Radiotherapy for Rectal Cancer:

An Exploration of Factors Associated with Symptoms and Tumour Response

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Thesis submitted in fulfilment of the requirement for the Degree of Doctor of Philosophy

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Submitted to Waterford Institute of Technology,

September 2014

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Abstract

Preoperative radiotherapy is widely accepted as standard treatment for rectal cancer as it is associated with remarkable improvements in locoregional control. However, both the illness and treatment lead to a number of side effects which may negatively influence QoL. Few studies have investigated this specifically in relation to rectal cancer patients, particularly regarding fatigue. KRAS is mutated in 30%-50% of rectal cancer cases and mediates the expression of the cytokines IL-6 and IL-8. Exploration of KRAS status and expression levels of IL-6 and IL-8 as factors associated with radiosensitivity, in terms of tumour response and symptom presentation would be helpful in guiding treatment protocols, as individuals react differently due to inherent radiosensitivity.

Therefore, the aim of this two armed study was to 1) investigate symptom presentation and 2) explore potential factors associated with radiosensitivity in rectal cancer patients receiving preoperative radiotherapy.

An exploratory, correlational design was adopted which used nonprobability, convenience sampling to select suitable participants. Data in relation to symptoms and QoL was collected at 4 time points over a 10 week period. Tumour tissue was analysed to determine KRAS status and expression levels of IL-6 and IL-8, with these cytokines also measured in blood plasma using micro-array technology. Data was analysed using SPSS.

The final sample consisted of 35 rectal cancer patients, with ages ranging from 34-82 years. One-way repeated measures ANOVA found that levels of fatigue, bowel function symptoms, nutrition, pain, dermatological issues and urinary function symptoms changed significantly over time. Lower levels of global QoL were associated with increases in fatigue, constipation, bloating, blood and mucous in stool, stool frequency, appetite loss, weight worry, dry mouth, pain, urinary frequency and lower levels of sexual interest. KRAS status was not associated with tumour response to treatment or expression of IL-6 and IL-8, although plasma levels of these cytokines were indicative of tumour size. Furthermore, both cytokines correlated with faecal incontinence.

These findings demonstrate the importance of the close monitoring and management of symptoms during radiotherapy for rectal cancer in order to optimise QoL and avoid interruptions to treatment. KRAS status is not indicative of tumour response, although further subtype analysis is required. In addition, IL-6 and IL-8 may be useful as potential biomarkers in terms of tumour size.

Acknowledgements

I wish to express my grateful appreciation to all of those that provided me with support and encouragement throughout my work for this thesis. Particularly, to my supervisors Dr. Suzanne Denieffe and Dr. Martina Gooney, who offered me invaluable advice and guidance, as well as thoughtful and constructive criticism in a manner that was so positive, practical and encouraging, it made this seemingly impossible journey possible.

I would like to thank my colleagues in the Department of Nursing WIT, especially Professor John Wells, for the continuous direction and assistance he provided with this work. In addition, I wish to acknowledge the contribution made to this project by Mr. Mark White, from the Nursing and Midwifery Planning and Development Unit, which allowed this research to be possible. Thank you also to my colleagues in South Tipperary General Hospital for their on-going support.

In particular, I wish to acknowledge the contribution made to this project by the team at UPMC Whitfield Cancer Centre, especially Dr. Wojciech Sasiadek and Dr. Amanda Barry. Also to Eimear and Lynda, Karen and Agnes, and of course Michelle, Clodagh and Ollie, thank you for the friendly and practical help that ye gave so readily.

To the members of the WIT / HSE / RCSI Biomedical Research Group for their assistance and guidance with this research, and in particular to Dr. Orla O'Donovan, who provided a listening ear and helpful advice when needed.

I wish to express my sincerest gratitude and appreciation to the patients that participated so willingly in the study, which made this research possible. The people I met gave their time and effort so selflessly during a difficult period in their lives, as they wanted to help those who may be in a similar situation in the future, and for this, I thank them.

Last but not least, a special thank you to my friends and family. Particularly to my parents Dave and Trish, whose unwavering love and support throughout my life has gotten me to where I am today. To William and Laura, for all the cups of tea, neighbourly chats and encouragement and of course, to little Emma whose sense of fun and mischievous smile puts everything back in perspective. Finally, to Declan, thank you for your wise advice, your unfailing belief in my potential, and for always being there for me.

Declaration

I, Claire O'Gorman, declare that this thesis is submitted in partial fulfilment of the requirement for the degree of Doctor of Philosophy (PhD) and is entirely my own work except where otherwise accredited. It has not at any time either whole or in part been submitted for any other educational award.

Signed: _____ (Candidate)

Claire O'Gorman

Date: _____

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Index of Abbreviations

% CV:	Coefficient of Variation
AIDS:	Acquired Immune Deficiency Syndrome
AJCC:	American Joint Committee on Cancer
ANOVA:	Analysis of Variance
BFI:	Brief Fatigue Inventory
BMI:	Body Mass Index
cDNA:	complementary Deoxyribonucleic Acid
CINAHL:	Cumulative Index to Nursing and Allied Health Literature
cm:	centimetre
CTCAE:	Common Terminology Criteria for Adverse Events
DEPC:	Diethylpyrocarbonate
DNA:	Deoxyribonucleic Acid
DOH:	Department of Health and Human Services
EGFR:	Epidermal Growth Factor Receptor
ELISA:	Enzyme Linked Immunosorbent Assay
EORTC QLQ:	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire
EORTC QLQ-C30:	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire – Core 30
EORTC QLQ-CR29:	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire – Colorectal 29
FACIT-F:	Functional Assessment of Chronic Illness Therapy – Fatigue
FACT-F:	Functional Assessment of Cancer Therapy Fatigue
FFPE:	Formalin Fixed Parrafin Embedded

GAP:	GTPase-activating protein
GDP:	Guanosine Diphosphate
GEF:	Guanine Exchange Factors
GTP:	Guanosine Triphosphate
Gy:	Gray
Hb:	Haemoglobin
HRAS:	Harvey rat sarcoma viral oncogene
HSE:	Health Service Executive
ICD-10:	International Statistical Classification of Diseases Criteria
IL-6:	Interleukin 6
IL-8:	Interleukin 8
KRAS:	V-Ki-ras2 Kirsten rat sarcoma viral oncogene
MAPK:	Mitogen-Activated Protein Kinase
MFI-20:	Multidimensional Fatigue Inventory-20
mg:	milligram
ml:	millilitre
MOS-SF:	Medical Outcomes Short-Form
mRNA:	messenger Ribonucleic Acid
MSD:	Meso Scale Discovery
ng:	nanogram
NRAS:	Neuroblastoma rat sarcoma viral oncogene
PI3K / AKT:	Phosphatidylinositol-3-Kinase and Protein Kinase B
pAKT:	phosphorylated Protein Kinase B
pERK:	phosphorylated Extracellular Signal-Regulated Kinase
PFS:	Piper Fatigue Scale

pg:	picogram
PKD:	Proteinase K Digestion
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QoL:	Quality of Life
RAF:	Rapidly Accelerated Fibrosarcoma
RAS:	Rat Sarcoma
RBC:	Red Blood Cell Count
RCT:	Randomised Controlled Trial
RM-ANOVA:	Repeated Measures Analysis of Variance
RNA:	Ribonucleic Acid
RTOG:	Radiation Therapy Oncology Group
RT-PCR:	Real Time Polymerase Chain Reaction
TNM:	Tumour Nodes Metastases
UPMC:	University of Pittsburgh Medical Centre
WBC:	White Blood Cell Count
WIT:	Waterford Institute of Technology
WRH:	Waterford Regional Hospital

Definition of Key Terms

Rectal Cancer: A neoplasm characterised by the uncontrolled growth of anaplastic cells that is present in the retroperitoneal area of the bowel which may be divided into the lower rectum, the midrectum or the upper rectum, with the upper limit occurring 12 – 15 cms from the anal verge.

Radiotherapy: The use of ionising radiation to damage DNA and cause cell death in order to preserve normal tissue function, whilst damaging the tumour.

KRAS: A type of oncogene, that when mutated, plays a key role in neoplastic progression.

Cytokines: A diverse group of soluble proteins produced by cells to act as chemical mediators of cell to cell communication, that possess the unifying feature of regulating the immune system against pathogens and / or the inflammatory response.

Fatigue: A distressing, continuous, subjective sense of physical, emotional and / or cognitive tiredness related to cancer or cancer treatment that is not relative to recent activity and disrupts usual functioning.

Symptoms: Any subjective change in function, condition or sensation as perceived by the patient.

Quality of Life: A measurement of the physical, psychological and social effects of illness or treatment.

Radiosensitivity: The relative susceptibility of cells, tissues, organs, organisms, or any other substances to the effects of radiation.

Tumour Response: The response of the tumour to treatment which may be complete (no viable cancer cells), moderate (single cells or small groups of cancer cells), minimal (minimal or no tumour kill) or poor (extensive residual cancer).

CHAPTER I: Introduction & Organisation of Thesis

1.0 Introduction and Background to Study

This chapter outlines the background of the study, which has been undertaken to examine relationships between V-Ki-ras2 Kirsten rat sarcoma viral oncogene (KRAS) and circulating cytokines with responses to treatment in rectal cancer patients receiving preoperative neoadjuvant radiotherapy, with a specific focus on symptoms, quality of life (QoL) and tumour response. This chapter will also provide the rationale for the study, the chosen methodology and the scope of the literature review, followed by a description of the organisation of the thesis.

1.1 Rectal Cancer

The rectum is the area of the bowel that extends from the recto sigmoid junction to the anorectal ring (Van Cutsem *et al.,* 2008). Cancerous tumours can occur in the rectum and are caused by an uncontrolled division of abnormal cells (Mosby, 2009). Tumours of the rectum can be high, mid or low and are categorised based on the distance between its distal edge and the anal verge (Van Cutsem *et al.,* 2008).

Worldwide, 1,234,000 cases of colorectal cancer were registered in 2008, with incidence being more common in males than females (World Health Organisation, 2008). More specifically, in relation to rectal cancer, 14,999 cases were registered per 100,000 in the United Kingdom and in Ireland, an annual average of 606 rectal cancer cases were recorded between 2005 and 2009 (Cancer Research UK, 2009; National Cancer Registry Ireland, 2011). Colorectal cancer incidence in Ireland is ranked 8th highest in Europe, with Irish female incidence being 15% higher and Irish male incidence being 11% higher than the European average (National Cancer Registry Ireland, 2011). Therefore, optimum management of this disease must be aspired to in order to improve outcomes.

Standard treatment for this illness is a neoadjuvant approach using preoperative radiotherapy in conjunction with chemotherapy followed by radical surgery within 4-8 weeks following completion of this treatment. Therefore, the effect of preoperative treatment in this patient group requires further discussion.

1.2 Significance of Preoperative Radiotherapy

It appears from the literature that preoperative radiotherapy has beneficial effects in terms of reducing risk of recurrence. A Dutch trial, which included a sample of 1861 patients with resectable rectal cancer, was carried out to determine whether preoperative radiotherapy increased the benefit of total mesorectal excision and was seminal in identifying the benefits of this treatment (Kapiteijn *et al.,* 2001). Results demonstrated that with preoperative radiotherapy, the rate of local recurrence was 2.4% after two years, versus 8.2% in the patients that underwent surgery alone (Kapiteijn *et al.,* 2001). The significant difference in local recurrence was still evident at long term follow up of participants in this study, with this treatment reducing 10 year local recurrence by more than 50% when compared to patients that received surgery alone (van Gijn *et al.,* 2011).

These findings correlate with that of an analysis performed by the Colorectal Cancer Collaborative Group (2001), which carried out a systematic overview of 8507 patients from 22 randomised trials to compare the outcomes of combined radiotherapy and surgery for rectal cancer patients with surgery alone. Results indicated that although rates of curative resection were not improved and survival was only slightly better in patients that received radiotherapy (62% versus 61%), the risk of local recurrence was significantly decreased, with a reduction of 46% in those who had preoperative radiotherapy and 37% in those who had postoperative treatment (Colorectal Cancer Collaborative Group, 2001). Similarly, a

considerable reduction in local recurrence was also determined in a meta-analysis of 14 Randomised Controlled Trials (RCT), where the use of preoperative radiotherapy plus surgery was compared to surgery alone in patients with rectal cancer (Camma *et al.*, 2000).

These findings are supported by results of a more recent quantitative study that was carried out in Sweden, which also demonstrated that patients who did not receive preoperative radiotherapy had significantly higher rates of local recurrence when compared with those that did receive this treatment (Jorgren *et al.*, 2010). Guckenberger *et al.* (2009) also found a significant reduction in local recurrence with preoperative radiotherapy when evaluating the outcome of 118 patients receiving this treatment for rectal cancer, with results indicating that at five year follow up, local control rate was at 92%.

In relation to the use of preoperative radiotherapy and survival rates, results of the long term follow up of participants in the Dutch Trial that has previously been discussed, indicated that radiotherapy improved cancer specific survival in patients with a negative circumferential resection margin who underwent surgery (van Gijn *et al.*, 2011). However, an increase in other causes of death nullified this benefit and resulted in the same overall survival rate in both patient groups (van Gijn *et al.*, 2011).

In the systematic overview of 22 RCT's that investigated the use of preoperative radiotherapy in rectal cancer patients, overall survival was only slightly better in those that received this treatment when compared to surgery alone with results showing a death rate of 62% versus 63% (Colorectal Cancer Collaborative Group, 2001). The rate of survival in this overview correlates well with that of a more recent study in Germany, where overall survival was 67% in this patient cohort (Guckenberger *et al.*, 2009).

These studies clearly indicate the importance of radiotherapy in the management of rectal cancer, particularly in relation to reducing risk of local recurrence. However, establishing the prevalence of side effects that occur with this treatment, as well identifying potential factors that may be associated with responses to neoadjuvant radiotherapy, in terms of its effect on the tumour and the prevalence of toxicities, would be helpful in determining whether the benefits of this treatment are greater than the adverse effects that may occur. This may enable healthcare professionals to guide treatment plans for this cohort and potentially individualise care, as patients react differently to radiotherapy, due to their inherent, genetic background, which may influence their sensitivity to this treatment (Flint-Richter *et al.,* 2007). Therefore, current knowledge regarding the prevalence of radiotherapy related side effects and their impact on the QoL of rectal cancer patients is outlined. This is followed by justification for the identification of KRAS, and KRAS mediated expression of the cytokines interleukin 6 (IL-6) and interleukin 8 (IL-8) as factors associated with radiosensitivity.

1.3 Rectal Cancer and Radiotherapy Related Symptoms

Preoperative radiotherapy for rectal cancer leads to a number of common adverse effects of which prevalence rates can vary significantly across publications. These include fatigue (31% - 67%), diarrhoea (10.2% - 81.8%), dermatological problems (11% - 29%), micturition problems (2% - 50%), sexual dysfunction and pain (7.6% - 19.6%; Wang *et al.,* 2001; Marijnen *et al.,* 2002; Guren *et al.,* 2003; Sauer *et al.,* 2004; Carlomagno *et al.,* 2009; Fiorica *et al.,* 2009; Swellengrebel *et al.,* 2011). The issue of the severity of these symptoms is an important point to consider as is the impact of such symptoms on QoL and levels of functioning. Interestingly, only two studies have been identified that specifically investigated fatigue in this patient cohort, thereby identifying a major dearth of literature in this area (Wang *et al.,* 2001; Guren *et al.,* 2003). Investigations have indicated that these effects can

lead to a decline in health related QoL during treatment, and may result in prolonged surgical recovery times but interestingly, do not cause any long term deterioration in QoL (Guren *et al.,* 2003; Marijnen *et al.,* 2005; Stephens *et al.,* 2010). In order to identify patients at greater risk for the development of radiotherapy related adverse effects, as well as tumour response to this treatment, factors associated with radiosensitivity are outlined.

1.4 Rectal Cancer and KRAS

KRAS is one of the most commonly studied oncogenes in rectal cancer as in its active state, cell growth and proliferation is promoted (Clancy *et al.,* 2013). The involvement of KRAS in the development of rectal cancer was first proposed in 1988, where mutated KRAS, as opposed to wild type KRAS, was described as an early event in the pathogenesis of this illness (Vogelstein *et al.,* 1988). Mutation of KRAS occurs in 30 - 50% of colorectal cancer cases and is due to missense mutations through single amino acid substitutions (De Roock *et al.,* 2011; van Krieken *et al.,* 2008; de Campos-Lobato *et al.,* 2010). These mutations impair the intrinsic regulatory activity of KRAS, leading to accumulation of KRAS proteins in an active form, thereby promoting downstream cell proliferation and growth leading to tumorigenesis (Arrington *et al.,* 2012).

Using cell lines, it has been established that activated KRAS decreases radiosensitivity (Bernhard *et al.,* 2000; Kim *et al.,* 2005). However, results of clinical studies are conflicting in terms of KRAS status and tumour response to radiotherapy (Bengala *et al.,* 2009; Zauber *et al.,* 2009; Gaedcke *et al.,* 2010; Davies *et al.,* 2011; Erben *et al.,* 2011; Garcia-Aguilar *et al.,* 2011; Russo *et al.,* 2014). On further examination, none of these studies included subtype analysis of KRAS status, which involves identifying the codon where the mutation occurs, thereby possibly explaining the conflicting results, as well as identifying a paucity of

literature. The signalling cascade associated with activated KRAS is facilitated by KRAS induced secretion of IL-6 and IL-8, which have been identified as mediators of cell to cell communication (Wislez *et al.,* 2006; Ancrile *et al.,* 2007).

1.5 Rectal Cancer and KRAS Mediated Expression of IL-6 and IL-8

Elevated serum and tumoral levels of IL-6 and IL-8 are associated with the presence of colorectal cancer and metastases, with expression of both of these cytokines increasing with radiation in a dose dependent manner. (Galizia *et al.,* 2002; Nikiteas *et al.,* 2005; Esfandi *et al.,* 2006; Malicki *et al.,* 2009; Ning *et al.,* 2011). Increased expression of IL-6 has been positively correlated with tumour stage, whereas interestingly, in relation to IL-8, one particular study reported a significant association between lower levels of this cytokine and advanced stage disease, although this requires further validation (Kheirelseid *et al.,* 2013). Regarding symptom presentation, neither IL-6 nor IL-8 was associated with the presence of fatigue. However, a major paucity has been identified in relation to studies investigating expression levels of these cytokines in rectal cancer patients receiving neoadjuvant radiotherapy and the other symptoms associated with this treatment.

1.6 Rationale for the Study

In summary, the key findings emanating from the above literature highlights that reported prevalence rates of radiotherapy related side effects vary greatly, and there is a dearth of publications pertaining to fatigue in this patient cohort. Regarding KRAS as a factor associated with radiosensitivity in terms of tumour response, results of studies are conflicting. However, no study was identified that performed subtype analysis of KRAS mutation, thereby highlighting a paucity of literature. Further to this, KRAS mediated expression of IL-6 and IL-8 has been correlated with the presence of colorectal tumours, although the

association of levels of IL-8 with tumour stage has yet to be definitively established. Regarding symptom presentation, a major dearth has been identified in relation to studies investigating expression levels of IL-6 and IL-8 with symptoms that occur in rectal cancer patients receiving neoadjuvant radiotherapy.

1.7 Methodology and Scope of the Literature Review

The purpose of the review is twofold, firstly to provide a background to the study and secondly to provide a focused systematic review of the literature. To identify relevant literature, computer database searches were conducted, as they are the most efficient way to identify published studies. The literature search used the databases of the Cumulative Index to Nursing and Allied Health Literature (CINAHL), PubMed, Science Direct, the Cochrane Library and Wiley Online Library. No limit was set on publication dates. Annotations were also sourced from relevant research studies as part of the literature search.

The search was structured in line with the principles of systematic reviewing as advocated by the Cochrane Collaboration and was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher *et al.,* 2009; Higgins and Green, 2011). A systematic review attempts to collate all empirical evidence that fits pre-specified eligibility criteria in order to answer a specific research question. It uses explicit, systematic methods that are selected with a view to minimising bias, thus providing more reliable findings from which conclusions can be drawn and decisions made (Oxman e*t al.,* 2011).

The key characteristics of a systematic review are:

- A clearly stated set of objectives with pre-defined eligibility criteria for studies
- An explicit, reproducible methodology
- A systematic search that attempts to identify all studies that would meet the eligibility criteria
- An assessment of the validity of the findings of the included studies, for example, through the assessment of risk of bias
- A systematic presentation and synthesis of the characteristics and findings of the included studies

The initial search objective was to locate and retrieve all research studies that had been undertaken on symptoms associated with radiotherapy in rectal cancer patients. Following this, a second search was undertaken to identify potential factors associated with radiosensitivity. The final search focused on KRAS and KRAS mediated expression of the cytokines IL-6 and IL-8, as well as their association with tumour development, responses to radiation and symptom presentation.

Inclusion and exclusion criteria were set to ensure that as many relevant papers as possible were identified. Inclusion criteria were papers that were of original design, good quality, peer reviewed and in the English language, that focused on rectal cancer, radiotherapy, symptoms, KRAS and cytokines. Exclusion criteria were commentaries, editorials and papers that did not address the relevant topics. The search objectives and terms used can be seen in Table 1.1.

Table 1.1: Search objectives & terms

Search Objective	Terms Used
Symptoms in patients receiving radiotherapy for rectal cancer	radiotherapy preoperative radiotherapy rectal cancer side effects acute symptoms quality of life health related quality of life
KRAS as a potential factor associated with radiosensitivity	radiosensitivity KRAS rectal cancer radiotherapy biomarkers tumour tumour response side effects acute symptoms
IL-6 and IL-8 as factors associated with radiosensitivity	cytokines IL-6; interleukin IL-8; interleukin; chemokine rectal cancer radiotherapy tumour tumour response side effects acute symptoms

All abstracts of papers that met the broad inclusion criteria were read. Primary papers were obtained where they seemed to be relevant to the search objectives. These papers were then critically analysed to determine relevance. A limit was not set on the years, but an exponential increase in the number of articles written could be seen from the late 1980's. Material prior to this time was limited but not excluded during the reviewing process in order to ensure that key seminal work was included.

While it is recommended by the Cochrane guidelines (Higgins and Green, 2011) that research papers be graded according to the level of evidence, this was not incorporated in this review.

Instead, this review focused on examining the nature of the study, the methodologies and methods used and the findings, all of which formed part of the critical appraisal of this literature review.

In addition, there were papers which did not fit the eligibility criteria, for example grey literature, but which were utilised to add important contextual information to the review. Likewise, reviews and discussion papers were also included where appropriate. In many instances, different searches resulted in the same papers being identified. It is important to note that some of the research studies did not focus on rectal cancer but their findings may have credibility in understanding issues relevant to this study.

1.8 Organisation of the Thesis

The literature review is presented as two chapters. The first chapter is an exploration of the literature on symptoms associated with radiotherapy, which particularly focuses on patients receiving this treatment for rectal cancer, as well as the impact of these symptoms on QoL (Chapter II). This identifies the range of symptoms that can occur with this treatment, their prevalence rates and influence on QoL, and also examines methodological issues that may occur when planning a study to examine this topic.

Chapter III explores the use of KRAS and KRAS mediated expression of the cytokines IL-6 and IL-8 as factors associated with radiosensitivity, in terms of the tumour and symptom presentation. A background is provided in relation to the biology of KRAS and these cytokines, which provides justification for their use as factors associated with radiosensitivity in this patient cohort. Their association with tumour development and radiation responses at a cellular and clinical level, as well as symptom presentation is discussed. Methodological

issues that occur when planning a study to examine the expression of KRAS, IL-6 and IL-8 as factors associated with radiosensitivity are also highlighted throughout this discussion.

Chapter IV details the methodology and methods used in the study. Specifically the rationale for using a prospective, exploratory, correlational approach is analysed. In the methods section the research hypotheses and objectives of the study are outlined and a description of the population and sampling methods and measures to be used are included. The main procedures used for gathering and analysing data are explained, together with the steps taken to ensure the ethical conduct of the research.

The results of the study can be found in Chapter V. The results are explored in relation to the objectives and hypotheses for the study as outlined in Chapter IV. The socio-demographic and clinical characteristics of the sample recruited are described. The prevalence of each of the symptoms which includes fatigue, bowel function, nutrition, pain, dermatological symptoms, urinary function and sexual function are examined whilst looking for changes during radiotherapy treatment and following this, the impact of fatigue on other symptoms is presented. Changes in QoL and functioning throughout treatment, which includes cognitive, emotional, physical, role and social functioning are also examined. In addition, the influence of symptoms on QoL and functioning is outlined. The results of the KRAS and cytokine analyses, as well as their relationship to symptoms and tumour response to treatment are provided. Finally, a summary of the results with reference to the study's objectives is provided.

Chapter VI provides a critical discussion on how this research relates to the empirical literature on symptoms of rectal cancer patients receiving radiotherapy, the impact of this on

QoL and the use of KRAS, IL-6 and IL-8 as factors associated with radiosensitivity, in terms of symptom presentation and the tumour.

The conclusions and recommendations arising from the study can be found in Chapter VII. The limitations of the investigation are outlined and the conceptual contribution of this study to the existing body of knowledge is considered. Following this, the theoretical and clinical implications of the study findings are discussed and recommendations for future research and clinical practice are suggested. Finally, conclusions in relation to the overall study are provided.

CHAPTER II: Side Effects of Radiotherapy and Influence on QoL

2.0 Introduction

This chapter forms part one of the literature review, and provides a critical analyses of current literature pertaining to side effects of radiotherapy and their influence on QoL. Through this analysis, a summary of current knowledge on this topic will be provided, thereby placing this study in context, while also informing its design.

The use of preoperative radiotherapy in rectal cancer is combined with chemotherapy and is now widely accepted as standard treatment as it is associated with remarkable improvements in locoregional control and slight improvements in survival rates (Kapiteijn *et al.,* 2001; Guckenberger *et al.,* 2009; Jorgren *et al.,* 2010; van Gijn *et al.,* 2011). Although studies have investigated the various adverse effects that can occur with radiotherapy, these have not yet been critically appraised and synopsised to form a comprehensive review of their prevalence and effects on QoL. While the main focus of this review is in relation to radiotherapy, it must be noted that patients often receive chemotherapy as part of standard protocols which may also influence the occurrence of adverse effects. Therefore, this will also be addressed in this chapter.

Firstly, an outline of the search strategy is provided in Section 2.1. Themes that emerged from the literature in relation to acute adverse effects were fatigue (Section 2.2), diarrhoea (Section 2.3), dermatological problems (Section 2.4), micturition problems (Section 2.5), sexual dysfunction (Section 2.6) and pain (Section 2.7) and each will be discussed in detail. Emerging themes regarding the impact of preoperative radiotherapy on QoL were acute effects which include fatigue and diarrhoea and long term effects such as sexual and anorectal dysfunction. These effects will also be included for discussion (Section 2.8). The adverse

effects of chemotherapy will be reported in Section 2.9, and finally methodological issues relating to the literature reviewed are considered in detail (Section 2.10).

2.1 Search Strategy

The literature search was conducted as outlined in Chapter I. Studies selected for inclusion were based on the PRISMA 27 item checklist and the PRISMA four phase flow chart (see Figure 2.1; Moher *et al.,* 2009). A total of 33 publications were included in the review for critical analysis. These papers were discussed in relation to symptoms that present with this treatment with 7 studies also discussed in relation to QoL.

The majority of publications discussed in relation to symptom presentation and QoL adopted quantitative methodologies (32), with just 1 adopting a qualitative methodology. Of the studies that referred to symptom presentation, 13 referred to the incidence of fatigue, 17 measured the prevalence of diarrhoea, 8 monitored dermatological effects, 6 assessed micturition problems, 8 referred to sexual dysfunction, and 8 included analysis of pain.

Two publications from one original large scale Dutch trial (Kapiteijn *et al.,* 2001) have been included for review (Marijnen *et al.,* 2002; Marijnen *et al.,* 2005). Most of the studies were conducted in Europe (20), 5 were carried out in America, 1 in Canada, 5 in Asia and 1 with combined data from the United Kingdom and Canada. All but 11 studies specifically included rectal cancer patients and the largest number of patients that participated in one research study was 1861 (Kapiteijn *et al.,* 2001) and the smallest was 20 (Musio *et al.,* 2010).

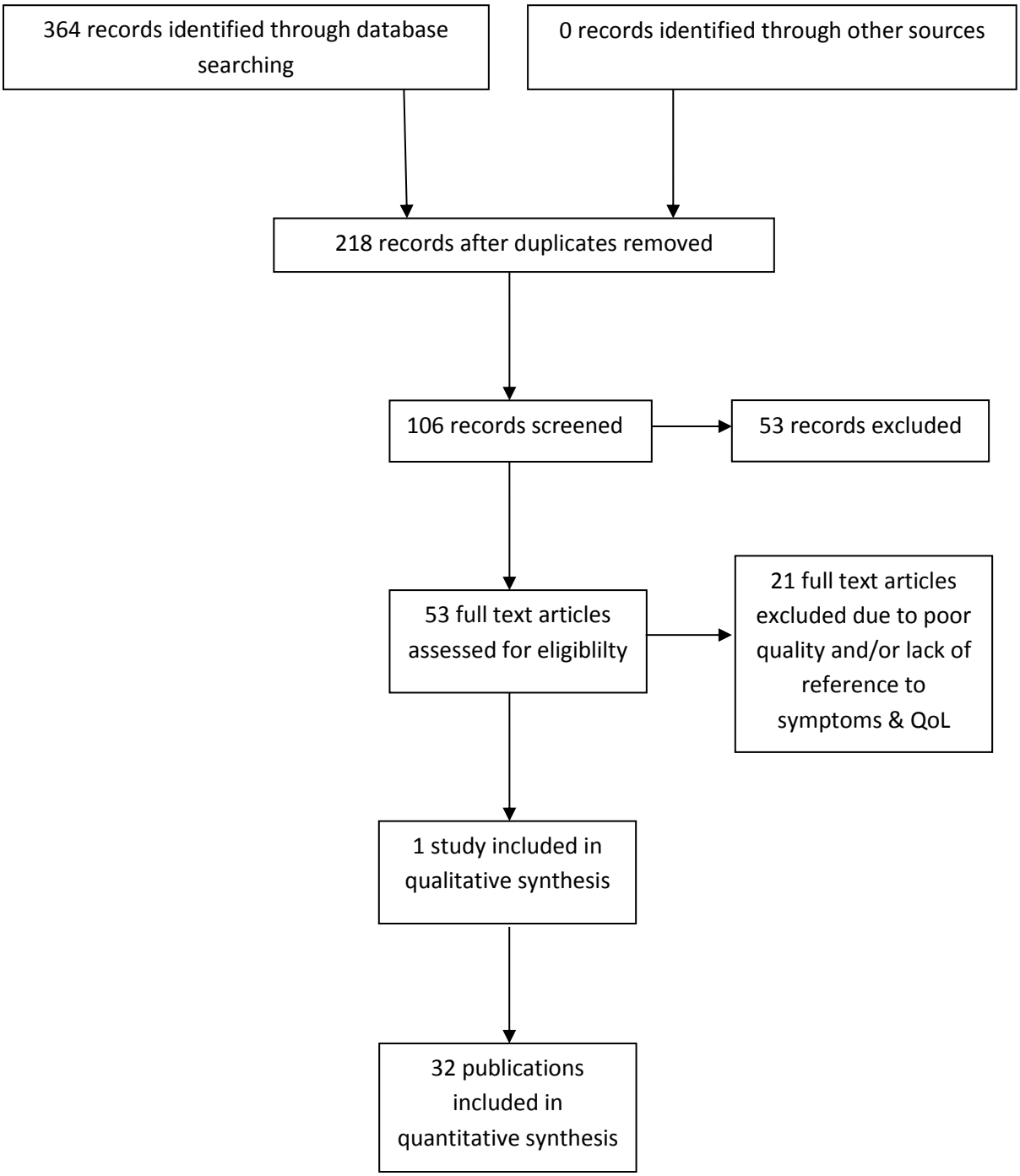

Figure 2.1: PRISMA Flow Chart

2.2 Fatigue

2.2.1 Cancer Related Fatigue

The concept of cancer related fatigue is difficult to formally define due its' subjectiveness, complexity and elusiveness (Narayanan *et al.,* 2009). In an attempt to capture the meaning of this multifaceted phenomenon it has been described by the National Comprehensive Cancer Network (2011, p. 5) as a "distressing persistent, subjective sense of physical, emotional and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning" .

Cancer related fatigue has been reported as one of the most common symptoms across a wide variety of cancer types (Cella *et al.,* 2001). In a retrospective survey that investigated the existence of this symptom in 379 cancer survivors, 6% of whom had gastrointestinal cancer, 80% of all participants reported problems with fatigue to some degree (Cella *et al.,* 2001). However, only 17% of these reached the threshold set by the International Statistical Classification of Diseases Criteria (ICD-10) and were recorded as having diagnosable fatigue, which could be misleading as many patients reporting fatigue related issues would be likely to benefit from clinical intervention (Cella *et al.,* 2001).

This study was included in a systematic review of research that analysed data from 9 separate investigations in order to determine the reliability and validity of the diagnostic criteria used (Donovan *et al.,* 2013). Results demonstrated good reliability and validity of the tool used although prevalence rates of fatigue varied widely between the studies, ranging from 9.2% - 56%, with the authors attributing this to the lack of consistency in how the criteria had been applied (Donovan *et al.,* 2013). Although, it must be noted that the studies included for

analysis demonstrated great variability regarding the nature of the samples, in relation to cancer type, stage and treatments received.

Despite this however, inconsistencies in clinical practice regarding the management of cancer related fatigue were also highlighted by results of a study that investigated adherence to treatment guidelines regarding cancer related fatigue (Hilarius *et al.,* 2011). In this prospective, observational Dutch study, 136 cancer patients attending a large community hospital completed questionnaires in relation to fatigue (Hilarius *et al.,* 2011). Findings indicated that information provided in relation to the management of fatigue was fragmented and inconsistent, as only 63% received counselling regarding this symptom, with 55% of participants given information on energy conservation, 18% receiving counselling on physical activity and less than 5% of patients receiving information in relation to sleep therapy, nutrition, and/or restorative therapy (Hilarius *et al.,* 2011).

Inadequate management of cancer related fatigue was also reported in a study that gathered data from patients attending outpatient departments and chemotherapy day units over a thirty day period at three regional cancer centres (Stone *et al.,* 2000). The prevalence, causes, management and impact of fatigue from the patients' perspective was investigated (Stone *et al.,* 2000). Five hundred and seventy six questionnaires were included for analysis, with 92 respondents having gastrointestinal cancer. Fatigue was prevalent in 58% of participants, with 52% of patients affected by this symptom never reporting it to a health care practitioner, only 14% receiving guidance about how it should be managed and 33% reporting that it was not managed adequately (Stone *et al.,* 2000).

Discrepancies between patient reporting and health care practitioner assessment of fatigue were also highlighted in a study that compared patient perception of symptoms with clinician reporting (Basch *et al.,* 2006). Patients and clinicians completed 400 paired surveys, with patients reporting greater differences in levels of fatigue than clinicians in 32% of cases and clinicians reporting greater levels of fatigue than patients in 13% of cases (Basch *et al.,* 2006).

It is clear from the evidence presented here that there are inconsistencies in the assessment and management of fatigue at a clinical level, despite the presence of this symptom in cancer patients being well documented. Patients have reported that treatment and medications more directly contributed to fatigue than their actual illness (Borneman *et al.,* 2007). Therefore, as preoperative radiotherapy is commonly used in the treatment of rectal cancer, it is necessary to examine literature that has specifically investigated the impact of this treatment on fatigue.

2.2.2 Radiotherapy Related Fatigue

The prevalence of fatigue has been shown to increase with radiotherapy, particularly in rectal cancer patients receiving preoperative treatment (Guren *et al.,* 2003). This symptom was measured using questionnaires at three different time points with results demonstrating that at the beginning of treatment, 17% of patients reported high levels of fatigue with this figure increasing to 31% at the end of treatment, and after 4-6 weeks, it had reduced to near baseline levels at 20% (Guren *et al.,* 2003). This correlates with findings of a study investigating the magnitude of fatigue in cancer patients receiving radiotherapy that included a sample of 90 patients, 10% of whom had rectal cancer, with results indicating that at follow up, fatigue scores were nearing pre-treatment levels (Janaki *et al.,* 2010).

Similarly, Ahlberg *et al.* (2005) discovered a significant increase in fatigue during radiotherapy treatment when examining the experience of fatigue, other symptoms and QoL for uterine cancer, with levels gradually increasing during treatment and peaking near its completion. In this study, baseline levels of fatigue were noted to be predictive when determining the variances in levels after completion of treatment (Ahlberg *et al.*, 2005).

In an investigation that examined the severity and patterns of fatigue in a sample of 72 rectal cancer patients receiving preoperative chemoradiotherapy, an increase in fatigue was also reported during radiotherapy treatment, with 67% of patients experiencing this effect (Wang *et al.*, 2001). However, levels of fatigue were not measured again after completion of treatment so it is difficult to determine whether this increase was transient.

Nonetheless, these results are further supported by those of an investigation to determine the impact of radiotherapy on the fatigue levels of 82 Jordanian cancer patients, where a correlational design was used to compare the prevalence of this symptom before and after radiotherapy treatment (Obead *et al.*, 2014). Unlike in the study by Wang *et al.* (2001), fatigue was measured at baseline and immediately after treatment, with levels increasing significantly, although these results would have been further strengthened if fatigue had been measured again at a third time point, in order to determine if the presence of this symptom was sustained over time, once treatment had been completed (Obead *et al.*, 2014).

Despite this, it could be concluded that radiotherapy certainly compounds fatigue, with levels gradually increasing throughout treatment and peaking near the end of treatment, although only two studies specifically included rectal cancer patients receiving preoperative

radiotherapy in their sample (Wang *et al.,* 2001; Guren *et al.,* 2003; Ahlberg *et al.,* 2005; Janaki *et al.,* 2010).

Fatigue has been reported to be more debilitating than pain or nausea, indicating the need for health care practitioners to develop and implement evidence based guidelines to improve the management of this symptom, particularly as it has been shown to correlate with and exacerbate other symptoms (Stone *et al.,* 2000; Hwang *et al.,* 2002).

2.2.3 Fatigue and Other Symptoms

A quantitative study was carried out to investigate fatigue in 180 male cancer patients, 15% of which had colorectal cancer, with findings indicating that varying levels of fatigue correspond with and may compound other symptoms, such as pain, constipation, feeling bloated and lack of appetite (Hwang *et al.,* 2002). It was also found that patients with higher levels of fatigue had lower levels of survival (Hwang *et al.,* 2002).

Results of a more recent investigation also indicated a correlation with fatigue and other symptoms (Oh *et al.,* 2011). A meta-analysis of 30 studies was performed to examine the relationship between symptoms and psychological distress with cancer related fatigue (Oh *et al.,* 2011). Findings demonstrated that the most significant correlation was with psychological distress (i.e. depression and anxiety), followed by nausea and vomiting and then pain and dyspnoea (Oh *et al.,* 2011).

The correlation between fatigue and symptom distress has also been confirmed in patients receiving treatment for uterine cancer, where loss of appetite, nausea and vomiting and diarrhoea were all associated with increased levels of fatigue (Ahlberg *et al.,* 2005). More

specifically, in relation to rectal cancer, pain and uncontrolled diarrhoea have been identified as predictors of fatigue, where the severity and patterns of fatigue were examined in a sample of 72 patients receiving preoperative chemoradiation (Wang *et al.,* 2001).

It has been established that although fatigue has not been adequately measured in rectal cancer patients during preoperative radiotherapy, it may be compounded by other symptoms such as diarrhoea, thereby warranting further discussion of this in order to establish prevalence rates (Wang *et al.,* 2001; Guren *et al.,* 2003).

2.3 Diarrhoea

When assessing the prevalence of diarrhoea, all studies discussed implemented either the Radiation Therapy Oncology Group (RTOG) scoring system or the Common Terminology Criteria for Adverse Events (CTCAE) to grade its severity. Both systems use a Likert type scale with the RTOG score ranging from 0 to 4 and the CTCAE scoring effects between 1 and 5. Milder grade 1 effects referred to the occurrence of diarrhoea requiring parasympatholytic drugs with more severe grade 2-5 effects requiring sanitary pads, parenteral nutrition and possibly leading to bowel obstruction, fistula or perforation (RTOG, 2011; National Cancer Institute, 2006).

In a quantitative study to analyse the effects of adjuvant radiotherapy in elderly patients with rectal cancer, diarrhoea was one of the most common symptoms, with it occurring in 81.8% of patients, 61% of these reporting it to be transient (grade 1), 24.4% had a tolerable toxicity level (grade 2) and 2.4% experienced grade 3 diarrhoea (Fiorica *et al.,* 2009).

Diarrhoea was also a common occurrence to some degree in most patients included in another quantitative study, which specifically investigated the acute side effects of preoperative radiotherapy combined with a total mesenteric excision (Marijnen *et al.*, 2002). Proctitis occurred in one patient in this study two months after radiotherapy (Marijnen *et al.*, 2002). Upon investigating QoL during radiotherapy for rectal cancer, Guren *et al.* (2003) also listed diarrhoea as a common complaint among most patients surveyed.

However, when comparing preoperative versus post-operative chemoradiotherapy for rectal cancer, Sauer *et al.* (2004) only found 12% of patients receiving preoperative treatment experienced diarrhoea. The difference in statistics may be attributed to the fact that Sauer *et al.* (2004) only included grade 3 and 4 toxic effects, whereas the other studies included all levels of toxicity (Marijnen *et al.*, 2002; Fiorica *et al.*, 2009).

Consensus regarding the prevalence of diarrhoea in this patient group is strengthened with a more recent study that investigated acute toxicity and surgical complications in patients receiving preoperative radiotherapy for rectal cancer, as it was found that diarrhoea continued to be reported as one of the most prominent acute effects of radiotherapy treatment, with 10.2% of patients reporting grade 3 toxicity (Swellengrebel *et al.*, 2011).

These results are supported by findings of a study by Wang *et al.* (2001), where diarrhoea was reported as a primary toxicity, with rates increasing from 10% during week 1 of treatment to 40% at week 4 and then decreased back to 26% at week 5, indicating that radiotherapy has a significant impact on prevalence rates.

However, even though diarrhoea causes obvious distress to patients, its occurrence was noted to be an independent predictor of improved relapse free survival after surgery in a study that examined the relationship between the adverse effects of preoperative radiotherapy in rectal cancer patients and their clinical outcomes (Ishihara *et al.,* 2011). In this, 75 Japanese patients with rectal cancer were included in the sample with 24% reporting diarrhoea to be a major adverse effect of treatment (Ishihara *et al.,* 2011).

It must be noted however, that rectal cancer patients often receive chemotherapy, in conjunction with radiotherapy treatment prior to surgery, and this has also been shown to influence the prevalence of diarrhoea (Bosset *et al.,* 2006). In a quantitative study to evaluate the addition of chemotherapy in the treatment of rectal cancer, findings indicated that diarrhoea (grade 2 or higher) was reported in 17.3% of patients receiving radiotherapy preoperatively, and 37.6% of patients receiving both radiotherapy and chemotherapy (Bosset *et al.,* 2006). This is a notable difference, particularly as participants in all the studies discussed in relation to diarrhoea, with the exception of Marijnen *et al.* (2002), included preoperative chemotherapy in their treatment protocol (see Table 2.1, Wang *et al.,* 2001; Guren *et al.,* 2003; Sauer *et al.,* 2004; Fiorica *et al.,* 2009; Ishihara *et al.,* 2011; Swellengrebel *et al.,* 2011).

However, as the chemotherapy regime differed in each study, it is difficult to accurately compare them, whereas the total radiotherapy dose given in all studies was 45 – 50 Gy, with the exception of Marijnen *et al.* (2002) where the total dose administered was 25 Gy (Wang *et al.,* 2001; Guren *et al.,* 2003; Sauer *et al.,* 2004; Bosset *et al.,* 2006; Fiorica *et al.,* 2009; Swellengrebel *et al.,* 2011).

Nonetheless, it is possible to conclude that preoperative radiotherapy certainly increases diarrhoea to some degree in rectal cancer patients, as it is a common theme that emerges from all studies discussed in relation to this adverse effect, even though it is difficult to put an exact figure on prevalence rates, due to differences in treatment protocols.

2.4 Dermatological Effects

Dermatological effects were also graded using either the RTOG Scoring System or the CTCAE criteria with grade 1 minimal effects referred to as faint erythema, dry desquamation and decreased sweating and more severe grade 2 to 5 effects classed as bright erythema, moist desquamation, ulceration and necrosis (RTOG 2011; National Cancer Institute 2006).

As previously stated, chemotherapy is often combined with radiotherapy in the treatment of rectal cancer. However, unlike the worsening effect this has on the prevalence of diarrhoea in this patient group, no major impact has been shown with the incidence of dermatological effects (Musio *et al.,* 2010).

In a quantitative study, to evaluate the addition of a chemotherapy drug (oxaloplatin) to the standard neoadjuvant protocol of radiotherapy and chemotherapy (5-fluorouracil) in the treatment of rectal cancer, the incidence of grade 3 radiation dermatitis was 23.8% in patients following the standard protocol and 20% in patients receiving oxaloplatin in addition to this (Musio *et al.,* 2010). It can therefore be concluded that the addition of this chemotherapy drug did not exacerbate the development of radiation dermatitis. However, it is difficult to generalise this finding to all rectal cancer patients receiving preoperative neoadjuvant chemoradiation due to differences in treatment protocols (see Table 2.1).

Marijnen *et al.* (2002) made no reference to the use of chemotherapy in the treatment protocol they implemented in their multicentre randomised trial, which investigated the acute side effects that occurred after short term preoperative radiotherapy for rectal cancer. Interestingly, few adverse dermatological effects were reported, but this was attributed to the fact that the majority of patients received surgery one week after completion of radiotherapy and were not seen by the radiation oncologist, who was scoring the acute side effects, until several weeks after their operation (Marijnen *et al.,* 2002).

The timing of the assessment of this side effect is significant as Wang *et al.* (2001) reported increased skin reactions during treatment and a reduction in irritation at the end of treatment. In this study, participants received radiotherapy over a 5 week period, with grade 2 to 3 skin reactions being reported in 10% of patients during week 3, 29% of patients at week 4 and 18% at week 5 (Wang *et al.,* 2001).

Grade 3 dermatological effects have also been reported as an acute adverse effect of radiotherapy in another quantitative study that evaluated acute toxicity and surgical complications in rectal cancer patients receiving this treatment preoperatively, with radiation dermatitis affecting 11.6% of this patient cohort (Swellengrebel *et al.,* 2011). Similar statistics were reported by Sauer *et al.* (2004), with this adverse effect occurring in 11% of patients in their study at grade 3 or 4 toxic levels.

Conversely, Fiorica *et al.* (2009) reported a much higher incidence of radiation dermatitis in their study, with all patients but one experiencing this side effect. Similar results were found by Ishihara *et al.* (2011). However, it must be noted that all grades of dermatological reactions were included in these two studies, whereas the previous studies that have been

discussed only reported more severe effects and also, there were differences in the tools used to assess skin toxicity (Sauer *et al.,* 2004; Fiorica *et al.,* 2009; Ishihara *et al.,* 2011; Swellengrebel *et al.,* 2011).

Li *et al.* (2012) assessed acute toxicities weekly in their study to determine whether optimising dose distributions, by improving the technique of delivering radiation, could achieve better outcomes for patients with rectal cancer, results of which indicated that just 3.2% of patients experienced grade 3 radiation dermatitis. This is significantly lower than the rate of grade 3 and 4 radiation dermatitis reported by Swellengrebel *et al.* (2011) and Sauer *et al.* (2004), which may indicate that the technique adopted by Li *et al.* (2012) to deliver radiation reduces this adverse effect. However, the difference in results may be explained further by the fact that the sample of patients that received preoperative radiotherapy in both of these studies (147 patients and 415 patients respectively) was much larger than the sample used by Li *et al.* (2012), which included 63 patients.

The studies reviewed here indicate radiation dermatitis occurs, to some degree, in the majority of patients receiving radiotherapy for rectal cancer, with more severe grade 3 and 4 toxic effects occurring less frequently, and symptoms improving once treatment has been completed (Wang *et al.,* 2001; Marijnen *et al.,* 2002; Sauer *et al.,* 2004; Fiorica *et al.,* 2009; Musio *et al.,* 2010; Ishihara *et al.,* 2011; Li *et al.,* 2012; Swellengrebel *et al.,* 2011). As patients with rectal cancer receive radiotherapy to the pelvic area, issues with micturition may occur due to the anatomical position and will therefore be discussed further.

Table 2.1: Treatment protocols

Study	Radiotherapy Dose	Chemotherapy Dose
Wang *et al.* (2001)	*Total Dose:* 45 Gy *Delivery:* 25 fractions of 1.8 Gy daily 5 times per week over 5 weeks	5-fluorouracil prescribed at a dose of 300 mg per m2 (of body surface area) per day and administered 5 days per week during the 5 week course of radiotherapy
Guren *et al.* (2003)	*Total Dose:* 50 Gy *Delivery:* 25 fractions of 2 Gy in daily fractions 5 days per week over 5 weeks	5-fluorouracil/leucovorin administered to 63% of patients– dose not stated
Li *et al.* (2012)	*Total Dose:* 41.8 Gy – 50.6 Gy *Delivery:* 22 fractions of 1.9Gy / 2.3 Gy 5 times per week over 30 days	Capecitabine administered at a dose level of 825mg/m2 orally twice daily, 5 days per week during radiotherapy
Marijnen *et al.* (2002)	*Total Dose:* 25 Gy *Delivery:* 5 fractions of 5 Gy during 5-7 days	None administered preoperatively
Musio *et al.* (2010)	*Total Dose:* 45 Gy *Delivery:* 25 fractions of 1.8 Gy; 3 fractions was delivered to the tumour mass with a 2cm margin for a total dose of 50.4 Gy; cT4 tumours received 2 additional fractions reaching a total dose of 50.4 Gy	Oxaliplatin was given weekly for 5-6 weeks in a 2-h infusion at a dose of 50 mg/m2
Sauer *et al.* (2004)	*Total Dose:* 50.4 Gy *Delivery:* 28 fractions of 1.8 Gy 5 times per week	Fluorouracil prescribed at a dose of 1000mg per m2 (of body surface area) per day and administered during the first and fifth weeks of radiotherapy
Bosset *et al.* (2006)	*Total Dose:* 45 Gy *Delivery:* 25 fractions of 1.8 Gy over 5 weeks	Fluorouracil prescribed at a dose of 350mg per m2 (of body surface area) and leucovorin at a dose of 20mg per m2 per day administered during the first and fifth weeks of radiotherapy
Fiorica *et al.* (2009)	*Total Dose:* 50 Gy *Delivery:* 25 fractions of 2 Gy over 5 weeks	Capecitabine – dose not stated. Not received by all patients preoperatively
Ishihara *et al.* (2011)	*Total Dose:*50 Gy or 50.4 Gy *Delivery:* 25 fractions of 2 Gy over 5 weeks or 1.8 Gy of 28 fractions over 6 weeks	44 out of 85 patients received tagafur-uracil (300-500mg/day) and leucovorin (75mg/day) with radiotherapy
Swellengrebel *et al.* (2011)	*Total Dose:* 50 Gy *Delivery:* 25 fractions of 2 Gy on weekdays	Capecitabine prescribed at a dose of 825mg/m2 and administered twice daily on days of radiotherapy and also at weekends

2.5 Micturition Problems

Due to the anatomical position of the treatment site, there is a risk of developing micturition problems. Grading of these problems in the studies was carried out once again using the RTOG Scoring System and the CTCAE criteria with mild grade 1 to 2 effects including increased frequency of urination, dysuria and bladder spasm and more severe grade 3 to 5 effects referred to as haematuria and bladder obstruction (National Cancer Institute 2006; RTOG 2011).

A common adverse effect of radiotherapy treatment is dysuria (Valentini *et al.*, 2008). In the study by Fiorica *et al.* (2009), which has previously been discussed, acute grade 1 urinary problems occurred in over 50% of patients undergoing treatment. At follow up, where late complications were recorded, this had reduced to less than 10%, indicating that although radiotherapy has a significant impact on bladder function, it occurs at a tolerable toxicity level (Fiorica *et al.*, 2009).

However, Sauer *et al.* (2004) reported that long term grade 3 and 4 bladder problems occurred at a rate of 2% in patients who had received preoperative radiotherapy. Unlike the study by Fiorica *et al.* (2009), no reference was made to acute problems with bladder function, which is likely to be due to the exclusion of grade 1 effects by Sauer *et al*. (2004).

Nonetheless, there is a significant difference in the reports of long term bladder problems between these two studies, with no grade 3 or 4 effects observed by Fiorica *et al.* (2009). On further examination, it was noted that only 75.6% of patients in the study by Fiorica *et al.* (2009) underwent surgery, compared with 95% in the study by Sauer *et al*. (2004). This is significant as when comparing the effects of preoperative radiotherapy and surgery with

surgery alone in patients with rectal cancer, problems with micturition were observed both in patients receiving radiotherapy and those that did not, thereby demonstrating that damage caused during surgery is more likely to lead to long term bladder problems (Marijnen *et al.*, 2005). This may explain the difference in the rate of long term bladder problems reported by Sauer *et al.* (2004) where comparing the effects of preoperative with post-operative chemoradiation demonstrated prevalence rates of 2% versus 4% respectively.

Marijnen *et al*. (2005) made no reference to acute toxicity in relation to bladder dysfunction as data in this study was gathered at baseline, between the second and third weeks after completion of preoperative treatment and then at 3, 6 and 12 months after surgery, with no measurement of bladder problems being recorded during preoperative treatment (Marijnen *et al.*, 2005).

Data was gathered during this time in the study by Guren *et al.* (2003), with results demonstrating just a small, non-significant increase in micturition problems. Swellengrebel *et al.* (2011) also assessed toxicity during treatment, with evaluation being carried out once or twice weekly, and reported no problems with micturition preoperatively. However, post operatively, 10.9% of patients presented with urological issues, ranging from grade 1 to grade 3 (Swellengrebel *et al.*, 2011). This supports the findings of Marijnen *et al.* (2005), which indicate bladder problems are more likely to occur due to surgery, rather than radiotherapy.

It can therefore be concluded that micturition problems in rectal cancer patients receiving radiotherapy occur to some extent, but are very tolerable, whereas more severe and more frequent problems have been reported in this cohort secondary to surgical intervention (Sauer *et al.*, 2004; Marijnen *et al.*, 2005; Fiorica *et al.*, 2009; Swellengrebel *et al.*, 2011). Issues

with sexual function have also been reported in patients receiving treatment for rectal cancer and therefore, require further exploration.

2.6 Sexual Dysfunction

In order to identify the risk factors associated with long term sexual dysfunction, sexually active participants in the original study by Kapiteijn *et al.* (2001), that determined whether preoperative radiotherapy increased the benefit of total mesorectal excision were analysed. It was reported that although preoperative radiotherapy had a diminutive effect on function, it was not an independent cause of sexual problems, with surgery being cited as the main risk factor (Lange *et al.,* 2009).

This is supported by results of a study that evaluated the impact of surgery related adverse effects on health related QoL in patients with rectal cancer (Vironen *et al.,* 2006). Findings indicated that there was a decrease in the sexual function of both men and women but this was attributed to surgery rather than preoperative radiotherapy, although this was not statistically significant (Vironen *et al.,* 2006).

However, in a large randomised trial, comparable outcomes were found, with preoperative radiotherapy having a minor adverse effect on sexual function whereas surgery was reported as having a major clinical impact (Stephens *et al.,* 2010). This study included both males and females, with both sexes completing the QoL forms in equal proportions (Stephens *et al.,* 2010). However, there was a very low female response rate to sexual function questions and therefore, these were excluded from further analysis due to lack of reliability (Stephens *et al.,* 2010).

Interestingly, difficulty in measuring sexual dysfunction in females was also present in the other studies that have been discussed, with Vironen *et al.* (2006) experiencing a much lower questionnaire response rate in females when compared to males (80% vs 98%) and Lange *et al.* (2009) citing the lack of simple end-points equivalent to ejaculation and potency as problematic, as sexual dysfunction may be still present even if intercourse is technically possible.

Similar problems were found in a multicentre prospective trial that described outcomes of patients with rectal cancer after receiving preoperative chemoradiotherapy, with less than 30% of females responding to the sexual problem scale, resulting in its exclusion from further analyses (Pucciarelli *et al.,* 2012). Data was collected at baseline, two to three weeks after completion of chemoradiotherapy and at six and twelve months after surgery, with male sexual function worsening significantly throughout the study period (Pucciarelli *et al.,* 2012). This supports the hypothesis that preoperative radiotherapy leads to sexual dysfunction to some degree, but it is the effects of surgery that compound this problem considerably.

Due to the difficulties in measuring female sexual dysfunction after preoperative radiotherapy, studies have a tendency to focus more on issues experienced by males. Upon evaluating the effect of radiotherapy on the sexual function of 201 males with rectal cancer, it was reported that this treatment had an adverse effect, but maximum deterioration occurred 8 months after surgery (Heriot *et al.,* 2005). Bonnel *et al*. (2002) reported comparable findings when they investigated the effects of preoperative radiotherapy for rectal cancer, concluding that this treatment may impair their sexual function to some degree.

The literature examined here indicates that sexual dysfunction is difficult to measure in females but in males, preoperative radiotherapy has a minor adverse effect, with long term problems mainly being attributed to surgery (Bonnel *et al.,* 2002; Heriot *et al.,* 2005; Vironen *et al.,* 2006; Lange *et al.,* 2009; Stephens *et al.,* 2010; Pucciarelli *et al.,* 2011).

2.7 Pain

According to Chapman (2011) cancer pain can be acute or chronic and can be tumour or treatment related. Although there was no significant difference in levels of pain during radiotherapy in the study by Guren *et al.* (2003), this was not the case in the Dutch study by Marijnen *et al.* (2002). It was reported that 53 patients had pain or discomfort in their legs or gluteal region, with treatment being interrupted in 13 of these patients due to its severity. This was quite a large scale trial and consisted of a sample of 1530 participants, with 695 in the radiotherapy group, and when calculated, participants that complained of pain included 7.6% of the total number of patients' surveyed (Marijnen *et al.,* 2002). Therefore, the difference in the findings by Guren *et al.* (2003) may be attributed to the small sample size (*n* = 42) when compared with this larger trial.

Upon evaluating the outcomes of using short course radiotherapy, which delivered a total dose of 25Gy in 5 fractions, with delayed surgery, in patients with non resectable rectal cancer, it was reported that many had less local symptoms, such as pain, after commencing this treatment (Radu *et al.,* 2008). As the protocol delivered the same total dose of radiation as the Dutch trial (Marijnen *et al.,* 2002), it is interesting that results regarding pain levels are conflicting, particularly as the patient cohort in the study by Guren *et al.* (2003), which reported no significant difference in pain levels, received a much higher total dose of 50Gy, although this was delivered over a longer period of time in daily fractions of 2Gy. Once

again, an explanation for the difference in results, when comparing these studies with that of Marijnen *et al.* (2002) may be the sample size, as Radu *et al*. (2008) used a similar number of patients (n = 46) to Guren *et al.* (2003).

Conversely, when investigating the effect of neoadjuvant therapy with combined chemotherapy and radiotherapy in a sample of 46 patients with rectal cancer, grade 1 – 2 anal pain occurred in 9 patients (19.6%) and grade 1 peripheral neuropathy occurred in 6 patients (13%; Carlomagno *et al.*, 2009). However, it is not stated whether this pain was present at baseline, prior to commencement of treatment, thereby making it difficult to determine if it was caused by the radiation and also, the degree of pain was not significant enough to interrupt treatment, enabling the authors to conclude that this was a tolerable level of toxicity (Carlomagno *et al.*, 2009).

In a separate study, which evaluated the severity and patterns of fatigue in rectal cancer patients during preoperative neoadjuvant treatment and determined predictive factors that may lead to the development of fatigue, pain scores were measured using the National Cancer Institute Common Toxicity Criteria (Wang *et al.,* 2001). At baseline, this symptom was reported as moderate to severe in 18% of patients prior to commencing preoperative radiotherapy (Wang *et al.,* 2001). Although no reference was made to whether this treatment had any impact on pain scores, a strong correlation was found between the development of fatigue and poor pain control prior to commencement of radiotherapy, thereby indicating the necessity of accurately managing this symptom (Wang *et al.*, 2001).

In general, the literature examined here demonstrates that preoperative radiotherapy does not seem to have a significant adverse effect on levels of pain in rectal cancer patients (Wang *et*

al., 2001; Guren *et al.,* 2003; Radu *et al.,* 2008; Carlomagno *et al.,* 2009). However, in the larger scale Dutch trial 7.6% of patients complained of significant pain, although it is difficult to accurately compare results of this study with those previously discussed in relation to pain, as the authors introduced their own measurement tool to assess this symptom. However, as treatment was interrupted in some participants in this study due to the presence of pain, it could be concluded that there may be a small number of patients that are more sensitive to radiotherapy, indicating that this symptom still requires close monitoring during treatment.

It is clear from the literature examined here that acute adverse reactions of preoperative radiotherapy include fatigue, diarrhoea, proctitis, nausea, skin reactions, pain, and dysuria (Cella *et al.,* 2001; Kapiteijn *et al.,* 2001; Hwang *et al.,* 2002; Marijnen *et al.,* 2002; Guren *et al.,* 2003; Sauer *et al.,* 2004; Fiorica *et al.,* 2009; Janaki *et al.,* 2010). A synopsis of the side effects measured in each study discussed here is available in Table 2.2.

Table 2.2: Side effects measured

Study	Sample	Diarrhoea	Dermatological Problems	Micturition Problems	Sexual Dysfunction	Pain	Fatigue
Stone *et al.* (2000)	-Varying cancers - 576 pts						X
Cella *et al.* (2001)	-Varying cancers -379 pts; 6% GI						**X**
Wang *et al.* (2001)	- Rectal cancer - 72 pts	**X**	**X**				**X**
Bonnel *et al.* (2002)	- Rectal cancer - 50 pts			**X**	**X**		
Hwang *et al.* (2002)	- Various cancers - 180 pts; 15% colorectal					**X**	**X**
Marijnen *et al.* (2002)	- Rectal cancer -1530 pts	**X**	**X**			**X**	
Guren *et al.* (2003)	- Rectal cancer - 42 pts	**X**		**X**		**X**	**X**
Sauer *et al.* (2004)	- Rectal cancer - 799 pts	**X**	**X**	**X**			
Ahlberg *et al.* (2005)	Uterine cancer -62 pts	**X**					**X**
Marijnen *et al.* (2005)	- Rectal cancer - 990 pts			**X**	**X**		
Heriot *et al.* (2005)	- Rectal cancer - 201 pts				**X**		
Basch *et al.* (2006)	- Various cancers - 400 pairs (pt/clinician)	**X**				**X**	**X**
Bosset *et al.* (2006)	- Rectal cancer -1011 pts	**X**					
Vironen *et al.* (2006)	- Rectal cancer - 94 pts	**X**		**X**	**X**		
Radu *et al.* (2008)	- Rectal cancer - 46 pts	**X**				**X**	
Carlomagno *et al.* (2009)	- Rectal cancer - 46 pts	**X**				**X**	
Fiorica *et al.* (2009)	- Rectal cancer - 41 pts	**X**	**X**	**X**			
Lange *et al.* (2009)	- Rectal cancer -1530pts				**X**		
Janaki *et al.* (2010)	-Varying cancers - 90 pts, 10% rectal						**X**
Musio *et al.* (2010)	- Rectal cancer - 20 pts	**X**	**X**			**X**	
Hilarius *et al.* (2011)	- Various cancers - 136 pts						**X**
Oh *et al.* (2011)	- Various cancers -30 studies					**X**	**X**
Stephens *et al.* (2010)	- Rectal cancer -1350 pts	**X**			**X**		
Ishihara *et al.* (2011)	- Rectal cancer - 75 pts	**X**	**X**				
Li *et al.* (2012)	- Rectal cancer - 63 pts	**X**	**X**				
Poirier *et al.* (2011)	- Varying cancers - 77 pt.;12% rt abdo/pelvis						**X**
Pucciarelli *et al.* (2011)	- Rectal cancer - 149 pts	**X**			**X**		
Swellengrebel *et al.* (2011)	- Rectal cancer - 147 pts	**X**	**X**		**X**		
Donovan *et al.* (2013)	- Systematic review - 9 studies						**X**
Obead *et al.* (2014)	- Various cancers - 82 pts						**X**

In order to determine how these adverse effects impact on patients' QoL, the current body of knowledge regarding patients' perception of treatment related toxicity must be established.

2.8 Preoperative Radiotherapy and QoL

Adverse effects as discussed in the previous section may be present during and after radiotherapy. In order to gain a more comprehensive understanding of the impact these symptoms have on patients' daily lives it is necessary to examine literature pertaining to this in relation to both acute and long term effects.

2.8.1 Acute Effects on QoL

Health related QoL can be defined as a measurement of the physical, psychological and social effects of illness or treatment (Ferrans *et al.,* 2005). Guren *et al.* (2003) examined health related QoL during radiotherapy for rectal cancer by assessing 42 patients at the start of treatment, the end of treatment and 4-6 weeks later. Findings indicated that although patients demonstrated a non-significant reduction in QoL at the end of radiotherapy, as well as increased levels of fatigue and diarrhoea, at follow up after 4-6 weeks, symptoms and levels of QoL had returned to near baseline levels (Guren *et al.,* 2003).

Similarly, Janaki *et al.* (2010) also found increased levels of fatigue during radiotherapy in a sample of 90 patients, with this returning to near pre-treatment levels after approximately one month following completion of therapy. Fatigue and QoL questionnaires were administered to patients and results indicated that during the period where fatigue levels had increased, a negative impact on the physical, cognitive and social functions of patients was seen (Janaki *et al.,* 2010).

An investigation to establish the impact of fatigue, site specific side effects and individual characteristics on functional status during radiotherapy indicated a decline in QoL during treatment (Poirier, 2011). This study included a sample of 77 patients, 12% of which

received treatment to the abdomen or pelvis, and demonstrated the multifactorial effects on QoL by identifying fatigue, severity of side effects, treatment site, living alone, age and multiple modes of treatment as influential (Poirier, 2011).

Fatigue was not mentioned as having any impact on health related QoL in three other trials, which gathered data over a longer period of time, thereby indicating the need to identify radiotherapy related effects that influence long term health related QoL (Marijnen *et al.,* 2005; Pietrzak *et al.,* 2007; Stephens *et al.,* 2010).

2.8.2 Long Term Effects on QoL

In the study by Marijnen *et al.* (2002), a separate analysis of the impact of preoperative radiotherapy for rectal cancer on health related QoL with the Rotterdam Symptom Checklist was performed at 3, 6, 12, 18 and 24 months after surgery using the same Dutch study group (Marijnen *et al.,* 2005). It was concluded from this that although patients that received radiotherapy preoperatively had lower health related QoL at 3 months, therefore requiring more time to recover after surgery, and also had reduced levels of sexual function than those who just had surgery alone, overall health related QoL was not significantly affected.

Conversely, in a Polish trial that collected data approximately one year after treatment for rectal cancer, in order to investigate QoL, anorectal and sexual function after preoperative radiotherapy, 20% of participants indicated anorectal function impairment negatively influenced their QoL (Pietrzak *et al.*, 2007). Pietrzak *et al.* (2007) attribute the variation in their findings when compared with that of Marijnen *et al.* (2005) to the difference in the QoL assessment tools used, stating theirs (the European Organisation for Research and Treatment

of Cancer QoL Questionnaire (EORTC-QLQ) was more sensitive at detecting differences in treatment toxicity levels.

However, McNair *et al.* (2009) included the Dutch study in their expert review of how health related QoL assessment can improve clinical practice and concluded that the tool used was validated and the trial was well designed, thereby providing clinicians with valuable information regarding treatment decisions for patients with rectal cancer.

Also, a study by Stephens *et al.,* (2010) which is comparable to the Dutch trial, the EORTC-QLQ was used to gather data in conjunction with the Medical Outcomes Short-Form (MOS-SF) and despite the application of a different assessment tool, results were similar to those in the study by Marijnen *et al.* (2005). Although there was a negative impact on sexual and anorectal dysfunction after completion of treatment, there was no evidence that this impacted on patients' QoL (Stephens *et al.,* 2010). It was hypothesised that these results may be due once again to lack of sensitivity of the instrument, or perhaps participants in the study accepted that the benefits of treatment outweighed its adverse effect on sexual dysfunction (Stephens *et al.,* 2010).

This finding is further supported by results of a qualitative study that investigated the physical, psychological, social and emotional experiences of 10 rectal cancer patients within 2 years of their treatment, with all participants besides 1 having received radiotherapy as part of their treatment protocol (Wright *et al.,* 2006). Unanticipated morbidity emerged as one of the main themes, with patients stating they had not anticipated the extent of treatment related adverse effects, despite discussions with health care providers regarding the risks, benefits and rationale for treatment (Wright *et al.,* 2006). Adverse effects had a negative impact on

mobility, activities of daily living and sexual function, and although patients would have appreciated a more realistic view in relation to the consequences of their treatment, this would not have changed the choices they made, as they were willing to accept that the negative effects of treatment outweighed the long term benefits (Wright *et al.*, 2006).

2.9 Adverse Effects of Chemotherapy

Although the main focus of this review is the side effects associated with radiotherapy and the impact of this on QoL, standard treatment for rectal cancer involves a neoadjuvant approach that also incorporates concomitant chemotherapy in protocols. Patients that were included in this study received a total dose of 50.4Gy of radiation, as well as 825mg/m2 of capecitabine twice daily. Therefore, the adverse effects of this drug will also be outlined, in order to form a more comprehensive review of issues patients receiving this neoadjuvant therapy may experience.

Capecitabine is an orally administered thymidine phosphorylase activated fluoropyrimidine carbamate and was developed due to the need for efficient, tolerable and convenient agents, which do not require continuous intravenous infusion (Saif *et al.*, 2008). Less toxic gastrointestinal and dermatologic effects have been reported with this drug when compared with its intravenous counterpart, 5-fluorouracil (Saif *et al.*, 2008). Side effects that may occur with oral capecitabine include diarrhoea, nausea, vomiting, stomatitis, skin reactions on the hands and feet, and fever (Chau *et al.*, 2004).

2.10 Methodological Issues

This review has presented a general overview and discussion of radiotherapy related side effects. However, it must be noted that accurate comparing and contrasting of studies is restricted due to differences in methodologies employed which include the wide range of data collection tools, the timing of data collection, variances in end points, and small sample sizes (see Table 2.3).

This is apparent in studies that measured the prevalence of fatigue, with a wide range of assessment tools used which include the Functional Assessment of Cancer Therapy-Fatigue (FACT-F) questionnaire, ICD-10 criteria, the Brief Fatigue Inventory (BFI), the Piper Fatigue Scale (PFS) and the Multidimensional Fatigue Inventory-20 (MFI-20), although it must be noted that all tools have proven validity and reliability (Stone *et al.,* 2000; Cella *et al.,* 2001; Wang *et al.,* 2001; Hwang *et al.,* 2002; Ahlberg *et al.,* 2005; Janaki *et al.,* 2010; Hilarius *et al.,* 2011; Obead *et al.,* 2014). Also, there is lack of consistency at the various time points fatigue levels were measured, with just two studies following up patients 4-6 weeks after completion of treatment, making it difficult to determine whether this symptom was sustained, particularly as both studies included small sample sizes (n = 42; n = 90), although both reported similar findings (Guren *et al.,* 2003; Janaki *et al.,* 2010).

In relation to the prevalence of diarrhoea, dermatological problems and issues with micturition, lack of consistency in reported findings emerged due to variances in end points measured. In studies where all grades of diarrhoea, dermatological problems and issues with micturition were included in analyses, prevalence was much higher, when compared with studies that only included more severe effects (Wang *et al.,* 2001; Bonnel *et al.,* 2002;

Marijnen *et al.,* 2002; Guren *et al.,* 2003; Sauer *et al.,* 2004; Marijnen *et al.,* 2005; Vironen *et al.,* 2006; Fiorica *et al.,* 2009; Musio *et al.,* 2010; Ishihara *et al.,* 2011).

Prevalence rates of diarrhoea may also be affected by differences in treatment protocols, as the study by Bosset *et al.,* (2006) reported higher rates of this symptom in patients that received chemotherapy and radiotherapy, rather than radiotherapy alone (37.6% vs 17.3%). (Bosset *et al.,* 2006; Musio *et al.,* 2010) However, the addition of chemotherapy did not have any impact on rates of dermatological issues (Musio *et al.,* 2010). Nonetheless, it must be noted that although doses of radiotherapy were similar in the majority of studies that assessed these symptoms, there were differences in the chemotherapy regime patients received, thereby making it difficult to accurately compare them (see Table 2.1).

Pain appears to occur to some extent in a small number of patients, although it seems to be at a tolerable level, as no interruption to treatment occurred in these instances, with the exception of one particular study. It must be noted however, that the sample size was much larger in this (n = 1530) than in the other investigations that monitored pain levels (highest n = 180), and also, the authors introduced their own tool to measure this symptom, without stating whether it had proven validity and reliability (Hwang *et al.,* 2002; Marijnen *et al.,* 2002; Guren *et al.,* 2003; Radu *et al.,* 2008; Carlomagno *et al.,* 2009; Musio *et al.,* 2010; Oh *et al.,* 2011).

Another interesting point of note is that all studies measuring symptom presentation adopted quantitative methodologies. In relation to the impact of these effects on QoL, only one study adopted a qualitative methodology. This highlights a major paucity in relation to the

qualitative measurement of patients' experiences of adverse effects and the impact this has on their QoL.

Table 2.3: Methodologies in studies investigating symptom presentation

Study	Methodology	Cancer Type	Sample Size	Tools	Timing of Measurement	Symptoms Assessed
Stone *et al.* (2000)	Quantitative	-Varying cancers	- 576 pts	FACT-F Questionnaire	1 time point – stage of tx not stated	Fatigue
Cella *et al.* (2001)	Quantitative	-Varying cancers 6% GI	-379 pts	ICD-10 Criteria	1 time point - post chemotherapy / chemoradiotherapy treatment	Fatigue
Wang *et al.* (2001)	Quantitative	- Rectal cancer	- 72 pts	BFI CTCAE Criteria	5 time points – weekly during treatment	Diarrhoea Dermatological Problems Fatigue
Bonnel *et al.* (2002)	Quantitative	- Rectal cancer	- 50 pts	Standardised Questionnaires that incorporated the I-PSS	1 time point - >/= 6 months post treatment	Micturition Problems Sexual Dysfunction
Hwang *et al.* (2002)	Quantitative	- Various cancers 15% colorectal	- 180 pts	FACT-F Questionnaire FACT-G Questionnaire BFI MSAS-SF Depression Scale	1 time point	Pain Fatigue
Marijnen *et al.* (2002)	Quantitative	- Rectal cancer	-1530 pts	RTOG Neurologic Complaints Scoring System	During radiotherapy – exact timing not stated	Diarrhoea Dermatological Problems Pain
Guren *et al.* (2003)	Quantitative	- Rectal cancer	- 42 pts	EORTC QLQ Symptom Diary	3 time points – beginning, end and 4-6 weeks after completion of treatment	Diarrhoea Micturition Problems Pain Fatigue
Sauer *et al.* (2004)	Quantitative	- Rectal cancer	- 799 pts	German Classification System	5 time points – symptoms measured weekly during treatment	Diarrhoea Dermatological Problems Micturition Problems
Ahlberg *et al.* (2005)	Quantitative	Uterine cancer	- 62 pts	EORTC QLQ MFI-20	3 time points - beginning, midway and on completion of treatment	Diarrhoea Fatigue
Marijnen *et al.*, (2005)	Quantitative	- Rectal cancer	- 990 pts	Rotterdam Symptom Checklist	6 time points - before treatment, 3, 6, 12, 18 & 24 months after surgery	Micturition Problems Sexual Dysfunction
Heriot *et al.*, (2005)	Quantitative	- Rectal cancer	- 201 pts	Sexual Functioning Questionnaire	7 time points - preop, 4, 8, 12, 24, 36 & 48 mts post surgery	Sexual Dysfunction

Study	Methodology	Cancer Type	Sample Size	Tools	Timing of Measurement	Symptoms Assessed
Basch *et al.* (2006)	Quantitative	- Various cancers	- 400 pairs (clinician / patient)	CTCAE Criteria	1 time point	Anorexia Constipation Diarrhoea Fatigue Nausea Pain Vomiting
Bosset *et al.* (2006)	Quantitative	- Rectal cancer	-1011 pts	WHO Criteria for Toxic Effects	5 time points - preop tx weekly for acute toxic effects during - plus post op every 3 wks (length of assessment not stated)	Diarrhoea
Vironen *et al.* (2006)	Quantitative	- Rectal cancer	- 94 pts	RAND 36 Questionnaire Urinary, Sexual & Bowel Dysfunction Questionnaire	1 time point - 1 year post op	Diarrhoea Micturition Problems Sexual Dysfunction
Radu *et al.* (2008)	Quantitative	- Rectal cancer	- 46 pts	Retrospective analysis of medical records	Not stated	Diarrhoea Pain
Carlomagno *et al.* (2009)	Quantitative	- Rectal cancer	- 46 pts	CTCAE Criteria	5 time points - weekly	Diarrhoea Pain
Fiorica *et al.* (2009)	Quantitative	- Rectal cancer	- 41 pts	WHO Criteria	5 time points - weekly	Diarrhoea Dermatological Problems Micturition Problems
Lange *et al.* (2009)	Quantitative	- Rectal cancer	-1530 pts	Sexual Dysfunction Questionnaires	Preop & 3, 6, 12, 18 & 24 months post op	Sexual Dysfunction
Janaki *et al.* (2010)	Quantitative	-Varying cancers - 10% rectal	- 90 pts	EORTC QLQ BFI	8 time points - At baseline, weekly x 6 wks & 1 month post treatment	Fatigue
Musio *et al.* (2010)	Quantitative	- Rectal cancer	- 20 pts	CTCAE Criteria	Not stated	Diarrhoea Dermatological Problems Pain
Hilarius *et al.* (2011)	Quantitative	- Varying cancers - % of rectal not stated	- 136 pts	FACIT-F	Up to 6 timepoints - at baseline & when attending routine clinic appointments	Fatigue
Oh *et al.* (2011)	Quantitative	- Various cancers	- 30 studies	Not Applicable	Not Applicable	Pain Fatigue

Study	Methodology	Cancer Type	Sample Size	Tools	Timing of Measurement	Symptoms Assessed
Stephens *et al.* (2010)	Quantitative	- Rectal cancer	-1350 pts	EORTC QLQ MOS SF-36	11 time points - at baseline, every 3 months for 1 yr & every 6 months for 3 years	Diarrhoea Sexual Dysfunction
Ishihara *et al.* (2011)	Quantitative	- Rectal cancer	- 75 pts	CTCAE Criteria	5 – 6 time points - weekly	Diarrhoea Dermatological Problems
Li *et al.* (2012)	Quantitative	- Rectal cancer	- 63 pts	CTCAE Criteria	5 – 6 time points - weekly	Diarrhoea Dermatological Problems
Pucciarelli *et al.* (2011)	Quantitative	- Rectal cancer	- 149 pts	EORTC QLQ Faecal Incontinence Score Questionnaire	4 time points - At baseline, 2-3 wks post tx, 6 months & 12 months after surgery	Diarrhoea Sexual Dysfunction
Swellengrebel *et al.* (2011)	Quantitative	- Rectal cancer	- 147 pts	CTCAE Criteria RTOG Criteria	5 time points - weekly	Diarrhoea Dermatological Problems Sexual Dysfunction
Obead *et al.* (2014)	Quantitative	- Rectal Cancer	- 82 pts	FACT-G PFS	2 time points - pre radiotherapy & post radiotherapy	Fatigue

2.11 Conclusion

Studies have indicated that preoperative radiotherapy in the management of rectal cancer leads to a number of common adverse effects which include fatigue, diarrhoea, dermatological problems, micturition problems, and to a lesser degree, pain and sexual dysfunction. These effects can lead to a decline in functional status and health related QoL during treatment, result in prolonged surgical recovery times but interestingly, do not cause any long term deterioration in QoL (Guren *et al.,* 2003; Marijnen *et al.,* 2005; Stephens *et al.,* 2010). A synopsis of all studies included for critical analysis is available in Table 2.4.

This review has enabled the identification of a paucity of literature that specifically examines fatigue and QoL, particularly in relation to rectal cancer patients during preoperative radiotherapy treatment, with results of the most recent investigation published in 2003 (Guren *et al.,* 2003). This is significant as fatigue has been reported to be the most common cause of reduced QoL in patients receiving radiotherapy, which is noteworthy, as lower QoL prior to surgery in patients with rectal cancer, has been associated with prolonged surgical recovery times (Guren *et al.,* 2003; Marijnen *et al.,* 2005; Janaki *et al.,* 2010; Poirer 2011).

Also, due to the methodological issues that have been highlighted, it is difficult to draw definitive conclusions from the literature reviewed. Therefore, further research is warranted in relation to symptom presentation and the impact this has on the QoL of rectal cancer patients undergoing preoperative radiotherapy. It can be seen from the literature here that such a study should attempt to ensure homogeneity with regard to disease type and stage and also, utilise validated symptom assessment tools at pre-determined time points, in order to obtain a comprehensive overview of patients' experiences during and immediately after their treatment.

It has also been shown that an individual's genetic background influences how they will respond to radiation and thus, may develop more toxic effects due to increased sensitivity (Flint-Richter *et al.*, 2007). Therefore, it must be established whether factors associated with the development of radiotherapy related side effects can be identified, so that their influence on patients' health related QoL can be minimised through individualisation of their nursing care, thus hastening post-operative recovery times for patients undergoing treatment for rectal cancer. The next chapter thus aims to identify and critically discuss factors that may be associated with reactions to radiotherapy treatment.

Table 2.4: Acute symptoms & side effects of radiotherapy / Impact on QoL
(*n* = 33 articles)

Author/Year/Country	Aim	Method/Sample	Type of Cancer	Key Findings
Ahlberg *et al.* (2005) Sweden	To investigate fatigue, other symptoms & QoL in patients receiving radiotherapy for uterine cancer	Longitudinal, descriptive correlational design using questionnaires with a sample of 62 patients	Uterine	Fatigue increased significantly during treatment. Nausea, diarrhoea & loss of appetite increased & were associated with fatigue. Worsening symptoms correlated with lower QoL.
Basch *et al.* (2006) USA	To compare the reporting of symptom severity reported by patients & clinicians	Quantitative analysis of 400 paired questionnaires based on CTCAE criteria	Various	Agreement was higher for symptoms that could be observed directly such as vomiting and diarrhoea, than for more subjective symptoms, such as fatigue and dyspnoea
Bonnel *et al.* (2002) France	To establish the impact of preop radiotherapy on the urinary and sexual function of pts undergoing TME.	Quantitative analysis of 50 pts using questionnaires adminsitered retrospectively.	Rectal	Sexual function may be impaired to some degree in pts that have received preop radiotherapy.
Bosset *et al.* (2006) France	To evaluate the addition of chemotherapy to preoperative radiotherapy & the use of postop chemotherapy in rectal cancer.	Quantitative analysis of 1011 pts randomly assigned to receive preop radiotherapy (252), preop chemoradiotherapy (253), preop radiotherapy & postop chemo (253) & preop chemoradiotherapy & postop chemoradiotherapy (253) with survival as the endpoint.	Rectal	Adding fluorouracil-based chemotherapy preop or postop had no significant effect on survival but is beneficial in improving local control. Grade 2 toxic effects occurred in 29.7% of pts receiving preop radiotherapy & 38.4% of pts receiving preop chemoradiotherapy.
Carlomagno *et al.* (2009) Italy	To determine the effects of capecitabine & oxaliplatin in combination with radiotherapy for the treatment of rectal cancer.	Quantitative analysis of 46 pts receiving neoadjuvant treatment who were monitored weekly with toxicities measured by using the National Cancer Institute Common Toxicity Criteria.	Rectal	Gastrointestinal adverse effects occurred at Grade 1 – 2, with only 2 pts having Grade 3 vomiting & diarrhoea. Proctitis & anal pain occurred in 2 pts & peripheral neuropathy was reported in 1 pt. A remarkable rate of complete or near complete response was documented.
Cella *et al.* (2001) United States	To evaluate the proposed cancer related fatigue diagnostic criteria in cancer survivors	Quantitative analysis of 379 cancer survivors	Various 6% GI cancer	80% of total sample reported problems with fatigue to some degree. 17% of these reached the threshold set by the ICD-10 criteria & were recorded as having diagnosable fatigue
Donovan *et al.* (2013) United Kingdom	To systematically review research that used specific criteria to diagnose cancer related fatigue	Systematic review of 9 studies with sample sizes ranging from 16-379	Various	Across the 9 studies, prevalence rates of fatigue ranged from 9.2% - 56%. The wide variances in results demonstrates lack of consistency in how the criteria have been applied

Author/Year/Country	Aim	Method/Sample	Type of Cancer	Key Findings
Fiorica *et al.* (2009) Italy	To evaluate the impact of radiotherapy in elderly patients with rectal cancer.	Quantitative analysis of 41 pts using WHO criteria to assess acute morbidities weekly during radiation.	Rectal	Diarrhoea was the most common acute toxicity, followed by urinary & skin complications. Despite this, it was concluded that the rate of toxicity was acceptable & treatment was well tolerated in elderly patients.
Guren *et al.* (2003) Norway	To assess symptoms and HRQoL in pts undergoing preop radiotherapy for rectal cancer.	Quantitative analysis of 42 pts using a 5 day symptom diary & the European Organisation for Research & Treatment of Cancer QoL Questionnaire (EORTC-QLQ) to assess QoL	Rectal	At the end of radiotherapy diarrhoea, fatigue, appetite & physical function scores had disimproved with negative effects noted on QoL. HRQoL scores had returned to near pre-treatment levels when reassessed 4-6 weeks after radiotherapy.
Heriot *et al.* (2005) Ohio	To evaluate to effect of radiotherapy on sexual function in rectal cancer pts undergoing resection & to develop a mathematical model for measuring the risk of sexual dysfunction in this pt cohort.	Quantitative analysis of 201 pts at 7 time points over 4 yrs after surgery.	Rectal	Radiotherapy had a negative influence on sexual function with maximum deterioration noted 8 months after surgery. A predictive model to quantify the risk of sexual dysfunction occurring was developed.
Hilarius *et al.* (2011) Netherlands	To investigate adherence to treatment guidelines on cancer related anaemia and fatigue	Quantitative analysis of 136 pts at up to 6 time points	Various Cancers % of rectal not stated	- Guidelines concerning the use of epoetin or blood transfusion in severe cancer related anaemia are adhered to in approx. half of cases. - 63% received counselling regarding fatigue, with 55% of participants given info on energy conservation, 18% receiving counselling on physical activity and less than 5% receiving info in relation to sleep therapy, nutrition and/or restorative therapy
Hwang *et al.* (2002) United States	To investigate independent predictors of fatigue	Quantitative analysis of 180 cancer pts using questionnaires	Various Cancers 15% Colorectal	Independent predictors of fatigue were analgesics, haemoglobin & serum sodium, feeling drowsy, dyspnoea, pain, feeling sad & irritable, & lack of appetite.
Ishihara *et al.* (2011) Japan	To establish the prognostic significance of acute toxicities associated with preop radiotherapy in rectal cancer patients.	Quantitative analysis of 75 pts using the Common Terminology Criteria for Adverse Events to score acute toxicities.	Rectal	Diarrhoea & leukocytopenia were reported as the most common acute adverse effects with their occurrence showing a better prognosis after surgery following radiotherapy.
Janaki *et al.* (2010) India	To identify the prevalence of fatigue & its impact on QoL during radiotherapy treatment.	Quantitative analysis of 90 pts with various cancers using the BFI & the EORTC questionnaires.	Various Cancers (10% Rectal)	Fatigue increased gradually over the course of radiotherapy, peaked at the last week but had returned to pre-treatment level at follow up.

Author/Year/Country	Aim	Method/Sample	Type of Cancer	Key Findings
Kapiteijn *et al.* (2001) Holland	To determine whether the addition of radiotherapy enhances the benefits of Total Mesorectal Excision (TME).	Quantitative analysis of 1861 rectal cancer pts. 924 pts received radiotherapy preop, 937 pts received surgery alone.	Rectal	Survival rates after 2 years was 82% in the group that received preop radiotherapy & 81.8% in the group that had only surgery. Local recurrence rates were 2.4% in the radiotherapy group and 8.2% in those that only had surgery.
Lange *et al.* (2009) Holland	To identify risk factors for sexual dysfunction after treatment for rectal cancer.	Quantitative analysis of 1530 pts using questionnaires to assess sexual dysfunction.	Rectal	Sexual dysfunction is common after treatment for rectal cancer & is mainly attributed to surgery with preoperative radiotherapy having an additional effect.
Li *et al.* (2012) Beijing / Singapore	To assess the safety and effectiveness of preoperative intensity-modulated radiotherapy (IMRT) with oral capecitabine in the treatment of rectal cancer.	Quantitative analysis of 63 pts using the National Cancer Institute Common Toxicity Criteria to measure acute toxicities & postop complications, toxicities, complete response, local recurrence & survival as endpoints.	Rectal	Grade 3 toxicities included diarrhoea, radiation dermatitis & neutropenia & 4 pts developed post op complications. There was a high rate of complete response.
Marijnen *et al.* (2002) Holland	To determine acute adverse effects of preop radiotherapy and to establish its influence on surgical parameters & postop morbidity & mortality in pts in the Dutch TME trial by Kapiteijn *et al.*, (2001) (see above).	Quantitative analysis of 1530 pts using the Radiation Therapy Oncology Group (RTOG) scoring system to measure acute effects.	Rectal	Low occurrence of toxicity during radiotherapy. Pts that received radiotherapy had more perineal complications. No difference in mortality rates between the groups was noted.
Marijnen *et al.* (2005) Holland	To assess the impact of preop radiotherapy & TME on the sexual functioning & HRQoL of rectal cancer pts. in the Dutch TME trial by Kapiteijn *et al. (*2001).	Quantitative analysis of 990 pts using the Rotterdam Symptom Checklist to assess symptoms & QoL.	Rectal	Pts that received preop radiotherapy had slower postop recovery times and increased sexual dysfunction. However, this did not affect HRQoL in the long term.
Musio *et al.* (2010) Italy	To compare intensified neoadjuvant chemoradiotherapy for rectal cancer with standard preoperative treatement.	Quantitative analysis of 20 pts that received intensified neoadjuvant treatment results of which were compared with a control group that received standard treatment & were evaluated retrospectively.	Rectal	Toxicity was increased with the addition of oxaliplatin but resulted in higher rates of sphincter preservation, down staging & complete response. Results require validation by a larger trial which was ongoing at the time of publication.

Author/Year/Country	Aim	Method/Sample	Type of Cancer	Key Findings
Obead *et al*. (2014) Jordan	To examine the impact of radiotherapy treatment on Jordanian cancer patients QoL & fatigue, and to explore the relationship between fatigue & QoL	Quantitative quasi-experimental correlational design with 82 pts using the FACT-G & PFS	Various	Significant differences noted between pre & post radiotherapy QoL & fatigue scores
Oh *et al.* (2011) Korea	To examine the association of symptom & psychological distress with cancer related fatigue using a literature review & meta analysis	Meta analysis of 30 publications	Various cancers	Symptoms & psychological distress were correlated with fatigue
Pietrzak *et al.* (2007) Poland	To determine whether large doses of radiotherapy per fraction of a short course protocol result in more severe anorectal & sexual dysfunction & impaired QoL.	Quantitative analysis of 316 pts using the EORTC questionnaire to assess QoL and also an anorectal & sexual function questionnaire.	Rectal	Approximately two thirds of pts reported anorectal function impairment with 20% stating that this significantly influenced their QoL.
Poirier *et al.* (2011) USA	To establish the impact of fatigue, side effects & individual characteristics on functional capacity during radiotherapy.	Quantitative analysis of 77 pts with varying cancers by grouping items from the PFS & BFI into primary, secondary & tertiary roles using the Roy Adaptation Model.	Various Cancers (12% received radiotherapy to abdo / pelvis)	Functioning declined during treatment. Living alone & an increase in the total side effects scores increased disruption to role functioning further.
Pucciarelli *et al.* (2012) Italy	To describe patient reported outcomes after preoperative neoadjuvant chemoradiotherapy for rectal cancer.	Quantitative analysis of 149 pts using fecal incontinence & EORTC questionnaires.	Rectal	Sexual dysfunction problems in males were noted to be greatly impaired throughout the study & 1 year after treatment. Physical & social functioning, fatigue & body image were suboptimal immediatly after treatment but had returned to baseline levels after 1 year.
Radu *et al*. (2008) Sweden	To describe the experience of using short course preop radiotherapy & delayed surgery in pts not eligible for standard long course treatment.	Quantitative analysis of 46 pts by retrospectively evaluating clinical records.	Rectal	Although the short course protocol was well tolerated in general with less local symptoms such as pain reported after commencing treatment, 3 elderly pts reported Grade 4 diarrhoea.
Sauer *et al*. (2004) Germany	To compare the effects of preop versus postop chemoradiotherapy in rectal cancer pts.	Quantitative anlysis of 799 pts (415 preop / 384 postop) with acute effects assessed using the German classification system.	Rectal	Survival rates were 76% (preop) & 74% (postop) at 5 yrs with local recurrence rates of 6% (preop) & 13% (postop). Grade 3 or 4 toxic effects occured in 27% of pts in preop group & 40% in postop group.

Author/Year/Country	Aim	Method/Sample	Type of Cancer	Key Findings
Stephens *et al.* (2010) United Kingdom / Canada	To determine whether the benefits of preoperative radiotherapy are balanced against any adverse effects this treatment may induce.	Quantitative analysis of 1350 pts using the MOS SF-36 & the EORTC questionnaires.	Rectal	The most common adverse effect for males was sexual dysfunction but this was mainly attributed to surgery with radiotherapy treatment having a less significant impact. Pt's that received radiotherapy & those that did not had similar levels of decreased function at 3 months but this then returned to baseline levels.
Stone *et al.* (2000) United Kingdom	To investigate cancer patients experience of fatigue, & their perceptions about the causes, management & impact of this symptom	Cross-sectional, questionnaire based survey of 576 cancer patients	Various cancers	The response rate was 44%. Fatigue was reported in 58% of cases. Fatigue not reported to doctor in 52% of cases. 14% received tx or advice to manage fatigue. Fatigue not well managed in 33% of cases.
Swellengrebel *et al.* (2011) Holland	To evaluate acute adverse effects & surgical complications in pts receiving preoperative chemoradiotherapy with capecitabine for rectal cancer.	Quantitative analysis of 147 pts using Common Terminology Criteria & Radiation Therapy Oncology Group scoring systems to measure acute toxicities. Surgical complications were assessed using the Clavien-Dindo classification	Rectal	Grade 3-5 toxicity occurred in 32 pts, particularly diarrhoea & dermatological problems. Anastomotic leakage & perineal wound complications were reported in 36 pts with surgical reintervention required in 30 pts.
Vironen *et al.* (2006) Finland	To determine the impact of surgery related adverse effects on the QoL of rectal cancer pts.	Quantitative analysis of 94 pts using the RAND-36 questionnaire & questionnaires to assess urinary, sexual & bowel dysfunction.	Rectal	Patients reported a good QoL after surgery which was comparable to that of the general population. Social functioning was adversely affected by bowel & urogenital dysfunction.
Wang *et al.* (2001) Texas	To evaluate the severity and pattern of fatigue in rectal cancer patients undergoing preop chemoradiotherapy and to establish factors that may predict the development of severe fatigue.	Quantitative analysis of 72 pts using the Brief Fatigue Inventory (BFI).	Rectal	Fatigue increased in 67% of patients undergoing treatment. Pain was a predictor of severe fatigue which was present in 18% of pts prior to commencing treatment. Uncontrolled diarrhoea was a predictor for increased fatigue during treatment and at the end of treatment, approximately one third of pts had severe fatigue that affected their activities.
Wright *et al.* (2006)	To describe the physical, psychological, social and emotional experiences of pts receiving tx for rectal cancer	Phenomenological qualitative analysis of interviews with 10 pts	Rectal	Unanticipated morbidity emerged as one of the main themes. Adverse effects had a negative impact on mobility, ADL's & sexual function

CHAPTER III: KRAS and Cytokines as Factors Associated with Radiosensitivity

3.0 Introduction

As outlined in the previous chapter, the presence of rectal cancer and its' treatment with radiotherapy can lead to a number of side effects. In order to establish whether there is a method to identify those that demonstrate greater radiosensitivity, in terms of tumour response and side effects, potential factors associated with this will be critically discussed. Therefore, this chapter will demonstrate that KRAS status and cytokine expression may be associated with tumour response to treatment and the development of treatment related toxicities.

Firstly, an outline of the search strategy is provided in Section 3.1. Themes that emerged from the literature in relation to factors associated with the development of rectal cancer, tumour response to radiotherapy and adverse effects were KRAS and KRAS mediated cytokine expression. Therefore, justification for examining KRAS as a factor associated with radiosensitivity is provided in Section 3.2, followed by an outline of the biology of KRAS (Section 3.2.1), the activating signal pathways of KRAS (Section 3.2.2), the influence of KRAS on the development of cancer (Section 3.2.3), the association between KRAS and Epidermal Growth Factor Receptor (EGFR; Section 3.2.4), the use of KRAS as an indicator of radiosensitivity (Section 3.2.4), and finally, its use as an indicator of neoadjuvant outcomes (Section 3.2.5).

Cytokines mediated by KRAS include IL-6 and IL-8. Therefore, an overview of cytokine function is provided in Section 3.3 and this is then followed by a review of literature in relation to KRAS mediated cytokine expression (Section 3.3.1) and a general overview of interleukins, which is provided in Section 3.3.2. Further detail in relation to IL-6 is outlined in Section 3.3.3, followed by a discussion of IL-6 in relation to the proliferation of colorectal

cancer cells (Section 3.3.3.1), colorectal cancer patients (Section 3.3.3.2), radiotherapy (Section 3.3.3.3) and symptom presentation (3.3.3.4). Similarly, further detail regarding IL-8 is provided in Section 3.3.4, followed by a discussion of IL-8 in relation to the proliferation of colorectal cancer cells (Section 3.3.4.1), colorectal cancer patients (Section 3.3.4.2), radiotherapy (Section 3.3.4.3) and symptom presentation (3.3.4.4). The chapter concludes with a synopsis of the key findings arising from the literature reviewed and highlights the necessity for the current study so that areas where there is limited knowledge are addressed.

3.1 Search Strategy

The literature search was conducted as outlined in Chapter I. Studies selected for inclusion were based on the PRISMA 27 item checklist and the PRISMA four phase flow chart (see Figure 3.1; Moher *et al.,* 2009). A total of 63 publications were included in the review for critical analysis. These papers were discussed in relation to KRAS, IL-6 and IL-8 as factors associated with radiosensitivity, with a specific focus on the tumour, response to neoadjuvant treatment and symptom presentation.

All publications discussed adopted quantitative methodologies. Of the 63 publications, 20 referred to KRAS, 27 referred to IL-6 and 17 referred to IL-8, with the article by Pasi *et al.* (2010) discussed in relation to both IL-6 and IL-8.

Most of the studies were conducted in America (27), 25 were carried out in Europe, 1 in Canada, 9 in Asia and 1 in Australia. Sample sizes in the studies ranged from 20 to 606 (Reyes-Gibby *et al.*, 2007; Nastase *et al.*, 2011).

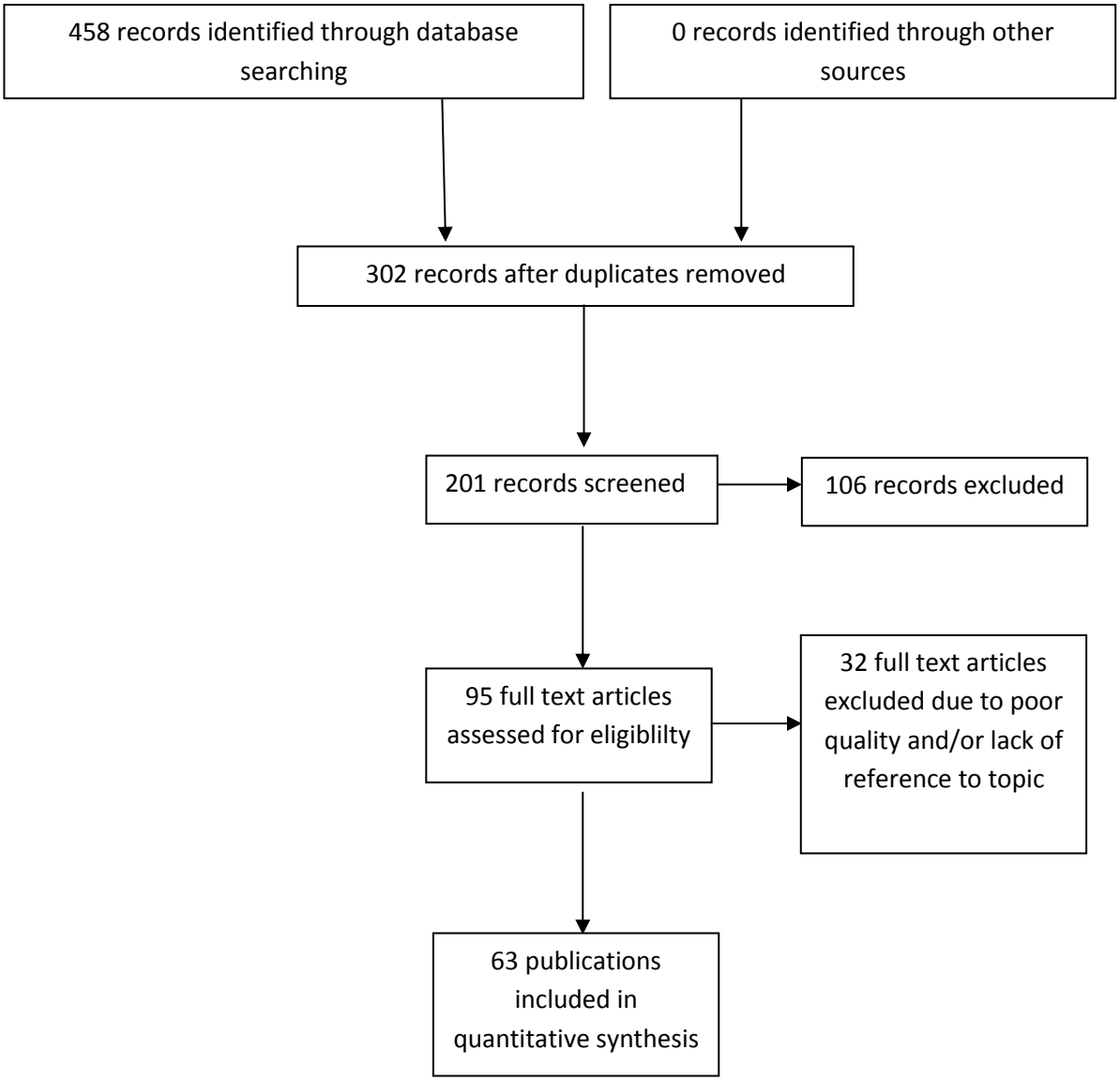

Figure 3.1: PRISMA Flow Chart

3.2 KRAS

The use of preoperative radiotherapy in rectal cancer is now widely accepted as standard practice as it is associated with remarkable improvements in locoregional control and slight improvements in survival rates (Kapiteijn *et al.,* 2001; Guckenberger *et al.,* 2009; Jorgren *et al.,* 2010; van Gijn *et al.,* 2011). As outlined in the previous chapter, this treatment may lead to a number of side effects that have a negative influence on QoL. This is significant as not all patients respond to preoperative treatment. Therefore, identification of factors associated with poor response to preoperative radiotherapy for rectal cancer could be used to select optimum management strategies and avoid significant morbidity in patients who will not benefit from this treatment. KRAS is one of the most commonly studied oncogenes in rectal cancer as in its active state, cell growth and proliferation is promoted (see Figure 3.2). Therefore, the association of KRAS with responses to preoperative radiotherapy in this patient group warrants further discussion.

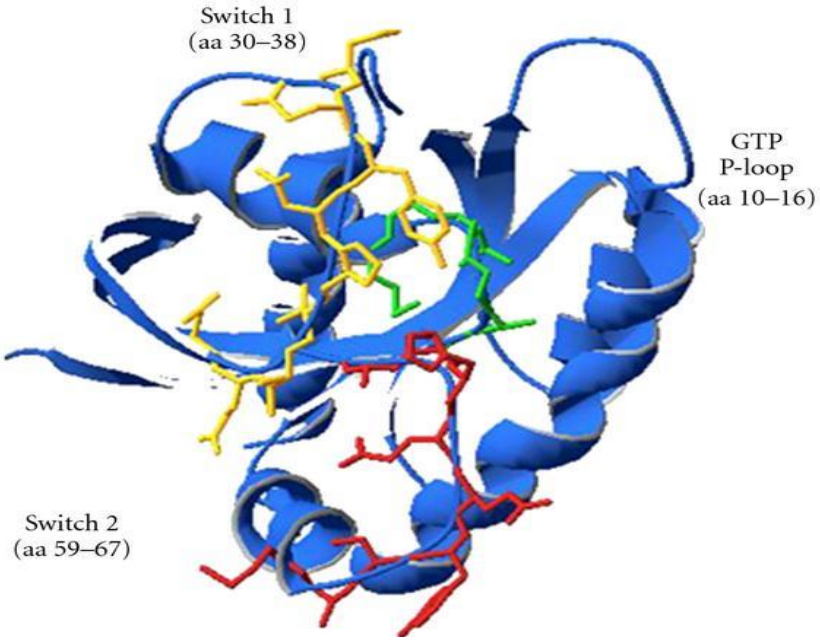

Figure 3.2: KRAS Gene
Jancík *et al.* (2010)

3.2.1 Biology of KRAS

The Rat Sarcoma (RAS) gene refers to a family of related oncogenes that promote cell growth and regulation (Arrington *et al.*, 2012). Three human isoforms of RAS have been identified – namely the Neuroblastoma rat sarcoma viral oncogene (NRAS), the Harvey rat sarcoma viral oncogene (HRAS) and KRAS (wild type and mutant), with KRAS mutations comprising of 86% of all RAS mutations and occurring more frequently than NRAS and HRAS in the presence of cancer (Bamford *et al.*, 2004).

The KRAS gene is located on chromosome 12 and encodes a protein that regulates cell growth, development and function (van Krieken *et al.*, 2008). KRAS usually cycles between an inactive and active state (see Figure 3.3). In its inactive form, KRAS is guanosine diphosphate (GDP) bound but guanine exchange factors (GEF) released during an intracellular signal cascade facilitate the activation of KRAS by replacing GDP with guanosine triphosphate (GTP), thereby leading to the release of a variety of downstream effectors (Arrington *et al.*, 2012). Deactivation of KRAS only occurs when GTPase-activating proteins (GAPs) convert the GTP molecule back to its GDP bound state (Arrington *et al.*, 2012).

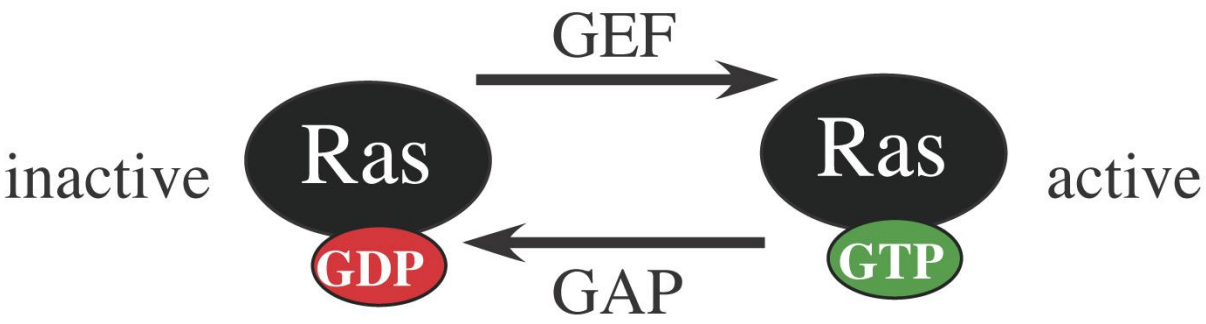

Figure 3.3: Inactive versus Active KRAS

3.2.2 KRAS and EGFR

The activation of KRAS is mediated through the EGFR signalling pathway (see Figure 3.4). EGFR signals through two main effector pathways which include the RAS / Rapidly Accelerated Fibrosarcoma (RAF) / Mitogen-Activated Protein Kinase (MAPK) pathway and the Phosphatidylinositol-3-Kinase and Protein Kinase B (P13K / AKT) pathway, both of which are paralleled and possess some overlap (Krasinskas *et al.,* 2011). Ligand binding activates EGFR, which in turn, activates RAS (i.e. KRAS). When active, KRAS consequently activates RAF, with subsequent phosphorylation of MEK and then, MAPK, leading to cell growth and proliferation (Krasinskas *et al.,* 2011). The KRAS pathway, which is activated upstream by EGFR overlaps with the P13K / AKT pathway as subunits of PI3K can be activated by KRAS (Krasinskas *et al.,* 2011). It is clear from this that oncogenic activation of the KRAS signalling pathways is implicated in the malignant process, as this is thought to control cell growth, differentiation, and survival. Therefore, further discussion is warranted in relation to KRAS and the development of cancer.

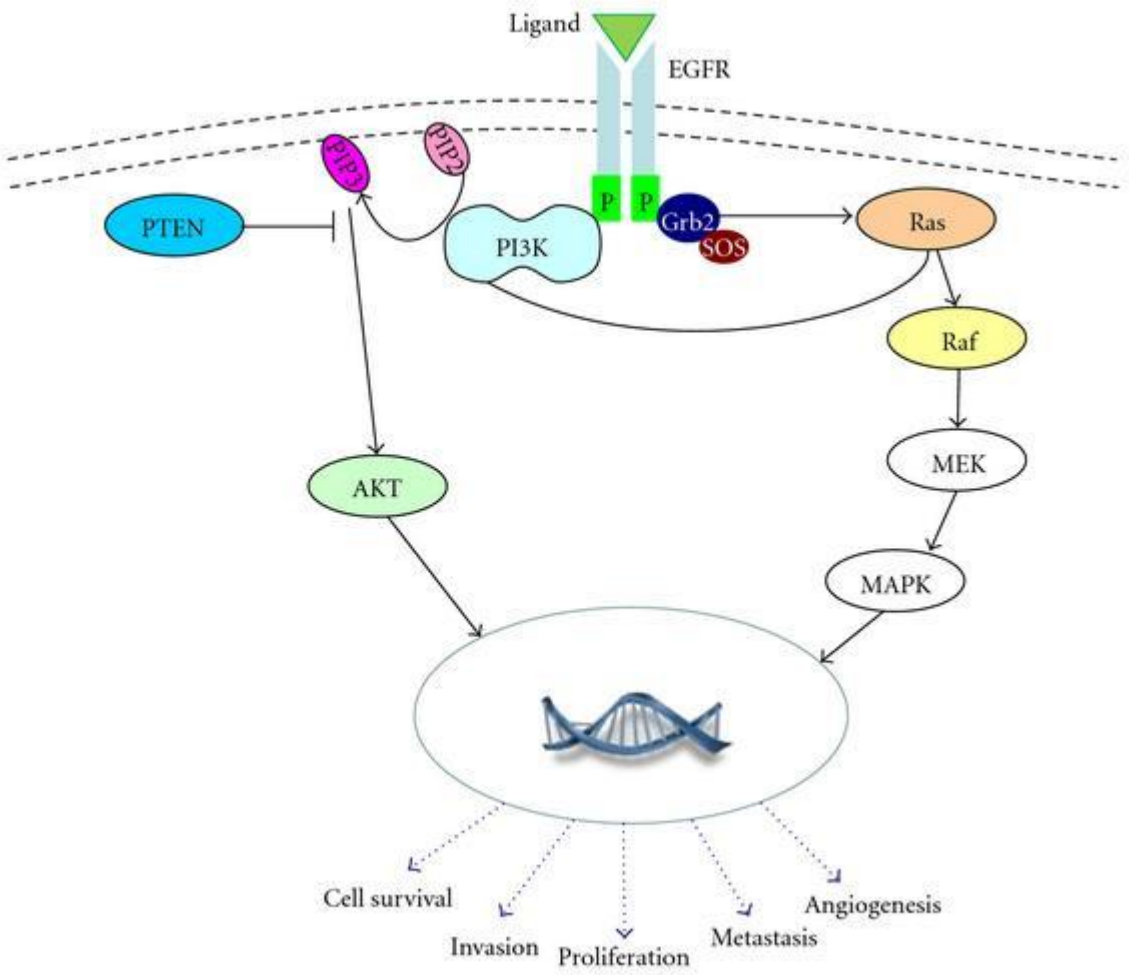

Figure 3.4: EGFR Signalling of KRAS
Krasinskas *et al.* (2011)

3.2.2 KRAS and the Development of Rectal Cancer

The involvement of KRAS in the development of rectal cancer was first proposed in 1988, where mutated KRAS, as opposed to wild type KRAS, was described as an early event in the pathogenesis of this illness (Vogelstein *et al.,* 1988). Mutation of KRAS occurs in 30 - 50% of colorectal cancer cases and is due to missense mutations through single amino acid substitutions (De Roock *et al.,* 2008; van Krieken *et al.,* 2008; de Campos-Lobato *et al.,* 2010).

In rectal cancer, KRAS mutations occur most commonly at codons 12 and 13, accounting for approximately 95% of all mutation types, with 80% occurring at codon 12, 15% occurring at codon 13 and the remaining 5% of mutations present at codons 61, 146 and 154 (Forbes *et al.,* 2006). These mutations impair the intrinsic GTPase activity of KRAS, leading to accumulation of KRAS proteins in the GTP bound, active form, thereby promoting downstream cell proliferation and growth leading to tumorigenesis (see Figure 3.5; Arrington *et al.,* 2012). This signalling cascade is facilitated by KRAS induced secretion of IL-6 and IL-8 which have been identified as mediators of cell to cell communication (Wislez *et al.,* 2006; Ancrile *et al.,* 2007).

In the presence of metastases, rectal tumours that harbour mutated KRAS, as opposed to wild type KRAS are unresponsive to EGFR antibody medications, as the KRAS protein is constitutively activated and the unregulated downstream signalling will not be blocked by treatments that target the EGFR receptor (Amado *et al.*, 2008; Krasinskas *et al.*, 2011). At present, this is the only clinically relevant application for the detection of KRAS status, as mutant KRAS tumours should not be prescribed EGFR antibody drugs in cases of metastases (Amado *et al.,* 2008). Therefore, discussion of literature that investigates KRAS status as an indicator of radiosensitivity is justified in order to determine its association with response to treatment, which may guide clinical management of this illness.

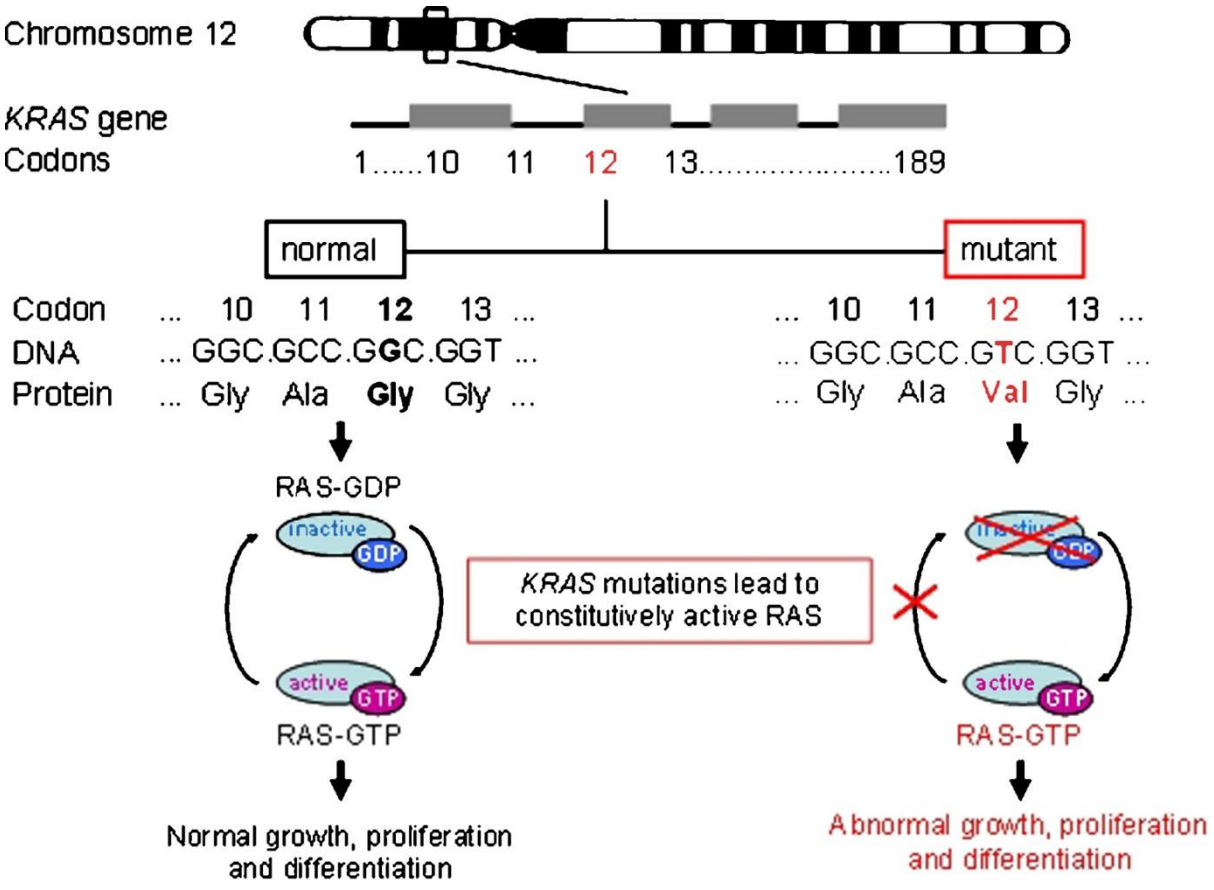

Figure 3.5: Wild Type versus Mutant KRAS
(van Krieken *et al.*, 2008)

3.2.3 KRAS as an Indicator of Radiosensitivity

Tumour cell survival after radiation is a complex process that is not fully understood but may be enhanced by the presence of activated oncogenes such as RAS (McKenna *et al.*, 2003; Cengel *et al.*, 2005). In order to establish the association of KRAS with radiosensitivity, 5 preclinical studies that have been carried out have demonstrated this and will be discussed in further detail.

Early preclinical studies assessed the effect of radiation on all RAS oncogenes (K, H and N) by transfecting them to NIH 3T3 murine cell lines and then determining the radiation survival curve using clonogenic survival curve assays (Sklar, 1988). All cell lines transfected with

RAS genes activated by a missense mutation showed a large increase in intrinsic radiation resistance, with no significant difference among the type of RAS gene or the site of activating mutation (Sklar, 1988). However, these results were limited to NIH 3T3 cell lines that were exposed to radiation in very specific conditions and required validation through further studies.

Ling *et al.* (1989) evaluated cellular radiosensitivity induced by oncogenic transfection using rat embryo cells obtained from rats that were 13 to 15 days pregnant. These cells were then cultured and transfected with the oncogene human c-Ha-RAS from EJ bladder carcinoma and exposed to radiation doses of up to 6Gy, with findings demonstrating greater radioresistance in transfected cells than in primary rat embryo cells (Ling *et al.*, 1989). This study confirmed and strengthened the results of the previous investigation, as utilising rat embryo cells avoided the need for established cell lines, as adopted by Sklar (1988), which would have already undergone unspecified genetic changes in the immortalisation process (Sklar 1988; Ling *et al.*, 1989).

More specifically, in relation to KRAS, an investigation that used human tumour cell lines derived from colon carcinoma to examine the contribution of this oncogene to intrinsic radiosensitvity, also confirmed that the presence of activated KRAS significantly increased cellular radioresistence (Bernhard *et al.*, 2000). Interestingly, these findings are more robust and strengthen those of the studies previously discussed as rather than introducing an activated oncogene into cells, the survival of cell lines was established from cells where the activated KRAS allele was disrupted by targeted knockout, thereby yielding a line expressing only the normal allele, and also, they were derived from human, colon carcinomas rather than murine models (Bernhard *et al.*, 2000).

These results were further confirmed in an investigation that measured the radiosensitivity of human tumour cell lines with oncogenic KRAS and HRAS mutations after treatment with prenyltransferase inhibitors, with findings demonstrating that inhibition of oncogenic RAS activity reduced the radiation survival of these cells (Bernhard *et al.,* 1998). In this, cell lines were derived from colon, bladder, breast and cervical tumours, exposed to radiation doses from 1Gy to 4Gy, treated with prenyltransferase inhibitors and clonogenic survival was then assessed using a clonogenic assay and limiting dilution cloning (Bernhard *et al.,* 1998).

Russell *et al.* (1999) also examined human tumour cell lines, which included cells from glioblastomas, pancreatic and colon tumours, and successfully enhanced their radiosensitvity, by compromising RAS protein activity through the transduction of an anti-RAS adenovirus, leading to a remarkable 40 – 50% reduction in tumour cell survival, thereby providing further evidence for the role of this oncogene as a marker of radiosensitivity. These results are further strengthened by findings of a more recent study that examined human colon, bladder and laryngeal cancer cell lines, where inhibition of KRAS led to a reduction in tumour cell survival (Kim *et al.,* 2005).

It is clear from the findings presented here that activated KRAS certainly decreases radiosensitivity at a preclinical, cellular level, with all investigations that included colon carcinoma cells confirming this. However, in order to establish the influence of KRAS mutation on the radiosensitivity of patients receiving neo-adjuvant treatment for colorectal cancer, relevant clinical investigations must be reviewed.

3.2.4 KRAS as an Indicator of Neoadjuvant Treatment Outcomes

In clinical studies, testing for KRAS mutations has been incorporated into the management plans of rectal cancer patients as it is now widely accepted that the presence of mutation is predictive of poor response to anti - EGFR monoclonal antibody treatments (De Roock *et al.,* 2008; Soulières *et al.,* 2010; Mao *et al.,* 2011). As the presence of KRAS mutation leads to tumour growth and proliferation, studies that have examined its value in relation to tumour response to neoadjuvant chemoradiotherapy warrant further discussion, particularly as 3 studies demonstrate an association between this and KRAS status and 5 studies have reported no association.

Studies that are discussed used varying definitions of tumour response to treatment, as well as treatment regimes, which are outlined in Tables 3.1 and 3.2.

Table 3.1: Definition of tumour response

Study	Definition of Tumour Response
Bengala *et al.,* (2009) Hu-Lieskovan et al., (2011)	TRG0 (no regression) TRG1 (minor regression – fibrosis in < 25% of tumour) TRG2 (moderate regression – fibrosis in 26-50% of tumour) TRG3 (good regression - >50% of tumour regression) TRG4 (total regression – no viable tumour cells, only fibrotic mass)
Davies *et al.,* (2011)	Limited response (gross residual disease present) Major response (only microscopically visible disease present) Pathologic Complete response (no pathological evidence of residual tumour cells)
Erben *et al.,* (2011)	TRG0/TRG1 (bad responders) TRG2/TRG3 (good responders)
Garcia-Aguilar *et al.,* (2011) Russo *et al.,* (2014)	Non pathologic complete response Pathologic complete response
Gaedcke *et al.,* (2010)	TRG0 (no fibrosis or regression) TRG1 (>50% viable tumour cells) TRG2 (regression of 50-70%) TRG3 (regression >70%)
Zauber *et al.,* (2009)	Stage 1 (no disease or minimal microscopic disease with marked fibrosis) Stage 3 (abundant macroscopic disease with little or no fibrosis)

A recent study investigated a sample of 132 rectal cancer patients to identify a biomarker profile associated with tumour response to treatment (Garcia-Aguilar *et al.*, 2011). Non-pathologic complete response occurred in only 24% of patients with KRAS mutation whereas 49% of patients without mutation had pathologic complete response (Garcia-Aguilar *et al.*, 2011).

This is supported by a separate investigation that included a sample of 79 rectal cancer patients and also used pathologic complete response and non-pathologic complete response as endpoints, with results demonstrating the presence of KRAS mutations in 43% of cases, and pathologic complete response occurring in 23.5% of wild type tumours versus just 3.3% in those with mutation (Russo *et al.*, 2014).

An Italian study performed to evaluate the relationship between EGFR expression and KRAS mutation also demonstrated the negative influence of KRAS mutation on tumour response to treatment (Bengala *et al.*, 2009). Although results were not statistically significant, total regression grade 3-4 occurred in only 11% of cases with mutant KRAS versus 36.7% of cases that harboured wild type KRAS (Bengala *et al.*, 2009).

All three studies described here indicate the presence of KRAS mutation may impede patient response to neoadjuvant chemoradiotherapy (Bengala *et al.*, 2009; Garcia-Aguilar *et al.*, 2011; Russo *et al.*, 2014). However, these results are contradicted by those of a study performed to explore the relationships of KRAS and BRAF mutations with phosphorylated Protein Kinase B (p-AKT) and phosphorylated Extracellular Signal-Regulated Kinase (p-ERK) with outcomes in 70 patients with rectal cancer (Davies *et al.*, 2011). In this, the

presence of wild type KRAS versus mutated KRAS, when correlated with tumour response was limited in an equal number of cases (67% versus 67%), major in 14% versus 21% of cases and complete in 19% versus 13% and thus, mutation was not correlated with radioresistance, even though higher rates of pathologic complete response was seen in wild type tumours (Davies *et al.,* 2011).

KRAS mutation was not significantly correlated with pathologic complete response in a study to identify molecular predictors for clinical outcome in rectal cancer patients receiving neoadjuvant chemoradiotherapy (Hu-Lieskovan *et al.,* 2011). These results are interesting as pathologic complete response was demonstrated in 15% of patients with KRAS mutant tumours versus just 10% of patients with KRAS wild type tumours (Hu-Lieskovan *et al.,* 2011). These findings are supported by those of two separate investigations that also correlated KRAS status with tumour response to treatment in this patient cohort (Zauber *et al.,* 2009; Erben *et al.,* 2011).

In an investigation to explore the predictive and prognostic value of KRAS mutation in 57 rectal cancer patients, the presence of this mutation was not significantly associated with poor response to treatment, although 39 patients with wild type tumours versus just 18 patients with KRAS mutant tumours demonstrated tumour regression (Erben *et al.,* 2011). Similarly, no significant association between KRAS status and tumour response to treatment was indicated in a study of 53 rectal cancer patients, despite 45% of the sample having a high level of regression (Zauber *et al.,* 2009).

A recently published systematic review of studies that evaluated the effect of KRAS status on treatment outcomes for rectal cancer patients used random effects methods to retrieve data

from 8 series that described 696 patients in total (Clancy *et al.,* 2013). Within this data set, a sub analysis of 4 studies (363 patients) that included results on tumour down staging was performed, with the authors concluding that the presence of KRAS mutation did not affect tumour response to treatment (Clancy *et al.,* 2013).

The consensus among the 5 studies described here appears to be that KRAS status is not correlated with tumour response to treatment (Clancy *et al.,* 2013; Erben *et al.,* 2011; Davies *et al.,* 2011; Zauber *et al.,* 2009; Hu-Lieskovan *et al.,* 2011). However, on further examination, it is difficult to accurately compare results of these investigations due to differences in the definition of the study endpoint, which is tumour response to treatment (see Table 3.1) and also, variances in treatment regimes that patients received in each study (see Table 3.2).

Table 3.2: Treatment regimes

Study	Treatment Regime
Bengala *et al.,* (2009)	- 50 Gy of radiation - 5-FU + / - Oxaliplatin + / - Capecitabine
Clancy *et al.,* (2013)	Various regimes
Davies *et al.,* (2011)	- Preoperative radiotherapy (dose not stated) - 5FU chemotherapy
Erben *et al.,* (2011)	- 50.4 Gy of radiation - Cetuximab + Irinotecan + Capecitabine
Gaedcke *et al.,* (2010)	- 50.4 Gy of radiation - Oxaliplatin and 5-FU
Garcia-Aguilar *et al.,* (2011)	- 50.4 Gy - 5-FU +/- additional (leucovorin + Oxaliplatin)
Hu-Lieskovan et al., (2011)	- 45 Gy -50.4 Gy of radiation - Cetuximab +/- Capecitabine +/- Oxaliplatin +/- 5-FU
Russo *et al.,* (2014)	- Preoperative radiotherapy (dose not stated) - Chemotherapy
Zauber *et al.,* (2009)	- 49.12 Gy of radiation (mean dose) - Fluorouracil +/- leucovorin rescue

Further to this, although KRAS status was not correlated with tumour response in an American study that established rates of KRAS and RAF mutation in pre-treatment rectal cancer biopsies, separate analysis of mutations based on amino acid exchange appeared to be associated with higher rates of tumour regression (Gaedcke *et al.,* 2010).

This is interesting as although KRAS status alone does not correlate with tumour response in the studies discussed here, there is an indication that subtype analysis of KRAS mutation may be more informative. None of the studies discussed above which reported no significant correlation between KRAS status and tumour response conveyed findings of mutation subtype analysis (Hu-Lieskovan et al., 2011; Zauber *et al.,* 2009; Gaedcke *et al.,* 2010; Davies *et al.,* 2011; Erben *et al.,* 2011; Clancy *et al.,* 2013). Similarly, no subtype KRAS mutation analysis was reported in the investigations that indicated a positive correlation between KRAS status and tumour response and thus, may explain the conflicting findings among these publications (Bengala *et al.,* 2009; Garcia-Aguilar *et al.,* 2011; Russo *et al.,* 2014).

Discussion of clinical studies included in this review has highlighted a major paucity in the literature in relation to subtype analysis of KRAS status and tumour response to treatment. This needs to be addressed, particularly as KRAS status has been clearly correlated with response to radiation at a preclinical level.

Previously, it has been identified that KRAS mutation leads to cell proliferation and growth through the activation of downstream effectors. This signalling cascade is facilitated by mediators of cell to cell communication and includes the expression of cytokines, thereby

warranting further discussion of KRAS mediated cytokine expression and the role of cytokines in both tumour growth and symptom presentation.

3.3 Cytokines

The study of cytokine biology was initiated originally through the laboratory investigation of white blood cell exudate that contained pus, which led to an interest in the examination of soluble factors (Dinarello, 2007). The biological manifestation of these soluble factors included fever, increased white blood cell count, death of cancer cells and migration of inflammatory cells (Dinarello, 2007). The development of knowledge in relation to cytokines was then underpinned by a paradigm that describes how a disease process leads to the secretion of soluble factors (cytokines) from cells and how the properties of these factors account for the manifestation of disease (Dinarello, 2007). Therefore, a cytokine can be defined as a soluble factor or protein produced by one cell to act on another cell, in order to initiate a change in the function of the target cell (Dinarello, 2007).

Cytokines are thereby an essential part of the immune system within the body as they act as chemical mediators of cell to cell communication. They possess the unifying feature of regulating the immune system against pathogens and / or the inflammatory response (Fitzgerald *et al.,* 2001). Cytokines are referred to as pleiotropic as they have more than one action - their receptors are expressed on multiple cell types and the signalling pathways that are activated increase gene expression specifically for the affected cell, thus demonstrating their complex nature (Fitzgerald *et al.,* 2001). This pleiotropic nature creates an environment where cytokines act in synergy, are released in succession and are often counter regulated by inhibitory cytokines or receptors (Fitzgerald *et al.,* 2001).

As cytokines are so pleiotropic, it is difficult to categorise them based on their function and they are therefore classified according to the cell types that produce them (see Table 3.3) (Fitzgerald *et al.*, 2001).

Table 3.3: Classification of cytokines

Cell	Cytokine Family	Members
Mononuclear cells / Macrophages	Monokines	IL-1; TNFα; IFN-α; IFN-β; CSF
T lymphocytes	Lymphokines	IL-2; IL-3; IL-4; IL-5; IL-6; GM-CSF; IFNγ
Regulate migration of other cells	Chemokines	CCL2; CCL3; CCL4; CCL5; CCL11; CCL14; CCL19; CCL20; CCL21; CCL25; CCL27; CXCL-8; CXCL10; CXCL12
Leukocytes	Interleukins	IL-1; IL-2; IL-3; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IL-10; IL-11; IL-12; IL-13; IL-14; IL-15

Fitzgerald *et al.* (2001)

With the exception of red blood cells, all cells within the body have the ability to produce and respond to cytokines (Dinarello, 2007). For the purpose of this review, KRAS induced cytokine expression will be discussed further, as this is associated with tumour growth and prolifieration (Ancrile *et al.*, 2007).

3.3.1 KRAS Mediated Cytokine Expression

KRAS induced cytokines that have been identified in the literature are IL-6 and IL-8. IL-6 is secreted by both lymphoid and non-lymphoid cells and regulates acute phase reactions, B and T cell function and haematopoiesis (Fitzgerald *et al.*, 2001). IL-8 is an inflammatory chemokine that is produced by many types of cells and functions as a neutrophil chemoattractant and activating factor but also attracts basophils and some lymphocytes

(Fitzgerald *et al.,* 2001). Literature pertaining to KRAS mediated expression of these cytokines will now be discussed in greater detail, with 5 studies demonstrating a positive association at a pre clinical level.

Higher concentrations of IL-6 were correlated with increased activation of KRAS in an American investigation that examined the activation of NRAS and KRAS induced by IL-6 using model cell lines (Rowley *et al.,* 2002). Findings demonstrated that 1 ng/ml of IL-6 activated approximately 18% of the KRAS pool and also, activated RAS in cells was correlated with levels of proliferation (Rowley *et al.,* 2002).

This is interesting as it supports the results of a separate investigation, where findings indicated that the expression of RAS induced IL-6 acts in a paracrine fashion and promotes tumour growth (Ancrile *et al.,* 2007). Cell lines were used to examine whether IL-6 plays a role in RAS mediated cancers, with higher concentrations of IL-6 being detected as cells became tumorigenic with the expression of RAS G12V fusion protein, when compared with uninduced nontumorigenic cells (Ancrile *et al.,* 2007). Also, knockdown of IL-6 and treatment with a neutralising IL-6 antibody retarded RAS driven tumorigenesis (Ancrile *et al.,* 2007).

Similarly, KRAS mediated expression of the cytokine IL-8 was also identified as acting in a paracrine fashion that promotes tumorigenesis (Wislez *et al.,* 2006). KRAS mice were transfected with an oncogenic version of KRAS mutation, which led to the development of KRAS G12D-driven lung adenocarcinomas (Wislez *et al.,* 2006). Oncogenic KRAS induced secretion of IL-8 homologs was detected in lung tissue homogenates of mice that harboured the activated KRAS allele when compared to mice with wild type KRAS (Wislez *et al.,*

2006). KRAS mediated secretion of IL-8 in the promotion of tumour growth was further proven in this study, as the number of lung lesions detected in mice decreased by 30% post injection with a neutralising antibody serum to receptors of this cytokine family (Wislez *et al.,* 2006).

These findings are validated further in an analysis of IL-8 as a transcriptional target of KRAS signalling using a mouse tumour xenograft model, where signalling from the KRAS isoform led to an increase in both IL-8 messenger Ribonucleic Acid (mRNA) and protein, with ablation of IL-8 function in KRAS induced tumours resulting in a substantial decrease in tumour vasculature and extensive tissue necrosis (Sparmann *et al.,* 2004).

A more recent investigation analysed 89 non-small cell lung cancer tumours, with findings also indicating that IL-8 plays a role in cell growth and migration in oncogenic KRAS driven tumours (Sunaga *et al.,* 2011). Cell lines harbouring KRAS mutations overexpressed IL-8 and knockdown with a neutralising antibody resulted in downregulation of this cytokine (Sunaga *et al.,* 2011).

It is clear from the 5 studies discussed here that KRAS mediates the expression of both IL-6 and IL-8 and both of these cytokines have been correlated with tumour growth and proliferation (Rowley *et al.,* 2002; Sparmann *et al.,* 2004; Wislez *et al.,* 2006; Ancrile *et al.,* 2007; Sunaga *et al.,* 2011). This indicates that further discussion is warranted in relation to interleukins, with a specific focus on IL-6 and IL-8, as expression of these cytokines are not only detected at tumoral level but they are also present in blood samples, where their expression has been correlated with response to radiotherapy treatment and symptom presentation, as well as tumorigenesis.

3.3.2 Interleukins

Interleukins are a group of cytokines that were initially seen to be expressed by white blood cells (leukocytes). The term interleukin derives from 'inter', which refers to a means of communication and 'leukin', which refers to the fact that many of these cytokines are produced by leukocytes and act on leukocytes. However, the name is erroneous as it has since been found that interleukins are produced by a wide variety of body cells (Doan *et al.,* 2007). A number of interluekins have been discovered but for the purpose of this review, IL-6 and IL-8 will be discussed in greater detail as literature has indicated their expression is mediated through KRAS.

3.3.3 IL-6

IL-6, also referred to as B-cell stimulatory factor-2 (BSF-2), is secreted by both lymphoid and non-lymphoid cells and regulates acute phase reactions, B and T cell function and haematopoiesis (Fitzgerald *et al.,* 2001). Once IL-6 has been released from the activated cell, it can act on target cells by binding to the membrane bound IL-6 receptor, thereby mediating cell to cell communication (Knupfer *et al.,* 2010). Therefore, further discussion of this cytokine is presented in relation to its association with colorectal cancer cells (3 studies), colorectal cancer patients (6 studies), radiotherapy (12 studies) and symptom presentation (8 studies). In relation to symptom presentation, the most commonly investigated symptom was fatigue, with all studies but 1 reporting no association between this and IL-6.

3.3.3.1 IL-6 and Colorectal Cancer Cells

Findings of an investigation of the biological effect of IL-6 and the expression of IL-6 receptors on human colorectal carcinoma cell lines indicated a positive association between secretion of this cytokine and the promotion of colorectal cancer cell growth in vitro (Schneider *et al.,* 2000). The association between IL-6 and cell growth occurred in a dose

dependent manner, as IL-6 doses of 100ng/ml significantly enhanced colony formation of cell lines (Schneider *et al.,* 2000).

Conversely, Hsu *et al.,* (2006) reported that neither physiological (10ng/ml) and pharmacological (50 and 100ng/ml) concentrations of IL-6 affected cell growth. The authors attributed this difference to the fact that IL-6 stimulates proliferation of only certain colorectal cell lines (Hsu *et al.,* 2006). However, in this study IL-6 (10ng/ml) significantly increased attachment to the basement membrane, with levels of 50ng/ml significantly increasing the chemotaxis, anchorage-independent growth and invasiveness of cell lines, thereby demonstrating that IL-6 increases invasiveness of colorectal cancer cells (Hsu *et al.,* 2006).

At a cellular level, Brozek *et al.,* (2005) also investigated the effect of IL-6 levels on growth and proliferation through analysis of tumour tissue, adjacent mucosa and human colon adenocarcinoma derived cell clones using an Enzyme Linked Immunosorbent Assay (ELISA) and Real Time Polymerase Chain Reaction (RT-PCR). Findings indicated that levels of IL-6 are low in normal human mucosa and rises only moderately during progression from adenomatous to low grade tumours, whereas higher levels of IL-6 were observed in more advanced tumours, thereby implying that IL-6 may accelerate tumour progression towards malignancy (Brozek *et al.,* 2005).

The results of the studies discussed above all demonstrate an association between levels of IL-6, cell growth and tumour proliferation in colorectal carcinoma cell lines. This indicates that further discussion of studies that have investigated the significance of IL-6 in colorectal cancer patients at a clinical level is warranted.

3.3.3.2 IL-6 and Colorectal Cancer Patients

A number of studies that examine levels of IL-6 in colorectal cancer patients associate their results with tumour stage as an endpoint. Colorectal tumours are mainly staged using two systems – Dukes staging and the American Joint Committee on Cancer (AJCC) Tumour Nodes Metastases (TNM) classification system. A definition of these staging systems can be seen in Table 3.4, with a legend to explain the abbreviations provided in Table 3.5.

Table 3.4: Tumour staging systems

Dukes' Staging	• Dukes' A: Invasion into but not through the bowel wall (90% 5-y survival) • Dukes' B: Invasion through the bowel wall but not involving lymph nodes (70% 5-y survival) • Dukes' C: Involvement of lymph nodes (30% 5-y survival) • Dukes' D: Widespread metastases		
AJCC TNM Classification System	Stage 0	Tis N0 M0	Tis: Tumor confined to mucosa; cancer-*in-situ*
	Stage I	T1 N0 M0	T1: Tumor invades submucosa
	Stage I	T2 N0 M0	T2: Tumor invades muscularis propria
	Stage II-A	T3 N0 M0	T3: Tumor invades subserosa or beyond (without other organs involved)
	Stage II-B	T4 N0 M0	T4: Tumor invades adjacent organs or perforates the visceral peritoneum
	Stage III-A	T1-2 N1 M0	N1: Metastasis to 1 to 3 regional lymph nodes. T1 or T2.
	Stage III-B	T3-4 N1 M0	N1: Metastasis to 1 to 3 regional lymph nodes. T3 or T4.
	Stage III-C	any T, N2 M0	N2: Metastasis to 4 or more regional lymph nodes. Any T.
	Stage IV	any T, any N, M1	M1: Distant metastases present. Any T, any N.

(AJCC, 2002; Kyriakos, 1985)

Table 3.5: TNM classification

Tumour (T)
Tis Tumour confined to mucosa; cancer *in-situ* **T1** Tumour invades submucosa **T2** Tumour invades muscularis propria **T3** Tumour invades subserosa or beyond **T4** Tumour invades adjacent organs or perforates the visceral peritoneum
Nodes (N)
N1 Metastasis to 1 to 3 regional lymph nodes **N2** Metastasis to 4 or more regional lymph nodes
Metastasis (M)
M1 Distant etastasis present

In an investigation to establish the role of IL-6 in the progression of colorectal cancer, serum concentrations of this cytokine were measured using an ELISA in 167 colorectal cancer patients, 67 of which were diagnosed with rectal cancer, and compared with samples from 20 healthy controls (Chung *et al.,* 2003). Results demonstrated that median levels of IL-6 were significantly higher in cancer patients when compared with the control group (11.89pg/ml versus 3.41 pg/ml) and higher levels also correlated with tumour size and stage (Chung *et al.,* 2003).

These findings are supported by those of a Polish study that examined the relationship between platelet activation and inflammation in 42 colorectal cancer patients, 15 of whom had rectal cancer, and 38 healthy controls (Dymicka-Piekarska *et al.,* 2007). Similarly, higher levels of IL-6 were measured in cancer patients than in healthy controls (mean 4.15pg/ml vs. 1.31pg/ml; Dymicka-Piekarska *et al.,* 2007).

A significant relationship between IL-6 levels, colorectal cancer patients, tumour stage and metastases was also reported in a separate investigation to establish the prognostic significance of this cytokine in this patient cohort (Galizia *et al.,* 2002). Preoperative serum IL-6 levels were on average 9.3 pg/ml in cancer patients (*n* = 50) versus 4.4 pg/ml in healthy controls (*n* = 25; Galizia *et al.,* 2002).

Likewise, Kaminska *et al.,* (2005) also found higher serum levels of IL-6 in their sample of 157 colorectal cancer patients when compared with 50 healthy controls (median 2.8pg/ml vs. 0.8pg/ml) and in addition to this, significant correlations between higher levels of this cytokine and tumour stage were evident (Stage 1 = 1.7pg/ml versus Stage 4 = 4.9pg/ml; Kaminska *et al.,* 2005). This is supported by findings of an earlier study that measured levels of IL-6 at three time points – 1 day, 10 days and 42 days post-surgery (Kaminska *et al.,* 2000). It was reported that there was an increase in IL-6 levels after surgery and in the sample, all had returned to normal by the final time point with the exception of patients diagnosed with stage D disease (Kaminska *et al.,* 2000).

Median levels of IL-6 were also significantly higher with the presence of cancer in a Greek study that investigated the role of IL-6 in the progression of colorectal cancer in a sample of 74 cancer patients, 24 of whom were diagnosed with rectal cancer (8.11pg/ml vs. 3.52 pg/ml; Nikiteas *et al.,* 2005). However, no significant association between tumour stage and IL-6 was reported, although higher levels were detected in cases where the tumour size was larger (Nikiteas *et al.,* 2005).

In an Iranian study, levels of IL-6 were measured at tumoral level, as well as in the serum of 50 colorectal cancer patients in order to determine whether there was a correlation between

both, as well as with tumour stage (Esfandi *et al.,* 2006). The overall mean serum level of IL-6 was 5.49pg/ml, with levels increasing with more severe illness as IL-6 ranged from 2.75pg/ml in patients with stage 1 tumours to 9.56 in those with stage 4 disease (Esfandi *et al.,* 2006). Similarly, tumoral levels of IL-6 also increased with stage and ranged from 230.88pg/ml in stage 1 disease to 785.16pg/ml in those with stage 4 tumours, with the mean level reported to be 230.19pg/ml (Esfandi *et al.,* 2006). These results provide evidence that indicate levels of IL-6 in serum and tumoral tissue in this patient cohort correlate significantly with each other and with the staging of the tumour, thereby demonstrating that levels of this cytokine may reflect the proliferative activity of the tumour itself (Esfandi *et al.,* 2006).

An overview of all the studies discussed above gives a strong indication that levels of IL-6 are certainly elevated in patients diagnosed with colorectal cancer and metastases. In relation to IL-6 and tumour stage, all but one investigation reported a positive association between higher levels of this cytokine and increasing tumour stage. However, a number of methodological issues were highlighted when analysing these publications.

When reporting levels of IL-6, either the median level or the mean level of this cytokine was stated in publications, making it difficult to accurately compare results in order to obtain a comprehensive picture of the average reading of this cytokine. Three studies reported the mean level of IL-6 to be 9.3pg/ml, 5.49pg/ml and 4.15pg/ml, with all investigations including similar sample sizes (n = 50, n = 50, n = 42 respectively) and using an ELISA technique to analyse serum samples (Galizia *et al.,* 2002; Esfandi *et al.,* 2006; Dymicka-Piekarska *et al.,* 2007). The other three studies reported median values of 11.89pg/ml, 2.8 pg/ml and 8.11pg/ml, with larger sample sizes used in all investigations (n =164, n = 157, n = 74 respectively) although similarly, all adopted the ELISA technique for cytokine analysis

(Chung *et al.*, 2003; Kaminska *et al.,* 2005; Nikiteas *et al.,* 2005). The study by Kaminska *et al.,* (2000) did not specify levels of IL-6 but rather displayed their findings in graph format. The range in reported levels may not have been as varied if all studies had reported their results as either the median or mean serum levels of IL-6, thereby enabling direct comparisons. It is an interesting point to note as all studies included for discussion used similar patient cohorts, sample sizes, timing of assessment and method of blood sample analysis. An explanation for the variance in IL-6 readings may therefore be the disease stage of the patients within each sample.

Staging of colorectal cancer tumours was used as an endpoint to correlate levels of IL-6 in a number of studies discussed, although a variety of definitions were used which included the Dukes staging system, the TNM classification system, TNM classification by Hutter and Sobin (1986) and the TNM classification as modified by the authors. The lack of consistency in end points of measurement throughout these studies also makes it difficult to accurately compare results. A comparison of these studies can be seen in Table 3.5.

Despite these methodological issues, it could however be concluded that elevated serum and tumoral levels of IL-6 are associated with the presence of colorectal cancer, tumour stage and metastases. Interestingly, all studies discussed included other cancers along with colorectal cancer patients in their samples, thereby highlighting a dearth in the literature that focuses specifically on patients diagnosed with rectal cancer.

Table 3.6: Comparison of studies measuring IL-6 in colorectal cancer patients

Study	Sample Size	Timing of Assessment/ Tumour Classification	IL-6 Level in Cancer Patients	IL-6 Level in Healthy Controls	IL-6 Cut Off Range
Chung *et al.,* (2003)	164 colorectal ca pts 67 rectal cancer pts 20 healthy controls	-Preoperatively -Dukes Staging	Median 11.89pg/ml Range 0-440pg/ml	Median 3.41pg/ml Range 0-9.12pg/ml	Low levels <12pg/ml High levels >12pg/ml
Dymicka-Piekarska *et al.,* (2007)	42 colorectal ca pts 15 rectal cancer pts 38 healthy controls	-Preoperatively -TNM Classification (Hutter and Sobin, 1986)	Mean (no mets) 2.98 +- 1.49pg/ml Mean (with mets) 4.15 +- 2.45pg/ml	Mean 1.31 +-0.55pg/ml	Not stated
Esfandi *et al.,* (2006)	50 colorectal ca pts Rectal ca pts not stated	-Preoperatively -TNM Staging	Mean Serum 5.49 +- 2.49pg/ml Stage 1: 2.75+-0.2pg/ml Stage 2: 4.14 +-0.51pg/ml Stage 3: 5.86 +- 1.68pg/ml Stage 4: 9.56 +- 0.99pg/ml - Mean Tumour 402.93 +-230.19pg/ml Stage 1: 230.88 +- 49.02pg/ml Stage 2: 282.5 +- 64.15pg/ml Stage 3: 394.16 +-197.03pg/ml Stage 4: 785.16 +-105.19pg/ml	Not measured	Not stated
Galizia *et al.,* (2002)	50 colorectal ca pts Rectal ca pts not stated 25 healthy controls	-5 timepoints: Preop, postop, 4, 8 and 16 days after surgery -TNM Classification	Preop Mean 9.3 +- 2.1pg/ml Range 6-13.5pg/ml	Mean 4.4 +- 0.8pg/ml Range 2.8-6.1pg/ml	Not stated
Kaminska *et al.,* (2000)	35 colorectal ca pts Rectal ca pts not stated 40 healthy controls	3 timepoints: 1, 10 and 42 days post operatively -Authors modified staging system	Specific levels not stated – displayed in graph format		
Kaminska *et al.,* (2005)	157 colorectal ca pts Rectal ca pts not stated 50 healthy controls	Timing not stated -TNM Classification	Median 2.8 pg/ml Range 0.7 -107pg/ml Stage 1: 1.7pg/ml Stage 2: 3.2pg/ml Stage 3: 2.8pg/ml Stage 4: 4.9pg/ml	Median 0.8pg/ml Range 0.7-2.4pg/ml	Not stated
Nikiteas *et al.,* (2005)	74 colorectal ca pts 24 rectal ca pts 25 contorls	- Preoperatively - Dukes Staging	Median 8.11pg/ml Range 1.09-188.42pg/ml	Median 3.52pg/ml Range 0.45-9.96pg/ml	Low levels <8pg/ml High levels >8pg/ml

Circulating levels of IL-6 are not only affected by the presence of a cancerous tumour, but also by treatment of this illness, particularly in relation to radiotherapy, thereby indicating the need for further discussion (Akmansu *et al.,* 2005).

3.3.3.3 IL-6 and Radiotherapy

IL-6 is thought to play an important role in the inflammatory reaction associated with exposure to radiation. Therefore, in order to investigate this phenomenon, two immortalised epithelial and keratinocyte cell lines were exposed to doses of up to 10Gy of radiation, resulting in apoptosis and rapid release of IL-6 from the cells (Petit-Frere *et al.,* 2000).

This correlation between radiotherapy treatment and levels of IL-6 was also demonstrated in a study that exposed murine hepatocellular cancer cell lines to 6 – 15Gy of radiation and examined radiation response of IL-6 by implementing clonogenic assays and tumour growth delay methods (Chen *et al.,* 2012). Results indicated that levels of IL-6 were significantly associated with radiation resistance and it has been suggested that blocking this cytokine may reduce recruitment of myeloid derived suppressor cells and tumour development (Chen *et al.,* 2012).

These results are supported by findings of a more recent investigation that used human dermal microvascular endothelial cells and human adipose derived stem cells to determine the effects of radiation in a co-culture model of these cells (Haubner *et al.,* 2013). Following culturing, cells were exposed to doses of 2 – 12 Gy of radiation and a dose-dependent increase of IL-6 was detected in the supernatants using an ELISA technique (Haubner *et al.,* 2013).

Pasi *et al.,* (2010) used much smaller doses of radiation (0Gy, 0.25Gy, 0.5Gy and 1Gy) in their study of the effects of radiation on IL-6 bystander signalling in human glioblastoma cells. Despite these lower doses, findings confirm the results of the three previous studies discussed as a dose dependant increase in levels of IL-6 was noted (Pasi *et al.,* 2010). Interestingly, no significant difference in levels of receptor expression in bystander cells was observed when compared with controls, thereby indicating that IL-6 may use a transignalling pathway rather than the classic pathway, which is similar to that taken by tumoral cells (Pasi *et al.,* 2010).

In relation to tumour growth, sub lethal doses of radiation prevented IL-6 protein expression and reduced tumour growth in an investigation to establish the role of IL-6 to the radiation response of murine prostate cancer cell lines and hormone resistant cell sublines, thereby suggesting a positive association between this cytokine and radiation resistance (Wu *et al.,* 2013). Radiation response was assessed in this study using in vitro clonogenic assays, where cells were exposed to 0, 3, 6 or 9Gy of radiation and in vivo using tumour growth delay models in mice, where they were exposed to 15Gy of radiation, with results demonstrating greater radioresistance in the hormone resistant cells and increased radiosensitivity with inhibition of IL-6 (Wu *et al.,* 2013).

Similarly, a study carried out in Tawain also reported a positive association between IL-6 and radiation resistance, as the suppressed ability of tumour growth in human oral carcinoma cells was attributed to down regulation of this cytokine (Chen *et al.,* 2012).

In order to establish the influence of radiotherapy on levels of IL-6 in head and neck cancer patients, an ELISA was performed with serum from 34 patients before and after radiotherapy

treatment (total dose 50Gy in 25 fractions), with 19 receiving curative, primary radiotherapy treatment and 15 receiving post-operative adjuvant treatment (Akmansu *et al.*, 2005). It was observed that mean IL-6 levels in the group that received primary curative radiotherapy reduced after completion of treatment (74.03pg/ml vs. 50.02pg/ml), whereas in the post-operative treatment group, mean levels of this cytokine increased (45.78pg/ml vs. 214.29pg/ml), with the authors attributing the difference in results to surgery that may have caused an acute phase inflammatory response (Akmansu *et al.*, 2005).

Conversely, levels of IL-6 increased during radiotherapy in a study that specifically focused on patients with rectal cancer, where levels of this cytokine were measured in 32 patients before, during and on completion of radiotherapy (Willet *et al.*, 2009). Increased median levels of IL-6 were detected at day 32 of treatment when compared to baseline, followed by a reduction immediately prior to surgery (1.48pg/ml vs 2.26pg/ml vs 1.56pgml; Willet *et al.*, 2009). This is interesting as tumour regression was noted for all study participants prior to surgery (Willet *et al.*, 2009). Further to this, lesser increases in IL-6 from baseline were seen in patients with minor or no lymph node disease following pre-operative treatment, thereby indicating that higher levels of IL-6 are associated with more severe illness (Willet *et al.*, 2009). This may explain the difference in findings by Akmansu *et al.* (2005), as IL-6 levels reduced in the group of patients that received curative radiotherapy for head and neck cancer.

These results are supported by findings of a separate investigation that also included rectal cancer patients receiving radiotherapy treatment in their sample (n = 35) with the aim of establishing the predictive value of cytokines in this cohort, where findings demonstrated that higher levels of IL-6 post treatment was associated with poor tumour response (Tada *et al.*, 2014).

Furthermore, plasma levels of IL-6 were also correlated with tumour response to radiotherapy, as well as tumour biopsy levels, in 52 patients with non-small cell lung carcinoma, thereby suggesting that the tumour is the major source of circulating cytokines in patients receiving radiotherapy (Rube *et al.,* 2008).

However, a study that investigated 37 patients receiving radiotherapy for prostate cancer demonstrated an immediate elevation of IL-6 after initial radiotherapy treatment, with levels peaking after 1-2 weeks of radiotherapy before returning toward pre-treatment levels (Rube *et al.,* 2008). This demonstrates the inflammatory response to radiotherapy, although IL-6 levels were not correlated with tumour response to treatment, thereby making it difficult to accurately determine that increases in this cytokine were solely due to radiation (Rube *et al.,* 2008).

These findings are supported by those of a separate investigation that also included prostate cancer patients in their sample (n = 20), as well as patients with breast cancer (n = 28; Bower *et al.,* 2009). In this, measurement of levels of IL-6 was intensive and included 6 time points - 5 days, 10 days, 20 days and during the final week of treatment, and also 2 weeks and 2 months after completion of treatment, with increases observed during treatment and a decline in levels noted two months after completion of treatment (Bower *et al.,* 2009).

In relation to colorectal (n = 50) and oesophageal cancer patients (n = 53) treated with radiotherapy, similar results were observed in that levels of IL-6 were 17.4 pg/ml pre-treatment, 23.4 pg/ml post treatment and 6.5 pg/ml one month after completion of treatment, thereby demonstrating the inflammatory response associated with radiotherapy (Wang *et al.,* 2012).

Based on the literature discussed here it could be concluded that in vitro, exposure of cell lines to radiation elicits an inflammatory response leading to a dose dependent increase in levels of IL-6. In vivo, higher levels of IL-6 are reported during radiotherapy, are associated with higher tumoral levels of this cytokine and may also be associated with radiation resistance and poor tumour response to treatment. However, results of these studies need to be interpreted with caution due to variances in methodologies applied which include sample size and type, as well as doses of radiation administered (see Table 3.6). Only two studies included in this section solely used patients with rectal cancer in their sample, thereby demonstrating the need for further investigation of IL-6 in relation to plasma concentration, tumoral levels and responses to radiation in this patient cohort.

Table 3.7: Comparison of studies investigating IL-6 & radiotherapy

Study	Sample	Dose of Radiation
Akmansu *et al.,* (2005)	34 patients Head and Neck cancer	Total dose of 50Gy
Bower *et al.,* (2009)	20 patients Prostate cancer 28 patients Breast cancer	Breast – 57.96Gy (50.4-66.4Gy) Prostate – 7165Gy (45 -75.6Gy)
Chen *et al.,* (2012)	Murine hepatocellular cancer cell lines	In vivo 6Gy In vitro 15Gy
Haubner *et al.,* (2013)	- Human dermal microvascular endothelial cells - Human adipose-derived stem cells	2 – 12 Gy
Pasi *et al.,* (2010)	Human glioblastoma cells	0, 0.25, 0.5 and 1Gy
Petit-Frere *et al.,* (2000)	- Immortalised epithelial cell lines - Immortalised keratinocyte cell lines	Up to 10Gy
Rube *et al.,* (2008)	52 patients NSCLC	Total dose of 32 - 66Gy
Tada *et al.,* (2014)	35 patients Rectal cancer	Total dose of 50.4Gy
Tamatani *et al.,* (2004)	Human carcinoma cells and nude mice	Not stated
Wang *et al.,* (2012)	103 patients - 50 colorectal cancer - 53 oesophageal cancer	Total dose 50.4 – 51.3Gy
Willet *et al.,* (2009)	32 patients Rectal cancer	Total dose of 50.4Gy
Wu *et al.,* (2013)	- Murine prostate cancer cell line - Hormone resistant cell subline	0, 3, 6, 9 and 15Gy

Response to radiation may also lead to symptom manifestation. In order to ascertain a molecular basis for the occurrence of symptoms during this treatment, studies that have investigated IL-6 in relation to this will be discussed.

3.3.3.4 IL-6 and Symptom Presentation

In order to determine whether radiation induced inflammation is associated with cancer related fatigue, patients receiving radiotherapy for breast (n = 28) and prostate (n = 20) cancer were assessed at 6 different time points throughout and upon completion of treatment using validated questionnaires to assess fatigue and immunoassay kits to measure cytokines (Bower *et al.,* 2009). Although there was a significant increase in fatigue during treatment and a higher dose of radiation was associated with significantly higher levels of IL-6, no correlation between both was observed (Bower *et al.,* 2009).

Similarly, a German investigation that assessed the level of fatigue in 41 patients with breast cancer during radiotherapy also reported an increase in this symptom during treatment, with levels returning to baseline measurement at follow up, two months later (Geinitz *et al.,* 2001). No association between this symptom and levels of IL-6, which were measured in parallel with fatigue assessments, was reported (Geinitz *et al.,* 2001).

These results are further confirmed in a cohort of breast cancer survivors that were investigated to define immunologic and inflammatory variables associated with persistent post treatment fatigue (Collado-Hidalgo *et al.,* 2006). Plasma cytokines were measured using ELISA and fatigue was assessed with validated questionnaires, with no correlation reported between IL-6 and this symptom (Collado-Hidalgo *et al.,* 2006). This is interesting as unlike the patient cohorts described in the two previous studies, the sample investigated here were not receiving radiotherapy at the time of assessment but still, findings offer support to the results that have been reported (Geinitz *et al.,* 2001; Collado-Hidalgo *et al.,* 2006; Bower *et al.,* 2009).

Likewise, in patients receiving chemotherapy alone for metastatic colorectal cancer (n = 80), fatigue was not significantly associated with IL-6, despite having higher scores for both at baseline (Rich *et al.*, 2005). However, a significant relationship was reported between this cytokine and the symptoms of nausea, vomiting and appetite loss (Rich *et al.*, 2005).

Interestingly, in a study that included 106 patients receiving radiotherapy at an outpatients clinic for gastrointestinal cancer, 50 of whom had colorectal cancer, no significant correlation was made between levels of fatigue and circulating IL-6 alone but there was however, a significant association between increased levels of this cytokine and the severity of a fatigue centred symptom cluster, thereby demonstrating that tumour or host generated IL-6 may be related to general symptom presentation (Wang *et al.*, 2012). A breakdown of correlations between this cytokine and specific symptoms was not reported (Wang *et al.*, 2012).

A positive correlation between levels of IL-6 and fatigue was noted in 52 breast cancer patients receiving radiotherapy for breast conservation, although this association seemed to be mediated by Body Mass Index (BMI), rather than fatigue as a stand-alone symptom (Wratten *et al.*, 2004).

Conversely, when a genetic variation of IL-6, rather than circulating levels of IL-6, was investigated in 168 oncology patients receiving radiotherapy for either breast, prostate, brain or lung cancer, findings indicated a positive genetic association between a functional promoter polymorphism in the IL-6 gene and the severity of fatigue (Miaskowski *et al.*, 2010). However, in a later study, that analysed the same data set in order to establish whether there was a relationship between the IL-6 gene and symptom clusters, no significant association was reported, with the authors attributing the discrepancy in results to the

measurement of a symptom cluster, rather than individual symptoms (Illi *et al.,* 2012). However, these results should be interpreted with caution as all other studies that reported a negative association between IL-6 and fatigue measured circulating levels of IL-6 rather than a genetic variation of this cytokine.

The general consensus among the studies discussed above appears to be that fatigue and levels of IL-6 are not associated with one another, despite the fact that radiotherapy leads to increases in both. However, it is necessary to be mindful that the investigations used a variety of patient cohorts that included those diagnosed with breast, prostate, colorectal, brain and lung cancer, thereby making it difficult to accurately compare results. Another limitation includes the small sample sizes utilised within each study, which ranged from 48 – 168 patients (Bower *et al.,* 2009; Illi *et al.,* 2012). Despite this, it has been highlighted that of all the symptoms experienced by cancer patients, particularly during radiotherapy, fatigue is the most common one studied in relation to expression of the cytokine IL-6. This has enabled the identification of a major paucity in published literature as although there seems to be no association between fatigue and IL-6, this cytokine has been linked with a fatigue centred symptom cluster in one study and also, nausea, vomiting and appetite loss in a separate investigation (Rich *et al.,* 2005; Wang *et al.,* 2012). Therefore, further research is required in relation to this cytokine and other radiotherapy related symptoms, which also include fatigue, particularly regarding rectal cancer as no study was identified that specifically focused on this patient cohort.

3.3.4 IL-8

IL-8 is an inflammatory chemokine that is produced by many types of cells and functions as a neutrophil chemoattractant and activating factor but also attracts basophils and some lymphocytes (Fitzgerald *et al.,* 2001). It is multifunctional in nature, but its primary function involves the induction of chemotaxis in cells (Nastase *et al.,* 2011). Through autocrine and paracrine mechanisms, IL-8 has an important role in angiogenesis, tumour growth, tumour progression and metastasis (Nastase *et al.,* 2011). Therefore, further discussion of this cytokine is presented in relation to its association with colorectal cancer cells (2 studies), colorectal cancer patients, radiotherapy and symptom presentation. A total of 7 investigations examined colorectal cancer patients, with 2 demonstrating no significance between IL-8 and tumour size and 2 indicating that lower levels of this cytokine were associated with more advanced disease. In relation to radiotherapy, 4 studies were included for review and for symptom presentation, 5 studies were examined, with 2 studies identifying a possible association between pain and memory.

3.3.4.1 IL-8 and Colorectal Cancer Cells

In an investigation to establish the effects of the tissue microenvironment on colon cancer progression and metastases, mouse and human colon cancer cell lines were evaluated (Lee *et al.,* 2012). Results indicated that elevated levels of IL-8 in both the serum and tumour profoundly enhanced the growth of cancer cells, and promoted metastases of these cells into the lung and liver (Lee *et al.,* 2012).

These findings are supported by results of another study that also used human colon cancer cell lines to determine the role of IL-8 in colorectal cancer cells (Ning *et al.,* 2011). Cells that overexpressed IL-8 demonstrated increased cellular proliferation, cell migration and

invasion and formed significantly larger tumours when compared with control cells (Ning *et al.,* 2011). Inhibition of this cytokine reversed the observed increases in tumorigenic functions (Ning *et al.,* 2011).

Results of these investigations demonstrate a positive correlation between increased levels of IL-8 and tumour growth and development, as well as metastases. However, in order to establish the role of this cytokine in patients at a clinical level, it is necessary to discuss studies that have examined IL-8 in vivo.

3.3.4.2. IL-8 and Colorectal Cancer Patients

The association between IL-8 and tumour formation has been established at a cellular level and was demonstrated further at a clinical level in a study that investigated the responses of this cytokine in patients diagnosed with colorectal cancer (Malicki *et al.,* 2009). Blood samples were collected from 25 healthy controls and 25 colorectal cancer patients, with tumour tissue collected from 6 of these patients at the time of surgery (Malicki *et al.,* 2009). Both tumoral and serum levels of IL-8 were elevated, with removal of the tumour resulting in a prompt reduction of this cytokine in blood serum 3 days after surgery (Malicki *et al.,* 2009). This study also established cell lines derived from colorectal cancer cells and confirmed their capability to produce IL-8, thereby further demonstrating that the colorectal cells may be the main source of serum IL-8 (Malicki *et al.,* 2009).

Increased levels of IL-8 in the presence of colorectal cancer were further confirmed in an investigation that examined the inflammatory microenvironment in 36 patients diagnosed with this illness (McLean *et al.,* 2011). Levels of IL-8 were much lower in normal colonic mucosa when compared with mucosa in adenoma and adenocarcinoma, thereby identifying this cytokine as a key inflammatory component in tumour progression (McLean *et al.,* 2011).

The use of IL-8 as a biomarker to identify colorectal cancer onset and progression was also established in another study that analysed tumour tissue from 20 patients that underwent surgery for this illness (Nastase *et al.,* 2011). Levels of IL-8 were higher in the presence of adenocarcinoma when compared with adenoma, thereby implying a direct correlation between levels of IL-8 and the progression of colorectal cancer (Nastase *et al.,* 2011). Interestingly however, no correlation was detected between the expression of IL-8 and tumour stage (Nastase *et al.,* 2011).

Similarly, no correlation between tumour stage and levels of IL-8 was discovered in a separate investigation that analysed the expression of this cytokine in blood plasma, tumour and paired normal tissue samples from 50 colorectal cancer patients, although patients with distant metastases demonstrated a significantly higher plasma level of this cytokine (Dimberg *et al.,* 2012). Significant differences were also observed in tumour tissue levels of IL-8 in comparison with normal tissue (median 419 pg/mg vs 77 pg/mg) and similarly, in the plasma of colorectal cancer patients versus healthy controls (median 18.7pg/ml vs 7.12 pg/ml; Dimberg *et al.,* 2012).

These results are supported by findings of an Irish study that examined the expression of IL-8 in 107 patients with colorectal cancer and correlated expression levels with clinic-pathological variables (Kheirelseid *et al.,* 2013). Expression levels of IL-8 were significantly upregulated in tumours compared to normal tissue, with levels increasing progressively from adenoma to adenocarcinoma (Kheirelseid *et al.,* 2013). Although the expression of IL-8 was greater in tumours than in normal tissue, reduced levels were significantly associated with advanced nodal stage and disease recurrence (Kheirelseid *et al.,* 2013). Also, a non-

significant trend of reduced IL-8 expression was associated with advanced disease stage, unlike the findings reported by the two previous studies (Nastase *et al.,* 2011; Dimberg *et al.,* 2012; Kheirelseid *et al.,* 2013).

This trend towards reduced IL-8 expression and correlation with disease stage was also indicated by results of another investigation that analysed blood serum from 105 colorectal cancer patients in order to identify diagnostic and prognostic indicators for disease progression (Berghella *et al.,* 2002). Statistical significance was reached in this case, with findings demonstrating that serum IL-8 levels < / = 339 pg/ml were indicative of AJCC stage III disease, with nodal involvement and levels > / = 339 pg/ml indicating stage I – II disease, with no nodal involvement (Berghella *et al.,* 2002). This confirms the findings of the study by Kheirelseid *et al.,* (2013), in that reduced levels of IL-8 are associated with advanced disease stage and nodal involvement.

The conflicting findings between the 4 studies that reported correlations between disease stage and expression levels may be due the differences in sample size. Studies that found no correlation included just 20 and 50 patients, respectively (Nastase *et al.,* 2011; Dimberg *et al.,* 2012), whereas the two investigations that demonstrated positive correlations included 105 and 107 patients, respectively (Berghella *et al.,* 2002; Kheirelseid *et al.,* 2013).

The literature discussed here provides consensus that increased expression of IL-8 promotes tumour progression in colorectal cancer, although results are conflicting regarding correlations between expression levels and tumour stage. Findings of studies that include larger sample sizes indicate that lower levels of IL-8 may be associated with more advanced disease and nodal involvement, despite levels of this cytokine increasing in the presence of

colorectal tumours. Expression levels of IL-8 are not only affected by the presence of a cancerous tumour, but also by treatment of this illness, particularly in relation to radiotherapy, thereby indicating the need for further discussion (Lambros *et al.*, 2011).

3.3.4.3 IL-8 and Radiotherapy

In order to identify cellular and molecular stress after radiation, oral keratinocytes were exposed to doses of 0, 2 and 12 Gy of radiation and expression levels of IL-8 were quantified, with results indicating that IL-8 was significantly upregulated 6 hours after exposure to 12 Gy of radiation (Lambros *et al.*, 2011). These findings are supported by those of an investigation that also assessed the effects of radiation on oral keratinocytes, by exposing these cells to 0, 1, 3 and 8 Gy of radiation, culturing them for 24 hours post irradiation and then measuring the expression of IL-8 with an ELISA (Tobita *et al.*, 2010). Findings indicated that levels of IL-8 increased in a radiation dose dependant manner, with significant differences occurring at 8 Gy when compared with the control (551.56 versus 237.46; Tobita *et al.*, 2010).

Conversely, Pasi *et al.*, (2010) reported a decrease in the release of IL-8 when compared with controls within 5 - 7 hours after irradiation. Human glioblastoma cells were exposed to doses of 0.25, 0.5 and 1 Gy of radiation, with expression levels of IL-8 measured using ELISA over a 20 hour period after exposure (Pasi *et al.*, 2010). However, following this initial decrease in expression levels, there was an increase noted with time, which was significant at the dose of 0.5 Gy and peaked 20 hours after irradiation (Pasi *et al.*, 2010). This may explain the conflicting findings reported in the two previous studies, as IL-8 levels were measured more than 5 hours after irradiation, at 6 and 24 hours, and also, cells were exposed to higher doses of radiation, respectively (1,3 and 8 Gy; 2 and 12 Gy; Tobita *et al.*, 2010; Lambros *et al.*, 2011). Therefore, IL-8 expression may be radiation induced and secreted as a defence signal

of the tumour to promote tumour progression in the surrounding non-irradiated cells (Pasi *et al.,* 2010).

Interestingly, this is supported by results of a study that examined the mechanisms underlying the enhancement of radiosensitivity in human oral carcinoma cells, where findings suggested that increased expression levels of IL-8 due to radiation exposure may induce radioresistance (Tamatani *et al.,* 2004). It was reported that the down-regulation of IL-8 resulted in a marked reduction of tumour progression and also, inhibiting radiation induced production of this cytokine augmented the effects of radiotherapy on the tumour progression (Tamatani *et al.,* 2004).

The general consensus among all of the studies reviewed in relation to the effects of radiation on the expression of IL-8 suggest that levels increase over time in a dose dependant manner, despite variances in the type of cancer cells that were examined. This may be a defence signal of the tumour cells to promote tumour progression in the surrounding non-irradiated cells (Pasi *et al.,* 2010). However, there is a major paucity in the literature in relation to the effects of radiation on colorectal cell lines and also, regarding the expression levels of IL-8 in patients treated with neoadjuvant radiotherapy for rectal cancer.

Response to radiation may also lead to symptom manifestation. In order to ascertain a molecular basis for the occurrence of symptoms during this treatment, studies that have investigated IL-8 in relation to this will be discussed.

3.3.4.4 IL-8 and Symptom Presentation

In order to investigate the relationship between functional impairments of cancer patients and circulating cytokines, 50 patients, 6% of whom had colorectal cancer, and 33 healthy controls completed QoL questionnaires and had serum cytokines analysed using multi-plex technology (Ishikawa *et al.,* 2012). No significant correlation between IL-8 and physical, emotional or cognitive function was reported (Ishikawa *et al.,* 2012). Similarly, no correlation between cognitive function and levels of IL-8 was demonstrated in another study that assessed the effect of circulating cytokines on cognition, memory and fatigue in patients with acute myelogenous leukemia and myelodysplastic syndrome (Meyers *et al.,* 2005). However, IL-8 levels were associated with better memory performance, although there was no correlation between this cytokine and fatigue (Meyers *et al.,* 2005).

Conversely, the expression of IL-8 was 42% lower in the presence of chronic fatigue syndrome when compared with controls, although this difference may be more indicative of immune activation and inflammation, rather than specific for chronic fatigue syndrome, thereby making it difficult to apply these findings to cancer patients (Fletcher *et al.,* 2009).

In relation to pain, IL-8 was significantly associated with severe pain in patients with non-small cell lung cancer, where cytokines were analysed in order to establish whether levels were indicative of self-reported pain (Reyes-Gibby *et al.,* 2007). Similar results were reported in patients with breast cancer, where changes in plasma levels of cytokines were correlated with changes in musculoskeletal symptoms before, during and on completion of chemotherapy (Pusztai *et al.,* 2004). Results indicated that IL-8 levels increased in patients that received higher doses of chemotherapy every 3 weeks, rather than lower doses at more

frequent weekly intervals, and this increase correlated with joint pain and flu like symptoms (Pusztai *et al.,* 2004).

Although the literature discussed here indicates that in the presence of cancer, there is no correlation between IL-8 and fatigue, emotional, physical and cognitive function, and a possible association between this cytokine and improved memory and pain, differences in methodologies, sample sizes and patient populations makes it difficult to accurately compare studies. Investigations focusing on the impact of IL-8 levels on symptom presentation in the presence and treatment of cancer is therefore fragmented and lacking, with no study identified that examined this in relation to rectal cancer patients receiving adjuvant radiotherapy, thereby warranting the need for further work in this area.

3.4 Conclusion

Chapter II identified that radiotherapy treatment for cancer can lead to a number of adverse effects that may be associated with a decline in QoL. The literature reviewed in this chapter demonstrates that KRAS and KRAS mediated IL-6 and IL-8 expression may be factors associated with radiosensitivity. A summary of these results can be seen in Tables 3.7, 3.8 and 3.9.

KRAS mutation promotes cell growth by activating signalling pathways and is associated with decreased radiosensitivity at a cellular level. However, results are conflicting in studies that have investigated whether KRAS status correlates with tumour response to treatment in colorectal cancer, although no study included in this review performed a subtype analysis of KRAS mutation, thereby identifying a gap in the literature that may explain the current conflicting findings. It has been identified that KRAS mediates the expression of the

cytokines IL-6 and IL-8. Both of these cytokines are associated with cell growth and proliferation at a preclinical, cellular level and radiation increases expression in a dose dependent manner. Increased levels of IL-6 have been positively correlated with increased tumour stage and may be associated with radiation resistance and poor response to treatment. Interestingly, in relation to IL-8, in some cases a significant association between lower levels of this cytokine and advanced disease has been reported, although this requires further validation. Regarding symptom presentation, neither IL-6 nor IL-8 was associated with the presence of fatigue. However, a major paucity has been identified in relation to studies investigating expression levels of these cytokines in rectal cancer patients receiving neoadjuvant radiotherapy and other symptoms associated with this treatment.

It is difficult to draw definitive conclusions from the literature reviewed as a result of differing methodologies applied, which include variances in sample sizes, type, radiation doses and none or few symptoms measured. It is clear therefore that further research is warranted on the effects of KRAS status and cytokine expression on patients receiving neoadjuvant radiotherapy for rectal cancer. The proposed study will address some of these methodological issues, ensuring that it will have a homogenous sample, identical radiation protocols and a comprehensive measurement of symptoms by using validated data collection tools. This will then address the dearth of literature that has been identified in this review in relation to KRAS, IL-6 and IL-8 as factors associated with radiosensitivity.

Table 3.8: Studies on KRAS
(*n* = 20 articles)

Study	Aim	Sample	Method	Results	Implications
Ancrile e*t al.* (2007) USA	To examine whether IL6 plays any role in Ras-mediated cancers	- Human embryonic kidney cells - Immunocomprimised mice	-Immunoblot - ELISA - RT-PCR	-IL-6 concentrations were elevated, and cells became tumorigenic upon expression of RAS G12V fusion protein, when compared to uninduced, nontumorigenic cells - Similar increase in IL-6 mRNA and protein was detected in tumorigenic fibroblasts, myoblasts, and mammary epithelial cells expressing H-Ras G12V as compared to the isogenic non-tumorigenic counterparts lacking HRasG12V. - Knockdown of IL6, genetic ablation of the IL6 gene, or treatment with a neutralizing IL6 antibody retard Ras-driven tumorigenesis - Knockdown of IL-6 in pancreatic cancer cell lines characterized by K-Ras oncogenic mutations also reduced their tumorigenic capacity when injected into immunocompromised mice	- IL6 appears to act in a paracrine fashion to promote angiogenesis and tumor growth - IL6 may have therapeutic utility for treatment of cancers characterized by oncogenic Ras mutations

Study	Aim	Sample	Method	Results	Implications
Bengala et al. 2009 Italy	To investigate the relationship between EGFR expression, EGFR gene copy number and KRAS mutation evaluated on diagnostic biopsy and pathological tumour response	39 patients	- EGFR immunohistochemistry expression, EGFR, gene copy number and KRAS mutation were evaluated on diagnostic tumour biopsy - Pt's received 50 Gy of radiation & 5-FU + / - Oxaliplatin + / - Capecitabine - Tumour response defined as: TRG0: No regression; TRG1: Minor regression – fibrosis in < 25% of tumour TRG2: Moderate regression – fibrosis in 26 – 50% of tumour; TRG3: Good regression – > 50% tumour regression TRG4: Total regression – no viable tumour cells, only fibrotic mass -KRAS codon 12 & 13	- Tumour Response: TRG1: 35%; TRG2: 30.8%; TRG3: 23.1%; TRG4: 7.7% - KRAS:77% WT vs 23%Mutant TRG 3-4: 36.7% WT vs 11% Mutant ($p>0.05$) - the coexpression of high level of EGFR genomic gain (EGFR/nuclei ratio ‡2.9) and wild-type K-ras was associated with a 58.8% of high pathologic tumour regression	The concomitant presence of wild-type K-ras and a high EGFR genomic gain seems to identify a subgroup of pts with the best chance of cetuximab (anti EGFR tx) benefit
Bernhard et al. 1998 USA	To measure radiosensitivity of human tumour cell lines with oncogenic mutations in HRAS and KRAS genes after treatment with prenyltransferase inhibitors	Human Tumour Cell Lines - T24 HRAS codon 12 (bladder) - HS578T HRAS codon 12 (breast) - SW480 KRAS codon 12 (colon) - A549 KRAS codon 12 (lung) - SKBr-3 wt RAS (breast) - HT29 wt RAS (colon) - HeLa wt RAS (cervix)	- Cells were cultured at 37°C in water saturated 5% CO_2. All media were supplemented with 10% fetal bovine serum, 100 units/mi penicillin,and 100 sg/ml streptomycin. - Clonogenic survival was determined by two methods :clonogenic assay at radiation doses from 1 to 4 Gy; and limiting dilution cloning to measure surviving fraction at 2 Gy	- Inhibition of oncogenic RAS activity in human tumour cells can reduce the radiation survival of these cells, suggesting that oncogenic RAS can contribute to radiation resistance in human tumours - tumour cells without RAS mutations were not sensitized	- This provides evidence that RAS mutations contribute to radiation resistance in human cell lines -These findings indicate that it is likely that activating RAS mutations are key contributors to the resistance of human tumours to radiotherapy
Bernhard et al. 2000 USA	To investigate the expression of activated RAS and its contribution to intrinsic radiation resistance in human tumour cells	HT1080 and DLD-1 (colon carcinoma) human tumour lines	- Cells were cultured at 37°C in a water-saturated 5% CO_2 incubator. - Cells were maintained in DMEM high glucose medium. Media were supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin - Clonogenic survival was determined at radiation doses from 1 to 8 Gy	- The activated KRAS allele cell line showed greater clonogenic survival than the wild type KRAS allele cells after irradiation - An association between the extent of apoptosis and clonogenic survival results was seen in cells derived from HT1080 but not DLD-1.	- Loss of activated KRAS allele is sufficient to significantly reduce radiation survival in cell lines - This study differs from previous studies in that the survival of the cell lines was established from cells lacking an endogenous, activated oncogene rather than from cells into which an activated oncogene was introduced.

Study	Aim	Sample	Method	Results	Implications
Clancy *et al.* (2013) Ireland	To systematically appraise the effect of KRAS mutation on outcomes following CRT for rectal cancer	Systematic review - Data was retrieved from 8 series describing 696 patients	Random-effects methods were used to combine data	- KRAS mutation was present in an average of 33.2 % of patients with rectal cancer. - KRAS mutation was not associated with decreased rates of pathological complete response (odds ratio (OR): 0.778, 95% confidence interval (CI): 0.424e1.428, P ¼ 0.418), tumour down-staging (OR: 0.846, 95% CI: 0.331e2.162, P ¼ 0.728) or an increase in cancer related mortality (OR: 1.239, 95% CI: 0.607e2.531, P ¼ 0.555)	- Based on these data, the presence of KRAS mutation does not affect tumour down-staging or cancer specific survival following neo-adjuvant CRT and surgery for rectal cancer - 4 studies (363 pts) included data re downstaging – no correlation with KRAS. Endpoints for downstaging used by Davies different (limited, major, complete). Other studies gave a more definitive definition of regression grades - All studies used different neoadjuvant treatment regimes
Davies *et al.* (2011) USA	To explore the relationships of KRAS and BRAF mutation status with p-AKT and p-ERK and outcomes in rectal cancer	- 70 patients – pre radiotherapy biopsies assessed - 67 tumour samples assessed for KRAS	- KRAS & BRAF mutations at codon 12, 13 & 61 assessed using pyrosequencing - p-AKT & p-ERK assessed using immunohistchemistry - Pt's received preoperative radiotherapy & 5FU chemotherapy (doses not stated), followed by surgery - Tumour response defined as Limited Response: gross residual disease present Major Response: only microscopically visible disease present Complete Response: no pathological evidence of residual tumour cells - KRAS codon 12 & 13	- 36% tumours harboured mutant KRAS; 64% wildtype Responses to CRT: - 64% limited - 19% major - 17% pathologic complete response - KRAS mutation did not correlate with radioresistance WT vs Mutant: Limited Response 67% vs 67% Major Response 14% vs 21% Complete Response 19% vs 13% -Patients with mutant KRAS tumours had higher p-AKT scores - Higher p-AKT scores associated with better tumour response	- Results suggest that activation of AKT may be beneficial for response to radiation therapy, and thus targeting these pathways in the setting of chemoradiotherapy could possibly be contraindicated - However, small sample size and study endpoint of complete response was 19% (WT) vs 13% (Mutant)

Study	Aim	Sample	Method	Results	Implications
Erben *et al.* (2011) Germany	To investigate the predictive and prognostic value of KRAS and BRAF mutations as well as PTEN expression in rectal cancer patients treated with Cetuximab based chemoradiotherapy	- 57 rectal cancer patients	- Pts received weekly administration of cetuximab & irinotecan and daily doses of capecitabine in conjunction with radiotherapy (50.4 Gy) - Status of KRAS & BRAF mutations was determined with direct sequencing, & PTEN expression status was determined with immunohistochemistry -FFPE tissue samples used - Tumour regression described by Japenese Society for Cancer of the Colon and Rectum Grading System. Good responders – TRG2/TRG3; Bad responders TR0/TRG1	-31.6% KRAS mutant (18pts) -10 cases at codon 12 & 6 cases at codon 13. KRAS mutations found at codons 21 & 60 - 31.5% pts had bad response (TRG0/TRG1) - 64.9% had good response (TRG2/TRG3) -7 pts with KRAS mutations had TRG0/TRG1; 11pts had TRG2/TRG3 -13 pts with KRAS wt had TRG0/TRG1; 26 pts had TRG2/TRG3	-No correlation between tumour regression/downstaging & KRAS status -No subtype analyses of KRAS status
Gaedcke *et al.* (2010) USA	To establish the mutation status of KRAS and BRAF in pre-treatment biopsies from rectal cancers	- 94 patients – pre radiotherapy biopsies assessed	- DNA was isolated from pre-treatment biopsies - Mutation status of KRAS exons 1–3 and BRAF exon 15 was established using the ABI PRISM Big Dye Sequencing Kit and subsequently correlated with tumour response, lymph node status and survival - Pt's received 50.4 Gy of radiation & Oxaliplatin & 5-FU - Tumour response defined as: TRG0: No fibrosis or regression TRG1: > 50% viable tumour cells TRG2: Regression of 50 – 70% TRG3: Regression > 70% -KRAS codon 12,13,61 & 146	- KRAS mutations found in 48% - 64% at codon 12; 22% at codon 13; 7% at codon 61 & 146; - KRAS mutations were not correlated with tumour response, lymph node status or with overall survival or disease-free survival. - When KRAS exon 1 mutations were separated based on the amino-acid exchange, there was no significant correlations - However, G12V mutations appeared to be associated with higher rates of tumor regression than G13D mutations	- Although the KRAS mutation status was not correlated with response, the subtle difference between G12V and G13D mutations warrants analysis of a larger patient population

Study	Aim	Sample	Method	Results	Implications
Garcia-Aguilar *et al.* (2011) Canada	To identify a biomarker profile associated with tumor response to chemoradiotherapy in rectal cancer	- 132 patients	- Tumour DNA from pre-treatment tumour biopsies and control DNA from paired normal surgical specimens was screened for mutations and polymorphisms in 23 genes. - Genetic biomarkers were correlated with tumour response to CRT - Pt's received 50.4 Gy radiation & 5-FU + / -additional (leucovorin + / -Oxaliplatin) - Tumour response defined as: Non Pathologic Complete Response Pathologic Complete Response: the complete absence of tumour cells from the rectal wall and regional lymph nodes -KRAS codon 12 & 13	Responses to CRT: - 25% pathologic complete response - 75% non-pathologic complete response - KRAS mutation was associated with non-pathologic complete response. KRAS mutations were more common in non-pCR patients compared to patients with a pCR (49% vs 24%)	- Sample size is relatively small - Study endpoint of pathologic complete response occurred in only 25% of patients
Hu-Lieskovan *et al.* (2001) USA	To evaluate functional germline polymorphisms of genes involved in EGFR pathway, angiogenesis, ADCC, DNA repair, and drug metabolism, for their potential role as molecular predictors for clinical outcome in rectal cancer patients treated with preoperative cetuximab based chemoradiation	- 130 patients - KRAS status available for 101 patients	- Genomic DNA was extracted from formalin-fixed paraffin-embedded tumor samples and genotyping was performed using PCR-RFLP assays. Fisher's exact test was used to examine associations between polymorphisms - Pt's received 45-50.4 Gy of radiation + / - Cetuximab + / - Capecitabine + / Oxaliplatin + / - 5-FU - Tumour response defined as: TRG0: no regression TRG1: minimal regression TRG2: moderate regression TRG3: good regression TRG4: total regression - KRAS codon 12 & 13	- KRAS WT 58% vs KRAS Mutant 42% - The mutation status (wild type vs. mutant) was not significantly correlated with pCR - KRAS mutant patients showed 15% (5/34) pCR to cetuximab based neoadjuvant CRT compared to KRAS wild type patients with 10% (5/52) pCR - EGF A+61G polymorphism has a potential to be a predictive marker for pCR, independent of KRAS mutation status, to cetuximab-based neoadjuvant chemoradiation	-Retrospective study - small sample size - All patients received cetuximab based therapy - no untreated control group

Study	Aim	Sample	Method	Results	Implications
Kim *et al.* (2005) USA	To establish whether selective inhibition of Ras, Phosphoinositide 3 Kinase, and Akt Isoforms increases the radiosensitivity of human carcinoma cell lines	- Human Carcinoma Cell Lines	RNA interference was used to selectively block expression of specific isoforms of Ras, phosphoinositide 3 (PI3) kinase, and Akt	- Inhibition of oncogenic Ras expression decreased both phospho-Akt and phospho-p42/44 mitogenactivated protein (MAP) kinase levels and reduced clonogenic survival	Ras signaling to the PI3 kinase–Akt pathway is an important contributor to survival, whether Ras activation results from mutation of ras or overexpression of epidermal growth factor receptor
Ling *et al.* (1989) USA	To evaluate level of radioresistance induced by oncogenic transformation	- Rat Embryo Cells	- Embryos from Fischer rats 13 to 15 days pregnant were minced, dispersed, trypsinized, and cultured in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum and appropriate antibiotics - For transfection of REC with a single oncogene human c-Ha-ras oncogene from the EJ bladder carcinoma & the human c-myc oncogene were used - Cells transfected with single oncogenes were irradiated with a dose of up to 6Gy - To assess the possible influence of cell cycle distribution on the radiosensitivity of the different cell lines cytofluorometric analysis was performed	- Transfection with the RAS oncogene induces alteration in radiosensitivity by demonstrating greater radioresistance than the primary rat embryo cells	The approach adopting rat embryo cells avoided the use of established cell lines, such as the NIH3T3, which have already undergone unspecified genetic changes in the immortalization process

Study	Aim	Sample	Method	Results	Implications
Rowley *et al.* (2002) USA	To investigate the activation of NRAS & KRAS induced by IL-6	- Model cell lines	GTP-bound Ras was measured and the percentage of the total Ras pool that was activated in response to IL-6 was calculated.	- IL-6 is able to transiently activate both N and K-RAS in the ANBL6 cell line. - Increasing concentrations of IL-6 are able to activate increasing levels of both N- and K-ras - One ng/ml of IL-6 is able to activate approximately 10% of the N-ras pool and 18% of the K-ras pool - The amount of Ras-GTP in the cells correlates with the level of proliferation at low levels, but proliferation plateaus when higher levels of Ras-GTP are present. concentration and Ras activation.	Higher concentrations of IL-6 are correlated with higher concentrations of KRAS
Russell *et al.* (1999) USA	To investigate the application of the AV1Y28 adenovirus as a strategy for compromising Ras protein activity and potentially enhancing the radiosensitivity of tumor cells	Four human tumour cell lines - U251 glioblastoma, - MIA PaCa-2 pancreatic carcinoma - colon carcinomas SW620 and HT29	Cultures were exposed to sufficient levels of AV1Y28 to transduce more than 90% of the cells; 24 h later, cultures were exposed to ionizing radiation, and clonogenic cell survival was determined	- Tumour cell survival was reduced by 40–50% when the tumour cell lines were exposed to AV1Y28 only. - For each tumour cell line, AV1Y28 exposure enhanced the level of radiation-induced cell killing. - In contrast to the results seen in tumour cells, the radiosensitivity of a normal human fibroblast cell line was not affected by AV1Y28	These data indicate that this anti-Ras adenovirus enhances the radiosensitivity of tumour cells but does not affect the radiosensitivity of normal cells
Russo *et al.* (2013) USA	To determine potential mutational and clinical predictors of pathological complete response in rectal cancer patients treated with chemoradiotherapy	79 rectal cancer patients	- A clinical cancer genotyping assay evaluated 140 hotspot mutation sites across 15 cancer genes in 47 patients with sufficient tissue. - Mutational profiles were compared in pre- and post-CRT specimens and with pCR rate. Clinical variables were evaluated using logistic regression.	-KRAS mutations present in 43% of cases -In the entire cohort, 21.5 % had a pCR. - pCR rate was 23.5 % (4/17) in wild-type tumors versus 3.3 % (1/30) in those with a mutation.	- Used pCR as endpoint - Patients without mutations in commonly mutated cancer genes may be associated with a higher likelihood of having a pCR after preoperative CRT

Study	Aim	Sample	Method	Results	Implications
Sklar (1988) USA	To assess the role of RAS oncogenes in inducing resistance to ionizing radiation	NIH 3T3 Cell Lines	- The effect of RAS oncogenes were evaluated by first adding them to the same NIH 3T3 subline by transfection, & then determining the radiation survival curve of the transfected cell lines by clonogenic survival curve assays -. Single cell suspensions were irradiated in isotonic phosphate-buffered saline in 15 ml corning centrifuge tubes with a Theratron 80 .' 60Co unit; a lateral field in a specially constructed Lucite block holder was used and cell lines were exposed to doses of 0-6Gy of irradation - RAS oncogenes were activated by missense mutations at codon 12 or 61.	- All cell lines transformed with RAS genes that had been activated by a missense mutation showed a large increase in intrinsic radiation resistance - There were no significant differences among ras genes in their effect on radiation resistance regardless of the type of ras gene (H, K, or N; viral or cellular) or the site of activating mutation	- Findings are limited to NIH 3T3 cells irradiated in vitro under specific conditions - If human tumours are similarly affected by activated RAS, then their presence or aberrant expression may help predict tumour response to radiation treatment
Sparmann *et al.* (2004) USA	To analyse the inflammatory mediator interleukin-8 as a transcriptional target of KRAS signalling	Mouse tumour xenograft model	- High-density oligoupregunucleotide-based microarray analyses in HeLa cell lines expressing activated H-RasG12→V transgenes under a tetracycline responsive promoter - RT-PCR - ELISA	- Signalling from the KRAS isoform led to an increase in both IL8 mRNA and protein - IL8 induction is necessary for RasV12- induced tumor growth, and ablation of CXCL-8 function in RasV12-expressing tumours leads to a substantial decrease in tumour vasculature and extensive tissue necrosis	These findings indicate a role for IL8 in RAS oncogene-dependent tumour angiogenesis.

Study	Aim	Sample	Method	Results	Implications
Sunaga *et al.* (2011) Japan	To describe the positive association between IL-8 expression, KRAS mutations and certain clinicopathological features and the therapeutic significance of IL-8 expression in KRAS mutated NSCLC	-89 tumour specimens - NCI-H1792 NSCLC cells	- RT-PCR - Microarray analysis	- IL-8 was the most downregulated gene by shRNA-mediated KRAS knockdown in NCI-H1792 NSCLC cells where IL-8 is overexpressed - NSCLC cell lines harbouring KRAS or EGFR mutations overexpressed IL-8, while IL-8 levels were more prominent in KRAS mutants compared to EGFR mutants - IL-8 expression was downregulated by shRNA-mediated KRAS knockdown in KRAS mutants	- Activating mutations of KRAS or EGFR upregulate IL-8 expression in NSCLC - IL-8 plays a role in cell growth and migration in oncogenic KRAS-driven NSCLC
Wislez *et al.* (2006) France	To investigate the role of IL8 in KRAS induced tumorigenesis	KRAS mice that develop lung adenocarcinoma through somatic activation of a KRAS allele carrying an activating mutation in codon 12	- KRAS mice were transfected with an oncogenic (G12D) version of the KRAS transgene inserted in the KRAS locus so they developed KRAS G12D-driven lung adenocarcinomas - elevated expression of macrophage inflammatory protein-2 (MIP-2) and keratinocyte chemoattractant (KC), the mouse functional homologs of IL-8, were detected in lung tissue homogenates of mice harboring the activated KRAS allele compared to wild type littermates - intraperitoneal injections of a neutralizing antibody serum to CXCR2, a receptor for the ELR+ CXC family of cytokines that includes MIP-2 and KC, were administered 3 times a wk for 3 wks -LKR-13and LKR-10 cell lines were derived by serial passaging of minced lung adenocarcinoma tissues isolated from KRAS mice	- Oncogenic KRAS induced secretion of mouse IL-8 homologs - Post injection with a neutralising antibody serum, the number of lesions in the lungs of mice decreased by 30% and more malignant lesions developed when compared to control mice injected with a control antibody	-Findings support a model whereby oncogenic KRAS mediated secretion of IL-8 acts in a paracrine fashion to promote tumorigenesis

Study	Aim	Sample	Method	Results	Implications
Zauber *et al.* (2009) UK	To investigate rectal cancers for the molecular changes of loss of heterozygosity of the APC nd DCC genes, KRAS mutations and microsatellite instability	53 rectal cancer patients	-PCR used to assess diagnostic biopsies from patients that received preoperative CRT, followed by single stranded conformation polymorphism and DNA sequencing. - Tumour regression assessed using surgically removed specimen -Specimens obtained from FFPE samples - Tumour regression score defined by Wheeler -Stage 1 no disease or minimal microscopic disease with marked fibrosis to Stage 3 abundant macroscopic disease with little or no fibrosis	- 45% had high degree of regression - None of the molecular changes were useful indicators of regression	- Pts that did not receive full course of radiation were excluded - Treatment protocol patients received not described

Table 3.9: Studies on IL-6
(*n* = 27 articles)

Study	Aim	Sample	Method	Results	Implications
Akmansu *et al.* (2005) Turkey	To establish the influence of radiotherapy on TNFα & IL6 in the serum of pts with head & neck cancer	34 head & neck cancer pts	- ELISA - Bloods taken before radiotherapy & on completion of treatment	- Pre / post tx mean IL-6 levels were 61.56 ± 14.32 and 122.45 ± 30.66 respectively.	Irradiation is likely to cause an acute phase response
Bower *et al.* (2009) USA	To determine whether radiation-induced inflammation might contribute to cancer-related fatigue	-28 Breast cancer pts - 20 Prostate cancer pts	- 6 time points: After 5 days, 10 days, 20 days, during final wk, 2 wks post tx & 2 months post tx - Fatigue Symptom Inventory - Medical Outcomes Study Sleep Scale - Center for Epidemiologic Studies–Depression scale - Quantikine High Sensitivity Immunoassay kits to measure cytokines - Mean radiation dose: Breast – 5796 (5040-6640); Prostate; 7165 (4500-7560)	- There was a significant increase in fatigue during radiation treatment - Changes in serum levels of inflammatory markers C-reactive protein and IL-1 receptor antagonist were positively associated with increases in fatigue symptoms - Serum levels of IL-1β and IL-6 were not associated with fatigue - A higher dose of radiation was associated with significantly higher levels of IL-6	Results suggest that activation of the proinflammatory cytokine network and associated increases in downstream biomarkers of proinflammatory cytokine activity are associated with fatigue during radiation therapy for breast and prostate cancer
Brozek *et al.* (2005) Austria	To determine the effect of IL6 on cellular proliferation	- Tumour tissue & adjacent mucosa - Human colon adenocarcinoma derived cell clones	- Cell culture - ELISA - RT PCR - Immunohistochemistry	- IL6 is low in normal human mucosa & rises only moderately during progression from adenomatous to low grade tumours - High levels of IL6 were noted in high grade tumours	- IL-6 may accelerate tumour progression towards malignancy

Study	Aim	Sample	Method	Results	Implications
Chen *et al.* (2012) Taiwan	To investigate the role of IL6 in biological sequelae & tumour regrowth after irradiation for hepatic malignancy which are critical for the clinical radiation response of liver tumours	Murine hepatocellular cancer cell lines	- Hepatocellular cancer cell lines used to examine radiation response by clonogenic assays & tumour growth delay in vivo - After irradiation of 6Gy in vitro &15Gy in vivo, biological changes including cell death & tumour regrowth were examined by experimental manipulation of IL6	- IL6 expression was positively linked to irradiation & radiation resistance - Irradiation induced IL6 & the subsequent recruitment of myeloid derived suppressor cells could be linked to tumour growth - Blocking of IL6 could overcome irradiation induced MDSC recruitment & tumour regrowth after treatment	IL6 levels significantly associated with radiation resistance
Chung *et al.* (2003) China	To investigate the role of IL6 in the progression of colorectal cancer	164 colorectal cancer pts - 67 rectal	-Serum concentrations of IL6 were measured before surgery using ELISA - Low levels of IL6 defined as < 12pg/ml - High levels of IL6 defined as > 12pg/ml - Dukes staging	- Median IL6, TNFα, & CRP levels were significantly higher in CRC pts vs normal controls 11.89pg/ml (range 0-440.64pg/ml) vs 3.41pg/ml (range 0-9.12) - Maximum size of tumour in high IL6 group 5.29 +- 0.27cm - Maximum size of tumour in low IL6 group 4.43 +- 0.19cm - High levels of serum IL6 were correlated with larger tumour size - Serum IL6 levels significantly correlate with cancer stage - Serum concentrations of IL6 in pts with lymph node & liver metastases were significantly higher than those in patients without metastases	- Serum IL6 levels correlated with disease status of colorectal cancer but could not be regarded as an independent predictor for prognosis

Study	Aim	Sample	Method	Results	Implications
Collado-Hidalgo *et al.* (2006) USA	To define immunologic & inflammatory variables associated with persistent post-treatment fatigue in breast cancer survivors.	- 50 breast cancer patients (32 fatigued vs. 18 non fatigued)	- SF-36 vitality scale used to differentiate fatigued / non-fatigued participants - Demographic questionnaires - Beck Depression Inventory - Leukocyte subsets & protein expression analysed with flow cytometry - Intracellular cytokine production measured using flow cytometry - Plasma cytokines measured using ELISA	- Fatigue-related differences in plasma IL-6 did not reach significance	- Plasma IL6 not correlated with fatigue
Dymicka-Piekarska *et al.* (2007) Poland	To investigate the relationship between platelet activation and inflammation in colorectal cancer patients	42 colorectal cancer pts -15 rectal 38 controls	- IL6, P selectin & CRP taken preop - ELISA - Immunoenzymetic methods - Turbidmetric immunoassay - TNM Classification based on Hutter & Sobin (1986): Stage I & II (T1-4; N0; M0) Stage III (T1-4; N1-3; M0)	- P-selectin, CRP and IL-6 levels were significantly increased when compared to the control group - Plasma levels of P-selectin, CRP and IL-6 were higher in those with metastases vs those without metastases - CRC patients had a positive correlation between IL-6 and CRP and between P-selectin and IL-6 - CRC without metastases mean 2.98 +-1.49 pg/ml (IL6) - CRC with metastases mean 4.15 +- 2.45 pg/ml(IL6) - Control group Mean 1.31 +- 0.55 pg/ml(IL6)	Enhanced platelet activation and inflammatory response in patients with colorectal cancer observed

Study	Aim	Sample	Method	Results	Implications
Esfandi *et al.* (2006) Iran	To determine levels of IL6 in serum & tumoral tissue of pts with staging of the tumour & whether serum IL6 levels in colorectal cancer pts correlates with tumoral tissue level of it	50 colorectal cancer pts - No. of rectal cancer pts not stated	- Blood samples collected preop - ELISA - TNM Staging	- Significant association with staging of tumour, tumoral tissue levels & serum IL6 levels - Mean serum level of IL6 5.49 +- 2.49pg/ml Stage 1: 2.75+-0.2pg/ml Stage 2: 4.14 +- 0.51pg/ml Stage 3: 5.86 +- 1.68pg/ml Stage 4: 9.56 +- 0.99pg/ml - Mean tumour level 402.93 +- 230.19pg/ml Stage 1: 230.88 +- 49.02 Stage 2: 282.5 +- 64.15 Stage 3: 394.16 +- 197.03 Stage 4: 785.16 +-105.19	The IL-6 amount of the serum and tumoral tissue in the patients with colorectal cancer correlate signifi-cantly with the staging of the tumour and with eachother. Serum IL-6 level may reflect the proliferate activity of the tumour in patients with colorectal carcinoma.
Galizia *et al.* (2002) Italy	To investigate the prognostic significance of IL10 and IL6 serum levels in colon cancer patients undergoing surgery	50 patients with colorectal cancer - No. of rectal cancer cases not stated	-Cytokine levels measured by ELISA - Blood samples obtained at 5 timepoints – preop, postop, 4, 8 & 16 days after surgery -Dukes Staging - TNM Classification	- Significant relationship detected between baseline IL6 serum levels and tumour status, stage & metastases - Baseline serum IL6 levels more elevated in cancer pts (range 6-13.5pg/ml; mean 9.3pg/ml) vs healthy controls (mean 4.4pg/ml)	-IL6 levels are associated with tumour status, stage & metastases

Study	Aim	Sample	Method	Results	Implications
Geinitz *et al.* (2001) Germany	To assess the level of fatigue during the course of adjuvant radiotherapy (RT) of breast cancer patients and its relation to anxiety, depression, serum cytokines, and blood count levels.	41 breast cancer patients	- Adjuvant RT post breast conserving surgery - Fatigue measured with Fatigue Assessment Questionnaire and a visual analog scale on fatigue intensity, before RT, during weeks 1-5 of RT, and 2 months after RT completion. - The anxiety and depression levels were assessed with the Hospital Anxiety and Depression Scale. - A differential blood cell count and the serum levels of the cytokines interleukin (IL)-1beta, IL-6, and tumour necrosis factor-alpha were determined in parallel to the fatigue assessments. - Analysis of cytokines performed using ELISA	- Fatigue intensity as assessed with the visual analog scale increased until treatment week 4 and remained elevated until week 5. - Two months after RT, the values had fallen to the pre-treatment levels. - Fatigue measured with the Fatigue Assessment Questionnaire did not increase significantly during treatment, but the subscores on physical ($p = 0.035$) and cognitive ($p = 0.015$) fatigue were elevated during treatment weeks 4 and 5. - Affective fatigue did not change significantly. - IL-1beta, IL-6, and tumour necrosis factor-alpha levels did not change during therapy and did not correlate with fatigue.	- There was an increase in fatigue during adjuvant RT of patients with breast cancer. - Fatigue returned to pre-treatment levels 2 months after treatment. - No evidence was found that anxiety, depression, serum levels of IL-1beta, IL-6, tumour necrosis factor-alpha, or declining haemoglobin levels were responsible for the treatment-induced fatigue.
Haubner *et al.* (2013) Germany	To investigate the effects of external radiation in a co-culture model of endothelial cells and adipose-derived stem cells	- Human dermal microvascular endothelial cells (HDMEC) - Human adipose-derived stem cells (ASC)	- Cells were cultured in a co-culture setting and irradiated with sequential doses of 2 to 12 Gy. - Cell count was determined 48 h after radiation using a semi-automated cell counting system. - Levels of IL6 were determined in the supernatant using ELISA - Irradiated HDMEC & ASC as well as non-irradiated co-cultures, HDMEC or ASC respectively were used as controls	- Cell count was significantly reduced in irradiated co-cultures of HDMEC and ASC compared to non-irradiated controls - ASC and co-cultures of HDMEC-ASC showed a dose dependent increase of IL6 in the supernatants.	- The increased expression of cytokines and adhesion molecules by HDMEC after external radiation is mitigated in the co-culture setting with ASC - These in vitro changes seem to support the clinical observation that ASC may have a stabilizing effect when injected into irradiated wounds.

Study	Aim	Sample	Method	Results	Implications
Hsu *et al.* (2006) Tawain	To investigate the role of IL-6 in colorectal carcinoma proliferation, chemotaxis and invasion	Colorectal carcinoma cell lines	- Proliferation assay - Adhesion assay - Invasion & chemotaxis assays	-Physiological (10 ng/ml) and pharmacological (50 and 100 ng/ml) concentrations of IL6 did not significantly affect cell growth as IL6 stimulates proliferation of only selected colorectal cell lines - IL6 (10 ng/ml) significantly increased attachment to basement membrane - IL-6 (50 ng/ml) significantly increased the chemotaxis, anchorage-independent growth and invasiveness of cell lines - IL-6 (100 ng/ml) resulted in negative feedback inhibition of these effects	- IL6 affects the adhesion, chemotaxis and invasion of colorectal carcinoma, suggesting that IL6 may be a useful clinical aid in the management of patients with this disease
Illi *et al.* (2012) USA	To determine if distinct classes of individuals could be identified based on their experience with pain, fatigue, sleep disturbance & depression; if these classes differed on demographic and clinical characteristics; and if variations in pro- and anti- inflammatory cytokine genes were associated with latent class membership.	168 oncology outpatients - 38% breast cancer - 49% prostate cancer, - - 7% brain cancer, and - 6% lung cancer 85 family caregivers	-Demographic questionnaire - Brief Pain Inventory - Lee Fatigue Scale - General Sleep Disturbance Scale - Center for Epidemiological Studies-Depression scale - Genomic DNA was extracted from archived buffy coats maintained by the UCSF Genomic Markers of Symptoms Tissue Bank - Radiotherapy dose not stated	No association between IL6 gene & symptom clusters	The discrepancy in study findings may be related to differences in symptom phenotypes (i.e. single symptoms versus a symptom cluster

Study	Aim	Sample	Method	Results	Implications
Kaminska *et al.* (2000) Poland	To investigate blood serum levels of TNFa, IL-1ra, IL-6, IL-8, IL-10 & CRP in colorectal cancer patients	35 colorectal cancer patients - No. of rectal cancer cases not stated 40 controls	- 3 timepoints: 1, 10 & 42 days postop - ELISA used to test for cytokine levels - Authors modified staging system	- There was an increase in IL6 levels 24 hours post surgery - IL6 returned to normal levels by the final timepoint, except in stage D disease	- IL6 levels higher in patients with more advanced stage disease
Kaminska *et al.* (2005) Poland	To exploit the potential clinical use of circulating cytokine measurements in colorectal cancer (CRC) patients.	157 colorectal cancer patients -No. of rectal cancer cases not stated 50 controls	- ELISA - TNM Staging	- Levels of IL6 significantly increased with the clinical stage of CRC - Levels of IL-6 were associated with tumour grade - IL8 associated with bowel wall invasion - Control IL6 median 0.8 pg/ml (range 0.7-2.4) - Pts IL6 median 2.8 pg/ml - Control IL8 median 10pg/ml - Pts IL8 median 18pg/ml - IL6/IL8 Tumour Stage 1 1.7/10.6pg/ml -IL6/IL8 Tumour Stage 2 3.2/18pg/ml -IL6/IL8 Tumour Stage 3 2.8/16.3pg/ml -IL6/IL8 Tumour Stage 4 4.9/43pg/ml - IL6/IL8 Tumour Grade I 2.2/13.1pg/ml -IL6/IL8 Tumour Grade II 2.7/17.2pg/ml -IL6/IL8 Tumour Grade III 9.2/42pg/ml	-Significant correlations of serum cytokine levels, with clinical stage and grade provide additional evidence for the direct or indirect involvement of some of these cytokines in the progression of CRC and also demonstrate their potential value as diagnostic and prognostic tools

Study	Aim	Sample	Method	Results	Implications
Miaskowski *et al.* (2010) USA	To investigate whether a genetic variation in IL6 is associated with mean ratings of evening fatigue, morning fatigue & sleep disturbance as well as with trajectories of these symptoms	168 oncology outpatients - 38% breast cancer - 49% prostate cancer, - - 7% brain cancer, and - 6% lung cancer 85 family caregivers	- Demographic questionnaire - General Sleep Disturbance Scale - Lee Fatigue Scale - Data collected at baseline, at the middle of RT, at the end of RT, and once a month for four months after the completion of RT - DNA was amplified by whole genome amplification from archived buffy coat specimens. The IL6 (rs4719714) was screened by TaqMan allelic discrimination assay - Radiotherapy dose not stated	- Common allele homozygotes reported higher levels of evening fatigue (P=0.003), morning fatigue (P=0.09), and sleep disturbance (P=0.003) than minor allele carriers.	Findings provide preliminary evidence of a genetic association between a functional promoter polymorphism in the IL6 gene and severity of evening fatigue, morning fatigue, and sleep disturbance in oncology patients and their FCs
Nikiteas *et al.* (2005) Greece	To investigate the role of IL6, TNFα and CRP levels in the progression of colorectal cancer	74 colorectal cancer pts - 24 cases rectal cancer	-Serum concentrations of IL6 & TNFα were measured before surgery using ELISA - CRP was measured using immunoturbinometric method - Low levels of IL6 defined as < 8pg/ml - High levels of IL6 defined as > 8pg/ml - Dukes staging	- Median IL6, TNFα, & CRP levels were significantly higher in CRC pts vs normal controls - Serum IL6 levels Pt vs Control: Median 8.11 Range 1.09-188.42pg/ml vs Median 3.52pg/ml Range 0.45 – 9.96pg/ml -Max. size of tumour in high IL6 group 4.8cm +- 1.9cm -Max. size of tumour in low IL6 group 3.8cm +-1.3cm - High levels of IL6, TNFα & CRP levels were correlated with larger tumour size - High IL6 & CRP levels were associated with reduced overall survival - No significant association between IL6 & tumour stage - Higher tumour stage in 19 pts with low levels of IL6 vs 11 pts with high levels of IL6	- Serum IL6, TNFα & CRP levels increase in CRC - Preop serum IL6 & CRP was found to be a predictor of prognosis in CRC pts

Study	Aim	Sample	Method	Results	Implications
Pasi *et al.* (2010) Italy	To investigate IL8 and IL6 bystander signalling in human glioblastoma cells exposed to gamma radiation	Human glioblastoma cells	- ELISA technique. - Immunocytochemistry was used to investigate the expression of corresponding cell membrane receptors in irradiated cells and in cells cultured with medium collected from irradiated cells - Cells exposed to doses of 0Gy, 0.25Gy, 0.5Gy, 1Gy of irradiation	- The exposure to radiation determined an increase of IL6 concentration which was dose dependent at 20 hrs, whereas IL8 release was lower than control shortly after irradiation but increased with time, in particular at the dose of 0.5Gy - the dose-dependent increase of IL6 release in the medium after irradiation may have caused the down-regulation of the receptor in the same irradiated cells. In the bystander cells, no significant difference in receptor expression was observed, compared to controls. This might suggest that IL6 seems to use the transignalling pathway instead of the classic pathway, which is less used by tumoral cells	Our data suggest that these cytokines are differently modulated by radiation and are likely to play a role in the transmission of radiation-induced response, probably orchestrating the inflammatory microenvironment of the tumour
Petit-Frere *et al.* (2000) United Kingdom	To compare the induction of apoptosis & cytokine release by irradiation in primary & in two immortalised epithelial/keratinocyte cell lines	-Two immortalised epithelial/keratinocyte cell lines - HaCaT & KB	- Cell culture - Irradiation 10Gy - ELISA used for cytokine measurement - RT PCR	- In both primary & immortalised cell lines apoptosis & release of IL6 were rapidly induced following irradiation - IL6 levels increased after exposure to 6Gys of irradiation 72hours after exposure	- Cytokines are thought to play an important role in the inflammatory reactions associated with exposure to radiation

Study	Aim	Sample	Method	Results	Implications
Rich *et al.* (2005) France	To evaluate the role of circulating cytokines in the production symptoms in cancer patients	80 patients with metastatic colorectal cancer	- Normal (group I, n = 40) or dampened (group II, n = 40) 24-hour rest//activity patterns measured by actigraphy were identified. - Actigraphy patterns were correlated with QOL indices, serum cortisol obtained at 8:00 a.m. and 4:00 p.m. and with serum levels of transforming growth factor-A, tumor necrosis factor-A and IL-6 obtained at 8:00 a.m. and analyzed in duplicate by ELISA. - Cytokine levels and survival were also correlated - QoL measured using EORTC QLQ-C30 - Hospital Anxiety and Depression Scale - Chronochemotherapy	- Group II patients had significantly higher pre treatment levels of all three cytokines, displayed significantly poorer emotional and social functioning, had higher fatigue, more appetite loss, and poorer performance status compared with group I patients. - IL6 significantly associated with nausea/vomiting and appetite loss	- Significant correlations were found between serum levels of TGF-a and IL-6, circadian patterns in wrist activity and serum cortisol and tumour related symptoms in patients with metastatic colorectal cancer. - These data support the hypothesis that some cancer patient's symptoms of fatigue, poor QOL, and treatment outcome are related to tumour or host generated cytokines and could reflect cytokine effects on the circadian timing system
Rube *et al.* (2008) Germany	To investigate the prognostic value of TNF-a, IL-1b, IL-6 and TGF-b1 plasma levels to predict radiation pneumonitis and to evaluate the impact of tumour-derived cytokine production on circulating plasma levels in patients irradiated for NSCLC	-52 NSCLC patients	- Cytokine plasma levels were investigated by ELISA before and weekly during RT, during follow-up (1/3/6/9 months after RT), and at the onset of RP. - Tumour biopsies were immunohistochemically stained for IL-6 and TGF-b1, and immunoreactivity was quantified (grade 1–4). RP was evaluated according to LENT-SOMA scale - Tumour response was assessed according to RECIST criteria by chest-CT during follow-up	- 21 out of 52 patients developed RP (grade I/II/III/IV: 11/3/6/1 patients). - Cytokine plasma levels measured before and during RT did not correlate with RP incidence - In most patients IL6 plasma levels were already elevated before RT and correlated significantly with the IL6 production in corresponding tumour biopsies. - IL6 plasma levels measured during follow-up were significantly associated with the individual tumour responses of these patients	-The clear correlations of IL6 plasma levels with the cytokine production in corresponding tumour biopsies and with the individual tumour responses suggest that the tumour is the major source of circulating cytokines in patients receiving RT for advanced NSCLC

Study	Aim	Sample	Method	Results	Implications
Schneider *et al.* (2000) Germany / Switzerland	To investigate the biological effect of IL6 and the expression of IL6 receptors on human colorectal carcinoma cell lines	Colorectal carcinoma cell lines	-Proliferation assay -Methylcellulose assay -RT PCR	- IL6 stimulated colony formation of primary & metastatic colorectal cancer cells - In the presence of IL-6 (100 ng/ml) colony formation of cell line was significantly enhanced	-IL6 may contribute to the proliferation of primary colorectal tumours and hepatic metastases
Tada *et al.* (2014) Tokyo	To characterise the predictive value of cytokines/chemokines in rectal cancer patients receiving chemoradiation therapy	35 rectal cancer patients	Blood samples were obtained pre- and post-CRT and the correlation between plasma levels of cytokines/chemokines and the response to CRT was analysed	-Higher post-CRT interleukin IL6 was associated with a poor response	Post treatment IL6 levels may indicate response to CRT
Wang *et al.* (2012) USA	To investigate the critical role of inflammation in promoting fatigue and related symptoms in patients with colorectal and oesophageal cancer	103 patients - 50 colorectal - 53 oesophageal	- M. D. Anderson Symptom Inventory - all patients contributed symptom data at baseline (week 0) and weekly during CXRT (weeks 1–6) and after CXRT (weeks 7–13). - Blood was drawn at baseline, at the patient's weekly routine clinic visits during the 5–6 weeks of CXRT, and at the 1-month clinic follow-up visit post-CXRT - Inflammatory markers analysed using ELISA - 50.4 – 51.3Gy of radiation administered	- Fatigue severity ratings increased significantly from pre-CXRT to the end of CXRT and decreased significantly from the end of CXRT to one month post-CXRT - IL6 levels in colorectal group: 17.4 (pre CXRT); 23.4 (post CXRT); 6.5 (one month post CXRT) - Fatigue was consistently the most-severe symptom over time for both colorectal and esophageal cancer patients treated with CXRT. - No significant association with fatigue alone and IL6 - Symptom severity consistently peaked around the end of treatment, as did serum IL-6 levels. - Significant association between the severity of a fatigue-centered symptom cluster & increased serum IL-6.	- This longitudinal study suggests a role for over-expressed sTNF-R1 and IL-6 in the development of fatigue and other severe sickness symptoms during CXRT in patients with colorectal or esophageal cancer.

Study	Aim	Sample	Method	Results	Implications
Willet *et al.* (2009) USA	To assess the safety and efficacy of neoadjuvant bevacizumab with standard chemoradiotherapy in locally advanced rectal cancer and explore biomarkers for response.	- 32 patients with rectal cancer	- Four cycles of therapy consisting of: bevacizumab infusion (5 or 10 mg/kg) on day 1 of each cycle; fluorouracil infusion (225 mg/m2/24 hours) during cycles 2 to 4; external-beam irradiation (50.4 Gy in 28 fractions over 5.5 weeks); and surgery 7 to 10 weeks after completion of all therapies. - Molecular, cellular, and physiologic biomarkers measured before treatment, during bevacizumab monotherapy, and during and after combination therapy using blood plasma and ELISA	- Tumours regressed from mass with mean size of 5 cm to an ulcer/scar with mean size of 2.4 cm in all 32 pts - IL-6 increased at day 32 - The pretreatment level of circulating cytokines showed no association with the degree of tumour regression after combination therapy - At day 32, lesser increases in IL6 from baseline were seen in patients with minor or no lymph node disease after combination therapy	- The potential biomarker candidates emerging from this study should be further evaluated in larger studies to validate them, with the goal of optimizing the outcome of combination of bevacizumab with FU/ radiation or other cytotoxic regimens.
Wratten *et al.* (2004) Australia	To investigate the underlying mechanisms of fatigue in patients receiving radiotherapy for breast conservation	52 breast cancer patients	- Functional Assessment of Cancer Therapy (FACT) fatigue subscale was administered before during and after radiotherapy. - Blood for analysis of a variety of circulating cytokines, coagulation factors, peripheral blood indices and biochemical factors was also collected at the same time points.	- Twenty-one patients (43%) developed significant fatigue during radiotherapy, whereas 28 (54%) developed minimal or no fatigue. - Fatigue appeared to plateau between week 4 of treatment and 2 weeks after treatment. The fatigue was beginning to settle by 6 weeks after treatment. - Fatigue was positively correlated with serum levels of IL-6 and other inflammatory markers, although this association seemed to be mediated by body mass index	- This study has shown that significant fatigue is common in patients receiving breast irradiation and is precipitated during radiotherapy in some patients but not others

Study	Aim	Sample	Method	Results	Implications
Wu *et al.* (2013) Taiwan	To investigate the role of IL6 in the radiation response of prostate cancer	Murine prostate cancer cell line (TRAMP-C1) and hormone-resistant cell sub-line (TRAMP-HR)	- Biological changes following irradiation were investigated by means of experimental manipulation of IL6 signalling - Correlations among IL-6 levels, tumour regrowth, angiogenesis and myeloid-derived suppressor cell (MDSC) recruitment were examined in an animal model - Cells exposed to doses of 0, 3, 6 & 9 Gy of irradiation	- HR prostate cancer cells had a higher expression of IL6 compared to TRAMP-C1 cells - IL6 levels positively linked to irradiation & radiation resistance - IL6 inhibition enhanced the radiation sensitivity of prostate cancer - When mice were irradiated with a sub lethal dose, inhibition of IL6 protein expression reduced tumour growth	These data demonstrate that IL6 is important in the biological responses following irradiation. Therefore, treatment with concurrent IL6 inhibition is a potential therapeutic strategy for increasing the radiation response of prostate cancer

Table 3.10: Studies on IL-8
(*n* = 17 articles)

Study	Aim	Sample	Method	Results	Implications
Berghella *et al.* (2002) Italy	To identify diagnostic and prognostic indices for disease progression in colorectal cancer	105 patients Colorectal cancer	- pTNM tumour classification using AJCC staging - ELISA for blood sample analysis (drawn 1hr pre surgery, centrifuged, frozen and then batch analysed)	-IL8 significantly correlated with disease stage - IL8 negatively associated to stage with significance noted for levels of IL8 from stage II to stage III - When IL8 > = 339pg/ml there is a 95% probability that the disease is in stage I or II where there is no infiltration of lymph nodes - When serum IL8 < = 339pg/ml 95% probability that disease is at stage III and the tumour has invaded the lymph nodes	- IL8 may be useful as a diagnostic biomarker for disease stage in colorectal cancer
Dimberg *et al.* (2012) Sweden	To analyse the protein expression of IL-8 in plasma, tumour and paired normal tissue and methylation status of the IL-8 gene to evaluate its impact on CRC	50 patients Colorectal cancer	- Tissue samples obtained during surgical resection - Tumour and normal tissue were obtained and frozen at -70 degrees - Two established human colon cancer cell lines Caco-2 and HT-29 were grown - 49 CRC patients gave blood samples before surgery - 51 blood donors with no history of CRC used as controls - Bloods centrifuged, frozen and plasma then batch analysed - Luminex bead based technology used to quantify concentrations of IL-8 in lysates of CRC tissue, paired normal tissue and plasma - Methylation specific PCR	- The CRC tissue levels of IL-8 (median,419 pg/mg) showed significant differences ($p<0.001$) in comparison with normal tissue (median, 77 pg/mg) - Evaluation of the relative expression (tumour vs. normal tissue) showed 94% (47/50) upregulation for IL-8 - IL-8 concentration in plasma from CRC patients (median, 18.7 pg/ml; range, 1.9–68.2 pg/ml) differed significantly from the healthy controls (median, 7.12 pg/ml, $P<0.001$ - No correlation with IL-8 in tissue and plasma samples and clinical (as age, gender, histological grading, location and stage) except for distant metastases	- Higher levels of IL8 in tumour tissue and plasma of CRC than in normal tissue and the plasma of healthy controls

Study	Aim	Sample	Method	Results	Implications
Fletcher *et al.* (2009) USA	To identify circulating biomarkers associated with chronic fatigue syndrome	- 50 female pts with CFS	- ELISA	- IL-8 was decreased in CFS when compared with controls (42% lower)	- The cytokine changes observed are likely to be more indicative of immune activation and inflammation, rather than specific for CFS
Ishikawa *et al.* (2012) Japan	To investigate the relation between functional impairments of cancer patients and circulating cytokines using a multiplex technique.	- 50 pts with cancer - 6% colorectal - 33 healthy volunteers	- Blood samples centrifuged & frozen at -80 degrees - EORTC QLQ-C30	- IL-8 was not significantly correlated with physical, emotional or cognitive function	- This study, in which 27 cytokines are simultaneously tested with cutting edge technology, demonstrates that plasma IL-6 and VEGF are significant independent determinants of functional impairments in patients with cancer.
Kheirelseid *et al.* (2013) Ireland	To quantitatively examine the expression of target genes in colorectal cancer and to correlate their expression levels with clinico-pathological variables	107 patients Colorectal cancer	- A detailed analysis of published CRC microarray data was performed to identify the most prominent genes - The selected genes were validated in fifty-two pairs of fresh colorectal tumour and associated normal tissue specimens by RT-PCR using TaqMan® assays - UICC tumour staging	- Levels of IL8 (P=0.000) was upregulated in tumours compared to normal tissues - There were significant associations of gene expression levels tumour size, grade, invasion and lymph node status - Levels of IL-8 increased progressively from tumour-associated normal, to polyps, to tumours - Although IL-8 increased in tumour vs normal colorectal tissues, its reduced expression was significantly associated with poor differentiation (P=0.008), advanced nodal stage (P=0.015) and disease recurrence (P=0.036) - A non-significant trend of reduced IL-8 expression was also associated with advanced Dukes' stage (P=0.425) and distal metastasis (P=0.062)	- A comprehensive list of genes were identified, with highly differential expression patterns in colorectal cancer that could serve as molecular markers to complement existing histopathological factors in diagnosis, follow up and therapeutic strategies for individualised care of patients

Study	Aim	Sample	Method	Results	Implications
Lambros *et al.* (2011) USA	To identify cell and molecular stress after radiation in a 3-D model of oral mucositis	- Normal human oral keratinocytes grown on the top of fibroblasts	- Tissues were exposed to gamma radiation at doses of 0, 2, and 12 Gy. - - Subsequent to irradiation, the tissues were incubated for 6 h at 37 degrees and 10% CO2. - Tissues were then harvested, and some were placed in 10% formalin for histopathologic studies; others were used for the extraction of total RNA	- IL-8 was significantly upregulated 6 h after 12 Gy irradiation	This model quantifies radiation damage and this is an important first step towards the development 3-D tissue as a screening tool.
Lee *et al.* (2012) USA	To evaluate the effects of tissue microenvironment-encoded IL-8 and CXCR2 on colon cancer progression and metastasis	- A mouse colon carcinoma cell line CT26 (BALB/c background) - A human colon cancer cell line HCT116	- A novel immunodeficient, skin-specific IL-8-expressing transgenic model was generated to evaluate colon cancer growth and metastasis. Syngeneic mouse colon cancer cells were grafted in CXCR2 knockout (KO) mice to study the contribution of CXCR2 in the microenvironment to cancer growth	- Elevated levels of IL-8 in the serum and tumour microenvironment profoundly enhanced the growth of human and mouse colon cancer cells with increased peri-tumoural angiogenesis, and also promoted the extravasation of the cancer cells into the lung and liver	- Increased expression of IL-8 in the tumour microenvironment enhanced colon cancer growth and metastasis - Demonstrates for the first time that the expression of IL-8/CXCR2 in the tumour microenvironment plays a critical role in colon cancer growth, progression and metastases
Malicki *et al.* (2009) Poland	To investigate the responses of IL-6 and IL-8 in colorectal cancer in vivo and in vitro cancer cells subjected to simvastatin	- In Vitro: 25 colorectal cancer patients; 25 healthy controls - In Vivo: Two cell lines derived from colon carcinoma; Caco-2 and HT-29	- Bloods collected from healthy volunteers and immediately before surgery and 8 days after surgery - Tumour tissue collected from 6 patients during surgery and frozen - ELISA & RT-PCR	- In case of IL-8, normal mucosa contains only traces of corresponding mRNA and, therefore, an increase observed in tumor tissue is statistically significant ($p<0.05$) - Serum levels of IL8 = mean values of 29.3 pg/ml - Surgical resection of CRC resulted in a strong reduction in serum IL-8 levels - Cells derived from the CRC are capable of producing IL-8 - The in vitro studies demonstrate growing colorectal tumor produces these cytokines and may contribute to increased plasma levels in CRC pts - The tumor removal resulted in the reduction in IL-8, where the decrease was prompt and clearly visible already after 3 days	- Colorectal carcinogenesis is accompanied by increased synthesis and release of proinflammatory cytokines such as IL-6 and IL-8 - The difference in the rate of reduction in IL-8 vs. IL-6 could be explained by the fact that the cells of CRC themselves are the main source of serum IL-8 so tumor removal leads to direct reduction of IL-8 in serum, whereas in case of IL-6 serum cytokine derives also from cells of the immune system, which is stimulated and remains active even after surgical operation

Study	Aim	Sample	Method	Results	Implications
McLean *et al.* (2011) United Kingdom	To investigate the inflammatory microenvironment in colorectal neoplasia	36 patients Colorectal cancer	- Inflammatory cell phenotype assessed by immunohistochemistry - Dukes staging	- IL-8 had increased expression in the adenoma and adenocarcinoma compared to normal colonic mucosa	This study has defined the stromal microenvironment of premalignant colorectal adenomas and identified key inflammatory components involved in adenoma progression to invasive malignancy
Meyers *et al.* (2005) USA	To assess the correlations between cognitive function, fatigue, quality of life, and circulating cytokine levels in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS).	- 54 pts with AML / MDS	- Pretreatment evaluation of cognitive function and symptoms. - 50% of the sample was reevaluated 1 month later, when response to protocol chemotherapy was assessed - Digit Span to measure attention span 2) Digit Symbol to measure graphomotor speed 3) Hopkins Verbal Learning Test for memory, including Total Recall, Immediate Recognition, and Delayed Recall 4) Controlled Oral Word Association for verbal Fluency 5) Trail Making Test Part A for visual-motor scanning speed 6) Trail Making Test Part B for executive function and 7) Grooved Pegboard for fine motor dexterity.	- A significant proportion of patients had impaired cognitive function prior to the institution of chemotherapy. - 65% of patients also experienced significant fatigue. - Higher IL-8 levels were associated with better memory performance	- Patients with AML/MDS are highly symptomatic and experience cognitive impairment and fatigue before the initiation of their treatment. – The current results indicated a correlation between these symptoms and levels of circulating cytokines, providing some support to the hypothesis that cancer-related symptoms are related at least in part to cytokine immunologic activation
Nastase *et al.* (2011) Romania	To identify potential biomarkers for colon cancer onset and progression	20 patients Colorectal cancer	- Tissue samples obtained during surgery - RT-PCR & immunohistochemistry	- IL8, irrespective of tumor stage, has a high mRNA level in adenocarcinoma ($p<0.05$) - IL8 has a high mRNA level in adenomas but higher in adenocarcinomas (p value <0.05) - No correlation between tumor stage and IL-8 expression - Results imply a direct correlation between IL-8 levels, onset and progression of colon cancer	- IL-8 could be used to diagnose an early stage colon cancer and to evaluate the prognostic of progression for colon tumors - Lack of correlation between IL-8 & tumour stage possibly due to sample size (Stage I 1; II 6; III 7; IV 6)

Study	Aim	Sample	Method	Results	Implications
Ning *et al.* (2011) USA	To determine the role of IL-8 overexpression in colorectal cancer cells in vitro and in vivo	- Two human colon cancer cell lines, HCT116 and Caco2 - BALB mice	- ELISA	- IL-8 mRNA was overexpressed in IL-8 transfectants vs parent cells - The IL-8 transfectants demonstrated increased cellular proliferation, cell migration and invasion based on functional assays - Inhibition of IL-8 overexpression with small interfering RNA reversed the observed increases in tumorigenic functions - IL-8-expressing cells formed significantly larger tumours than the control cells with increased microvessel density	- These findings indicate that overexpression of IL-8 promotes tumour growth, metastasis, chemoresistance and angiogenesis
Pasi *et al.* (2010) Italy	To investigate IL-8 and IL-6 bystander signalling in human glioblastoma cells exposed to gamma radiation	- Human glioblastoma cells	- The release of IL-6 and IL-8 in the culture medium of irradiated human glioblastoma cells was investigated using an ELISA technique. - Immunocytochemistry was used to investigate the expression of corresponding cell membrane receptors in irradiated cells and in cells cultured with medium collected from irradiated cells - Dose rate of radiation 0.83 Gy/min in doses of 0.25, 0.5 and 1 Gy	- IL-8 release was lower than control shortly after irradiation but increased with time, in particular at the dose of 0.5.	- Likely to play a role in the transmission of radiation-induced response, probably orchestrating the inflammatory microenvironment of the tumour - IL-8 signalling appears to be involved in radiation-induced effect and in the tumoral progression of unirradiated cells and this cytokine may be secreted in response to radiation as a defence signal of the tumour to promote tumourigenesis and induce cancer progression in the surrounding non-irradiated cells

Study	Aim	Sample	Method	Results	Implications
Pusztai *et al.* (2004) USA	To assess changes in plasma levels of interleukin (IL)-1b, IL-6, IL-8, IL-10, IL-12, and TNF-a during chemotherapy and to correlate these changes with musculoskeletal symptoms.	- 90 breast cancer pts - 15 healthy controls	- 70 patients received single agent paclitaxel either weekly or every 3 weeks and 20 received FAC (5-FU, doxorubicin, cyclophosphamide) chemotherapy - Cytokines and symptoms were measured before starting therapy, on day 3 and on the last day of one treatment cycle - Bloods centrifuged and frozen at -80 degrees until analysis - The Brief Fatigue Inventory;The Hospital Anxiety and Depression Scale; Linear Analog Score to measure the intensity of nausea, muscle aches, joint pain, and the subjective feeling of having a flu - Daily toxicity diary during the course of treatment	- At baseline, all subjects had measurable levels of IL-8, with no difference in baseline levels of cancer pts when compared with healthy controls - In the every 3-week paclitaxel group IL-8 increased - Fatigue and flu-like symptoms were worse on day 3. - In the every 3-week paclitaxel group, increase in IL-8 level correlated positively with flu-like symptoms	- Every 3-week higher dose treatments induce IL-8 in the plasma. These changes correlate with joint pain and flu-like symptoms
Reyes-Gibby *et al.* (2007) USA	To explore if polymorphisms in candidate cytokine genes could explain variability in self-reported pain in lung cancer patients of all stages	- 606 patients with non – small cell lung cancer - 446 Whites; 125 African-Americans; 35 Hispanics	- Pain, clinical, and demographic variables were assessed at presentation and before any cancer treatment - Trained M.D. Anderson Cancer Center staff interviewers collected data on demographics, smoking history, and history of cancer - Brief Pain Inventory - Bloods drawn, centrifuged & frozen at -80 degrees until analysis	- IL-8 was significantly associated with severe pain among White patients	- Results provide evidence of the influence of cytokine genes on pain in White patients with lung cancer

Study	Aim	Sample	Method	Results	Implications
Tamatani *et al.* (2004) Japan	To examine the mechanisms underlying the enhancement of radiosensitivity and chemosensitivity to - irradiation and 5-FU in human oral carcinoma cells (B88) in which NF-kB activity was constitutively suppressed	- Human oral carcinoma cells - BALB nude mice	- Flow cytometry analysis - Annexin V staining - ELISA	- Constitutive inhibition of NF-kB activity in human oral squamous carcinoma (B88) cells resulted in a marked reduction of tumorigenicity through the down-regulation of IL-8 - A novel finding of the present study was that radiotherapy and chemosensitivity to IR and 5-FU were augmented in B88mI cell clones by preventing IR- and 5-FU-induced production of IL-6 and IL-8 - Regarding inhibition of IR- and 5-FU-induced production of IL-6 and IL-8, no specific inhibitors for these cytokines were required in our experimental system; i.e., by simply blocking the NF-kB pathway, IR- and 5-FU-induced production of IL-6 and IL-8 was significantly decreased in B88 cells - IR and 5-FU markedly enhanced the production of IL-6 and IL-8 in B88 and B88neo,expression of these cytokines was inhibited in B88mI cell clones; and that srIB- significantly reduced in vivo tumor growth of B88 cells in response to IR and 5-FU	These findings suggest that constitutive suppression of NF-kB not only inhibits the tumorigenicity of oral cancer cells but also sensitizes tumour cells to radiotherapy and chemotherapy - These findings suggest that production of angiogenic factors and growth factors in response to radiotherapy and chemotherapy is a principal mechanism of inducible radioresistance and chemoresistance in human oral cancers, and establish the inhibition of NFkB as a rational approach to improve conventional radiotherapy and chemotherapy outcomes.
Tobita *et al.* (2010) USA	To assess the development of an in vitro model for radiation-induced effects on oral keratinocytes	Oral mucosa keratinocytes 6 surgically discarded samples	- Organotypic culture system as an in vitro model to study the effects of radiation on oral keratinocytes - Colony forming efficiency (CFE) assay - Dose rate of 3Gy/min and irradiated with 0, 1, 3 and 8 Gy	- The production IL-8 tended to increase in a radiation dose dependent manner - There were significant differences at 8 Gy (551.56 + / - 44.45 pg/ml) ($p < 0.01$) compared with the control (237.46 + / - 17.91 pg/ml)	The fact that the IL-8 concentration was shown to be greater than IL-1a post-irradiation may be a novel way of modulating the cascade that leads to oral mucositis by controlling the release of specific pro-inflammatory cytokines

CHAPTER IV: Methodology and Methods

4.0 Introduction

This chapter describes the research design and methods applied in this study. The aim, objectives and hypotheses are identified in Section 4.1. The theoretical framework that underpins this research is discussed and an explanation of how the concepts that were derived from the literature review were developed into a conceptual framework is offered (Section 4.2). A rationale for the choice of research design adopted is outlined (Section 4.3) and this is followed by a description of the population and sampling techniques used (Section 4.4). Also described are the data collection measures and procedures (Section 4.5), ethical considerations (Section 4.6) and statistical methods of data analysis, including reliability, validity and scientific rigour (Section 4.7).

4.1 Research Aim

A review of literature pertaining to symptoms, QoL and the association of KRAS, IL-6 and IL-8 with responses to treatment for cancer has been presented in the preceding chapters. This has identified a dearth of knowledge in relation to fatigue and other symptoms present during preoperative radiotherapy for the treatment of rectal cancer, and their impact on patients QoL during this time. Also, findings in relation to the association of KRAS, IL-6 and IL-8 with responses to treatment are fragmentary and in some cases conflicting. Consequently, it is indicated that a study to address these gaps in knowledge is necessary and this has led to the development of the aims of this research. Therefore, the study has two primary purposes which aim to:

1) investigate symptom presentation and effects on QoL

2) investigate the association of KRAS, IL-6 and IL-8 with responses to treatment in rectal cancer patients receiving preoperative radiotherapy

4.1.1 Objectives

The primary and secondary objectives of the study in relation to newly diagnosed rectal cancer patients receiving preoperative radiotherapy are to:

1. Describe the changes in fatigue and symptoms experienced by rectal cancer patients during preoperative radiotherapy.

- Determine the impact of fatigue on other symptoms.
- Determine the impact of fatigue and symptom presentation during preoperative radiotherapy on the QoL of rectal cancer patients.

2. Determine the association between KRAS status and radiosensitivity in terms of tumour response to treatment.

- Correlate KRAS status with levels of specific cytokines.

3. Determine the association between levels of specific cytokines in tumour and blood plasma samples and tumour response to treatment.

- Determine gene and protein expression levels of cytokines in pre-treatment and post treatment tumour tissue samples and pre-treatment blood plasma samples respectively.
- Compare gene expression levels of specific cytokines in pre-treatment tumour samples with the protein levels of cytokines in pre-treatment blood plasma samples.
- Compare levels of specific cytokines with symptom presentation.

4.1.2 Hypotheses

Research hypotheses predict an expected relationship between constructs and are therefore more specific than research questions (Heppner and Heppner, 2004). In terms of establishing a focused enquiry, hypotheses consist of a null hypothesis (H_0) and an alternative hypothesis (H_a), (Polit and Beck, 2006). The null hypothesis states that there is no relationship between the independent and dependent variables, whereas the alternative hypothesis predicts the expected outcome (Heppner and Heppner, 2004; Polit and Beck, 2006). In research, the presumed cause of a phenomenon is referred to as the independent variable, and the presumed effect is known as the dependent variable (Polit and Beck, 2006). The research hypotheses and variables pertaining to this study are outlined in Table 4.1.

Table 4.1: Research hypotheses and variables

Null Hypotheses	Alternative Hypotheses	Independent Variables	Dependent Variables
1. The level of fatigue and symptom presentation does not change in rectal cancer patients receiving preoperative radiotherapy – $H0_1$ ➢ Fatigue is not associated with other symptoms present in rectal cancer patients receiving preoperative radiotherapy – $H0$ ➢ Levels of fatigue and symptom presentation are not related to QoL in rectal cancer patients receiving preoperative radiotherapy – $H0$	1. The level of fatigue and symptom presentation changes significantly in rectal cancer patients receiving preoperative radiotherapy – HA_1 ➢ Fatigue is associated with other symptoms present in rectal cancer patients receiving preoperative radiotherapy – HA ➢ Levels of fatigue and symptom presentation are related to QoL in rectal cancer patients receiving preoperative radiotherapy – HA	Preoperative radiotherapy and rectal cancer patients	EORTC QLQ C-30 EORTC QLQ CR-29 FACIT – Fatigue Scale
2. KRAS status is not associated with tumour response to treatment in rectal cancer patients receiving preoperative radiotherapy – $H0_2$ ➢ KRAS status is not associated with levels of specific cytokines in rectal cancer patients receiving preoperative radiotherapy – $H0$	2. KRAS status is associated with tumour response to treatment in rectal cancer patients receiving preoperative radiotherapy – HA_2 ➢ KRAS status is associated with levels of specific cytokines in rectal cancer patients receiving preoperative radiotherapy – HA	Preoperative radiotherapy and rectal cancer patients KRAS status	Tumour response Expression levels of specific cytokines
3. Levels of specific cytokines are not associated with tumour response to treatment in rectal cancer patients receiving preoperative radiotherapy – $H0_3$ ➢ Levels of specific cytokines are not significantly altered in pre-treatment tumour samples when compared with post treatment tumour samples of rectal cancer patients – $H0$ ➢ Levels of specific cytokines are not associated with pre-treatment tumour samples when compared with pre-treatment blood plasma samples of rectal cancer patients – $H0$ ➢ Levels of specific cytokines are not associated with symptom presentation in rectal cancer patients receiving preoperative radiotherapy – $H0$	3. Levels of specific cytokines are associated with tumour response to treatment in rectal cancer patients receiving preoperative radiotherapy – HA_3 ➢ Levels of specific cytokines are significantly altered in pre-treatment tumour samples when compared with post treatment tumour samples of rectal cancer patients – HA ➢ Levels of specific cytokines are associated with pre-treatment tumour samples when compared with pre-treatment blood plasma samples of rectal cancer patients – HA ➢ Levels of specific cytokines are associated with symptom presentation in rectal cancer patients receiving preoperative radiotherapy – HA	Preoperative radiotherapy and rectal cancer patients	Expression levels of specific cytokines EORTC QLQ C-30 EORTC QLQ CR-29 FACIT – Fatigue Scale Tumour response

As outlined in Table 4.1, these hypotheses are not directional in nature. However, the anticipated outcomes would be that rectal cancer patients may experience higher levels of fatigue and worsening symptoms during preoperative radiotherapy and this may negatively influence their QoL. KRAS status may be associated with tumour response to treatment and may also correlate with gene expression levels of specific cytokines. In addition, cytokines may be correlated with tumour response to treatment, and may be altered in pre-treatment tumour tissue samples when compared with post treatment tumour tissue samples. Pre-treatment tumour tissue levels of cytokines may correlate with pre-treatment blood plasma levels, and finally, expression levels of cytokines may correlate with symptom presentation.

4.2 Conceptual Development

Analysis of the literature pertaining to patients receiving preoperative radiotherapy for the treatment of rectal cancer revealed that symptoms differ at various times during radiotherapy. This may be influenced by KRAS status and KRAS mediated expression levels of IL-6 and IL-8, which may also correlate with tumour response to treatment. These concepts have guided the current research and provide a conceptual framework in order to help define the parameters of the overall study.

4.2.1 Development of a Conceptual Framework

A conceptual framework uses deductive reasoning to make predictions about how phenomena would behave if the theory were true, with research being implemented to test these specific predictions and the results then used to reject, alter or lend credibility to the theory (Polit and Beck, 2006). Therefore, this enables the cohesion of certain emerging theories that can consequently serve as a springboard for generating research hypotheses.

The conceptual framework that has been developed for this study represents a visual map to merge the proposed concepts together and can be seen in Figure 4.1.

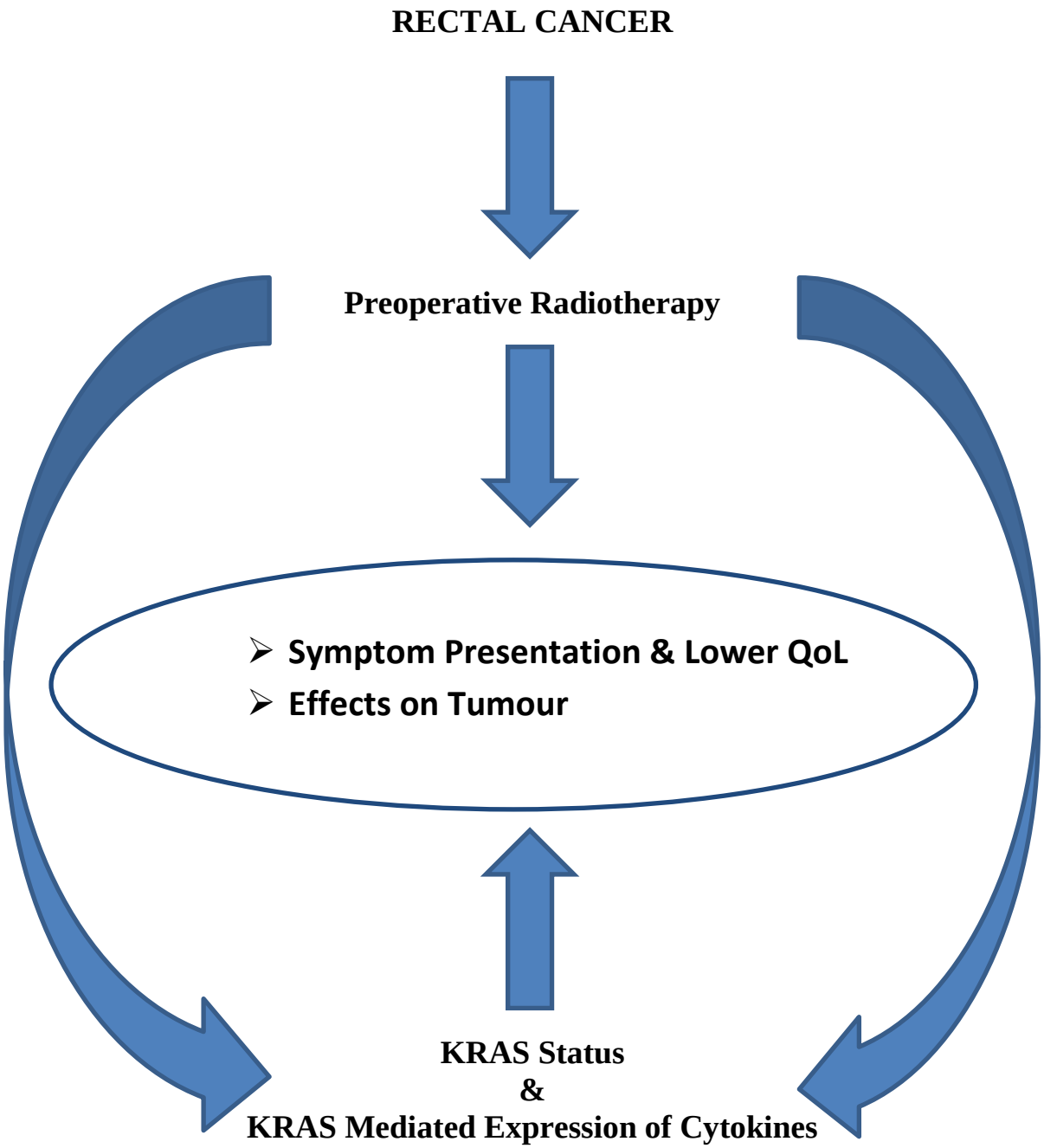

Figure 4.1: Conceptual Framework

As illustrated in the conceptual map (Figure 4.1), the presence of rectal cancer leads to treatment with preoperative radiotherapy, the response to which is influenced by a person's inherent radiosensitivity, which may be determined by KRAS status as this gene is mutated in 30% – 50% of rectal cancer patients. Also, KRAS mediates the expression of certain cytokines which may also indicate radiosensitvity. This inherent radiosensitivity determines the way in which a person will respond to treatment, including the severity of side effects they may experience, which could negatively influence their QoL during this time, as well as the tumour response. The following definitions clarify the concepts outlined in this framework more clearly. The conceptual definitions refer to the abstract or theoretical meaning of the concepts being studied and the operational definitions refer the operations that are performed to collect the required information (Polit and Beck, 2006).

4.2.2 Conceptual Definitions

- Rectal Cancer: A neoplasm characterised by the uncontrolled growth of anaplastic cells that is present in the retroperitoneal area of the bowel which may be divided into the lower rectum, the midrectum or the upper rectum, with the upper limit occurring 12 – 15 cms from the anal verge (Anderson *et al.,* 1998; DeVita *et al.,* 2008).
- Radiotherapy: The use of ionising radiation to damage deoxyribonucleic acid (DNA) and cause cell death in order to preserve normal tissue function, whilst damaging the tumour (Corner and Bailey 2008).
- KRAS: A type of oncogene, that when mutated, plays a key role in neoplastic progression (Mosby, 2009).
- Cytokines: A diverse group of soluble proteins produced by cells to act as chemical mediators of cell to cell communication, that possess the unifying feature of regulating the immune system against pathogens and / or the inflammatory response (Fitzgerald *et al.* 2001).

4.2.3 Operational Definitions

- Fatigue: A distressing, continuous, subjective sense of physical, emotional and / or cognitive tiredness related to cancer or cancer treatment that is not relative to recent activity and disrupts usual functioning (National Comprehensive Cancer Network, 2011). For the purpose of this study fatigue will be measured using the fatigue subscale of the EORTC Quality of Life Questionnaire-Core 30 (QLQ-C30) and the Functional Assessment of Chronic Illness Therapy - Fatigue (FACIT – F) 13 item subscale.
- Symptoms: Any subjective change in function, condition or sensation as perceived by the patient (Anderson *et al.,* 1998). For the purpose of this study symptoms will be measured using the EORTC QLQ-C30 and the EORTC Quality of Life Questionnaire-Colorectal 29 (QLQ-CR29).
- Quality of Life: A measurement of the physical, psychological and social effects of illness or treatment (Ferrans *et al.,* 2005). For the purpose of this study quality of life will be measured using the EORTC QLQ-C30 and the EORTC QLQ-CR29.
- Radiosensitivity: The relative susceptibility of cells, tissues, organs, organisms, or any other substances to the effects of radiation (Mosby, 2009). For the purpose of this study, radiosensitivity will be measured by determining KRAS status and expression levels of certain cytokines in tumour tissue and blood plasma, and then this will be correlated with symptom presentation and tumour response to treatment.
- Tumour Response: The response of the tumour to treatment. For the purpose of this study, tumour response will be measured in accordance with the criteria of the AJCC (Edge *et al.,* 2010) and will be defined as as complete (no viable cancer cells), moderate (single cells or small groups of cancer cells), minimal (minimal or no tumour kill) or poor (extensive residual cancer).

The researcher is aware that there are other concepts extraneous to the hypotheses underpinning this study that may also influence side effects and symptoms rectal cancer patients receiving preoperative radiotherapy may experience, particularly other treatments such as chemotherapy, the illness itself, as well as personal characteristics and coping mechanisms. However, the focus of this study is on the identification of factors that may be associated with radiosensitivity and this correlation with symptom presentation as well as effects on QoL.

4.3 Research Design

This research is a two armed study and will be undertaken using a quantitative and exploratory design which includes elements of correlation and will examine 1) symptom presentation in rectal cancer patients receiving preoperative radiotherapy and the effect of this on their QoL and 2) the relationship between KRAS, certain cytokines, tumour response and symptom presentation. A quantitative design is the most appropriate methodology to adopt when carrying out this research as a systematic format will be applied using formal instruments to collect the required information (Polit and Beck, 2006). This objective data can then be statistically analysed and interpreted so that conclusions can be made on a factual basis (Parahoo, 1997).

The focus of this study is theory verification. Literature that has been examined has indicated that symptoms increase during preoperative radiotherapy for rectal cancer which may negatively influence QoL, and that there may be a link between KRAS status, certain cytokines, tumour response and symptom presentation in this patient cohort. The above hypothesis was deduced from this theory, thereby signifying the need for its verification (Polit and Beck, 2006). Variables must not be manipulated, as the objectives of this study are

to establish 1) the prevalence and effect of symptoms on the quality of life of rectal cancer patients while receiving preoperative radiotherapy and 2) whether KRAS status and certain cytokines can act as potential indicators of tumour response and symptom presentation. Therefore, the hypothesis will be tested using a nonexperimental research design.

Elements of the study are correlational in nature due to the necessary use of correlational statistics, although it must be noted that differences will also be detected using other non-correlational tests. This ensures that it can be determined whether a change in the independent variables (radiotherapy, KRAS status) leads to a related change occurring in the dependent variables (symptom presentation, cytokine protein and gene expression levels and tumour response), thereby enabling one to quantify the strength of the relationship between the variables (Polit and Beck, 2006). A descriptive correlational design will also be incorporated within this study as the relationship among variables (symptom presentation and QoL) will be established (Polit and Beck, 2006). A disadvantage of performing a correlational study is the fact that cause-and-effect conclusions are not warranted due to the lack of manipulation and control of the independent variable (Polit and Beck, 2006). Despite this, however, a correlational study is the most appropriate selection in this instance, as it is an efficient and effective method of collecting the required data to test the hypothesis that has been deduced from the literature review (Parahoo, 1997). Also, it provides a framework for exploring relationships between variables that cannot be manipulated (Parahoo, 1997).

4.4 Population and Sample

A population is defined as the total number of units from which data can be potentially collected and may include individuals, organisations, events or artefacts (Polit and Beck,

2006). The target population of this study comprised of patients greater than or equal to 18 years who were diagnosed with locally advanced rectal cancer.

Sampling involves the process of choosing representative units of a population that are included in a study and includes selecting a subset of the target population or sample frame (Polit and Beck, 2006). The sample that was selected for this study included patients with newly diagnosed locally advanced rectal cancer attending a regional cancer centre who were followed prospectively and selected consecutively.

Therefore nonprobability, convenience sampling was used to select study participants (Polit and Beck, 2006). In probability sampling, every unit of the target population has a more than zero chance of being selected for study participation (Polit and Beck, 2006). Nonprobability sampling is adopted when probability sampling is not feasible, as was the case in this study, and when the purpose of the investigation is to learn about individuals in the population (Saks and Allsop, 2007). This method of sampling is acceptable when the findings of the study are intended to add to the body of current knowledge, rather than for generalisation (Burns and Grove, 2001). Therefore, further justification for the use of nonprobability sampling is the exploratory nature of the study as it is hoped to contribute to knowledge pertaining to symptoms, QoL and factors associated with radiosensitivity in patients receiving treatment for rectal cancer. The inability of the researcher to statistically generalise findings due to the sampling method selected is recognised as a limitation, although as the study is exploratory, it was deemed to be the most appropriate choice.

This sample selection method was greatly strengthened through the use of a sampling frame, which attempts to ensure that a homogenous sample is obtained. A sampling frame is an

objective list of the population from which the study participants are selected (Polit and Beck, 2006). Homogeneity was maintained in this study through the definition of strict eligibility criteria which clearly identified all possible participants. Steps were put in place during the recruitment stage in order to ensure all potential participants were approached about the study.

Convenience sampling refers to using the most conveniently available people as participants (Polit and Beck, 2006). Convenience sampling is the most widely used form of sampling in quantitative studies, although it is the weakest, due to the risk of introducing bias (Polit and Beck, 2006). However, it was deemed to be the most appropriate method of sampling in this study due to restricted resources which included time and finance.

Justification for the use of a nonprobability method, using a convenience homogenous sampling technique with a sampling frame in this study has been provided. Based on this, the final sample consisted of 35 patients that were newly diagnosed with rectal cancer. Those that were eligible for entry into the study were selected based on criteria as outlined below.

Inclusion Criteria

- Pathologically proven diagnosis of adenocarcinoma of the rectum (located up to 14 cm from the anal verge on flexible endoscopy)
- Diagnosis of rectal adenocarcinoma must be obtained by biopsy technique that does not completely excise the lesion (e.g., fine needle aspiration, core needle biopsy)
- Clinically determined to be stage T3 or T4,N0-N2, and M0, based upon the following minimum diagnostic workup:

  - Colonoscopy within 56 days prior to registration

-History/physical examination (including medication history to screen for contraindications)

- Contrast-enhanced imaging of the abdomen and pelvis

- Zubrod Performance Status 0-2
- Age ≥ 18
- Complete Blood Count obtained within 14 days prior to registration on study, with adequate bone marrow function defined as follows:

  -Absolute neutrophil count ≥ 1,800 cells/mm3

  -Platelets ≥ 100,000 cells/mm3

  -Haemoglobin ≥ 8.0 g/dl (Note: The use of transfusion or other intervention to achieve Hb ≥ 8.0 g/dl is acceptable)
- Metabolic panel within 28 days prior to registration on study, with adequate liver and renal function defined as follows:

  -alkaline phosphatase < 2.5 x upper limit of normal

  -Bilirubin ≤ 1.5

  -Calculated creatinine clearance > 50 ml/min
- Patient must provide study-specific informed consent prior to study entry

Exclusion Criteria

- Prior invasive malignancy (except non-melanomatous skin cancer) unless disease free for a minimum of 3 years
- Prior systemic chemotherapy for colorectal cancer; note that prior chemotherapy for a different cancer is allowable

- Prior radiotherapy to the region of the study cancer that would result in overlap of radiation therapy fields
- Severe, active comorbidity, defined as follows:

  -Unstable angina and/or congestive heart failure requiring hospitalization within the last 12 months

  -Transmural myocardial infarction within the last 6 months

  -Acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration

  -Chronic obstructive pulmonary disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy within 30 days prior to registration.

  -Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects

  -Acquired immune deficiency syndrome (AIDS); The need to exclude patients with AIDS from this protocol is necessary because the treatments involved in this protocol may be significantly immunosuppressive.

  -Evidence of uncontrolled seizures, central nervous system disorders, or psychiatric disability judged by the investigator to be clinically significant, precluding informed consent, or interfering with compliance of oral drug intake.

  -Known, existing uncontrolled coagulopathy. Patients on therapeutic anticoagulation may be enrolled provided that they have been clinically stable on anti-coagulation for at least 2 weeks.

  -Evidence of grade 2 or greater peripheral neuropathy

  -Major surgery within 28 days of study enrollment (other than diverting colostomy)
- Pregnancy or women of childbearing potential and men who are sexually active and not willing/able to use medically acceptable forms of contraception (this exclusion is

necessary because the treatment involved in this study may be significantly teratogenic)

- Prior allergic reaction to capecitabine (this is the drug administered based on the standard treatment protocol)
- Any evidence of distant metastases (M1)
- A synchronous primary colon carcinoma (the aim of the study is to investigate only patients with rectal cancer)
- Extension of malignant disease into the anal canal
- Lack of physical integrity of the gastrointestinal tract (i.e., severe Crohn's disease that results in malabsorption; significant bowel resection that would make one concerned about the absorption of capecitabine) or malabsorption syndrome that would preclude feasibility of oral chemotherapy (capecitabine)

4.4.1 Access to Sample

Prior to commencing this study, ethical approval was sought and granted from Waterford Institute of Technology (WIT) and the Health Service Executive (HSE) (see Appendices I and II), which allowed access to all newly diagnosed rectal cancer patients that were referred for treatment in the South East.

4.4.2 Setting

All newly diagnosed rectal cancer patients in the South East receive a standard protocol of treatment at the University of Pittsburgh Medical Centre (UPMC) Whitfield, in Waterford. This treatment involves the administration of a total dose of 50.4Gy of preoperative

radiotherapy, divided into 28 fractions and delivered over 6 weeks, as well as concomitant oral chemotherapy which includes 825mg/m2 of capecitabine twice daily. Patients were met when they attended fth clinic for routing appointments, informed of the study and invited to participate.

4.4.3 Recruitment procedure

The eligibility of every patient that attended UPMC Whitfield Cancer Centre for treatment was assessed at Multidisciplinary Team Meetings and those that met the criteria were approached and given a verbal and written description of the study (see Appendix III). When informed consent was obtained, patients were met at the clinic when attending for routine appointments and brought to a private room where a blood sample was obtained and the questionnaire was completed.

4.4.4 Sampling and Planning Issues

As the study progressed, it was decided to seek consent to access tumour tissue samples that were stored in Waterford Regional Hospital (WRH) as part of normal treatment in order to establish KRAS status and perform gene expression analysis. This involved seeking approval from the ethics committee to gain consent for this access from previously recruited patients (see Appendix IV). Follow up letters were posted to study participants explaining the extension to the existing study and if in agreement, they were asked to complete the enclosed consent form and return it by post in an enclosed stamped addressed envelope (see Appendix V). Tumour tissue samples were then accessed in WRH.

4.5 Data Collection

When commencing data collection in research the first consideration is to identify the best methods to meet the objectives of the study and answer the research questions and hypotheses (Polit and Beck, 2006). The task of selecting methods for gathering data is among the most challenging in the research process, as the validity of the study conclusions can be easily challenged with the use of inappropriate data collection methods (Polit and Beck, 2006).

The quantitative, correlational design of this study was guided by collecting data prospectively. The quantitative design of the research is advantageous as it was possible to adopt a systematic fashion from defining the problem, selecting the concepts upon which to focus and determining the best measures to adopt in order to resolve the problem or hypotheses (Polit and Beck, 2006). Measures selected for use in this study have been determined to have the properties necessary to address the study objectives and hypotheses, as well as possessing proven psychometric properties. Methods of data collection included the use of questionnaires, as well as obtaining and analysing blood plasma and tumour tissue samples.

4.5.1 Personal Data and Medical History Questionnaire

All participants were administered a detailed questionnaire to obtain relevant personal and disease related information (see Appendix VI). This questionnaire was modelled on Armes (2004) personal data form, and following permission, information was obtained on age, weight, height, BMI, marital status, menopausal status and employment status.

BMI was calculated using the following metric BMI formula: BMI (kg/m^2) = weight in kilograms / height in $meters^2$. The metric BMI formula accepts weight measurements in

kilograms and height measurements in either centimetres or meters. This information was obtained from patients nursing notes.

As different nations and organisations use different BMI ranges to classify weight status, the weight status categories range recognised by the Department of Health and Human Services (DOH, 2005) is used (see Table 4.2).

Table 4.2: BMI weight status categories

BMI	Weight Status
Below 18.5	Underweight
18.5 – 24.9	Normal
25 – 25.9	Overweight
30 and above	Obese

4.5.2 Symptoms and QoL Questionnaires

Previous studies investigating symptoms and QoL, as discussed in Chapter II, used validated instruments to collect data, with obvious variances noted between the studies (see Table 4.3). This informed the selection of instruments chosen for application in the current study.

Table 4.3: Tools used to measure symptoms & QoL in previous studies

Study	Tools to Measure Side Effects	Timing of Measurement
Stone et *al.* (2000)	FACT-F Questionnaire	1 time point – stage of tx not stated
Cella et *al.* (2001)	ICD-10 Criteria	1 time point - post chemotherapy / chemoradiotherapy treatment
Wang *et al.* (2001)	BFI CTCAE Criteria	5 time points – weekly during treatment
Bonnel *et al.* (2002)	Standardised Questionnaires that incorporated the I-PSS	1 time point - >/= 6 months post treatment
Hwang et *al.* (2002)	FACT-F Questionnaire FACT-G Questionnaire BFI MSAS-SF Depression Scale	1 time point – 74 inpatients and 106 outpatients with diagnosis of cancer
Marijnen *et al.* (2002)	RTOG Neurologic Complaints Scoring System	During radiotherapy – exact timing not stated
Guren *et al.* (2003)	EORTC QLQ Symptom Diary	3 time points – beginning, end and 4-6 weeks after completion of treatment
Sauer *et al.* (2004)	German Classification System	5 time points – symptoms measured weekly during treatment
Ahlberg *et al.* (2005)	EORTC QLQ MFI-20	3 time points - beginning, midway and on completion of treatment
Marijnen *et al.* (2005)	Rotterdam Symptom Checklist	6 time points - before treatment, 3, 6, 12, 18 and 24 months after surgery
Heriot *et al.* (2005)	Sexual Functioning Questionnaire	7 time points - preop, 4, 8, 12, 24, 36 and 48 months after surgery
Bosset *et al.* (2006)	WHO Criteria for Toxic Effects	5 time points - preop tx weekly for acute toxic effects during - plus post op every 3 wks (length of assessment not stated)
Vironen *et al.* (2006)	RAND 36 Questionnaire Urinary, Sexual and Bowel Dysfunction Questionnaire	1 time point - 1 year post op
Radu *et al.* (2008)	Retrospective analysis of medical records	Not stated
Carlomagno *et al.* (2009)	CTCAE Criteria	5 time points - weekly
Fiorica *et al.*(2009)	WHO Criteria	5 time points - weekly
Lange *et al.* (2009)	Sexual Dysfunction Questionnaires	Preop and 3, 6, 12, 18 and 24 months post op
Janaki *et al.* (2010)	EORTC QLQ BFI	8 time points - At baseline, weekly x 6 wks and 1 month post treatment
Musio *et al.* (2010)	CTCAE Criteria	Not stated
Stephens *et al.* (2010)	EORTC QLQ MOS SF-36	11 time points - at baseline, every 3 months for 1 yr and every 6 months for 3 years
Ishihara *et al.* (2011)	CTCAE Criteria	5 – 6 time points - weekly
Li *et al.* (2011)	CTCAE Criteria	5 – 6 time points - weekly
Pucciarelli *et al.* (2011)	EORTC QLQ Faecal Incontinence Score Questionnaire	4 time points - At baseline, 2-3 wks post tx, 6 months and 12 months after surgery
Swellengrebel *et al.* (2011)	CTCAE Criteria RTOG Criteria	5 time points - weekly

In this study, three assessment tools were used to measure symptoms and QoL and these included the EORTC QLQ-C30, the EORTC QLQ-CR29 and the FACIT-F questionnaires (see Appendix VII). Validity and reliability of these tools has been established and permission has been sought for their use in this study. Cronbach's Alpha coefficient was used to determine internal consistency, where values above 0.7 were considered acceptable (Pallant, 2007). All questionnaires were combined into a user friendly booklet. They were administered by the researcher at the following time points (TP).

- TP 1: At baseline, prior to commencement of treatment
- TP 2: Midway through treatment (at approximately three weeks)
- TP 3: Upon completion of treatment
- TP 4: Within 14 days prior to surgery, or 4-6 weeks after completion of treatment

Questionnaires are advantageous as they allow respondents to answer in their own time and ensure participant anonymity (Parahoo, 1997). However, they do not provide an opportunity to ask respondents to elaborate or illustrate their answers (Parahoo, 1997). According to Polit and Beck (2006) postal questionnaires tend to elicit a low response rate. Therefore, questionnaires used in this study were administered by the researcher when the patient attended clinic appointments. On occasion, at the final time point, it was necessary to contact the patient at home to complete the questionnaire as they were not attending the clinic at the time the questionnaire was due for completion. Permission to make contact in this way had been previously sought from participants at the time of obtaining consent.

4.5.2.1 EORTC QLQ C-30

Symptoms and QoL were assessed using the EORTC QLQ-C30. This is a cancer-specific, self-administered, structured questionnaire designed for assessing symptoms and QoL (Aaronson *et al.,* 1993; Aaronson *et al.,* 1999). It contains 30 questions, 24 of which form nine multi-item scales that represent various dimensions of health related QoL. These include a two-item global scale, five functional scales (physical, role, emotional, cognitive and social), and three symptom scales (fatigue, pain and nausea). The remaining six items include one item each and describe relevant cancer specific symptoms (dyspnoea, insomnia, appetite, constipation, diarrhoea, financial difficulties). Participants are asked to rate each item on a four point scale.

This questionnaire has been reported to have good psychometric properties with known validity across different cancer populations (Aaronson, 1993; Stone *et al.,* 1998; Knobel e*t al.,* 2003; Cankurtaran *et al.,* 2007). One particular Turkish study reported a Cronbachs α-coefficient for multi-item scales of 0.56 – 0.85, with all scales, except cognitive functioning scoring greater than the acceptable level of 0.7 (Cankurtatan e*t al.* 2007; Pallant 2007). An explanation for the low score in this instance may be that the cognitive scale contains only 2 items.

4.5.2.2 EORTC QLQ-CR29

The EORTC QLQ-CR29 tool was also selected as an instrument for data collection in this study as it is designed to be used specifically for colorectal cancer patients with varying disease stages and treatment modalities and should always be used in conjunction with the EORTC QLQ-C30 (Whistance e*t al.,* 2009). This questionnaire consists of a total of 29 items, which includes four functioning scales (anxiety, body image, sexual interest and

weight), three symptom scales (stool frequency, urinary frequency and blood and mucous in stool) and 14 single-item scales (abdominal pain, bloating, buttock pain, dry mouth, dysuria, dyspareunia, embarrassment, faecal incontinence, flatulence, hair loss, impotence, sore skin, taste and urinary incontinence (Gujral e*t al.*, 2007). The layout is similar to the EORTC QLQ-C30, with participants asked to rate each item on a four point scale.

A large international study that included a sample of 351 patients across 7 different countries investigated the validity and reliability of this questionnaire and reported it to have good psychometric properties with a Cronbach's α-coefficient ranging between 0.69 – 0.84 (Whistance e*t al.*, 2009).

4.5.2.3 EORTC QLQ Scoring Guidelines

The items on both the EORTC QLQ-C30 and the EORTC QLQ-CR29 were scored using the EORTC scoring guidelines, with raw scores transformed to a linear scale ranging from 0-100 (see Tables 4.4 and 4.5). A higher score demonstrates a higher level of functioning or higher levels of symptoms.

In relation to clinical significance, a change of 5-10 points between time points is considered to be a small clinically meaningful change, a change of 10-20 points is considered to be a moderate clinical meaningful change and a change of greater than 20 points is classified as a large clinical meaningful change (Osoba *et al.* 1998; Guren *et al.*, 2003).

According to the scoring procedures, it is also possible to use scores in order to determine the proportion of patients that are experiencing more severe symptoms (Fayers, 2001). As these questionnaires are based on a 4 item scale, that is scored from 0-100, a score of > 50

identifies patients that experience a particular symptom ‘quite a bit’ or ‘very much’, whereas a score of < 50 identifies patients that report a particular symptom as being ‘not at all’ present or there ‘a little’ (Fayers, 2001; Guren *et al.*, 2003).

Table 4.4: Linear transformation formula for EORTC QLQ-C30

Subscale QLQ-C30	Linear Transformation Formula
Global QoL	Raw Score: Q29 + Q30 / 2 Score: (Raw Score – 1/6) x 100
Physical Functioning	Raw Score: Q1 + Q2 + Q3 + Q4 + Q5 / 5 Score: (Raw Score – 1/3) x 100
Role Functioning	Raw Score: Q6 + Q7 / 2 Score: (Raw Score – 1/3) x 100
Emotional Functioning	Raw Score: Q21 + Q22 + Q23 + Q24 / 4 Score: (Raw Score – 1/3) x 100
Cognitive Functioning	Raw Score: Q20 + Q25 / 2 Score: (Raw Score – 1/3) x 100
Social Functioning	Raw Score: Q26 + Q27 / 2 Score: (Raw Score – 1/3) x 100
Fatigue Symptoms	Raw Score: Q10 + Q12 + Q18 / 3 Score: (Raw Score – 1/3) x 100
Nausea Symptoms	Raw Score: Q14 + Q15 / 2 Score: (Raw Score – 1/3) x 100
Pain Symptoms	Raw Score: Q9 + Q19 / 2 Score: (Raw Score – 1/3) x 100
Dyspnoea Symptoms	Raw Score: Q8 / 1 Score: (Raw Score – 1/3) x 100
Insomnia Symptoms	Raw Score: Q11 / 1 Score: (Raw Score – 1/3) x 100
Appetite Loss	Raw Score: Q13 / 1 Score: (Raw Score – 1/3) x 100
Constipation	Raw Score: Q16 / 1 Score: (Raw Score – 1/3) x 100
Diarrhoea	Raw Score: Q17 / 1 Score: (Raw Score – 1/3) x 100
Financial Difficulties	Raw Score: Q28 / 1 Score: (Raw Score – 1/3) x 100

Table 4.5: Linear transformation formula for EORTC QLQ-CR29

Subscale QLQ-CR29	Linear Transformation Formula
Anxiety	Raw Score: Q13 / 1 Score: (Raw Score – 1/3) x 100
Body Image	Raw Score: Q15 + Q16 + Q 17 / 3 Score: (Raw Score – 1/3) x 100
Sexual Interest	Raw Score: Q26 or Q28 / 1 Score: (Raw Score – 1/3) x 100
Weight	Raw Score: Q14 / 1 Score: (Raw Score – 1/3) x 100
Abdominal Pain	Raw Score: Q5 / 1 Score: (Raw Score – 1/3) x 100
Bloating	Raw Score: Q7 / 1 Score: (Raw Score – 1/3) x 100
Blood and Mucous in Stool	Raw Score: Q8 + Q9 / 2 Score: (Raw Score – 1/3) x 100
Buttock Pain	Raw Score: Q6 / 1 Score: (Raw Score – 1/3) x 100
Dry Mouth	Raw Score: Q10 / 1 Score: (Raw Score – 1/3) x 100
Dyspareunia	Raw Score: Q29 / 1 Score: (Raw Score – 1/3) x 100
Dysuria	Raw Score: Q4 / 1 Score: (Raw Score – 1/3) x 100
Embarrassment	Raw Score: Q24 / 1 Score: (Raw Score – 1/3) x 100
Faecal Incontinence	Raw Score: Q20 / 1 Score: (Raw Score – 1/3) x 100
Flatulence	Raw Score: Q19 / 1 Score: (Raw Score – 1/3) x 100
Hair Loss	Raw Score: Q11 / 1 Score: (Raw Score – 1/3) x 100
Impotence	Raw Score: Q27 / 1 Score: (Raw Score – 1/3) x 100
Sore Skin	Raw Score: Q21 / 1 Score: (Raw Score – 1/3) x 100
Stool Frequency	Raw Score: Q22 + Q23 / 2 Score: (Raw Score – 1/3) x 100
Taste	Raw Score: Q12 / 1 Score: (Raw Score – 1/3) x 100
Urinary Frequency	Raw Score: Q1 + Q2 / 2 Score: (Raw Score – 1/3) x 100
Urinary Incontinence	Raw Score: Q3 / 1 Score: (Raw Score – 1/3) x 100

4.5.2.4 FACIT-F 13 Item Subscale

As this study aimed to specifically investigate fatigue and its impact on the QoL of rectal cancer patients, the FACIT-F 13 item subscale was also administered to patients. This scale assesses self-reported fatigue and its impact upon daily activities and function. It is specifically formatted for ease of self-administration, as it takes 2-3 minutes for an average patient to complete (FACIT-F, 2007). The psychometric properties of this scale are well documented as it has been used on over 20,000 people, with published data presented in Table 4.6 demonstrating its validity and reliability (FACIT-F, 2007).

Table 4.6: Internal consistency and reproducibility of the FACIT-F Scale

Source	Group	Cronbach's Coefficient Alpha	Test-Retest Correlation Coefficient
Boogaerts et al, 2003	Lymphoid or solid tumor malignancies	0.93	
Cella et al, 2002 Sample 1	FACT-An validation sample (Yellen et al, 1997)	0.93	0.90
Cella et al, 2002 Sample 2	Cancer chemotherapy outpatients	0.95	
Cella et al, 2002 Sample 3	Anemic cancer patients (Demetri et al, 1998)	0.94	
Cella et al, 2003	US general population (Internet survey)	0.93	
Hwang et al, 2003	Veterans Administration cancer patients	0.94	
Kallich et al, 2001	Solid tumorcancer chemotherapy patients Lung cancer chemotherapy patients	0.86 0.87	
Yellen et al, 1997	initial retest	0.93 0.95	0.90

4.5.2.5 FACIT-F Scoring Guidelines

Scores for the FACIT-F 13 item subscale can range from 0-52, with a higher score indicating lower levels of fatigue and better QoL (see Table 4.7). As recommended by the scale developers, a minimally important difference of 3-4 points in scores between time points is used to determine significant clinical changes (FACIT-F, 2007).

Table 4.7: Scoring guidelines for FACIT-Fatigue 13 item subscale

Instructions: 1. Record answers in "item response" column. If missing, mark with an X 2. Perform reversals as indicated, and sum individual items to obtain a score 3. Multiply the sum of the item scores by the number of items in the subscale, Then divide by the number of items answered-this produces the subscale score 4. The higher the score, the better the quality of life				
Subscale	**Item Code**	**Reverse Item**	**Item Response**	**Item Score**
Fatigue Subscale *Score range:* 0-52	H17	4 -		
	H12	4 -		
	An1	4 -		
	An2	4 -		
	An3	4 -		
	An4	4 -		
	An5	0 +		
	An7	0 +		
	An8	4 -		
	An12	4 -		
	An14	4 -		
	An15	4 -		
	An16	4 -		
	Sum individual item scores:			
	Multiply by 13:			
	Divide by number of items answered:			

4.5.3 Tumour Tissue Samples

Pre and post-operative tumour tissue samples are stored in the histology department in WRH, in line with standard patient care. These tissue samples were accessed and sent to St. James's Hospital for KRAS testing.

4.5.3.1 KRAS Analysis

KRAS analysis was performed in the molecular diagnostics laboratory of St. James's Hospital. The Roche Cobas® 4800 KRAS assay detects mutations at codons 12/13 and 61 of the KRAS gene using melt-curve analysis. Reports are returned with an indication that a mutation has been detected at codon 12/13, codon 61 or that no mutation has been detected.

4.5.3.2 Gene Expression

Two further sections were cut from the tumour tissue block in St. James's Hospital using microtomes. Gene expression analysis was performed on these sections in the Biomedical laboratory in WIT. RNA was extracted using the following protocol.

RNA Extraction

The elimination of RNases is imperative prior to an RNA extraction. Solutions were treated with diethylpyrocarbonate (DEPC) and surfaces, and pipettes were treated with RNaseZAP® (Ambion, Cat. No. AM9780). Isolation of total RNA was performed using the 'RNeasy FFPE Kit (Qiagen, Cat. No. 73504.)'.

Assay Procedure

- Using a scalpel, excess paraffin was trimmed off the sample block and 2 sections of 10μm thickness were cut. The sections were immediately placed in a 1.5 ml microcentrifuge tube and the lid was closed.
- 160 μl Deparaffinization Solution was added and vortexed for 10 s, then centrifuged.
- The sample was incubated at 56°C for 3 min, and then allowed to cool at room temperature.150 μl Buffer PKD was added and vortexed. Centrifuged for 1 min at 11,000 *g* (10,000 rpm).
- 10 μl proteinase K was added to the lower, clear phase.
- The sample was incubated at 56°C for 15 min, then at 80°C for 15 min.
- The lower, uncoloured phase was transferred into a new 2 ml microcentrifuge tube. Incubated on ice for 3 min. Then, centrifuged for 15 min at 20,000*g* (13,500 rpm).
- The supernatant was transferred to a new microcentrifuge tube DNase Booster Buffer equivalent to a tenth of the total sample volume (approximately 16 μl) and 10 μl DNase I stock solution was added and mixed by inverting the tube.

- Incubated at room temperature for 15 min. 320 µl Buffer RBC was added, and the lysate was mixed thoroughly.
- 720 µl ethanol (100%) was added to the sample, and mixed well by pipetting.
- 700 µl of the sample was transferred to an RNeasy MinElute spin column placed in a 2 ml collection tube. Centrifuged for 15 s at ≥8000 *g* (≥10,000 rpm). The last step was repeated until the entire sample had passed through the RNeasy MinElute spin column.
- 500 µl of Buffer RPE was added to the RNeasy MinElute spin column. The lid was closed gently, and centrifuged for 15 s at ≥8000 *g* (≥10,000 rpm). The RNeasy MinElute spin column was placed in a new 2 ml collection tube. The lid of the spin column was opened, and centrifuged at full speed for 5 min.
- The RNeasy MinElute spin column was placed in a new 1.5 ml collection tube. 14µl RNase-free water was added directly to the spin column membrane. The lid was closed gently, and centrifuged for 1 min at full speed to elute the RNA.

The integrity, purity and concentration of the isolated RNA samples were assessed via a Nanodrop ND-1000 Spectorophotometer. RNA samples were stored at -70°C until required.

cDNA Synthesis

Each RNA sample was reverse transcribed using the 'High-Capacity complementary DNA (cDNA) Reverse Transcription Kit (Applied Biosystems, U.K. Cat. No. 4368814)'. 10µl of 2X reverse transcription master mix was pipetted into individual tubes. Following quantification, 10µl of RNA sample was then pipetted into each tube. Samples were briefly centrifuged for 1 minute to spin down the contents. The tubes were then loaded on the thermal cycler. The PCR amplification protocol was carried out as outlined in Table 4.8. cDNA samples were subsequently stored at -20°C until required.

Table 4.8: PCR amplification protocol for reverse transcription (Applied Biosystems)

Step	Temperature (°C)	Duration (minutes)	No. of Cycles
1. Denaturation	25	10	1
2. Annealing	37	120	1
3. Elongation	85	5	1
4. Holding	4		1

Real-Time PCR Relative Quantification

The objective of the study was to compare the expression levels of two genes pre- and post-treatment. The two genes of interest were IL-6 and IL-8. The pre-treatment samples served as the calibrator. Real time relative quantification analysis was carried out on the generated cDNA samples using the 7300 System Sequence Detection Software (SDS) v1.2.3 (Relative Quantification study application) by Applied Biosystems. The SDS sets gene expression levels for the calibrator samples to one. Consequently, if more IL-6 and IL-8 are found in the post-treatment than in the pre-treatment samples, the gene expression level of IL-6 and IL-8 is greater than one. Because relative quantification (RQ) is based on PCR, the more template in a reaction, the more PCR product and the greater the fluorescence. To adjust for possible differences in the amount of template added to the reaction, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) served as an endogenous control (expression levels of the endogenous control were subtracted from expression levels of IL-6 and IL-8).

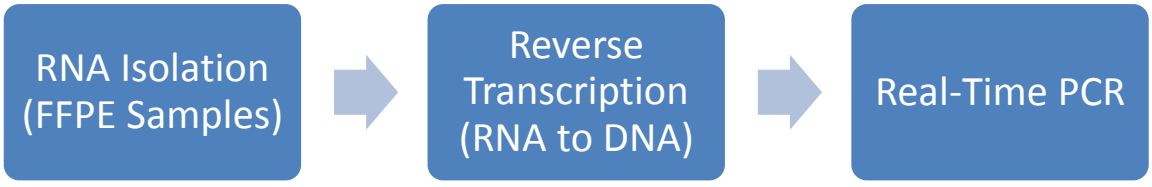

Figure 4.2: Workflow for gene expression analysis using Formalin Fixed Parrafin Embedded (FFPE) samples

4.5.4 Blood Samples

The collection of blood samples were necessary for both haematological and cytokine analysis.

4.5.4.1 Collection and Storage of Blood Samples

Blood-taking was performed by personnel trained in venepuncture into blood tubes with unique identifiers assigned to individual patients who were not fasting. Whole blood samples were collected in Vacutainer® Plus 4.5ml tubes K3 EDTA 15% (Beck Dickinson VACUTAINER Systems, UK. Cat. # 366452). Within 1 hour of collection samples were analysed using the AcT Diff 2 Haematology Analyser to measure haematological parameters. These samples were not chilled and were immediately centrifuged at 4°C for 10 minutes at 3000 rpm (1500 *g*) and plasma from the sample was pipetted into eppendorfs and stored at -70°C until further analysis was performed.

4.5.4.2 Haematological Analysis

The Beckman and Coulter AcT Diff 2 analyser was standardised against a 4C plus blood control and used for whole-blood analysis of haematological parameters. Haematological analysis was carried out in order to rule out infection and anaemia in participants. Within 1 hour of blood collection, each sample was analysed, before the sample clotted which could skew the result. The machine automatically diluted a whole blood sample of 29.6µl and gave results of absolute numbers of White Blood Cell Count (WBC) 10^9/µl, Red Blood Cell Count (RBC) 10^{17}/µl, platelets 10^9/µl, mononuclear cells 10^9/µl and granulocytes 10^9/µl. Differentiation between neutrophils, eosinophils and basophils was not made. In addition, Hb g/dl was measured by the analyser. For the purpose of this study, WBC, platelets and Hb were recorded for each participant and interpreted by the parameters in Table 4.9.

Table 4.9: Haematological reference values

Blood Cells	Normal Parameters
WBC (10^9/µl)	4.5 – 10.4
Platelets (10^9/µl)	136 – 451
Hb (g/dl)	11.6 – 14.9

Wakeman e*t al.* (2007)

4.5.4.3 Cytokine Measurements

Specified cytokine levels were measured by Meso Scale Discovery's (MSD)'s Multi-Array technology. This system enables the detection of biomarkers in multiplex formats allowing the measurement of several cytokines in a single plasma sample. The cytokines that were assessed included IL-6 and IL-8. The cytokine measurement system as developed by MSD Systems, Gaithersburg, Maryland requires that certain cytokines are tested concurrently. Therefore, 7 plex plates were required in order to ensure the cytokines of interest were tested.

V-PLEX™ Custom Human Cytokine 7-PLEX Kit

Reagent Preparation

- All reagents were brought to room temperature. The calibration standards were prepared.

Sample Preparation

- All plasma samples were thawed completely at room temperature, vortexed and centrifuged at 3000 g for 15 minutes to remove any clotted material.

Assay Procedure

- 50µl of calibrators and samples were pipetted into each well and the plate was sealed again and incubated at room temperature (18-28°C) for 2 hours with vigorous shaking at 500rpm to ensure thorough mixing.

- The wash solution was then prepared 10 minutes before the end of the incubation period.
- Each well was then aspirated and washed 3 times with 150µl of the wash solution and blotted dry by inverting the plate on absorbent material.
- Following this extensive washing, 25µl of detection antibody solution was pipetted into each well and the plate was sealed and incubated at room temperature (18-28°C) for 2 hours with vigorous shaking at 500rpm to ensure thorough mixing.
- Each well was then aspirated and washed 3 times with 150µl of the wash solution and blotted dry by inverting the plate on absorbent material.
- 150µl of 2X read buffer was then pipetted into each well.
- The plate was read within 10 minutes of adding the read buffer solution and analysed using a SECTOR PR^{TM} Reader (MSD, 2013).

Quantification

All samples were assayed in duplicate, and all samples for given participants were run in parallel to minimise inter-assay variability. The specificity of the assay is measured by the coefficient of variation (% CV). The % CV is calculated by dividing the standard deviation by the mean and multiplying by 100. Typically, a % CV that is < 10% is considered acceptable (Reed *et al.,* 2002), although results where the % CV is < 15% can also be utilised. Samples with higher concentrations tend to have lower % CV (Gilbertson-White *et al.,* 2010). The inter-assay variability can be seen in Table 4.10.

Table 4.10: Cytokine analyses inter-assay variability and lower limits of detection (LLOD)

Cytokine	% CV	LLOD (pg/ml)
IL-6	8.8	0.12
IL-8	8.4	0.10

4.5.5 Measurement of Tumour Stage, Size and Regression

Staging and sizing of the tumour was performed at two distinct time points, the first of which was immediately after diagnosis, before any treatment had been given. Radiological imaging, clinical examination, and biomarker assessment were carried out by a radiologist, histopathologist and an oncologist to assign a stage and determine the size of the tumour, which was used to make decisions about primary treatment and management. The second time point of this assessment occurred after preoperative treatment and for some patients included radiological imaging prior to surgery in order to determine whether the tumour responded to treatment, with tumours from all patients assessed after surgical removal, and included assessment of level of regression, as well as tumour stage and size. Once again, these assessments were performed by a radiologist, histopathologist and oncologist, who had not been blinded to the pretreatment assessment. TNM staging and level of regression were determined by AJCC criteria and are outlined in Table 4.11 (AJCC, 2002).

Table 4.11: TNM Classification and Level of Regression

Tumour (T)
Tis Tumour confined to mucosa; cancer *in-situ* **T1** Tumour invades submucosa **T2** Tumour invades muscularis propria **T3** Tumour invades subserosa or beyond **T4** Tumour invades adjacent organs or perforates the visceral peritoneum
Nodes (N)
N1 Metastasis to 1 to 3 regional lymph nodes **N2** Metastasis to 4 or more regional lymph nodes
Metastasis (M)
M1 Distant metastasis present
Level of Regression
Complete No viable cancer cells **Moderate** Single cells or small groups of cancer cells **Minimal** Minimal or no tumour kill **Poor** Extensive residual cancer

4.6 Ethical Considerations

Ethical approval to carry out this study was granted from WIT Ethics Committee and the HSE Ethics Committee in April / May 2012 (see Appendices I & II). As changes were made in relation to procedures during the study, amendments were sought and granted by the relevant ethics committees (see Appendix IV). Ethical considerations when carrying out this research took precedence over the expected benefits to knowledge (Robson, 2006). The ethical principles of respect for human dignity and informed consent, beneficence, and justice were all addressed in this study.

4.6.1 Respect for Human Dignity and Informed Consent

The right to self-determination and the right to full disclosure are upheld in the principle of respect for human dignity (Polit and Beck, 2006). Participants were in no way coerced into taking part in this study. The nature of the study was fully described to individuals in order to ensure their consent to participate was comprehensively informed.

Informed consent to carry out the study was obtained from each participant. Both verbal and written information about the study was given to patients that were newly diagnosed with rectal cancer and they were invited to participate. Patients then signed a consent form indicating that they agreed to take part in the study (see Appendix III). No inducements or rewards, monetary or otherwise were offered to participants. The information sheet and consent form made it clear that it was entirely up to the participants to take part and they could withdraw consent at any time or choose not to respond to any items in the questionnaires.

4.6.2 Beneficence

The principle of beneficence requires one to do no physical or psychological harm (Polit and Beck, 2006). Therefore, great sensitivity was exercised when approaching patients to participate in the study and obtaining data. If a participant felt uncomfortable or vulnerable they could withdraw from the study at any stage.

Distress and support measures were put in place if any participant became upset. If such an instance arose, the person was listened to until they became less distressed. Then an explanation was given that the researcher was not the best person to talk to about these issues and they were encouraged to express their feelings to a supportive friend, family member,

oncology nurse or clinician. However, where appropriate, permission was sought to make contact on their behalf.

Should the study highlight that a participant had high levels of certain symptoms, such as fatigue, and might benefit from further medical or psychological treatment, the participant would receive a telephone call to reiterate this. In such an event, it would be explained to the participant that the researcher has a professional duty as a practicing general nurse to inform the clinician in charge of their care of such disclosures. The clinician would be asked to assess the participant and, where appropriate, make a referral to a relevant professional and/or services.

Should the study highlight that a participant has alterations in their haematological parameters indicating infection or anaemia, the participant would have received a telephone call to reiterate this. In such a case, the participant would be encouraged to contact their clinician to seek medical advice and treatment. It would also be explained to the participant that the researcher has a professional duty as a registered nurse to inform the clinician in charge of their care of such findings.

4.6.3 Justice

Each participant has the right to fair treatment and a right to privacy, thus addressing the principle of justice (Polit and Beck, 2006). Participants in any research study have the right to anonymity and confidentiality and they should expect that any information provided is treated confidentially (Gillon, 2003). All information gathered during this study was treated in the strictest of confidence and coded to ensure anonymity and will be stored in line with WIT's policies. This identifier code was recorded on all study documentation and any

personally identifying material was removed. All original data was stored in a locked filing cabinet, whereby the researcher only had access to this filing cabinet.

4.7 Data Analysis

Data analysis is described as the systematic organisation and synthesis of research data aimed to address the research question (Polit and Beck, 2006). Quantitative data analysis was performed using the Statistical Package for the Social Sciences (SPSS) Version 19. Prior to discussing the particular methods of data analysis used, issues in relation to data entry and the management of missing data are addressed.

4.7.1 Data Entry

Data obtained from questionnaires were scored as described previously and were entered into the database systematically, along with the results of cytokine analysis, KRAS testing and tumour response to treatment. All data were double checked during this process to avoid any errors. Visual examination of a frequency table that was generated using descriptive statistics allowed for the screening of outlying values, which were then examined for accuracy, thereby ensuring accurate preparation of data for later analyses, as outlying values may significantly impact on the results of statistical tests, particularly in smaller samples (Pallant, 2007).

4.7.2 Missing Data

When carrying out research, particularly with people, it is rare that complete data will be obtained for every case (Pallant, 2007). Missing data for some participants can occur due to refusals, researcher error or skip patterns in the chosen instrument (Polit and Beck, 2006).

If data is missing, it is important to deal with this in the most appropriate manner when performing statistical analyses, as this can have a dramatic influence on results (Pallant, 2007). The statistical package used for data analysis in this study allows the researcher to exclude cases listwise, or exclude cases pairwise (Pallant, 2007). To exclude cases listwise, cases will only be included for analysis if there is full data on all variables, whereas excluding cases pairwise will only exclude the person if they are missing data required for specific analysis (Pallant, 2007). It is clear from this that excluding cases listwise can severely, and unnecessarily limit the sample size. Therefore, in this study, where possible, the option to exclude cases pairwise was selected as the case was still included in any of the analyses for which they had necessary information (Pallant, 2007).

In this study, missing data was minimal. Questionnaires were completed by all participants except two, at all time points. In the cases where the questionnaire was missed, it was at the final time point and this was followed up by telephoning the participants and leaving a message. However, the participants did not respond and it was deemed inappropriate to contact them on a second occasion. Within the questionnaire itself, some participants skipped questions, particularly in relation to sexual function and this was dealt with during analysis by excluding these cases pairwise (Pallant, 2007). Pre-treatment blood samples were obtained from all participants. Tumour tissue samples were accessed for all consented patients (28), with pre and post treatment samples available for 21 of these.

4.7.3 Level of Significance

In order to test the hypotheses as previously outlined, analysis of variance procedures were undertaken. However, this type of analysis may lead to type 1 error which rejects the null

hypotheses when it is, in fact, true (Pallant, 2007). The risk of performing a type 1 error is minimised by selecting an appropriate alpha level. Therefore, for the purposes of this study an alpha level of 0.05 (5%) was considered as appropriate.

4.7.4 Analysis Plan

Data comprised of both continuous and categorical variables. Continuous data included age, weight, height, BMI, questionnaire responses, haematological data, tumour size and cytokine levels. Categorical data included gender, smoking status, marital status, employment status, tumour stage, grade and level of regression, and KRAS status.

Histograms were produced to assess the distribution of data. The normality assumption of the distribution of scores for subscales on each continuous measure was verified using the *Kolmogorow-Smirnov* and *Shapiro-Wilk* tests (Pallant, 2007). Normally distributed continuous variables (age, weight, height, BMI, Hb, WCC and platelets) were presented as mean and standard deviation (SD). Variables that were non-normally distributed (IL-6, IL-8 and tumour size) were presented as median and interquartile range (IQR), with mean levels also reported. Categorical data (gender, smoking status, marital status, employment status, tumour stage, grade and level and KRAS status) were presented using numbers and percentages.

Internal consistency of the questionnaires was assessed using Cronbach's alpha coefficient and Standardised Cronbach's alpha coefficient. As no difference was detected between the Cronbach's alpha coefficient and Standardised Cronbach's alpha coefficient, Cronbach's alpha coefficient was used to present the results of the study. Cronbach's alpha was presented for the EORTC QLQ-C30, EORTC QLQ-CR29 and the FACIT-F. These were tested for internal consistency using the total sample (n = 35) with a Cronbach's Alpha

coefficient above 0.7 considered acceptable (Pallant, 2007). The EORTC QLQ also contained individual sub scales which were short as they consisted of less than 10 items and therefore, a low Cronbach's Alpha value was expected in some cases. To address any low scores that may occur, the mean inter-item correlation was also reported in such cases, as recommended by Pallant (2007), where results of 0.2 – 0.4 are considered to be within the optimal range.

Changes in symptoms and QoL over time were assessed using one-way Analysis of Variance with repeated measures (RM-ANOVA), with post-hoc tests performed using Bonferroni correction to identify the time points at which any significant changes occurred. This is a commonly used statistical approach to repeated measure designs where the same subjects are observed under different conditions (Hager, 2007). In some cases, it has been recommended that this test should only be performed with data that is normally distributed, whereas for non-normally distributed data, the Friedman test, which is the non-parametric alternative, should be used (Pallant, 2007). However, RM-ANOVA was chosen for this study despite non-normally distributed data in relation to symptoms and QoL, as detected by the Shapiro-Wilk test, as it has been established that the Friedman test has only modest statistical power (Zimmerman and Zumbo, 1993). Therefore, RM-ANOVA can be used to replace its non-parametric alternative with this type of data as it provides a more robust test with greater statistical power (Zimmerman and Zumbo, 1993; Hager, 2007). Also, it has been previously demonstrated that the false positive rate detected by ANOVA using non-normal distributions is not significantly affected by violating the assumption of normality (Lix *et al.*, 1996). In addition, outputs from the Mauchly's Test of Sphericity were not violated for the dataset in this study. Based on this, RM-ANOVA was the most appropriate choice for testing changes in symptoms and QoL across time points.

In order to assess the relationships between continuous variables, Spearman Rank correlation coefficients (rho's) were calculated (two-tailed). This is the non-parametric equivalent of the Pearson's Product Moment Correlation Coefficient (r) for measuring the degree of association between the values of two variables (Pallant, 2007). In order to assess the relationships between continuous and categorical variables, the Kruskal-Wallis Test was performed (Pallant, 2007). Other statistical tests used in this study can be seen in Table 4.11.

Table 4.12: Variables and statistical tests

Purpose	Age, Weight, Height & BMI	Gender, Smoking Status, Marital Status, Employment Status	Hb, WCC, Platelets	Tumour Stage, Grade, Level of Regression and Size	EORTC QLQ-C30 EORTC QLQ-CR29	FACIT-F	Blood Plasma & Tumoral Cytokine Levels	KRAS Status	Statistical Methods
Examination of normal distribution in variables	✓	✓	✓	✓	✓	✓	✓	✓	*Kolmogorow-Smirnov test* *Shapiro-Wilk* test
Examination of internal consistency of questionnaires in an Irish population					✓	✓			*Cronbach's alpha coefficient* *Standardised Cronbach's alpha coefficient*
Examination of symptoms, QoL & functioning across time points					✓	✓			*RM -ANOVA*
Examination of the relationship between fatigue and other symptoms					✓				*Spearman's* rank correlation
Examination of relationship between KRAS status & tumour regression				✓				✓	*Chi-Square* test

Examination of relationship between KRAS status & levels of cytokines							✓	✓	*Mann-Whitney U* test
Examination of relationship between cytokines & tumour stage, size & level of regression							✓		*Spearman's* rank correlation *Kruskal-Wallis* test
Examination of relationship between cytokines in tumour tissue samples between time points							✓		*Wilcoxon Signed-Rank* test
Examination of relationship between cytokines in pre-treatment tumour tissue samples and pre-treatment blood plasma samples							✓		*Spearman's* rank correlation
Examination of relationship between cytokines & symptom presentation					✓		✓		*Spearman's* rank correlation *Mann-Whitney U* test

4.8 Conclusion

This chapter provides a rationale for the research methods applied in this study. Issues in relation to the population and sample, the choice of instrument, data collection and statistical analyses were critically discussed and justification for the choice of research design that was adopted has been provided. A large amount of quantitative data has been obtained through this study, which has been used to address the aims, objectives and research hypotheses outlined in this chapter. The results obtained through analysis of this data are outlined and discussed in the following chapters.

CHAPTER V: Results

5.0 Introduction

This chapter presents the results of the research study. The study accrual and attrition is outlined in Section 5.1, and this is followed by a description of the socio-demographic and clinical characteristics of the study participants (Section 5.2). The internal consistency of each of the assessment tools used to measure symptoms and QoL is then reported in Section 5.3.

The results of each of the research hypothesis are discussed. The prevalence and changes in symptom presentation, including fatigue, bowel function, nutrition, pain, dermatological symptoms, urinary function and sexual function are examined looking for changes over time (Section 5.4). The impact of fatigue on other symptoms is presented in Section 5.5. Changes in QoL and functioning over the 4 time points, including cognitive, emotional, physical, role and social functioning are examined in Section 5.6. The influence of symptoms on QoL and functioning at the 4 time points is then outlined in Section 5.7. Following this, the results of the KRAS and cytokine analyses, as well as their relationship to tumour response and symptom presentation are provided (Section 5.8). Finally, section 5.9 provides a summary of the results with reference to the study's objectives.

5.1 Study Accrual

Recruitment was undertaken over a 19 month period (1/06/12 – 31/01/2014), with Figure 5.1 providing an overview of the flow of participants throughout the study. Accrual of patients that had been newly diagnosed with rectal cancer took place in a regional cancer centre, and those that met the study criteria were identified at routine multidisciplinary team meetings. These patients were then approached and given both verbal and written information about the study when they attended a clinic appointment and were invited to participate. Upon obtaining informed consent, 36 patients met the eligibility criteria and were entered into the study. However, data was analysed for 35 patients as 1 patient was excluded due to the development of metastases.

Attrition of the participants was not an issue in relation to obtaining bloods and completing questionnaires as they were met at routine clinic appointments, with all 35 patients giving blood samples and all patients completing questionnaires at the necessary time points, except 2, who failed to return one questionnaire at time point 4. In some cases, information was missing from parts of the questionnaire as patients had not answered all questions. In such instances, data were analysed by excluding cases pairwise, thereby excluding the person only if they were missing the data required for the specific analysis. This ensured their results were still included in any of the analyses for which they had necessary information. In relation to accessing tumour tissue samples to perform KRAS and cytokine analysis, following ethical approval, letters were sent to the first 31 study participants explaining the extension to the study and seeking their permission for this access. Consent for such access was not sought from the final 4 participants of the study as their surgery was due to be performed after the scheduled time for tumour tissue analysis. Following informed consent, tumour tissue samples were obtained for 28 participants. Pre-operative tumour tissue

samples were available for 26 participants and post-operative samples were available for 23 participants as 3 had complete response to treatment, with no tumour left to surgically remove. Of the 23 pre and post operative tumour samples, 21 were available for testing.

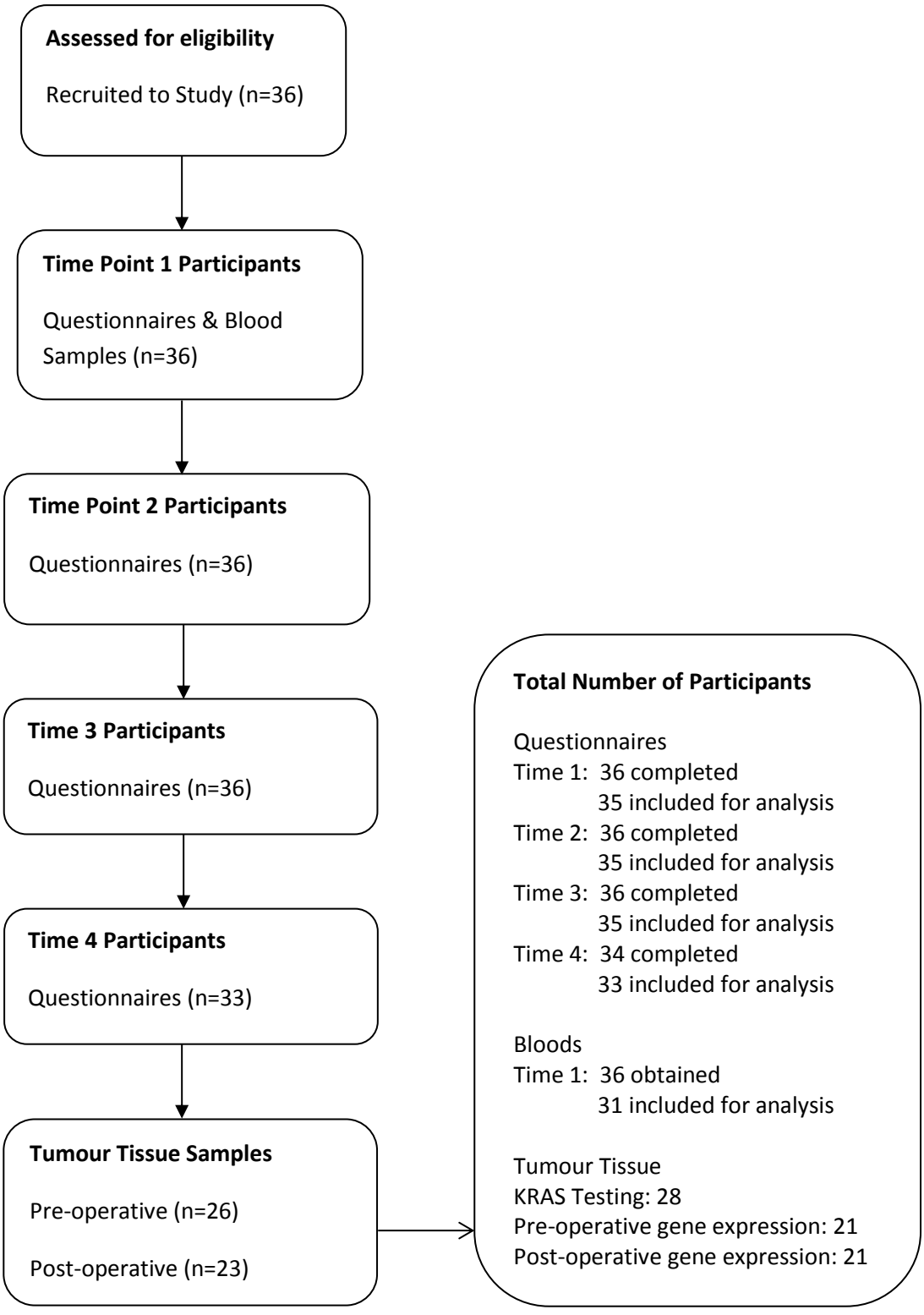

Figure 5.1: Study Participants

5.2 Socio-Demographic and Clinical Characteristics of the Sample

The socio-demographic and clinical characteristics of the study participants are summarised in Table 5.1. The total number of participants whose questionnaires were included for analysis was 35, 68.6% (*n* = 24) of which were male and 31.4% (*n* = 11) of which were female, with ages ranging from 34 - 82 years (mean 61.69, SD 11.15). Most participants were in a relationship (91.2%, *n* = 31), with 58.8% (*n* = 20) retired and 29.4% (*n* = 10) in paid employment. With regard to living arrangements, most (91.2%, *n* = 31) did not live alone. In relation to pathological disease status, 7.1% (*n* = 2) had stage 0 tumours, 7.1% (*n* = 2) had stage 1 tumours, 7.1% (*n* = 2) had stage 2 tumours, 60.7% (*n* = 17) had stage 3 tumours and 17.9% (*n* = 5) had stage 4 tumours. After pre-operative treatment tumour regression was noted to be poor in 3.1% (*n* = 1), minimal in 35.7% (*n* = 10), moderate in 50% (*n* = 14) and complete in 10.7% (*n* = 3) of cases. In relation to KRAS status, 19 patients had wild type KRAS and 9 had mutant KRAS, with these mutations occurring at codon 12/13 in 8 cases and at codon 61 in 1 case. Of the 9 patients that harboured mutant type KRAS, information in relation to the tumour response to treatment of 8 patients was available. Data indicated that 1 had minimal response, 5 had moderate response and 2 had complete response to treatment.

Table 5.1: Socio-demographic and disease characteristics of sample

Variable	Number (%)	Range	Mean (Std. Dev.)
Age (years)	35	34 – 82	61.69 (11.15)
Gender: M	24 (68.6%)		
F	11 (31.4%)		
Weight (kg)	25	58 – 108	79.23 (15.37)
Height (cms)	25	153 – 185	169.72 (9.15)
BMI (kg/m^2)	25	19 - 39	27.21 (5.48)
Smoking Y	4 (11.8%)		
N	30 (88.2%)		
Bloods			
Platelets	31	53 – 384	241.65 (69.41)
WCC	31	4.1 - 13.5	7.36 (2.10)
Hb	31	8.7 – 14.9	11.89 (1.64)
KRAS Status			
Wild Type	18		
Mutant	9		
Codon 12/13	8		
Codon 61	1		
Employment Status			
Employed	10 (29.4%)		
Unemployed	4 (11.8%)		
Retired	20 (58.8%)		
Habitation			
Lives Alone: Y	3 (8.8%)		
N	31 (91.2%)		
Marital Status			
Unmarried:	3 (8.8%)		
Married:	27 (79.4%)		
Other:	4 (11.8%)		
Tumour			
Pathological Tumour Stage:			
0	2 (7.1%)		
1	2 (7.1%)		
2	2 (7.1%)		
3	17 (60.7%)		
4	5 (17.9%)		
AJCC Tumour Regression:			
Poor	1 (3.1%)		
Minimal	10 (35.7%)		
Moderate	14 (50%)		
Complete	3 (10.7%)		
Tumour Size (cms):			
0-4	24 (88.8%)		
4-9	3 (11.2%)		

Tumour Regression & KRAS:

	Wild Type	*Mutant*
Poor	1	0
Minimal	9	1
Moderate	9	5
Complete	1	2

5.3 Reliability of Scales

Three assessment tools were used to measure symptoms and QoL and these included the EORTC QLQ-C30, the EORTC QLQ-CR29 and the FACIT-F questionnaires. These were tested for internal consistency using the total sample (n = 35) with Cronbach's Alpha coefficient above 0.7 considered acceptable (Pallant, 2007).

5.3.1 Internal Consistency of the EORTC QLQ-C30

The EORTC QLQ-C30 scored a Cronbach's Alpha value of 0.88, thereby demonstrating good internal consistency. Separate analysis of the sub scales within this questionnaire (those with two or more items) was performed to establish their individual reliability with results ranging from 0.41 – 0.93. These individual sub scales were short as they consisted of less than 10 items and therefore a low Cronbach's Alpha value was expected in some cases. However, all subscales analysed scored a Cronbach's Alpha value of greater than 0.7 with the exception of the nausea and vomiting scale. To address this low score, the mean inter-item correlation is also reported, as recommended by Pallant (2007) and demonstrated a result of 0.31 for this subscale, which is acceptable, as results of 0.2 – 0.4 are considered to be within the optimal range (Pallant, 2007). Results of this analysis are demonstrated in Table 5.2.

For the purpose of comparison and measurement of the internal consistency of the QLQ-C30 questionnaire, this Irish study was compared with a Turkish study that investigated the reliability and validity of this scale in 114 cancer patients, as well as an American study performed by the scale developers (Aaronson *et al.*, 1993; Cankurtaran *et al.*, 2007; see Table 5.2).

These results demonstrate that the QLQ-C30 displayed very good internal consistency on all items and was similar to the results of the Turkish and American investigations, thereby indicating its suitability for use in this study.

Table 5.2: Cronbach's alpha & mean inter-item correlation of EORTC QLQ-C30 in the sample, compared to cronbach's alpha of published data

Scale	No. of Items	Mean Inter-Item Correlation	Cronbach's Alpha (Irish Study)	Cronbach's Alpha (Cankurtaran *et al.* 2007)	Cronbach's Alpha Scale Developers (Aaronson *et al.*, 1993)
EORTC QLQ-C30	**30**		**0.88**		
Functional Scales:					
- Cognitive	2	0.60	0.75	0.56	0.60
- Emotional	4	0.61	0.86	0.85	0.70
- Physical	5	0.43	0.81	0.81	0.70
- Role	2	0.66	0.78	0.83	0.50
- Social	2	0.56	0.71	0.74	0.70
Symptom Scales:					
- Fatigue	3	0.71	0.88	0.84	0.80
- Nausea & Vomiting	2	0.31	0.41	0.77	0.70
- Pain	2	0.63	0.77	0.74	0.80
QoL:					
- Global QoL	2	0.87	0.93	0.81	0.90

5.3.2 Internal Consistency of the EORTC QLQ-CR29

There was a very low response rate to questions pertaining to sexual function in the EORTC QLQ-CR29 and these items had to be omitted to carry out reliability testing of this scale. Despite this however, the scale still scored a Cronbach's Alpha value of 0.82. The individual sub scales of this questionnaire were also tested separately, with the mean inter-item correlation reported due to their limited number of items. Results of this analysis are outlined in Table 5.3.

The results of tests for internal consistency in this study were compared with that of an international study that examined the measurement properties of the EORTC QLQ-CR29 (see

Table 5.3; Whistance *et al.* 2009). The international investigation reported a higher Cronbach's Alpha score than this Irish study in the scale addressing body image, stool frequency and urinary frequency, thereby strengthening the validation of its use in this investigation as Whistance *et al.* (2009) analysed data from a larger sample (n = 351) across 7 different countries.

Table 5.3: Cronbach's alpha co-efficient & mean inter-item correlation of the EORTC QLQ-CR29 in the sample compared to cronbach's alpha of published data

Scale	Number of Items	Cronbach's Alpha	Mean Inter-Item Correlation (Irish Study)	Cronbach's Alpha (Whistance et *al.*, 2009)
EORTC QLQ-CR29		**0.82**		
Functional Scales:				
- Body Image	3	0.80	0.56	0.84
Symptom Scales:				
- Blood & Mucous in Stool	2	0.72	0.57	0.70
- Stool Frequency	2	0.66	0.50	0.70
- Urinary Frequency	2	0.66	0.49	0.75

5.3.3 Internal Consistency of the FACIT - F (13 item subscale)

Although the QLQ-C30 contains questions in relation to fatigue, in order to obtain a greater insight into this symptom in rectal cancer patients the FACIT-F 13 item subscale was included in the final questionnaire. The FACIT-F scale demonstrated good internal consistency, as when data from all 35 patients were analysed, this resulted in a Cronbach's Alpha value of 0.96. This scale also demonstrated good internal consistency when it was used in a study to examine fatigue in patients with cancer (n = 297) with results indicating a similar Cronbach's Alpha Value of 0.96 (Butt *et al.*, 2013).

5.3.4 Comparing the FACIT-F with the Fatigue Sub-scale of the QLQ-C30

To further examine the reliability of the questionnaires being used in the study, Spearman's correlation co-efficient was used to compare the relationship between the FACIT-F and the sub-scale for fatigue from the QLQ-C30, using the data from the 35 patients recruited to the study at all 4 time points.

The correlation between the FACIT-F and the fatigue items on the QLQ-C30 was - 0.69 at time point 1, - 0.83 at time point 2, -0.93 at time point 3 and – 0.80 at time point 4, with all results demonstrating statistical significance ($p<0.01$). As can be seen, the correlations are all negative, in that as fatigue scores decrease on the FACIT-F scale, indicating higher levels of fatigue, they increase on the fatigue subscale of the QLQ-C30, which on this particular scale, demonstrates greater rates of fatigue. The strength of the relationships, using the criteria as recommended by Cohen (1988) were all large, as the correlation value at each time point was greater than 0.5. (see Table 5.4).

Table 5.4: Relationship between fatigue measurements of the FACIT-F & the EORTC QLQ-C30

Time Point	*r* Value	Number	*p* Value	Coefficient of Determination
1	-0.69	34	< 0.01	48%
2	-0.83	33	< 0.01	69%
3	-0.93	34	< 0.01	86%
4	-0.80	32	< 0.01	64%

5.4 Changes and Prevalence of Symptoms over Time

To examine the changes in symptoms over time, one-way repeated measures analysis of variance (RM-ANOVA) was conducted to compare the symptoms at time point 1 (pre-treatment), time point 2 (midway through treatment), time point 3 (on completion of treatment) and time point 4 (4-6 weeks after completion of treatment). Where statistically significant changes are reported, the clinical significance of this is also stated, with changes of 5-10 points denoting a small change, 10-20 points a medium change and > 20 points a large change (Osoba e*t al.*, 1998). Where statistically significant changes occur between time points, these are demonstrated on the bar charts using $^{*}p<0.05$ or $^{**}p<0.01$ symbols, with placement on the bar indicating changes between that time point & the preceding time point. The proportion of patients reporting symptom scores of > 50 (i.e. 'quite a bit' or 'very much') as measured by the EORTC QLQ is also reported.

5.4.1. Changes in Fatigue over Time

Fatigue was measured using two separate tools – the FACIT – F and the fatigue subscale of the EORTC QLQ-C30. In relation to the measurement of fatigue using the FACIT-F, it was found that mean fatigue scores were 40.67 (SD 11.98) at time point 1. This score fell to 38.86 (SD 12.02) at time point 2, 35.94 (SD 14.06) at time point 3 and then increased to 40.35 (SD 11.44) at time point 4. There was a significant effect for time, Wilks' Lambda = 0.60, F (3, 28) = 6.11, $p<0.01$, multivariate partial eta squared = 0.40. Post-hoc tests using Bonferroni correction revealed a change between fatigue scores at time point 1 (40.67) and time point 3 (35.94) which was statistically significant ($p=0.02$). These changes can be seen in Figure 5.2

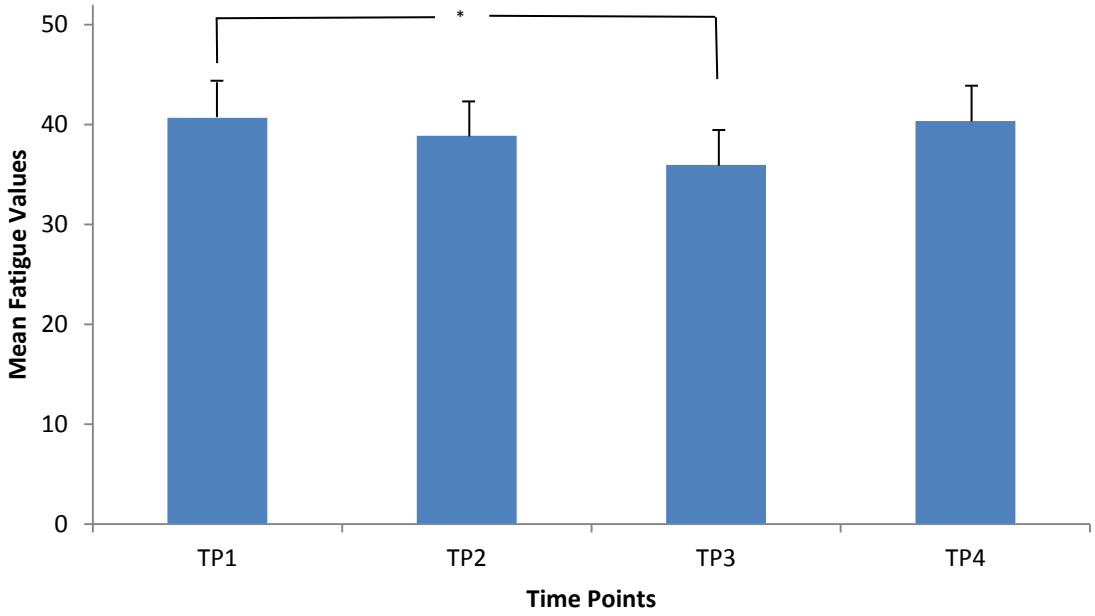

Figure 5.2: Changes in fatigue as measured by the FACIT F scale over a period of 6 weeks in rectal cancer patients (n=35) receiving radiothereapy

*$p<0.05$

Regarding the mean fatigue scores as measured by the EORTC QLQ-C30 fatigue subscale, results indicated scores of 20.55 (SD 24.89) at time point 1, which increased to 32.59 (SD 24.40) at time point 2, 35.56 (SD 30.80) at time point 3 and then decreased to 27.40 (SD 22.17) at time point 4. The fatigue sub scale of the EORTC QLQ-C30 also reported a significant effect for time, Wilks' Lambda = 0.65, F (3, 27) = 4.90, $p<0.01$, multivariate partial eta squared = 0.35. Post-hoc tests using Bonferroni correction revealed a change between fatigue scores at time point 1 (20.55) and time point 2 (32.59) which was statistically significant (p = 0.007), as well as time point 1 (20.55) and time point 3 (35.56; p=0.028). These changes were also clinically significant, with a score difference of 12.04 noted between time point 1 and time point 2, and 15.01 between time point 1 and time point 3. The changes over time for fatigue, as measured by the EORTC QLQ-C30 fatigue subscale can be seen in Figure 5.3.

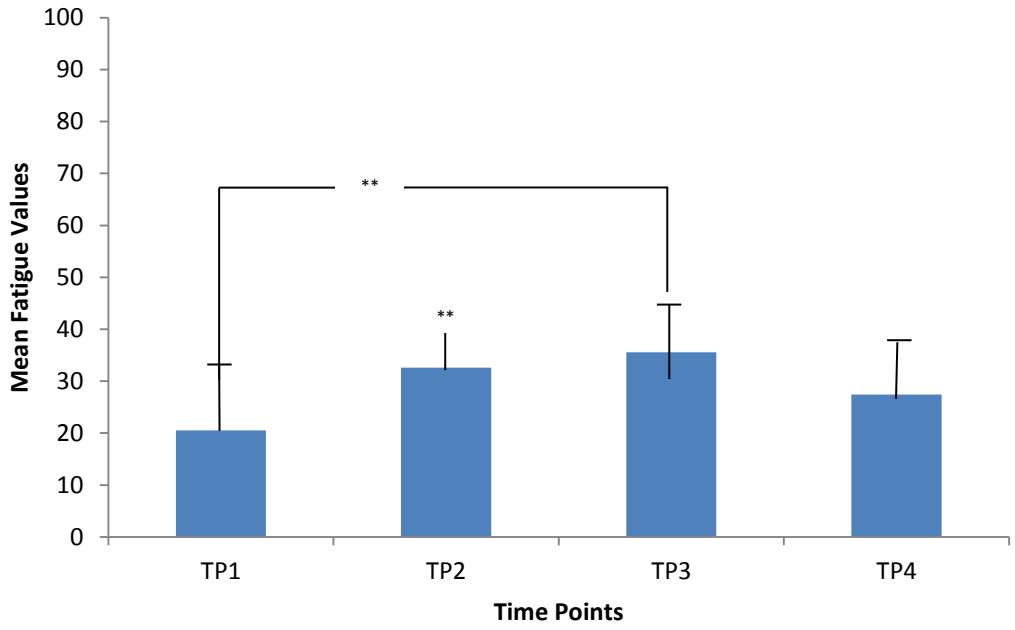

Figure 5.3: Changes in fatigue as measured by the EORTC fatigue subscale over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

The proportion of patients with a fatigue score of > 50 (i.e. 'quite a bit' or 'very much') was 5.7% at time point 1, increased to 18.2% at time point 2, increased further to 32.4% at time point 3 and then decreased to 6.1% at time point 4. The presence of fatigue over time can be seen in Figure 5.4.

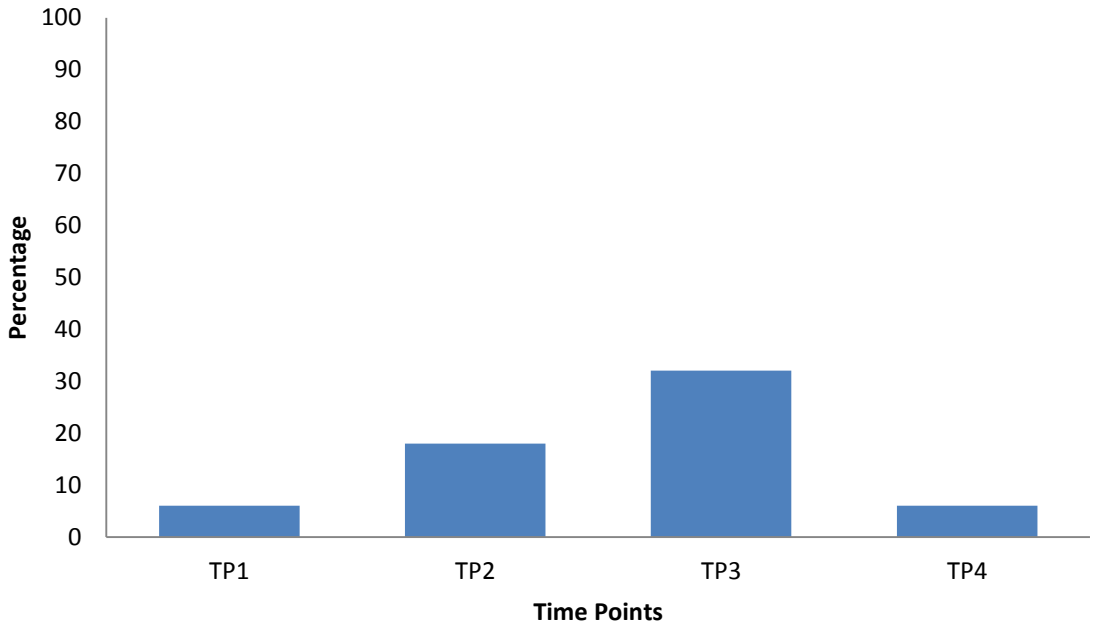

Figure 5.4: Percentage of rectal cancer patients (n=35) with high fatigue scores while receiving radiotherapy over a 6 week period

5.4.2 Changes in Bowel Function over Time

The symptoms of constipation, diarrhoea, flatulence, bloating, blood & mucous in stool, faecal incontinence and stool frequency were examined to determine issues with bowel function during radiotherapy.

Symptoms that showed no significant changes over time were constipation, flatulence and bloating. However, diarrhoea, blood and mucous in stool, stool frequency and faecal incontinence all demonstrated significant changes over time.

Mean scores for constipation were 14.94 (SD 24.54) at time point 1 and then increased to 17.24 (SD 21.12) at time point 2, were 19.54 (SD 22.74) at time point 3 and similarly, 19.54 (SD 24.42) at time point 4. There was no significant effect for time for this symptom, Wilks' Lambda = 0.98, F (3, 26) = 0.29, p=0.77, multivariate partial eta squared = 0.03. In relation

to flatulence, mean scores were 31.30 (SD 31.11) at time point 1, increased to 37.37 (SD 33.08) at time point 2, increased further to 39.39 (SD 33.80) at time point 3 and then fell to 27.27 (SD 30.57) at time point 4. No significant effect for time was noted in relation to flatulence, Wilks' Lambda = 0.84, F (3, 30) = 1.85, p=0.16, multivariate partial eta squared = 0.16. For bloating, at time point 1 mean scores were 15.56 (SD 24.34) and this increased to 18.89 (SD 25.79) at time point 2, to 21.11 (SD 23.95) at time point 3 and then fell to 15.56 (SD 24.34) at time point 4. There was also no significant effect for time in terms of bloating, Wilks' Lambda = 0.93, F (3, 27) = .67, p=0.58, multivariate partial eta squared = 0.07.

For diarrhoea, mean scores were 31.11 (SD 28.95) at time point 1, and then increased to 37.78 (SD 27.31) at time point 2, remained at 37.78 (SD 32.44) at time point 3 and fell to 19.99 (SD 28.50) at time point 4. Conversely, the presence of diarrhoea demonstrated a significant effect for time, Wilks' Lambda = 0.71, F (3, 27) = 3.64, p=0.03, multivariate partial eta squared = 0.29. Post-hoc tests using Bonferroni correction revealed a decrease in diarrhoea at time point 3 (37.78) and time point 4 (19.99) which was statistically significant (p=0.02). These changes were also clinically significant, with a score difference of 17.79 noted between time point 3 and time point 4. Mean scores for blood and mucous in stool were 23.33 (SD 27.30) at time point 1, with this decreasing to 19.70 (SD 18.84) at time point 2, followed by an increase to 25.25 (SD 25.39) at time point 3 and at time point 4 mean scores decreased to a further 10.10 (SD 14.99). In relation to this symptom, there was a significant effect for time, Wilks' Lambda = 0.58, F (3, 30) = 7.35, p<0.01, multivariate partial eta squared = 0.342. Post-hoc tests using Bonferroni correction revealed a decrease in blood and mucous in stool scores occurred at time point 1 (23.23) and time point 4 (10.10) which was statistically significant (p=0.05), with a significant change over time also detected at time point 3 (25.25) and time point 4 (10.10; p=0.01). These changes were also clinically

significant, with a score difference of 13.13 noted between time point 1 and time point 4, 15.15 between time point 3 and time point 4. Mean scores for stool frequency were 29.57 (SD 22.65) at time point 1, increased to 34.41 (SD 23.54) at time point 2, followed by a further increase to 44.62 (SD 27.69) at time point 3 and then decreased to 24.19 (SD 19.64) at time point 4. In relation to stool frequency, a strong significant effect for time was evident, Wilks' Lambda = 0.52, F (3, 28) = 8.69, p<0.01, multivariate partial eta squared = 0.48. Post-hoc tests using Bonferroni correction revealed a change between stool frequency scores at time point 1 (29.57) and time point 3 (44.62), as well as time point 2 (34.41) and time point 3 (44.62) and also, between time point 3 (44.62) and time point 4 (24.19), which was statistically significant (p=0.003; p=0.05; p<0.001), with this symptom worsening between time point 1 and 2 and time point 2 and 3, and then improving between time point 3 and 4. These changes were also clinically significant, with a score difference of 12.04 noted between time point 1 and time point 3, 10.21 between time point 2 and time point 3, and 20.43 between time point 3 and time point 4. In relation to faecal incontinence, mean scores were 12.12 (SD 23.30) at time point 1, 15.15 (SD 18.80) at time point 2 and increased further to 23.23 (SD 29.44) at time point 3, followed by a decrease to 10.10 (SD 17.65) at time point 4. Regarding this symptom, there was a significant effect for time, Wilks' Lambda = 0.75, F (3, 30) = 3.33, p<0.05, multivariate partial eta squared = 0.25. Post-hoc tests using Bonferroni correction revealed a decrease in faecal incontinence scores at time point 3 (23.23) and time point 4 (10.10) which was statistically significant (p=0.02). These changes were also clinically significant, with a score difference of 13.13 noted between time point 3 and time point 4. The changes over time for bowel function can be seen in Figure 5.5.

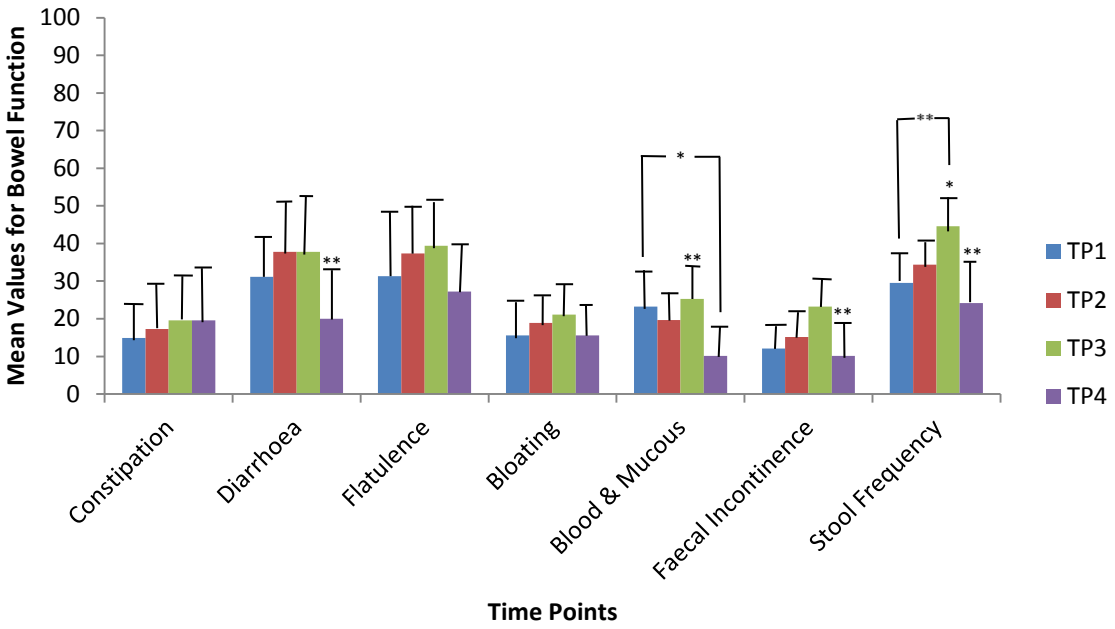

Figure 5.5: Changes in bowel function over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

$^{**}p<0.01$ $^{*}p<0.05$

The proportion of patients with a constipation score of > 50 (i.e. 'quite a bit' or 'very much') was 8.6% at time point 1, decreased to 6.2% at time point 2, increased to 8.8% at time point 3 and then decreased to 6% at time point 4. The proportion of patients with a diarrheoa score of > 50 (i.e. 'quite a bit' or 'very much') was 22.9% at time point 1, increased to 33.3% at time point 2, decreased to 26.5% at time point 3 and then decreased further to 9.1% at time point 4. The proportion of patients with a flatulence score of > 50 (i.e. 'quite a bit' or 'very much') was 28.6% at time point 1, increased to 37.1% at time point 2, increased to 40% at time point 3 and then decreased to 27.3% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for bloating was 17.6% at time point 1, which decreased to 11.8% at time point 2, decreased further to 5.9% at time point 3 and continued to decrease to 3.1% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for blood and mucous in stool was 11.4% at time point 1, increased to 14.3% at time point 2, increased further to 17.1% at time point 3 and then decreased to 6.1%

at time point 4. The proportion of patients a score of > 50 (i.e. 'quite a bit' or 'very much') for faecal incontinence was 5.7% at time point 1, which decreased to 2.9% at time point 2, followed by an increase to 14.3% at time point 3 which then decreased to 3% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for stool frequency was 25.7% at time point 1, increased to 37.1% at time point 2, increased further to 51.4% at time point 3 and then decreased to 18.8% at time point 4. The presence of symptoms over time in relation to bowel function can be seen in Figure 5.6.

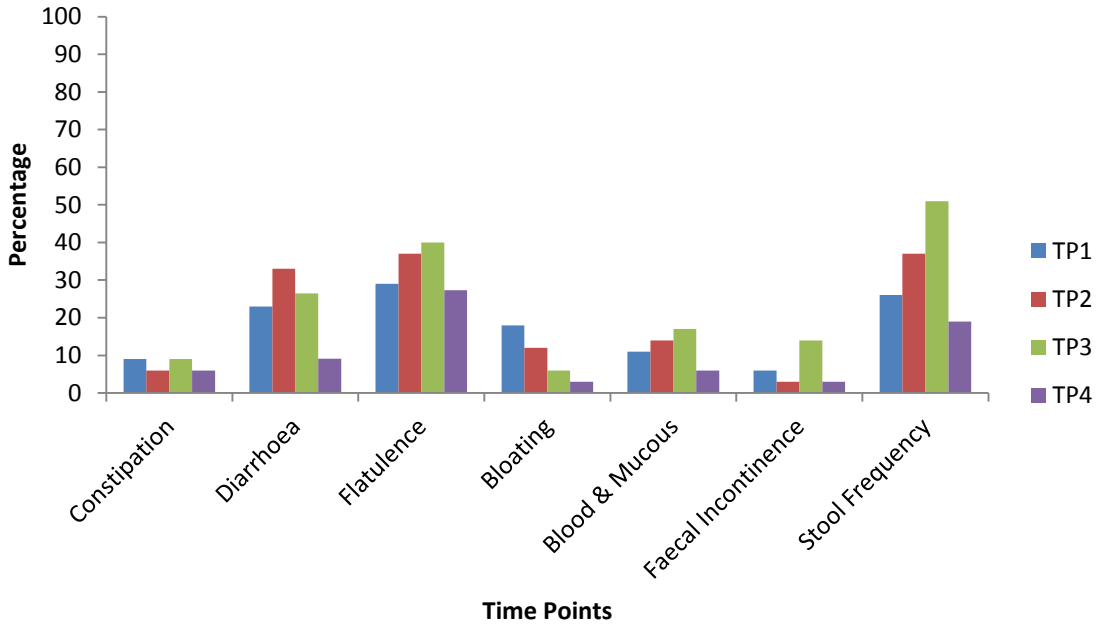

Figure 5.6: Percentage of rectal cancer patients (n=35) with high bowel symptom scores while receiving radiotherapy over a 6 week period

5.4.3 Changes in Nutrition over Time

The symptoms of worry about weight, taste, dry mouth, appetite loss and nausea and vomiting were examined to determine issues with nutrition during radiotherapy. All symptoms showed significant changes over time except for worry about weight.

In relation to weight worry, mean scores were 12.50 (SD 21.99) at time point 1, decreased slightly to 11.45 (SD 21.77) at time point 2, increased to 16.67 (SD 23.95) at time point 3 and remained at 16.67 (SD 25.40) at time point 4. Regarding this symptom, there was no significant effect for time, Wilks' Lambda = 0.89, F (3, 29) = 1.08, p=0.37, multivariate partial eta squared = 0.10.

Mean scores for alterations in taste were 8.33 (SD 18.93) at time point 1, increased to 18.74 (SD 26.69) at time point 2, to 22.92 (SD 32.17) at time point 3 and then fell to 14.58 (SD 25.31) at time point 4. Conversely, regarding alteration in taste, there was a significant effect for time, Wilks' Lambda = 0.75, F (3, 29) = 3.20, p<0.05, multivariate partial eta squared = 0.25. Post-hoc tests using Bonferroni correction revealed a change between taste scores occurred at time point 1 (8.33) and time point 2 (18.74), which was statistically significant (p=0.03), with this worsening between these time points. These changes were also clinically significant, with a score difference of 10.41 noted between time point 1 and time point 2. Mean scores for dry mouth were 18.28 (SD 25.59) at time point 1, increased slightly to 20.43 (SD 23.85) at time point 2, increased further to 23.65 (SD 26.10) at time point 3 and then fell to 12.90 (SD 18.61) at time point 4. There was a significant effect for time in relation to this symptom, Wilks' Lambda = 0.72, F (3, 28) = 3.62, p<0.05, multivariate partial eta squared = 0.28. Post-hoc tests using Bonferroni correction revealed a change between dry mouth scores occurred at time point 3 (23.65) and time point 4 (12.90), which was statistically significant (p =0.01), where problems with dry mouth improved between these time points. These changes were also clinically significant, with a score difference of 10.75 noted between time point 3 and time point 4. Mean scores for appetite loss were 13.33 (SD 27.12) at time point 1, increased to 22.22 (SD 26.74) at time point 2, increased further to 41.11 (SD 39.81) at time point 3 and then fell to 19.99 (SD 29.81) at time point 4. Regarding this symptom, there was

also a significant effect for time, Wilks' Lambda = 0.55, F (3, 27) = 0.55, p<0.01, multivariate partial eta squared = 0.45. Post-hoc tests using Bonferroni correction revealed a change between appetite loss scores occurred at time point 1 (13.33) and time point 3 (41.11), time point 2 (22.22) and time point 3 (41.11), as well as time point 3 (41.11) and time point 4 (19.99) which was statistically significant (p<0.001; p=0.01; p=0.01), with it worsening during treatment and then improving 4-6 weeks after completion of treatment. These changes were also clinically significant, with a score difference of 27.78 noted between time point 1 and time point 3, 18.89 between time point 2 and time point 3, and 21.12 between time point 3 and time point 4. For nausea and vomiting, mean scores were 3.89 (SD 7.17) at time point 1, increased to 11.11 (SD 15.98) at time point 2, fell slightly to 10.56 (SD 11.97) at time point 3 and decreased further to 6.11 (SD 14.17) at time point 4. There was a significant effect for time in relation to this symptom, Wilks' Lambda = 0.64, F (3, 27) = 4.99, p<0.01, multivariate partial eta squared = 0.36. Post-hoc tests using Bonferroni correction revealed a change between nausea and vomiting scores occurred at time point 1 (3.89) and time point 3 (10.56) which was statistically significant (p=0.01), with this symptom worsening between these time points. These changes were also clinically significant, with a score difference of 6.67 noted between time point 1 and time point 3. The changes for nutrition over time can be seen in Figure 5.7.

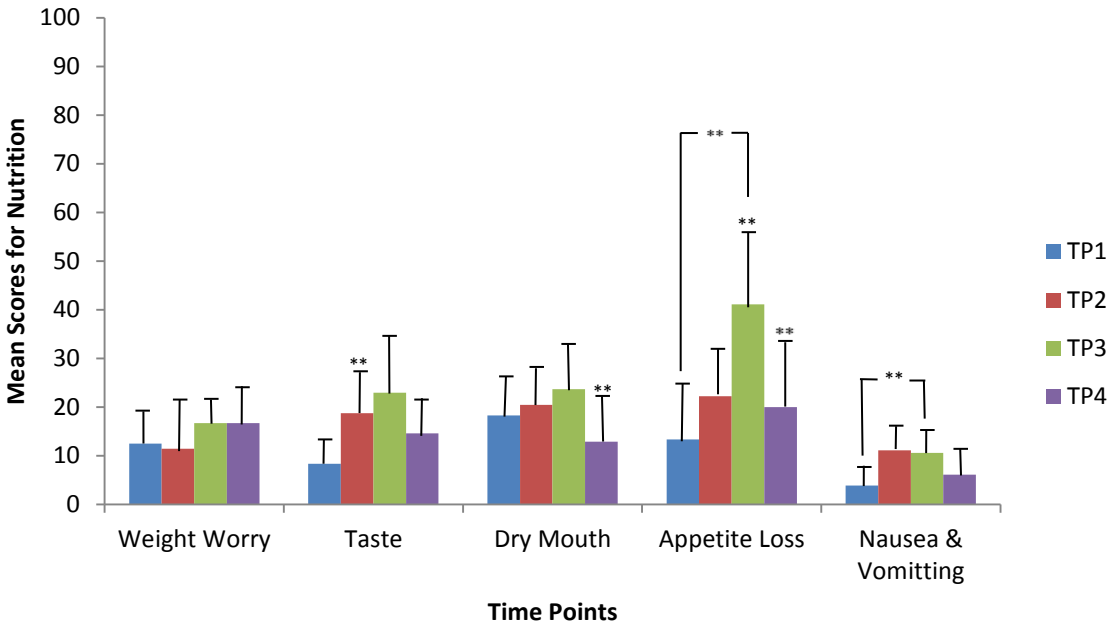

Figure 5.7: Changes in nutrition over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

$^{**}p<0.01$

The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for worry about weight was 8.8% at time point 1, which decreased to 5.7% at time point 2, remained at 5.7% at time point 3 which then increased to 15.2% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for alteration in taste was 5.7% at time point 1, which increased to 11.4% at time point 2, followed by an increase to 20% at time point 3 which then decreased to 9.4% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for dry mouth was 9.2% at time point 1, which increased to 14.6% at time point 2, followed by an increase to 18% at time point 3 which then decreased to 6% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for appetite loss was 11.4% at time point 1, which increased slightly to 12.1% at time point 2, followed by an increase to 31.4% at time point 3 which then decreased to 12.1% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for nausea and vomiting was 0% at time point 1, 5.9% at time point 2, 0% at

time point 3 which then increased to 3% at time point 4. The presence of symptoms over time in relation to nutrition can be seen in Figure 5.8.

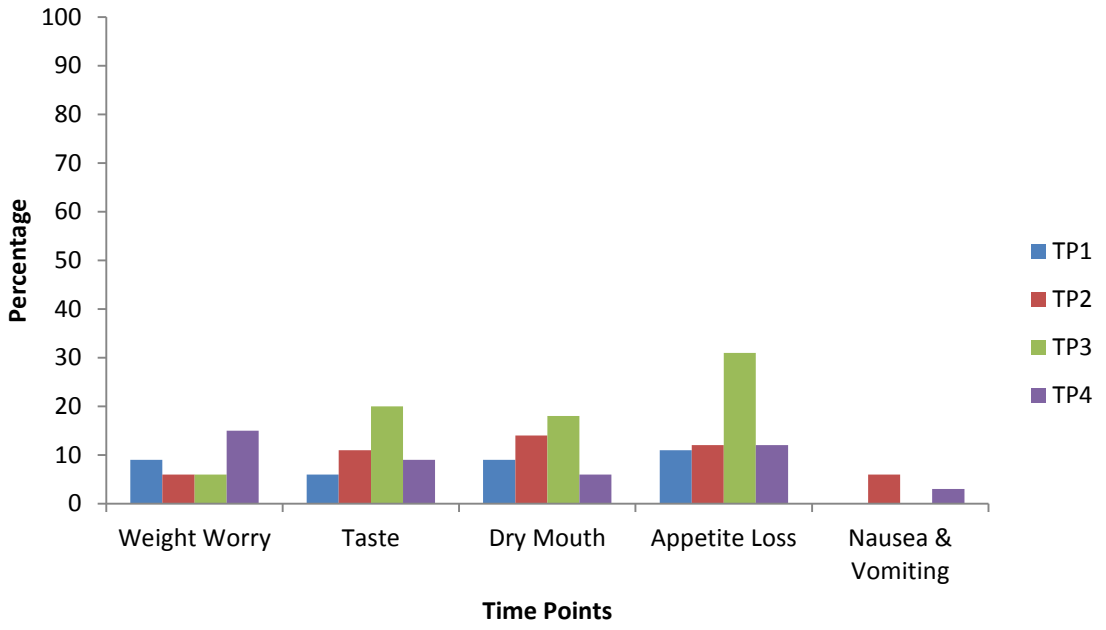

Figure 5.8: Percentage of rectal cancer patients (n=35) with high nutritional symptom scores while receiving radiotherapy over a 6 week period

5.4.4 Changes in Pain over Time

The symptoms of general pain, buttock pain and abdominal pain were examined to determine issues with pain during radiotherapy. All symptoms in relation to pain demonstrated significant changes over time.

Mean scores for general pain were 14.94 (SD 20.09) at time point 1, increased to 21.84 (SD 20.94) at time point 2, increased further to 34.48 (SD 30.19) at time point 3 and then fell to 17.24 (SD 18.08) at time point 4. Regarding this symptom, there was a significant effect for time, Wilks' Lambda = 0.53, F (3, 26) = 7.75, p<0.01, multivariate partial eta squared = 0.47. Post-hoc tests using Bonferroni correction revealed a change between general pain scores at time point 1 (14.94) and time point 3 (34.48), time point 2 (21.84) and time point 3 (34.48),

as well as time point 3 (34.48) and time point 4 (17.24), which was statistically significant (p=0.001; p=0.01; p=0.001), with this symptom worsening between time points 1, 2 and 3, and then improving at time point 4. These changes were also clinically significant, with a score difference of 19.54 noted between time point 1 and time point 3, 12.64 time point 2 and time point 3, and 17.24 between time point 3 and time point 4. Mean scores for buttock pain were 18.18 (SD 25.13) at time point 1, increased to 34.34 (SD 26.93) at time point 2, increased further to 46.46 (SD 36.27) at time point 3 and then fell to 16.16 (SD 22.24) at time point 4. Similarly, there was a significant effect for time in relation to buttock pain, Wilks' Lambda = 0.42, F (3, 30) = 13.84, p<0.01, multivariate partial eta squared = 0.58. Post-hoc tests using Bonferroni correction revealed a change between buttock pain scores at time point 1 (18.18) and time point 2 (34.34), time point 1 (18.18) and time point 3 (46.46), time point 2 (34.34) and time point 4 (16.16), as well as time point 3 (46.46) and time point 4 (16.16), which was statistically significant (p=0.003; p<0.001; p=0.004; p<0.001), with this symptom worsening between time points 1, 2 and 3, and then improving at time point 4. These changes were also clinically significant, with a score difference of 16.16 noted between time point 1 and time point 2, 28.28 between time point 1 and time point 3, and 18.18 between time point 2 and time point 4. In relation to abdominal pain, mean scores were 13.13 (SD 18.52) at time point 1, increased to 23.23 (SD 22.80) at time point 2, fell slightly to 19.19 (SD 27.68) at time point 3 and fell further to 12.12 (SD 21.76) at time point 4. There was also a significant effect for time regarding abdominal pain, Wilks' Lambda = 0.70, F (3, 30) = 4.30, p=0.01, multivariate partial eta squared = 0.30. Bonferroni correction revealed a change between abdominal pain scores at time point 1 (13.13) and time point 2 (23.23), which was statistically significant (p<0.001), with this symptom worsening between these time points. These changes were also clinically significant, with a score difference of 10.10

noted between time point 1 and time point 2. The changes over time for pain can be seen below in Figure 5.9.

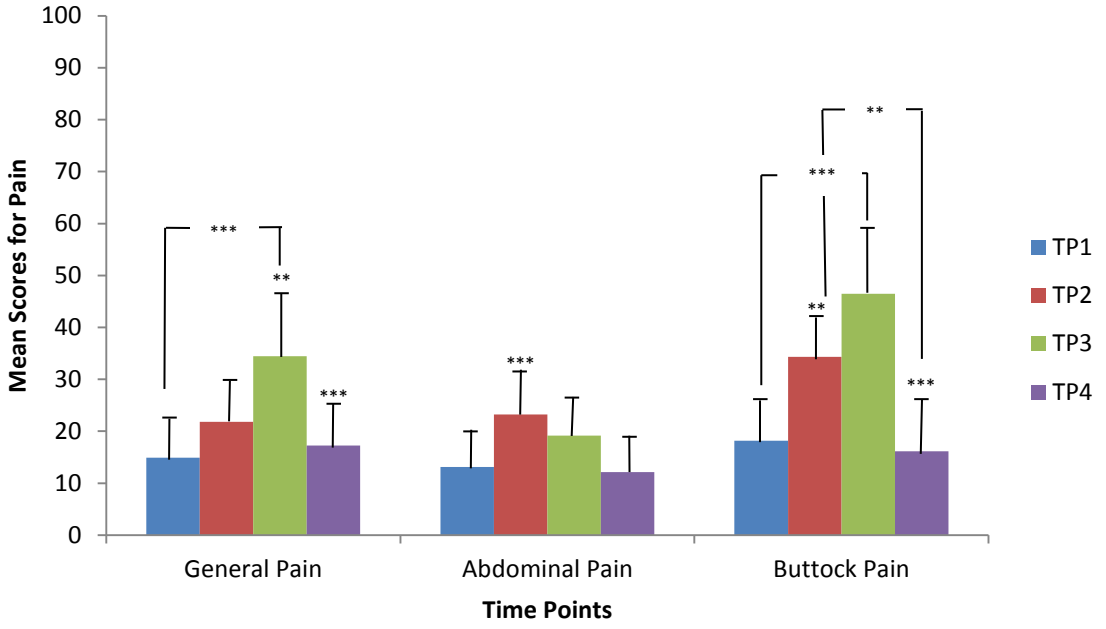

Figure 5.9: Changes in pain over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

$^{**}p<0.01$ $^{***}p<0.001$

The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for general pain was 8.6% at time point 1, which increased to 15.2% at time point 2, followed by an increase to 32.4% at time point 3 which then a decrease to 12.1% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for abdominal pain was 2.9% at time point 1, which increased to 11.4% at time point 2, followed by an increase to 14.3% at time point 3 which then decreased to 9.1% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for buttock pain was 8.6% at time point 1, which increased to 20% at time point 2, followed by a further

increase to 48.6% at time point 3 which then decreased to 9.1% at time point 4. The presence of symptoms over time in relation to pain can be seen in Figure 5.10.

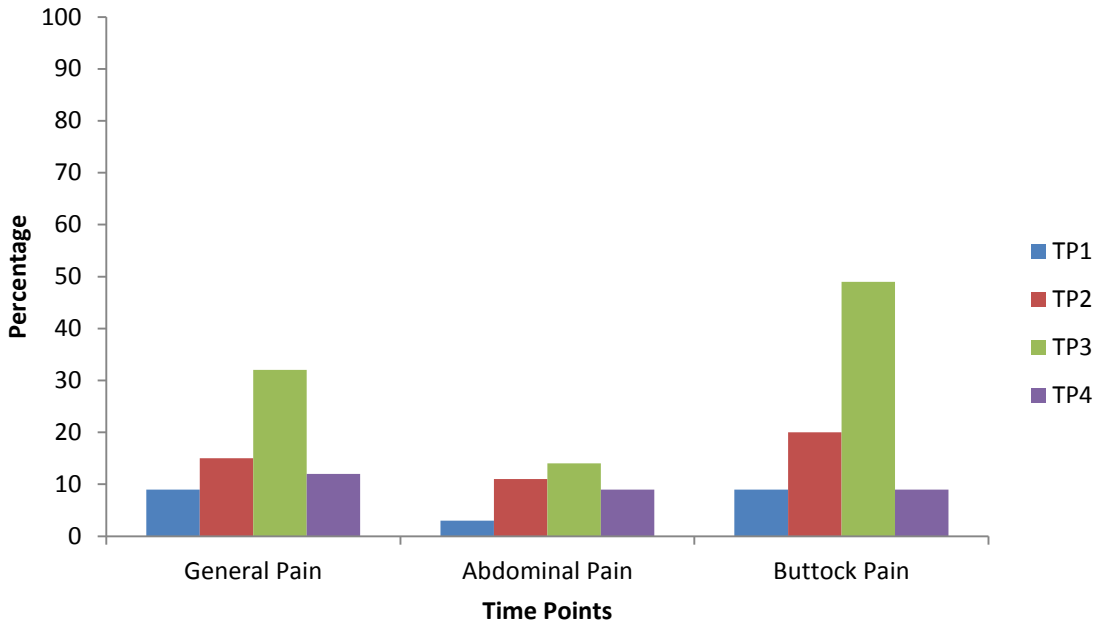

Figure 5.10: Percentage of rectal cancer patients (n=35) with high pain scores while receiving radiotherapy over a 6 week period

5.4.5 Changes in Dermatological Issues over Time

Regarding changes over time, mean scores for sore skin were 11.46 (SD 21.77) at time point 1, increased to 35.41 (SD 30.45) at time point 2, increased further to 47.91 (SD 33.80) at time point 3 and then fell to 18.75 (SD 22.30) at time point 4. There was a significant effect for time for sore skin, Wilks' Lambda = 0.47, F (3, 29) = 10.77, p<0.01, multivariate partial eta squared = 0.53. Post-hoc tests using Bonferroni correction revealed a change between sore skin scores at time point 1 (11.46) and time point 2 (35.41), time point 1 (11.46) and time point 3 (47.91), time point 2 (35.41) and time point 3 (47.91), time point 2 (35.41) and time point 4 (18.75), as well as time point 3 (47.91) and time point 4 (18.75), which was statistically significant (p=0.001; p<0.001; p=0.03; p=0.04; p<0.001), with this symptom worsening at time points 1, 2 and 3, and then improving at time point 4. These changes were

also clinically significant, with a score difference of 23.95 noted between time point 1 and time point 2, 36.45 between time point 1 and time point 3, 12.5 between time point 2 and time point 3, 16.66 between time point 2 and time point 4, and 29.16 between time point 3 and time point 4. The changes over time for dermatological issues can be seen below in Figure 5.11.

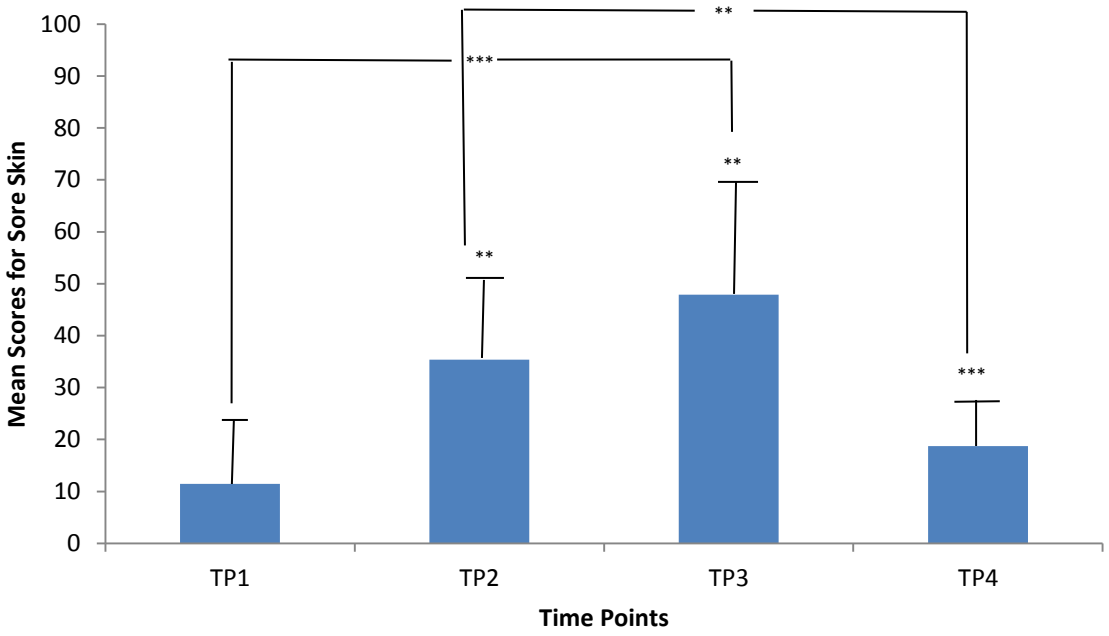

Figure 5.11: Changes in sore skin over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

$^{**}p<0.01$ $^{***}p<0.001$

The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for sore skin was 5.7% at time point 1, which increased to 31.4% at time point 2, followed by a further increase to 51.4% at time point 3 which then decreased to 9.4% at time point 4. The presence of sore skin over time can be seen in Figure 5.12.

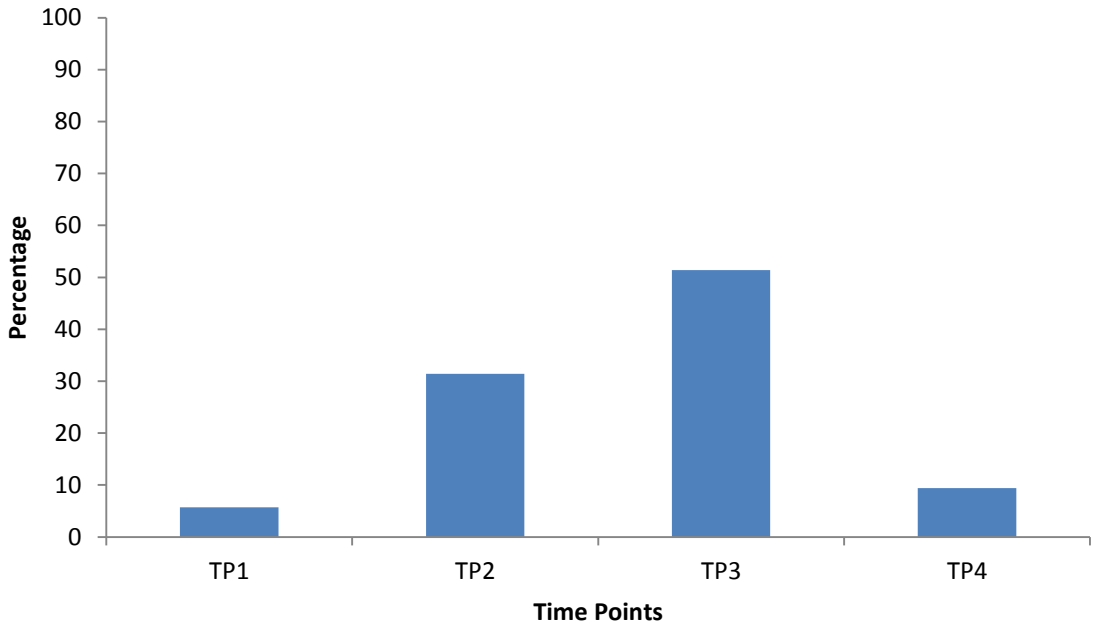

Figure 5.12: Percentage of rectal cancer patients (n=35) with high sore skin scores while receiving radiotherapy over a 6 week period

5.4.6 Changes in Urinary Function over Time

The symptoms of dysuria, urinary frequency and urinary incontinence were examined to determine issues with urinary function during radiotherapy. All symptoms demonstrated significant changes over time.

Mean scores for dysuria were 0.00 (SD 0.00) at time point 1, increased to 7.53 (SD 14.17) at time point 2, increased further to 27.96 (SD 31.15) at time point 3 and then decreased to 2.15 (SD 8.32) at time point 4. Regarding this symptom, there was a significant effect for time, Wilks' Lambda = 0.49, F (3, 28) = 9.71, p<0.01, multivariate partial eta squared = 0.51. Post-hoc tests using Bonferroni correction revealed a change between dysuria scores at time point 1 (0.00) and time point 2 (7.53), time point 1 (0.00) and time point 3 (27.96), time point 2 (7.53) and time point 3 (27.96), as well as time point 3 (27.96) and time point 4 (2.15), which was statistically significant (p=0.03; p<0.001; p=0.01; p=0.001), with this symptom

worsening at time points 1, 2 and 3 and then improving at time point 4. These changes were also clinically significant, with a score difference of 7.53 noted between time point 1 and time point 2, 27.96 between time point 1 and time point 3, 20.43 between time point 2 and time point 3, and 29.16 between time point 3 and time point 4. In relation to urinary frequency, mean scores were 33.33 (SD 18.57) at time point 1, increased to 48.88 (SD 22.72) at time point 2, increased further to 61.11 (SD 26.02) at time point 3 and then fell to 34.44 (SD 19.04) at time point 4. Similarly, there was a significant effect for time in relation to urinary frequency, Wilks' Lambda = 0.39, F (3, 27) = 13.83, p<0.01, multivariate partial eta squared = 0.61. Post-hoc tests using Bonferroni correction revealed a change between urinary frequency scores at time point 1 (33.33) and time point 3 (61.11), time point 2 (48.88) and time point 3 (61.11), time point 2 (48.88) and time point 4 (34.44), as well as time point 3 (61.11) and time point 4 (34.40), which was statistically significant (p<0.001; p=0.02; p=0.02; p<0.001), with this symptom worsening at time points 1, 2 and 3, and then improving at time point 4. These changes were also clinically significant, with a score difference of 27.78 noted between time point 1 and time point 3, 12.23 between time point 2 and time point 3, 14.44 between time point 2 and time point 4, and 26.71 between time point 3 and time point 4. Mean scores for urinary incontinence were 2.15 (SD 8.32) at time point 1, remained at 2.15 (SD 15.05) at time point 2, increased to 15.05 (SD 22.51) at time point 3 and then fell to 9.68 (SD 21.42) at time point 4. A significant effect for time was also noted in relation to urinary incontinence, Wilks' Lambda = 0.68, F (3, 28) = 4.46, p<0.05, multivariate partial eta squared = 0.32. Post-hoc tests using Bonferroni correction revealed a change between urinary incontinence scores at time point 1 (2.15) and time point 3 (15.05), as well as time point 2 (2.15) and time point 3 (15.05), which was statistically significant (p=0.01; p=0.03), with this symptom worsening between these time points. These changes were also clinically significant, with a score difference of 12.9 noted between time point 1 and time point 3, and

12.9 between time point 2 and time point 3. The changes for urinary function over time can be seen in Figure 5.13.

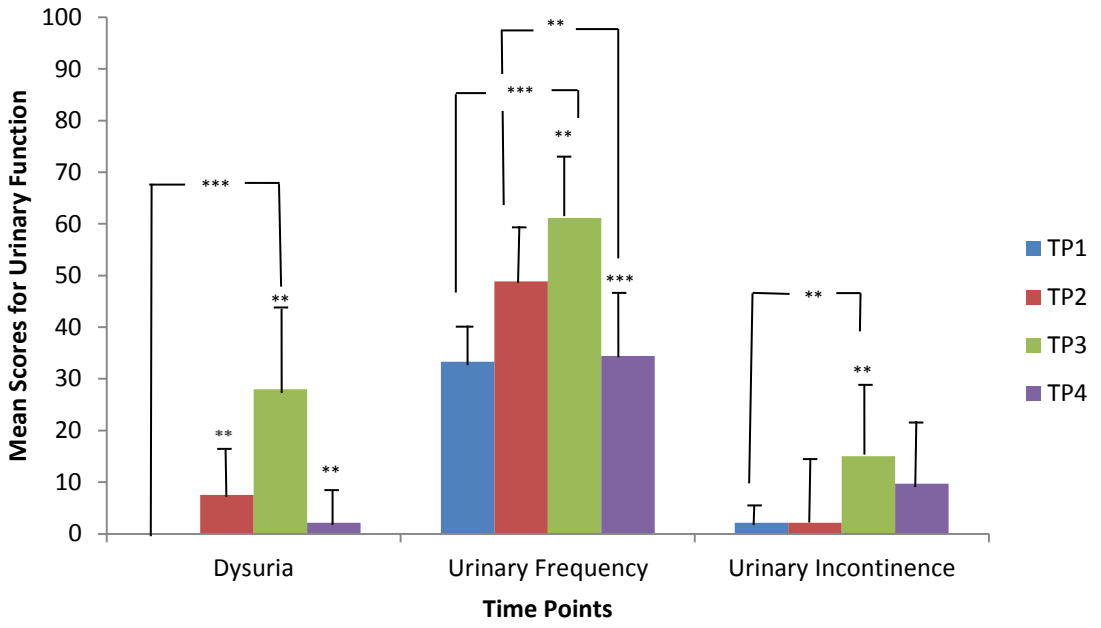

Figure 5.13: Changes in urinary function over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

** $p<0.01$ *** $p<0.001$

The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for dysuria was 0% at time point 1, remained at 0% at time point 2, increased to 21.2% at time point 3 and then returned to 0% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for urinary frequency was 31.4% at time point 1, which increased to 57.6% at time point 2, then increased further to 65.7% at time point 3, followed by a decrease to 36.4% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for urinary incontinence was 0% at time point 1, remained at 0% at time point 2, then increased to 8.6% at time point 3 and 9.4% at time point 4. The presence of symptoms over time in relation to urinary function can be seen in Figure 5.14.

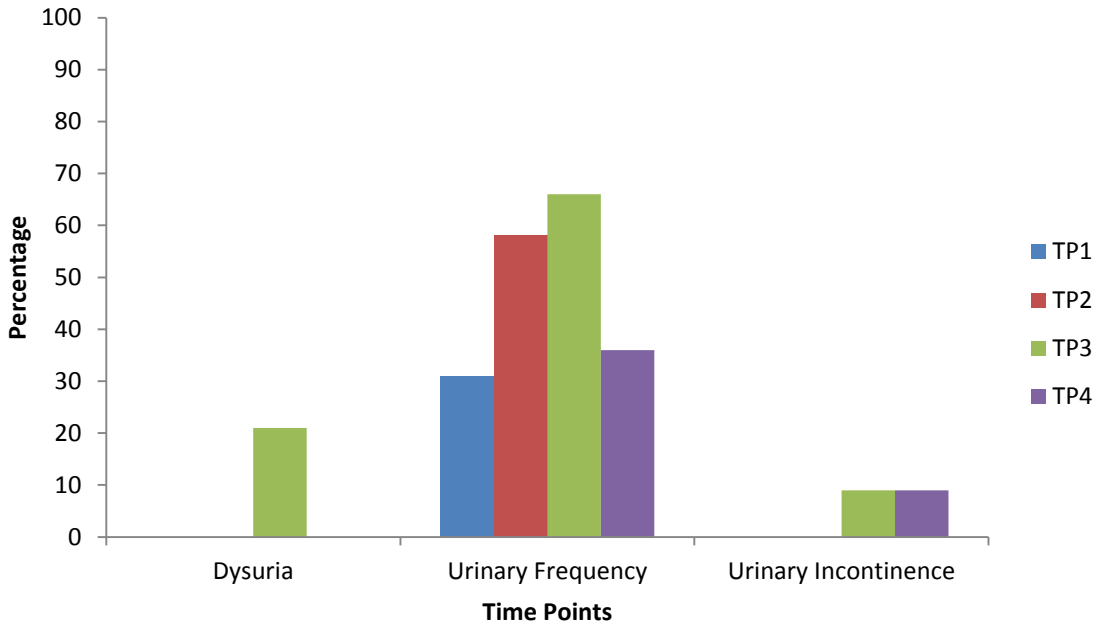

Figure 5.14: Percentage of rectal cancer patients (n=35) with high urinary function symptom scores while receiving radiotherapy over a 6 week period

5.4.7 Changes in Sexual Function over Time

Sexual interest, impotence and dyspareunia were examined to determine issues with sexual function during radiotherapy. There was a very low response rate to questions in relation to dyspareunia (n = 3) and therefore, analysis of this data was omitted. All symptoms showed no significant changes over time.

Mean scores for sexual interest were 29.63 (SD 26.69) at time point 1, decreased to 25.92 (SD 23.27) at time point 2 and then increased to 27.16 (SD 22.72) at time point 3, followed by a further increase to 30.86 (SD 24.33) at time point 4. Regarding sexual interest, there was no significant effect for time, Wilks' Lambda = 0.92, F (3, 24) = 0.66, p=0.59, multivariate partial eta squared = 0.08. In relation to impotence, mean scores were 31.11 (SD 29.46) at time point 1, increased to 37.78 (SD 35.34) at time point 2, increased further to 39.99 (SD 38.21) at time point 3 and then decreased to 31.11 (SD 36.66) at time point 4.

Similarly, there was no significant effect for time for this symptom, Wilks' Lambda = 0.79, F (3, 12) = 1.08, p=0.39, multivariate partial eta squared = 0.21. The changes for sexual function over time can be seen in Figure 5.15.

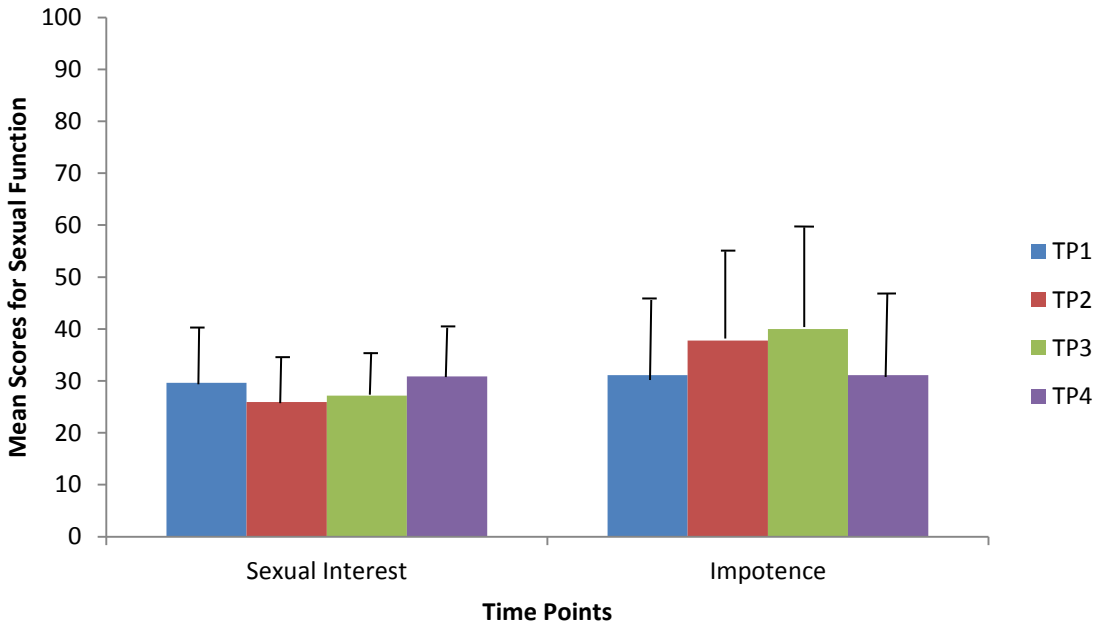

Figure 5.15: Changes in sexual function over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for sexual interest was 25.8% at time point 1, which decreased to 11.8% at time point 2, remained at 11.8% at time point 3, and then increased to 19.4% at time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for impotence was 20% at time point 1, which increased to 33% at time point 2, increased further to 34.8% at time point 3, and then decreased to 26.3% at time point 4.The presence of symptoms over time in relation to sexual function can be seen in Figure 5.16.

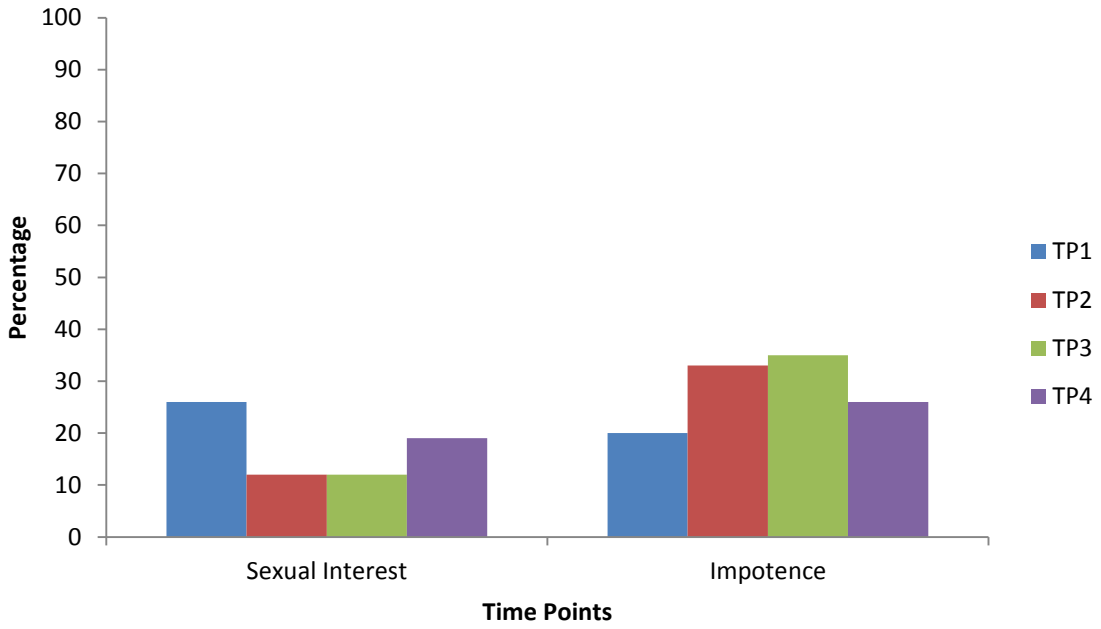

Figure 5.16: Percentage of rectal cancer patients (n=35) with more severe sexual function symptom scores while receiving radiotherapy over a 6 week period

A summary of changes in symptoms over time can be seen in Table 5.5.

Table 5.5: Changes in symptoms over time

Symptom	Time Point 1	Time Point 2	Time Point 3	Time Point 4
Fatigue				
- FACIT F*	**40.67 (SD 11.98)**	↓ 38.86 (SD 12.02)	**↓ 35.94 (SD 14.06)**	↑ 40.35 (SD 11.44)
- QLQ-C30*	**20.55 (SD 24.89)**	**↑ 32.59 (SD 24.40)**	**↑ 35.56 (SD 30.80)**	↓ 27.40 (SD 22.17)
% Patients > 50	***5.7%***	***18.2%***	***32.4%***	***6.1%***
Bowel Function				
Constipation	14.94 (SD 24.54)	↑ 17.24 (SD 21.12)	↑ 19.54 (SD 22.74)	19.54 (SD 24.42)
Diarrhoea*	31.11 (SD 28.95)	↑ 37.78 (SD 27.31)	**37.78 (SD 32.44)**	**↓ 19.99 (SD 28.50)**
% Patients > 50			***26.5%***	***9.1%***
Flatulence	31.30 (SD 31.11)	↑ 37.37 (SD 33.08)	↑ 39.39 (SD 33.80)	↓ 27.27 (SD 30.57)
Bloating	15.56 (SD 24.34)	↑ 18.89 (SD 25.79)	↑ 21.11 (SD 23.95)	↓ 15.56 (SD 24.34)
Blood & Mucous*	**23.23 (SD 27.30)**	↓ 19.70 (SD 18.84)	**↑ 25.25 (SD 25.39)**	**↓ 10.10 (SD 14.99)**
% Patients > 50	***11.4%***		***17.1%***	***6.1%***
Faecal Incontinence*	12.12 (SD 23.30)	↑ 15.15 (SD 18.80)	**↑ 23.23 (SD 29.44)**	**↓ 10.10 (SD 17.65)**
% Patients > 50			***14.3%***	***3%***
Stool Frequency*	**29.57 (SD 22.65)**	**↑ 34.41 (SD 23.54)**	**↑ 44.62 (SD 27.69)**	**↓ 24.19 (SD 19.64)**
% Patients > 50	***25.7%***	***37.1%***	***51.4%***	***18.8%***
Nutrition				
Appetite Loss*	**13.33 (SD 27.12)**	**↑ 22.22 (SD 26.74)**	**↑ 41.11 (SD 39.81)**	**↓ 19.99 (SD 29.81)**
% Patients > 50	***11.4%***	***12.1%***	***31.4%***	***12.1%***
Weight Worry	12.50 (SD 21.99)	↑ 11.45 (SD 21.77)	↑ 16.67 (SD 23.95)	16.67 (SD 25.40)
Taste*	**8.33 (SD 18.93)**	**↑ 18.74 (SD 26.69)**	↑ 22.92 (SD 32.17)	↓ 14.58 (SD 25.31)
% Patients > 50	***5.7%***	***11.4%***		
Nausea & Vomiting*	**3.89 (SD 7.17)**	↑ 11.11 (SD 15.98)	**↓ 10.56 (SD 11.97)**	↓ 6.11 (SD 14.17)
% Patients > 50	***0%***		***0%***	
Dry Mouth*	18.28 (SD 25.59)	20.43 (SD 23.85)	**↑ 23.65 (SD 26.10)**	**↓ 12.90 (SD 18.61)**
% Patients > 50			***17.6%***	***6.1%***
Pain				
General Pain*	**14.94 (SD 20.09)**	**↑ 21.84 (20.94)**	**↑ 34.48 (SD 30.19)**	**↓ 17.24 (SD 18.08)**
% Patients > 50	***8.6%***	***15.2%***	***32.4%***	***12.1%***
Abdominal Pain*	**13.13 (SD 18.82)**	**↑ 23.23 (SD 22.80)**	↓ 19.19 (SD 27.68)	↓ 12.12 (SD 21.76)
% Patients > 50	***2.9%***	***11.4%***		
Buttock Pain*	**18.18 (SD 25.13)**	**↑ 34.34 (SD 26.98)**	**↑ 46.46 (SD 36.27)**	**↓ 16.16 (SD 22.24)**
% Patients > 50	***8.6%***	***20%***	***48.6%***	***9.1%***
Dermatological Issues				
Sore Skin*	**11.46 (SD 21.77)**	**↑ 35.41 (SD 30.45)**	**↑ 47.91 (SD 33.80)**	**↓ 18.75 (SD 22.30)**
% Patients > 50	***5.7%***	***31.4%***	***51.4%***	***9.4%***
Urinary Function				
Dysuria*	**0**	**↑ 7.53 (SD 14.17)**	**↑ 27.96 (SD 31.15)**	**↓ 2.15 (SD 8.32)**
% Patients > 50	***0%***	***0%***	***21.2%***	***0%***
Urinary Frequency*	**33.33 (SD 18.57)**	**↑ 48.88 (SD 22.72)**	**↑ 61.11 (SD 26.02)**	**↓ 34.44 (SD 19.04)**
% Patients > 50	***31.4%***	***57.6%***	***65.7%***	***36.4%***
Urinary Incontinence*	**2.15 (SD 8.32)**	**2.15 (SD 8.32)**	**↑ 15.05 (SD 22.51)**	↓ 9.68 (SD 21.42)
% Patients > 50	***0%***	***0%***	***8.6%***	
Sexual Function				
Sexual Interest	29.63 (SD 26.69)	↓ 25.92 (SD 23.27)	↑ 27.16 (SD 22.72)	↑ 30.86 (SD 24.33)
Impotence	31.11 (SD 29.46)	↑ 37.78 (SD 35.34)	↑ 39.99 (SD 38.21)	↓ 31.11 (SD 36.66)

* Significant change over time – time points that demonstrated change denoted in **red**

5.5 Correlation between Fatigue, Haemoglobin and Other Symptoms

The relationships between fatigue, as measured by the EORTC fatigue subscale, haemoglobin and other symptoms were examined using Spearman's Rank order correlation co-efficient at each time point. The strength of these relationships is determined using criteria as recommended by Cohen (1988) which included small (0.10 -0.29), medium (0.30 -0 .49) and large (0.50 – 1.0) ranges. There was no correlation between fatigue and haemoglobin levels. Table 5.6 identifies the strength of the correlation between fatigue and other symptoms related to bowel function, nutrition, pain, dermatological issues, urinary function and sexual function.

5.5.1 Correlation between Fatigue and Symptoms Related to Bowel Function

Fatigue was correlated with the symptoms of constipation, diarrhoea, flatulence, bloating, blood and mucous in stool, faecal incontinence and stool frequency. All correlations were positive indicating that an increase in symptom presentation was associated with an increase in levels of fatigue.

At time point 1, there were no large correlations noted. Statistically significant medium correlations occurred for constipation ($p<0.01$), flatulence ($p<0.05$), bloating ($p<0.05$) and stool frequency ($p<0.05$). Small correlations that did not reach statistical significance were noted in relation to diarrhoea, blood and mucous in stool and faecal incontinence.

At time point 2, there were no large correlations noted. A medium correlation occurred for diarrhoea ($p<0.05$). Small correlations were associated with constipation, flatulence, bloating, faecal incontinence and stool frequency. Non-significant results occurred for blood and mucous in stool. Statistical significance was reached for diarrhoea.

At time point 3 there was a large correlation for stool frequency ($p<0.01$). Medium correlations occurred for constipation ($p<0.05$), flatulence ($p<0.05$), bloating ($p<0.05$) and blood and mucous in stool ($p<0.05$). A small correlation was noted in relation to diarrhoea and faecal incontinence. Statistical significance was reached for constipation, flatulence, bloating, blood and mucous in stool and stool frequency.

At time point 4 a large correlation was noted for bloating ($p<0.01$). A medium correlation occurred for flatulence ($p<0.05$). Small correlations were noted in relation to constipation, diarrhoea, faecal incontinence and stool frequency. There was a non-significant result for blood and mucous in stool. Statistical significance was reached for flatulence and bloating.

5.5.2 Correlation between Fatigue and Symptoms Related to Nutrition

Fatigue was correlated with the symptoms of appetite loss, weight worry and nausea and vomiting. All correlations were positive indicating that an increase in symptom presentation was associated with an increase in levels of fatigue.

At time point 1, there were no large correlations. Medium correlations occurred for appetite loss ($p<0.01$), weight worry ($p<0.01$) and nausea and vomiting ($p<0.05$), with statistical significance reached for all symptoms.

At time point 2, a large correlation was noted for appetite loss ($p<0.01$), a medium correlation occurred for nausea and vomiting and there was a small correlation for weight worry.

At time point 3 statistically significant large correlations occurred for appetite loss and nausea and vomiting ($p<0.01$). There was a small correlation for weight worry.

At time point 4, there was a medium correlation for appetite loss ($p<0.05$) and nausea and vomiting ($p<0.05$). There was a small correlation in relation to weight worry. Statistical significance was reached for nausea and vomiting and appetite loss.

5.5.2 Correlation between Fatigue and Symptoms Related to Pain

Fatigue was correlated with the symptoms of general pain, abdominal pain and buttock pain. All correlations were positive indicating that an increase in symptom presentation was associated with an increase in levels of fatigue.

At time point 1, there was a large correlation for general pain ($p<0.01$), a medium correlation for buttock pain ($p<0.01$) and a small correlation for abdominal pain. Statistical significance was reached for general and buttock pain.

At time point 2, there was a large correlation for general pain ($p<0.01$), a medium correlation for buttock pain ($p<0.05$), and a small correlation for abdominal pain. Statistical significance was reached for general and buttock pain. At time point 3, large statistically significant correlations occurred for general, and buttock pain ($p<0.01$). A statistically significant medium correlation was noted for abdominal pain ($p<0.01$).

At time point 4, there was a statistically significant medium correlation for general pain ($p<0.05$) and small correlations occurred for abdominal and buttock pain.

5.5.3 Correlation between Fatigue and Symptoms Related to Dermatological Issues

Fatigue was correlated with the symptom of sore skin. Correlations at time point 4 were negative but results were non-significant. All other correlations were positive indicating that an increase in symptom presentation was associated with an increase in levels of fatigue.

At time point 1, time point 2 and time point 4, the correlation between fatigue and sore skin was non-significant. At time point 3, there was a small correlation. No correlation was statistically significant.

5.5.4 Correlation between Fatigue and Symptoms Related to Urinary Function

Fatigue was correlated with the symptoms of dysuria, urinary frequency and urinary incontinence. The correlation for urinary frequency at time point 2 was negative (-0.003), and yielded a non-significant result. All other correlations were positive indicating that an increase in symptom presentation was associated with an increase in levels of fatigue.

At time point 1, there were no statistically significant correlations. Dysuria was associated with a non-significant result and small correlations occurred for urinary frequency and urinary incontinence.

At time point 2, similarly, no statistically significant correlations are reported. Non-significant results occurred for dysuria and urinary frequency and there was a small correlation for urinary incontinence.

At time point 3, there was a medium correlation for urinary frequency ($p<0.01$). Small correlations occurred for dysuria and urinary incontinence.

At time point 4, there were no statistically significant correlations. A medium correlation occurred for dysuria and small correlations were associated with urinary frequency and urinary incontinence.

5.5.5 Correlation between Fatigue and Symptoms Related to Sexual Function

Fatigue was correlated with the symptoms of sexual interest and impotence. The correlations for sexual interest were all negative, indicating that lower levels of sexual interest were associated with higher levels of fatigue. In relation to impotence, all results are positive, indicating that an increase in symptom presentation was associated with an increase in levels of fatigue.

At time point 1, there were small correlations between fatigue, sexual interest and impotence. At time point 2, a medium correlation was noted in relation to sexual interest ($p<0.05$) and a small correlation occurred for impotence. At time point 3 there were small correlations for sexual interest and impotence and at time point 4, medium correlations were evident for both, with sexual interest reaching statistical significance ($p<0.05$).

Table 5.6: Correlations between fatigue & other symptoms

Symptom	Time Point 1	Time Point 2	Time Point 3	Time Point 4
Bowel Function				
Constipation	0.477**	0.265	0.422*	0.216
Diarrhoea	0.125	0.440*	0.299	0.135
Flatulence	0.385*	0.207	0.360*	0.370*
Bloating	0.347*	0.163	0.389*	0.618**
Blood & Mucous	0.195	0.011	0.346*	0.059
Faecal Incontinence	0.194	0.157	0.236	0.272
Stool Frequency	0.392*	0.178	0.620**	0.148
Nutrition				
Appetite Loss	0.478**	0.574**	0.734**	0.368**
Weight Worry	0.406*	0.273	0.269	0.148
Nausea & Vomiting	0.416*	0.302	0.730**	0.440*
Pain				
General Pain	0.590**	0.543**	0.676**	0.369*
Abdominal Pain	0.238	0.208	0.497**	0.275
Buttock Pain	0.491**	0.401*	0.581**	0.178
Dermatological Issues				
Sore Skin	0.047	0.088	0.278	-0.070
Urinary Function				
Dysuria	0	0.070	0.165	0.300
Urinary Frequency	0.260	-0.003	0.478**	0.160
Urinary Incontinence	0.252	0.258	0.242	0.203
Sexual Function				
Sexual Interest	-0.149	-0.368*	-0.201	-0.385*
Impotence	0.104	0.256	0.284	0.364

** $p<0.01$
* $p<0.05$

5.6 Changes in QoL and Functioning over Time

To examine the changes in QoL and functioning over time, RM-ANOVA was conducted to compare changes at time point 1 (pre-treatment), time point 2 (midway through treatment), time point 3 (on completion of treatment) and time point 4 (4-6 weeks after completion of treatment).

5.6.1 Changes in Global QoL over Time

Mean scores for global QoL were 72.31 at time point 1 (SD 26.65), fell to 66.13 (SD 18.63) at time point 2, remained at 66.13 (SD 26.43) at time point 3 and then increased to 71.77 (SD 19.68) at time point 4. There was no significant effect for time, Wilks' Lambda = 0.81, F (3, 28) = 2.09, p=0.12, multivariate partial eta squared = 0.84. The changes for global QoL can be seen in Figure 5.17.

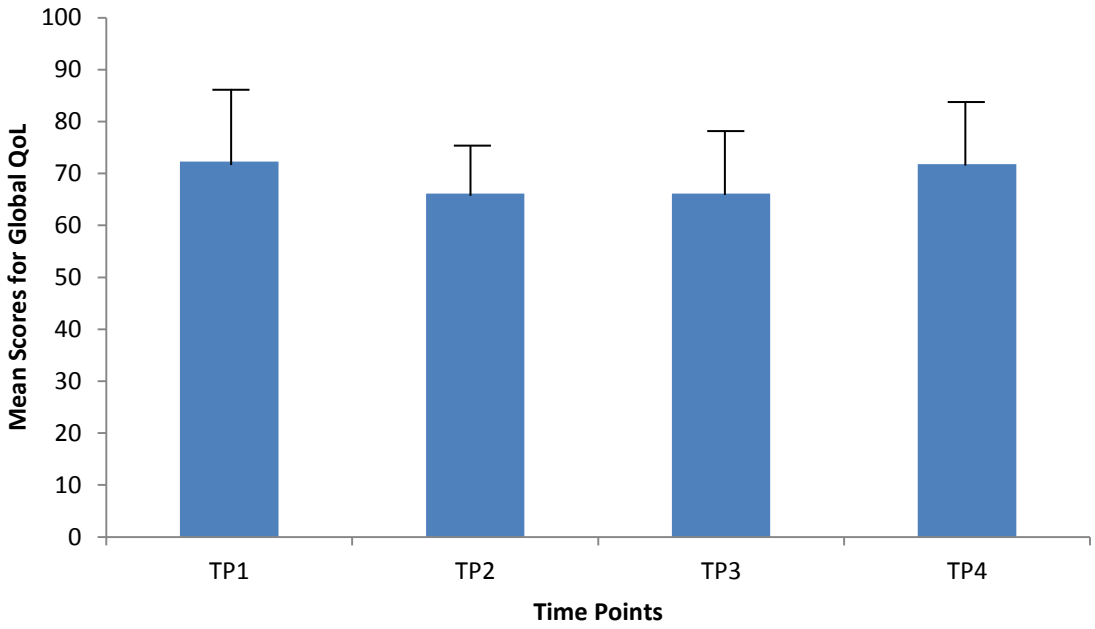

Figure 5.17: Changes in global QoL over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

5.6.2 Changes in Cognitive Functioning over Time

Mean scores for cognitive function were 88.02 (SD 21.27) at time point 1, decreased to 82.29 (SD 23.93) at time point 2, fell further to 79.69 (SD 29.85) at time point 3 and then increased to 85.94 (SD 19.91) at time point 4. There was no significant effect for time for cognitive function, Wilks' Lambda = 0.82, F (3, 29) = 2.15, p=0.12, multivariate partial eta squared = 0.18. The changes for cognitive functioning over time can be seen below in Figure 5.18.

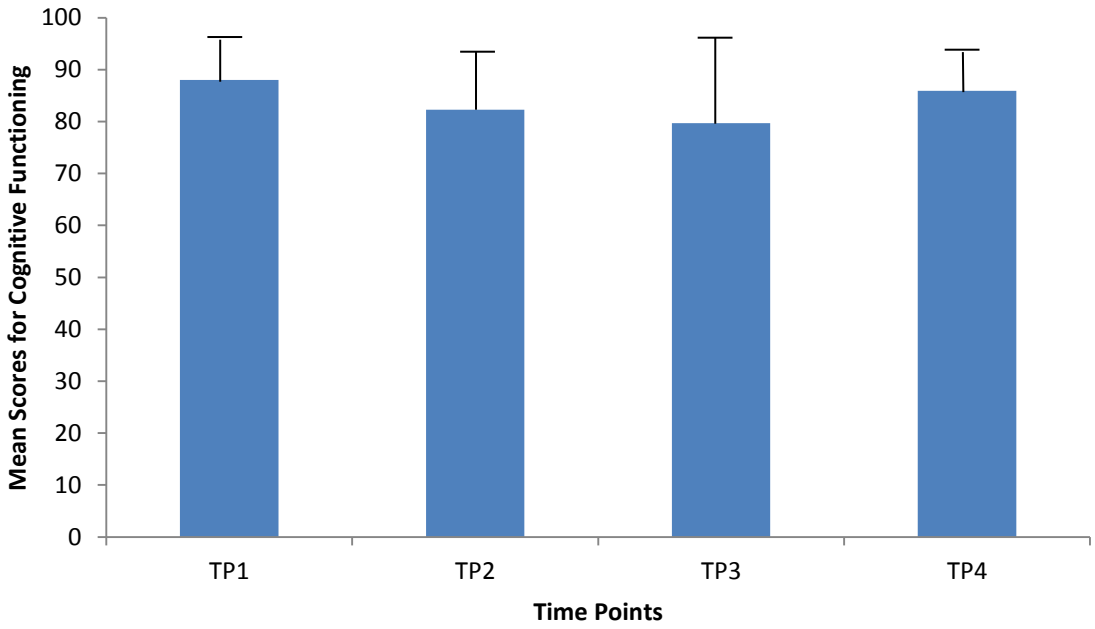

Figure 5.18: Changes in cognitive functioning over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

5.6.3 Changes in Emotional Functioning over Time

Emotional function, anxiety, body image, insomnia and embarrassment were examined to determine issues with emotional functioning during radiotherapy. All symptoms showed no significant changes over time.

In relation to emotional function, mean scores were 78.49 (SD 22.23) at time point 1 and increased slightly to 78.50 (SD 23.45) at time point 2, followed by decrease to 76.62 (SD 24.47) at time point 3 and then increased slightly to 78.49 (SD 19.93) at time point 4. Regarding this, there was no significant effect for time, Wilks' Lambda = 0.99, F (3, 2) = 0.14, p=0.94, multivariate partial eta squared = 0.01. Mean scores for anxiety were 38.38 (SD 29.01) at time point 1 and decreased slightly to 36.36 (SD 31.58) at time point 2, increased to 39.39 (SD 32.76) at time point 3 and remained at 39.39 (SD 31.68) at time point 4. Similarly, levels of anxiety also demonstrated no significant effect for time, Wilks'

Lambda = 0.99, *F* (3, 30) = 0.14, *p*=0.93, multivariate partial eta squared = 0.01. Mean scores for body image were 89.93 (SD 16.30) at time point 1, fell to 88.55 (SD 18.60) at time point 2, were 86.63 (SD 18.39) at time point 3 and then increased slightly to 88.54 (SD 15.83) at time point 4. There was no significant effect for time noted in relation to body image, Wilks' Lambda = 0.95, *F* (3, 29) = 0.53, *p*=0.66, multivariate partial eta squared = 0.05. In relation to insomnia, mean scores were 27.78 (SD 26.38) at time point 1, increased to 33.33 (SD 29.03) at time point 2, increased further to 37.78 (SD 31.24) at time point 3 and then fell to 33.33 (SD 29.03) at time point 4. Similarly, there was no significant effect for time in relation to this symptom, Wilks' Lambda = 0.90, *F* (3, 27) = .1.01, *p*=0.40, multivariate partial eta squared = 0.10. Mean scores for embarrassment were 22.22 (SD 31.91) at time point 1, fell to 21.21 (SD 24.75) at time point 2, increased to 30.30 (SD 32.66) at time point 3 and then fell again to 23.33 (SD 22.80) at time point 4. Regarding embarrassment, as with all other scales, no significant effect for time was noted, Wilks' Lambda = 0.85, *F* (3, 30) = 1.74, *p*=0.18, multivariate partial eta squared = 0.15. The changes for emotional functioning over time can be seen in Figure 5.19.

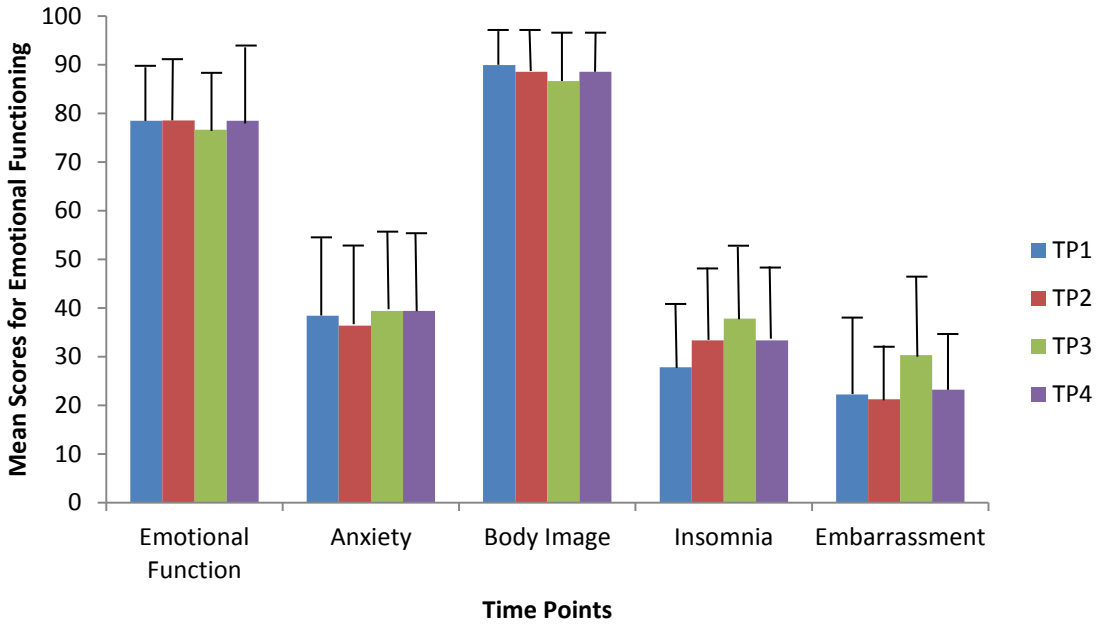

Figure 5.19: Changes in emotional functioning over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

5.6.4 Changes in Physical Functioning over Time

For physical function, mean scores were 86.44 (SD 18.39) at time point 1, fell slightly to 84.60 (SD 17.38) at time point 2, fell further to 80.23 (SD 22.50) at time point 3 and then increased to 85.52 (SD 17.37) at time point 4. There was no significant effect for time, Wilks' Lambda = 0.88, F (3, 26) = 1.23, p=0.32, multivariate partial eta squared = 0.12. The changes for physical functioning over time can be seen in Figure 5.20.

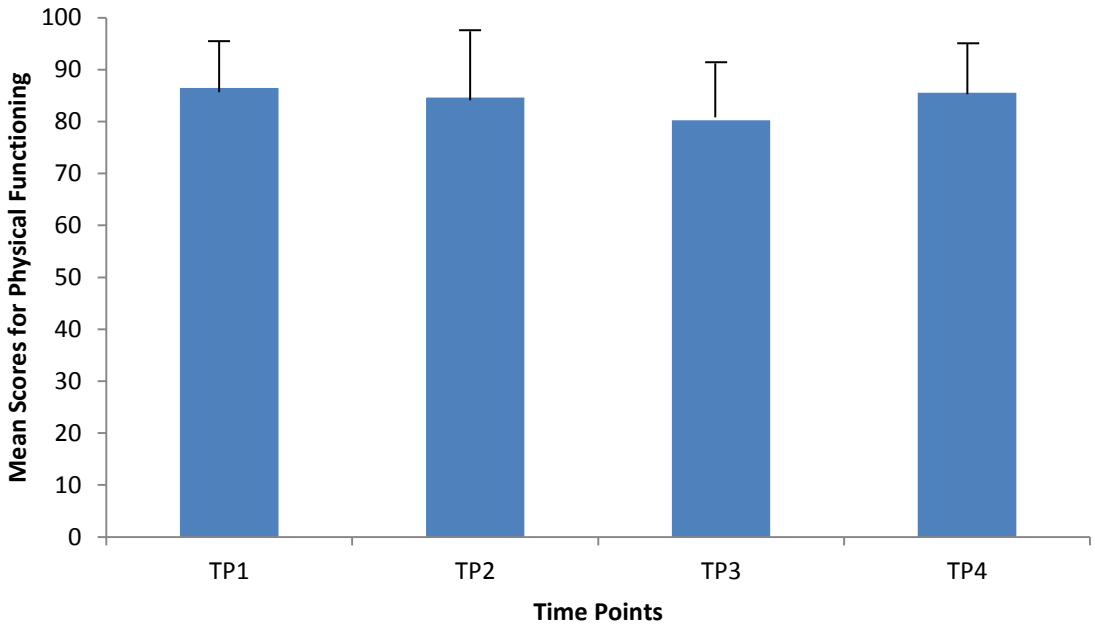

Figure 5.20: Changes in physical functioning over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

5.6.5 Changes in Role Functioning over Time

Mean scores for role function were 80.46 (SD 28.55) at time point 1, fell to 77.01 (SD 25.36) at time point 2, fell further to 60.92 (SD 34.00) at time point 3 and then increased to 77.01 (SD 24.56) at time point 4. There was a significant effect for time, Wilks' Lambda = 0.57, F (3, 26) = 6.16, p<0.01, multivariate partial eta squared = 0.43. Post-hoc tests using Bonferroni correction revealed a change between role function scores at time point 1 (80.46) and time point 3 (60.92), time point 2 (77.01) and time point 3 (60.92), as well as time point 3 (60.92) and time point 4 (77.01), which was statistically significant (p=0.003; p=0.02; p=0.002), with this symptom worsening at time points 1, 2 and 3, and then improving at time point 4. The changes for role functioning over time can be seen in Figure 5.21.

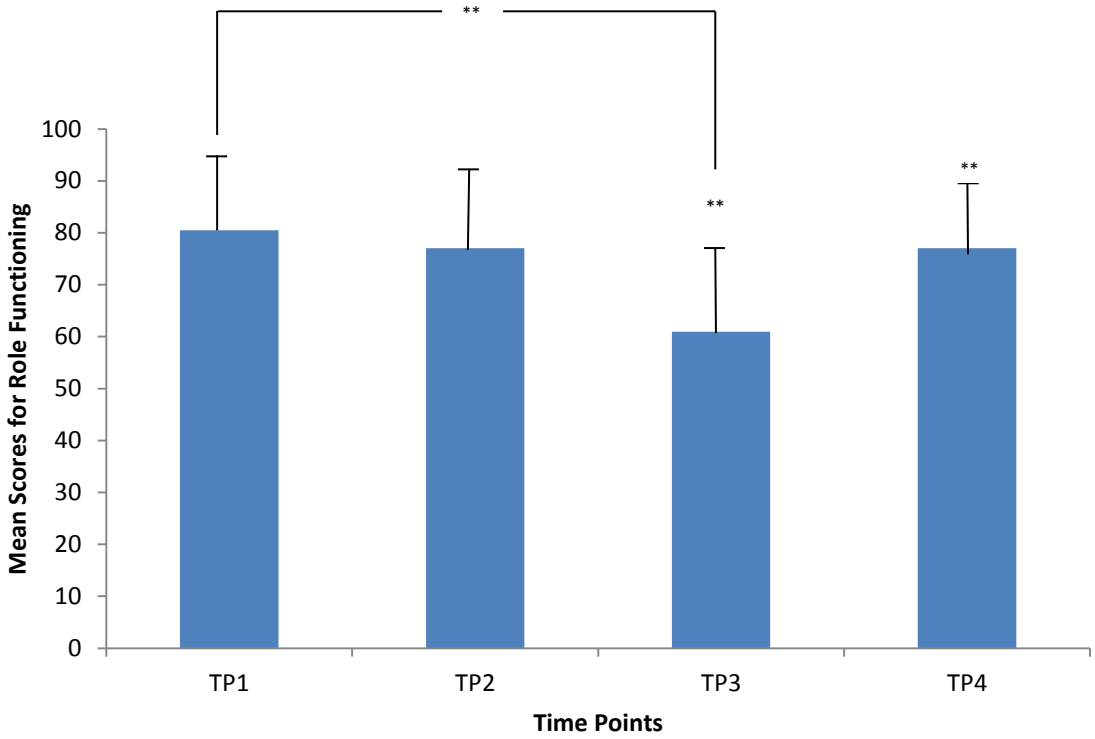

Figure 5.21: Changes in role functioning over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

** $p<0.01$

5.6.6 Changes in Social Functioning over Time

Regarding social function, mean scores were 76.34 (SD 27.81) at time point 1, was 76.88 (SD 22.64) at time point 2, fell further to 70.97 (SD 28.53) at time point 3 and then increased to 76.34 (SD 23.48) at time point 4. There was no significant effect for time, Wilks' Lambda = 0.92, F (3, 28) = 0.80, p=0.51, multivariate partial eta squared = 0.08. The changes for social functioning over time can be seen in Figure 5.22.

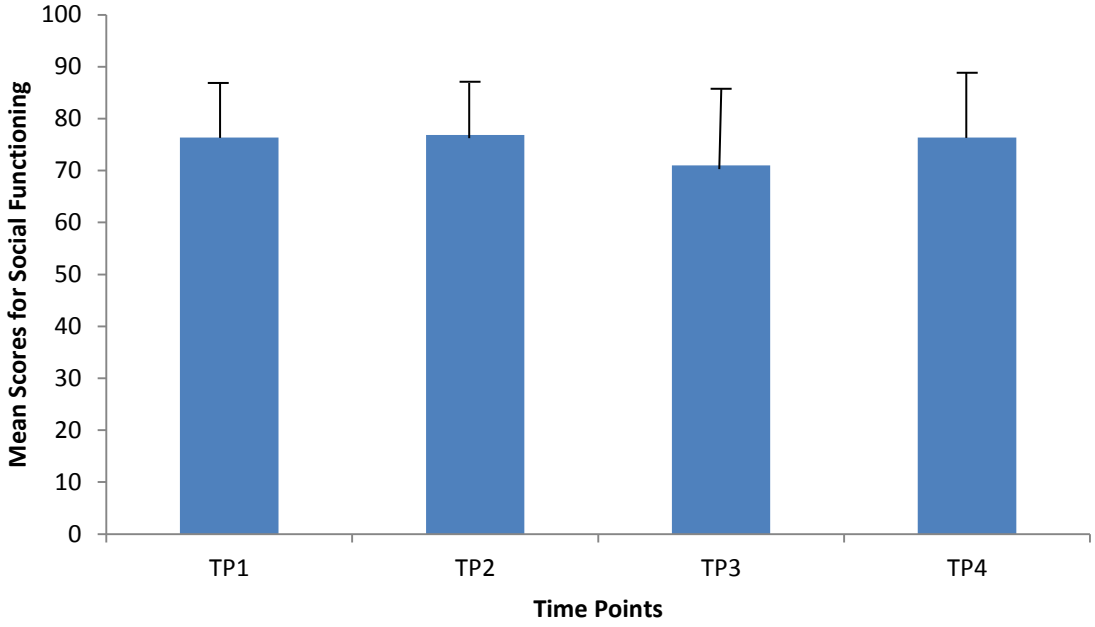

Figure 5.22: Changes in social functioning over a period of 6 weeks in rectal cancer patients (n=35) receiving radiotherapy

A summary of changes in global QoL and functioning can be seen in Table 5.7

Table 5.7: Changes in global QoL & functioning over time

Function	Time Point 1	Time Point 2	Time Point 3	Time Point 4
Cognitive Function	88.02 (SD 21.27)	↓ 82.29 (SD 23.92)	↓ 79.69 (SD 29.85)	↑ 85.94 (SD 19.91)
Emotional Function	78.49 (SD 22.23)	↑ 78.50 (SD 23.45)	↓ 76.62 (SD 24.47)	↑ 78.49 (SD 19.93)
Anxiety	38.38 (SD 29.01)	36.36 (SD 31.58)	↑ 39.39 (SD 32.76)	↑ 39.39 (SD 31.68)
Body Image	89.93 (SD 16.30)	↓ 88.55 (SD 18.60)	↓ 86.63 (SD 18.39)	↑ 88.54 (SD 15.83)
Insomnia	27.78 (SD 26.38)	↑ 33.33 (SD 29.03)	↑ 37.78 (SD 31.24)	↓ 33.33 (SD 29.03)
Embarrassment	22.22 (SD 31.91)	↓ 21.21 (SD 24.75)	↑ 30.30 (SD 32.66)	↓ 23.23 (SD 22.80)
Physical Function	86.44 (SD 18.39)	↓ 84.60 (SD 17.38)	↓ 80.23 (SD 22.50)	↑ 85.52 (SD 17.37)
Role Function*	**80.46 (SD 28.55)**	**↓ 77.01 (SD 25.36)**	**↓ 60.92 (SD 34.00)**	**↑ 77.01 (SD 24.56)**
Social Function	76.34 (SD 27.81)	↓ 76.88 (SD 22.64)	↓ 70.97 (SD 28.35)	↑ 76.34 (SD 23.48)
Global QoL	72.31 (SD 26.65)	↓ 66.13 (SD 18.63)	66.13 (SD 26.43)	↑ 71.77 (SD 19.68)

* Significant change over time – time points that demonstrated change denoted in **red**

5.7 Correlation between Symptoms, QoL and Functioning

The relationships between symptoms and patients' QoL and levels of functioning were examined using Spearman's Rank order correlation co-efficient at each time point. The strength of these relationships is determined using criteria as recommended by Cohen (1988) which included small (0.10 - 0.29), medium (0.30 - 0 .49) and large (0.50 – 1.0) ranges.

5.7.1 Fatigue

The strength of correlations between the symptoms of fatigue, QoL and functioning across the 4 time points is identified in Table 5.8. The strength of the relationships were small (0.10 – 0.29), medium (0.30 - 0.49) and large (0.50 – 1.0).

In relation to the FACIT-F scale, higher scores were associated with lower levels of fatigue, thereby explaining the positive correlations, as when scores are higher, quality of life and functioning increases. The majority of correlations between fatigue, as measured by the FACIT-F, QoL and functioning were large and statistically significant (p<0.01), with the exception of role and social functioning at time point 1, which both demonstrated non-significant medium correlations.

In relation to the fatigue subscale of the EORTC QLQ C-30, higher scores indicate higher fatigue levels. Therefore, all correlations reported are negative, as higher fatigue scores are associated with lower QoL and levels of functioning. The majority of correlations were large and statistically significant (p<0.01), except for global QoL at time point 1, which demonstrated a medium correlation (p<0.05). Significant medium correlations were also evident in relation to cognitive functioning (p<0.01) and social functioning at time point 4 (p<0.05). A medium correlation occurred for emotional functioning at time point 1, which

did not reach statistical significance, and non-significant small correlations were also evident for role and social functioning at time point 1.

Table 5.8: Correlations between fatigue, QoL & functioning

Symptom		GQoL	Cognitive	Emotional	Physical	Role	Social
FACIT-F	TP1	0.650**	0.635**	0.572**	0.666**	0.324	0.325
(Fatigue)	TP2	0.654**	0.571**	0.527**	0.661**	0.714**	0.682**
	TP3	0.874**	0.800**	0.673**	0.857**	0.739**	0.644**
	TP4	0.819**	0.707**	0.559**	0.785**	0.748**	0.600**
EORTC	TP1	-0.400*	-0.529**	-0.309	-0.558**	-0.225	-0.235
(Fatigue)	TP2	-0.614**	-0.625**	-0.577**	-0.737**	-0.647**	-0.693**
	TP3	-0.820**	-0.777**	-0.638**	-0.908**	-0.721**	-0.626**
	TP4	-0.680**	-0.495**	-0.583**	-0.716**	-0.625**	-0.402*

** $p<0.01$

* $p<0.05$

5.7.2 Bowel Function

The strength of correlations between the symptoms in relation to bowel function, QoL and functioning across the 4 time points is identified in Table 5.9. Symptoms measured include constipation, diarrhoea, flatulence, bloating, blood and mucous in stool, faecal incontinence and stool frequency. In the majority of cases, a negative correlation is reported, indicating that higher scores on the symptom scales are associated with lower QoL and functioning scores. Exceptions to this include diarrhoea and role functioning at time point 1, blood and mucous in stool and cognitive and emotional functioning at time point 2, as well as stool frequency and emotional function at time point 4, where non-significant results occurred.

In relation to global QoL, there were large correlations between this and constipation at time point 1 ($p<0.01$) and time point 3 ($p<0.01$), bloating at time point 1 ($p<0.01$) and time point 4 ($p<0.01$), and stool frequency at time point 3 ($p<0.01$). All correlations were statistically significant. Medium correlations were noted between global QoL and diarrhoea at time point

3, flatulence at time point 4, bloating at time point 3 (p<0.05), blood and mucous in stool at time point 1 (p<0.05), and stool frequency at time point 1 (p<0.05). Statistical significance was reached for bloating and blood and mucous in stool and stool frequency. Small correlations occurred between global QoL and constipation at time point 2 and time point 4, diarrhoea at time point 1 and time point 2, flatulence at time point 1, time point 2 and time point 3, bloating at time point 2, blood and mucous in stool time point 3 and time point 4, faecal incontinence at all time points, and stool frequency at time point 2 and time point 4. Correlations were non-significant in relation to diarrhoea at time point 4 and blood and mucous in stool at time point 2.

Regarding cognitive functioning, a large, statistically significant correlation was noted between this and stool frequency at time point 3 (p<0.01). Medium correlations between cognition and constipation were reported at time point 2 (p<0.05) and time point 3, for diarrhoea at time point 2 (p < 0.05) and time point 3, for bloating at time point 1 (p<0.05) and time point 4 (p < 0.01), for blood and mucous in stool at time point 1 (p<0.05), for faecal incontinence at time point 3 and time point 4, and for stool frequency at time point 1 (p<0.05). Small correlations were seen for constipation at time point 1, diarrhoea at time point 1, flatulence at all time points, bloating at time point 2 and time point 3, blood and mucous in stool at time point 3 and time point 4, and stool frequency at time point 2 and time point 4. Non-significant results were noted in relation to constipation at time point 4, diarrhoea at time point 4, blood and mucous in stool at time point 2, and faecal incontinence at time point 1 and time point 2.

In relation to emotional functioning, no large correlations between any symptoms are reported. Medium correlations were seen between this and constipation at time point 1,

diarrhoea at time point 2 (p<0.05) and stool frequency at time point 2 and time point 3 (p<0.05). Statistical significance was reached for diarrhoea at time point 2 and stool frequency at time point 3. Small correlations occurred with constipation at time point 2 and time point 3, diarrhoea at time point 1 and time point 3, flatulence at time point 1, time point 2 and time point 3, bloating at time point 2, time point 3 and time point 4, blood and mucous at time point 1, time point 2 and time point 3, and faecal incontinence at time point 3. Non-significant results were seen in relation to emotional functioning and constipation at time point 4, diarrhoea at time point 4, flatulence at time point 4, bloating at time point 1, blood and mucous in stool at time point 4, faecal incontinence at time point 1, time point 2 and time point 4, and stool frequency at time point 1 and time point 4.

Regarding physical functioning, large correlations were noted between this and bloating at time point 4 (p<0.01) and for stool frequency at time point 3 (p<0.01). Medium correlations occurred with constipation at time point 3 (p<0.05) and time point 4, diarrhoea at time point 2 (p<0.05), bloating at time point 3 (p<0.01), blood and mucous in stool at time point 3 (p<0.05), and stool frequency at time point 4. Small correlations were noted for constipation at time point 1, diarrhoea at time point 1, time point 3 and time point 4, flatulence at time point 1, time point 3 and time point 4, bloating at time point 1, blood and mucous in stool at time point 1, faecal incontinence at all time points, and stool frequency at time point 1 and time point 2. Non-significant results occurred for constipation at time point 2, flatulence at time point 2, bloating at time point 2, and blood and mucous in stool at time point 2 and time point 4.

In relation to role functioning, large correlations were noted between this and diarrhoea at time point 2 (p<0.01) and for stool frequency at time point 3 (p<0.01). Medium correlations

occurred for constipation at time point 1 and time point 4 ($p<0.05$), diarrhoea at time point 3, flatulence at time point 4 ($p<0.05$), bloating at time point 3 ($p<0.05$) and time point 4 ($p<0.01$), and stool frequency at time point 2 ($p<0.05$). Small correlations were evident for constipation time point 2 and time point 3, diarrhoea at time point 4, flatulence at time point 1, time point 2 and time point 3, bloating at time point 1 and time point 2, blood and mucous in stool at all time points, faecal incontinence at time point 1, time point 2 and time point 4 and stool frequency at time point 1 and time point 4. Non-significant results occurred for diarrhoea at time point 1 and faecal incontinence at time point 3.

Regarding social functioning, large correlations were observed for diarrhoea at time point 2 ($p<0.01$) and stool frequency at time point 1 ($p<0.01$). Medium correlations were noted for constipation at time point 1 ($p<0.05$) and time point 3, diarrhoea at time point 3 ($p<0.05$), bloating at time point 3 ($p<0.05$) and time point 4 and stool frequency at time point 2 ($p<0.05$) and time point 3 ($p<0.01$). Small correlations occurred for constipation at time point 2 and time point 4, diarrhoea at time point 1, flatulence at time point 1 and time point 4, bloating at time point 1 and time point 2, blood and mucous in stool at time point 4, faecal incontinence at time point 1, time point 2 and time point 4 and stool frequency at time point 4. Non-significant results were noted for diarrhoea at time point 4, flatulence at time point 2 and time point 3, blood and mucous in stool at time point 1, time point 2 and time point 3, and faecal incontinence at time point 3.

Table 5.9: Correlations between bowel function, QoL & functioning

Symptom		GQoL	Cognitive	Emotional	Physical	Role	Social
Constipation	TP1	-0.456**	-0.121	-0.311	-0.208	-0.306	-0.350*
	TP2	-0.272	-0.419*	-0.228	-0.067	-0.277	-0.233
	TP3	-0.570**	-0.318	-0.114	-0.390*	-0.251	-0.315
	TP4	-0.254	-0.056	-0.050	-0.302	-0.380*	-0.214
Diarrhoea	TP1	-0.264	-0.258	-0.129	-0.256	0.058	-0.218
	TP2	-0.116	-0.376*	-0.373*	-0.344*	-0.569**	-0.669**
	TP3	-0.336	-0.325	-0.282	-0.263	-0.304	-0.431*
	TP4	-0.068	-0.084	-0.066	-0.235	-0.217	-0.072
Flatulence	TP1	-0.195	-0.218	-0.155	-0.168	-0.161	-0.152
	TP2	-0.154	-0.228	-0.248	-0.093	-0.235	-0.047
	TP3	-0.130	-0.251	-0.232	-0.237	-0.267	-0.017
	TP4	-0.310	-0.133	-0.053	-0.226	-0.357*	-0.210
Bloating	TP1	-0.468**	-0.427*	-0.058	-0.256	-0.288	-0.117
	TP2	-0.146	-0.155	-0.148	-0.086	-0.190	-0.217
	TP3	-0.342*	-0.271	-0.145	-0.456**	-0.419*	-0.435*
	TP4	-0.547**	-0.450**	-0.232	-0.556**	-0.461**	-0.314
Blood & Mucous	TP1	-0.390*	-0.418*	-0.160	-0.226	-0.147	-0.089
	TP2	-0.078	0.067	0.183	-0.070	-0.156	-0.024
	TP3	-0.107	-0.265	-0.144	-0.348*	-0.221	-0.032
	TP4	-0.118	-0.210	-0.048	-0.096	-0.251	-0.196
Faecal Incontinence	TP1	-0.191	-0.065	-0.057	-0.283	-0.188	-0.256
	TP2	-0.250	-0.033	-0.016	-0.187	-0.131	-0.149
	TP3	-0.138	-0.318	-0.180	-0.189	-0.065	-0.085
	TP4	-0.254	-0.343	-0.003	-0.299	-0.233	-0.232
Stool Frequency	TP1	-0.375*	-0.427*	-0.062	-0.168	-0.149	-0.504**
	TP2	-0.205	-0.275	-0.311	-0.146	-0.376*	-0.426*
	TP3	-0.610**	-0.560**	-0.345*	-0.519**	-0.552**	-0.481**
	TP4	-0.108	-0.231	0.033	-0.340	-0.260	-0.213

** $p<0.01$

* $p<0.05$

5.7.3 Nutrition

In relation to nutrition, Table 5.10 identifies the strength of correlations between these symptoms, QoL and functioning across the 4 time points. Symptoms measured include appetite loss, weight worry, taste, nausea and vomiting and dry mouth. In most cases, a negative correlation is reported, indicating that higher scores on the symptom scales are associated with lower QoL and functioning scores. Exceptions to this include the correlation between taste with role and social functioning at time point 1 which were .039 (non-significant) and .250 (small) respectively.

In relation to global QoL, there were large correlations between this and appetite loss across all the 4 time points ($p<0.01$), for weight worry at time point 1 ($p<0.01$), for nausea and vomiting at time point 3 ($p<0.01$), and for dry mouth at time point 4 ($p<0.01$), all of which were statistically significant. Medium correlations were observed for taste at time point 3 ($p<0.05$) and time point 4 ($p<0.05$), as well as nausea and vomiting at time point 1 ($p<0.05$) and time point 4 ($p<0.05$). Small correlations occurred for weight worry at time point 3 and time point 4, taste at time point 1 and time point 2, nausea and vomiting at time point 2 and dry mouth at time point 1 and time point 2. Non-significant findings were noted for weight worry at time point two.

Regarding cognitive function large correlations occurred with appetite loss at time point 1 ($p<0.01$), time point 2 ($p<0.05$) and time point 3 ($p<0.01$), weight worry at time point 1 ($p<0.01$), and nausea and vomiting at time point 3 ($p<0.01$), with all correlations demonstrating statistical significance. Medium correlations were noted in relation to appetite loss at time point 4 ($p<0.05$); weight worry at time point 2; taste at time point 2 ($p<0.05$) and time point 3 ($p<0.05$); nausea and vomiting at time point 1, time point 2 ($p<0.05$) and time

point 4 (p<0.05) and dry mouth at time point 3 (p<0.05) and time point 4 (p<0.05). Small correlations occurred for weight worry at time point 3 and time point 4, for taste at time point 1 and time point 4, and for dry mouth at time point 1. There was a non-significant correlation between cognitive function and dry mouth at time point 2.

In relation to emotional functioning, large correlations were noted for appetite loss at time point 1 (p<0.01). Medium correlations occurred for appetite loss at time point 2 (p<0.01), time point 3 (p<0.01) and time point 4 (p<0.01), weight worry at time point 1 (p<0.05), time point 2 (p<0.01), time point 3 (p<0.05) and time point 4 (p<0.05), and for nausea and vomiting at time point 2 (p<0.05), time point 3 (p<0.01) and time point 4 (p<0.05). Small correlations were observed for taste at all time points, for nausea and vomiting at time point 1 and for dry mouth at all time points.

Regarding physical functioning, large correlations were observed for appetite loss at time point 1 (p<0.01), time point 2 and time point 3 (p<0.01), and for nausea and vomiting at time point 3 (p<0.01), all of which were statistically significant. Medium correlations occurred for appetite loss at time point 4 (p<0.01), for taste at time point 1 (p<0.01), time point 2 (p<0.01) and time point 3 (p<0.05), for nausea and vomiting at time point 4 (p<0.05) and for dry mouth at time point 1, time point 3 (p<0.05) and time point 4. Statistical significance was reached for appetite loss, taste at time point 1, time point 2 and time point 3, nausea and vomiting and for dry mouth at time point 3. Small correlations were noted for weight worry at all time points, for taste at time point 4, nausea and vomiting at time point 1 and time point 2 and for dry mouth at time point 2.

In relation to role functioning, large correlations were noted for appetite loss at time point 2 (p<0.01) and time point 3 (p<0.01), nausea and vomiting at time point 3 (p<0.01) and for dry mouth at time point 4 (p<0.01), with all results reaching statistical significance. Medium correlations were observed for appetite loss at time point 1 (p<0.05) and time point 4 (p<0.05); weight worry at time point 2 (p<0.01) and time point 3; taste at time point 2 (p<0.05); nausea and vomiting at time point 1 (p<0.01), time point 2 and time point 4 (p<0.01) and for dry mouth at time point 1 (p<0.05) and time point 3 (p<0.05). Statistical significance was reached for appetite loss, weight worry at time point 2, taste, nausea and vomiting at time point 1 and time point 4, and for dry mouth. Small correlations occurred for weight worry at time point 1 and time point 4, taste at time point 3 and time point 4, and dry mouth at time point 2. A non-significant correlation was noted for taste at time point 1.

Regarding social functioning, large correlations occurred for appetite loss at time point 2 (p<0.01) and for nausea and vomiting at time point 3 (p<0.01), with both results demonstrating statistical significance. Medium correlations were noted for appetite loss at time point 1 (p<0.05), time point 3 (p<0.01), and time point 4 (p<0.01), for weight worry at time point 1 (p<0.01), time point 2 (p<0.05) and time point 4 (p<0.05), for taste at time point 2 (p<0.01), for nausea and vomiting at time point 2 (p<0.05) and time point 4, and for dry mouth at time point 1 (p<0.05), time point 3 (p<0.05) and time point 4 (p<0.05). Statistical significance was reached for appetite loss, weight worry, taste, for nausea and vomiting at time point 2 and for dry mouth. Small correlations were observed for weight worry at time point 3, taste at time point 1, time point 3 and time point 4 and nausea and vomiting at time point 1. A non-significant result occurred for dry mouth at time point 2.

Table 5.10: Correlations between nutrition, QoL & functioning

Symptom		GQoL	Cognitive	Emotional	Physical	Role	Social
Appetite Loss	TP1	-0.547**	-0.505**	-0.500**	-0.538**	-0.351*	-0.368*
	TP2	-0.603**	-0.676*	-0.494**	-0.503**	-0.510**	-0.566**
	TP3	-0.749**	-0.702**	-0.484**	-0.618**	-0.516**	-0.471**
	TP4	-0.466**	-0.381*	-0.433**	-0.388*	-0.370*	-0.457**
Weight Worry	TP1	-0.540**	-0.542**	-0.404*	-0.129	-0.236	-0.465**
	TP2	-0.025	-0.332	-0.476**	-0.154	-0.454**	-0.426*
	TP3	-0.237	-0.279	-0.364*	-0.149	-0.303	-0.244
	TP4	-0.280	-0.118	-0.397*	-0.271	-0.266	-0.404*
Taste	TP1	-0.101	-0.231	-0.189	-0.449**	0.039	0.250
	TP2	-0.232	-0.391*	-0.299	-0.415**	-0.402*	-0.465**
	TP3	-0.383*	-0.367*	-0.167	-0.396*	-0.179	-0.141
	TP4	-0.402*	-0.273	-0.208	-0.202	-0.139	-0.187
Nausea & Vomiting	TP1	-0.425*	-0.306	-0.222	-0.164	-0.445**	-0.228
	TP2	-0.231	-0.382*	-0.363*	-0.197	-0.318	-0.395*
	TP3	-0.693**	-0.748**	-0.466**	-0.696**	-0.520**	-0.553**
	TP4	-0.403*	-0.347*	-0.369*	-0.408*	-0.455**	-0.338
Dry Mouth	TP1	-0.278	-0.211	-0.158	-0.300	-0.362*	-0.355*
	TP2	-0.112	-0.022	-0.109	-0.241	-0.239	-0.050
	TP3	-0.411*	-0.410*	-0.185	-0.446*	-0.477**	-0.412*
	TP4	-0.537**	-0.399*	-0.243	-0.336	-0.591**	-0.438*

** $p<0.01$ * $p<0.05$

5.7.4 Pain

The strength of correlations between the symptoms in relation to pain, QoL and functioning across the 4 time points is identified in Table 5.11. Symptoms measured include general pain, abdominal pain and buttock pain. In all cases, a negative correlation is reported, indicating that higher scores on the symptom scales are associated with lower QoL and functioning scores.

Regarding global QoL, large correlations were noted for general pain at time point 3 ($p<0.01$) and buttock pain at time point 3 ($p<0.01$), with statistical significance reached for both results. Medium correlations were observed for general pain at time point 1 ($p<0.01$), time point 2 ($p<0.05$) and time point 4, abdominal pain at time point 1 ($p<0.05$), time point 2

(p<0.05) and time point 3 (p<0.01) and for buttock pain at time point 1 (p<0.05), time point 2 (p<0.05) and time point 4. A small correlation occurred between global QoL and abdominal pain at time point 4.

For cognitive functioning, large correlations were observed for general pain at time point 1 (p<0.01), time point 2 (p<0.01) and time point 3 (p<0.01), and for buttock pain at time point 3 (p<0.01), with statistical significance reached for all results. Medium correlations were noted in relation to general pain at time point 4 (p<0.05), abdominal pain at time point 3 (p<0.05) and time point 4 (p<0.05) and buttock pain at time point 1 (p<0.05), time point 2 (p<0.01) and time point 4 (p<0.05), with statistical significance reached for all results. Small correlations occurred between cognitive functioning and abdominal pain at time point 1 and time point 2.

In relation to emotional functioning, a large, statistically significant correlation occurred for general pain at time point 3 (p<0.01). Medium correlations were noted in relation to general pain at time point 2 and buttock pain at time point 3 (p<0.05), with statistical significance reached in this instance. Small correlations occurred for general pain at time point 1 and time point 4, for abdominal pain at time point 2 and time point 4, and for buttock pain at time point 1 and time point 2. Non-significant results occurred for abdominal pain at time point 1 and time point 3, and buttock pain at time point 4.

Regarding physical functioning, large correlations occurred for general pain at all 4 time points and were statistically significant (p<0.01). Medium correlations occurred for abdominal pain at time point 2 (p<0.05), time point 3 (p<0.01) and time point 4 (p<0.05) and for buttock pain at time point 1 (p<0.05), time point 2, time point 3 (p<0.01) and time point 4

(p<0.05). Statistical significance was reached for abdominal pain and for buttock pain at time point 1 and time point 3. A non-significant result occurred for abdominal pain at time point 1.

For role function, large correlations were seen for general pain at time point 2 (p<0.01) and time point 3 (p<0.01), with results demonstrating statistical significance. Medium correlations were noted for general pain at time point 1 (p<0.01) and time point 4 (p<0.05), abdominal pain at time point 2 and time point 3 (p<0.01) and for buttock pain at time point 1 (p<0.05), time point 2 (p<0.01), time point 3 (p<0.01) and time point 4 (p<0.05). Small correlations were observed for abdominal pain at time point 1 and time point 4.

In relation to social function a large correlation was noted for general pain at time point 3 (p<0.01), which reached statistical significance. Medium correlations occurred for general pain at time point 1 (p<0.05), time point 2 (p<0.05) and time point 4 (p<0.05), abdominal pain at time point 1 (p<0.05) and time point 3 (p<0.05), and buttock pain at time point 1 (p<0.05), time point 2 (p<0.05) and time point 3 (p<0.05), with statistical significance reached for all results. A small correlation occurred for abdominal pain at time point 2 and time point 4, and for buttock pain at time point 4.

Table 5.11: Correlations between pain, QoL & functioning

Symptom		GQoL	Cognitive	Emotional	Physical	Role	Social
General Pain	TP1	-0.493**	-0.533**	-0.275	-0.593**	-0.459**	-0.372*
	TP2	-0.411*	-0.595**	-0.318	-0.573**	-0.512**	-0.411*
	TP3	-0.725**	-0.695**	-0.576**	-0.698**	-0.571**	-0.660**
	TP4	-0.316	-0.409*	-0.183	-0.534**	-0.435*	-0.410*
Abdominal Pain	TP1	-0.353*	-0.105	-0.015	-0.092	-0.249	-0.417*
	TP2	-0.392*	-0.252	-0.186	-0.375*	-0.327	-0.279
	TP3	-0.459**	-0.341*	-0.079	-0.451**	-0.497**	-0.419*
	TP4	-0.295	-0.417*	-0.266	-0.376*	-0.206	-0.236
Buttock Pain	TP1	-0.407*	-0.352*	-0.182	-0.398*	-0.370*	-0.401*
	TP2	-0.378*	-0.449**	-0.219	-0.324	-0.452**	-0.383*
	TP3	-0.686**	-0.504**	-0.383*	-0.480**	-0.439**	-0.348*
	TP4	-0.307	-0.407*	-0.019	-0.349*	-0.371*	-0.230

** $p<0.01$

* $p<0.05$

5.7.5 Dermatological Issues

Regarding dermatological issues, Table 5.12 identifies the strength of correlations between sore skin, QoL and functioning across the 4 time points. In most cases, a negative correlation is reported, indicating that higher scores on the symptom scales are associated with lower QoL and functioning scores. An exception to this is in relation to sore skin and global QoL at time point 1, physical function at time point 2 and role function at time point 1 and time point 4, where non-significant results were noted.

In relation to global QoL and sore skin, there was no large correlation across any of the time points. Medium correlations were noted at time point 2 ($p<0.05$) and time point 3, with a non-significant result occurring at time point 4. For cognitive function, a medium correlation occurred at time point 3, with small correlations occurring for time point 1, time point 2 and time point 4. No statistical significance was reached. In relation to emotional function, a statistically significant medium correlation was observed at time point 3 ($p<0.05$), with small

correlations observed at time point 1 and time point 2 and a non-significant result yielded for time point 4. In the case of physical function, small correlations were noted at time point 1, time point 3 and time point 4, with a non-significant result observed at time point 2. In relation to role function, there was a medium, statistically significant correlation for this and sore skin at time point 3 ($p<0.01$), with results at all other time-points being non-significant. Regarding social function, small correlations were evident at time point 1, time point 3 and time point 4, with time point 2 yielding a non-significant result.

Table 5.12: Correlations between dermatological issues, QoL & functioning

Symptom		GQoL	Cognitive	Emotional	Physical	Role	Social
Sore Skin	TP1	-0.106	-0.268	-0.216	-0.263	-0.006	-0.281
	TP2	-0.403*	-0.195	-0.179	-0.013	-0.032	-0.052
	TP3	-0.321	-0.305	-0.358*	-0.273	-0.478**	-0.253
	TP4	-0.078	-0.160	0.031	-0.238	-0.012	-0.109

** $p<0.01$

* $p<0.05$

5.7.6 Urinary Function

The strength of correlations between the symptoms in relation to urinary function, QoL and functioning across the 4 time points is identified in Table 5.13. Symptoms measured were dysuria, urinary frequency and urinary incontinence. In cases where a negative correlation is reported, higher scores on the symptom scales are associated with lower QoL and functioning scores. In relation to dysuria and all levels of functioning at time points 1 and 2, as well as emotional function at time point 3, where neutral or positive scores are reported, the results are non-significant. Non-significant results also occurred for emotional and role function and urinary incontinence at time point 1. Correlations for urinary frequency with emotional function at time point 4 are positive but very small, which is also the case for urinary

incontinence and physical function at time point 2 and social function at time point 1, with no statistical significance reached in all cases.

Regarding global QoL, no large correlations are reported. There was a statistically significant medium correlation for urinary frequency at time point 1 ($p<0.05$) and time point 3 ($p<0.01$). Small correlations were observed for dysuria at time point 3 and time point 4, urinary frequency for time point 4, and urinary incontinence at time point 1, time point 3 and time point 4. Non-significant results were yielded for dysuria at time point 1 and time point 2 and for urinary frequency and urinary incontinence at time point 2.

In relation to cognitive function, similarly, no large correlations are reported. Medium correlations occurred for dysuria at time point 3 and time point 4 ($p<0.05$), urinary frequency at time point 3 ($p<0.05$) and urinary incontinence at time point 1. Statistical significance was reached for dysuria at time point 4 and urinary frequency at time point 3. Small correlations were observed for urinary frequency at time point 1, time point 2 and time point 4 and for urinary incontinence at time point 2, time point 3 and time point 4. Non-significant results are reported for dysuria at time point 1 and time point 2.

For emotional function, a large statistically significant correlation was noted for urinary frequency at time point 1 ($p<0.01$). Small correlations were observed for dysuria at time point 4, urinary frequency at time point 3 and time point 4, and urinary incontinence at time point 2. Non-significant results occurred for dysuria at time point 1, time point 2 and time point 3, for urinary frequency at time point 2, and for urinary incontinence at time point 1, time point 3 and time point 4.

In the case of physical function, a large statistically significant correlation was observed for urinary frequency at time point 3 (p<0.01). A medium correlation occurred at time point 4 for urinary incontinence. Small correlations were noted for dysuria at time point 3 and time point 4, urinary frequency at time point 1, time point 2 and time point 4 and for urinary incontinence at time point 1, time point 2 and time point 3. A non-significant result is reported for dysuria at time point 1 and time point 2.

Regarding role function, a medium correlation occurred for urinary frequency at time point 2, time point 3 (p<0.01) and time point 4, with statistical significance demonstrated at time point 3. Small correlations were observed for dysuria at time point 3 and time point 4, for urinary frequency at time point 1 and time point 4, and for urinary incontinence at time point 4. Non-significant results are reported in the cases of dysuria at time point 1 and time point 2, and urinary incontinence at time point 1, time point 2 and time point 3.

In relation to social function, a medium correlation occurred for urinary frequency at time point 1, time point 2 and time point 3 (p<0.05). Small correlations were noted for dysuria at time point 3 and time point 4, urinary frequency at time point 4 and for urinary incontinence at time point 1, time point 2 and time point 3. Non-significant results occurred for dysuria at time point 1 and time point 2, and for urinary incontinence at time point 2, time point 3 and time point 4.

Table 5.13: Correlations between urinary function, QoL & functioning

Symptom		GQoL	Cognitive	Emotional	Physical	Role	Social
Dysuria	TP1	0	0	0	0	0	0
	TP2	0	0.038	0.004	-0.070	-0.029	-0.016
	TP3	-0.204	-0.305	0.077	-0.175	-0.200	-0.136
	TP4	-0.213	-0.374*	-0.210	-0.205	-0.276	-0.183
Urinary Frequency	TP1	-0.424*	-0.288	-0.513**	-0.238	-0.230	-0.308
	TP2	-0.073	-0.128	-0.051	-0.150	-0.335	-0.319
	TP3	-0.446**	-0.429*	-0.232	-0.513**	-0.453**	-0.379*
	TP4	-0.143	-0.153	0.134	-0.215	-0.241	-0.228
Urinary Incont.	TP1	-0.247	-0.326	0.081	-0.297	0.079	0.257
	TP2	-0.009	-0.169	-0.256	0.100	-0.095	-0.127
	TP3	-0.234	-0.269	-0.096	-0.267	-0.015	-0.123
	TP4	-0.197	-0.260	-0.022	-0.340	-0.249	-0.068

** $p<0.01$

* $p<0.05$

5.7.7 Sexual Function

The strength of correlations between the symptoms in relation to sexual function, QoL and functioning across the 4 time points is identified in Table 5.14. Symptoms measured include sexual interest and impotence. Scores for dyspareunia were excluded due to low response rates. In all cases regarding sexual interest, a positive correlation is reported, indicating that higher scores are associated with higher QoL and functioning scores. In relation to impotence, a negative correlation is reported in the majority of cases, indicating that higher levels of impotence are associated with lower QoL and functioning scores. Positive and scores are reported in relation to physical function at time point 1 (0.183) and also for emotional function at time point 1 (0.116). However these scores indicate small correlations with impotence and did not reach statistical significance.

In relation to global QoL, a medium correlation occurred for sexual interest at time point 1 ($p<0.05$), time point 2 and time point 4 ($p<0.01$), with statistical significance evident at time

point 1 and time point 4. A medium correlation for impotence was noted at time point 4, although statistical significance was not reached. Small correlations were observed in relation sexual interest at time point 3, and impotence at time point 1, time point 2 and time point 3, and did not reach statistical significance.

Regarding cognitive function, a large, statistically significant correlation occurred for sexual interest at time point 4 ($p<0.01$) and for impotence at time point 3 ($p<0.01$). Medium correlations were noted for sexual interest at time point 1 ($p<0.01$) and for impotence at time point 4. Small correlations that did not reach statistical significance occurred for sexual interest at time point 2 and time point 3, and for impotence at time point 2. A non-significant result was yielded for impotence at time point 1.

For emotional function, there was a large statistically significant correlation for impotence at time point 3 ($p<0.05$). Medium correlations for this symptom are reported at time point 2 and time point 4. There was a small correlation for sexual interest at time point 2, time point 3 and time point 4, and for impotence at time point 1. A non-significant result was yielded for sexual interest at time point 1 and time point 4.

In relation to physical function, a medium correlation occurred for sexual interest at time point 1 and at time point 4 ($p<0.05$), with statistical significance reached at time point 4. Small correlations were observed for sexual interest at time point 3 and impotence at time point 1 and time point 3. Non-significant results were noted for sexual interest at time point 2 and impotence at time point 2 and time point 4.

Regarding role function, a medium correlation was observed for sexual interest and impotence at time point 4. Small correlations were noted for sexual interest at time point 3 and for impotence at time point 2 and time point 3. Non-significant results occurred for sexual interest at time point 1 and time point 2, and for impotence at time point 1.

For social function, a medium correlation was noted for impotence at time point 3 and time point 4. There was a small correlation for sexual interest at time point 2, time point 3 and time point 4 and also, for impotence at time point 2. Non-significant results were noted for sexual interest at time point 1 and for impotence at time point 1.

Table 5.14: Correlations between sexual function, Qol & functioning

Symptom		GQoL	Cognitive	Emotional	Physical	Role	Social
Sexual Interest	TP1	0.411*	0.485**	0.089	0.321	0.077	0.083
	TP2	0.303	0.295	0.162	0.021	0.025	0.156
	TP3	0.280	0.189	0.239	0.146	0.285	0.226
	TP4	0.475**	0.595**	0.128	0.415*	0.308	0.223
Impotence	TP1	-0.111	-0.036	0.116	0.183	-0.040	-0.061
	TP2	-0.199	-0.185	-0.326	0.054	-0.188	-0.256
	TP3	-0.159	-0.579**	-0.511*	0.180	-0.200	-0.315
	TP4	-0.362	-0.452	-0.337	-0.089	-0.434	-0.333

** $p<0.01$

* $p<0.05$

5.8 KRAS and Cytokine Results

KRAS, tumoral IL-6 and IL-8 gene expression levels, and plasma IL-6 and IL-8 protein levels were measured. Results of these measurements are reported, as well as their analysis in relation to the tumour and symptom presentation.

5.8.1 KRAS Analysis Results

Of the 27 patients tested for KRAS status, 18 harboured wild type KRAS and 9 had mutant KRAS. Subtype analysis demonstrated that of these 9 patients, KRAS was mutated at codon 12/13 in 8 cases and at codon 61 in 1 case.

5.8.2 Cytokine Analysis Results

The expression of the cytokines IL-6 and IL-8 were analysed at the gene level in the tumour tissue of 21 patients and at the protein level in the blood plasma of 31 patients.

5.8.2.1 mRNA Purity

Due to fixation and embedding conditions, nucleic acids in FFPE samples are usually heavily fragmented and chemically modified by formaldehyde. The ratio of readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminates. To establish if the RNA was intact and could subsequently be used for gene expression studies, the A_{260}/A_{280} ratio was determined for each sample on the Thermo Scientific NanoDrop 3300 fluorospectrometer. Pure RNA has an A_{260}/A_{280} ratio of 1.9 – 2.1. The mean (±SD) A_{260}/A_{280} for the pre-treatment tumour samples was 1.94 (±0.12) and for the post-treatment tumour samples was 2.14 (±0.77).

5.8.2.2 Cytokine Gene Expression

Relative quantification of IL-6 in pre-treatment tumour tissue samples had a median of 0.35 and a mean of 0.48(±1.68). In post treatment tumour tissue samples relative quantification of IL-6 had a median of 0.45 and a mean of 0.75 (±0.91).

Relative quantification of IL-8 in pre-treatment tumour tissue samples had a median of 0.00 and a mean of -0.36 (2.10). In post treatment tumour tissue samples relative quantification of IL-8 had a median of 0.04 and a mean of 0.37 (±1.02).

Plasma levels of IL-6 had a median of 1.58 pg/ml (IQR = 1.18-2.28), with and a mean of 1.81 pg/ml (±0.97). Plasma levels of IL-8 had a median of 10.92 pg/ml (IQR = 6.89-14.88) and a mean of 11.15 pg/ml (±6.76).

5.8.3 Association between KRAS, Tumour Response and Cytokine Expression

The relationships between KRAS status and the tumour, in terms of response to treatment, stage, grade and size were examined using the Chi-Square test for independence and the Mann-Whitney *U* Test. The Mann-Whitney *U* test was also used to examine the differences between KRAS status and cytokine expression.

5.8.3.1 KRAS Status and the Tumour

Of the 9 patients that harboured mutant type KRAS, information in relation to the tumour response to treatment of 8 patients was available. Data indicated that 1 had minimal response, 5 had moderate response and 2 had complete response to treatment. Results from the Chi-Square test for independence indicated no significant association between KRAS status and tumour response to treatment, χ^2 (3, n = 26) = 7.46, p=0.06, phi = 0.55. Further subtype analysis could not be performed to determine whether the codon at which the

mutation occurred was an influencing factor, as all patients but one had similar mutations, and the information in relation to this patient's tumour response was unavailable (see Table 5.15).

Table 5.15: Tumour response and KRAS status

	Poor Response	Minimal Response	Moderate Response	Complete Response
Wild Type KRAS	3 (12%)	8 (31%)	7 (27%)	0
Mutant KRAS	0	1 (4%)	5 (18%)	2 (8%)

In relation to KRAS status and tumour stage no significant results were detected χ^2 (1, n = 26) = 1.15, p=0.28, phi = 0.21. These results can be seen in Table 5.16.

Table 5.16 Tumour stage and KRAS status

	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Wild Type KRAS	0	1 (4%)	2 (8%)	11 (42%)	4 (15%)
Mutant KRAS	2 (8%)	0	0	5 (19%)	1 (4%)

No significant results were detected for tumour grade χ^2 (3, n = 26) = 2.50, p=0.40, phi = 0.31. These results can be seen in Table 5.17.

Table 5.17 Tumour grade and KRAS status

	Grade 0	Grade 1	Grade 2	Grade 3
Wild Type KRAS	0	0	17 (64%)	1 (4%)
Mutant KRAS	2 (8%)	0	6 (24%)	0

Similarly, in relation to tumour size, a Mann-Whtney *U* test revealed no significant difference between those with mutant KRAS (*Md* = 3, *n* = 7) versus wild-type KRAS (*Md* = 2, *n* = 17), *U* = 50, *z* = -0.59, *p* = 0.58, *r* = -0.12. These results can be in Figure 5.23.

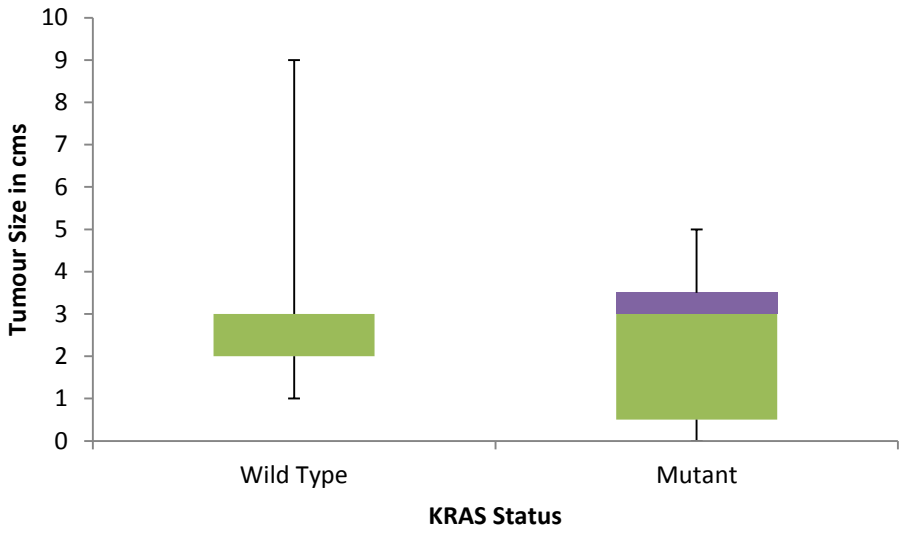

Figure 5.23 Tumour size and KRAS status

5.8.3.2 KRAS Status and Cytokine Expression

The differences between KRAS status and cytokine expression were examined using a Mann-Whitney *U* test. This demonstrated no significant difference between blood plasma levels of IL-6 and those with mutant KRAS (*Md* = 1.97, *n* = 9) versus wild-type KRAS (*Md* = 1.52, *n* = 18), *U* = 64, *z* = -0.87, *p* = 0.38, *r* = -0.12. Plasma concentration of IL-6 in relation to KRAS status can be seen in Figure 5.24.

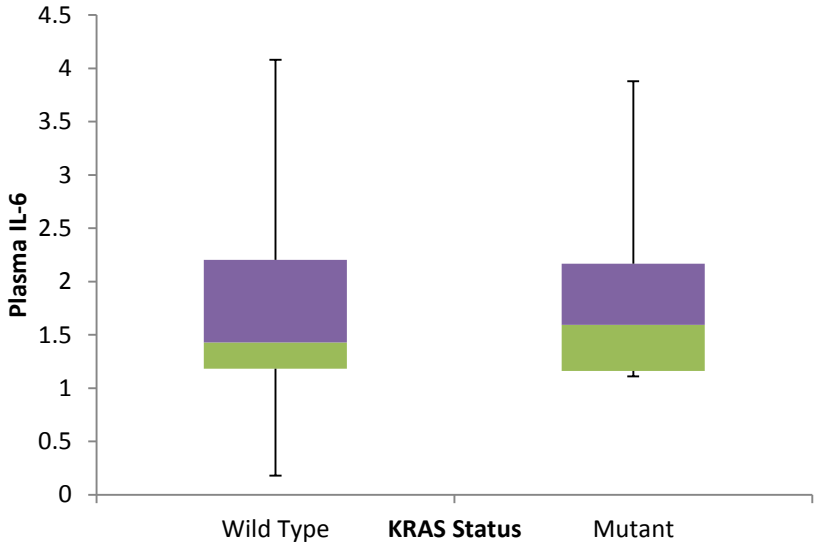

Figure 5.24: KRAS status and plasma concentration of IL-6

Similarly, there was no significant association between blood plasma levels of IL-8 and those with mutant KRAS (*Md* = 16.05, *n* = 9) versus wild-type KRAS (*Md* = 10.52, *n* = 18), *U* = 59, *z* = -0.11, *p* = 0.28, *r* = -0.02. Plasma concentration of IL-6 in relation to KRAS status can be seen in Figure 5.25.

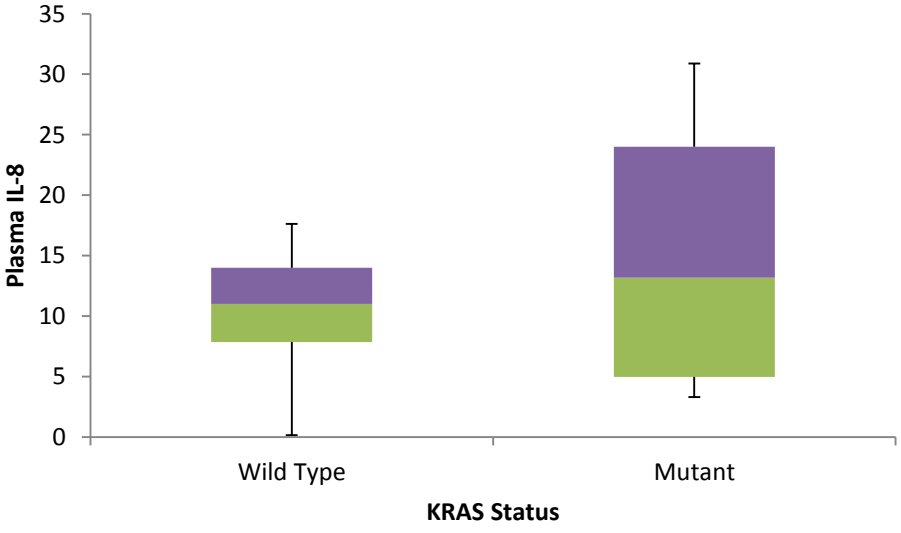

Figure 5.25 KRAS status and plasma concentration of IL-8

In relation to pre-treatment gene expression levels of IL-6, there was no significant association between levels of this cytokine and those with mutant KRAS (*Md* = 0.00, *n* = 5) versus wild-type KRAS (*Md* = 0.46, *n* = 16), *U* = 35, *z* = -0.42, *p* = 0.68, *r* = -0.09. Likewise, no significant association occurred for post treatment mRNA levels of IL-6 and those with mutant KRAS (*Md* = 0.17, *n* = 6) versus wild-type KRAS (*Md* = 0.65, *n* = 15), *U* = 36, *z* = -0.71, *p* = 0.41, *r* = -0.15. Gene expression levels of IL-6 in relation to KRAS status can be seen in Figure 5.26.

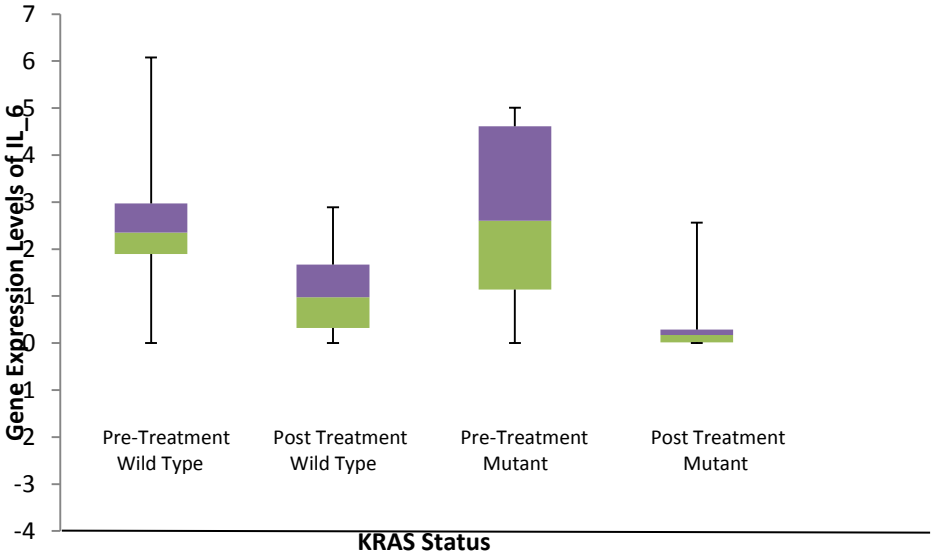

Figure 5.26: Pre-treatment and post treatment gene expression levels of IL-6 in relation to KRAS status

Regarding pre-treatment gene expression levels of IL-8, there was no significant association between levels of this cytokine and those with mutant KRAS (*Md* = 0.00, *n* = 5) versus wild-type KRAS (*Md* = -0.90, *n* = 16), *U* = 22.5, *z* = -1.46, *p* = 0.44, *r* = -0.32. Similarly, no significant association occurred for post treatment mRNA levels of IL-8 and those with mutant KRAS (*Md* = 0.01, *n* = 6) versus wild-type KRAS (*Md* = 0.07, *n* = 15), *U* = 27, *z* = -1.41, *p* = 0.16, *r* = -0.31. Gene expression levels of IL-8 in relation to KRAS status can be seen in Figure 5.27.

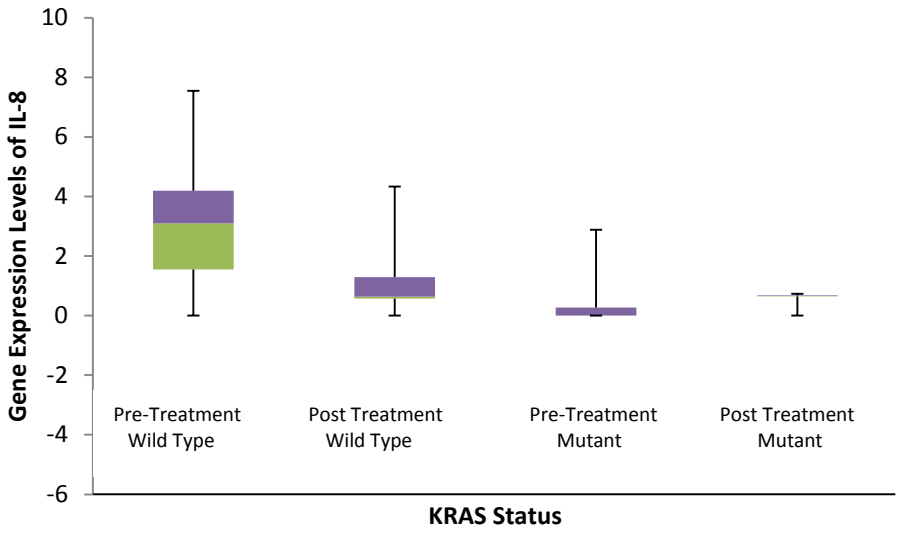

Figure 5.27: Pre-treatment and post treatment gene expression levels of IL-8 in relation to KRAS status

5.8.4 Associations between Cytokine Expression, the Tumour and Symptom Presentation

The relationships between cytokines and tumour size were examined using Spearman's Rank order correlation co-efficient. The strength of these relationships is determined using criteria as recommended by Cohen (1988) which included small (.10 -.29), medium (.30 - .49) and large (.50 – 1.0) ranges. The Kruskal-Wallis test was also performed to explore relationships between cytokines and tumour stage, grade and level of regression. Spearman's Rank order correlation co-efficient was used to establish the strength of relationships between cytokine expression & symptom presentation.

5.8.4.1 Cytokine Expression Levels and the Tumour

There were no statistically significant findings between pre-treatment plasma levels of IL-6 or IL-8 across the five radiological tumour stages (stage 0-4), three tumour grades (1-3) and four tumour regression categories (poor-complete). However, plasma levels of both IL-6 and IL-8 demonstrated large (p<0.01) and medium (p<0.05) correlations with tumour size respectively (0.72 & 0.47).

There were no statistically significant findings between pre-treatment and post treatment mRNA levels of IL-6 or IL-8 across the five radiological tumour stages (stage 0-4), three tumour grades (1-3), four tumour regression categories (poor-complete) or with tumour size.

5.8.4.2 Cytokine Expression Levels in Tumour Tissue Samples and Blood Plasma

The Wilcoxon Signed Rank test was performed to establish whether there was any relationship between expression levels of IL-6 and IL-8 in pre-treatment tumour tissue samples and post treatment tumour tissue samples.

No statistically significant results were demonstrated for IL-6, z = -0.724, p=0.47. Gene expression levels of IL-6 in pre-treatment and post treatment tumour tissue samples are displayed in Figure 5.28.

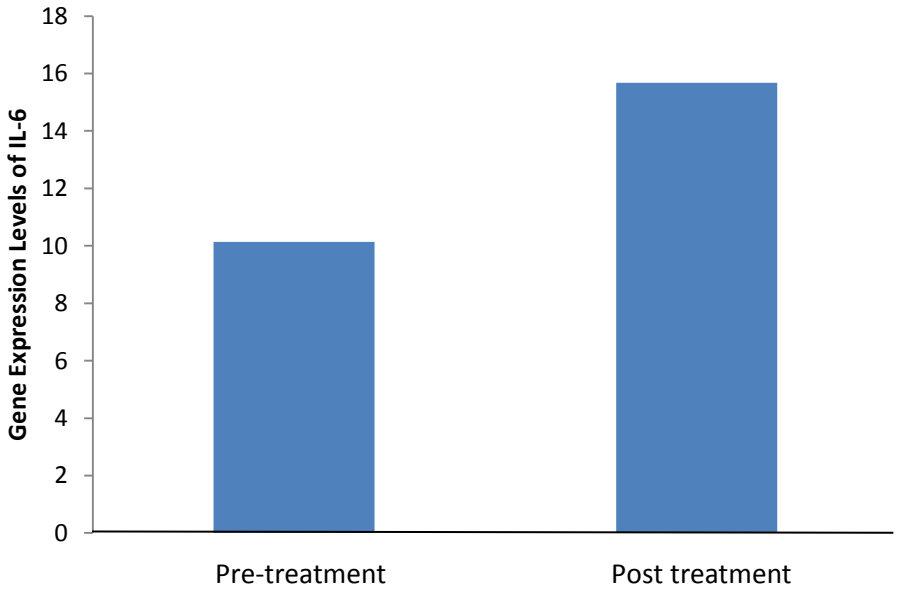

Figure 5.28: IL-6 gene expression levels in pre-treatment and post treatment tumour tissue samples

Similarly, no statistically significant differences occurred for pre-treatment and post treatment levels of IL-8, z = -.063, p=0.53. Gene expression levels of IL-8 in pre-treatment and post treatment tumour tissue samples are displayed in Figure 5.29.

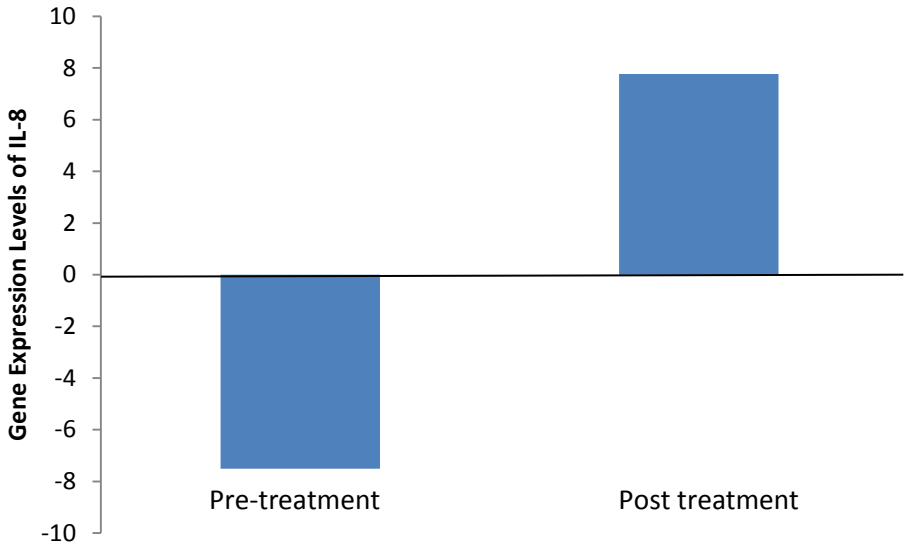

Figure 5.29: IL-8 gene expression levels in pre-treatment and post treatment tumour tissue samples

The data which is visually presented in Figure 5.23 was captured on the 7300 System SDS, which sets the pre-treatment samples as the calibrator. The pre-treatment samples were therefore set at a value of 1 and the IL-8 gene expression level was calculated as 11.49. This demonstrates that levels of IL-8 were overexpressed in post treatment samples when compared with pre-treatment samples.

Spearman's rank order correlation co-efficient was used to determine whether there was a correlation between blood plasma and tumour tissue levels of cytokines. Results demonstrate no statistically significant association between pre-treatment blood plasma levels and pre-treatment tumour tissue gene expression levels of either IL-6 (p=0.33, rho=0.14) or IL-8 p=0.56, rho=0.21).

5.8.4.3 Correlation between Plasma Cytokines and Symptoms

The relationships between plasma cytokines and symptoms were examined using Spearman's Rank order correlation co-efficient at the first time point. The strength of these relationships is determined using criteria as recommended by Cohen (1988).

As can be seen from Table 5.18, results demonstrated mostly small or non-significant correlations for both IL-6 and IL-8 and levels of symptoms at time point 1. Medium correlations were noted for IL-6 and diarrhea (rho=0.34), sore skin (rho=0.33) and impotence (rho=0.32), although these did not reach statistical significance. However, a large statistically significant correlation was noted in relation to IL-6 and faecal incontinence ($p<0.01$, rho=0.61). Similarly, a statistically significant correlation was observed between this symptom and IL-8 ($p<0.05$, rho=0.40), although this association was not as strong as for IL-6 (0.61 vs 0.40). IL-8 also demonstrated a statistically significant medium correlation with impotence ($p<0.05$, rho=0.48).

Table 5.18: Correlations between cytokines & symptoms

Symptom	IL-6	IL-8
Fatigue		
FACIT-F	0.130	0.220
EORTC QLQ-C30	0.037	-0.140
Bowel Function		
Constipation	-0.145	-0.206
Diarrhoea	0.343	0.219
Flatulence	0.193	0.139
Bloating	0.011	0.046
Blood & Mucous	-0.110	0.105
Faecal Incontinence	0.608**	0.400*
Stool Frequency	0.257	0.183
Nutrition		
Appetite Loss	-0.076	0.094
Taste	0.082	-0.127
Nausea & Vomiting	-0.198	-0.254
Dry Mouth	0.017	-0.186
Pain		
General Pain	0.002	-0.247
Abdominal Pain	-0.039	0.013
Buttock Pain	-0.045	0.002
Dermatological Issues		
Sore Skin	0.332	0.223
Urinary Function		
Dysuria	0.00	0.00
Urinary Frequency	-0.263	-0.222
Urinary Incontinence	0.117	0
Sexual Function		
Impotence	.319	.480*

** $p<0.01$

* $p<0.05$

5.9 Symptoms, QoL, Cytokines and Additional Variables

Variables of interest that may have an influence on symptoms, QoL and cytokine expression may include age and BMI, thereby warranting further analysis. In addition, the stage and grade of cancer was compared with symptoms, QoL and levels of functioning.

5.9.1 Symptoms, QoL, Cytokines and Age

The age of the sample ranged from 34 to 82 years (mean 61.69, SD 11.15). Age was examined with symptoms and QoL across the 4 time points using Spearman's correlation co-efficient, to identify relationships that reached significance level ($p<0.05$). In addition, the relationships between age and tumoral IL-6 and IL-8 gene expression levels, and plasma IL-6 and IL-8 protein levels were also examined using Spearman's correlation co-efficient. The strength of these relationships is determined using criteria as recommended by Cohen (1988).

Symptoms that were correlated with age include fatigue, bowel function issues, nutrition, pain, dermatological issues, issues with urinary function and issues with sexual function. There was a medium negative correlation between age and fatigue at time point 1, *rho* = -0.41, $n = 32$, $p<0.05$, with those with lower age levels experiencing greater levels fatigue. In relation to bowel function, a medium negative correlation occurred for constipation at time point 1, *rho* = -0.36, $n = 32$, $p<0.05$, and at time point 2, *rho* = -0.46, $n = 30$, $p<0.05$, with those with lower age levels experiencing greater levels of constipation. For bloating, there was a medium negative correlation between this and age at time point 4, *rho* = -0.46, $n = 29$, $p<0.05$, with those with lower age levels experiencing more bloating. There was a medium negative correlation between age and blood and mucous in stool at time point 4, *rho* = -0.44, $n = 30$, $p<0.05$, with those with lower age levels experiencing more blood and mucous in stool. In relation to nutrition, there was a medium negative correlation between age and appetite loss at time point 4, *rho* = -0.39, $n = 30$, $p<0.05$, with those with lower age levels

experiencing greater levels of appetite loss. Similarly, there was a medium negative correlation for age and weight worry at time point 1, *rho* = -0.41, *n* = 31, $p<0.05$, with those with lower age levels experiencing greater levels of weight worry. For nausea and vomiting, there was a medium negative correlation for this and age at time pint 4, *rho* = -0.42, *n* = 30, $p<0.05$, with those with lower age levels experiencing greater levels of nausea and vomiting. Regarding pain, there was a medium negative correlation for this and age at time point 1, *rho* = -0.37, *n* = 32, $p<0.05$, at time point 2, *r* = -0.44, *n* = 29, $p<0.05$, as well as at time point 4, *rho* = -0.43, *n* = 31, $p<0.05$, with those with lower age levels experiencing greater levels of pain. For sexual function, a large positive correlation occurred for impotence and age at time point 1, Regarding pain, there was a medium negative correlation for this and age at time point 1, *rho* = -0.514, *n* = 20, $p<0.05$, with those with higher age levels reporting more impotence.

In order to establish the influence of age on QoL and functioning, this was correlated with scores for cognitive, emotional, physical, role and social function, as well as global QoL. There was a medium negative correlation between age and anxiety at time point 3, *rho* = -0.43, *n* = 32, $p<0.05$, with those with lower age levels experiencing greater levels of anxiety. There was a medium positive correlation between age and body image at time point 4, *rho* = -0.44, *n* = 30, $p<0.05$, with those with higher age levels experiencing better body image. Similarly, a medium negative correlation occurred for embarrassment at time point 4, *rho* = -0.42, *n* = 30, $p<0.05$, with those with lower age levels experiencing greater levels of embarrassment.

For age and cytokine expression as measured in blood plasma, there was a medium positive correlation with IL-6, *rho* = 0.45, *n* = 31, $p<0.05$, where higher levels occurred with greater

age. In relation to IL-8, a large positive correlation occurred between this cytokine and age, *rho* = 0.53, *n* = 32, $p<0.01$, with higher levels of this cytokine associated with greater age levels. However, no significant correlations occurred for age and tumoral IL-6 and IL-8 gene expression levels and plasma IL-6 and IL-8 protein levels. These results can be seen in Table 5.19.

Table 5.19: Correlation between symptoms, QoL, cytokines & age

	Time Point 1	Time Point 2	Time Point 3	Time Point 4
Symptoms				
Fatigue	**-0.414***	-0.179	-0.235	-0.154
Bowel Function				
Constipation	**-0.359***	-0.169	-0.318	**-0.456***
Diarrhoea	-0.074	-0.152	0.207	0.171
Flatulence	-0.019	-0.023	-0.094	0.048
Bloating	-0.155	-0.227	-0.175	**-0.458***
Blood & Mucous	-0.227	0.131	0.143	**-0.441***
Faecal Incontinence	0.092	-0.062	0.133	-0.271
Stool Frequency	-0.067	-0.241	-0.199	-0.282
Nutrition				
Appetite Loss	-0.213	-0.140	-0.024	**-0.387***
Weight Worry	**-0.413***	-0.217	-0.121	-0.126
Taste	0.037	0.074	0.264	-0.065
Nausea & Vomiting	-0.289	-0.135	-0.012	**-0.415***
Dry Mouth	-0.145	-0.116	-0.107	-0.109
Pain				
General Pain	**-0.372***	**-0.438***	-0.264	**-0.432***
Abdominal Pain	-0.192	-0.303	-0.089	-0.221
Buttock Pain	-0.290	-0.236	-0.303	-0.299
Dermatological Issues				
Sore Skin	0.342	0.022	-0.214	-0.270
Urinary Function				
Dysuria	-	-0.287	-0.242	-0.270
Urinary Frequency	-0.069	-0.044	-0.001	-0.073
Urinary Incontinence	0.147	0.191	-0.012	-0.319
Sexual Function				
Sexual Interest	-0.343	-0.318	-0.108	-0.268
Impotence	**0.514***	0.231	-0.072	0.303
QoL / Functioning				
Cognitive Function	0.127	0.260	0.063	0.086
Emotional Function	0.181	0.134	0.175	0.279
Anxiety	-0.074	-0.179	**-0.429***	-0.287
Body Image	0.100	0.193	-0.182	**0.441***
Insomnia	-0.144	-0.102	-0.251	-0.275
Embarrassment	-0.090	-0.005	-0.116	**-0.421***
Physical Function	0.159	0.196	0.219	0.333
Role Function	0.239	0.200	0.269	0.341
Social Function	0.236	0.066	0.330	0.322
Global QoL	0.091	0.113	0.193	0.251
Cytokines				
IL-6				
Blood Plasma	**0.450***			
Pre-Treatment Tumour	0.041			
Post Treatment Tumour	0.130			
IL-8				
Blood Plasma	**0.532****			
Pre-Treatment Tumour	0.214			
Post Treatment Tumour	0.179			

** $p<0.01$ * $p<0.05$

5.9.2 Symptoms, QoL, Cytokines and BMI

The BMI of the sample ranged from 19 to 39 kg/m^2 (mean 27.21, SD 5.48). BMI was correlated with symptoms, QoL and cytokines at time point 1 using Spearman's correlation co-efficient to identify relationships that reached significance level ($p<0.05$). The strength of these relationships is determined using criteria as recommended by Cohen (1988) which included small (0.10 -0.29), medium (0.30 -0 .49) and large (0.50 – 1.0) ranges. No significant correlations occurred for symptoms, QoL or cytokines and BMI.

5.9.3 Symptoms, QoL and Disease Stage and Grade

Stage 2 tumours occurred in 6.7% of the total sample and stage 3 tumours occurred in 93.3% of the total sample. In relation to tumour grade, this was noted to occur at grade 0 in 3.3%, grade 1 in 6.7%, grade 2 in 86.7% and grade 3 in 3.3% of the total sample. Tumour stage and grade were correlated with symptoms, QoL and functioning at time point 1 using Spearman's correlation co-efficient to identify relationships that reached significance level ($p<0.05$). No significant correlations occurred for symptoms, QoL or functioning and disease stage and grade.

5.10 Key Findings

The key findings of the study as they relate to the objectives of the study can be seen in Table 5.20.

Table 5.20: Key findings

1. Study Objective: Determine the changes in fatigue and symptoms experienced by rectal cancer patients during preoperative radiotherapy.
Key Findings: *Fatigue:* There was a significant increase in fatigue between time point 1, time point 2 and time point 3. The proportion of patients that reported more severe effects was 5.7% at time point 1, 18.2% at time point 2, 32.4% at time point 3 and 6.1% at time point 4. *Bowel Function:* There were significant changes over time for diarrhoea, blood and mucous in stool, faecal incontinence and stool frequency. In relation to diarrhoea, there was a significant decrease between time point 3 and time point 4. The presence of blood and mucous in stool decreased significantly between time point 1 and time point 4, as well as time point 3 and time point 4. Faecal incontinence also decreased significantly between time point 3 and time point 4. There was a significant increase in stool frequency at between time point 1 and time point 3, time point 2 and time point 3, followed by a significant decrease in this symptom between time point 3 and time point 4. The proportion of patients that reported more severe effects for diarrhoea was 22.9% at time point 1, 33.3% at time point 2, 26.5% at time point 3 and 9.1% at time point 4. For blood and mucous in stool more severe effects occurred for 11.4% at time point 1, 14.3% at time point 2, 17.1% at time point 3 and 6.1% at time point 4. Regarding faecal incontinence, more severe effects were reported in 5.7% of patients at time point 1, 2.9% at time point 2, 14.3% at time point 3 and 3% at time point 4. Finally, for stool frequency, the proportion of patients reporting more severe effects was 25.7% at time point 1, 37.1% at time point2, 51.4% at time point 3 and 18.8% at time point 4. *Nutrition:* There were significant changes over time for appetite loss, taste, nausea and vomiting and dry mouth. Appetite loss increased significantly from time point 1 to time point 3, time point 2 to time point 3, followed by a decrease between time point 3 and time point 4. Problems with taste increased between time point 1 and time point 2 and there was a significant increase in nausea and vomiting from time point 1 to time point 3. Reporting of dry mouth decreased significantly between time point 3 and time point 4. The proportion of patients that reported more severe effects for appetite loss was 11.4% at time point 1, 12.1% at time point 2, 31.4% at time point 3 and 12.1% at time point 4. For alteration in taste, more severe effects occurred for 5.7% at time point 1, 11.4% at time point 2, 20% at time point 3 and 9.4% at time point 4. Regarding nausea and vomiting the proportion of patients reporting more severe effects was 0% at time point 1, 5.9% at time

point 2, 0% at time point 3 and 3% at time point 4.

Pain: There were significant changes over time for general pain, abdominal pain and buttock pain. General pain increased significantly between time point 1 and time point 3, time point 2 and time point 3 and then decreased between time point 3 and time point 4. Abdominal pain increased significantly between time point 1 and time point 2. Buttock pain also increased significantly between time point 1 and time point 2, between time point 1 and time point 3, followed by a decrease from time point 2 to time point 4 and from time point 3 to time point 4.

The proportion of patients that reported more severe effects for general pain was 8.6% at time point 1, 15.2% at time point 2, 32.4% at time point 3 and 12.1% at time point 4. In relation to abdominal pain, more severe scores occurred for 2.9% of patients at time point 1, 11.4% at time point 2, 14.3% at time point 3 and 9.1% at time point 4. For buttock pain, more severe reports of this symptom occurred for 8.6% of patients at time point 1, 20% at time point 2, 48.6% at time point 3 and 9.1% at time point 4.

Dermatological Effects: Rates of sore skin increased significantly between time point 1 and time point 2, time point 1 and time point 3, time point 2 and time point 3, followed by a decrease between time point 3 and time point 4.

The proportion of patients that reported more severe effects for sore skin was 5.7% at time point 1, 31.4% at time point 2, 51.4% at time point 3 and 9.4% at time point 4.

Urinary Function: There were significant changes over time for dysuria, urinary frequency and urinary incontinence. Dysuria increased significantly from time point 1 to time point 2, time point 1 to time point 3 and time point 2 to time point 3, followed by a decrease between time point 3 and time point 4. Urinary frequency increased significantly from time point 1 to time point 3, time point 2 to time point 3 and then decreased from time point 2 to time point 4 and from time point 3 to time point 4. Urinary incontinence increased significantly from time point 1 to time point 3, as well as from time point 2 to time point 3.

The proportion of patients that reported more severe effects for dysuria was 0% at time point 1 and 2, 21.2% at time point 3 and 0% at time point 4.

Secondary Objective:
Establish the impact of fatigue on other symptoms.

Key Findings:

Increased levels of fatigue were associated with increased levels of constipation, diarrhoea, flatulence, bloating, blood and mucous in stool, stool frequency, appetite loss, weight worry, nausea and vomiting, pain, urinary frequency and lower levels of sexual interest.

Secondary Objective:
Explore the impact of fatigue and symptom presentation during preoperative radiotherapy on the QoL of rectal cancer patients.

Key Findings:

Fatigue: There was a medium to large correlation between increased levels of fatigue and lower levels of global QoL, cognitive function, emotional function, physical function, role function and social function.

Bowel Function: There was a medium to large correlation between increased levels of constipation and lower levels of global QoL, physical functioning, role functioning and social function.

There was a medium to large correlation between increased levels of diarrhoea and stool frequency and lower levels of cognitive, emotional, physical, role and social function.

There was a medium correlation between increased levels of flatulence and lower levels of role function.

There was a medium to large correlation between increased levels of bloating and global QoL, cognitive, physical, role and social function.

There was a medium correlation between increased levels of blood and mucous in stool and lower levels of global QoL, cognitive and physical function.

There was a medium to large correlation between increased levels of stool frequency and lower levels of global QoL, cognitive, emotional, physical, role and social function.

Nutrition: There was a medium to large correlation between increased levels of appetite loss, and lower levels of global QoL, cognitive, emotional, physical, role and social function.

There was a medium to large correlation between increased levels of weight worry and lower levels of global QoL, cognitive, emotional, role and social function.

There was a medium correlation between alteration in taste and lower levels of global QoL, cognitive, physical and social function.

There was a medium to large correlation between increased levels of nausea and vomiting and lower levels of global QoL, cognitive, emotional, physical, role and social function.

There was a medium to large correlation between increased levels of dry mouth and lower levels of global QoL, cognitive, physical, role and social function.

Pain: There was a medium to large correlation between increased levels of general pain and lower levels of global QoL, cognitive, emotional, physical, role and social function.

There was a medium to large correlation between increased levels of abdominal pain and lower levels of global QoL, cognitive, physical, role and social function.

There was a medium to large correlation between increased levels of buttock pain and lower levels of global QoL, cognitive, emotional, physical, role and social function.

Dermatological Issues: There was a medium to large correlation between increased levels of sore skin and lower levels of global QoL, emotional and role function.

Urinary Function: There was a medium correlation with lower levels of cognitive function and global QoL.

There was a medium to large correlation between increased levels of urinary frequency and lower levels of global QoL, cognitive, emotional, physical, role and social function.

Sexual Function: There was a medium to large correlation between increased levels of sexual interest and increased levels of global QoL, cognitive and physical function.

There was a large correlation between increased levels of impotence and lower levels of cognitive and emotional function.

2. Study Objective:
Establish whether KRAS status is associated with tumour response to treatment.

Key Findings:

There was no significant association between KRAS status and tumour response to treatment.

Secondary Objective:
Correlate KRAS status with levels of specific cytokines.

Key Findings:

There was no significant association between KRAS status and expression levels of IL-6 and IL-8 in either blood plasma or tumour tissue.

3. Study Objective:
Establish whether levels of specific cytokines in tumour and blood plasma samples are associated with tumour response to treatment.

Key Findings:

Pre-Treatment Tumoral Cytokines & the Tumour:

There was no significant association between pre-treatment expression levels of IL-6 and IL-8 and the tumour.

Post-Treatment Tumoral Cytokines & the Tumour:

There was no significant association between post treatment expression levels of IL-6 and IL-8 and the tumour.

Blood Plasma Cytokines & the Tumour:

There was a statistically significant large correlation between levels of IL-6 and tumour size. There was a statistically significant medium correlation between levels of IL-8 and tumour size.

Secondary Objective:
Determine gene and protein expression levels of cytokines in pre-treatement and post treatment tumour tissue samples and pre-treatment blood plasma samples respectively.

Key Findings:

- IL-6 was overexpressed in post treatment tumour tissue samples when compared with pre-treatment tumour tissue samples
- IL-8 was overexpressed in post treatment tumour tissue samples when compared with pre-treatment tumour tissue samples

Secondary Objective:
Correlate gene expression levels of specific cytokines in pre-treatment tumour samples with the protein levels of cytokines in pre-treatment blood plasma samples.

Key Findings:

No significant association between levels of IL-6 and IL-8 in pre-treatment tumour samples and pre-treatment blood plasma samples.

Secondary Objective:
Correlate levels of specific cytokines with symptom presentation.

Key Findings:

There was a statistically significant large correlation between IL-6 and faecal incontinence. There was a statistically significant medium correlation between IL-8, faecal incontinence and impotence.

5.11 Conclusion

A critical discussion of these results is provided in the next chapter, whereby the researcher will interpret these findings in relation to the literature and their clinical significance.

CHAPTER VI: Discussion

6.0 Introduction

This chapter will discuss the implications of the study findings as presented in Chapter V. An examination of the findings in relation to the literature will be provided. The reliability of the assessment tools used is discussed in section 6.1. Section 6.2 considers the symptoms present during preoperative radiotherapy for rectal cancer. QoL during this treatment is discussed in Section 6.3, with Section 6.4 considering KRAS and cytokines as potential factors associated with radiosensitivity.

6.1 Reliability of Assessment Tools

A key component of any study is the reliability of the tools used to collect data (Polit and Beck, 2006). Reliability in terms of the internal consistency of the assessment tools used in this study, which included the EORTC QLQ C-30, the EORTC QLQ CR-29 and the FACIT-F scale, when assessed using Cronbach's α, was satisfactory when compared to other studies using these tools.

The QLQ C-30 is used to assess global QoL and levels of functioning, as well as including questions that specifically examine fatigue. In order to ensure fatigue was comprehensively measured in this study, participants also completed the FACIT-F scale. Upon comparing these scales, it was noted that the correlations between the fatigue subscale of the EORTC QLQ C-30 and the FACIT-F scale were quite strong.

Both scales measured increased levels of fatigue during radiotherapy treatment, with significant changes occurring between time point 1 and time point 3. However, the fatigue subscale of the EORTC QLQ C-30 also detected a significant change between time point 1 and time point 2, which was not evident in the FACIT-F scale. This is interesting as both

scales demonstrated good internal consistency, although the Cronbach's α was higher for the FACIT-F scale when compared with the fatigue subscale of the EORTC QLQ C-30 (0.96 versus 0.88). An explanation for this may be the difference in the number of items in each scale, with the FACIT-F including 13 items and the EORTC QLQ C-30 including just 3 items in relation to fatigue. Despite this however, the internal consistency for the EORTC QLQ C-30 fatigue subscale was still quite high in this sample, particularly when compared to other studies that used this tool to assess fatigue across a variety of cancer populations (0.80 and 0.84; Aaronson *et al.*, 1993; Cankurtaran *et al.*, 2007). Therefore, in this study, results from both the FACIT-F scale and the EORTC QLQ C-30 fatigue subscale may be considered reliable.

Consistency in relation to the measurement of fatigue for these tools is supported by findings of correlations between this symptom, global QoL and functioning. Similar results in relation to both scales were detected with this correlation across all time points, except in the case of emotional functioning at time point 1, where statistical significance was not reached for the EORTC QLQ C-30. When correlating fatigue with other symptoms, the EORTC QLQ C-30 fatigue subscale was used in order to ensure consistency, as all other symptoms were measured by the EORTC QLQ.

6.2 Symptom Presentation during Preoperative Radiotherapy for Rectal Cancer

The first hypothesis of the study was that the presence of fatigue and other symptoms change significantly during preoperative radiotherapy for rectal cancer. The anticipated outcome was that the level of fatigue and other symptoms would be significantly different between time points. This was supported by the study results for fatigue, diarrhoea, blood and mucous in stool, faecal incontinence, stool frequency, alteration in taste, dry mouth, appetite loss, nausea

and vomiting, pain, dermatological issues and urinary function. Symptoms that demonstrated no significant changes over time included constipation, flatulence, bloating, weight worry and sexual function.

6.2.1 Fatigue

The study hypothesis was that the level of fatigue would be significantly different between the time points. This finding was confirmed by the results. Fatigue, when examined using RM-ANOVA for the EORTC QLQ C-30 fatigue subscale identified that the mean fatigue scores differed significantly between time points. The mean fatigue score at time point 1 was 20.55, rising to a mean fatigue score of 32.59 at time point 2, increasing further to a mean fatigue score of 35.56 at time point 3, followed by a decrease at time point 4 with a mean fatigue score of 27.40. Post-hoc tests using Bonferroni correction revealed a significant increase in fatigue between time point 1 and time point 2, as well as time point 1 and time point 3, which was also clinically significant. The significant increase in this symptom between time point 1 and time point 3 was also detected by the FACIT-F when examined using RM-ANOVA, following post-hoc tests using Bonferroni correction.

The proportion of patients in this study with a fatigue score of > 50 (i.e. 'quite a bit' or 'very much') was 5.7% at time point 1. At time point 2, this increased to 18.2% and continued to increase to 32.4% at time point 3. This was followed by a decrease to 6.1% at time point 4, which was 4-6 weeks after completion of treatment.

These results are similar to findings of other studies that examined changes in fatigue with radiotherapy. Guren *et al.* (2003) also investigated patients with rectal cancer and detected a statistical and clinically significant increase in fatigue levels from the start of radiotherapy, to the end of treatment, with the proportion of patients reporting a fatigue score of > 50, as

measured by the EORTC QLQ C-30, being 17% at baseline and increasing to 31% at the end of treatment. These results are comparable to those of the current study, although higher fatigue levels were reported by Guren *et al.* (2003) in a greater proportion of patients at baseline (17% versus 5.7%), which is interesting, as findings of both studies in relation to this were similar at the end of treatment (31% versus 32.4%). At follow up, 4-6 weeks later, both studies reported a decrease in the proportion of patients reporting higher fatigue to near baseline levels (20% and 6.1%; Guren *et al.,* 2003). No significance was reported in relation to the decrease in fatigue between the end of treatment and at follow up in either study (Guren e*t al.,* 2003).

Increased fatigue with preoperative radiotherapy was also reported in an investigation that examined the severity and patterns of fatigue in 72 rectal cancer patients, with 67% of patients experiencing this effect (Wang *et al.,* 2001). In this, fatigue was measured using the Brief Fatigue Inventory, but despite the differences in the data collection tools, the prevalence of severe fatigue reported in this study at the end of treatment was comparable to the findings of the current study and Guren e*t al.* (2003), with this detected in 31% of patients (Wang *et al.,* (2001). Unlike the current study, levels of fatigue were not measured again after completion of treatment (Wang e*t al.,* 2001).

Nonetheless, these results are further supported by those of an investigation to determine the impact of radiotherapy on the fatigue levels of 82 Jordanian cancer patients, with levels increasing significantly with this treatment. Unlike the current study, fatigue was only measured at two time points, thereby making it difficult to determine if the presence of this symptom was sustained once treatment had been completed (Obead *et al.*, 2014).

However, like the findings of the current study and those reported by Guren *et al.* (2003), baseline levels of fatigue were noted to be associated with variances in levels after completion of treatment, in patients receiving radiotherapy for uterine cancer (Ahlberg *et al.*, 2005). In this study, fatigue also increased significantly during treatment and peaked near its completion (Ahlberg *et al.*, 2005).

Despite differences in relation to data collection tools and timing of assessments, results of this study regarding fatigue are in broad agreement with the literature. Fatigue scores in rectal cancer patients' increase during treatment, peak at the end of treatment and then return to near baseline levels 4-6 weeks after completion of treatment, with statistical and clinical significance reached for changes in this symptom from the start of treatment to midway through treatment, and on completion of treatment.

6.2.1.2 Fatigue and Other Symptoms

The second hypothesis was that fatigue impacts on other symptoms present in rectal cancer patients receiving preoperative radiotherapy. This finding was confirmed by the results. The relationships between fatigue, as measured by the EORTC fatigue subscale and other symptoms were examined using Spearman's Rank order correlation co-efficient at each time point. In relation to bowel function, there were correlations between fatigue and constipation at time point 1 and time point 3, diarrhoea at time point 2, flatulence at time point 1 and time point 4, bloating at time point 1, time point 3 and time point 4, blood and mucous in stool at time point 3, and stool frequency at time point 1 and time point 3. Regarding nutrition, correlations occurred with fatigue and appetite loss across all time points, weight worry at time point 1 and nausea and vomiting at time point 1, time point 3 and time point 4. For pain, correlations were noted between fatigue and general pain across all time points, abdominal

pain at time point 3, and buttock pain at time point 2 and time point 3. In relation to urinary function, there was a correlation with fatigue and urinary frequency at time point 3 and finally, for sexual function, there was a correlation with fatigue and reduced sexual interest at time point 2 and time point 4.

These results are supported by findings in the literature. An investigation that examined fatigue during preoperative chemoradiotherapy for rectal cancer also correlated this with other symptoms and reported an association with pain and diarrhoea (Wang *et al.,* 2001). Symptoms were measured weekly and despite using different questionnaires to those in this current study, the presence of diarrhoea correlated significantly with fatigue only at week 3 of treatment, as was the case in this study (Wang e*t al.,* 2001). The presence of diarrhoea and its association with fatigue was further confirmed in patients receiving pelvic radiotherapy for uterine cancer (Ahlberg *et al.,* 2005).

In the study by Wang *et al.* (2001), at the start of treatment, severe fatigue was also positively associated with uncontrolled pain (Wang *et al.,* 2001). The current study detected a positive correlation between fatigue and pain at the start of treatment, as well as at all other time points. As the investigation by Wang *et al.* (2001) did not report levels of pain during treatment in the results, it is difficult to determine if this symptom remained present throughout radiotherapy and compounded levels of fatigue. However, the positive association between fatigue and pain was also reported in two separate investigations despite the lack of measurement of these symptoms at multiple time points and with heterogeneous cancer populations (Hwang e*t al.,* 2002; Oh *et al.,* 2011). A quantitative study that was carried out to investigate fatigue in 180 male cancer patients, 15% of which had colorectal cancer, reported that varying levels of fatigue correlate with pain (Hwang *et al.,* 2002).

Similarly, a meta-analysis of 30 studies that examined the relationship between symptoms with cancer related fatigue, also demonstrated a significant correlation with this and pain (Oh *et al.,* 2011).

Other symptoms that correlated with fatigue in the investigation by Hwang *et al.* (2002) include constipation, feeling bloated and lack of appetite, all of which were also positively associated with fatigue in the current study. Interestingly, nausea and vomiting was also measured by Hwang *et al.* (2002), but no significant correlation was detected between this and fatigue, which contradicts the findings of the current study, the meta-analysis by Oh e*t al.* (2011), as well as another investigation that examined fatigue in patients receiving radiotherapy for uterine cancer (Ahlberg *et al.,* 2005). The difference in results may be due to the variances in the sample type and treatment protocols, as in the study by Hwang *et al.* (2002), just 10% of the sample received radiotherapy.

In the current study, other symptoms that correlated with fatigue include flatulence, blood and mucous in stool, stool frequency, urinary frequency and reduced sexual interest. In the literature discussed, these symptoms were either not reported in the findings (Wang *et al.*, 2001) or not investigated (Hwang *et al.* 2002; Ahlberg *et al.* 2005; Oh *et al.* 2011).

The literature discussed in relation to the correlation of fatigue with other symptoms supports the findings of the current study, in that there is a positive association between the presence of fatigue and the symptoms of constipation, diarrhoea, bloating, appetite loss, nausea and vomiting and pain. Results of this study contribute further to empirical knowledge as the symptoms of flatulence, blood and mucous in stool, stool frequency, urinary frequency and

reduced sexual interest were also positively correlated with fatigue, and had not been measured in the studies that have been discussed.

6.2.1.3 Fatigue, Age and Other Symptoms

Interestingly, higher levels of fatigue also correlated significantly with lower age levels at time point 1. Other symptoms that demonstrated correlations with lower age levels at time point 1 included constipation, appetite loss and pain, all of which were also associated with fatigue. However, the correlations between fatigue and these symptoms were larger and had greater statistical significance than when they were correlated with age, thereby demonstrating that fatigue may be more of a significant variable than age in relation to this.

6.2.2 Bowel Function

The study hypothesis was that symptoms associated with bowel function, which include constipation, diarrhoea, flatulence, bloating, blood & mucous in stool, faecal incontinence and stool frequency would be significantly different between the time points. This finding was confirmed by the results in relation to diarrhoea, blood and mucous in stool, stool frequency and faecal incontinence. Symptoms that showed no significant changes over time were constipation, flatulence and bloating.

For diarrhoea, mean scores were 31.11 (SD 28.95) at time point 1, and then increased to 37.78 (SD 27.31) at time point 2, remained at 37.78 (SD 32.44) at time point 3 and fell to 19.99 (SD 28.50) at time point 4, with a statistically and clinically significant change occurring from the time of completion of treatment to follow up, 4-6 weeks later. The proportion of patients with a diarrheoa score of > 50 (i.e. ‘quite a bit’ or ‘very much’) was 22.9% at time point 1, increased to 33.3% at time point 2, decreased to 26.5% at time point 3 and then decreased further to 9.1% at time point 4.

The importance of monitoring this symptom during treatment was highlighted in the current study, as for one patient, it became intolerable and led to an interruption in radiotherapy at week 4. However, in relation to changes in this symptom over time, the results of this study differ slightly when compared with the literature. Rather than significant changes in diarrhoea from the end of treatment to follow up, findings from the investigation by Guren e*t al.* (2003) indicated that prevalence rates increased significantly from commencement of radiotherapy to the end of treatment. The current study demonstrated only a small clinically significant change between these time points, which did not reach statistical significance. However, Guren *et al.* (2003) reported a large clinically significant decrease to near pretreatment levels on follow up 4-6 weeks later. In relation to more severe diarrhoea, the proportion of patients reporting this in the current study was 23% at the beginning of treatment, 27% at the end and 9% at follow up, whereas Guren *et al.* (2003) reported rates of 11%, 20% and 16% at these times. The lower prevalence rates of this symptom in the current study at follow up may be due to differences in treatment protocols, as although both patient groups received similar doses of radiation (50Gy and 50.4Gy), those in the current study also received concomitant chemotherapy, which may have improved tumour response to radiotherapy, resulting in improvements in symptom presentation prior to surgery. However, as Guren *et al.* (2003) did not report tumour response in the study findings it is difficult to compare this end point for each study. Nonetheless, both investigations demonstrate a transient increase in diarrhoea with radiotherapy, which improves 4-6 weeks after completion of treatment, despite discrepancies in relation to the significance of the timing of this change. These results are supported by findings of a study by Wang *et al.* (2001), where diarrhoea was found to be a primary toxicity, with rates increasing from 10% during week 1 of treatment to 40% at week 4 and then decreased back to 26% at week 5, indicating that radiotherapy has a significant impact on prevalence rates.

Although information in relation to specific time points was not provided, results of a quantitative study that analysed the effects of adjuvant radiotherapy in elderly patients with rectal cancer indicate that this was one of the most common symptoms reported during treatment, with it occurring to some degree in 81.8% of patients (Fiorica *et al.,* 2009). Diarrhoea was also a common occurrence in most patients included in another quantitative study, which specifically investigated the acute side effects of preoperative radiotherapy combined with a total mesenteric excision (Marijnen *et al.,* 2002). When comparing preoperative versus post-operative chemoradiotherapy for rectal cancer, Sauer *et al.* (2004) found 12% of patients receiving preoperative treatment experienced higher grades of diarrhoea. Consensus regarding the prevalence of diarrhoea in this patient group is strengthened with a more recent study that investigated acute toxicity and surgical complications in patients receiving preoperative radiotherapy for rectal cancer, as it was found that diarrhoea continued to be reported as one of the most prominent acute effects of radiotherapy treatment, with 10.2% of patients indicating higher grades of toxicity (Swellengrebel *et al.,* 2011). Furthermore, diarrhoea was reported to be a major adverse effect of treatment in 24% of patients receiving radiotherapy for rectal cancer (Ishihara *et al.,* 2011). Despite the lack of information in relation to prevalence rates of diarrhoea at specific time points, combined results of these studies indicate that this symptom is common in rectal cancer patients and is compounded with preoperative radiotherapy, which supports the findings of the current investigation.

Therefore, although in current literature differences exist in relation to measurement tools and reported timing of assessments, which may explain the discrepancies in the prevalence rates of this symptom, in general results of this investigation are in consensus with existing studies. Although not statistically significant, a small clinically significant increase in diarrhoea

occurred during treatment, with a noteworthy reduction in this symptom occurring 4-6 weeks after completion of treatment.

Other symptoms in relation to bowel function that demonstrated significant changes over time include blood and mucous in stool, stool frequency and faecal incontinence. Mean scores for blood and mucous in stool were 23.33 (SD 27.30) at time point 1, with this decreasing to 19.70 (SD 18.84) at time point 2, followed by an increase to 25.25 (SD 25.39) at time point 3 and at time point 4 mean scores decreased to a further 10.10 (SD 14.99). There was a statistically and clinically significant change in this symptom between time point 1 (23.23) and time point 4 (10.10), as well as time point 3 (25.25) and time point 4 (10.10). Mean scores for stool frequency were 29.57 (SD 22.65) at time point 1, increased to 34.41 (SD 23.54) at time point 2, followed by a further increase to 44.62 (SD 27.69) at time point 3 and then decreased to 24.19 (SD 19.64) at time point 4. In relation to stool frequency, a strong significant effect for time was evident between time point 1 (29.57) and time point 3 (44.62), as well as time point 2 (34.41) and time point 3 (44.62) and also, between time point 3 (44.62) and time point 4 (24.19), which was statistically significant and clinically significant, as this symptom worsened between time point 1 and 2 and time point 2 and 3, and then decreased between time point 3 and 4. Regarding faecal incontinence, mean scores were 12.12 (SD 23.30) at time point 1, 15.15 (SD 18.80) at time point 2 and increased further to 23.23 (SD 29.44) at time point 3, followed by a decrease to 10.10 (SD 17.65) at time point 4, with a clinically and statistically significant change occurring between time point 3 (23.23) and time point 4 (10.10).

In the literature, little is reported in relation to these specific symptoms. However, the investigation by Guren *et al.* (2003) measured gastrointestinal symptoms prior to

commencement of radiotherapy for rectal cancer, on completion and 4-6 weeks later, with a small, statistically significant increase observed in mean scores between the first and second time point, and a reduction in symptoms noted at follow up, although this was not significant. While not reported at specific time points, Marijnen *et al.* (2002) observed that prevalence rates of gastrointestinal symptoms occurred for 13% of patients, with Sauer *et al.* (2004) noting this to be at 9% when investigating toxicities in this patient population. Although it is difficult to accurately compare these findings with results of the current study, as information in relation to specific symptoms is not reported, consensus is provided in broad terms, in that generally, issues with bowel function may increase with radiotherapy.

In relation to blood and mucous in stool, the results of the current study concur with one particular investigation that performed an exploratory analysis of specific symptoms related to bowel function which were measured at baseline and 3, 6 and 12 months later, in that symptoms were worse initially, and then improved after treatment (Stephens *et al.,* 2010). However, in relation to stool frequency and faecal incontinence, findings are conflicting, as these symptoms were worse 3 months after treatment, whereas in the current study, symptoms increased initially, with improvements noted at follow up, 4-6 weeks later (Stephens e*t al.,* 2010). An explanation for this may be that Stephens *et al.* (2010) did not measure these symptoms after completion of preoperative treatment and before surgery, but rather after surgical intervention which may have further affected bowel function. This was evident in a separate investigation of rectal cancer patients receiving preoperative radiotherapy, followed by surgery, that measured symptoms at baseline, 2-3 weeks post treatment but before surgery, and 6 and 12 months after surgery (Pucciarelli e*t al.,* 2009). Findings indicated a decrease in stool frequency before surgery from baseline, which increased 6 months post operatively (Pucciarelli e*t al.,* 2009). Similarly, rates of faecal

incontinence also increased at the 6 month follow up, when compared with baseline scores, with a small non-significant increase noted in this before surgery (Pucciarelli *et al.,* 2009).

Therefore, findings of the current study are in general agreement with the literature, although direct comparisons are difficult due to variances in methodologies adopted. Despite this, it could be concluded that radiotherapy compounds symptoms related to bowel function, which improve once treatment is completed. Nonetheless, in the current study, the majority of symptoms occurred at a tolerable level, with the exception of diarrhoea, which led to an interruption in treatment for one particular patient.

6.2.3 Nutrition

The study hypothesis was that symptoms associated with nutrition, which include appetite loss, weight worry, taste, nausea and vomiting and dry mouth would be significantly different between the time points. This finding was confirmed by the results in relation to appetite loss, taste, nausea and vomiting and dry mouth. Weight worry was the only symptom that did not demonstrate any significant change over time.

Mean scores for appetite loss were 13.33 (SD 27.12) at time point 1, increased to 22.22 (SD 26.74) at time point 2, increased further to 41.11 (SD 39.81) at time point 3 and then fell to 19.99 (SD 29.81) at time point 4, with statistically and clinically significant changes occurring between time points 1 and 3, 2 and 3, as well as 3 and 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for appetite loss was 11.4% at time point 1, which increased slightly to 12.1% at time point 2, followed by an increase to 31.4% at time point 3 which then decreased to 12.1% at time point 4.

There is general consensus between the findings of this current study and those in the literature. Guren *et al.* (2003) measured appetite loss using the same tool and also reported an increase in this symptom with radiotherapy. Results of this study demonstrated a score of 13 for appetite loss prior to treatment, which then increased to 26 at the time of completion and then returned to near baseline levels at follow up, 4-6 weeks later, with results demonstrating statistical and clinical significance (Guren e*t al.,* 2003). Similar findings were also reported by Ahlberg *et al.* (2005) in their study to investigate the impact of radiotherapy on patients with uterine cancer, which used the same tool as the current study and Guren *et al.* (2003) to measure appetite loss. Findings demonstrated a score of 8.9 before commencing treatment, which increased to 22.7 midway through treatment and was 25.2 on completion of treatment (Ahlberg e*t al.,* 2005). It must be noted however, that although results of all studies indicate an increase in this symptom with radiotherapy, scores were much higher at the end of treatment in this current study when compared with the two previously discussed studies (26 and 25.3 versus 41.11; Guren e*t al.,* 2003; Ahlberg e*t al.,* 2005). This may be due to variances in treatment protocols, as the patients in the current study also received concomitant chemotherapy, whereas patients in the other studies received radiotherapy alone.

For nausea and vomiting, mean scores were 3.89 (SD 7.17) at time point 1, increased to 11.11 (SD 15.98) at time point 2, fell slightly to 10.56 (SD 11.97) at time point 3 and decreased further to 6.11 (SD 14.17) at time point 4, with statistical and clinical significance reached for changes between time point 1 and time point 3. The proportion of patients with a score of > 50 (i.e. ‘quite a bit’ or ‘very much’) for nausea and vomiting was 0% at time point 1, 5.9% at time point 2, 0% at time point 3 which then increased to 3% at time point 4.

Conversely, no significant changes between time points occurred for this symptom in the study by Guren *et al.* (2003). However, on closer examination, nausea and vomiting seemed to present at a very tolerable level in this current study as more severe effects only occurred in patients at time point 2 and time point 4, with it affecting a very small proportion of patients (5.9% and 3%). Similar results were reported by Carlomagno *et al.* (2009) in their investigation of rectal cancer patients receiving neoadjuvant chemoradiotherapy, with more severe nausea occurring in 2% of patients and more severe vomiting present in just 4% of patients (Carlomagno *et al.*, 2009). As the time points at which these symptoms occurred were not reported in this study, it is difficult to compare changes over time between this and the current study. Despite this, it could be concluded that although nausea and vomiting can increase with radiotherapy, more severe effects occur in a very minimum number of patients, which did not interrupt treatment, thereby making it a very tolerable toxicity for patients receiving this treatment.

The symptoms of alterations in taste and dry mouth also demonstrated significant changes over time in this current study. Mean scores for problems with taste were 8.33 (SD 18.93) at time point 1, increased to 18.74 (SD 26.69) at time point 2, to 22.92 (SD 32.17) at time point 3 and then fell to 14.58 (SD 25.31) at time point 4, with statistical and clinical significance demonstrated for changes in this symptom between time point 1 and time point 2. Mean scores for dry mouth were 18.28 (SD 25.59) at time point 1, increased slightly to 20.43 (SD 23.85) at time point 2, increased further to 23.65 (SD 26.10) at time point 3 and then fell to 12.90 (SD 18.61) at time point 4. Statistical and clinical significance was reached in relation to this symptom between time point 3 and time point 4. Little is reported in the literature in relation to changes in either of these symptoms over time during radiotherapy, with these

results contributing to the body of knowledge in relation to effects of this treatment on patients' nutrition.

The findings of this current study in relation to appetite loss are by in large, in agreement with the literature, in that radiotherapy is associated with increases in this symptom. Regarding nausea and vomiting, results are conflicting in relation to significance, although on closer examination, more severe effects are very minimal and do not lead to treatment interruptions, thereby demonstrating the tolerability of these symptoms.

6.2.4 Pain

The study hypothesis was that symptoms associated with pain, which include general pain, abdominal pain and buttock pain would be significantly different between the time points. This finding was confirmed by the results in relation to general pain, abdominal pain and buttock pain.

Mean scores for general pain were 14.94 (SD 20.09) at time point 1, increased to 21.84 (SD 20.94) at time point 2, increased further to 34.48 (SD 30.19) at time point 3 and then fell to 17.24 (SD 18.08) at time point 4. Statistical and clinical significance was reached for changes between time points 1 and 3, 2 and 3, as well as 3 and 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for general pain was 8.6% at time point 1, which increased to 15.2% at time point 2, followed by an increase to 32.4% at time point 3 which then decreased to 12.1% at time point 4.

In the study by Guren e*t al.* (2003), pain levels increased during treatment, although this was neither statistically nor clinically significant. However, results of the investigation by Ahlberg *et al.* (2005) that examined the effects of radiotherapy on patients with uterine

cancer indicated a statistically significant increase in pain midway through treatment. This was followed by a small decrease at the end of treatment, which did not reach statistical significance (Ahlberg e*t al.*, 2005). Similarly, significant pain levels were reported in 7.6% of the total number of patients' surveyed in a study by Marjinen *et al.* (2002) that investigated the effects of radiotherapy in 1530 rectal cancer patients, which led to interruptions in treatment for some patients. The findings of these studies are in broad agreement with those of the current investigation, although greater significance was reached in relation to changes in pain over time in this study, and higher pain scores were present in a greater number of patients, (32.4% versus 7.6%), although it must be noted that those in the current study received nearly double the amount of radiation (50.4Gy versus 25 Gy; Marjinen *et al.*, 2002). This is remarkable as pain became so severe in one particular patient participating in this study that it led to an interruption in treatment at week 5. Also, results of this investigation differ slightly from those of Ahlberg e*t al.* (2005), in that at the end of treatment, scores were slightly lower than midway through, whereas scores continued to increase at the end of treatment in the current investigation, followed by a significant decrease at follow up, 4-6 weeks later. The difference in results may be due to the sample type as Ahlberg e*t al.* (2005) investigated patients with uterine cancer.

Therefore, it appears that although results of this study are in general consensus with these investigations, pain was a more significant issue in the current investigation when compared with previous literature. This is interesting as the sample size was smaller than that in the study by Guren e*t al.* (2003; 35 versus 42) and although like the study by Ahlberg e*t al.* (2005) pain increased midway through treatment, it was not so severe to interrupt treatment, as was the case in this current investigation. Where it was reported that treatment was interrupted due to pain, although a different pain assessment tool was used, the sample size

was much larger than in this current study and still only occurred in 7.6% of the total number of patients (1530 versus 35), although these received much lower doses of radiation (25Gy versus 50.4Gy; Marijnen *et al.* 2002).

6.2.5 Dermatological Issues

The study hypothesis was that sore skin would be significantly different between the time points. This finding was confirmed by the results.

Regarding changes over time, mean scores for sore skin were 11.46 (SD 21.77) at time point 1, increased to 35.41 (SD 30.45) at time point 2, increased further to 47.91 (SD 33.80) at time point 3 and then fell to 18.75 (SD 22.30) at time point 4, with clinical and statistical significance occurring for changes between time point 1 and time point 2, time point 1 and time point 3, time point 2 and time point 4, as well as time point 3 and time point 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for sore skin was 5.7% at time point 1, which increased to 31.4% at time point 2, followed by a further increase to 51.4% at time point 3 which then decreased to 9.4% at time point 4.

These results are in broad agreement with the literature, in that the prevalence of sore skin due to radiation dermatitis increases during radiotherapy, followed by improvements in this symptom after completion of treatment. In the study by Wang *et al.* (2001) participants receiving radiotherapy reported more severe skin reactions in 10% of patients' midway through treatment and 29% of patients near the end of treatment. Although specific time points were not reported in other studies, more severe cases of this symptom occurred in 11%, 23.8%, 11.6% and 6% of cases respectively (Sauer *et al.,* 2004; Musio *et al.,* 2010; Swellengrebel *et al.,* 2011; Wolff *et al.,* 2011). Although the results of the current study are

supported by these findings, it must be noted that prevalence rates of this symptom were much higher than those reported in the literature, with more severe effects occurring in 51.4% of the total number of patients assessed at the end of treatment. However, as different assessment tools were used in this study, it is difficult to accurately compare results, although these findings may attribute somewhat to the higher scores for pain that were also detected in this investigation.

6.2.6 Urinary Function

The study hypothesis was that symptoms associated with urinary function, which include dysuria, urinary frequency and urinary incontinence would be significantly different between the time points. This finding was confirmed by the results in relation to all symptoms.

Mean scores for dysuria were 0.00 (SD 0.00) at time point 1, increased to 7.53 (SD 14.17) at time point 2, increased further to 27.96 (SD 31.15) at time point 3 and then decreased to 2.15 (SD 8.32) at time point 4, with clinical and statistical significance reached for changes in this symptom between time points 1 and 2, 1 and 3, 2 and 3, as well as 3 and 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for dysuria was 0% at time point 1, remained at 0% at time point 2, then increased to 21.2% at time point 3 and then returned to 0% at time point 4. In relation to urinary frequency, mean scores were 33.33 (SD 18.57) at time point 1, increased to 48.88 (SD 22.72) at time point 2, further increased to 61.11 (SD 26.02) at time point 3 and then fell to 34.44 (SD 19.04) at time point 4, with clinical and statistical significance reached for changes in this symptom between time points 1 and 3, 2 and 3, 2 and 4, as well as 3 and 4. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for urinary frequency was 31.4% at time point 1, which increased to 57.6% at time point 2, then increased further to 65.7% at time point 3, followed

by a decrease to 36.4% at time point 4. Mean scores for urinary incontinence were 2.15 (SD 8.32) at time point 1, remained at 2.15 (SD 15.05) at time point 2, increased to 15.05 (SD 22.51) at time point 3 and then fell to 9.68 (SD 21.42) at time point 4, with clinical and statistical significance reached for changes in this symptom between time points 1 and 3, and 2 and 3. The proportion of patients with a score of > 50 (i.e. 'quite a bit' or 'very much') for urinary incontinence was 0% at time point 1, remained at 0% at time point 2, then increased to 8.6% at time point 3 and 9.4% at time point 4.

It is difficult to accurately compare these results with findings in the literature as issues with urinary function were reported on a more general basis and did not specify the occurrence of dysuria, urinary frequency or urinary incontinence. Despite this, general consensus was reached with results of the current study and findings in previous literature. Micturition problems also increased in the study by Guren *et al.* (2003) at the end of treatment and then decreased to below baseline levels at follow up 4-6 weeks after completion of treatment, although these results did not reach significance. In the study by Fiorica *et al.* (2009), that investigated the safety and efficacy of radiotherapy treatment in older patients with rectal cancer, lower grade urinary problems occurred in over 50% of patients undergoing treatment. A separate investigation that also examined the effect of radiotherapy on issues with urinary function reported that inflammation of the bladder due to infection occurred in 34.6% of patients during treatment (Wolff *et al.*, 2011). Therefore, it could be concluded that findings of this study are in broad agreement with results of these publications, in that radiotherapy can compound issues with urinary function, although these symptoms occur at a tolerable level.

6.2.7 Sexual Function

The study hypothesis was that symptoms associated with sexual function, which include sexual interest, impotence and dyspareunia would be significantly different between the time points. This finding was not confirmed by the results.

Mean scores for sexual interest were 29.63 (SD 26.69) at time point 1, decreased to 25.92 (SD 23.27) at time point 2 and then increased to 27.16 (SD 22.72) at time point 3, followed by a further increase to 30.86 (SD 24.33) at time point 4, with no clinical or statistical changes noted over time. Similarly, no statistical changes over time were noted for impotence with results demonstrating mean scores were 31.11 (SD 29.46) at time point 1, which increased to 37.78 (SD 35.34) at time point 2, increased further to 39.99 (SD 38.21) at time point 3 and then decreased to 31.11 (SD 36.66) at time point 4. Analyses in relation to dyspareunia were not performed due to low response rates to this question.

Results of this study are confirmed by findings of previous investigations. Data in relation to sexual function was analysed from an investigation to determine whether radiotherapy enhanced the benefit of surgery for rectal cancer, with results demonstrating that although preoperative radiotherapy had a diminutive effect on function, it was not an independent cause of sexual problems, with surgery being cited as the main risk factor (Lange *et al.*, 2009). Similarly, upon evaluating the effect of radiotherapy on the sexual function of 201 males with rectal cancer, results demonstrated that this treatment had an adverse effect, but maximum deterioration occurred 8 months after surgery (Heriot *et al.*, 2005). This is supported by findings of a large randomised trial, where comparable outcomes were found, with preoperative radiotherapy having a minor adverse effect on sexual function whereas surgery was cited as having a major clinical impact (Stephens *et al.*, 2010). The authors of

this study also reported difficulty in measuring symptoms with sexual dysfunction in women due to low response rates to questions, as was the case in the current investigation (Stephens *et al.,* 2010). Similar problems were also reported by Lange *et al.* (2009), as well as in a separate multicenter prospective trial that described outcomes of patients with rectal cancer after receiving preoperative chemoradiotherapy, with less than 30% of females responding to the sexual problem scale, resulting in its exclusion from further analyses (Pucciarelli *et al.,* 2012).

Therefore, the findings of this current study are in general agreement with the literature, in that radiotherapy does not have a significant impact on sexual function, with difficulty in accurately assessing this in female patients due to low response rates.

6.3 QoL during Preoperative Radiotherapy for Rectal Cancer

The next hypothesis was whether the level of fatigue and symptom presentation in rectal cancer patients would impact on their QoL during preoperative radiotherapy. The anticipated outcome was that greater levels of symptom presentation would lead to lower levels of QoL. Results of the analysis supported this hypothesis with large correlations demonstrated in relation to fatigue at all time points, constipation at time point 3, bloating at time point 4, stool frequency at time point 3, appetite loss at time points 1, 2 and 3, weight worry at time point 1, nausea and vomiting at time point 3, dry mouth at time point 4, as well as general and buttock pain at time point 3. Despite these positive correlations, no significant changes over time were noted in relation to QoL specifically, or any other functions (cognitive, emotional, physical, social) except role function. Mean scores for this were 80.46 (SD 28.55) at time point 1, fell to 77.01 (SD 25.36) at time point 2, fell further to 60.92 (SD 34.00) at time point

3 and then increased to 77.01 (SD 24.56) at time point 4, with clinically and statistically significant changes noted between time points 1 and 3, 2 and 3, as well as 3 and 4.

Results of the study by Guren *et al.* (2003) support the findings of the current study, in that there were no significant changes in QoL for rectal cancer patients receiving pre-operative radiotherapy. However, there was a small significant change over time in relation to physical function in this investigation which was not detected in the current study (Guren *et al.*, 2003). Janaki *et al.* (2010) used the same QoL data collection tool as this study and the current investigation, when the magnitude of fatigue was examined in cancer patients receiving radiotherapy and its impact on QoL, with findings confirming the results of both these studies, in that a significant decline in role and physical function was noted with this treatment. Unlike Guren *et al.* (2003), the authors assessed the impact of fatigue on QoL, with results confirming findings of the current study, in that deterioration in QoL was noted with increased levels of fatigue. In a separate investigation, to establish the impact of fatigue, site specific side effects and individual characteristics on functional status during radiotherapy, a decline in QoL during treatment was also detected, which specifically identified fatigue as being influential (Poirier, 2011).

Therefore, the results of this investigation are in general consensus with the literature, in that although QoL does not change significantly during preoperative radiotherapy for rectal cancer, role function is affected. In addition, there is a strong association with higher levels of fatigue and deterioration in QoL, with this study adding to the body of knowledge by also identifying the symptoms of constipation, bloating, stool frequency, appetite loss, weight worry, nausea and vomiting, dry mouth and pain as influencing factors.

6.4 KRAS as a Factor Associated with Radiosensitivity

The next hypothesis was to explore whether KRAS status is associated with tumour response to treatment in patients with rectal cancer. The anticipated outcome was that patients with KRAS mutation, as opposed to wild type KRAS would demonstrate greater radioresistance in terms of tumour response. As findings in the literature are conflicting in relation to this, further subtype analysis to identify the codon at which the mutation occurred was proposed, which may indicate which mutation type demonstrates greater resistance to radiotherapy. Results of the analysis did not support this hypothesis.

Of the 27 patients tested for KRAS in the current study, 33% harboured mutations. Subtype analysis indicated that 89% of these had mutations at codon 12/13 and the mutation occurred at codon 61 in 11% of the sample. This is similar to results of previous studies which have indicated that mutation of KRAS arises in 30 – 50% of colorectal cancer cases, with this occurring most commonly at codons 12 and 13 (Forbes *et al.,* 2006; De Roock *et al.,* 2008; van Krieken *et al.,* 2008; de Campos-Lobato *et al.,* 2010).

Preclinical studies have demonstrated that activated KRAS can decrease radiosensitivity at a cellular level with this confirmed by studies that have investigated colon carcinoma cell lines (Bernhard *et al.,* 1998; Russell *et al.,* 1999; Bernhard *et al.,* 2000). However, at a clinical level, results in relation to KRAS status as an indicator of radiosensitivity are conflicting. Some studies have indicated that the presence of KRAS mutation may impede patient response to neoadjuvant chemoradiotherapy (Bengala *et al.,* 2009; Garcia-Aguilar *et al.,* 2011; Russo *et al.,* 2014). Two studies that used pathologic complete response and non-pathologic complete response as endpoints of measurement reported a much higher rate of favourable response to treatment in patients without KRAS mutation (Garcia-Aguilar *et al.,*

2011; Russo *et al.*, 2014). Contrary to this, findings of the current study demonstrated pathologic complete response occurred in 3 patients (11%), with 2 of these patients harbouring mutant KRAS at codon 12/13, and further analysis indicating no significant relationship between KRAS status and tumour response. Despite this, it must be noted that these previous studies included larger sample sizes (132 and 79 patients) than the current investigation (Garcia-Aguilar *et al.*, 2011; Russo *et al.*, 2014).

However, findings of the current study are strengthened by results of an investigation that included 130 rectal cancer patients, which indicate that pathologic complete response was demonstrated in 15% of patients with KRAS mutant tumours versus just 10% of patients with KRAS wild type tumours (Hu-Lieskovan *et al.*, 2011). These findings are supported by those of two separate investigations that also correlated KRAS status with tumour response to treatment in this patient cohort (Zauber *et al.*, 2009; Erben *et al.*, 2011). A systematic review that analysed data from a total of 696 rectal cancer patients also indicated that the presence of KRAS mutation did not affect tumour response to treatment (Clancy *et al.*, 2013).

Due to the conflicting findings in the literature, it was proposed in the current study that carrying out further subtype KRAS analysis may establish whether the codon at which the mutation occurs has any influence on tumour response to treatment. However, as just 1 patient harboured a mutation at a different codon to the other 8, it was not possible to carry out further analysis in relation to this.

6.4.1 KRAS Mediated Expression of Cytokines

The secondary hypothesis in relation to KRAS was that this mediates the expression of the cytokines IL-6 and IL-8 and it was anticipated that there would be significant correlations

between mutated KRAS and expression of these cytokines. However, the results did not support this hypothesis. At a pre-clinical level, literature has indicated that active KRAS mediates the expression of both of these cytokines and this has been correlated with tumour growth and proliferation (Rowley *et al.,* 2002; Sparmann *et al.,* 2004; Wislez *et al.,* 2006; Ancrile *et al.,* 2007; Sunaga *et al.,* 2011). In this study, no significant correlation occurred for KRAS status and tumour tissue and blood plasma levels of either IL-6 or IL-8. The pre-clinical studies that examined these cytokines in relation to KRAS and tumour growth resulted in positive correlations when KRAS was in its active state. However, it the current study there was no association between KRAS status and tumour stage, grade or response to treatment, despite mutations being present in 9 cases, making it difficult to determine whether KRAS was active and therefore, mediating in the expression of IL-6 and IL-8.

6.5 Cytokines as Factors Associated with Radiosensitivity

The next hypothesis was to establish whether levels of specific cytokines in tumour tissue and blood plasma samples are associated with tumour response to treatment. It was proposed that specific cytokines would be correlated with response of the tumour to pre-operative treatment. The results did not however, support this hypothesis, although blood plasma levels of IL-6 and IL-8 were significantly correlated with tumour size.

Similar findings in relation to IL-6 and tumour size were reported in an investigation to establish the role of this cytokine in the progression of colorectal cancer, which also adopted an ELISA technique, although blood serum was analysed in this study, rather than blood plasma (Chung *et al.,* 2003). Previous literature has demonstrated that IL-6 is significantly correlated with tumour stage. Based on this, it was proposed that levels of IL-6 may be indicative of tumour response to treatment. However, neither pre-treatment nor post

treatment mRNA levels of this cytokine demonstrated any significant correlation with tumour response to treatment, tumour stage or tumour grade. This non-significant finding may be due to the small sample size. In the examination of previous literature in relation to IL-8, findings were conflicting in relation to this cytokine and tumour stage which may also have been due to the differences in sample size. Studies that found no correlation included just 20 and 50 patients, respectively (Nastase *et al.,* 2011; Dimberg *et al.,* 2012), whereas two investigations that demonstrated positive correlations included 105 and 107 patients, respectively (Berghella *et al.,* 2002; Kheirelseid *et al.,* 2013). As the current study examined cytokine expression levels in the blood plasma of 31 patients and pre-treatment and post treatment tumour tissue samples in 21 patients, this may explain the non-significant findings in relation to IL-6, IL-8 and tumour response, stage and grade.

A secondary hypothesis in relation to cytokine expression involved determining the gene expression levels of cytokines in pre-treatment and post treatment tumour tissue samples and protein expression levels in pre-treatment blood plasma samples. Quantifying these levels enabled comparisons between tumoral cytokine expression before and after radiotherapy treatment, as well as correlations between pre-treatment tumoral and blood plasma cytokine levels.

Regarding IL-6, results demonstrated that levels were overexpressed in post treatment tumour tissue samples when compared with pre-treatemtent tumour tissue samples. This is in consensus with the findings of current literature as in vitro, exposure of cell lines to radiation elicits an inflammatory response leading to a dose dependent increase in levels of IL-6 (Pasi *et al.* 2010; Chen *et al.,* 2012; Haubner *et al.,* 2013). In vivo, higher levels of IL-6 were also

reported during radiotherapy and in addition, may be associated with radiation resistance and poor tumour response to treatment (Rube *et al.,* 2008; Bower *et al.,* 2009; Willet *et al.,* 2009). In relation to IL-8, results demonstrated that levels were overexpressed in post treatment tumour tissue samples when compared with pre-treatment tumour tissue samples. This is in consensus with findings of previous studies that have investigated the effects of radiation on the expression of IL-8, with levels increasing over time in a dose dependant manner. This may be a defence signal of the tumour cells to promote tumour progression in the surrounding non-irradiated cells, which may explain why levels remain elevated, despite tumour downstaging after treatment.

Regarding cytokine expression in pre-treatment tumour tissue and blood plasma, there was no significant association between these variables in the current study. Conversely, results of an investigation that measured IL-6 in the serum and tumoral tissue of 50 rectal cancer patients, demonstrated significant correlations, which may have been due to the larger sample size (Esfandi *et al.,* 2006). The conflicting results may be due to differences in relation to sample analysis. In the current study mRNA levels of IL-6 were quantified with RT-PCR from FFPE samples, whereas Esfandi *et al.* (2006) used frozen tumour tissue samples and performed an ELISA.

The final secondary hypothesis in relation to cytokine expression was the correlation of levels of specific cytokines with symptoms that present in this patient group during pre-operative treatment. The anticipated outcome was that alterations in the expression of IL-6 and IL-8 would be significantly associated with certain symptoms. Results of the analysis supported this hypothesis in relation to the symptoms of faecal incontinence and impotence.

No significant correlation occurred for fatigue and either IL-6 or IL-8, which is in consensus with investigations that have previously examined this symptom. However, in relation to IL-6, associations were noted between this and the symptoms of nausea, vomiting and appetite loss in one particular study, which were not evident in this particular investigation (Wang *et al.*, 2012). There is a paucity of literature in relation to symptom presentation and cytokine expression in rectal cancer patients receiving pre-operative treatment, with results of the current study adding to the body of knowledge.

6.6 Chapter Conclusion

This chapter considered the key findings of the study and critically compared these with those in the literature. It was seen that where comparable literature was available, there was overall agreement with the results in this study. However, it was also noted that for some of the results there was limited literature with which to compare findings. This however, is not unexpected as the study was exploratory in nature and was addressing a topic about which there were many unanswered questions in the literature.

CHAPTER VII: Conclusion and Recommendations

7.0 Introduction

This chapter considers the relevance and significance of the study findings. Limitations of the study are discussed in Section 7.1. The clinical and theoretical implications are identified and discussed in Section 7.2 and following this, the future directions for research and recommendations for practice are outlined in Section 7.3. Finally, an overall summary of the study is provided in Section 7.5.

7.1 Limitations of the Study

The main limitations of this study include issues in relation to the design, the sample and confounding variables.

7.1.1 Research Design

This study adopted a prospective, exploratory, correlational design as this was the most appropriate method in this instance. However, a disadvantage of implementing this type of study design is that it is difficult to establish cause-and-effect conclusions. Due to this, it is necessary to acknowledge that KRAS status and cytokine expression may not be the proximal cause of symptom presentation and tumour response to treatment. In addition, the risk of Type I error is greater due to the high number of correlations that were performed in order to address the study objectives.

Also, although data was collected in relation to symptoms at 4 different time points, cytokines in peripheral blood were measured just at baseline and therefore, could only be correlated with symptoms at this time point, making it difficult to establish whether changes in cytokines during treatment were associated with changes in symptom presentation. It would have been more comprehensive to measure plasma cytokines at all time points and

correlate these with symptoms, particularly as fatigue and other symptoms demonstrate significant changes at time point 3 when compared with baseline. In order to address this limitation, tumoral cytokine expression levels were also measured before and after treatment and were correlated with peripheral cytokine expression levels, as well as symptom presentation. Despite this, however, the results of this study remain descriptive, as they cannot provide direct evidence for cause and effect relationships between symptoms and tumour response, KRAS status and changes in cytokine levels.

7.1.2 Sample

The study was conducted at one regional cancer centre, where participants were selected using nonprobability, convenience sampling, thereby affecting the generalisability of the study findings. The inability of the researcher to statistically generalise findings due to the sampling method selected is recognised as a limitation, although as the study was exploratory in nature, it was deemed as the most appropriate choice. The study included a sample of 35 rectal cancer patients, which was similar to sample sizes in comparable studies, although this was still relatively small. Therefore, there is a possibility of Type II errors in the analyses of this study.

In addition, the small sample size prevented the performance of multiple regression analysis, which is a family of techniques that explores the relationship between one continuous variable, such as fatigue, and a number of independent variables or predictors, such as flatulence, blood and mucous in stool, stool frequency, urinary frequency and reduced sexual interest. It is recommended that a minimum of 15 subjects per predictor are required for reliable analyses (Stevens, 1996). Although the objective of the current study was to explore

correlations, not predictors, if the sample size had been larger, it would have allowed for more sophisticated analyses in terms of multiple regression.

7.1.3 Confounding Variables

The effect of confounding variables on symptom presentation and the tumour, such as chemotherapy regimens and drugs used for symptom management were not analysed in this study. In the future, a larger study may be able to evaluate the effect of these confounding variables. The lack of a control group to compare results with is also recognised as a limitation.

Also, the influence of psychological processes on symptom presentation was not taken into consideration. However, including a data collection instrument in relation to this would have lengthened the questionnaire further, thereby increasing questionnaire burden. Although results of this may have been of interest, it would have been beyond the focus of the study, which was to explore the physiological cause of symptom presentation.

In addition, younger age demonstrated significant correlations with some symptoms. However, age could not be controlled for as patients that were enrolled in the study met the specific selection criteria, which included age > 18 years. The sample size would have been limited if the study focused on a more specific age group. Also, the objective of the investigation was to explore the symptom of fatigue in particular, and on further examination, it was noted that although greater fatigue was associated with lower age levels, other symptoms that correlated with fatigue, which include constipation, appetite loss and pain, were not as strongly or as significantly correlated with age. Therefore, it could be concluded that fatigue was more of an influencing variable on these symptoms than age.

7.2 Implications of the Research Findings

The conclusions drawn from the research findings and review of the literature have implications for theory and clinical practice. In an effort to advance our understanding of symptom presentation, tumour response to treatment, KRAS status and cytokine activity, theoretical and clinical implications will be discussed.

7.2.1 Theoretical Implications

As the literature review informed the concepts of this study, it is necessary to consider the implications of the results in relation to this theoretical framework. This framework proposed that symptoms occur in patients with rectal cancer due to preoperative radiotherapy which may negatively influence QoL, and the presentation of these symptoms, as well as tumour response to treatment, may be influenced by KRAS status and cytokine expression.

In general, in relation to symptoms that occur with radiotherapy, the findings of the current study are in broad agreement with the literature, as well as the theoretical framework, in that there is an increase in presentation during treatment, which peaks at the end of treatment and then returns to near baseline levels at follow up 4-6 weeks later. Regarding fatigue and other symptoms, there is consensus between findings of this study and existing literature, with these results contributing further to the body of knowledge by also associating this symptom with flatulence, blood and mucous in stool, stool frequency, urinary frequency and reduced sexual interest. Interestingly, no significant changes over time in global QoL were detected, although role functioning decreased significantly between time points 1 and 3, as well as time points 2 and 3, which was followed by an increase between time points 3 and 4. However, in relation to specific symptoms, decreased global QoL was significantly correlated with higher levels of fatigue, constipation, bloating, blood and mucous in stool, stool frequency, appetite loss, weight worry, dry mouth, pain and urinary frequency. Lower levels of sexual interest

were also associated with lower levels of global QoL. These results are in agreement with previous literature in relation to fatigue and QoL, with little published regarding the influence of these other symptoms, thereby further adding to the body of knowledge. Therefore, in relation to the theoretical implications of these findings global QoL does not change significantly across the time points, it is influenced by certain symptoms during radiotherapy treatment for rectal cancer.

Regarding factors associated with tumour response to radiotherapy, KRAS status and KRAS mediated expression of IL-6 and IL-8 demonstrated potential value in the theoretical framework. KRAS status was not indicative of tumour response to treatment in this study, which is in agreement with results of some previous investigations. It was proposed that subtype gene analysis may be more informative but it was not possible to perform this analysis due to low numbers. Tumoral mRNA levels of specific cytokines also failed to correlate with KRAS status, tumour response, stage and grade and pre-treatment plasma cytokine levels. This may have been due to the small sample size in the current investigation. However, in agreement with the literature, there was a significant correlation between plasma levels of both cytokines and tumour size, thereby indicating their possible value as potential biomarkers in relation to the tumour. However, it must be acknowledged that as this study is correlational and exploratory, these results do not definitively prove causation.

Studies that have investigated the association between cytokines and symptoms in cancer patients have reported no correlation between levels of IL-6 and fatigue, although there is a paucity of literature in relation to investigations that have included rectal cancer patients in their sample. However, despite examining this patient cohort, the current study also failed to identify any significant correlation between this cytokine and fatigue. It was suggested that

IL-6 may be associated with appetite loss, nausea and vomiting although this has not been confirmed by results of this study, with the only significant positive correlation occurring for faecal incontinence, which has not been reported previously in the literature examined. Similarly, IL-8 was also associated with this symptom in the current study, although no correlation occurred for this cytokine and pain, which was reported in a previous study that investigated patients with lung cancer (Reyes-Gibby *et al.*, 2007). Therefore, although the theoretical framework had suggested an association between IL-6, IL-8 and symptom presentation, the issue of aetiology as it relates to cytokine activity is still uncertain.

7.2.2 Clinical Implications

As this study was exploratory and correlational in nature, it is difficult to propose definitive implications for practice. However, there were changes in symptoms during treatment, with significant correlations noted for lower levels of QoL and a number of these symptoms, which included fatigue, pain, issues with bowel function and nutrition, as well as increased urinary frequency and lower levels of sexual interest. In addition, findings of the current study indicate that fatigue correlated with other symptoms that had not been reported or investigated in previous literature and these include flatulence, blood and mucous in stool, stool frequency, urinary frequency and reduced sexual interest.

Therefore, the importance of symptom management during treatment is highlighted, in order to optimise QoL and reduce fatigue in patients during this time. Close monitoring of symptoms may also assist in avoiding interruptions to treatment, as in this study, the symptoms of pain and diarrhoea became so severe that it resulted in treatment breaks for 2 patients. The findings of the current investigation indicate greater significance was reached in relation to changes in pain when compared with previous literature, and higher pain scores

were present in a greater number of patients. Also, there was a small clinically significant increase in diarrhoea during treatment, with a noteworthy reduction in this symptom occurring 4-6 weeks after completion of treatment. Consequently, these results have indicated that close monitoring of symptom presentation during treatment would enable the implementation of timely interventions, thereby enhancing the QoL of patients and minimising the risk of interruptions to treatment.

KRAS status and mRNA levels of IL-6 and IL-8 were not indicative of tumour response to treatment, although these should not be completely disregarded as factors associated with radiosensitivity, particularly as plasma levels of these specific cytokines were significantly correlated with tumour size. In addition, further subtype analysis of KRAS status may provide more information in terms of future studies.

Findings of the current study indicate plasma levels of IL-6 and IL-8 are significantly correlated with faecal incontinence, although there was no other significant association for these cytokines and other symptoms measured. As literature pertaining to symptoms and cytokines is fragmented and lacking, with the current study the only one to identify an association with faecal incontinence, it is difficult to identify definitive clinical implications based on these findings.

7.3 Recommendations Arising from the Study

A number of recommendations are made based on the findings of this study which focus on both clinical practice and future research.

7.3.1 Recommendations for Clinical Practice

- Rectal cancer patients receiving preoperative radiotherapy must be closely monitored for symptoms that may arise so that timely interventions are implemented in order to prevent interruptions in their treatment.
- Nursing assessment and care provision must consider how symptoms affect the QoL and functioning of these patients so that care is planned accordingly.
- The use of IL-6 and IL-8 as potential biomarkers of disease stage in this patient group requires further consideration.

7.3.2 Recommendations for Future Research

- Replication of this study in other settings, using the same methodology and methods, so that findings can be generalised to a larger population.
- Undertake qualitative research to investigate symptoms and QoL in rectal cancer patients.
- Retrospective analysis of KRAS status, which includes subtype analysis, and tumour response in rectal cancer patients that have received the same treatment in order to increase the sample size and improve significance of results.
- Building on the current study and previous studies, continue to explore the use of IL-6 and IL-8 as potential biomarkers for disease stage in rectal cancer patients.
- Exploration of the value of fatigue management strategies in this patient cohort.

7.4 Study Conclusion

This study investigated symptom presentation and effects on QoL in patients receiving preoperative radiotherapy for rectal cancer, as well as the use of KRAS status and expression

levels of IL-6 and IL-8 as factors associated with radiosensitivity, in terms of tumour response and symptoms. The study was correlational and exploratory in nature and adopted a nonprobability, convenience sampling technique to examine symptoms throughout treatment at 4 different time points. Symptoms and tumour response were also examined in relation to KRAS status and cytokine activity using micro-array technology. Relationships between symptoms and QoL, tumour response and KRAS status, tumoral cytokine expression and plasma cytokine expression, as well as tumour response and cytokine expression were then examined.

The current study advanced on previous investigations as it examined symptom presentation in this patient cohort, with a specific focus on fatigue, as there was a paucity of literature in relation to this. Although findings regarding symptoms present during radiotherapy were in general consensus with previous studies, knowledge was added in relation to the influence of fatigue on other symptoms. Increased levels of this symptom were associated with increased levels of constipation, diarrhoea, flatulence, bloating, blood and mucous in stool, stool frequency, appetite loss, weight worry, nausea and vomiting, pain, urinary frequency and lower levels of sexual interest. In addition, lower levels of QoL were associated with specific symptoms, including fatigue. In relation to clinical implications, this highlights the importance of the need for thorough symptom assessment and management to ensure optimum QoL and to avoid interruptions in treatment through timely intervention.

KRAS status was not indicative of tumour response to treatment, with the presence of mutation evident in 33% of cases, which is in general consensus with previous studies. Due to the small sample size, it was not possible to perform further subtype analysis of this mutation in terms of tumour response. Tumoral levels of IL-6 and IL-8 were not significantly

correlated with tumour response to treatment or KRAS status, although this may be due to the presence of KRAS in an inactive state. Quantification of these cytokines demonstrated that post treatment levels of IL-6 and IL-8 were over expressed when compared with pre-treatment levels, which may be a defence signal of the tumour cells to promote tumour progression in the surrounding non-irradiated cells, thus explaining why levels remained elevated, despite tumour downstaging after treatment. Although the theoretical framework of the study had implied that cytokine activity may explain the aetiology of symptom presentation, results were insignificant. This may be due to the small sample size, but could also be a finding in itself. However, the use of IL-6 and IL-8 as factors associated with tumour size warrants further investigation in a larger sample, as significant correlations were evident. Future studies should also focus on further subtype analysis of KRAS status in order to establish whether the codon at which the mutation occurs is indicative of radiosensitivity. In addition, to gain a comprehensive understanding of the influence of symptom presentation of QoL during treatment, further studies that adopt a qualitative methodology are required.

7.5 Study Overview

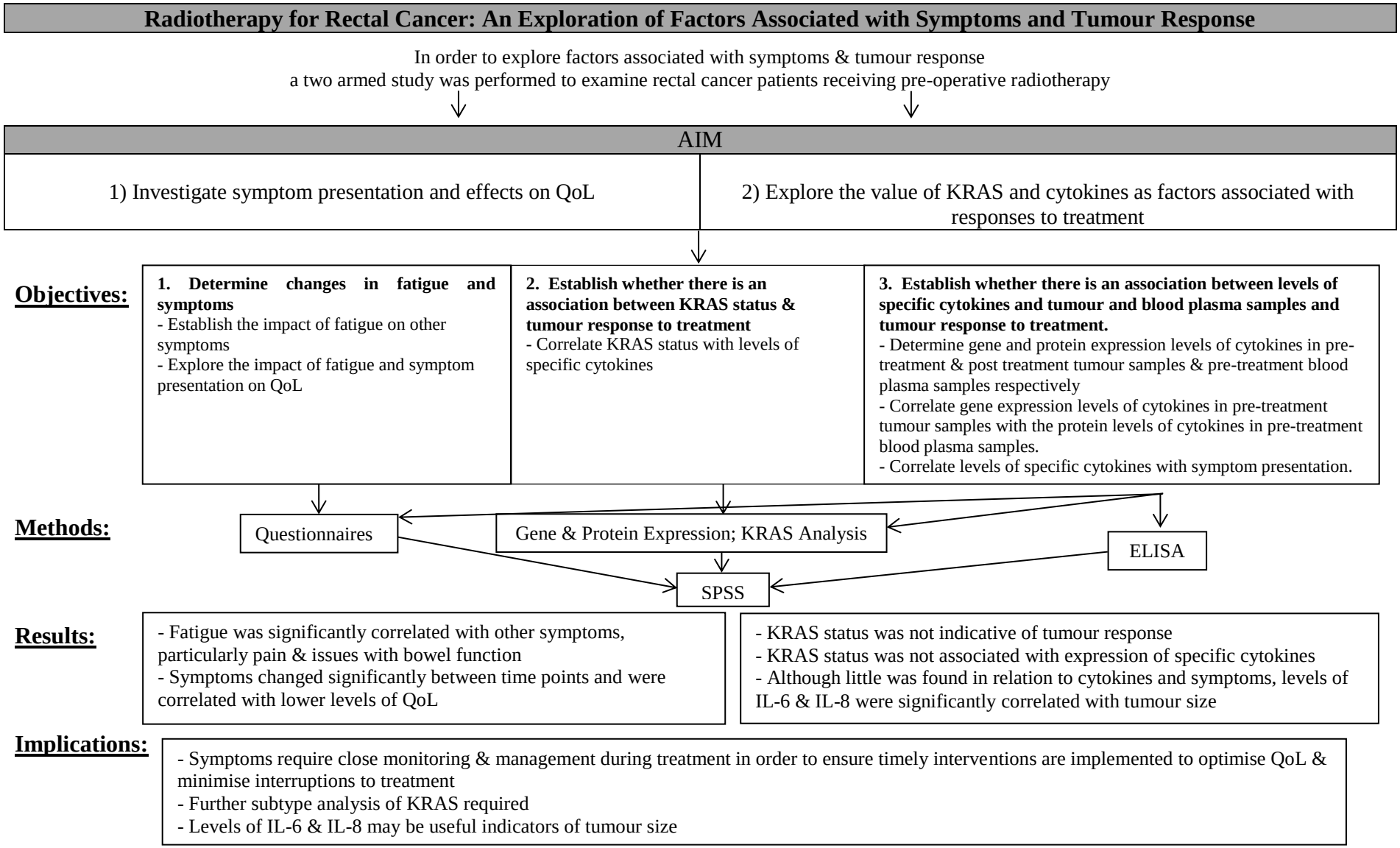

Figure 7.1 Study Overview

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Appendices

Appendix I: WIT Ethical Approval Letter

Ref: 12/NURIO 1

17th May, 2012.

Ms. Claire O'Gorman,
Suttonrath,
Cahir,
Co. Tipperary.

Dear Claire.

Thank you for-submitting your amended documentation in relation to your project *'Chemoradiotherapy and rectal cancer: Impact on symptoms, quality of life and radiosensitivity of lymphocytes as a prognostic indicator'* to the WiT Research Ethics Committee.

I am pleased to inform you that we fully approve WIT's participation in this project and we will convey this to Academic Council.

We wish you well in the work ahead.

Yours sincerely,

D ,

Dr. John Wells
Chairperson,
Research Ethics Committee

cc: Dr. Martina Gooney
Ms. Suzanne Denieffe

Appendix II: HSE Ethical Approval Letter

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

HSE South,
Waterford Regional Hospital,
Dunmore Road,
Waterford,
Ireland.

Telephone 051 848000
Fax 051 848572

NAME **Dr Martina Gooney**

ADDRESS **School of Health & Science**
Waterford Institute of Technology
Cork Road
Waterford

DATE **23rd May 2012**

RESEARCH ETHICS COMMITTEE.
HEALTH SERVICE EXECUTIVE, SOUTH EASTERN AREA

Study Title:
"Chemoradiotherapy and rectal Cancer: Impact on Symptoms, Quality of Life and Radiosensitivity of Lymphocytes as a Prognostic Indicator"

Study Status: APPROVED

Dear Dr Gooney

The Research Ethics Committee, HSE, South East reviewed the above study at their meeting held on Monday 16th April 2012.

The following documents were received:

1. Research Proposal
2. Standard Application Form
3. Information Sheet
4. Patient Consent Form
5. Computer Network Security Policy document W.I.T.
6. Appendix 1 Questionnaire
7. W.I.T. Record Retention Policy
8. UPMC Cancer Centers: Memorandum
9. Signed Declaration Page
10. C.V. Dr Gooney/Dr Sasiadek
11. Indemnity cover letter: Willis.

Please notify the Research Ethics Committee Office, Old School of Nursing, Waterford Regional Hospital on completion of Research.

Version 2 13/10/10 CL

Yours sincerely,

Pp:_____

Dr. Paula Lane
Chairperson
Research Ethics Committee,
Health Service Executive South Eastern Area

The Research Ethics Committee, HSE, South East is a recognized Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human use) Regulations 2004 and as such is authorized to undertake ethical review of clinical trials of all descriptions and classes for the Republic of Ireland.

The Research Ethics Committee, HSE South East issues ethical approval on the basis of information provided. It is the responsibility of the researcher to notify the Research Ethics Office of any changes to a study to ensure that the approval is still relevant.

c.c. Dr Wojciech Sasiadek, Consultant Radiation Oncologist, UPMC Whitfield Cancer Centre, Butlerstown North, Cork Road, Waterford
Ms Claire O'Gorman, School of Health Sciences, Department of Nursing, Waterford Institute of Technology, Cork Road, Waterford

Appendix III: Patient Information Sheet & Consent Form

Chemoradiotherapy and Rectal Cancer: Impact on Symptoms, Quality of Life and Prognostic Indicators

INFORMATION SHEET

What we are trying to do?

Many people with rectal cancer receive pre operative radiotherapy. This radiotherapy may lead to fatigue and other symptoms. By using validated questionnaires, we are hoping to examine how common fatigue is and how this fatigue, and other symptoms may affect your quality of life.
We also wish to obtain a blood sample. We want to see if changes in certain blood components, called lymphocytes in addition to some other bio-markers, will tell us if some patients are more sensitive to radiotherapy treatment than others. By doing this study, in the future, we may be able to predict the symptoms each patient may suffer from and how they will respond to treatment. This would allow us to individualise care, reduce symptoms and improve quality of life.

What does it involve?

We will ask you to complete a simple questionnaire to see what radiotherapy related symptoms you are suffering from and how these are affecting your quality of life at four different times.

1) Before commencing treatment.
2) Halfway through your treatment.
3) Immediately after you have completed your treatment.
4) Four to eight weeks after you have completed your treatment (before surgery).

We need to collect this information at these different times as symptoms may get better or worse at different stages. For convenience, we will ask you to complete the questionnaire at your routine clinic appointments, and then maybe once, by telephone, four to eight weeks after completion of pre operative treatment. The blood sample will be collected once, before you start any radiotherapy treatment. Information will be kept strictly confidential and will allow us to build a picture of how you are affected with this treatment. The researchers involved in this study may also require information from your medical notes in order to oversee your symptoms and your response to treatment.

Consenting to participate?

It is entirely up to you whether you take part and to what extent. You can change your mind at any time without giving any reason. None of this will affect your treatment or medical care in any way.

Confidentiality

All information is kept strictly confidential and your name will never be used with this information. We will not give information to anyone other than the research team.

Any questions?

If you have any questions at any time, please feel free to write or telephone us.

Dr. Martina Gooney
Department of Nursing
Waterford Institute of Technology
Tel: 051-302194
email: mgooney@wit.ie
clairefogorman@eircom.net

Dr. Wojciech Sasiadek
UPMC Whitfield Cancer Centre
Waterford
Tel: 051-337444
email: sasiadekw@upmc.edu

Ms Claire O'Gorman
Department of Nursing
Waterford Institute of Technology
Tel: 087-7794162
email:

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Waterford Institute of Technology
INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

UPMC University of Pittsburgh Medical Center
Whitfield Cancer Centre

PATIENT CONSENT FORM

Please insert your initials in the box opposite each statement:

- I have read the patient information form and understand fully the purpose of this study, and what my participation will involve.	
- I am satisfied that any queries, which I had, were answered satisfactorily.	
- I am participating in this study of my own free will.	
- I am aware that I can withdraw from the study at any time.	
- I am aware that I will be asked to provide a blood sample for the study.	
- I am aware that those involved in this study may require information from my medical notes in the future.	
- I am aware that all information I give will be in confidence and that my name will never be used with this information.	
- I agree that I may be contacted by telephone by the researcher at the final timepoint of the study.	

_____ _____
Participants Signature Date

_____
Name in Block Capitals

_____ _____
Researchers Signature Date

_____
Name in Block Capitals

Appendix IV: HSE Ethical Approval Letter for Study Extension

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

HSE South,
Waterford Regional Hospital,
Dunmore Road,
Waterford,
Ireland.

Telephone 051 848000
Fax 051 848572

RESEARCH ETHICS COMMITTEE.
HEALTH SERVICE EXECUTIVE, SOUTH EASTERN AREA

18th March 2014

Ms. Claire O' Gorman, RGN/Dip/BSc/PGDip
Department of Nursing
Waterford Institute of Technology
Cork Road
Waterford

STUDY TITLE: Chemoradiotherapy and Rectal Cancer: Impact on Symptoms, Quality of Life and Factors that influence Radiosensitivity

STUDY STATUS: *APPROVED*

Dear Ms. O' Gorman,

The Research Ethics Committee, HSE, South East reviewed the above study and are happy to grant you Full Ethical Approval.

The following documents were reviewed and approved:

1. Standard R.E.C. Application Form
2. Research Protocol Form
3. P.I.L
4. Participant Consent Form

The following documents were received:

1. Copy of Insurance documentation
2. C.V. Of Chief Investigator – Ms. Claire O' Gorman

Please notify the Research Ethics Committee Office, Old School of Nursing, Waterford Regional Hospital on completion of Research.

Yours sincerely,

Caroline Lamb

Ms Caroline Lamb
A/Research Ethics Committee Coordinator
Health Service Executive, South Eastern Area

The Research Ethics Committee, HSE, South East is a recognized Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human use) Regulations 2004 and as such is authorized to undertake ethical review of clinical trials of all descriptions and classes for the Republic of Ireland.

The Research Ethics Committee, HSE South East issues ethical approval on the basis of information provided. It is the responsibility of the researcher to notify the Research Ethics Office of any changes to a study to ensure that the approval is still relevant.

Appendix V: Patient Information Sheet & Consent Form for Study Extension

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Waterford Institute of Technology
INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

UPMC University of Pittsburgh Medical Center
Whitfield Cancer Centre

Waterford Institute of Technology
Cork rd.
Co. Waterford

03 March 2014

Dear

I am writing in relation to the ongoing research which is being undertaken at Waterford Institute of Technology / UPMC Whitfield Cancer Centre, that you kindly agreed to participate in at the beginning of your treatment.

As part of your standard treatment, tissue samples were routinely taken and stored in the hospital if required for further analysis. As an extension to the study you are taking part in, with your permission, we would like to access a portion of these samples and test them for certain components or molecules that could tell us how your tumour reacted to the treatment you received before your surgery.

As these samples are taken routinely anyway, the extension to the current study requires no further intervention or additional treatment, just your permission for access to the tissue. If you agree to this, I would appreciate it if you could sign the consent form overleaf and return it to me in the enclosed stamped addressed envelope.

This information could potentially guide the treatment of future patients diagnosed with this illness and lead to improvements in healthcare provision. Therefore, your assistance in this matter would be gratefully received.

I hope this letter finds you well and I would like to take this opportunity to thank you for your participation in the research study so far, and to wish you luck with your treatment. Please do not hesitate to contact me if you have any further queries in relation to the above.

Kindest regards

_____

Wojciech Sasiadek
Consultant Radiation Oncologist
UPMC Whitfield Cancer Centre

All information is strictly confidential and participants may withdraw from the study at any time

Chemoradiotherapy and Rectal Cancer: Impact on Symptoms, Quality of Life and Prognostic Indicators

PATIENT CONSENT FORM – STUDY EXTENSION

I am aware and give permission to the researchers of this study to access tissue samples that are taken routinely and as part of my treatment

Signed: _____

Date: _____

All information is strictly confidential and participants may withdraw from the study at any time

Appendix VI: Personal Information Form

SECTION A: ABOUT YOURSELF

Study Identity Number: _____

Date of Study Entry: _____

Name: _____

Telephone Number: _____

Date of Birth: _____

Weight: _____

Height: _____

Smoking: □O Yes □O No

Menopausal Status: □O Pre-menopausal □O Post-menopausal

Marital Status □O Married / living with partner

O Never married

□O Other (please specify) _____

Lives alone: □O Yes □ O No

Employment Status □O Employed □ O Full Time □ O Part Time

□O Unemployed

□O Other / Pension (please specify) _____

Appendix VII: Study Questionnaire

EORTC QLQ-C30
(version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:
Your birthdate (Day, Month, Year):
Today's date (Day, Month, Year):

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

EORTC QLQ – CR29

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
31. Did you urinate frequently during the day?	1	2	3	4
32. Did you urinate frequently during the night?	1	2	3	4
33. Have you had any unintentional release (leakage) of urine?	1	2	3	4
34. Did you have pain when you urinated?	1	2	3	4
35. Did you have abdominal pain?	1	2	3	4
36. Did you have pain in your buttocks/anal area/rectum?	1	2	3	4
37. Did you have a bloated feeling in your abdomen?	1	2	3	4
38. Have you had blood in your stools?	1	2	3	4
39. Have you had mucus in your stools?	1	2	3	4
40. Did you have a dry mouth?	1	2	3	4
41. Have you lost hair as a result of your treatment?	1	2	3	4
42. Have you had problems with your sense of taste?	1	2	3	4

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
43. Were you worried about your health in the future?	1	2	3	4
44. Have you worried about your weight?	1	2	3	4
45. Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
46. Have you been feeling less feminine/masculine as a result of your disease or treatment?	1	2	3	4
47. Have you been dissatisfied with your body?	1	2	3	4
48. Do you have a stoma bag (colostomy/ileostomy)? (please circle the correct answer)	Yes		No	

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
Answer these questions ONLY IF YOU HAVE A STOMA BAG, if not please continue below:				
49. Have you had unintentional release of gas/flatulence from your stoma bag?	1	2	3	4
50. Have you had leakage of stools from your stoma bag?	1	2	3	4
51. Have you had sore skin around your stoma?	1	2	3	4
52. Did frequent bag changes occur during the day?	1	2	3	4
53. Did frequent bag changes occur during the night?	1	2	3	4
54. Did you feel embarrassed because of your stoma?	1	2	3	4
55. Did you have problems caring for your stoma?	1	2	3	4
Answer these questions ONLY IF YOU DO NOT HAVE A STOMA BAG:				
49. Have you had unintentional release of gas/flatulence from your back passage?	1	2	3	4
50. Have you had leakage of stools from your back passage?	1	2	3	4
51. Have you had sore skin around your anal area?	1	2	3	4
52. Did frequent bowel movements occur during the day?	1	2	3	4
53. Did frequent bowel movements occur during the night?	1	2	3	4
54. Did you feel embarrassed because of your bowel movement?	1	2	3	4

During the past 4 weeks:

	Not at All	A Little	Quite a Bit	Very Much
For men only:				
56. To what extent were you interested in sex?	1	2	3	4
57. Did you have difficulty getting or maintaining an erection?	1	2	3	4
For women only:				
58. To what extent were you interested in sex?	1	2	3	4
59. Did you have pain or discomfort during intercourse?	1	2	3	4

FACIT Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important**. Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.**

		Not at all	A little bit	Some-what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4
An2	I feel tired.....	0	1	2	3	4
An3	I have trouble <u>starting</u> things because I am tired.....	0	1	2	3	4
An4	I have trouble <u>finishing</u> things because I am tired	0	1	2	3	4
An5	I have energy.....	0	1	2	3	4
An7	I am able to do my usual activities.....	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat.....	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired.....	0	1	2	3	4

Conferences

O'Gorman, C., Denieffe, S. and Gooney, M. (2014) Chemoradiotherapy & rectal cancer: symptoms, quality of life & tumour response - the influence of CEA & circulating cytokines. 14th Healthcare Interdisciplinary Research Conference & Student Colloquium 2013, Trinity College Dublin, Dublin 5th – 7th November 2013 (Oral Paper).

O'Gorman, C., Denieffe, S. and Gooney, M. (2013) Preoperative Radiotherapy & Rectal Cancer: Impact on Acute Symptom Presentation & Quality of Life. Faculty of Nursing & Midwifery 32nd Annual International Nursing Research Conference 2013, Royal College of Surgeons in Ireland, Dublin 20th - 21st February 2013 (Oral Paper).

O'Gorman C., Denieffe, S. and Gooney, M. (2012) Radiotherapy and rectal cancer: side effects, quality of life & predicting outcomes during treatment. Waterford Institute of Technology Research Day, Waterford 2nd – 3rd May 2012 (Poster).

Publications

O'Gorman, C. (2014) 'Bridging the theoretical gap', *Nursing Standard,* 28(33), pp. 66.

O'Gorman, C., Denieffe, S. and Gooney, M. (2014) 'Literature review: preoperative radiotherapy and rectal cancer - impact on acute symptom presentation and quality of life', *Journal of Clinical Nursing,* 23(3-4), pp.333-351.

O'Gorman, C., Sasiadek, W., Denieffe, S. and Gooney, M. (2014) 'Predicting Radiotherapy-Related Clinical Toxicities in Cancer: A Literature Review', *Clinical Journal of Oncology Nursing,* 18(3), pp. 1-8.