Surgery. 25(5S) Supplement 1:S184-S327, September/October 2019.
[Abstract] [Abstracts]
AN: 01436319-201909001-00006.

8. Oral Abstracts.
Journals@Ovid Full Text Journal of Psychosocial Oncology Research and Practice. 1(1S) Supplement 1:e10, September 2019.
[Abstract] [Supplement Abstracts]
AN: 02091979-201909001-00002.

9. Abstracts - Oral and Poster Presentations from the XII International Congress of Dermatology April 18-22, 2017 - Buenos Aires, Argentina.
Journals@Ovid Full Text International Journal of Dermatology. 56(11):1204-1316, November 2017.
[Abstract] [Abstracts]
AN: 00004342-201711000-00022.

10. Systematic Review: The Impact of Cancer Treatment on the Gut and Vaginal Microbiome in Women With a Gynecological Malignancy.
Muls, Ann RN MSc *; Andreyev, Jervoise MA, PhD, FRCP *; Lalondrelle, Susan MD, BM, BSc, MRCP, FRCR *; Taylor, Alexandra MBBS, MRCP, FRCR MD *; Norton, Christine MA, RGN, DMS PhD +; Hart, Ailsa BMBCh FRCP, PhD ++
Journals@Ovid Full Text International Journal of Gynecological Cancer. 27(7):1550-1559, September 2017.
[Miscellaneous Article] [General]
AN: 00009577-201709000-00033.

Appendix 9. Ethical approval document

NHS
Health Research Authority
NRES Committee London - Dulwich
Health Research Authority
Skipton House
80 London Road
London
SE1 6LH

Telephone: 020 7972 2582

01 May 2015

Dr Jervoise Andreyev
Consultant in Pelvic Radiation Disease
The Royal Marsden NHS Foundation Trust
Fulham Road
SW3 6JJ

Dear Dr Andreyev

Study title:	**Using an electronic nose to predict gastrointestinal consequences of pelvic radiotherapy**
REC reference:	**15/LO/0436**
Protocol number:	**4201**
IRAS project ID:	**159187**

Thank you for your letter of 15 April 2015, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Stephanie Hill, nrescommittee.london-dulwich@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Appendix 10: Expanded Prostate cancer Index Composite Bowel Assessment

EPIC

The Expanded Prostate Cancer Index Composite

Bowel Assessment

This questionnaire is designed to measure Quality of Life issues in patients with Prostate cancer. To help us get the most accurate measurement, it is important that you answer all questions honestly and completely.

Remember, as with all medical records, information contained within this survey will remain strictly confidential.

Today's Date (please enter date when survey completed): Month_____Day_____Year_____

Name (optional): _____

Date of Birth (optional): Month_____Day_____Year_____

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Do Not Mark in This Space

BOWEL HABITS

The next section is about your bowel habits and abdominal pain.
Please consider **ONLY THE LAST 4 WEEKS**.

1. How often have you had rectal urgency (felt like I had to pass stool, but did not) **during the last 4 weeks**?

   More than once a day..... 1
   About once a day..... 2
   More than once a week..... 3 (Circle one number) 42/
   About once a week.....4
   Rarely or never..... 5

2. How often have you had uncontrolled leakage of stool or feces?

   More than once a day..... 1
   About once a day..... 2
   More than once a week..... 3 (Circle one number) 43/
   About once a week.....4
   Rarely or never..... 5

3. How often have you had stools (bowel movements) that were loose or liquid (no form, watery, mushy) **during the last 4 weeks**?

   Never..... 1
   Rarely..... 2
   About half the time..... 3 (Circle one number) 44/
   Usually..... 4
   Always.....5

4. How often have you had bloody stools **during the last 4 weeks**?

   Never.....1
   Rarely..... 2
   About half the time..... 3 (Circle one number) 45/
   Usually..... 4
   Always.....5

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Do Not Mark in This Space

5. How often have your bowel movements been painful **during the last 4 weeks**?

Never..... 1
Rarely..... 2
About half the time..... 3 (Circle one number) 46/
Usually..... 4
Always.....5

6. How many bowel movements have you had on a typical day **during the last 4 weeks**?

Two or less..... 1
Three to four.....2 (Circle one number) 47/
Five or more.....3

7. How often have you had crampy pain in your abdomen, pelvis or rectum **during the last 4 weeks**?

More than once a day..... 1
About once a day..... 2
More than once a week..... 3 (Circle one number) 48/
About once a week.....4
Rarely or never..... 5

8. How big a problem, if any, has each of the following been for you? (Circle one number on each line)

	No Problem	Very Small Problem	Small Problem	Moderate Problem	Big Problem	
a. Urgency to have a bowel movement	0	1	2	3	4	49/
b. Increased frequency of bowel movements.....	0	1	2	3	4	50/
c. Watery bowel movements.....	0	1	2	3	4	51/
d. Losing control of your stools.....	0	1	2	3	4	52/
e. Bloody stools	0	1	2	3	4	53/
f. Abdominal/ Pelvic/Rectal pain...	0	1	2	3	4	54/

9. Overall, how big a problem have your bowel habits been for you **during the last 4 weeks**?

No problem..... 1
Very small problem..... 2
Small problem.....3 (Circle one number) 55/
Moderate problem..... 4
Big problem..... 5

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Appendix 11. PREDICT Study Protocol

Protocol Template for non-CTIMP Interventional studies

Title: Using an electronic nose to predict gastrointestinal consequences of pelvic radiotherapy

Short Title: The PREDICT Study

Investigators:

Chief Investigator:	Dr. Alex Taylor
Principal Investigator:	Ann Muls
Co-investigators:	Dr. Susan Lalondrelle Dr. Jervoise Andreyev Professor James Covington Professor Ramesh Arasaradnam
Project Manager:	Amyn Lalji
Statistician:	Kabir Mohammad

Sponsor: **The Royal Marsden NHS Foundation Trust**

Address: Fulham Road, London SW3 6JJ

Management: Clinical Research & Development, The Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey SM2 5PT

Site Address:
The Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey SM2 5PT
The Royal Marsden NHS Foundation Trust, Fulham Rd, London, SW3 6JJ
The Royal Marsden NHS Foundation Trust, Kingston Hospital, Surrey

Protocol Reference:	CCR 4201
Version Number & Date:	Version 5 9th May 2017
Effective Date:	TBD
Superseded Version Number & Date (If applicable)	Version 1 – February 17th 2015 Version 2- April 09th 2015 Version 3 – August 13th 2015 Version 4 – February 11th 2016

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12.	Data Handling, Record Keeping	13
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1.0 Rationale

There have been enormous improvements in outcomes for patients diagnosed with cancer in recent years. They survive longer and the number of survivors after cancer therapy increases 3% annually in the UK and 11% in the USA. There will be 4 million cancer survivors in the UK by 2030 (Maher et al., 2011).

Patients after cancer treatments have many unmet needs (Armes et al., 2009) which are often caused by the very therapies given to cure or control the cancer. Of all the possible chronic physical side-effects of cancer treatment, gastrointestinal (GI) symptoms are the most common and not only have the greatest impact on daily activity but also frequently carry serious financial, psychological and social implications (Bacon et al., 2002, Abayomi et al., 2009).

During treatment for the cancer, GI symptoms are common and their causes are poorly researched despite their impact on patients' quality of life and the fact that when they occur they often require dose-reduction or cessation of chemotherapy and/or radiotherapy with potential impact on outcome. In addition, the GI side effect may not settle once anti-cancer treatment is stopped. In those receiving radiotherapy for a tumour in the pelvis, more severe acute reactions predispose to worse long term side effects (Wedlake et al., 2011).

17,000 British patients are treated annually with radiotherapy for pelvic cancer. Up to 80% of these patients are left with chronic alteration in GI function, 50% state that this affects their daily activity and 30% that this change in function has a moderate or severe effect (Andreyev, 2007). While progress has been made in defining optimal management of chronic changes in bowel function after cancer treatment and UK multi-professional guidance to aid clinicians has been published (Adreyev et al., 2013) it would be extremely valuable to be able to predict those in advance who might go on to develop serious problems as a result of radiotherapy so as to have the opportunity to provide them with pre-treatment counseling, potentially modify the cancer treatment and introduce toxicity modifying therapies at the earliest opportunity.

It is widely believed that technical advances in the delivery of radiotherapy will abolish GI problems however, recent compelling data demonstrate that 30% of patients treated with "perfect" radiotherapy – meeting every described constraint - still develop significant unpredicted problems (Gulliford et al., 2010). While mechanisms by which gastrointestinal symptoms occur after cancer treatment are starting to be understood and personal parameters which change the risk profile for individuals are being identified (body mass index, concomitant chemotherapy, use of a statin or ACE inhibitor, the presence of diabetes mellitus, hypertension, connective tissue disorders or HIV disease) the reasons why some patients remain symptom-free whilst others experience severe side-effects remains under-researched.

It has become clear that development of GI toxicity is not solely related to the dose and way the radiotherapy is delivered, and a phenomenon independent of the radiation called

the ‘the consequential effect’ - is a second reason why some people get chronic side effects after radiotherapy. An important driver of the ‘consequential effect’ is increasingly believed to be the microbiota, the vast numbers of bacteria that live in our guts.

The gastrointestinal microbiota are a complex ecosystem of up to 1,000 bacterial species in any one individual (Quigley et al., 2006). The species vary greatly between individuals but within each individual, the flora composition remains stable for the majority of the species over time. The diversity of the microbiota is high in healthy people and low in people with GI side effects after pelvic radiotherapy (Maninchanh et al., 2008; Floch, 2010), a process which very closely parallels findings in people with other much more intensively researched inflammatory conditions of the bowel (Ferreira et al., 2014).

Normal gut flora produce gases from their metabolites (Volatile Organic Compounds or VOCs). The gold standard for analysing volatile organic compounds is gas chromatography/mass spectrometry (GC-MS). GC-MS analyses gasses to identify all chemical components of that gas. However, this is very expensive and requires a specialised laboratory to process the samples hence is not a viable option in day-to-day clinical practice (Probert et al., 2009; Arasaradnam et al., 2014).

Two alternative techniques can be applied to analyse those VOCs: electronic sensing (e-nose) or High Field Asymmetric Ion Mobility Spectrometry (FAIMS).

Electronic sensing techniques are applied via an instrument (electronic nose) that attempts to replicate the biological olfactory system, by investigating samples as a whole, instead of identifying specific chemicals within a complex sample. The air above the sample (headspace) is drawn into the e-nose and passed across an array of chemical sensors. The size of the array varies, but most are between 6 and 32 sensors. As each sensor is dissimilar, the interaction between the sensor and the sample is unique and an olfactory signature for this complex odour can be created (Covington et al., 2012).

Fields Asymmetric Waveform Ion Mobility Spectometry (FAIMS) is a new technology capable of separating gas phase ions at atmospheric pressure and at room temperature. As with the electronic nose, FAIMS can be used for the real-time analysis of complex chemical components, looking at the total chemical composition of a sample. Differences in the way the ionised molecules move in high electric fields are used to draw a mobility signature of a complex sample (Covington et al., 2012).

The e-nose and FAIMS techniques have been used successfully in small studies to diagnose lung disease, diabetes, bladder cancer, tuberculosis, cyanide poisoning, renal failure and schizophrenia. Focusing on gastrointestinal disorders, consistent changes in the VOCs produced by patients with inflammatory bowel disease, bile acid malabsorption, gastrointestinal cancer, coeliac disease, Clostridium difficile infection can be identified compared to healthy individuals (Covington et al., 2012; Arasaradnam et al., 2014).

In 2012, we published a retrospective blinded pilot study using the e-nose in 23 patients in whom we investigated stool samples collected immediately before the start of radiotherapy. These patients were selected because 11 subsequently had minimal or no side effects from radiotherapy and 12 had severe toxicity. The e-nose analysed the samples and astonishingly identified with 100% accuracy two completely distinct groups of patients: those who would develop severe toxicity during radiotherapy and those who would not (Covington et al., 2012).

The results of this study suggest that the substantial differences in the volatile gases produced by stool samples in the two populations, have a critical impact on toxicity and the reason that different fermentation products occur is either because the microbiological composition of stool differs between the two groups, or because bacterial function is somehow different. Whatever the reason, our findings suggesting that the microbiota plays a critical role in the initiation of radiation-induced inflammation are completely consistent with findings from other studies using very complex methodologies and with studies investigating the role of the microbiota in other GI conditions (Fereira et al., 2014).

2.0 Study Plan

Study participation will be offered to women, newly diagnosed with a gynaecological malignancy who are due to be treated with pelvic radiotherapy.

<u>The recruitment period will be 3 years s and participants who are recruited in the last year will be followed up for 1 year after completion of their radiotherapy. Participants recruited in year 1 and 2 will complete 2 year follow-up.</u>

There will be 5 or 6 main time points of data and sample collection: one before treatment (if patients are treated with a course of chemotherapy, there will be the option to collect samples after chemotherapy but before the start of radiotherapy), 2 during treatment (beginning and end of radiotherapy) and 3 after treatment (at 6, 12 and 24 months). The 6th time point at 24 months after completing treatment will be omitted for those recruited in year 3.

The samples will be analysed using metabonomics, testing the electronic sensing technique and the FAIMS technique. A subset will be compared against the golden standard of GC-MS. The test results will be analysed to identify changes in olfactory signatures in relation to the GI toxicity scores of the participants.

The interpretation of the results will show whether there is a predictive value in measuring GI toxicity with electronic sensing or the FAIMS technique and whether this can be applied before treatment with pelvic radiation starts.

The findings of the study will be disseminated via published journal articles, abstracts and conference presentations.

For a schematic representation of the study plan, please refer to appendix 1.

3.0 Study Objectives

Primary objective:

To identify the predictive marker of chronic radiation induced GI toxicity using the olfactory signatures.
Secondary objectives:

- To identify the optimal time point to predict acute and chronic GI toxicity.

- To determine which of the samples (rectal swab, stool sample, urine sample, saliva sample) will best predict chronic GI toxicity.

Over 2 years we will recruit 100 patients undergoing radical radiotherapy for a new, histologically proven, gynaecological malignancy at the Royal Marsden Hospital. Patients will be followed up for 24 months. Patients will be asked to provide blood, saliva, urine, stool samples and a rectal and vaginal swab (optional). They will also complete symptom assessment questionnaires at each time point: baseline (before treatment), twice during radiotherapy, 6 months after completion of radiotherapy and 12 and 24 months after completion of treatment. The 6th time point at 24 months after treatment will be omitted for those recruited in year 3.

Participants will be requested to complete a 7-day food diary and GI toxicity will be assessed using the Inflammatory Bowel Disease Questionnaire subset B (IBDQ-B), the St. Mark's Incontinence score and the Bristol Stool Chart. The Common Terminology Criteria for Adverse Events (CTCAE) (version 3.0) scores will be completed by the clinician.

4.0 Inclusion/ Exclusion Criteria

Inclusion criteria:

- Patients aged 18 years or above able to give informed consent
- Patients to be treated with radical, adjuvant or neo-adjuvant radiotherapy for a diagnosis of a gynaecological cancer.
- Ability of the patient to provide informed consent.

Exclusion criteria:

- Patients aged less than 18 years
- Patients who are pregnant
- Patients unable to give informed consent
- Patients due to have their post-treatment follow-up elsewhere in the country
- Patients on studies with conflicting end-points

5. 0 End Points

Patients will be assessed pre-treatment (baseline), twice during radiotherapy, and at 6 months, 12 months and 24 months after completion of radiotherapy.

Primary endpoint:
To describe the differences in olfactory signatures in different samples (rectal swab, stool sample, urine, sample saliva sample) between patients who develop severe toxicity* and those with no or minimal toxicity±.

GI toxicity will be determined by:
- Change in the score of the Inflammatory Bowel Disease Questionnaire Subset B (IBDQ-B)
- Change in St Mark's Incontinence Score
- Change in Common Terminology Criteria for Adverse Events (CTCAE) (version 3.0)
- Change in the Bristol Stool Chart parameters (stool consistency)

*± please refer to point 6.0 for a definition of severe and minimal or no toxicity.

Secondary endpoints:

- the olfactory signatures of all time points will be analysed to identify the timing of best predictive ability.

- Signatures of different sampling methods will be analysed separately to identify the highest predictive yield for chronic GI toxicity.

- ***Rationale for selection of questionnaires to measure outcomes***

<u>1) Inflammatory Bowel Disease Questionnaire - Bowel subset (IBDQ-B)</u>
The IBDQ is a quality of life (QoL) tool which groups 32 questions into 4 dimensions: bowel, systemic, social, and emotional (32). ten of these 32 questions make up the bowel subset questionnaire score which can range from 10 (poorest QoL) to 70 (best QoL). Higher scores indicate a better QoL. The IBDQ has a good internal consistency, with a Cronbach α of 0.85 for the bowel-related quality of life items (Palis et al., 1999).

Previous research using the (IBDQ-B) to assess GI symptoms in patients undergoing pelvic radiotherapy has shown that the IBDQ-B is a more sensitive tool than the more commonly used RTOG (Radiation Therapy Oncology Group) questionnaire in the acute setting and the RTOG and LENT SOMA questionnaires in the chronic setting after radiotherapy (Khalid et al., 2006). Pelvic radiotherapy induces consistent patterns of toxicity. A change in IBDQ-B score measured prospectively in a recent cohort of 60 patients before pelvic radiotherapy and during the 4th week of treatment ranged from -35 to +8. More than one quarter (25-35%) of patients developed severe toxicity (fall of >10 points) and between one third and a half (30-55%) developed moderate but still clinically significant toxicity (IBDQ-B fall of >6).

<u>2) St Mark's Incontinence Questionnaire</u>
The St. Mark's Incontinence Questionnaire assesses the severity of faecal incontinence and contains items about the type (gas, fluid, solid) and frequency of incontinence, in addition

to addressing social invalidation, the need to wear a pad or plug, the use of constipating medication and the presence of urgency. The total score on the St. Mark's scale ranges from 0 (complete continence) to 24 (complete incontinence) (Vaizey et al., 1999). Previous studies showed that the St. Mark's Incontinence Questionnaire is a more sensitive tool than all other currently available incontinence scales (Khalid et al., 2006; Olopade et al., 2005). Table 1 demonstrates the scoring method and a score of 12 or higher out of 24 will be considered as severe.

	Never	*Rarely*	*Sometimes*	*Weekly*	*Daily*
Incontinence for solid stool	0	1	2	3	4
Incontinence for liquid stool	0	1	2	3	4
Incontinence for gas	0	1	2	3	4
Alteration in lifestyle	0	1	2	3	4
				No	*Yes*
Need to wear a pad or plug				0	2
Taking constipating medicines				0	2
Lack of ability to defer defecation for 15 minutes				0	4

Never, no episodes in the past four weeks; rarely, 1 episode in the past four weeks; sometimes, >1 episode in the past four weeks but <1 a week; weekly, 1 or more episodes a week but <1 a day; daily, 1 or more episodes a day.
Add one score from each row: minimum score = 0 = perfect continence; maximum score = 24 = totally incontinent.

Table 1. The St Mark's Incontinence questionnaire.

3) The Common Terminology Criteria for Adverse Events (CTCAE)

The Common Terminology Criteria for Adverse Events (CTCAE), is a standardised classification of side effects used in assessing drugs for cancer therapy developed by the National Cancer Institute. Currently version 3.0 is in use at the Royal Marsden (Trotti 2003). Most US and UK drug trials base their observations on this system which has a range of grades from 1-5. Specific conditions and symptoms may have values or descriptive comment for each level, but the general guideline is:

1 - Mild
2 - Moderate
3 - Severe
4 - Life threatening
5 – Death

The subset relating to gastrointestinal toxicity of treatment for cancer will be used. A score of 3 or above is considered as severe toxicity.

4) The Bristol Stool Chart

The Bristol stool chart categorises the types of stool a person passes into 7 categories (Lewis et al., 1997). It is a helpful tool to assess stool consistency in combination with other tools assessing GI symptoms (Heaton, 2009). It does not describe the severity of toxicity so this tool has no cut-off value.

Study measurements

	Baseline before treatment	***If having chemotherapy, before radiotherapy (optional)***	***During radiotherapy (1)***	***During radiotherapy (2)***	***6 months after completion of treatment***	***12 months after completion of treatment***	***24 months after completion of treatment for those recruited in year 1 and 2***
Patient characteristics	***X***						
Treatment characteristics	***X***						
Stool sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Urine sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Rectal swab (optional)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Blood sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Saliva sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Vaginal swab	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Inflammatory Bowel Disease Questionnaire	***X***	***X***	***X***	***X***	***X***	***X***	***X***
St. Mark's incontinence score		***X***	***X***	***X***	***X***	***X***	***X***
GI section of the Common Terminology Criteria for Adverse Events (CTCAE) (version 3.0) score	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Bristol Stool Chart parameters	***X***	***X***	***X***	***X***	***X***	***X***	***X***
7-day food diary	***X***	***X***	***X***	***X***	***X***	***X***	***X***

6.0 Sample Size and Statistical Consideration

In the last year, just under 1,000 patients were referred to the Royal Marsden Hospital (RMH) with gynaecological malignancies and referral rates are increasing. Of the 780 patients treated and alive at one year, 129 would have been eligible for this study. The recruitment rate for many previous studies in this population group performed by our team has consistently been between 30-40% of eligible women. This suggests that annual recruitment of 60 patients (or 5 per month), 100 patients over 2 years may be feasible.

Our pilot study in 23 patients showed 100% accuracy in identifying from their olfactory signatures according to severity of toxicity. Toxicity was determined using the bowel subset score of the modified Inflammatory Bowel Disease Questionnaire (IBDQ).

The IBDQ was developed for use in monitoring disease activity in Ulcerative Colitis and Crohn's Disease (Guyatt et al., 1989). It has been validated for use in primary, secondary and tertiary care and is useful in different ethnic groups (Yoshida, 1999; Cheung et al., 2000; Rubin et al., 2004). We have shown that the IBDQ is a simpler, and more sensitive measure of quality of life and gastrointestinal symptomatology than the RTOG toxicity scale (a standard tool for measuring toxicity) in patients undergoing radiotherapy (Khalid et al., 2006) and is simpler to use and more sensitive than LENT SOMA in patients with chronic radiation-induced bowel symptoms (Olopade et al., 2005). The IBDQ scores severity of disease on bowel, emotional and social function. The IBDQ-B is that part of the questionnaire which scores bowel function. A change in IBDQ-B score of more than 6 points is clinically significant from a patient perspective (Hlavaty et al., 2006).

A recent cohort of IBDQ-B scores in 60 patients undergoing pelvic radiotherapy for a gynaecological malignancy showed that 59% of patients experienced significant worsening of bowel function of more than 6 points and for 21% this did not return to baseline 1 year after completion of radiotherapy. Before radiotherapy, the mean IBDQ-B score was 67 (range: 39-70, SD 5.9), at the end of pelvic radiotherapy 58.8 (range: 33-70, SD 9.3), one year after radiotherapy, 62.8 (range: 19-70 SD 10.3) [a lower score indicates greater toxicity].

This proposal is for an observational study, which will not be powered to a specific end-point but the following assumptions are made to justify the number of patients we will recruit. With data on 100 patients available to analyse (we expect 15% to be lost to follow up), 25-33% will develop acute severe GI toxicity, 30-55% moderate toxicity and 12-45% little or no toxicity. Based on the retrospective pilot study results and assuming at least 80% discriminatory ability, this prospective study will be able to demonstrate that the olfactory signatures created by the electronic sensing technique can discriminate levels of acute toxicity and how that relates to long-term toxicity at 1 and 2 years after completion of treatment.

In addition to obtaining signature profiles of analysed emitted volatiles and gases produced by gut microflora fermentation processes, the volatile gases will be subjected to Gas Chromatography Mass Spectometry (GCMS). Profiles obtained will provide the identity of those individual metabolite components of the microbiota which discriminate between patients with severe vs. patients with the least or no GI toxicity (Covington et al., 2012).

Statistical analysis of the differences in signature profiles between groups (severe vs. least/no GI toxicity) will be performed by calculating diversity indices in collaboration with

the engineering department at Warwick University. Multivariate statistical analysis such as Linear Discriminant Analysis will be employed to associate the individual metabolite components with all available metadata.

Summary statistics will summarise categorical data. Exploratory sub-group analysis will allow for the impact of concomitant chemotherapy and surgical interventions affecting bowel motility. For testing of statistical hypotheses a significance level of 5% will be used and all statistical tests will be two sided.

7.0 Data acquisition

The study has been designed with input from patients and carers who will be invited to take part in the stakeholder meetings. All patient literature produced in conjunction with this protocol has additionally been reviewed for readability by the Patient and Carers Advisory Group (PCAG) and the Patient Liaison Service (PALS). The trial will be conducted in compliance with the protocol, standard operating procedures, RMH Trust policies, local R&D management guidance, Good Clinical Practice including the Research Governance Framework 2005 (2nd edition) and other applicable regulatory requirement(s) including but not limited to the Human Tissue Act 2004, Human Tissue (Quality and Safety for Human Application) Regulations 2007, the Medical Devices Regulations 2002, Ionising Radiation (Medical Exposures) Regulations 2000 as amended from time to time.

8.0 Data analysis

In addition to study toxicity data, the study will also collect the following:

- Observational (study measurement data) data
- Recruitment data (rate of acceptance and reasons for declining study entry)
- Adverse events (in accordance with RMH required reporting Standard Operating Procedures)
- Withdrawals (investigator and patient-initiated)

Signature profiles of analysed emitted volatiles and gases produced by gut microflora fermentation processes will be obtained by using the electronic sensing technique and FAIMS. This data will provide the identity of individual metabolite components of the microbiota able to discriminate the different patient groups (patients with severe vs. patients with the least or no GI toxicity) (Covington et al., 2012).

Statistical analysis of the differences in signature profiles between both patient groups (with severe vs. with the least or no GI toxicity) will be performed by calculating diversity indices. Multivariate statistical analyses: Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) or Fisher Discriminant Analysis (FDA) will be employed to associate the individual metabolite components with all available metadata (Covington et al., 2012). Principal components calculations will use correlation matrices. Statistical relationships between data sets and disease state will be examined by General Linear Modeling of response (metabolite components) and explanatory (disease state, time since treatment) variables. This will enable comparison of the signature profiles of the

microbiotic metabolites between patients with severe GI toxicity after pelvic radiotherapy and patients with the least or no GI toxicity.

Summary statistics such as mean, standard deviation, median, minimum and maximum will be used to summarise quantitative data. Where appropriate, means will be presented together with 95% confidence intervals. Summary statistics such as number of events and percentages will be used to summarise categorical data. Where appropriate, proportions will be presented together with 95% confidence intervals. For testing of statistical hypotheses a significance level of 5% will be used and all statistical tests will be two sided.

The IBDQ-B scores at 12 and 24 months will be used to determine toxicity level for those recruited in year 1 and 2. For those recruited in year 3, the 12 month time point will be used.
The ability of the electronic sensing technique and FAIMS in discriminating the patients based on signature profiles assessments at baseline, 2 and 6 weeks will be assessed using PCA factors plots.

9.0 Study Organisation

The following study-related responsibilities have been defined:

- Overall responsibility: Chief Investigator (CI)
- Responsibility for patients: Principal investigator (PI) / Research Nurse
- Monitoring study progress: Chief Investigator, Principal investigator (PI)
- Day-to-day running: Principal investigator (PI) / Research Nurse
- Data collection: Principal Investigator (PI) / Research Nurse
- Consent forms: Principal investigator (PI) / Research Nurse
- Data analysis: Project manager and Statistician
- Data storage: Chief Investigator / Project Manager
- Intellectual property: Chief Investigator, Principal investigator (PI)

There will be a quarterly progress meeting for all stakeholders in the study to discuss all aspects of the study's progress and any issues encountered. There will be monthly progress meetings run by the Chief Investigator to review the day to day running and progress of the study. All study personnel have received their Good Clinical Practice (GCP) certification and is updated every three years. The Project Manager will maintain a Study Site File in which all critical study documentation including study protocol, correspondence with Local Research and Ethics Committee (LREC) and the RMH Committee for Clinical Research (CCR), study amendments, copies of Adverse Event forms, CVs and GCP certificates for all study personnel will be held. The study has also been adopted in the NIHR research portfolio.

Following notification in writing to the PI of CCR/Research & Development (R&D) approval the study will be activated on the Hospital Information System and the PIs and study coordinator notified that the study is open for recruitment. This will be classified as the

start date for the R&D database. The study will be deemed to have reached completion at 51 months after the last patient has been recruited. The final questionnaires are due at 48 months and an extra three months has been added to allow for scheduling delays and completion of the investigations and treatment.

10.0 Adverse Events

It is anticipated that Serious Adverse Events will not occur since this is a non-Clinical Trial (non-CTIMP) study. However, all adverse events defined as those resulting in death, as being life-threatening, requiring hospitalisation or prolongation of hospitalisation, resulting in persistent or significant disability or incapacity to a study participant will be reported in line with Trust's Generic SOP for Adverse Events Reporting for Non-CTIMP Trials sponsored and hosted by RMH/ICR (gSOP-03-03)(gSOP-03-03).

Withdrawals from the study may be investigator-led or patient-led. Patients expressing a wish to withdraw from the study will be asked if data up to that point can be used and they will be withdrawn in line with RMH procedures for reporting study withdrawal. Participants will be assured that they can withdraw from the study without giving a reason and that this will have no impact on any aspect of their current or future care. Reasons for withdrawal will be captured within the study database and reported, if volunteered by the participant.

11.0 Ethical Considerations

After gaining CCR approval this protocol will be submitted to the LREC for consideration and will not proceed unless approval has been granted.

12.0 Data Handling, Record Keeping and Patient Confidentiality

Data collection will be rigorously undertaken in an organised and clearly documented way in accordance with the trust policy for the use of computerised databases for storing and processing personal and business critical data (policy 1758) and the Data Protection Act 1998. Documentation will be backed up on the main computer server in a password protected area to ensure no data are lost.

Patients will be allocated a study number. All information which is collected during the course of the research will be coded and kept strictly confidential. Patients will not be identified in any report or publication as per the Data Protection Act 1998 and also in accordance with the confidentiality code of practice and data protection policy and procedure. Sensitivity to the patient's needs and wishes will be paramount, and all details will be recorded in confidence and discussed within a quiet, closed room.

13.0 Financing, Indemnity & Insurance

This study is funded by a NIHR clinical fellowship grant.

For this study, the Royal Marsden NHS Foundation Trust is the sponsoring organisation. The NHS Litigation Authority will cover standard clinical negligence by employees, staff and health professionals. Further information is available at the following website: www.clinical-medical-negligence-injuries.co.uk

There is unlimited liability and no excess. Insurance is provided under the Clinical Negligence Scheme for Trusts and there is no cover for non-negligence claims. For all notification of claims please contact the Board Secretariat.

14.0 Dissemination of findings

This study will be eligible for inclusion in the NIHR portfolio and this will allow dissemination of the results to health professionals in the UK using NIHR publications and websites. The results of this research will also be submitted for presentation at key oncology conferences and for publication in multidisciplinary academic journals.

We also feel it is crucial that people considering pelvic radiotherapy are made aware of new advances. Since 2005, our team has brought together and been meeting quarterly with a national coalition of charities (Bowel Cancer UK, CORE, CRUK, Jo's Trust, Macmillan Cancer Support, Prostate Cancer UK, and PRDA) to promote improvements in management of consequences of cancer treatment. One result of this coalition is that the British Society of Gastroenterology has recently designated GI consequences of cancer treatment as one of 3 national priorities. We will help this group produce user-friendly lay summaries for their websites, helplines and patient information sheets.

References

Abayomi J, Kirwan J, Hackett A. The prevalence of chronic radiation enteritis following radiotherapy for cervical or endometrial cancer and its impact on quality of life. Eur J Oncol Nurs. 2009;13:262-7.

Andreyev HJN. Gastrointestinal problems following pelvic radiotherapy: the past, the present and the future. Clinical Oncology. 2007;19(10):790-9.

Andreyev HJN, Davidson SE, Gillespie C, Allum WH, Swarbrick E. Practice guidance on the management of acute and chronic gastrointestinal problems arising as a result of treatment for cancer. Gut. 2012;61:179-92.

Andreyev HJN, Benton BE, Lalji A, Norton C, Mohammed K, Gage H, et al. Algorithm-based management of patients with gastrointestinal symptoms in patients after pelvic radiation treatment (ORBIT): a randomised controlled trial. The Lancet. 2013;382(9910):2084-92.

Arasaradnam RP, Covington JA, Harmston C, Nwokolo CU. Review article: next generation diagnostic modalities in gastroenterology - gas phase volatile compound biomarker detection. Aliment Pharmacol Ther. 2014;Feb 20.

Armes J, Crowe M, Colbourne L, Morgan H, Murrells T, Oakley C, et al. Patients' supportive care needs beyond the end of cancer treatment: a prospective, longitudinal survey. J Clin Oncol. 2009;27(36):6172-9.

Bacon C, Giovannucci E, Testa M, Glass T, Kawachi I. The association of treatment-related symptoms with quality-of-life outcomes for localized prostate carcinoma patients. Cancer. 2002;94(3):862-71.

Cheung W, Garratt A, Russell I, Williams J. The UK IBDQ-a British version of the inflammatory bowel disease questionnaire. development and validation. J Clin Epidemiol. 2000;53(3):297-306.

Covington J, Wedlake L, Andreyev HJN, Ouaret N. The detection of patients at risk of gastrointestinal toxicity during pelvic radiotherapy, by electronic nose and FAIMS: a pilot study. Sensors. 2012;12:1-9.

Ferreira MR, Muls AM, Dearnaley DP, Andreyev HJN. Microbiota and radiation-induced bowel toxicity: lessons from inflammatory bowel disease for the radiation oncologist. Lancet Oncol. 2014;15(3):e139-47.

Floch M. Fecal Bacteriotherapy, Fecal Transplant, and the Microbiome. Journal of Clinical Gastroenterology. 2010;44(8):529-30.

Gulliford SL, Partridge M, Sydes MR, Andreyev J, Dearnaley DP. A comparison of dose-volume constraints derived using peak and longitudinal definitions of late rectal toxicity. Radiother Oncol. 2010;94(2):241-7.

Guyatt G, Mitchell A, Irvine E, Singer J, Williams N, Goodacre R, et al. A new measure of health status for clinical trials in inflammatory bowel disease. Gastroenterology. 1989;96:804-10.

Heaton K. Response to Constipation in Adults, BMJ; 2009. Accessed March 2014 via: http://www.bmj.com/rapid-response/2011/11/02/bristol-stool-chart-and-bristol-stool-form-scale

Hlavaty T, Persoons P, Vermeire S, Ferrante M, Pierik M, Van Assche G, et al. Evaluation of short-term responsiveness and cutoff values of inflammatory bowel disease questionnaire in Crohn's disease. Inflamm Bowel Dis. 2006;12(3):199-204.

Khalid U, McGough C, Hackett C, Blake P, Harrington KJ, Khoo VS, et al. A modified Inflammatory Bowel Disease Questionnaire and the Vaizey Incontinence Questionnaire are more sensitive measures of acute gastrointestinal toxicity during pelvic radiotherapy than RTOG grading. Int J Radiat Oncol Biol Phys. 2006;64(5):1432-41.

Lewis S & Heaton K. Stool form scale as a useful guide to intestinal transit time. Scand. J. Gastroenterol. 1997; 32 (9): 920–4.

Maher J, McConnell H. New pathways of care for cancer survivors: adding the numbers. British Journal of Cancer. 2011;105:S5-10.

Manichanh C, Varela E, Martinez C, Antolin M. The gut microbiota predispose to the pathophysiology of acute postradiotherapy diarrhea. Am J Gastroenterol. 2008;103(7):1754-61.

Olopade FO, Norman AR, Blake P, Dearnaley DP, Harrington KJ, Khoo V, et al. The inflammatory bowel disease questionnaire and the Vaizey incontinence questionnaire are useful to identify gastrointestinal toxicity after pelvic radiotherapy. British Journal of Cancer. 2005;92(9):1663-70.

Palis AG, Mouzas IA, et al. The Inflammatory Bowel Disease Questionnaire: a review of its national validation studies. Inflamm Bowel Dis. 2004; 10(3): 261-9.

Probert CS, Ahmed I, Khalid T, Johnson E, Smith S, Ratcliffe N. Volatile organic compounds as diagnostic biomarkers in gastrointestinal and liver diseases. J Gastrointestin Liver Dis. 2009;18(3):337-43.

Quigley E, Quera R. Small Intestinal bacterial overgrowth: role of antibiotics, prebiotics and probiotics. Gastroenterology. 2006;130:S78-90.

Rubin G, Hungin A, Chinn D, Dwarakanath D. Quality of life in patients with established inflammatory bowel disease: a UK general practice survey. Aliment Pharmacol Ther. 2004;19(5):529-35.

Trotti A, Byhardt R, Stetz J, Gwede C, Corn B, Fu K, Gunderson L, McCormick B, Morris M, Rich T, Shipley, W and Curran W. Common toxicity criteria: version 2.0. An improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. International Journal of Radiation Oncology Biology Physics 2000; 47: 13-47.

Vaizey CJ, Carapeti E, Cahill JA, Kamm MA. Prospective comparison of faecal incontinence grading systems. Gut. 1999; 44: 77–80.

Wedlake LJ, Andreyev HJN. Manipulating the consequential effect: an alternative approach to reducing pelvic radiation disease other than dose reduction. Curr Opin Support Palliat Care. 2011;5(1):25-8.

Yoshida E. The Crohn's disease activity index, its derivatives and the Inflammatory Bowel Disease Questionnaire: a review of instruments to assess Crohn's disease. Canadian J Gastroenterology. 1999;13(1):65-73.

Appendix 1: Schematic representation of the PREDICT study plan.

Women newly diagnosed with a gynaecological malignancy

Treatment with pelvic radiotherapy

Before treatment		Optional before radiotherapy if having chemotherapy	during radiotherapy (1)	during radiotherapy (2)	6 months after completion of radiotherapy	12 months after completion of radiotherapy	24 months after completion of radiotherapy for those recruited year 1-2.

Clinical & treatment characteristics
+
Sample collection: blood + rectal swab + stool + urine + saliva
+
GI toxicity: severity + impact on QoL
(IBDQ-B, St Mark's Incontinence Score, CTCEA v3, Bristol Stool Chart)
+
Metabonomic sample analysis: electronic sensing + FAIMS
(+ subset of GC-MS to verify with gold standard)
+
Identification of differences in olfactory signatures of samples

Predictive value + risk stratification

Dissemination of study results

Appendix 12. Study information leaflet for participants

The ROYAL MARSDEN
NHS Foundation Trust

PATIENT INFORMATION SHEET

The PREDICT Study: Using an electronic nose to predict gastrointestinal consequences of pelvic radiotherapy

Introduction

We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. Your doctor will be happy to go through the information sheet with you and answer any questions you have or give you more information about the study. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Why have I been invited to take part?

You have been invited to take part in this study because you are a patient at The Royal Marsden NHS Foundation Trust, have a diagnosis of a gynaecological cancer and will receive radiotherapy as part of your treatment. This study aims to recruit 120 women who have been diagnosed with gynaecological cancer and who will be treated with radiotherapy with or without other treatments

What is the purpose of the study?

Scientists have developed an instrument which works like an "electronic nose". It is able to "sniff" smells and separate different smells by their electronic "signature". Studies using an electronic nose strongly suggest that smelling samples taken from humans (eg urine/ stool/ sweat/ tears) can identify different electronic smell signature from people with different diseases and in the future might be a new and easier way to diagnose serious conditions at an earlier stage.

In a very small study, we showed that using an electronic nose to sniff a stool sample does seem to identify people - before they have had any radiotherapy – who will go on to get serious bowel side effects of radiotherapy. If this finding is correct, this is very important as it would allow the cancer doctors the option to change the way they give radiotherapy if they knew that a person was at very high risk of serious side effects from the treatment and to start treatment for the side effects at a much earlier stage.

In this study we want to confirm in a larger study whether our previous findings are correct, see whether we get the same result by sniffing urine rather than stool (that would be much easier for everyone) and identify exactly which part of the complicated "smell" signature is different in the people who will get side effects. If we know this, we might be able to identify why people's urine and faeces emit this smell and then do something about changing the smell before treatment starts. The likeliest cause for the production of a smell which predisposes to side effects is a specific group of bacteria living in the bowel. If we can identify which bacteria these are, there are many possible ways of changing these bacterial populations in advance of radiotherapy.

Enormous improvements have been made in treating cancer in recent years leading to hugely improved survival, however, treatment not infrequently can lead to side effects. Of all the possible long-term physical side-effects of cancer treatment, gastrointestinal (GI) symptoms are the most common and can have a great impact on daily activity. It is becoming increasingly clear that development of side effects in the bowel is not just related to the dose and way the radiotherapy is delivered. An important finding in this field is the role of bacteria that live in our guts (called microbiota). The gastrointestinal microbiota are very important in keeping us well. Up to 1,000 bacterial types will be found in any one individual. The species vary greatly between individuals but for each individual person, the types of bacteria which are living in their bowels remains largely the same over time. We already know that the variability in the types of bacteria is high in healthy people and much lower in people with GI side effects after pelvic radiotherapy. This study will help us better understand how the bacteria in the gut impact on changes in bowel function after radiotherapy to a tumour in the pelvis and how we can predict who will experience these changes. Your participation will significantly improve our ability to help people avoid side effects of radiotherapy in the future.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to change your mind or withdraw at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

If you agree to take part, there will not be any extra visits to the hospital in this study. The researchers will ensure they see you at visits already scheduled for your treatment or follow-up.

We will request some samples and complete some questionnaires at different time points throughout the study.

For the collection of the samples we will provide a simple collection kit together with instructions as to when and how to collect the samples, at home immediately before your next appointment. All samples will be frozen for immediate storage in our hospital tissue bank and will be sent for analysis when we have collected enough samples. Some of the analysis of the samples will be done with scientists working in laboratories not at the Royal Marsden. All samples will be coded and will not have your name on it when it goes for analysis. Results of the analysis of all samples will be strictly confidential and no one working on the samples will know that they come from you.

What do I have to do?

We will request some samples and complete some questionnaires at different time points throughout the study.

We will ask you to provide us with a blood sample, a saliva sample, a swab from your bottom and vagina (we will teach you how to do this), stool and urine samples at different occasions.

The time points are: before you start your radiotherapy, at the beginning and end of your course of radiotherapy, and then 6 months and finally 12 months after finishing your radiotherapy.

In addition to that we will also request you to complete 3 short questionnaires which ask about how you feel and how your bowels are working and a diary of the things you have eaten over a week (our diets influence the type of bacteria which live in our bowels). The 7- day food diary will only take a few minutes per day at the time points when you are noting down what you are eating. The questionnaires are unlikely to take more than 20 minutes of your time and will be completed in private in one of our clinic rooms at each time point.

What are the possible disadvantages and risks of taking part?
There are no disadvantages in taking part except that it will take up a little bit of your time. Providing us with the samples can sometimes be slightly inconvenient.

What are the possible benefits of taking part?
There are no direct benefits for you from taking part in this study. However, by taking part, it will allow us to investigate a promising new test which might help people in the future.

What happens when the research study stops?
When, we have recruited all the patients, all the answers from the questionnaires will be compared to the 'smells' detected from the samples. We will look to see whether there are trends which help us predict who will run into problems. We will try to develop simple protocols to help doctors treat people receiving radiotherapy who have new stomach or bowel problems more efficiently. This may alter the way we treat patients in the future. In the meantime, your normal care will continue in the usual way.

What if something goes wrong?

If you are concerned about an issue regarding this study, please speak directly to Ms Ann Muls, Macmillan Nurse Consultant who is responsible for day to day running of the study or Dr. Alex Taylor Consultant Clinical Oncologist who is in overall charge of the study.

Alternatively, you can contact the Patient Advice and Liaison Service (PALS) on 0800 783 7176 or visit the team in person between 9am and 5pm, Monday to Friday. Outside these hours, you can contact the PALS team by:

- email using the contact form or feedback form
- writing to The Royal Marsden Help Centre at either Chelsea or Sutton.

In the unlikely event you are harmed during the research and this is due to someone's negligence, you may have grounds for legal action for compensation against the Royal Marsden NHS Foundation Trust but you may have to pay the legal cost. The normal NHS complaints procedure will still be available to you.

Will my taking part in this study be kept confidential?
All information, including documentation and samples, which is collected about you during the course of the research will be kept strictly confidential and will be coded with a unique study number which will not be interpretable to any personnel other than the named study investigators. Any information about you which leaves the hospital will have your name and address removed. You will not be identified in any report or publication that arises

What will happen to the results of the research study?
Your involvement in the study will be from just before you start your treatment until 2 years after the completion of your radiotherapy. It will take us 4-5 years to complete the study. The results will be available after the end of the study and we will send you a summary of our findings if you indicated you would like this on your consent form.

Who is organising and funding the research?
This research is being funded by a grant from the National Institute of Health Research (NIHR).

Who has reviewed the study?
The Royal Marsden Hospital's Committee for Clinical Research has reviewed and approved the scientific basis and content of this study. The study has also been reviewed and approved by the Research and Ethics Committee established by the Health research Authority (NRES Committee London- Dulwich).

Further Information
Before you make a decision about your participation in this study, remember that you can ask us any questions. Allow yourself as much time as you need to think through your decision. If you then decide that you still wish to take part, you will be asked to confirm in writing that you have read and understand this patient information and that all of your questions have been answered completely and that you wish to continue in the study.

If you would like further information about the study, or have any concerns during the study, please contact Ann Muls, Macmillan Nurse Consultant Tel. **0207 352 8171** or Dr. Alex Taylor**,** Consultant Clinical Oncologist Tel: 020 7811 2581 , who will be happy to discuss the study with you.

Date given to patient: _____

Appendix 13. PREDICT consent form

The ROYAL MARSDEN
NHS Foundation Trust

Centre Number:
Study Protocol Number: CCR4201
Ethics Protocol Number:
Patient Identification Number for this trial:

Consent Form

Study title: The PREDICT Study: Using an electronic nose to predict gastrointestinal consequences of pelvic radiotherapy

Name of Chief Investigator: Dr. Alex Taylor

Please initial box

1. I confirm that I have read and understand the information sheet dated _____for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and my medical care and legal rights will not be affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from The Royal Marsden Hospital, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree for my GP to be notified of my participation in this study. ☐
5. I agree to offer my blood, saliva, stool, rectal &vaginal swabs and urine samples and understand the risks associated with the process of acquiring these samples. These samples will be stored and used for the study. ☐
6. I have been assured strict confidentiality will be maintained. ☐
7. I agree to take part in the above study. ☐
8. I would/ would not like to be informed of the results of this study. (Please circle one)

Please sign here:

---	---	---
Name of Participant	Date	Signature
---	---	---
Name of Researcher	Date	Signature

(When completed, 1 copy for participant; 1 copy for research site file, 1 copy for medical notes.)

Patient Consent Form – *Version 4 9th May 2017*

Appendix 14. Study withdrawal letter

Withdrawal Letter

Ann Muls
GI Unit
The Royal Marsden NHS Foundation Trust
Tel 020 8661 3851 Ext: 4658

PREDICT study (CCR 4201)

Dear Ann:

I want to end my participation in this study. Ending my participation means the research team may only use and share the information as indicated below:

I want to (please choose one):

[ ] End my participation in the study and not let the research team collect any more information about me (revoke my Authorisation). My future health information may not be used by the research team. In rare cases, the research team may need to use my information even after I revoke my authorisation, such as to notify me of safety concerns.

[ ] End my active participation in the study, but let the research team continue to collect my information. The research team may continue collecting information from my medical record as needed for the study.

Optional:
I am ending my participation in this study because:

I will receive confirmation of this notice.

Signature of Participant Date

Appendix 15. Case Report Form

The Royal Marsden NHS Foundation Trust

Gastrointestinal Unit: GI research

PREDICT study (CCR 4201)
Case report Form

CASE REPORT FORM

Section 1: **Consent**

Date consented: / / CCRPAT: / / Clindocs/EPR: / /

Section 2: **Patient details**

DOB: Gender: F Site: Chelsea / Sutton / Kingston

RMH Consultant: Dr A Taylor Dr S Lalondrelle Other:

Tumour site: ☐ Cervical ☐ Endometrial ☐ Uterine
☐ Vulvar ☐ Vaginal ☐ Other

Tumour stage (see guidance list):

Stage 1	Stage 2	Stage 3	Stage 4	Stage X

Histology (see guidance list):

Treatment pathway:

Surgery: no planned yes (see guidance list) date:

Chemotherapy: no yes (see guidance list)
Start date: / /
End date: / /

Biological treatment: no yes (see guidance list)

Hormone treatment: no yes (see guidance list)

Radiotherapy: ☐ EBRT

Start – end date: / / to / /
Dose:
Number of fractions:
Number of phases:

☐ brachytherapy
Start – end date: / / to / /
Dose:
Number of fractions:
Number of phases:

Demographics: date: / /

Age (years):

Relationship status: ☐ single ☐ married ☐ divorced ☐ co-habiting

☐ nullipara ☐ unipara ☐ multipara (number): ☐ normal delivery ☐ C-section

Last menstruation: / /

Smoking status: ☐ never smoked
☐ ex-smoker: quit (date)
☐ current smoker: number of years smoked:
average of no of cigarettes/day:

Alcohol consumption: no yes: units/week:

Diet: ☐ Diet includes meat ☐ Lactose 'free' diet
☐ Vegetarian ☐ Gluten free diet
☐ Pescetarian ☐ Other:
☐ Vegan

Previous surgery (unrelated to cancer): yes no

if yes, number of procedures:
type of surgery: date: / /
type of surgery: date: / /
type of surgery: date: / /

permanent stoma: yes no

if yes, date of formation: / /
type of stoma:

GI co-morbidities: yes no

If yes, list (see guidance list):

Other co-morbidities: (use ICD-10 codes)

Baseline: date: / /

Current treatment details: pre-treatment

Height (m): Weight (kg): BMI (kg/m^2):

Current medication:

Medication	Start date	Dose	End date

Recent use of antibiotics	Start date	Dose	End date

Herbal supplements	Start date	Dose	End date
Vitamin supplements	Start date	Dose	End date
Laxatives	Start date	Dose	End date
Anti-diarrhoeal medication	Start date	Dose	End date
Pre / probiotics	Start date	Dose	End date

Questionnaires completed:

IBDQ-B	no	yes	score:
Vaizey score	no	yes	score:
BSC	no	yes	stool consistency type:
CTCAE-3	no	yes	toxicity grade:
Food diary	no	yes	

Physical Activity Index: ☐ inactive ☐ moderately inactive

☐ active ☐ moderately active

ECOG performance status 0 1 2 3 4 5

Samples collected:

Stool sample for faecal calprotectin	no	yes	result:
Saliva sample	no	yes	date stored:
Urine sample	no	yes	date stored:
Stool sample	no	yes	date stored:
Rectal swab	no	yes	date stored:

Translational samples:

Blood for inflammatory markers	no	yes	date stored:
Blood for further sequencing	no	yes	date stored:

Other screening results:

Hb	g/L	(normal range: 120-150 g/L)	Not done ☐
WBC	$x10^9$/L	(normal range: 4.0-10.0 $x10^9$/L)	Not done ☐
Neutrophils	$x10^9$/L	(normal range: 2.0-7.0 $x10^9$/L)	Not done ☐
CRP	mg/L	(normal range: < 10 mg/L)	Not done ☐
ESR	mm/Hr	(normal range: < 20 mm/Hr **age)	Not done ☐
CA 125	U/ml	(normal range: < 35 U/ml)	Not done ☐
CA15-3	U/ml	(normal range: < 28 U/ml)	Not done ☐
CA 19-9	U/ml	(normal range: < 37 U/ml)	Not done ☐
CEA	Ug/L	(non-smoker: <3 ug/L, smoker: <5 ug/L)	Not done ☐

Post Chemotherapy date: / / ☐ No chemo →
FUP 1

Current treatment details: post chemo – pre radiotherapy

Height (m): Weight (kg): BMI (kg/m^2):

Changes in current medication:

Medication	Start date	Dose	End date

Recent use of antibiotics	Start date	Dose	End date

Herbal supplements	Start date	Dose	End date
Vitamin supplements	Start date	Dose	End date
Laxatives	Start date	Dose	End date
Anti-diarrhoeal medication	Start date	Dose	End date
Pre / probiotics	Start date	Dose	End date

Questionnaires completed:

IBDQ-B	no	yes	score:
Vaizey score	no	yes	score:
BSC	no	yes	
CTCAE-3	no	yes	toxicity grade:
Food diary	no	yes	

PAI (researcher) ☐ inactive ☐ moderately inactive

☐ active ☐ moderately active

ECOG performance status 0 1 2 3 4 5

Samples collected:

Stool sample for faecal calprotectin	no	yes	result:
Saliva sample	no	yes	date stored:
Urine sample	no	yes	date stored:
Stool sample	no	yes	date stored:
Rectal swab	no	yes	date stored:

Translational samples:

Blood for inflammatory markers	no	yes	date stored:
Blood for further sequencing	no	yes	date stored:

Other screening results:

Hb	g/L	(normal range: 120-150 g/L)	Not done ☐
WBC	$x10^9$/L	(normal range: 4.0-10.0 $x10^9$/L)	Not done ☐
Neutrophils	$x10^9$/L	(normal range: 2.0-7.0 $x10^9$/L)	Not done ☐
CRP	mg/L	(normal range: < 10 mg/L)	Not done ☐
ESR	mm/Hr	(normal range: < 20 mm/Hr **age)	Not done ☐
CA 125	U/ml	(normal range: < 35 U/ml)	Not done ☐
CA15-3	U/ml	(normal range: < 28 U/ml)	Not done ☐
CA 19-9	U/ml	(normal range: < 37 U/ml)	Not done ☐
CEA	Ug/L	(non-smoker: <3 ug/L, smoker: <5 ug/L)	Not done ☐

If experiencing GI symptoms is referral to gastroenterologist by team wanted?

no yes date referred: / /

if yes (see list possible new diagnoses made by GIANT service)

Additional notes:

Follow- up 1 date: / /

Current treatment details: beginning of radiotherapy

Height (m): Weight (kg): BMI (kg/m^2):

Changes in current medication:

Medication	Start date	Dose	End date

Recent use of antibiotics	Start date	Dose	End date

Herbal supplements	Start date	Dose	End date
Vitamin supplements	Start date	Dose	End date
Laxatives	Start date	Dose	End date
Anti-diarrhoeal medication	Start date	Dose	End date
Pre / probiotics	Start date	Dose	End date

Questionnaires completed:

IBDQ-B	no	yes	score:
Vaizey score	no	yes	score:
BSC	no	yes	stool consistency type:
CTCAE-3	no	yes	toxicity grade:
Food diary	no	yes	

PAI (researcher) ☐ inactive ☐ moderately inactive

☐ active ☐ moderately active

ECOG performance status 0 1 2 3 4 5

Samples collected:

Stool sample for faecal calprotectin	no	yes	result:
Saliva sample	no	yes	date stored:
Urine sample	no	yes	date stored:
Stool sample	no	yes	date stored:
Rectal swab	no	yes	date stored:

Translational samples:

Blood for inflammatory markers	no	yes	date stored:
Blood for further sequencing	no	yes	date stored:

Other screening results:

Hb	g/L	(normal range: 120-150 g/L)	Not done ☐
WBC	$x10^9$/L	(normal range: 4.0-10.0 $x10^9$/L)	Not done ☐
Neutrophils	$x10^9$/L	(normal range: 2.0-7.0 $x10^9$/L)	Not done ☐
CRP	mg/L	(normal range: < 10 mg/L)	Not done ☐
ESR	mm/Hr	(normal range: < 20 mm/Hr **age)	Not done ☐
CA 125	U/ml	(normal range: < 35 U/ml)	Not done ☐
CA15-3	U/ml	(normal range: < 28 U/ml)	Not done ☐
CA 19-9	U/ml	(normal range: < 37 U/ml)	Not done ☐
CEA	Ug/L	(non-smoker: <3 ug/L, smoker: <5 ug/L)	Not done ☐

If experiencing GI symptoms is referral to gastroenterologist by team wanted?

no yes date referred: / /

if yes (see list possible new diagnoses made by GIANT service)

Additional notes:

Follow- up 2 date: / /

Current treatment details: end of radiotherapy

Height (m): Weight (kg): BMI (kg/m^2):

Changes in current medication:

Medication	Start date	Dose	End date

Recent use of antibiotics	Start date	Dose	End date

Herbal supplements	Start date	Dose	End date
Vitamin supplements	Start date	Dose	End date
Laxatives	Start date	Dose	End date
Anti-diarrhoeal medication	Start date	Dose	End date
Pre / probiotics	Start date	Dose	End date

Questionnaires completed:

IBDQ-B	no	yes	score:
Vaizey score	no	yes	score:
BSC	no	yes	stool consistency type:
CTCAE-3	no	yes	toxicity grade:
Food diary	no	yes	

PAI (researcher) ☐ inactive ☐ moderately inactive

☐ active ☐ moderately active

ECOG performance status 0 1 2 3 4 5

Samples collected:

Stool sample for faecal calprotectin	no	yes	result:
Saliva sample	no	yes	date stored:
Urine sample	no	yes	date stored:
Stool sample	no	yes	date stored:
Rectal swab	no	yes	date stored:

Translational samples:

Blood for inflammatory markers	no	yes	date stored:
Blood for further sequencing	no	yes	date stored:

Other screening results:

Hb	g/L	(normal range: 120-150 g/L)	Not done ☐
WBC	$x10^9$/L	(normal range: 4.0-10.0 $x10^9$/L)	Not done ☐
Neutrophils	$x10^9$/L	(normal range: 2.0-7.0 $x10^9$/L)	Not done ☐
CRP	mg/L	(normal range: < 10 mg/L)	Not done ☐
ESR	mm/Hr	(normal range: < 20 mm/Hr **age)	Not done ☐
CA 125	U/ml	(normal range: < 35 U/ml)	Not done ☐
CA15-3	U/ml	(normal range: < 28 U/ml)	Not done ☐
CA 19-9	U/ml	(normal range: < 37 U/ml)	Not done ☐
CEA	Ug/L	(non-smoker: <3 ug/L, smoker: <5 ug/L)	Not done ☐

If experiencing GI symptoms is referral to gastroenterologist by team wanted?

no yes date referred: / /

if yes (see list possible new diagnoses made by GIANT service)

Additional notes:

Follow- up 3 date: / /

Current treatment details: 6 months after radiotherapy

Height (m): Weight (kg): BMI (kg/m^2):

Changes in current medication:

Medication	Start date	Dose	End date

Recent use of antibiotics	Start date	Dose	End date

Herbal supplements	Start date	Dose	End date
Vitamin supplements	Start date	Dose	End date
Laxatives	Start date	Dose	End date
Anti-diarrhoeal medication	Start date	Dose	End date
Pre / probiotics	Start date	Dose	End date

Questionnaires completed:

IBDQ-B	no	yes	score:
Vaizey score	no	yes	score:
BSC	no	yes	stool consistency type:
CTCAE-3	no	yes	toxicity grade:
Food diary	no	yes	

PAI (researcher) ☐ inactive ☐ moderately inactive

☐ active ☐ moderately active

ECOG performance status 0 1 2 3 4 5

Samples collected:

Stool sample for faecal calprotectin	no	yes	result:
Saliva sample	no	yes	date stored:
Urine sample	no	yes	date stored:
Stool sample	no	yes	date stored:
Rectal swab	no	yes	date stored:

Translational samples:

Blood for inflammatory markers	no	yes	date stored:
Blood for further sequencing	no	yes	date stored:

Other screening results:

Hb	g/L	(normal range: 120-150 g/L)	Not done ☐
WBC	$x10^9$/L	(normal range: 4.0-10.0 $x10^9$/L)	Not done ☐
Neutrophils	$x10^9$/L	(normal range: 2.0-7.0 $x10^9$/L)	Not done ☐
CRP	mg/L	(normal range: < 10 mg/L)	Not done ☐
ESR	mm/Hr	(normal range: < 20 mm/Hr **age)	Not done ☐
CA 125	U/ml	(normal range: < 35 U/ml)	Not done ☐
CA15-3	U/ml	(normal range: < 28 U/ml)	Not done ☐
CA 19-9	U/ml	(normal range: < 37 U/ml)	Not done ☐
CEA	Ug/L	(non-smoker: <3 ug/L, smoker: <5 ug/L)	Not done ☐

If experiencing GI symptoms is referral to gastroenterologist by team wanted?

no yes date referred: / /

if yes (see list possible new diagnoses made by GIANT service)

Additional notes:

Follow- up 4 date: / /

Current treatment details: ie 1 year after radiotherapy

Height (m): Weight (kg): BMI (kg/m^2):

Changes in current medication:

Medication	Start date	Dose	End date

Recent use of antibiotics	Start date	Dose	End date

Herbal supplements	Start date	Dose	End date
Vitamin supplements	Start date	Dose	End date
Laxatives	Start date	Dose	End date
Anti-diarrhoeal medication	Start date	Dose	End date
Pre / probiotics	Start date	Dose	End date

Questionnaires completed:

IBDQ-B	no	yes	score:
Vaizey score	no	yes	score:
BSC	no	yes	stool consistency type:
CTCAE-3	no	yes	toxicity grade:
Food diary	no	yes	

PAI (researcher) ☐ inactive ☐ moderately inactive

☐ active ☐ moderately active

ECOG performance status 0 1 2 3 4 5

Samples collected:

Stool sample for faecal calprotectin	no	yes	result:
Saliva sample	no	yes	date stored:
Urine sample	no	yes	date stored:
Stool sample	no	yes	date stored:
Rectal swab	no	yes	date stored:

Translational samples:

Blood for inflammatory markers	no	yes	date stored:
Blood for further sequencing	no	yes	date stored:

Other screening results:

Hb	g/L	(normal range: 120-150 g/L)	Not done ☐
WBC	$x10^9$/L	(normal range: 4.0-10.0 $x10^9$/L)	Not done ☐
Neutrophils	$x10^9$/L	(normal range: 2.0-7.0 $x10^9$/L)	Not done ☐
CRP	mg/L	(normal range: < 10 mg/L)	Not done ☐
ESR	mm/Hr	(normal range: < 20 mm/Hr **age)	Not done ☐
CA 125	U/ml	(normal range: < 35 U/ml)	Not done ☐
CA15-3	U/ml	(normal range: < 28 U/ml)	Not done ☐
CA 19-9	U/ml	(normal range: < 37 U/ml)	Not done ☐
CEA	Ug/L	(non-smoker: <3 ug/L, smoker: <5 ug/L)	Not done ☐

If experiencing GI symptoms is referral to gastroenterologist by team wanted?

no yes date referred: / /

if yes (see list possible new diagnoses made by GIANT service)

Additional notes:

Follow- up 5 date: / /

Current treatment details: 2 years after radiotherapy

Height (m): Weight (kg): BMI (kg/m^2):

Changes in current medication:

Medication	Start date	Dose	End date

Recent use of antibiotics	Start date	Dose	End date

Herbal supplements	Start date	Dose	End date
Vitamin supplements	Start date	Dose	End date
Laxatives	Start date	Dose	End date
Anti-diarrhoeal medication	Start date	Dose	End date
Pre / probiotics	Start date	Dose	End date

Questionnaires completed:

IBDQ-B	no	yes	score:
Vaizey score	no	yes	score:
BSC	no	yes	stool consistency type:
CTCAE-3	no	yes	toxicity grade:
Food diary	no	yes	

PAI (researcher) ☐ inactive ☐ moderately inactive

☐ active ☐ moderately active

ECOG performance status 0 1 2 3 4 5

Samples collected:

Stool sample for faecal calprotectin	no	yes	result:
Saliva sample	no	yes	date stored:
Urine sample	no	yes	date stored:
Stool sample	no	yes	date stored:
Rectal swab	no	yes	date stored:

Translational samples:

Blood for inflammatory markers	no	yes	date stored:
Blood for further sequencing	no	yes	date stored:

Other screening results:

Hb	g/L	(normal range: 120-150 g/L)	Not done ☐
WBC	$x10^9$/L	(normal range: 4.0-10.0 $x10^9$/L)	Not done ☐
Neutrophils	$x10^9$/L	(normal range: 2.0-7.0 $x10^9$/L)	Not done ☐
CRP	mg/L	(normal range: < 10 mg/L)	Not done ☐
ESR	mm/Hr	(normal range: < 20 mm/Hr **age)	Not done ☐
CA 125	U/ml	(normal range: < 35 U/ml)	Not done ☐
CA15-3	U/ml	(normal range: < 28 U/ml)	Not done ☐
CA 19-9	U/ml	(normal range: < 37 U/ml)	Not done ☐
CEA	Ug/L	(non-smoker: <3 ug/L, smoker: <5 ug/L)	Not done ☐

If experiencing GI symptoms is referral to gastroenterologist by team wanted?

no yes date referred: / /

if yes (add separate section listing new diagnoses made by GIANT service)

Additional notes:

Section 3: **Withdrawal**

Participant voluntarily withdraws from study conduct: ☐ Yes

Participant voluntarily withdraws from study conduct **and** voluntarily withdraws access to protected health information (PHI): ☐ Yes

Participant related reason:

☐ Unable to complete study questionnaires
☐ Unable to provide study samples
☐ Participant's condition or disease has progressed/deteriorated
☐ Participant has a change in personal circumstances
☐ Treatment has been relocated to another treatment centre
☐ Follow-up has been relocated to another centre

Other reason:

☐	
☐	
☐	

Date withdrawn: / /

Other:

☐ Participant died date: / /

Researcher related reason:

☐ Study is terminated at a preset date and some follow-up assessments are not completed

date: / /

Guidance notes

Tumour staging

Appendix 1 FIGO Staging

Stage	Characteristics
0	Carcinoma in situ, intraepithelial carcinoma; cases of stage 0 should not be included in any therapeutic statistics for invasive carcinoma
I	The carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded)
IA	Invasive cancer identified only microscopically. All gross lesions, even with superficial invasion, are stage IB cancers. Invasion is limited to measured stromal invasion with a maximum depth of 5 mm taken from the base of the epithelium, either surface or glandular, from which it originates. Vascular space involvement, either venous or lymphatic, should not alter the staging)
IA1	Measured invasion of stroma no greater than 3 mm in depth and no wider than 7 mm
IA2	Measured invasion of stroma > 3 mm and no greater than 5 mm in depth and no wider than 7 mm
IB	Clinical lesions confined to the cervix or preclinical lesions > IA
IB1	Clinical lesions no greater than 4 cm in size
IB2	Clinical lesions > 4 cm in size
II	The carcinoma extends beyond the cervix, but has not extended onto the pelvic wall; the carcinoma involves the vagina, but not as far as the lower third
IIA	No obvious parametrial involvement
IIB	With parametrial involvement
III	The carcinoma has extended onto the pelvic wall; on rectal examination there is no cancer-free space between the tumour and the pelvic wall; the tumour involves the lower third of the vagina; all cases with a hydronephrosis or nonfunctioning kidney should be included, unless they are known to be due to other causes
IIIA	No extension onto the pelvic wall, but involvement of the lower third of the vagina
IIIB	Extension onto the pelvic wall or hydronephrosis or nonfunctioning kidney
IV	The carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder or rectum
IVA	Spread of the growth to adjacent organs
IVB	Spread to distant organs

(1) FIGO staging is based on clinical data (clinical examination and colposcopy), chest x-rays, IVP, biopsy and D&C.
(2) Cystocopy and sigmoidoscopy may be used for clinical stage (bladder and/or rectal mucosal biopsy).
(3) Lymphangiogram, CT, MRI, laparotomy, laparoscopy cannot be used for clinical staging.
(4) Pathological IVP defines a cancer as stage IIIB.
(5) Paracervical, parametrial, hipogastric, obturator, internal, external and common iliac, presacral and sacral are the regional nodes.

Regional Lymph Nodes (N)
- NX – Regional lymph nodes cannot be assessed
- N0 – No regional lymph node metastasis
- N1 – Unilateral regional lymph node metastasis;
- N2 – Bilateral regional lymph node metastases.

Distant Metastasis (M)
- MX– Distant metastasis cannot be assessed
- M0 – No distant metastasis
- M1 – Distant metastasis

Tumour grading

	Tick
Grade 1 – The cancer cells look very similar to normal cells and are growing slowly	
Grade 2 – The cells look unlike normal cells and are growing more quickly than normal	
Grade 3 – The cancer cells look very abnormal and are growing quickly.	
GX means that the grade can't be assessed = undetermined grade	

Tumour histology

	Tick
Squamous cell carcinoma	
Adenocarcinoma	
Serous	
Mucinous	
Germ cell	
Sarcoma	

ECOG score

ECOG Performance Status Developed by the Eastern Cooperative Oncology Group,
Robert L. Comis, MD, Group Chair.*

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

*Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982;5:649-655.

Surgery

		Tick
Total abdominal hysterectomy (TAH)		
Wertheim's hysterectomy: removal of uterus, cervix, top part of the vagina, fallopian tubes, ovaries, supporting tissues and lymph nodes.		
Laparoscopic assisted vaginal hysterectomy (LAVH)		
Bilateral salpingo-oophorectomy (BSO)		
Unilateral salpingo-oophorectomy Left ☐ Right ☐		
Radical trachelectomy		
Pelvic exenteration	Anterior exenteration + urostomy	
	Posterior exenteration + colostomy	
	Total exenteration + uro & colostomy	
Partial vulvectomy		
Radical vulvectomy		
Partial vaginectomy		
Total vaginectomy		
Wide local excision	site:	

Chemotherapy

	Tick
Carboplatin	
Cisplatin	
Cyclophosphamide	
Doxorubicin	
Paclitaxel	

Biological therapy

	Tick
Bevacizumab (Avastin)	

Hormone treatment

	Tick
Tamoxifen	
Letrozole	
Megestrol acetate	
Medroxyprogesterone	

Radiotherapy

		Tick
External beam radiotherapy (EBRT) phase 1	Gy	
External beam radiotherapy (EBRT) phase 2	Gy	
Boost		
Brachytherapy	Gy	

PHYSICAL ACTIVITY INDEX (PAI) Score

Patients can be classified into four categories based on the original EPIC index from
which the GPPAQ was developed.

Inactive	Sedentary job and no physical exercise or cycling
Moderately inactive	Sedentary job and some but < 1 hour physical exercise and / or cycling per week OR Standing job and no physical exercise or cycling
Moderately active	Sedentary job and 1-2.9 hours physical exercise and / or cycling per week OR Standing job and some but < 1 hour physical exercise and / or cycling per week OR Physical job and no physical exercise or cycling
Active	Sedentary job and ≥ 3 hours physical exercise and / or cycling per week OR Standing job and 1-2.9 hours physical exercise and / or cycling per week OR Physical job and some but < 1 hour physical exercise and / or cycling per week OR Heavy manual job

Note: Questions concerning Walking, Housework/Childcare and Gardening/DIY have
been included to allow patients to record their physical activity in these categories, however these questions have not been shown to yield data of a sufficient reliability
to contribute to an understanding of overall physical activity levels.

References:

Cust A, Smith B, Chau J, van der Ploeg H et al., Validity and repeatability of the EPIC physical activity questionnaire: a validation study using accelerometers as an objective measure. *International Journal of Behavioral Nutrition and Physical Activity* 2008, 5:33 doi:10.1186/1479-5868-5-33

Physical Activity Policy, Health Improvement Directorate (2009). General Practise Physical Activity Questionnaire: A screening tool to assess adult physical activity levels, within primary care. 22pp. Accessed via:
http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/@ps/documents/digitalasset/dh_112134.pdf

GI co-morbidities

	ICD-10 code	Tick
Oesophagitis	K20	
Gastro-oesophageal reflux disease with oesophagitis	K21.1	
Gastro-oesophageal reflux disease without oesophagitis	K21.9	
Oesophageal ulcer	K22.1	
Barrett's oesophagus	K22.7	
Gastric ulcer	K25	
Duodenal ulcer	K26	
Acute appendicitis (with generalised peritonitis)	K35	
Inguinal hernia	K40	
Umbilical hernia	K42	
Crohn's disease	K50	
Ulcerative colitis	K51	
Paralytic ileus and intestinal obstruction without hernia	K56	
Diverticular disease of intestine	K57	
Irritable bowel syndrome	K58	
Fissure and fistula of anal and rectal regions	K60	
Abscess of anal and rectal regions	K61	
Haemorrhoids	K64	
Liver disease	K70-K77	
Disorders of gallbladder, biliary tract and pancreas	K80-87	
Intestinal malabsorption- coeliac disease	K90.0	
Intestinal malabsorption- fat	K90.4	
Lactose intolerance	E73	

General co-morbidities

	ICD-10 code	Tick
Hypothyroidism	E03	
Thyrotoxicosis [hyperthyroidism]	E05	
Diabetes mellitus type 1	E10	
Diabetes mellitus type 2	E11	
Vitamin D deficiency	E55	
Obesity	E66	
Lactose intolerance	E73	
Hypercholesterolaemia	E78	
Depression	F32-33	
Multiple sclerosis	G35	
Glaucoma	H40	
Hypertension (essential)	I10	
Angina	I20	
Acute myocardial infarction	I21	
Chronic ischaemic heart disease	I25	
Pulmonary embolism	I26	
Pericarditis	I30	
Cardiomyopathy	I40	
Atrial fibrillation	I48	
Heart failure	I50	
Cerebral infarction (CVA)	I63	
Transient cerebral ischaemic attacks (TIA)	G45	
Raynaud syndrome	I73.0	
Phlebitis and thrombophlebitis	I80	
Hypotension	I95	
Emphysema	J43	
COPD	J44	
Asthma	J45	
Arthritis	M00	
Rheumatoid arthritis	M06	
Osteoporosis without pathological fracture	M81	
Chonic kidney disease	N18	
Cystitis	N30	
Urinary tract infection (general: site not specified)	N39	
Inflammatory diseases of female pelvic organs (specify)	N70-N77	
Endometriosis	N80	
Female genital prolapse	N81	
Cervical intraepithelial neoplasia [CIN], grade III, with or without mention of severe dysplasia	D06	

Possible GI diagnoses if referred to GIANT service

	Tick
Anal sphincter defect	
Barrett's oesophagus	
Bile acid malabsorption SeHCAT result: %	
Carbohydrate malabsorption	
Coeliac disease	
Dehydration	
Alcohol induced	
Dietary fibre excess	
Diverticular disease	
Drug –induced list:	
Duodenitis	
Faecal loading	
Fissure	
Fistula (site)	
Free fatty acid malabsorption	
Gastritis: Biliary ☐ Helicobacter related ☐ Other☐	
GORD	
Haemorrhoids	
Hiatus hernia	
Inflammatory bowel disease: Crohn's disease ☐ Ulcerative colitis ☐	
Iron deficiency	
Lymphoedema	
Diabetes	
Thyroid problems: Hypothyroidism ☐ Thyrotoxicosis ☐	
Neoplasma: new cancer	
Neoplasma: relapse	
Oesophagitis	
Pancreatic insufficiency FE result: pg/g	
Peg / tube insertion / problems	
Polyp(s)	
SIBO	
Stricture (site)	
Teleangiectasia no rectal bleeding	
Teleangiectasia with rectal bleeding	
Trace element deficiency	
Ulceration (site)	
Vitamin B12 deficiency B12 level: pg/ml	
Vitamin D deficiency Vit D level: nmol/L	
Weak pelvic floor musculature	

Study measurements

	Baseline before treatment	***If having chemotherapy, before radiotherapy (optional)***	***During radiotherapy (1)***	***During radiotherapy (2)***	***6 months after completion of treatment***	***12 months after completion of treatment***	***24 months after completion of treatment***
Patient characteristics	***X***						
Treatment characteristics	***X***						
Saliva sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Urine sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Rectal swab (optional)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Stool sample	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Faecal calprotectin	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Blood sample (translational)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Blood sample (fibrotic markers)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Inflammatory Bowel Disease Questionnaire (IBDQ)	***X***	***X***	***X***	***X***	***X***	***X***	***X***
St. Mark's incontinence score	***X***	***X***	***X***	***X***	***X***	***X***	***X***
GI section of the Common Terminology Criteria for Adverse Events (CTCAE) (version 3.0) score	***X***	***X***	***X***	***X***	***X***	***X***	***X***
Bristol Stool Chart parameters	***X***	***X***	***X***	***X***	***X***	***X***	***X***
7-day food diary	***X***	***X***	***X***	***X***	***X***	***X***	***X***

Appendix 16. Material Transfer Agreement between the Royal Marsden NHS Foundation Trust and Warwick University.

COLLABORATION AGREEMENT

This **COLLABORATION AGREEMENT** effective from date of last signature is made between

(1) **The Royal Marsden Hospital NHS Foundation Trust** whose administrative offices are at Fulham Road , London WS3 6JJ (hereinafter "the RM")

and

(2) **The University of Warwick** whose administrative offices are at University House, Coventry, CV4 8UW (hereinafter "the UW")

each a "Party" and collectively "the Parties".

WHEREAS

A. The RM is sponsoring the study entitled: "Using an electronic nose to predict gastrointestinal consequences of pelvic radiotherapy - Predict"(the "Study"), which will be conducted according to the Protocol (as amended from time to time during the Study), a copy of which is attached to this Agreement as Schedule 1 and incorporated herein by reference.

B. The UW is interested in undertaking the research laboratory work for the study pursuant to the terms and provisions of this Agreement.

C. The UW will receive a payment ("the Contract Price") for its collaboration details of which are attached herewith as Schedule 3.

This Collaboration Agreement sets out the terms under which the Parties shall perform the Allocated Work:

1. DEFINITIONS

1.1 The following expressions shall have the following meanings in this Collaboration Agreement including its recitals, unless the context requires otherwise:

'Allocated Work'	shall mean the work allocated to the UW as defined in Schedule 2 of this Agreement and in the Protocol.
'Arising Intellectual Property'	shall mean any Intellectual Property that is generated or first reduced to practice by any Party or Parties directly as a result of the work undertaken in accordance with this Collaboration Agreement.
'Background Intellectual Property'	shall mean any Intellectual Property excluding Arising Intellectual Property owned or controlled by any Party prior to commencement of this Collaboration Agreement or developed independently from the Collaboration Agreement, and which the owning Party contributes or uses in the course of performing the Allocated Work.

'Confidential information'	shall mean any Background intellectual Property disclosed by one Party to the other for use in the Allocated Work (and identified as confidential before or at the time of disclosure) and any Arising Intellectual Property in which that Party owns the Intellectual Property.
Material	shall mean any human biological material derived from Participant supplied by the RM to the UW or the RM's nominee (as the case may be) as outlined under Schedule 4 to this Collaboration Agreement.
'Metadata'	shall mean (i) anonymised data associated with the Materials which is limited to age, gender, ethnicity, and geographic location of the individuals from whom the Materials were collected, and (ii) the specifics of sampling protocols (including nurse collection data),symptoms questionnaire and equipment, storage and shipping parameters.
'Effective Date'	shall mean the date of the last Party to sign this Collaboration Agreement.
'Intellectual Property'	shall mean intellectual property of any description including but not limited to all data, inventions, designs, information, specifications, formulae, improvements, discoveries, know-how, processes, methods, techniques and the intellectual property rights therein, including but not limited to, patents, copyrights, database rights, design rights (registered and unregistered), trade-marks, trade names and service marks, applications for any of the above.
Key Personnel	Shall mean Dr Jervoise Andreyev employed at the RM and Professor James Covington employed at the UW.
Know-how	shall mean all technical and other information which is not in the public domain (other than as a result of a breach of confidence), including but not limited to information comprising or relating to concepts, discoveries, data, designs, formulae, ideas, inventions, methods, models, procedures, designs for experiments and tests and results of experimentation and testing, processes, specifications and techniques, laboratory records, clinical data, manufacturing data and information contained in submissions to regulatory authorities, whether or not protected by Intellectual Property Rights or any applications for such rights
Participant	means patient, service user, carer, relative of the deceased, professional carer, employee, or member of the public, who consents to take part in the Study. All references to Participants refer to those recruited to take part in the Study.
Processed Data	shall mean the data generated as a result of the UW's processing of the Raw Data.

Raw Data	shall mean the primary data collected and derived from the Materials provided by the RM and generated directly from analytical instrumentation as a result of the Allocated Work
Study Protocol	The description of the Study as contained in Schedule 1 and all amendments thereto. Such amendments will form part of this Collaboration Agreement

In this Collaboration Agreement, references to Clauses and Schedules refer to clauses and schedules of this Collaboration Agreement; and the singular form of any word includes the plural, and vice versa, as required by the context.

THE PARTIES HEREBY AGREE

2. OBLIGATIONS OF THE PARTIES

2.1 The Parties will each use their reasonable endeavours to collaborate to perform the Allocated Work including any modifications, deletions or expansions approved in writing by both Parties.

2.2 The Allocated Work shall be performed by or under the direction and supervision of the Key Personnel.

2.3 Each Party will use its reasonable endeavours to provide adequate facilities; to obtain any requisite materials, equipment and personnel and to carry out the work diligently. Although each Party will use its reasonable endeavours to perform the Allocated Work, no Party undertakes that work carried out under or pursuant to this Collaboration Agreement will not lead to any particular result, nor is the success of such work guaranteed. For the avoidance of doubt, nothing in this clause purports to permit any Party to reverse engineer or otherwise analyse any of the materials provided to it under this Collaboration Agreement except in accordance with the provisions of this Collaboration Agreement and to the extent applicable by law.

2.4 On completion of the Allocated Work the UW will provide to the RM summarised Processed Data so they can be used for further analysis as well as the UW will help interpret the signatures.

3 PAYMENT

3.1 The UW shall invoice the RM for the Contract Price as set out in Appendix 3 and, provided that the Allocated Work has been performed in accordance with this Collaboration Agreement the RM shall pay such invoice within 30 days of receipt. Each invoice should quote: "PREDICT".

3.2 In case of early termination of the Study or termination of this Collaboration Agreement, for any circumstance other than the RM's or the UW's breach of the Agreement, the financial support will be pro-rated according to the Study progress at time of termination, conditional to the provision included in the clause 2.4 of this Collaboration Agreement and information about the reasons for cancelling the Study.

4. PUBLICATION AND CONFIDENTIALITY PROCEDURES

4.1 Subject to Clauses 4.4 and 4.5, each Party will use all reasonable endeavours not to disclose to any third party any Confidential Information nor use for any purpose except as expressly permitted by this Collaboration Agreement, any of another Party's Confidential Information.

4.2 No Party shall incur any obligation under clause 4.1 with respect to information which:

4.2.1 is known to the receiving Party before the start of the Allocated Work, and not impressed already with any obligation of confidentiality to the disclosing Party; or

4.2.2 is or becomes publicly known without the fault of the receiving Party; or

4.2.3 is obtained by the receiving Party from a third party in circumstances where the receiving Party has no reason to believe that there has been a breach of an obligation of confidentiality owed to the disclosing Party; or

4.2.4 is independently developed by the receiving Party without reference to the Confidential Information as demonstrated by written notice; or

4.2.5 is approved for release in writing by an authorised representative of the disclosing Party; or

4.2.6 the receiving Party is specifically required to disclose in order to fulfil an order of any Court of competent jurisdiction provided that, in the case of a disclosure under the Freedom of Information Act 2000, none of the exemptions in that Act applies to the Confidential Information.

4.3 If any Party receives a request under the Freedom of Information Act to disclose any Confidential Information, it will notify and consult with the other Party. The other Party will respond within five working (5) days after receiving notice if the notice requests assistance in determining whether or not an exemption in that Act applies.

4.4 All publication should be made in compliance with the publication rules described in the Study Protocol.

4.5 Each Party will use all reasonable endeavours to submit material intended for publication to the other Party. Abstracts should be submitted by one Party to the other in writing not less than seven (7) days in advance of the external deadline. Larger publications must be submitted in writing not less than thirty (30) days in advance of the submission for publication. The publishing Party may be required to delay submission for publication if in the other Party's opinion such delay is necessary in order for that other Party to seek patent or similar protection for material in respect of which it is entitled to seek protection, or to modify the publication in order to protect Confidential Information. A delay imposed on submission for publication as a result of a requirement made by the other Party shall not last longer than is absolutely necessary to seek the required protection; and therefore shall not exceed three (3) months from the date of receipt of the material by such Party, although the publishing Party will not unreasonably refuse a request from the other Party for additional delay in the event that intellectual property rights would otherwise be lost. Notification of the requirement for delay in submission for publication must be received by the publishing Party within thirty (30) days after the receipt of the material by the other Party, failing which the publishing Party shall be free to assume that the other Party has no objection to the proposed publication. Each party shall give due acknowledgement to the other Party in any publication in accordance with standard scientific practice. Authorship, including senior authorship, of any publication must be agreed between the Parties and will reflect the relative intellectual and tangible contribution of the Parties to the Study.

4.6 Notwithstanding any other provision in this Collaboration Agreement, no Background Intellectual Property, and/or Confidential Information existing prior to or independently of the Collaboration Agreement and introduced by the other Party, may be published without the express prior written consent of that Party.

4.7 The provisions of Clause 4.1 and 4.2 shall survive for a period of three (3) years from the date of termination of this Collaboration Agreement.

5. INTELLECTUAL PROPERTY RIGHTS

5.1 For the avoidance of doubts all Background Intellectual Property used in connection with the Allocated Work shall remain the property of the Party introducing the same. No Party will make any representation or do any act which may be taken to indicate that it has any right, title or interest in or to the ownership or use of any of the Background Intellectual Property of the other Party except under the terms of this Collaboration Agreement. Each Party acknowledges and confirms that nothing contained in this Collaboration Agreement shall give it any right, title or interest in or to the Background Intellectual Property of the other Parties save as granted by this Collaboration Agreement. The Parties agree that any improvements or modifications to a Party's Background Intellectual Property arising from the Allocated Work which are not severable from that Background Intellectual Property will be deemed to form part of that Party's Background Intellectual Property.

5.2 Each Party grants the other a royalty-free, non-exclusive licence for the duration of this Collaboration Agreement to use its Background Intellectual Property for the sole purpose of carrying out the Allocated Work. No Party may grant any sub-licence over or in respect of the other's Background Intellectual Property.

5.3 Each Party shall own the Arising Intellectual Property generated by its employees, students and/or agents under the Study and shall ensure that it secures ownership of such Arising Intellectual Property from its employees, students and agents. Subject to the terms of this Collaboration Agreement, the Party owning any Arising Intellectual Property shall be entitled to use and exploit such Arising Intellectual Property as that Party sees fit, and subject always to Clauses 5.5 and 5.6.

5.4 Each Party shall promptly disclose to each other all Arising Intellectual Property generated by them and the Parties shall co-operate, where required, in relation to the preparation and prosecution of patent applications and any other applications relating to Arising Intellectual Property.

5.5 Where any Arising Intellectual Property is created or generated by the Parties jointly ("Co-owners") and it is impossible to segregate each Party's intellectual contribution to the creation of the Arising Intellectual Property, the Arising Intellectual Property will be jointly owned by those Parties in equal shares ("Joint Intellectual Property"). The Co-owners may take such steps as they may decide from time to time, to register and maintain any protection for that Joint Intellectual Property, including filing and prosecuting patent applications for any Joint Intellectual Property and taking any action in respect of any alleged or actual infringement of that Joint Arising Intellectual Property. If one owner does not wish to take any such step or action, the other owner may do so at their expense, and the owner not wishing to take such steps or action will provide, at the expense of the owner making the request, any assistance that is reasonably requested of it.

5.6 The process of commercialising the Joint Intellectual Property shall be the responsibility of the Party that made the greatest inventive contribution to such Arising Intellectual Property as determined by the Co-owners ("Commercialisation Lead") immediately after the Joint Intellectual Property is disclosed to the Parties. In any case where the Co-owners make an equal inventive contribution, the Co-owners shall discuss and agree which of them shall be the Commercialisation Lead. The Commercialisation Lead shall take such steps, as may be necessary from time to time, at the Commercialisation Lead's expense, to register and maintain any protection for that Joint Intellectual Property, including filing and prosecuting patent applications for any Joint Intellectual Property, and taking any reasonable action in respect of any alleged or actual infringement of that Intellectual Property. In return a fair and reasonable royalty will be paid to the Co-owners taking into consideration the respective financial, technical and intellectual contributions of the Co-owners concerned to the development of the of the Joint Intellectual Property, the expenses incurred in securing the intellectual property protection thereof and the costs of its commercial exploitation and any use of Background Intellectual Property.

5.7 Each Party is hereby granted an irrevocable, non-transferable, royalty-free right to use all Arising Intellectual Property and Joint Intellectual Property generated in the course of the Study for internal (non-

commercial) research purposes, including research involving projects funded by third parties provided that those parties gain or claim no rights to such Arising Intellectual Property and/ or Joint Intellectual Property.

5.8 If any Party (the "Exercising Party") requires the use of Background Intellectual Property of any other (the "Other Party") in order to exercise its rights in Arising Intellectual Property (whether solely or jointly owned) then, provided the Other Party is free to license the Background Intellectual Property in question, the Other Party will not unreasonably refuse to grant or delay granting a licence to the Exercising Party so that the Exercising Party may use such Background Intellectual Property for the purpose of exercising its rights in Arising Intellectual Property. Any such licence will be negotiated in good faith and granted on fair and reasonable terms to be agreed.

5.9 Neither Party shall have the right to assign or license its respective rights in any Joint Intellectual Property to any third party without the other Party's prior written consent.

5.10 The Metadata and the Raw Data generated or developed from the Study shall be owned by the RM.

5.11 The Processed Data generated or developed from the Study as well as any results deriving from it shall be jointly owned by the UW and the RM.

5.12 Both Parties hereby grant each other a royalty-free, irrevocable, non-transferable, non-exclusive, right and licence to use the Processed Data for internal (non- commercial) research and teaching purposes including research involving projects funded by third parties provided that those parties gain or claim no rights to the Processed Data.

5.13 In addition, the RM hereby grants to the UW a royalty-free, irrevocable, non-transferable, non-exclusive, right and licence to use the Metadata and the Raw Data for internal (non-commercial) research and teaching purposes including research involving projects funded by third parties provided that those parties gain or claim no rights to the Metadata and the Raw Data.

6. ASSIGNMENT

Neither Party will assign this Collaboration Agreement without the prior written consent of the other Party, such consent not to be unreasonably withheld, denied or delayed.

7. TERMINATION

7.1 The Agreement will come into effect on the Effective Date and will continue for the period of four years or until complete fulfilment of the Parties' obligations under this Collaboration Agreement.

7.2 This Collaboration Agreement may be terminated by one Party giving a minimum of ninety (90) days prior written notice to the other Party, receipt of which must be acknowledged in writing.

7.3 If a Party shall commit a breach of, or be in default in respect of, any provisions of this Collaboration Agreement (the "Party in Breach"), and also shall fail to remedy such default or breach within sixty (60) days after the receipt of written notice thereof from the other Party, the other Party may, at its option and in addition to any other remedies which it may have at law or in equity, terminate this Collaboration Agreement by sending notice of termination in writing to the Party in Breach to such effect, and such termination shall be effective as of the date of the receipt of such notice.

7.4 In case of early termination of the Allocated Work or termination of this Collaboration Agreement, for any circumstances other than the RM's or the UW's breach of the Collaboration Agreement, the financial support will be pro-rated according to Allocated Work progress at the time of termination.

8. LIMITATION OF LIABILITY

8.1 No Party makes any representation or warranty directly or implied that advice or information given by any of its employees, students, agents or appointees who work on the Allocated Work , or the content or use of any materials, works or information provided in connection with this Collaboration Agreement, will not constitute or result in infringement of third-party rights.

8.2 No Party accepts any responsibility for any use which may be made of any work carried out under or pursuant to this Collaboration Agreement, or of the results of the Allocated Work, nor for any reliance which may be placed on such work or results, nor for advice or information given in connection with them.

8.3 The Parties undertake to make no claim in connection with this Collaboration Agreement or its subject matter against any employees, students, agents or appointees of the other Parties (apart from claims based on fraud or wilful misconduct). This undertaking is intended to give protection to individual researchers: it does not prejudice any right which a Party might have to claim against any other Party.

8.4 The liability of any Party for any breach of this Collaboration Agreement, or arising in any other way out of the subject-matter of this Collaboration Agreement, will not extend to loss of business or profit, or to any indirect or consequential damages or losses.

8.5 The aggregate liability of each Party to the other for all and any breaches of this Collaboration Agreement, any negligence or arising in any other way out of the subject matter of this Collaboration Agreement, the Allocated Work and its results, will not exceed the total of the Contract Price defined in schedule 3.

8.6 Nothing in this Collaboration Agreement limits or excludes either Party's liability for:

8.6.1 death or personal injury resulting from negligence; or

8.6.2 any fraud or for any sort of other liability which, by law, cannot be limited or excluded.

8.7 If any sub-clause of this Clause 8 is held to be invalid or unenforceable under any applicable statute or rule of law then it shall be deemed to be omitted, and if as a result any Party becomes liable for loss or damage which would otherwise have been excluded then such liability shall be subject to the remaining sub-clauses of this Clause 8.

9. NOTICES

9.1 Until further notice, the following shall be the Parties' representatives for the purpose of receiving, statements, requests, legal notices and other documents in relation to this Agreement:

For the RM	**For the UW**
Head of Clinical Trial Contracts The Institute of Cancer Research 15 Cotswold Road Belmont Sutton Surrey SM2 5NG Email: ClinicalTrialContracts@icr.ac.uk	Director of Research and Impact Services University of Warwick, University House Coventry CV4 8UW

10. FORCE MAJEURE

10.1 A Party shall not be liable for failure to perform its obligations under this Collaboration Agreement, nor be liable to any claim for compensation or damage, nor be deemed to be in breach of this Collaboration Agreement, if such failure arises from an occurrence or circumstances beyond the reasonable control of that Party (excluding an obligation to make payment).

10.2 If a Party affected by such an occurrence causes a delay of three (3) months or more, and if such delay may reasonably be anticipated to continue, then the Parties discuss whether continuation of the Allocated Work is viable, or whether the Allocated Work and this Collaboration Agreement should be terminated.

11. GENERAL

11.1 Clause headings are inserted in this Collaboration Agreement for convenience only, and they shall not be taken into account in the interpretation of this Collaboration Agreement.

11.2 Nothing in this Collaboration Agreement shall create, imply or evidence any partnership or joint venture between the Parties or the relationship between them of principal and agent.

11.3 Each Party shall ensure that it has well defined arrangements for investigating and resolving allegations of research misconduct. Where an allegation of research misconduct arises in respect of an individual Party's participation in relation to this Collaboration Agreement and leads to a subsequent formal investigation, the relevant Party shall inform the other Party in accordance to section 9 of the investigation and its outcome. Where an allegation of research misconduct arises in respect of both Parties' participation in the Allocated Work, the Parties will work together to determine how the allegation will be investigated and reported.

11.4 No Party shall use the name or any trademark or logo of any other Party or the name of any of its staff or students in any press release or product advertising, or for any other commercial purpose, without the prior written consent of the Party(s). Any publicity, press release or the like related to the Allocated Work, which is issued or sourced by one Party, is subject to approval by the institutions' representatives for both Parties.

11.5 Except as otherwise expressly provided for herein, the Parties confirm that nothing in this Collaboration Agreement shall confer or purport to confer on any third party any benefit or any right to enforce any term of this Collaboration Agreement for the purposes of the Contracts (Rights of Third Parties) Act 1999.

11.6 This Collaboration Agreement and its Schedules (which are incorporated into and made a part of this Collaboration Agreement) constitute the entire agreement between the Parties for the Allocated Work and no statements or representations made by any Party have been relied upon by the other in entering into this Collaboration Agreement. Any variation shall be in writing and signed by authorised signatories for each Party.

11.7 This Collaboration Agreement shall be governed by English Law and the English Courts shall have exclusive jurisdiction to deal with any dispute which may arise out of or in connection with this Collaboration Agreement.

11.8 If any dispute arises out of this Collaboration Agreement the Parties will first attempt to resolve the matter informally through designated senior representatives of each Party to the dispute, who are not otherwise involved with the Allocated Work. If the Parties are not able to resolve the dispute informally within a reasonable time not exceeding two (2) months from the date the informal process is requested by notice in writing they will attempt to settle it by mediation in accordance with the Centre for Effective Dispute Resolution (CEDR) Model Mediation Procedure.

11.9 If any one or more clauses or sub-clauses of this Collaboration Agreement would result in this Collaboration Agreement being prohibited pursuant to any applicable competition law then it or they shall

be deemed to be omitted. The Parties shall uphold the remainder of this Collaboration Agreement, and shall negotiate an amendment which, as far as legally feasible, maintains the economic balance between the Parties.

11.10 Each of the parties agree to comply with all applicable UK and EU anti-corruption, anti-bribery and local employment laws, as well as any other applicable legislation, laws and regulations in connection with their performance under this Collaboration Agreement, (including that relating to import and export control, transportation of hazardous materials, anti-money laundering, and tax laws). Either party's failure to comply with any provision of this Clause 11.10 is considered to be a breach of this Collaboration Agreement.

11.11 This Collaboration Agreement may be executed in any number of counterparts, each of which when executed (and delivered) will constitute an original of this Collaboration Agreement, but all counterparts will together constitute the same agreement. No counterpart will be effective until each party has executed at least one counterpart.

[*signatures follow on a separate page*]

EXECUTED as an Agreement:

Signed on behalf of the University of Warwick by:

Name: Dr Navdeep Bains
Head of Research Support, Funding & Contracts

Position:

Signature: Date: 18/12/2015.

Signed on behalf of the Royal Marsden NHS Foundation Trust by:

Name:

Position:

Signature: Date:

Schedule 1 – The Study Protocol

Protocol version 3.0 dated [August 13th 2015] supplied as separate document with this Collaboration Agreement, and as amended during the conduct of the Study.

Schedule 2 - Allocated Work

1. Details and scope of Allocated Work for the PREDICT Study

The UW is to provide statistical analysis of the differences in signature profiles. Therefore, the following Materials will be sent to the UW:

- Stool
- Saliva
- Urine
- Rectal Swab

In total 3360 samples would be sent to UW over a period of 4 years for 120 patients.

Frequency:

The Materials will be sent every quarter packed in PI650 boxes using a UN373 courier service (insured)

All the samples will be tagged and anonymised at the RM before sending to the UW.

For the avoidance of doubt, all shipping costs of sending the samples will be borne by RM.

2. Documentation that the UW must hold: (please insert if any)

A record (computerised or manual) of the Material that has been transferred according to this Agreement.

Schedule 3 - Contract Price

The Sponsor will pay the Laboratory a gross sum of £ 22,289 during this Collaboration Agreement as detailed below.

Description of the Resources	Totals associated with each Resource (£)
Lab Technician	9,539
Computer Programme	1,500
Electronic Sensors	5,000
Filters	2,500
Tubing Connectors and Sampling pots	3,750
Grand Total	22,289

Following is the payment schedule (in line with NIHR disbursement schedule)

Payment No.	**Month**	**Amount (£)**
1.	December 2015	5,533
2.	December 2016	4,094
3.	October 2017	4,156
4.	October 2018	4,218
5.	November 2019	4,288
	Total	22,289

Schedule - 4

Materials Transfer

1. The RM shall remain custodian of the Materials.
2. The Materials shall not be removed from the UW premises, unless the UW informs the RM and enters into an agreement with the third party under terms equivalent to those set out in this Agreement.
3. The UW will use the Material solely and exclusively for the purposes as described in the Allocated Work and in accordance with the Protocol of the Study.
4. Any Material delivered pursuant to this Collaboration Agreement is understood to be experimental in nature and may have hazardous properties.
5. The UW shall ensure that any disposal of the Material is in accordance with prior written instructions given by the RM (if any) and all applicable regulations and best practice recommendations.
6. The UW and the RM shall each be responsible for keeping its own record of the Material that has been transferred according to this Collaboration Agreement.
7. No modification shall be made to the title and the acronym of the Material.

Appendix 17. Modified Inflammatory Bowel Disease Questionnaire (IBDQ)

Table 1 The modified IBDQ

	In the last 2 weeks please tell us how often you have:	More than ever before	Extremely frequently	Very frequently	Moderate increase in frequency	Some increase in frequency	Slight increase in frequency	Not at all/ normal
1	Had your bowel open?							
2	Felt tired and worn out?							
3	Felt frustrated, impatient or restless?							
4	Been unable to do what you want because of your bowels?							
5	Had loose bowel movements?							
6	Worried about your energy levels?							
7	Worried about having to have something done about your bowels?							
8	You had to cancel an engagement because of your bowels?							
9	Been troubled by pain in your bottom?							
10	Felt generally unwell?							
11	Worried about not being able to find a lavatory?							
12	Been prevented doing leisure or sports by your bowels?							
13	Been troubled by pain in your tummy or bottom?							
14	Been waking at night or having difficulty sleeping?							
15	Been depressed or discouraged?							
16	Not gone somewhere because there is no lavatory nearby?							
17	Passed large amounts of gas?							
18	Worried about getting to the weight you would like?							
19	Worried about your illness?							
20	Been troubled by bloating?							
21	Been relaxed and free of tension?							
22	Had a problem with bleeding from your bottom?							
23	Been embarrassed about your bowels?							
24	Felt like you need to have your bowels open but nothing happens?							
25	Felt tearful and upset?							
26	Been troubled by accidental soiling?							
27	Felt angry as a result of your bowel problems?							
28	Felt limited in sexual activity because of your bowels?							
29	Felt disgusted about your bowel problems?							
30	Felt irritable?							
31	Experienced lack of understanding from others?							
32	Felt satisfied, happy or pleased with your life?							

Appendix 18. IBDQ- Bowel subset

How often have you…	More than ever before	Extremely frequently	Very frequently	Moderate increase in frequency	Some increase in frequency	Slight increase in frequency	Normal / Not at all
Score	1	2	3	4	5	6	7
1. had your bowel open?							
2. had loose bowel movements?							
3. been troubled by pain in your bottom?							
4. been troubled by cramps in your tummy or bottom?							
5. passed a large amount of gas?							
6. been troubled by bloating?							
7. had a problem with bleeding from your bottom?							
8. felt like you need to have your bowels open but nothing happens?							
9. been troubled by accidental soiling?							
10. felt disgusted about your bowel problems?							

Appendix 19. St Mark's Incontinence Score

St Mark's incontinence score
(never: no episode in the last 4 weeks, rarely: 1 episode in the last 4 weeks, sometimes: more than one episode in the last 4 weeks but less than weekly, weekly: 1 or more episodes in a week but not daily, daily: 1 or more episodes per day). Min score: 0, max score: 24.

	Never (0)	Rarely (1)	Sometimes (2)	Weekly (3)	Daily (4)
How often are you incontinent of solid stool?					
How often are you incontinent of liquid stool?					
How often are you incontinent for gas?					
How often does incontinence alter your life style?					

	No (0)	Yes (2)
Do you need to wear a pad or a plug?		
Do you need to take constipating medicines?		
Can you defer for 15 minutes?	(4)	(0)

Total:

Appendix 20. CTCAE version 3.0

The PREDICT Study	Hospital no: Initials:
CCR NO. 4201	Date:

Please record the grade of disorders in the right-hand column: if no symptoms, leave the box blank.

	Gastrointestinal Disorders - Grade					
	Adverse Event	**1**	**2**	**3**	**4**	**Grade**
1	**Abdominal Pain**	Mild Pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	
	Definition: A disorder characterized by a sensation of marked discomfort in the abdominal region.					
2	**Bloating**	No change in bowel function or oral intake	Symptomatic, decreased oral intake; change in bowel function	-	-	
	Definition: A disorder characterized by subject-reported feeling of uncomfortable fullness of the abdomen.					
3	**Constipation**	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	
	Definition: A disorder characterized by irregular and infrequent or difficult evacuation of the bowels.					
4	**Diarrhoea**	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	
	Definition: A disorder characterised by frequent and watery bowel movements.					
5	**Faecal Incontinence active = urge incontinence passive = leakage**	Occasional use of pads required	Daily use of pads required	Severe symptoms; elective operative intervention indicated	-	
	Definition: A disorder characterised by the inability to control the escape of stool from the rectum.					
6	**Flatulence**	Mild symptoms; intervention not indicated	Moderate; persistent; psychosocial sequelae	-	-	
	Definition: A disorder characterized by a state of excessive gas in the alimentary canal.					

The PREDICT Study	Hospital no: Initials:
CCR NO. 4201	Date:

	Gastrointestinal Disorders - Grade					
	Adverse Event	**1**	**2**	**3**	**4**	**Grade**
7	**Frequency**	Mild, intervention not indicated	Moderate symptoms, limiting instrumental ADL	Severe symptoms; limiting self care ADL	-	
	Definition: A disorder characterised by the need to pass stool much more frequently than is normal.					
8	**Rectal bleeding**	Mild; intervention not indicated	Moderate symptoms; medical intervention or minor cauterization indicated.	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	
	Definition: A disorder characterized by bleeding from the rectal wall and discharged from the anus.					
9	**Rectal pain**	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	
	Definition: A disorder characterised by a sensation of marked discomfort in the rectal region.					
10	**Tenesmus**	Mild, intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL	-	
	Definition: The sensation of feeling the rectum is still not empty even after passing a stool.					
11	**Urgency**	Mild, intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL	-	
	Definition: A disorder characterised by the desperate need to open the bowel.					
12	**Gastrointestinal disorders –** **Other, specify below :**	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or non-invasive intervention indicated; limited age-appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of existing hospitalisation indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	

ADL – activities of daily living Instrumental ADL refers to preparing meals, shopping etc Self care ADL refers to bathing, dressing, feeding self etc

Completed by: Name _____ **Signature** _____ **Date** _____

Appendix 21. Bristol Stool Chart (BSC)

The PREDICT Study	Study ID:	Initials:
CCR No: 4201	Date:	

1. Please tick the box(es) which best describe(s) the stool you pass:

Bristol Stool Chart

Type 1	Separate hard lumps, like nuts (hard to pass)	☐
Type 2	Sausage-shaped but lumpy	☐
Type 3	Like a sausage but with cracks on its surface	☐
Type 4	Like a sausage or snake, smooth and soft	☐
Type 5	Soft blobs with clear-cut edges (passed easily)	☐
Type 6	Fluffy pieces with ragged edges, a mushy stool	☐
Type 7	Watery, no solid pieces. **Entirely Liquid**	☐

Appendix 22. 7-day food diary

7 DAY FOOD DIARY

This diary is designed to obtain accurate information about the type and quantity of food that you eat.

Please return to: _____ (Name)

_____ (Title)

The Royal Marsden Hospital
Sutton, Surrey, SM2 5PT

(Delete as appropriate)

The Royal Marsden Hospital
Fulham Rd, London, SW3 6JJ

For research personnel use only:

DIETPLAN Registration no.: _____

Date of entry: _____

Assessment type: _____

Activity level (recreational): _____

Activity level (occupational): _____

Patient Details

Name: ……

Date: ……

Weight: ……

Height: ……

Hospital number: ……

GENERAL QUESTIONS

Please answer the following questions:

Which type of bread do you **usually** eat?

White	☐
Brown	☐
Granary	☐
Wholemeal	☐
None	☐

Do you **usually** buy large or small loaves, sliced or unsliced?

Large	☐
Small	☐
Sliced	☐
Unsliced	☐

If you eat any type of biscuit regularly, please specify which brands:

……
……
……

Which type of milk do you **usually** use?

Full cream milk	☐
Semi-skimmed milk	☐
Skimmed milk	☐
Soya milk	☐
Lactose-free	☐
None	☐

How much milk do you **usually** use?

1-2 pints daily	☐
½ -1 pint	☐
¼ - ½ pint	☐
None	☐

How many tablespoons of milk do you take in tea and coffee?

…… tablespoons in a cup of tea

…… tablespoons in coffee

None: Tea/Coffee* taken black

*please circle if applicable

Which kind of fat do you usually use on bread, toast etc?

Butter	☐	
Margarine	☐	
Low fat	☐	spread

Which brand do you usually use?

……..

Which kind of fat do you usually use when cooking?

Butter	☐
Olive Oil	☐
Vegetable oil	☐
Lard	☐
Other ……..	☐

What do you do with the visible fat on meat?

Eat most of the fat	☐
Eat as little as possible	☐
Eat some of the fat	☐
Don't eat meat	☐

Do you drink alcoholic drinks?

YES ☐ NO ☐

If the answer is YES, please indicate the serving size below:

Wine: Large glass ☐ Small glass ☐

Beer: Half pint glass ☐ Pint glass ☐

Spirits: Single ☐ Double ☐

FOOD RECORD

Read these instructions and the example carefully before you start.

We would like you to record, as accurately as possible, what you eat and drink for 7 days.

- Please record **ALL** food and drink consumed. Record at the time of eating and **NOT** from memory at the end of the day. Keep this record sheet with you throughout the day.

- You should include all meals and snacks, plus sweets, drinks etc. When recording food eaten at meals, please include any sauces, dressings or extras, e.g. gravy, salad dressing, pickles as well as the main food.

If you do not eat a particular meal or snack simply draw a line across the page at this point.

Guidelines for describing food & drink:

1. Please give details of the cooking method i.e. grilled, boiled, roasted, fried.

2. Give as many details as possible about the food:

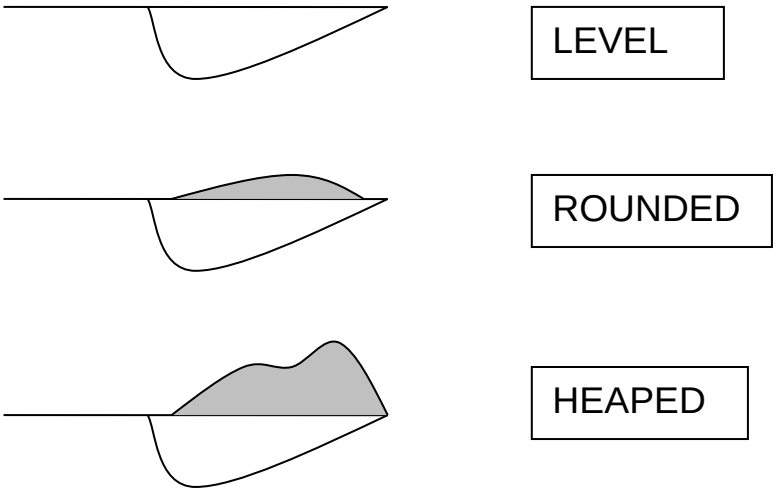
   a) State brand name if applicable
      → i.e. 'Heinz' baked beans OR
      'John West' tuna chunks in brine

   b) Name the type of biscuit, cake or cereal
      → i.e. Chocolate Hob-Nob, Madeira, Branflakes

   c) Name the type of cheese, fish or meat
      → i.e. Red Leicester cheese, smoked haddock fillet, lamb chop

3. Suggestions for recording quantity of food and drink:

   a) For many foods (vegetables, cereals and some fruit) a household measure is adequate, i.e. state the number of teaspoons (tsp), tablespoons (tbsp) or cups. Also whether it is level, rounded or heaped.



   b) All convenience foods have their weight on the packaging and this can be quoted i.e. 150g carton Ski raspberry yoghurt OR ½ 440g tin of Heinz Tomato soup.

   c) Bread, fruit loaves etc. please indicate the size of loaf and thickness of the slice i.e. 1 thick slice granary bread, small loaf.

   d) Cheese, fish, meat: please describe your portion as accurately as you can, for example:

      - 2 large thin slices ham
      - 2 small pork chops (no fat)
      - Matchbox sized cube of cheddar cheese.

Remember to include everything you eat and drink including snacks, nibbles and food eaten whilst out of the house.

When recording home-made foods, please list all ingredients used in recipe and estimate how much of each ingredient was consumed in the portion of food you ate.

The example on the following pages may be useful as a guide but please remember that although we have included space to record all possible meals and snacks, this does not mean that you have to eat at these specific times.

If you do not have very regular meals, please try to record the food you eat throughout the day, starting with the first items you eat and continuing to the last item consumed before bedtime.

THANK YOU VERY MUCH FOR YOUR HELP.

DIETARY RECORD SHEET – EXAMPLE

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning	1 cup 1 tbsp 1 tsp	Tea Semi-Skimmed milk Granulated sugar	
Breakfast	3 heaped tbsp ¼ pint 1 thick slice 1 rounded tsp 1 heaped tsp 2 mugs	Branflakes (Kellogg's) Full cream milk (for cereal & drink) Poppy seed bread (small loaf, self cut) 'Olivio' margarine Thick cut marmalade (Tesco's own) Coffee	
During morning	85g 330ml can	Vanilla flavour bio-yoghurt (Shape) Coca cola	
Lunch	1 standard 2 tbsp 2 thin strips 1 large 1 large mug	Onion bagel, toasted Full fat cream cheese (Philadelphia) Smoked salmon Banana Hot chocolate (Cadbury's)	

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon	1 tall glass 1 small	Orange squash (Robinson's) Blueberry muffin (Sainsbury's)	
Evening meal	4 heaped tbsp 2 drumsticks 1 level tbsp 1 level tbsp 3 heaped tbsp 3 heaped tbsp 1 heaped tbsp 2 cups (teacup sized) 2 small glasses	Chicken casserole (homemade with tomato/wine based sauce) containing: Chicken, baked (no skin) Olive oil Red wine Chopped fresh tomatoes Courgettes Carrots Boiled white long grain rice Orange juice	
During evening	4 squares 2 cups 2 tbsp 2 level tsp	Cadbury's Fruit & Nut chocolate Tea Semi-skimmed milk Granulated sugar	
Bedtime			

DIETARY RECORD SHEET

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

DAY 1: ……..... **DATE: ……..**

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning			
Breakfast			
During morning			
Lunch			

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon			
Evening meal			
During evening			
Bedtime			

DIETARY RECORD SHEET

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

DAY 2: ……..... **DATE: ……..**

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning			
Breakfast			
During morning			
Lunch			

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon			
Evening meal			
During evening			
Bedtime			

DIETARY RECORD SHEET

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

DAY 3: ……..... **DATE: ……..**

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning			
Breakfast			
During morning			
Lunch			

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon			
Evening meal			
During evening			
Bedtime			

DIETARY RECORD SHEET

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

DAY 4: ……..... **DATE: ……..**

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning			
Breakfast			
During morning			
Lunch			

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon			
Evening meal			
During evening			
Bedtime			

DIETARY RECORD SHEET

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

DAY 5: ……..... **DATE: ……..**

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning			
Breakfast			
During morning			
Lunch			

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon			
Evening meal			
During evening			
Bedtime			

DIETARY RECORD SHEET

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

DAY 6: ……..... **DATE: ……..**

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning			
Breakfast			
During morning			
Lunch			

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon			
Evening meal			
During evening			
Bedtime			

DIETARY RECORD SHEET

- Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.
- Record method of cooking, type and quantity of food.
- For home-made meals, please record all ingredients and the quantity of these consumed.

DAY 7: ……..... **DATE: ……..**

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
Early morning			
Breakfast			
During morning			
Lunch			

MEAL/ SNACK	QUANTITY EATEN	DETAILS	Food Code: (leave blank)
During Afternoon			
Evening meal			
During evening			
Bedtime			

Notes

- Use this page to make any notes or comments that may be helpful in interpreting your food diary.

Appendix 23. Sample collection instructions for participants

Instructions for collecting your stool sample

Thank you so much for agreeing to do this. Many bacteria live in the lower part of the gut. The are involved in many important functions such as digesting food, producing vitamins and fighting infections. Each one of us has a unique combination of bacteria. The purpose of this stool sample is to obtain a sample of the bacteria living in the gut.

Instructions as follows:

- Please wear a pair of disposable gloves
- Use the card tray to collect the stool before it drops into the pan
- Decant enough stools to fill the yellow-top container using the wooden spatula.
- Screw the yellow lids shut firmly and place the container in the transport bag.
- **Seal it back in the clear transport bag provided** (to avoid spills or contamination)
- Dispose of any surplus stool and collection items
- Remember to wash your hands thoroughly
- Bring the sample with you when you come to your next appointment and please ask the reception desk to call me when you arrive (ext 4658) or contact your Gynae CNS.

Please fill the yellow topped container as otherwise we will not have enough stool sample to perform a complete analysis.

Do not hesitate to contact me if you need any further details.
I look forward to collecting your sample and many thanks again.

With best wishes,

Ann Muls
Research Nurse
Tel: 020 7352 8171
Ext: 4658
Monday-Wednesday

Instructions for collecting your urine sample

Thank you so much for agreeing to do this. Your kidneys' main job is to filter wastes and extra water out of your blood to make urine. Normally, there are no actual bacteria in your urine (unless you have a urinary tract infection) but the bacteria in the gut produce waste products which we can detect in your urine. The purpose of this urine sample is to look more closely at the waste products of the gut bacteria.

We need a **mid-stream** urine sample. This means that you don't collect the first part of urine that comes out or the last part. This reduces the risk of the sample being contaminated with bacteria from your hands or the skin around the urethra.

Instructions as follows:

- Please wash your hands first
- Wash your genitals, including between the labia
- Start to urinate but do not collect the first part of urine that comes out
- Collect a sample of urine "mid-stream" in yellow-top container
- Screw the lid of the container shut
- **Seal it back in the clear sealable transport bag** to avoid spills or contamination
- Please wash your hands thoroughly
- Bring the sample with you when you come to your next appointment and please ask the reception desk to call me when you arrive (ext 4658) or contact your Gynae CNS.

Please do not hesitate to contact me if you need any further details.
I look forward to collecting your sample and many thanks again.

With best wishes,

Ann Muls
Research Nurse
Tel: 020 7352 8171
Ext. 4658
Monday-Wednesday

Instructions for collecting your saliva sample

Thank you so much for agreeing to do this. There are many bacteria living in our mouth. This is normal. The purpose of taking this saliva sample is to obtain a sample of the bacteria living in your mouth.

A few things to do in preparation for collecting the sample:
- Please do not eat, drink, smoke, chew gum, brush your teeth, or use mouthwash for at least 1 hour prior to providing your sample.
- Your sample is also not suitable if you have had any dental work within 24 hours prior to sample collection.

It may be the most convenient to do this in the hospital whilst you are waiting for me to collect your other samples.

If providing adequate sample volume is a challenge for you or someone you are assisting, the following may help increase saliva production:

Gently rubbing the outside of your cheeks
Making chewing motions with your mouth
Smelling or imagining sour foods such as lemons
Thinking about your own favourite food

Instructions as follows:

- Please wash your hands.
- Unscrew the yellow-top container.
- Let the saliva pool in the bottom of your mouth
- Spit it in the yellow-top container. You may need to do this a few times.
- Screw the yellow lids shut firmly and place the container in the transport bag.
- **Seal it back in the clear transport bag provided** (to avoid spills or contamination)
- Ask the reception desk to call me when you arrive (ext 4658) or contact your Gynae CNS.

Please fill the yellow topped container as much as possible otherwise we will not have enough saliva to perform a complete analysis.

Please do not hesitate to contact me if you need any further details.
I look forward to collecting your sample and many thanks again.

With best wishes,

Ann Muls
Research Nurse
Tel: 020 7352 8171
Ext: 4658
Monday-Wednesday

Instructions for collecting your rectal swab

Thank you so much for agreeing to do this. You have lots of bacteria which live in the stool. This is normal. Some bacteria in the bowel live mainly attached to the bowel wall and not in the stool. The purpose of taking a rectal swab is to obtain a sample of the bacteria living on the bowel wall. We need 3 swabs in total.

Instructions as follows:

Use the long white swab sample kit which contains 3 separate tubes.

- Please wash your hands.
- Find a comfortable position to be in

Find a comfortable position, either sitting or standing.

- Remove the cap from the swab tube and pull out the stick with the swab at the end
- Gently insert the swab about 3-5 cm into your bottom. Stop immediately if it hurts.
- Rotate swab against the side of the bowel wall at least 3 times.
- Withdraw the swab carefully. Swabs that are contaminated with stools should be discarded and the collection repeated.
- Immediately place the swab into the tube.
- Re-cap the swab specimen tube tightly
- Repeat this with swab 2 and 3.
- **Seal the tubes back in the clear transport bag provided** (to avoid spills or contamination)
- Ideally this should be done no longer than 6 hours before your hospital appointment. Store sample at room temperature.
- Ask the reception desk to call me when you arrive (ext 4658) or contact your Gynae CNS.

If you feel uncomfortable doing this yourself, I am always happy to take the swab for you when you are in the hospital.

Do not hesitate to contact me if you need any further details.
I look forward to collecting your sample and many thanks again.

With best wishes

Ann Muls
Research Nurse
Tel: 020 7352 8171
Ext: 4658
Monday-Wednesday

Appendix 24. PRDA support letter

Pelvic Radiotherapy Disease Association
PO Box 602 Epsom KT17 9JB
Phone: 01372 744338
www.prda.org.uk email: info@prda.org.uk

Ann Muls
Macmillan Nurse Consultant
Gastrointestinal Unit
The Royal Marsden NHS Foundation Trust
Fulham Road
London SW3 6JJ

April 16th 2014

Dear Ann,

As Chair if the Pelvic Radiation Disease Association (PRDA) I am pleased to be able to tell you that we unreservedly support the proposal for your study: "Is it possible to predict cancer patients at risk of developing gastrointestinal symptoms after treatment with pelvic radiotherapy?".

As you know, PRDA is a patient-led group whose aims are to provide support for people of all ages experiencing consequences of treatment with pelvic radiation, and to campaign for better recognition of pelvic radiation disease as well as improved facilities for treatment within the NHS.

Many members of PRDA are familiar with the clinical work that your team do and have heard you speak at conferences about the development of the service and future plans for innovation.

Your project fits within the Association's expectation to influence the disease trajectory and promote consideration of long-term consequences earlier in the disease trajectory. The experience of many patients is that they are never (or at best inadequately) warned about the potential chronic change to their bowel function and how this would affect their quality of life after cancer treatment, and the Association is concerned that this lack of information prejudices the concept of 'informed consent'. In terms of patient information provision and counselling before treatment starts, the outcomes of study could inform the current patient pathway and retune the patient experience in a more anticipatory way.

PRDA is dedicated to promoting the development of service provision and clinical advances that can help patients who receive pelvic radiotherapy and this project is congruent to PRDA's desire to inculcate wider awareness of consequences of pelvic radiotherapy among health professionals and patients alike. Furthermore, the findings of this study will promote your team's application for interventional research in the future.

We hope that your proposal receives a favourable review and look forward to hear about the progress on this foundational project.

With every best wish for your successful application
Yours sincerely

Richard Simmon

Chair, the Pelvic Radiation Disease Association

Pelvic Radiation Disease Association: registered charity No. 1147802, registered company No. 7998409

Appendix 25. Statistical Analysis Plan (SAP)

Statistical Analysis Plan	
CCR #:	4201
Version Number:	Version 1
Date	05/02/2020
Study protocol	Version 5
EUDRAC #:	<< >>

Predicting patients at risk of developing gastrointestinal symptoms after treatment with pelvic radiotherapy: a longitudinal cohort study.

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List of abbreviations

Abbreviation or special term	Explanation
BMI	Body Mass Index
CTCAE	Common Terminology Criteria Adverse Events
GC-IMS	Gas Chromatography- Ion Mobility Spectometry
Gy	Gray
IBDQ	Inflammatory Bowel Disease Questionnaire
IBDQ-B	Inflammatory Bowel Disease Questionnaire- Bowel subset
IBDQ-E	Inflammatory Bowel Disease Questionnaire- Emotional impact
IBDQ-SOC	Inflammatory Bowel Disease Questionnaire- Social function
SPSS	Study Package for Social Sciences
PhD	Doctorate in Philosophy

Amendment history

Date	Brief description of change	Justification for change
Nov 2019	First draft	
Feb 2020	Peer reviewed	

1. Study Details

1.1 Brief synopsis and background

The PREDICT study is a prospective observational cohort study including women undergoing radiotherapy for a new gynaecological malignancy and the development of long-term gastrointestinal symptoms under the care of participating consultants at Royal Marsden NHS Foundation Trust in Sutton and Fulham Road. The study has 6 time points: baseline (before treatment), beginning of treatment, end of treatment, 6 months, 12 months and 24 months after the end of treatment.

1.2 Study objectives

Primary objective:
To identify potential predictive markers of chronic radiation induced GI toxicity in women treated for gynaecological cancer using the metabolomic signatures of the microbiota in stool, urine, saliva and rectal swabs.
Secondary objectives:
- To identify the optimal time point to predict chronic GI toxicity.
- To determine which of the samples (rectal swab, stool sample, urine sample, saliva sample) will best predict chronic GI toxicity.

1.3 Study design

The study design used is a longitudinal observational cohort study. At baseline and follow-up time points patients complete questionnaires measuring gastrointestinal toxicity (IBDQ-B, Bristol Stool Chart, St Mark-s incontinence score, CTCAE) in addition to the sample and clinical data collection.

1.4 Number of subjects

This observational cohort study was not powered to a specific end-point but the following assumptions were made to estimate the number of patients feasible to recruit.

In preparation of the study protocol, the number of women referred to the organisation during 2012 were requested from our information department: 918 patients were referred with a gynaecological cancer (cervix, ovary, endometrium, vagina, and uterus). Of the 780 patients that were treated and alive within the year, 29 received pelvic radiotherapy alone (3 after surgery) and a further 100 received brachytherapy in combination with pelvic radiotherapy (Unpublished data). Of the total patients referred (n=918), 15% (n= 129) met the eligibility criteria for this study. The recruitment rate for studies within our team has been consistent between 30 and 40%. This suggested a likely annual recruitment of 55 patients (or 5 per month), 110 patients over two years.

However, these figures did not differentiate between patients with new diagnoses or those treated with radiotherapy for disease recurrence or progression. It also did not make it possible to anticipate the number of patients who met other exclusion criteria. For instance,

those unable to speak or read English, or patients living abroad but coming to the organisation for overseas treatment.

During the two year recruitment period, 635 patients were assessed for eligibility. 111 were eligible for recruitment and 41 (39%) consented to participate. A total of 24 participants completed the study one and two years after the end of treatment. Screening, eligibility, recruitment and reasons for declining participation will be listed in a CONSORT diagram.

1.5 Randomisation methods (if applicable)

There is no intervention in this observational study and no randomisation.

2. Analysis Sets

2.1 Definition of analysis sets

All patients recruited, those who withdrew before or at baseline, those who withdrew before one year after completion of treatment will be included in descriptive analysis of clinical and demographic data.

PEA (Primary Endpoint Analysis) set: All participants who complete time point 4 (one year after completions of treatment) and/ or time point 5 (two years after completion of treatment) will be included in the analysis.

2.2 Violations and deviations

Lost to follow-up or unable to contact the participant are violations or deviations expected in this observational cohort study.

3. Primary and Secondary endpoints

Primary endpoint:

To compare the development of new GI symptoms, measured with the IBDQ-B symptom scale between baseline, beginning and end of treatment, 6 months after treatment to 12 and 24 months after treatment with pelvic radiotherapy with the signatures of analysed emitted volatiles and gases as products of fermentation caused by gut microbiota using the electronic sensing technique and GC-IMS at each of the 6 time points.

Primary outcome:
- gastrointestinal toxicity measured by the IBDQ-B subscale at baseline and all subsequent time points. The minimum score is 10, the maximum score is 70.

Secondary endpoints:

- to examine the relationship between demographic, clinical and treatment characteristics on the development of long-term gastrointestinal symptoms as measured by IBDQ-B, the St Mark's Incontinence score, the CTCAE version 3.0. and stool consistency, measured on the Bristol Stool Chart.

- to describe changes in dietary intake before, during and after treatment in women receiving treatment for gynaecological malignancies using 7-day food diaries analysed by the Nutritics analysis system. This analysis will be performed at King's College London and will not be described further in this SAP.

- to correlate the faecal calprotectin results with the IBDQ-B-scores

Secondary data include:
- clinical demographic data as presented at baseline:
    age: years
    cancer tumour site: Cervix , Endometrium, Vagina, Vulva
    histology: Adenocarcinoma, Adenosquamous carcinoma, Carcinosarcoma, Clear cell carcinoma, Endometrioid, Mucinous carcinoma, Serous carcinoma, Squamous cell carcinoma
    the presence of lymphovascular space invasion: yes or no
    surgery: yes or no
    type of surgery: hysterectomy, bilateral salpingooophorectomy, lymph node dissection, local excision
    chemotherapy: yes or no
    type of chemotherapy: Cisplatin, Carboplatin, Carboplatin + placlitaxel, None
    radiotherapy (Gy and number of fractions)
    marital status: Single, Co-habiting, Married, Divorced, Widowed
    number of children: Nullipara, Unipara, Multipara
    mode of delivery: normal delivery, C-section
    smoking status: Current smoker, Ex-smoker, Never smoked
    number of cigarettes per day
    alcohol intake: yes or no
    Number of units per week
    type of diet: Includes meat, Vegetarian, Pescetarian
    weight: kg
    height: m
    BMI: as calculated with NHS BMI calculator
    performance status: active, moderately active, moderately inactive, inactive
    ECOG score: 0- 5
    co-morbidities: coded by IDC-10
    use of medications: generic names as listed in the British National Formulary.
- analysis of 7- food diaries: macronutrients, micronutrients, fat, protein, carbohydrate and fibre
- faecal calprotectin: mcg/g

Any missing data for the 24 participants who completed the one and two year post treatment time points will be handled by taking the average of the previous and subsequent time point if the missing value is in between time points or by taking the value of the last time point if the missing value occurs at the two-year time-point.

4. Analysis Methods

4.1 General principles

Primary hypothesis:
The null hypothesis is that there is no difference in the signatures created by the electronic sensing technique, FAIMS and GC-IMS in the emitted volatiles and gas profiles of the gut microbiota between cancer patients treated with pelvic radiotherapy who develop gastrointestinal symptoms and those who do not.
The alternative hypothesis is that the signatures created by the electronic sensing technique, FAIMS and GC-IMS will show there is a difference in the volatiles and gas profiles emitted by the gut microbiota between cancer patients treated with pelvic radiotherapy who develop GI symptoms and those who do not. These signature profiles can be used to predict risk of GI toxicity.

Secondary hypothesis:
Indications of differences between cancer patients treated with pelvic radiotherapy who develop GI symptoms and those who do not will be described using patient and clinical characteristics.

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. All confidence intervals presented will be 95% and two-sided.

4.2 Analysis methods

Primary outcome analysis:
- gastrointestinal toxicity (measured by the IBDQ-B subscale) at baseline and all subsequent time points will be compared with the metabolic profiles of the microbiota in the samples using Linear Discriminant Analysis and Principal Component Analysis as described in Covington et al. (2012). The samples were analysed as a single group and then post-categorised in a group with GI toxicity and a group without GI toxicity. Those with a reduction of 10 or more points in the IBDQ-B score between baseline and the fourth and final time point will be the group of participants who developed long-term gastrointestinal symptoms.

Secondary outcomes:
Participants will be described with respect to age, cancer tumour site, histology, the presence of lymphovascular space invasion, surgery, chemotherapy, radiotherapy (Gy and number of fractions), marital status, number of children, mode of delivery, smoking status, alcohol intake, type of diet, weight at baseline, BMI, performance status, co-morbidities and use of medications both for all who provided baseline data, those who provided data at more than 1 time point and those who completed the study.

Categorical data will be summarised by numbers and percentages. Continuous data will be summarised by mean, SD and range if data are normal and median, IQR and range if data are skewed. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

Depending on normality of the data, differences within patients between all time points will be analysed using general linear models with repeated measures or a non-parametric alternative test.

- analysis of food diaries is required in research involving the gut microbiome as this is a vital factor in determining a healthy microbiome. At least 50% of the diaries of those who completed all time points will be analysed and differences in macronutrients, micronutrients, fat, protein, carbohydrate and fibre will be associated with the primary outcome.

- faecal calprotectin, a marker of intestinal inflammation in stool, results will be correlated with the IBDQ-B scores using repeated measures correlation analysis.

Results will be presented with graphs, tables and figures.

Initial data analysis will be carried out using Statistical package for Social Sciences (SPSS version 24) and will be conducted under the supervision of the RMH Study Statistician and King's College London Statistician in collaboration with the Principle Investigator, academic supervisors and PhD researcher. Additional analysis of samples and IBDQ-B scores will be done in collaboration with Professor Covington at Warwick University using a bespoke data analysis programme in R Pack.

5. Interim Analyses

No statistical interim analysis was planned.

6. Changes of Analysis from Protocol

The PREDICT study is a small cohort study with only 24 participants who provided data at the 1- and 2-year post treatment time points. Green et al (1991) suggests $N > 50 + 8m$ (where m is the number of independent variables) for testing the multiple correlation and $N > 104 + m$ for testing individual predictors. Therefore, based on these criteria, predictive modeling of the data as was planned in the study protocol should not be considered. Rather the study should be treated as a feasibility study which provides correlations that may suggest potential predictive indicators which could be tested in a larger cohort as the study sample size is not large enough for a sufficiently powered statistical analysis of the predictive markers of GI toxicity amongst gynecological cancer patients.

7. Timing of Statistical Analysis

Final analysis will take place after all data has been collected and outcomes will be reported collectively.

8. References

Green, S. B. (1991). How many subjects does it take to do a regression analysis? Multivariate Behavioral Research, 26, 499-510.

Covington, James A., 1973-, Wedlake, Linda, Andreyev, Jervoise, Ouaret, Nathalie, Thomas, Matthew G., Nwokolo, Chuka U., Bardhan, Karna Dev and Arasaradnam, Ramesh P. (2012) The detection of patients at risk of gastrointestinal toxicity during pelvic radiotherapy by electronic nose and FAIMS : a pilot study. Sensors, Vol.12 (No.12). pp. 13002-13018.

9. Data checking plan

Data will be checked after completion of data collection by the researcher against the case report forms used in the study. The database will be updated by an independent data manager before locking. 100% of primary data will be checked. 10% of secondary data will be checked.

10. Appendix

Predicting patients at risk of developing gastrointestinal symptoms after treatment with pelvic radiotherapy: a longitudinal cohort study.

Peer Reviewer	Trevor Murrells	12/2/2020
	Trevor Murrells	Date
Study Statistician		12/02/2020
	Kabir Mohammed	Date
NIHR Clinical Doctoral Fellow		12/02/2020
	Ann Muls	Date

Predicting patients at risk of developing gastrointestinal symptoms after treatment with pelvic radiotherapy: a longitudinal cohort study.

Chief Investigator		17/02/2020
	Dr Alexandra Taylor	Date

Appendix 26. STROBE checklist of items that should be included in reports of cohort studies (von Elm et al., 2008)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	30-71, 75-102
Objectives	3	State specific objectives, including any prespecified hypotheses	72, 106-107
Methods			
Study design	4	Present key elements of study design early in the paper	106
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	108
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	110-112
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	113-151
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	113-151
Bias	9	Describe any efforts to address potential sources of bias	159
Study size	10	Explain how the study size was arrived at	160
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	163
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	163-165
		(*b*) Describe any methods used to examine subgroups and interactions	163-165
		(*c*) Explain how missing data were addressed	164
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	142-143, App. 24
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	167-168
		(b) Give reasons for non-participation at each stage	168-169
		(c) Consider use of a flow diagram	169

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	170-172
		(b) Indicate number of participants with missing data for each variable of interest	175
		(c) Summarise follow-up time (eg, average and total amount)	110
Outcome data	15*	Report numbers of outcome events or summary measures over time	171-239
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	171-239
		(*b*) Report category boundaries when continuous variables were categorised	171-239
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	215
Discussion			
Key results	18	Summarise key results with reference to study objectives	242-247
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	248-252
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	253-255
Generalisability	21	Discuss the generalisability (external validity) of the study results	257-259
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	258-259

*Give information separately for exposed and unexposed groups.

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

Appendix 27. Dietary Habits of women with gynecological cancer before, during and after treatment: a long-term prospective cohort study.

Nutrition and Cancer: An International Journal

Dietary habits of women with gynecological cancer before, during and after treatment: a long-term prospective cohort study.

Journal:	*Nutrition and Cancer: An International Journal*
Manuscript ID	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Cirillo Sanchez, Claudia; King's College London, Czuber-Dochan, Wladzia; King's College London, Cox, Selena; King's College London, Murrells, Trevor; King's College London, Norton, Christine; King's College London, Muls, Ann; Royal Marsden NHS Foundation Trust, ; King's College London,
Keywords:	Cervical cancer, Diet, Endometrial cancer
Manuscript Specific Keywords:	Gynecological cancer, Chemoradiation, Gastrointestinal toxicity, Prospective cohort study

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Manuscripts

Dietary habits of women with gynecological cancer before, during and after treatment: a long-term prospective cohort study.

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Ethical/legal considerations: This article is an original contribution not previously published and is not under consideration for publication elsewhere. Each author has contributed significantly in this research. Cirillo Sanchez C and Muls A have conducted primary research, drafted, written and revised the article. Murrells T has provided significant guidance on the statistical analysis, interpretation and presentation of results, Czuber-Dochan W, Cox S and Norton C have written and revised the article. The authors declare no conflict of interest.

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Key words: gynecological cancer, dietary intake, chemoradiation, gastrointestinal toxicity, prospective cohort study.

Word count: 3, 803

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Abstract

Background and aim: Over 21,000 new cases of gynecological cancer are diagnosed annually in the United Kingdom. There is evidence of cancer patients altering their eating habits before and during treatment. Some women with gynecological cancer make conscious decisions to change their diet as self-management for their cancer symptoms and to adopt a healthier life style. Little is known about the impact of treatment on dietary habits. This study aimed to identify and describe the dietary habits of women with gynecological cancer before, during and after treatment.

Methods: This was a longitudinal prospective cohort study using seven-day food diaries to collect dietary intake data before treatment and up to two years after gynecological cancer treatment. Nutritics© software was used for analysis of the diaries. A general linear mixed model was used for the statistical analysis, adjusted for multiple comparisons.

Results: 15 women with gynecological cancer participated; 69 food diaries were analyzed. There were no statistically significant changes in dietary habits or weight for this cohort during the two-year follow-up, except for caffeine intake which increased at 2 years ($p<0.05$).

Conclusions: Symptoms relating to gastrointestinal toxicity are more likely due to other factors rather than dietary changes.

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Introduction

Over 360,000 people are newly diagnosed with cancer every year in the United Kingdom (UK) and annually there are over 21,000 women diagnosed with a gynecological malignancy (1). Gynecological cancers include cancer of the endometrium (or uterus), cervix, ovary, vagina and vulva. In the UK in 2014-2016, there were about 9,300 new cases of endometrial cancer, 7,470 women were newly diagnosed with ovarian cancer, around 3,200 new cases of cervical cancer; about 1,300 new diagnoses of vulvar cancer and 250 new cases of vaginal cancer (1).

Obesity is a significant risk factor for adenocarcinoma of the endometrium, especially in post-menopausal women (2). Excessive weight also has an impact on cancer recurrence and second cancer development (3). Undergoing cancer treatment and living with cancer can impact the eating habits of patients especially in those women treated for gynecological cancer with radiotherapy who develop long-term changes in gastrointestinal function (4-9).

Surgery, chemotherapy and radiotherapy for gynecological cancer potentially impact on bowel function both acutely or long-term. However, research to date has mainly focused on acute gastrointestinal toxicity during radiotherapy as diarrhea and vomiting are the main treatment and dose limiting factors (10).

Although the incidence of severe complications such as fistula formation, obstruction, strictures or chronic radiation proctopathy has decreased since the introduction of modern radiotherapy techniques, even low or moderate gastrointestinal toxicity can significantly impact on quality of life (11-15). In general, clinicians underestimate the prevalence of moderate to severe gastrointestinal toxicity following pelvic radiotherapy for gynecological cancer compared to patients' reports of symptoms (10). In a retrospective review of women treated for gynecological cancer, long-term bowel problems were reported in 28% women with the most common symptoms being fecal urgency, diarrhea, fecal incontinence and rectal bleeding (16).

Increasingly, research in this area focusing on the prevention or reduction of long-term gastrointestinal toxicity following cancer treatment is exploring interventions

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to change diet during treatment (17) and recent studies explore the role of the gut microbiome in the development of both acute and long-term gastrointestinal toxicity and how this could be manipulated by changes in diet or the use of probiotics (18-22).

Long-term survival in women treated for gynecological cancer varies depending on the primary site of disease, age and stage of cancer presentation (1). Advances in multimodal treatments offer better chances of survival but can still impact in multiple ways including gastrointestinal symptoms, urinary problems, sexual difficulties, heart problems, reduced bone health, body image, psychological problems and financial concerns, not only during treatment but long-term (23-24).

Several studies have explored the dietary habits of people with various types of cancer including head and neck, breast, prostate or colorectal cancer (25-29) but only three studies have included women treated for a gynecological cancer (30-32). Bruning et al. (30) and Pía de la Maza et al. (31) reported a trend of on-going weight gain after treatment with an increase of Body Mass Index (BMI) in patients, who already tended to be overweight before the start of their treatment. The BMI of gynecological cancer survivors remains within the overweight or obese category for more than half of the women after treatment (8, 33-34). Studies report short follow-up periods, often only until the end of treatment or a few months after completion of radiotherapy and so do not capture long-term changes in dietary habits.

This study aimed to describe the dietary habits of women treated for gynecological cancer before, during and 2 years after completion of treatment.

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Design and methods

This was a longitudinal prospective cohort study. The rationale for employing this design was the lack of studies assessing dietary intake over time (30-32).

Ethical approval was obtained from National Research Ethics Committee in August 2015 (NREC 15/LO/0436). Participants were recruited between November 2015 and March 2017 when they attended the gynecology oncology clinic with a new

diagnosis of gynecological cancer at the Royal Marsden NHS Foundation Trust. Those who confirmed they wanted to participate, provided their written consent for data collection, including a 7-day food diary, within a larger study of gastrointestinal toxicity in patients with gynecological cancer undergoing radiotherapy.

Women aged 18 years or above, newly diagnosed with a gynecological cancer and due to be treated with radical, adjuvant or neo-adjuvant radiotherapy were eligible to participate. Patients were excluded if: they were pregnant at the time of diagnosis; they had recurrent disease as they would have been treated with pelvic radiotherapy previously; they had received treatment for another cancer in the pelvis within the last 5 years; they were taking part in other studies with conflicting end-points such as dietary interventions which might alter their diet; or were due to have their post-treatment follow-up in another hospital as they would not be returning for routine oncology follow-up appointments.

There were five time points for data collection for each participant: before the start of radiotherapy (baseline), at the end of radiotherapy, 6 months, a year and two years after completion of radiotherapy.

All data were anonymized in accordance to the General Data Protection Regulation (35) and Data Protection Act (36). This study was carried out in line with the Declaration of Helsinki's ethical principles for research involving human participants (37).

Weight was measured on calibrated electronic hospital scales in kg (Marsden, The Weighing Company©: DP2400V5BMI Indicator), height was measured on calibrated hospital stadiometer (cm). Body Mass Index (BMI) was calculated using the online NHS BMI calculator (38).

A seven-day food diary was used to assess habitual dietary intake of the participants as this can be analyzed in a systematic manner (39-40). Participants were not specifically advised to make any changes in their diet during or after cancer treatment in routine clinical practice. The food diaries used in this study are standard in clinical practice at the host hospital. Participants were asked to record everything they consumed at breakfast, lunch and dinner time as well as recording any in-

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between snacks and beverages. On the first pages of the diary, participants indicated usual portion sizes and types of food for instance: white or brown bread, type of milk, butter, margarine or low fat spread which aided in food diary analysis. Prospective food diaries were chosen to assess dietary intake as this method reduces the risk of memory bias, is independent of interviewer skills and is less resource heavy than food frequency questionnaires or 24hr diet recalls (39-41). Repeated assessments are most useful to measure changes over time (39). The completed food diaries were collected at baseline, at the end of treatment and all follow-up time points.

The information recorded in the food diaries was entered into Nutritics©, a regularly updated, cloud-based nutritional analysis software that allows the input of brand-specific ingredients and composite meals consumed by participants across the food diary and generates a 7-day average dietary intake report (42). The data were exported into Excel and analyzed using SPSS version 25 (43). The selection of 22 dietary parameters was informed by the studies described in a systematic literature review from this team (44). Twenty-two dietary parameters were assessed over time. Examples of the parameters included weight, BMI, energy intake (kcal), macro- and micronutrient intake.

A general linear mixed model (GLMM) was used for the statistical analysis. GLMMs allow for a variety of correlation patterns over time to be incorporated into the model and can estimate fixed and random effects (45). Furthermore, a GLMM is able to fit a model in the presence of missing data under the assumption that the data are missing-at-random (46). The p-value was adjusted for multiple tests (n=22) and a p-value of <0.0023 (0.05/22) was used as statistically significant, rather than $p<0.05$. Results were reported using numbers, percentage, means, standard deviation, medians, 25th and 75th percentiles and interquartile range (IQR) depending on variable type (categorical or continuous). The UK government recommendations for energy and nutrients for males and females aged 1- 18 years and 19+ years were used as reference (47).

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Results

Fifteen women participated in this study with a median age of 59 years (range: 27-78; SD 13.9). All had been diagnosed with a new gynecological cancer (cervical (n=5), endometrial (n= 9) and vaginal (n=1)) at various stages and grades. The majority (80%) had undergone surgery. All of the women had received External Beam Radiotherapy (EBRT) and brachytherapy, and 60% (n= 9) received concomitant chemotherapy. A median of 45Gy (range: 45-58) was given during EBRT and a median of 8 Gy (range: 8-20) during brachytherapy. There were six missing diaries out of a possible 75, making the total number of food diaries analyzed 69. Three diaries were missing at the end of treatment, one 6 months after completion of radiotherapy and two a year after completion of treatment. Further clinical and demographic baseline characteristics are in Table 1.

Changes in weight, BMI and energy intake

A median weight decrease of 1 kg was seen at the end of treatment compared to baseline (Table 2). Weight increased 6 months after completion of treatment but decreased by nearly 2 kg below baseline weight by 1 and 2 year after radiotherapy (not significant (NS), p= 0.59).

At all time points, the median BMI was above the UK recommendation for a healthy BMI (18.1 to 24.9) (38). BMI decreased by 1.2 kg/m^2 at the end of radiotherapy and was at its lowest one year following treatment (median: 27.4, range: 23.9 - 33). This decline was not statistically significant. Two years after completion of pelvic radiotherapy, BMI was 0.4 kg/m^2 below baseline BMI (NS, p=0.58). From baseline, daily caloric intake decreased by a median of 140 kcal one year after radiotherapy and increased above baseline to nearly 1500 kcal/ day two years after completion of treatment, but these changes were not statistically significant (p= 0.89).

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Changes in macronutrient intake

Baseline intakes of fat, carbohydrate and fiber intake were below the UK daily recommendation of 78 g/day, 267 g/day and 30 g/day respectively, but protein intake was adequate above 45 g/day (Table 2) (47).

Fat intake increased by a median of 1 g/day two years after completion of radiotherapy (53.18 g, IQR 38.68- 68.87 g) from baseline (52.06 g, IQR 42.71- 78.34 g). Carbohydrate intake reduced by a median of 10 g/day from baseline (175.65 g, IQR 143.88- 201.08 g), two years after completion of radiotherapy (164.68 g, IQR 147.16- 194.49 g). None of these changes were statistically significant. Median daily intake of fiber fluctuated across the time points (range: 14- 18 g/ day) but remained well below the recommended 30 g/day. Whilst initially the changes in fiber intake were statistically significant, this was no longer the case when adjusted for multiple testing.

Changes in micronutrient intake

Micronutrient intake across all time points is shown in Table 2. None of the changes in daily intake across time points of the micronutrients examined were statistically significant either before or after adjusting for multiple testing.

At baseline, the median iron intake was 8.72 mg/day (IQR 6.90- 11.25 mg); this reduced slightly to a median of 7.33 mg/day (IQR 6.15- 10.76 mg) at the end of the study. Only one woman was taking ferrous sulphate at baseline, but none took iron supplements during or after treatment.

Baseline Vitamin D intake through diet or supplements was a median of 3.43 µg/day (IQR 2.09- 4.63 µg). At that time, three women were taking a vitamin D supplement. All participants stopped their vitamin and herbal supplements during treatment. Intake through diet was at its lowest 6 months after radiotherapy (1.85 µg, IQR 1.02- 5.61 µg) and increased slightly to a median of 3.62 µg/day (IQR 1.67- 4.51 µg) two years after the end of treatment. At that time, only one woman was still taking a Vitamin D supplement.

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In contrast, at all time points, median vitamin B_{12} intake was higher than the recommended dose of 1.5 µg/day (47). One woman was taking a vitamin B_{12} supplement at baseline but discontinued this and one other participant was taking an over the counter multivitamin two years after treatment. Baseline intake was a median of 3.95 µg/day (IQR 3.43- 5.11 µg) and increased by an additional 1 µg/day at the end of the study (4.90 µg, IQR 3.40- 5.64 µg). Dietary folate intake remained below the recommended 200 µg/day throughout the study.

Alcohol and caffeine intake

There was an increase in alcohol intake across the five time points in those who drank alcohol; 40% of all participants did not consume any alcohol (Table 1). Median baseline caffeine intake was (68.89 mg, IQR 47.83- 187.69 mg). Caffeine intake decreased at the end of treatment (39.62 mg, IQR 10.78- 114.49 mg), peaked one year after completion of treatment (122.95 mg, IQR 60.79- 231.49 mg) and decreased again a year later (84.40 mg, IQR 59-04- 172.93 mg) (p= 0.02).

Discussion

The aim of this study was to describe the dietary habits of women with gynecological cancer before, during and after treatment. The analyses show that the changes in weight, BMI and dietary intake of macronutrients and micronutrients across time were not statistically significant when the p-value was adjusted for multiple testing. This is possibly because the study was underpowered to detect differences, with a cohort of only 15 participants. Nonetheless, decreasing trends can be observed in most of the parameters analyzed between baseline and one year after completion of treatment, with the exception of alcohol intake (Table 2).

Several studies have shown that cancer treatment can impact the eating habits of patients especially in those women treated for gynecological cancer with radiotherapy who develop long-term changes in gastrointestinal function (4-9).

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However, data correlating long-term gastrointestinal toxicity in the women included in this cohort was not included in this study.

Women did lose some minimal weight over time, but this was not statistically significant. Similarly, BMI decreased. Previous studies showed that weight and BMI initially decreased at the end of radiotherapy but increased long-term (31, 48). The small reduction in weight and BMI, whilst not statistically significant, may indicate a greater awareness of the increased risk of disease recurrence or a second cancer and the impact of weight on future prognosis (3, 49). However, these small changes in weight and BMI suggest that women treated for gynecological cancer may struggle to lose weight if they are already overweight and additional advice and support from a specialist dietitian may be needed.

A systemic inflammation syndrome is frequently active in people with cancer. This can vary in degree but impacts all relevant metabolic pathways (50). Baseline calculations of fat, carbohydrate and fiber intake were below the UK daily recommendation. There are no specific recommendations regarding fat intake for cancer patients beyond the general UK recommendation of a maximum daily fat intake of 78 g/day for women aged between 19-64 years (47) but in weight-losing cancer patients with insulin resistance the European Society for Parenteral and Enteral Nutrition (ESPEN) guidelines recommend an increase in the ratio of energy from fat to energy from carbohydrates. This is intended to increase the energy density of the diet and to reduce the glycemic load, especially when enteral feeding is required (50). None of the women in this study required enteral feeding up to two years after completion of radiotherapy.

As systemic inflammation is associated with altered protein turnover, a loss of fat and muscle mass and an increase in the production of acute phase proteins, the ESPEN guidelines recommend a protein intake above 1g/kg/day up to 1.5 g/kg/day for people with cancer (50). In this study, women consumed just below the recommended 1 g/kg/day of protein at all time points.

Carbohydrate intake was also consistently below the UK recommendation of 267 g/day with less than 27 g/day of free sugars and at least 30 g of fiber (47) and

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reduced further by a median of 10 g/day from baseline, two years after completion of radiotherapy. None of these changes were statistically significant. Systemic inflammation is frequently associated with insulin resistance and impaired glucose tolerance and this may increase the difficulty to lose weight and increases the risk of developing co-morbidities such as diabetes and cardiovascular disease (50-51).

Fiber intake fluctuated across the time points but remained well below the UK daily recommendation of 30g/ day. The slight increase in fiber intake one year after completion of treatment may have been a conscious shift in the participants' awareness that fiber is protective against cancer development (52-53). It may have also been an attempt to alleviate the bowel symptoms the cancer treatments may have caused, such as bloating or constipation (54-57). A recent randomized controlled clinical trial including 60 women receiving treatment for gynecological cancer showed however, that neither a low fiber diet or a high fiber diet had any impact on BMI or GI symptom burden at the end of treatment compared to people in the control group (17).

None of the changes in daily intake of micronutrients across time points of the micronutrients examined were statistically significant.

The main source of vitamin D is exposure to sunlight but up to a fifth comes from dietary intake. In this study, Vit D intake through diet remained well below a vitamin D intake of 10 µg/ day recommended in the UK (47). In the UK, people of all age groups are reported to have a low vitamin D status, as indicated by serum 25-hydroxyvitamin D (25[OH]D) concentrations below 25 nmol/L (47). Vitamin D in diet does not directly reflect blood serum levels but is important for bone health and is also known to have immunomodulatory characteristics: deficiency results in translocation of endotoxins and inflammation within the gut barrier (58). Furthermore, vitamin D absorption may be impaired in women who develop bile acid malabsorption following cancer treatment and supplementation is important to prevent insufficiency fractures (59-60).

In contrast, vitamin B_{12} intake via diet was higher than the recommended dose of 1.5 µg/day (47). Baseline intake was a median of 3.95 µg/day and increased by an

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additional 1 µg/day at the end of the study. Vitamin B_{12} in diet does also not directly reflect blood values as this is dependent on absorption but there is an increased risk of developing cancer, particularly liver, pancreas, and myeloid malignancies, in people with high vitamin B_{12} blood levels (Arendt et al., 2019). However, vitamin B_{12} deficiency is a potent cause for tiredness and is linked to memory problems, anemia and breathlessness. Levels are often low in those with bile acid malabsorption and small intestinal bacterial overgrowth (62-64) which are common in women with gynecological cancer and GI symptoms (65). Folate is also linked to B_{12} absorption but in this cohort, dietary folate intake remained below the recommended 200 µg/day throughout the study. Cancer-related fatigue is reported in 53% of the women treated for gynecological cancers (66) and nearly 90% of women with long-term GI toxicity describe fatigue (65).

Caffeine intake decreased at the end of treatment but peaked one year after completion of treatment and decreased again a year later. The increased caffeine consumption during and immediately after treatment may have been instigated by side effects of the cancer treatments, such as fatigue and weakness (54, 67). Coffee can stimulate colonic activity in some but not all people and suggesting decreased intake or decaffeinated beverages may not be of benefit in people with long-term gastrointestinal toxicity (68-69).

There was an increasing trend in alcohol intake across the five time points, however, 40% of participants did not consume any alcohol. These findings, however, were not statistically significant after correcting for multiple testing. Whilst Fassier et al. (34) reported a decrease in alcohol intake after diagnosis, recent research has highlighted a persistent trend in increasing alcohol intake among cancer survivors over the past decade, including women treated for gynecological cancer (70). Little is known about the factors contributing to this trend and further investigation is warranted.

It was unexpected to find that the dietary habits of the women in this cohort did not change statistically significantly across time as there is mounting evidence showing changes in dietary habits of patients undergoing treatment for different cancers (6). Soon after being diagnosed, cancer patients reflect and make changes to their lifestyle by developing health-promoting behaviors, such as following a balanced diet

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and exercising regularly (71-74). One older study exploring dietary habits of women with gynecological cancer (30) also found that gynecological cancer patients did not alter their eating habits during and after treatment. In contrast, Pía de la Maza et al. (31) described significant increases in weight, BMI, fat-, protein- and vitamin B_{12} intake two years after treatment in women treated for gynecological cancer.

Although a cancer diagnosis and undergoing cancer treatment is often thought of as a teachable moment, many cancer survivors do not adhere to behavioral recommendations that might improve their health (71-73, 75). Healthcare providers can play an important role in promoting healthy diet among cancer survivors and including this in health and wellbeing events to support individuals to self-manage their condition following completion of their treatment (23-24, 74). The women may not have shifted towards healthier dietary habits long-term because they regarded these habits as comfort eating in compensation for the disease and treatment related symptoms. Pía de la Maza et al. (31) also highlighted that some participants increased their food intake as compensation for their disease. In addition, participants may have chosen to maintain their weight or not lose weight due to the societal perspectives regarding weight loss during cancer treatment. Cancer patients affected by weight loss often feel weak and self-conscious and worry about the response they might receive from others regarding the change in weight (76).

There are several limitations to this study. This was a small cohort of 15 participants who may not be representative of the wider population of women with gynecological cancer. In addition, participants were recruited from a single specialist cancer hospital and may not be representative for people elsewhere in the UK.

Although food diaries are the gold standard method of measuring dietary intake, estimating portion sizes can be a source of significant error. In addition, 8% of diaries were not completed.

Examining correlation with other factors such as physical activity, GI symptomatology or impact on quality of life was not undertaken and this would add to our current understanding.

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In summary, the women in this study did not make significant changes to their diet long-term and maintained a high BMI. Various factors may have influenced these results, including psychological and social factors or comfort eating and drinking triggered by the diagnosis and treatment of cancer. Understanding if and how dietary habits are affected by cancer treatments is an important part of improving clinical cancer care for women treated for a gynecological malignancy, improving long-term survival and reducing the risk of developing cancer recurrence. Further research and larger studies are necessary in order to reach conclusive and generalizable findings. Qualitative research would also better our understanding of how a cancer diagnosis and treatment impact people's lives.

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References

1. Cancer Research UK. *Women's cancers (gynecological cancer)* [online]. Cancer Research UK. 2017. [Accessed February 2020]. Available from: https://www.cancerresearchuk.org/about-cancer/womens-cancer

2. Saso S, Chatterjee J, Georgiou E, Ditri A, Smith R et al. Clinical Review: Endometrial cancer. *BMJ* **342**, d3954, 2011.

3. Demark-Wahnefried W, Platz E, Ligibel J, Blair C, Courneya K et al. The Role of Obesity in Cancer Survival and Recurrence. Cancer Epidemiol Biomarkers Prev 21(8), 1244- 1259, 2012.

4. Henson C, Burden S, Davidson S and Lal S. Nutritional interventions for reducing gastrointestinal toxicity in adults undergoing radical pelvic radiotherapy. *Cochrane Database of Systematic Reviews* Issue 11, Art. No.: CD009896, 2013.

5. van de Wetering F, Verleye L, Andreyev HJN, Maher J, Vlayen J et al. Non-surgical interventions for late rectal problems (proctopathy) of radiotherapy in people who have received radiotherapy to the pelvis. *Cochrane Database of Systematic Reviews* **4**, Art. No.: CD003455, 2016.

6. Dias Custódio I, Da Costa Marinho E, Araujo Gontijo C, Silva Pereira T, Paiva C et al. Impact of Chemotherapy on Diet and Nutritional Status of Women with Breast Cancer: A Prospective Study. *PLoS One* **11(6)**, e0157113, 2016.

7. de Vries Y, van den Berg M, de Vries J, Boesveldt S, de Kruif J et al. Differences in dietary intake during chemotherapy in breast cancer patients compared to women without cancer. *Support Care Cancer.* **25(8)**, 2581-2591, 2017.

8. Lind H, Waldenstrom AC, Dunberger G, al-Abany M, Alevronta E et al. Late symptoms in long-term gynecological cancer survivors after radiation therapy: a population-based cohort study. *Br J Cancer* **105**, 737–745, 2011.

9. Lawrie T, Green J, Beresford M, Wedlake L, Burden S et al. Interventions to reduce acute and late adverse gastrointestinal effects of pelvic radiotherapy for primary pelvic cancers. *Cochrane Database Syst Rev.* **1**, CD012529, 2018.

For Peer Review Only

10. Taylor A, Fernandes A, Kumar P and Muls A. Chapter 179. Pelvic Irradiation: Acute and Late Toxicity. 1425- 1436. *European Society of Gynecological Oncology. Textbook of gynecological oncology.* Third Edition. 2016.

11. McGough C, Baldwin C, Frost G and Andrejev HJN. Role of nutritional intervention in patients treated with radiotherapy for pelvic malignancy. *British Journal of Cancer* **90**, 2278–2287, 2004.

12. Abayomi J, Kirwan J and Hackett A. The prevalence of chronic radiation enteritis following radiotherapy for cervical or endometrial cancer and its impact on quality of life. *Eur J Oncol Nurs.* **13**, 262-267, 2009.

13. Gillespie C, Goode C, Hackett C and Andreyev J The clinical needs of patients with chronic gastrointestinal symptoms after pelvic radiotherapy. *Aliment Pharmacol Ther* 26, 555-563, 2007.

14. Berveling M, Langendijk J, Beukema J, Mourits M, Reyners A et al. Health-related quality of life and late morbidity in concurrent chemoradiation and radiotherapy alone in patients with locally advanced cervical carcinoma. *J Gynecol Oncol* **22(3)**, 152-160, 2011.

15. Viswanathan A, Lee L, Eswara J, Horowitz N, Konstantinoloulous P, Mirabeau-Beale K et al. Complications of pelvic radiation in patients treated for gynecologic malignancies. *Cancer* **120**, 3870–3883, 2014.

16. Kuku S, Fragkos C, McCormack M, Forbes A. Radiation-induced bowel injury: the impact of radiotherapy on survivorship after treatment for gynecological cancers. *Br J Cancer* **109(6)**, 1504-1512, 2013.

17. Wedlake L, Shaw C, McNair H, Lalji A, Mohammed K et al. Randomized controlled trial of dietary fiber for the prevention of radiation-induced gastrointestinal toxicity during pelvic radiotherapy. *Am J Clin Nutr.* **106(3)**, 849-857, 2017.

18. Touchefeu Y, Montassier E, Nieman K, Gastinne T, Potel G et al. Systematic review: the role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis - current evidence and potential clinical applications. *Aliment Pharmacol Ther.* **40(5)**, 409-421, 2014.

For Peer Review Only

19. Ciorba M, Hallemeier C, Stenson W and Parikh P. Probiotics to prevent gastrointestinal toxicity from cancer therapy: An interpretive review and call to action. *Curr Opin Support Palliat Care.* **9(2)**, 157–162, 2015.

20. Garcia-Peris P, Velasco C, Hernandez M, Lozano M, Paron L et al. Effect of inulin and fructo-oligosaccharide on the prevention of acute radiation enteritis in patients with gynecological cancer and impact on quality-of-life: a randomized, double-blind, placebo-controlled trial. *European Journal of Clinical Nutrition* **70(2)**, 170-174, 2016.

21. Kumagai T, Rahman F and Smith AM. The Microbiome and Radiation Induced-Bowel Injury: Evidence for Potential Mechanistic Role in Disease Pathogenesis. *Nutrients* **10(10)**, pii: E1405, 2018.

22. Ferreira M, Andreyev HJN, Mohammed K, Truelove L, Gowan S et al. Microbiota- and Radiotherapy-Induced Gastrointestinal Side-Effects (MARS) Study: A Large Pilot Study of the Microbiome in Acute and Late-Radiation Enteropathy. *Translational Cancer Mechanisms and Therapy*, published online first, 2019.

23. Macmillan Cancer Support. Throwing light on cancer and its consequences. 136pp, 2013. https://www.macmillan.org.uk/.../throwinglightontheconsequencesofcanceranditstreatment. (Accessed February 2020)

24. National Cancer Survivorship Initiative. Living with and beyond cancer: taking action to improve outcomes. London, National Cancer Survivorship Initiative, 2013. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/181054/9333-TSO-2900664-NCSI_Report_FINAL.pdf (Accessed February 2020)

25. Farhangfar A, Makarewicz M, Ghosh S, Jha N, Scrimger R et al. Nutrition impact symptoms in a population cohort of head and neck cancer patients: Multivariate regression analysis of symptoms on oral intake, weight loss and survival. *Oral Oncology* **50**, 877–883, 2014.

For Peer Review Only

26. Ceccatto V, Faria Di Pietro P, Nogueira Previdelli A, Vieira F, Cesa Schiavon C et al. Brazilian Healthy Eating Index Revised (BHEI-R) of women before and during adjuvant treatment for breast cancer. *Nutr Hosp.* **30(5)**, 1101-1109, 2014.

27. Boltong A, Aranda S, Keast R, Wynne R, Francis P et al. A Prospective Cohort Study of the Effects of Adjuvant Breast Cancer Chemotherapy on Taste Function, Food Liking, Appetite and Associated Nutritional Outcomes. *PLoS ONE* **9(7)**, e103512, 2014.

28. Avery K, Donovan J, Gilbert R, Davis M, Emmett P et al. Men with prostate cancer make positive dietary changes following diagnosis and treatment. *Cancer causes & control* **24(6)**, 1119-1128, 2013.

29. Shaffer K, Kim Y, Llabre M and Carver C. Dyadic associations between cancer-related stress and fruit and vegetable consumption among colorectal cancer patients and their family caregivers. *Journal of Behavioral Medicine* **39(1)**, 75-84, 2016.

30. Bruning P, Egger R, Gooskens A, Hermus R, Hulshof K et al. Dietary Intake, Nutritional Status and Well-being of Cancer Patients: a Prospective Study. *European Journal of Cancer and Clinical Oncology* **21(12)**, 1449-1459, 1985.

31. Pía de la Maza M, Agudelo G, Yudin T, Gattas V, Barrera G et al. Long-Term Nutritional and Digestive Consequences of Pelvic Radiation. *Journal of the American College of Nutrition* **23(2)**, 102-107, 2004.

32. Sanchez M, Castro-Eguiluz D, Luvian-Morales J, Jimenez-Lima R, Aguilar-Ponce J et al. Deterioration of nutritional status of patients with locally advanced cervical cancer during treatment with concomitant chemoradiotherapy. *Journal of Human Nutrition and Dietetics* **32**, 480-491, 2019.

33. Von Gruenigen V, Waggoner S, Frasure H, Cavanagh M, Janata J et al. Lifestyle challenges in endometrial cancer survivorship. *Obstet Gynecol* **117**, 93–100, 2011.

34. Fassier P, Zelek L, Lécuyer L, Bachmann P, Touillaud M et al. Modifications in dietary and alcohol intakes between before and after cancer diagnosis: Results from the prospective population based NutriNet-Sante cohort. *International Journal of Cancer* **141**, 457-470, 2017.

For Peer Review Only

35. European Union. Regulation (EU) 2016/679 of the European parliament and of the council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation). *Official Journal of the European Union* 2016; 88pp. Accessed via https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0679 December 2019.

36. UK Government. Data Protection Act 2018. UK Public General Acts 2018 Chapter 12. https://www.legislation.gov.uk/ukpga/2018/12/contents. Accessed December 2020.

37. World Medical Association. Declaration Of Helsinki – Ethical Principles For Medical Research Involving Human Subjects. 2013. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ Accessed December 2019.

38. National Health Service. BMI calculator. 2020. https://www.nhs.uk/live-well/healthy-weight/bmi-calculator/ Accessed February 2020.

39. Shim J-S, Oh K and Chang Kim H. Dietary assessment methods in epidemiologic studies. *Epidemiology and Health* **36**, 8pp, 2014.

40. Naska A, Lagiou A and Lagiou P. Dietary assessment methods in epidemiological research: current state of the art and future prospects [version 1; referees: 3 approved] *F1000Research* **6**(F1000 Faculty Rev), 926, 2017.

42. Bingham S, Gill C, Welch A, Day K, Cassidy A et al. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *The British journal of nutrition* **72(4)**, 619-643, 1994.

43. Nutritics©. About Nutritics©. 2019 https://en-gb.nutritics.com/p/home. Accessed November 2019.

44. IBM Corporation. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp, 2017

For Peer Review Only

45. Muls A, Cirillo Sanchez C, Norton C and Czuber-Dochan W. A systematic review of the literature examining dietary habits of women with gynecological cancer before, during and after treatment. Submitted for publication.

46. McLean R, Sanders W and Stroup W. A Unified Approach to Mixed Linear Models. *The American Statistician* **45(1)**, 54-64, 1991.

47. Seltman HJ. Experimental Design and Analysis @http://www.stat.cmu.edu/~hseltman/309/Book/Book.pdf, 2018.

47. Public Health England. Government recommendations for energy and nutrients for males and females aged 1 –18 years and 19+ years. (Accessed February 2020). https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/618167/government_dietary_recommendations.pdf

48. Hulshof KF, Gooskens AC, Wedel M and Bruning PF. Food intake in three groups of cancer patients. A prospective study during cancer treatment. *Hum Nutr Appl Nutr.* **41(1)**, 23-37, 1987.

49. Jochems S, Van Osch F, Bryan R, Wesselius A, van Schooten F et al. Impact of dietary patterns and the main food groups on mortality and recurrence in cancer survivors: a systematic review of current epidemiological literature. *BMJ Open* **8**,e014530, 2017.

50. Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H et al. ESPEN guidelines on nutrition in cancer patients. *Clin Nutr.* **36(1)**, 11-48, 2017.

51. Kitson S, Lindsay J, Sivalingam V, Rutter M and Crosbie E. High prevalence of metabolic syndrome in women newly diagnosed with endometrial cancer. *Gynecol Oncol Rep.* **26**, 109–110, 2018.

52. Slavin J. Why whole grains are protective: biological mechanisms. *The Proceedings of the Nutrition Society* **62(1)**, 129-134, 2003.

53. Doyle C, Kushi L, Byers T, Courneya K, Demark-Wahnefried W et al. Nutrition, Physical Activity and Cancer Survivorship Advisory Committee; American Cancer Society.Nutrition and Physical Activity During and After Cancer Treatment: An

For Peer Review Only

American Cancer Society Guide for Informed Choices. *CA: A Cancer Journal for Clinicians* **56(6)**, 323-353, 2006.

54. Preedy V and Watson R. *Handbook of Disease Burdens and Quality of Life Measures*: Volume 4. USA: Springer. 2010.

55. Brandner S, Müller-Nordhorn J, Stritter W, Fotopoulou C, Sehouli J et al. Symptomization and triggering processes: ovarian cancer patients' narratives on pre-diagnostic sensation experiences and the initiation of healthcare seeking. *Social Science and Medicine* **119**, 123-130, 2014.

56. Low E, Whitaker K, Simon A, Sekhon M and Waller J. Women's interpretation of and responses to potential gynecological cancer symptoms: a qualitative interview study. *BMJ Open* **5**, e008082, 2015.

57. Flanagan J, Skrobanski H, Shi X and Hirst Y. Self-Care Behaviors of Ovarian Cancer Patients Before Their Diagnosis: Proof-of-Concept Study. *Journal of Medical Internet Research* **5(1)**, e10447, 2019.

58. Luthold R, Fernandes G, Franco-de-Moraes A, Folchetti L and Ferreira S. Gut microbiota interactions with the immunomodulatory role of vitamin D in normal individuals. *Metabolism Clinical and Experimental* **69**, 76-86, 2017.

59. Park S-H, Kim J-C, Lee J-E and Park I-K. Pelvic insufficiency fracture after radiotherapy in patients with cervical cancer in the era of PET/CT. *Radiation Oncol J.* **29(4)**, 269–276, 2011.

60. Bolland M, Grey A, Avenell A. Effects of vitamin D supplementation on musculoskeletal health: a systematic review, metaanalysis, and trial sequential analysis. Lancet Diabetes Endocrinol **6**, 847–858, 2018.

61. Arendt J, Sørensen H, Horsfall L and Petersen I. Elevated Vitamin B_{12} Levels and Cancer Risk in UK Primary Care: A THIN Database Cohort Study. *Cancer Epidemiol Biomarkers Prev* **28(4)**, 814-821, 2019.

62. Camilleri M. Advances in understanding of bile acid diarrhea. *Expert Rev Gastroenterol Hepatol* **8(1)**, 49–61, 2014.

For Peer Review Only

63. Ghoshal U, Shukla R and Ghoshal U. Small intestinal bacterial overgrowth and irritable bowel syndrome: a bridge between functional organic dichotomy. Gut and Liver **11(2)**, 196–208, 2017.

64. Green R. Vitamin B_{12} deficiency from the perspective of a practicing hematologist. *Blood* **129**, 2603–2611, 2017.

65. Muls A, Taylor A, Lalondrelle S, Mohammed K, Norton C et al. A proposed tailored investigational algorithm for women treated for gynaecological cancer with long-term gastrointestinal consequences. *Support Care Cancer* published online first, 2020.

66. Sekse R, Hufthammer K and Vika M. Fatigue and quality of life in women treated for various types of gynecological cancers: a cross-sectional study. *Journal of Clinical Nursing* **24**, 546–555, 2014.

67. Kwok A, Palermo C and Boltong A. Dietary experiences and support needs of women who gain weight following chemotherapy for breast cancer. *Supportive Care in Cancer* **23(6)**, 1561-1568, 2016.

68. Boekema P, Samsom S, van Berge Henegouwen G and Smout A. Coffee and Gastrointestinal Function: Facts and Fiction: A Review, *Scandinavian Journal of Gastroenterology* **34(230)**, 35-39, 1999.

69. Papakonstantinou E, Kechribari I, Sotirakoglou K, Tarantilis P, Gourdomichali T et al. Acute effects of coffee consumption on self-reported gastrointestinal symptoms, blood pressure and stress indices in healthy individuals. *Nutrition Journal* **15**, 26- 37, 2016.

70. Sanford N, Sher D, Xu X, Ahn C, D'Amico A et al. Alcohol Use Among Patients With Cancer and Survivors in the United States, 2000-2017. *J Natl Compr Canc Netw.* 18(1), 69-79, 2020.

71. Bell, K. Remaking the Self: Trauma, Teachable Moments, and the Biopolitics of Cancer Survivorship. *Culture, Medicine and Psychiatry* **36**, 584-600, 2012.

For Peer Review Only

72. Bluethmann S. Grasping the 'Teachable Moment': Time Since Diagnosis, Symptom Burden and Health Behaviors in Breast, Colorectal and Prostate Cancer Survivors. *Psychooncology*, **24(10)**, 1250-1257, 2015.

73. Frazelle M, and Friend P. Optimizing the Teachable Moment for Health Promotion for Cancer Survivors and Their Families. *Journal of the Advanced Practitioner in Oncology* **7**, 422-433, 2016.

74. Sandsund C, Towers R, Thomas K, Tigue R, Lalji A, et al. Holistic needs assessment and care plans for women with gynaecological cancer: do they improve cancer-specific health- related quality of life? A randomised controlled trial using mixed methods. *Supportive & Palliative Care* **0**, 1–14, 2017.

75. Coa K, Clegg Smith C, Klassen A, Caulfield L, Helzlsouer K et al. Capitalizing on the 'teachable' moment to promote healthy dietary changes among cancer survivors: the perspectives of healthcare providers. *Support Care Cancer.* **23(3)**, 679–686, 2015.

76. Macasa Escamilla D and Jarrett P. The impact of weight loss on patients with cancer. *Nursing Times* **112(11)**, 20-22, 2016.

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Table 1: Clinical and demographic characteristics of the cohort (n= 15)

Characteristics	Total n=15
Age (years) at baseline mean (SD; range)	55.8 (SD 13.43; range 27-78)
Height (cm) at baseline mean (SD; range)	161.63 (SD 7.86; range 145-175)
Weight (kg) at baseline mean (SD; range)	71.76 (SD 18.25; range 51.5-95.25)
BMI (kg/m2) at baseline mean (SD; range)	28.66 (SD 5.44; range 17-35.4)
Overweight or obese at baseline	
BMI > 24.9 kg/m2 (n; %)	5 (33%)
BMI > 29.9 kg/m2 (n; %)	7 (47%)
Cancer diagnosis n (%)	
Cervical	5 (33)
Endometrial	9 (60)
Vaginal	1 (7)
Tumour stage n (%)	
Stage 1	6 (40)
Stage 2	5 (33)
Stage 3	3 (6)
Stage 4	1 (7)
Surgery n (%)	
Yes	12 (80)
No	3 (20)
Chemotherapy n (%)	9 (60)
Cisplatin	7 (78)
Carboplatin + placlitaxel	2 (22)
EBRT n (%)	15 (100)
Dose Gy median (range)	45 (45-58)
N fractions n (%)	
25	12 (80)
28	3 (20)
Brachytherapy n (%)	15 (100)
Dose Gy median (range)	8 (8-20)
N fractions median (range)	2 (2-4)
Hormone Replacement Therapy started n (%)	
Yes	3 (20)
No	12 (80)
Smoking status n (%)	
Never smoked	14 (93)
Ex-smoker	1 (7)
Alcohol drinkers n (%)	9 (60)
Units/week mean (SD)	2.27 (2.64)
Diet n (%)	
Meat-eaters	14 (93)
Vegetarian diet	1 (7)

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Table 2: Weight, BMI, energy and nutrient intake per time point, median (25th percentile, 75th percentile)

* statistically significant level adjusted for multiple testing at $p < 0.023$

Time point	Baseline	End of treatment	6 months post RT	1 year post RT	2 years post RT	p-value
Weight (kg)	73.00 (67.95, 84.90)	72.00 (61.34, 85.45)	74.35 (61.73, 86.70)	71.10 (63.50, 83.00)	71.10 (56.68, 82.90)	p= 0.59
BMI (kg/m^2)	29.10 (26.15, 31.70)	27.90 (24.65, 31.63)	28.30 (24.93, 31.68)	27.40 (23.90, 33.00)	28.70 (23.75, 33.45)	p= 0.58
Energy (kcal)	1466.87 (1216.66, 1846.80)	1455.93 (1077.42, 1824.85)	1429.31 (1212.50, 1860.03)	1306.53 (1241.77, 1643.83)	1498.39 (1134.87, 1806.18)	p= 0.89

Macronutrients and fiber	Baseline	End of treatment	6 months post RT	1 year post RT	2 years post RT	p-value
Fat (g)	52.06 (42.71, 78.34)	60.89 (38.02, 73.00)	53.19 (42.65, 75.69)	52.19 (41.75, 58.95)	53.18 (38.68, 68.87)	p= 0.73
Saturated Fat (g/d)	18.94 (13.73, 25.98)	22.01 (15.08, 26.01)	19.10 (16.22, 26.21)	16.90 (12.54, 21.15)	18.46 (13.23, 27.60)	p= 0.24
Omega-3 (Total) (g/d)	0.94 (0.51, 1.48)	0.87 (0.52, 1.45)	0.48 (0.25, 1.26)	0.75 (0.56, 1.18)	0.66 (0.37, 1.35)	p= 0.39
Omega-6 (Total) (g/d)	3.26 (2.73, 4.68)	3.03 (1.53, 4.24)	2.66 (1.67, 3.43)	2.78 (2.05, 3.43)	2.44 (2.19, 3.23)	p= 0.24
Carbohydrate (g/d)	175.65 (143.88, 201.08)	156.44 (131.36, 191.33)	156.18 (127.58, 194.72)	147.24 (128.52, 155.53)	164.68 (147.16, 194.49)	p= 0.41
Free Sugars (g/d)	14.80 (12.17, 25.27)	15.95 (12.23, 19.82)	10.46 (8.95, 24.58)	9.68 (7.85, 22.11)	19.93 (5.84, 24.38)	p= 0.36
Protein (g/d)	70.72 (58.23, 90.75)	63.62 (49.68, 73.87)	72.50 (58.09, 84.47)	69.42 (57.13, 75.55)	66.55 (55.70, 88.68)	p= 0.092
Protein intake per kg body weight (g/kg/d)	0.96 (0.85, 1.06)	0.88 (0.81, 0.86)	0.97 (0.94, 0.97)	0.98 (0.90, 0.91)	0.94 (0.98, 1.07)	-
Fiber (g/d)	16.51 (13.90, 22.70)	13.82 (13.11, 16.62)	15.63 (11.19, 20.00)	17.75 (12.69, 19.21)	16.42 (10.96, 20.60)	p= 0.004*

For Peer Review Only

Appendix 28. PREDICT study CCR 4201 timing schedule (blue = withdrawn participants)

Study number	Consented	BL	FUP 1	FUP 2	FUP 3	FUP 4	FUP 5
42010001	06.11.2015	No	No	No	No	No	No
42010002	27.11.2015	27.11.2016	04.01.2016	25.01.2016	31.08.2016	08.03.2017	13.03.2018
42010003	15.12.2015	07.01.2016	18.01.2016	23.02.2016	07.11.2016	16.01.2017	05.02.2018
42010004	06.01.2016	No	No	No	No	No	No
42010005	21.01.2016	28.01.2016	13.05.2016	No	No	No	No
42010006	19.01.2016	02.02.2016	16.02.2016	08.03.2016	20.09.2016	07.02.2017	27.02.2018
42010007	04.02.2016	05.02.2016	17.02.2016	No	No	No	No
42010008	04.02.2016	24.02.2016	07.03.2016	31.03.2016	10.10.2016	No	No
42010009	08.02.2016	26.02.2016	10.03.2016	06.04.2016	17.10.2016	No	No
42010010	26.02.2016	16.03.2016	11.04.2016	22.04.2016	12.10.2016	17.05.2017	18.06.2018
42010011	14.03.2016	21.03.2916	08.04.2016	05.05.2016	25.10.2016	09.05.2017	25.04.2018
42010012	21.03.2016	No	No	No	No	No	No
42010013	30.03.2016	12.04.2016	22.04.2016	16.05.2016	24.11.2016	31.05.2017	26.04.2018
42010014	06.05.2016	19.05.2016	02.06.2016	22.06.2016	20.12 2016	20.06.2017	03.07.2018
42010015	10.05.2016	23.05.2016	02.06.2016	28.06.2016	04.04.2017	27.06.2017	15.05.2018
42010016	12.05.2016	19.05.2016	01.06.2016	22.06.2016	22.12.2016	27.07.2017	06.09.2018
42010017	18.05.2016	19.05.2016	No	No	No	No	No
42010018	20.05.2016	27.05.2016	24.06.2016	27.07.2016	14.02.2017	No	No
42010019	24.05.2016	26.05.2016	No	No	No	No	No
42010020	08.06.2016	No	No	No	No	No	No
42010021	14.07.2016	03.08.2016	15.08.2016	13.09.2016	24.04.2017	20.11.2017	04.02.2019
42010022	19.07.2016	21.07.2016	04.08.2016	30.08.2016	14.02.2017	22.08.2017	04.09.2018
42010023	16.08.2016	01.09.2016	No	No	No	No	No
42010024	17.08.2016	No	No	No	No	No	No
42010025	25.08.2016	30.08.2016	13.09.2016	04.10.2016	No	03.10.2017	11.03.2019
42010026	23.08.2016	12.09.2016	23.09.2016	14.10.2016	08.05.2017	06.11.2017	05.11.2018
42010027	08.09.2016	20.09.2016	04.10.2016	20.10.2016	11.05.2017	17.10.2017	19.02.2019
42010028	26.10.2016	27.10.2016	08.11.2016	29.11.2016	13.06.2017	No	No
42010029	24.11.2016	12.12.2016	No	No	No	No	No
42010030	08.12.2016	03.01.2017	16.01.2017	07.02.2017	17.11.2017	09.02.2018	04.02.2019
42010031	22.12.2016	13.01.2017	No	No	No	No	No
42010032	02.03.2017	14.03.2017	03.04.2017	26.04.2017	19.12.2017	19.06.2018	18.06.2019
42010033	07.03.2017	23.03.2017	06.04.2017	27.04.2017	07.11.2017	08.05.2018	23.04.2019
42010034	13.04.2017	21.04.2017	18.05.2017	06.06.2017	Missed	16.05.2018	26.03.2019
42010035	06.06.2017	19.06.2017	06.07.2017	20.07.2017	Missed	Missed	Missed
42010036	12.06.2017	16.06.2017	13.07.2017	07.08.2017	14.05.2018	06.08.2018	01.04.2019
42010037	13.06.2017	26.06.2017	12.07.2017	25.07.2017	07.02.2018	08.08.2018	12.06.2019
42010038	20.06.2017	28.06.2017	04.10.2017	31.10.2017	23.04.2018	22.10.2018	20.05.2019
42010039	07.07.2017	10.07.2017	31.07.2017	16.08.2017	08.02.2018	30.08.2018	13.06.2019
42010040	19.07.2017	03.08.2017	24.08.2017	12.09.2017	27.03.2018	09.10.2018	14.05.2019
42010041	03.08.2017	14.08.2017	17.11.2017	12.12.2017	18.06.2018	05.11.2018	10.06.2019

Appendix 29. Sensitivity analysis IBDQ Total and IBDQ-E with and without Question 21 and 32.

1. Sensitivity analysis for adjusted IBDQ Total subscale scores (n= 41)

```
DATASET ACTIVATE DataSet2.
DATASET CLOSE DataSet1.
T-TEST PAIRS=TotalIBDQscoreadjusted WITH TotalIBDQscoreoriginal (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Total IBDQ score adjusted	190.42	166	28.950	2.247
	Total IBDQ score original	188.12	166	28.783	2.234

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Total IBDQ score adjusted & Total IBDQ score original	166	.974	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Total IBDQ score adjusted - Total IBDQ score original	2.295	6.547	.508	1.292	3.298	4.517	165	.000

- Adjusted total IBDQ scores and original total IBDQ scores are strongly and positively correlated (r = 0.974, p < 0.000).
- There was a small average difference between adjusted IBDQ-E scores and original IBDQ-E scores (t_{397} = 4.517, p < 0.000).

2. Sensitivity analysis for adjusted total IBDQ-E scores (n= 41)

```
T-TEST PAIRS=IBDQemotionaladjusted WITH IBDQemotionaloriginal (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	IBDQ emotional adjusted	68.42	166	11.536	.895
	IBDQ emotional original	66.37	166	10.309	.800

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	IBDQ emotional adjusted & IBDQ emotional original	166	.931	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	IBDQ emotional adjusted - IBDQ emotional original	2.048	4.235	.329	1.399	2.697	6.231	165	.000

- Adjusted IBDQ-E scores and original IBDQ-E scores are strongly and positively correlated ($r = 0.931$, $p < 0.000$).
- There was a small average difference between adjusted IBDQ-E scores and original IBDQ-E scores ($t_{397} = 6.231$, $p < 0.000$).

  A t-score of 6 means the original IBDQ-E scores and adjusted IBDQ-E scores are 6 times as different from each other as they are within each other.

- On average, adjusted IBDQ-E scores were 2.04 points higher than the original IBDQ-E scores (95% CI [1.399, 2.697]).

Appendix 30. Medication use in the PREDICT study per time point.

Medication use (n)	Baseline	Beginning of RT	End of RT	6 months post RT	1 year post RT	2 years post RT
median (range)	1 (0-3)	0 (0-5)	0 (0-6)	1 (0-6)	1 (0-7)	0 (0-3)
Analgesia	4	7	6	6	3	4
Antibiotics	6	1	1	1	6	9
Anti-depressant	1	1	1	1	1	0
Anti-emetic	3	0	1	2	3	1
Anti-hypertensive	2	2	2	2	4	0
Anti-viral	1	1	1	1	1	1
Blood thinner	2	0	2	1	1	0
HRT	3	1	1	2	0	1
Probiotic	4	1	0	5	2	7
PPI	2	3	2	3	0	0
Statin	3	3	1	1	0	0
Steroid	4	0	1	2	1	1
Vitamin supplementation						
B12	1	0	0	0	0	1
Calcium	3	1	1	0	4	0
Magnesium	0	0	0	0	0	1
Vitamin C	2	1	0	1	0	3
Vitamin D	7	2	0	1	3	2
Multivitamin	5	0	0	2	0	2
Zinc	2	0	0	0	0	0
Herbal supplement	7	1	0	4	3	3
Other medication	6	2	1	4	4	0