AN INVESTIGATION INTO THE RELATIONSHIP BETWEEN THE PERIOPERATIVE SYSTEMIC INFLAMMATORY RESPONSE AND POSTOPERATIVE COMPLICATIONS IN PATIENTS UNDERGOING SURGERY FOR COLORECTAL CANCER

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Abstract

Colorectal cancer is the second most common cause of cancer death in the western world. Despite improvements in diagnosis and treatment, 50% of patients still die from this disease. It is now recognised that postoperative infective complications contribute to poor cancer specific survival following resection for colorectal cancer. The basis of this observation is not clear. One hypothesis is that the presence of a raised systemic inflammatory response may be responsible. Whether a raised postoperative inflammatory response is the result of an early underlying infection at a preclinical stage, or whether a raised inflammatory response leads to increased susceptibility to subsequent infection is not known. If the former proves true, it is possible that targeting at risk patients with pre-emptive antibiotics may reduce infective complications and improve patient outcomes. Conversely, if the latter is the case, perioperative intervention to reduce the postoperative inflammatory response may reduce infective complications and hence improve outcomes, both short and long term, for patients undergoing colorectal cancer resection.

The work presented in this thesis further examines the relationship between the systemic inflammatory response and postoperative infective complications following resection for colorectal cancer, determines predictive thresholds for the development of postoperative infective complications, assesses the impact of the peak systemic inflammatory response on these thresholds and investigates the determinants of the peak response. Finally, the question as to whether a raised postoperative systemic inflammatory response is the cause or consequence of infective complications is examined.

Patients with colorectal cancer who have a raised systemic inflammatory response prior to surgery have been shown to have poorer long term and short term outcomes. The presence of an ongoing systemic inflammatory response in these patients may be due to impaired cortisol production. Chapter 3 examines the relationship between the perioperative systemic inflammatory response and endogenous cortisol production by assessment of adrenocortical function preoperatively in 80 patients undergoing resection for colorectal cancer.

Infective complications particularly in the form of surgical site infections including anastomotic leak represent a serious morbidity after colorectal cancer surgery. Systemic inflammation markers, including C-reactive protein and white cell count, have been reported to provide early detection. However their relative predictive value is unclear. Chapter 4 examines the diagnostic accuracy of serial postoperative white cell count, albumin and C-reactive protein in detecting infective complications in 454 patients undergoing surgery for colorectal cancer. It demonstrates that postoperative C-reactive protein measurement, particularly a threshold of 170 mg/l on day 3 postoperatively, is clinically useful in predicting surgical site infective complications, including an anastomotic leak, in patients following colorectal cancer resection.

Chapter 5 compares the value of daily C-reactive protein concentrations in the prediction of postoperative infective complications in patients undergoing open versus laparoscopic resection for colon cancer. Although the magnitude of the systemic inflammatory response, as evidenced by C-reactive protein, following surgery was greater in open compared with laparoscopic resection, the threshold concentrations of C-reactive protein for the development of postoperative infective complications were remarkably similar on days 3 and 4.

The postoperative systemic inflammatory response, as evidenced by C-reactive protein on days 3 and 4, is shown in chapters 4 and 5 to be associated with the development of infective complications following surgery for colorectal cancer. However, patients in enhanced recovery after surgery programmes require earlier assessment at day 2, at the peak inflammatory response to surgery. Chapter 6 assesses the impact of day 2 C-reactive protein, on concentrations at days 3 and 4. A day 2 C-reactive protein concentration >190 mg/L was associated with day 3 and 4 concentrations above established thresholds for the development of infective complications.

Chapter 7 examines the clinicopathological determinants of the postoperative systemic inflammatory response, as evidenced by C-reactive protein concentrations on day 2, day 3 and day 4 in patients following resection of colorectal cancer. Chapter 7 demonstrates that several clinical factors are independently associated with the peak systemic inflammatory response, as evidenced by postoperative day 2 C-reactive protein concentration and threshold, following resection of colorectal cancer. In particular, emergency presentation, socioeconomic deprivation and preoperative systemic inflammation are associated with a higher peak systemic inflammatory response. In contrast, laparoscopic surgery is associated with a lower peak systemic inflammatory response.

Enhanced Recovery After Surgery (ERAS) programmes aim to attenuate the stress response to surgery, reduce the length of hospital stay and have been proposed to be associated with reduced morbidity and mortality. However, data on the effect of enhanced recovery on the systemic inflammatory response and infective complications remains limited. Chapter 8 examines the impact of enhanced recovery on the systemic inflammatory response and the rate of infective complications following elective surgery

for colorectal cancer. Enhanced recovery was associated with a significant reduction in length of hospital stay. In contrast, the postoperative systemic inflammatory response was similar to that of conventional care. Overall complication rates, both non-infective and infective, were also similar.

Chapter 9 examines the relationships between postoperative predictive thresholds of C-reactive protein and infective complications, in the context of the administration of pre-emptive antibiotic therapy, for patients undergoing resection for colorectal cancer. The administration of pre-emptive antibiotics guided by C-reactive protein thresholds predictive of infective complications did not reduce infective complication rates or the magnitude of the postoperative inflammatory response following elective resection for colorectal cancer.

In summary, the objective measurement of the postoperative systemic inflammatory response and its relationship with postoperative outcomes has profound implications for assessment and treatment of the surgical stress response in patients with colorectal cancer.

Table of Contents

List of Tables

List of Figures

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Author's Declaration

The work presented in this thesis was undertaken during a period of research between 2010 and 2013 in the University Department of Surgery at Glasgow Royal Infirmary. The work has been completed whilst working as a Clinical Fellow in General Surgery at Glasgow Royal Infirmary.

I declare that the work presented in this thesis was undertaken by myself, except where indicated below:

- Recruitment of patients and Short Synacthen testing was carried out by myself and Mr Campbell Roxburgh (Chapter 3).
- Co-scoring of histopathology slides for Klintrup-Makinen grade and Tumour Necrosis was carried out by myself, Mr Graeme Guthrie and Mr Campbell Roxburgh (Chapter 3).
- Data collection with regards to postoperative infective complications was carried out by Mr Jonathan Platt and Mr Graham MacKay for cases prior to 2010 (Chapters 4 - 8).

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Publications

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Impact of day 2 C-reactive protein on day 3 and 4 thresholds associated with infective complications following curative surgery for colorectal cancer.
Ramanathan ML, Mackay G, Platt J, Horgan PG, McMillan DC.
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Platt JJ, Ramanathan ML, Crosbie RA, Anderson JH, McKee RF, Horgan PG, McMillan DC.
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Clinicopatholigical determinants of an elevated systemic inflammatory response following potentially curative resection for colorectal cancer.

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Submitted to **Annals of Surgery**

Dedication

To James.

1 Introduction

1.1 Epidemiology of Colorectal Cancer

In the UK, the incidence of colorectal cancer is increasing. It is the fourth most common cancer with approximately 41,600 people diagnosed each year (CRUK, 2014). Around 16,000 deaths occur annually from colorectal cancer in the UK, second only to lung cancer as a cause of cancer death in the combined male and female population. Incidence increases with age with over 80% of cases occurring in patients over 60 years old. Colorectal cancer is the second most common cancer to affect women and the third most common to affect men. Men in the UK have a lifetime risk of 1 in 14 of developing colorectal cancer, and women a risk of 1 in 19 (CRUK, 2014). Survival from colorectal cancer has improved over the past 30 years in the UK (Shack et al., 2007, Mitry et al., 2008), mainly owing to improved treatment and increased surgical specialisation, and to a lesser extent, earlier presentation and diagnosis. However, outcome following diagnosis remains poor, with around half of those undergoing potentially curative procedures surviving to 5 years (CRUK, 2014).

Worldwide, colorectal cancer is the third most common cancer with a prevalence of over 3 million people in 2006 (Kamangar et al., 2006). The annual incidence is estimated at over 1.2 million with the highest rates seen in Australasia, Western Europe and North America. The African nations have the lowest incidence although countries with a rapid "westernisation" of diet and lifestyle, such as Japan, have seen a substantial increase in the number of new cases of colorectal cancer. Worldwide, the disease accounts for more than 600,000 deaths each year, making it the fourth commonest cause of cancer death (Parkin et

al., 1999). Despite the increased number of new cases diagnosed each year, mortality from colorectal cancer has fallen since the 1970's, decreasing more rapidly since the 1990's.

1.2 Aetiology of Colorectal Cancer

The aetiology of colorectal cancer is poorly understood. The majority of colorectal tumours (>90%) are termed “sporadic” and are thought to result from complex interactions between host and environmental factors. Approximately 10% represent well defined hereditary cancer syndromes. A number of factors have been implicated in the development of sporadic colorectal cancer.

1.2.1 Colorectal carcinogenesis pathways

There are thought to be at least three molecular pathways in colorectal carcinogenesis. The first occurs due to chromosomal instability and allelic losses (the adenoma-carcinoma sequence) causing the mucosa to undergo malignant transformation resulting in sporadic colorectal cancer. It is believed to develop gradually over a period of time through the sequential accumulation of genetic alterations due to environmental and lifestyle factors (CRUK, 2014). The second occurs in approximately 15% of all colorectal cancers and is due to microsatellite instability. Microsatellites are repetitive sequences of DNA randomly distributed throughout the genome. Microsatellite instability is caused by mutations in the genes that are involved in DNA repair (mismatch repair genes). This leads to base-pair mismatches during DNA replication, ultimately leading to protein truncations (Boland et al., 1998). In addition to chromosomal and microsatellite instability, a third carcinogenic pathway, known as hypermethylation, has been described. The precursor lesions for development of carcinomas via this route are not adenomas but serrated polyps. These tumours are thought to develop along a pathway where hypermethylation rather than genetic mutation is responsible for the inactivation of tumour suppressor gene function (Ferracin et al., 2008).

1.2.2 Age

Age remains the single biggest risk factor for the development of colorectal cancer, with over 85% of tumours diagnosed in patients aged over 60 years (CRUK, 2014). Possible reasons behind this association are the increased length of exposure to environmental risk factors, as well as increased time for chromosomal mutations to develop. As infectious diseases have waned, and healthcare has improved, we are faced with diseases that occur at ages not previously attained.

1.2.3 Deprivation

Socioeconomic deprivation has been shown to be a risk factor associated with colorectal cancer, particularly in males, with those in more affluent categories having 20% lower incidence compared to those in the most deprived (Oliphant et al., 2011). Furthermore, there is also evidence that patients with colorectal cancer who are more socioeconomically deprived have poorer short term outcomes, as well as cancer specific and overall survival (Hole and McArdle, 2002). The underlying causes for socioeconomic inequalities in survival from colorectal cancer remain unclear.

1.2.4 Diet and lifestyle

The highest rates of colorectal cancer are found in western countries. Studies on migrant populations have demonstrated that the incidence rates of the host country are adopted within a generation (Haenszel and Kurihara, 1968, Potter et al., 1993). This has led to a widely held belief that a western lifestyle is responsible for the development of colorectal cancer in many cases.

A “westernised” diet has been associated with the development of colorectal cancer, in particular consumption of red meat and diets low in fibre. It is thought to be the reason for increasing incidence in countries such as Japan, where a “western” diet and lifestyle has been adopted over recent years (CRUK, 2014). Epidemiological studies have consistently observed that countries with a high intake of red meat and animal fat have a higher incidence of colorectal cancer (Armstrong and Doll, 1975, Graham and Mettlin, 1979), however information on the mechanism underlying this relationship is sparse and overall the association between red meat intake and the development of colorectal cancer is unclear. With reference to fibre, a pooled analysis of over 13 prospective studies (>700,000 men and women) concluded that, after accounting for other dietary risk factors, high fibre intake was not associated with a reduced risk of colorectal cancer (Park et al., 2005).

Individuals with high levels of daily activity have a significantly lower risk than those who have sedentary lifestyles (Samad et al., 2005). Regular exercise has been shown to reduce the risk of colon cancer by almost 25% (Wolin et al., 2007). Furthermore, this affect appears to be independent of potentially confounding variables such as cardiovascular health, diet and obesity (Colditz et al., 1997).

1.2.5 Obesity

Obesity is a well established risk factor for the development of colorectal cancer. A Body Mass Index (BMI) $\geq$30 kg/m^2 has been shown to confer a 20% greater risk of developing colorectal cancer compared to normal BMI. In particular, in men with central obesity, for every 2cm increment in waist circumference the risk of colorectal cancer increased by 4% (Moghaddam et al., 2007). Mechanisms are poorly understood but it is suggested that

adipocytes produce pro-inflammatory cytokines resulting in a chronic systemic inflammatory response predisposing to cancer (McMillan et al., 2006).

1.2.6 Smoking and alcohol

Cigarette smoking is well known to be associated with an increased risk of colorectal cancer. This is thought to be due to the increase in the likelihood of cancer precursor adenomas following years of exposure to cigarette smoking.

Studies have shown that both lifetime and baseline alcohol intake can increase the risk of colorectal cancer (Ferrari et al., 2007). The mechanisms through which alcohol leads to tumour development have yet to be determined. One hypothesis is that metabolites of alcohol (e.g. acetaldehyde) may be carcinogenic and may generate free radicals (Poschl and Seitz, 2004). Heavy alcohol consumption is also associated with a systemic inflammatory response which may influence cancer risk (Imhof et al., 2001).

1.2.7 Medication

A number of medications have been shown to confer a protective effect regarding the risk of developing colorectal cancer. A large meta-analysis reported that the risk of developing colorectal cancer was significantly lower in postmenopausal women who had taken Hormone Replacement Therapy compared to those who had never received such treatment (Grodstein et al., 1999).

There is also good evidence that patients taking non-steroidal anti-inflammatory drugs (NSAIDs) reduce their risk of developing colorectal cancer. A randomised control trial in

2003 concluded that taking aspirin daily reduced the risk of colorectal adenoma formation in patients with a history of polyps (Baron et al., 2003). Precise mechanisms to explain these effects have yet to be elucidated, however one hypothesis is that these drugs work by modulating the local and systemic inflammatory responses, recognised to be associated with the development and progression of colorectal cancer (McMillan et al., 2003b).

Preoperative administration of glucocorticoids has been shown to reduce postoperative length of stay and systemic inflammation, as evidenced by serum Interleukin-6 following hepatic resection, and reduced length of stay following colorectal surgery. The proposed mechanism of action to explain this is that preoperative administration of glucocorticoids attenuates the postoperative systemic inflammatory response (Srinivasa et al., 2011).

1.2.8 Systemic inflammatory response

A number of studies have suggested that the risk of colorectal cancer is higher in individuals with evidence of a pre-existing systemic inflammatory response (Crozier et al., 2007, Roxburgh and McMillan, 2010). In two studies of over 22,000 patients, plasma C-reactive protein concentrations were consistently elevated among people who subsequently developed colorectal cancer (Erlinger et al., 2004, Proctor et al., 2010). It is of particular interest that inflammation has been associated with many other individual risk factors for colorectal cancer and raises the possibility that a final common pathway is responsible for both tumour development and the generation of a systemic inflammatory response. It remains to be established whether inflammation is a cause or consequence of cancer development, but their intimate relationship has led to inflammation being proposed as an inherent hallmark of cancer (Colotta et al., 2009).

1.2.9 Pre-existing conditions

In a small number of cases the pathogenesis of colorectal cancer can be attributed to specific aetiological factors such as inherited genetic mutations or inflammatory bowel disease (Ponz de Leon et al., 2004). The natural history of colorectal cancer differs in individuals with a hereditary predisposition: with an abbreviated length of tumorigenesis, often presenting at an earlier age.

1.2.9.1 Inflammatory bowel disease

Patients with inflammatory bowel disease, such as Crohn's disease or ulcerative colitis, have an increased risk of developing colorectal cancer. A meta-analysis of over 60,000 patients concluded that cumulative risk of colorectal cancer in patients with Crohn's disease was 2.9% at ten years (Canavan et al., 2006). The risk of colorectal cancer in patients with ulcerative colitis is related to the severity and duration of symptoms and is estimated at 2% after 10 years, 8% after 20 years and 18% after 30 years (Eaden et al., 2001). The predisposition to cancer in patients with inflammatory bowel disease does not appear to have a specific genetic basis but instead is assumed to be the result of chronic inflammation as the precursor of tumour development (Triantafillidis et al., 2009).

1.2.9.2 Hereditary Non-Polyposis Colon Cancer

Hereditary non-polyposis colon cancer (HNPCC), also referred to as Lynch syndrome, is the most common autosomal dominant cancer predisposition syndrome responsible for at least 50% of hereditary disease and about 3% of all cancer cases and results from a defect in one of the mis-match repair genes (mainly hMSH2 on chromosome 2p and hMLH1 on chromosome 3p). Incidence is approximately 1:1000 of the general population. A marked

70-80% increase in proximal colon cancers is observed in carriers (Lynch et al., 1977). Colorectal cancers are the most frequent cancers associated with HNPCC; endometrial cancers have been identified as the second-leading cancer associated with the syndrome. Patients with HNPCC have an 80% lifetime risk of colorectal cancer and women have a 60% risk of endometrial cancer. In addition they have an elevated risk of other cancers including stomach, biliary, ovarian and urogenital cancers. Cardinal features of Lynch syndrome colorectal cancer include early age of onset, proximal colon involvement, increased incidence of synchronous and metachronous colon cancers, and an autosomal dominant inheritance pattern. Tumours tend to be poorly differentiated with an increased frequency of local inflammatory reaction around the tumour termed “Crohns-like reaction” alongside an abundance of tumour infiltrating lymphocytes (Jass, 1998). With current detection and treatment options, it is felt that no one with HNPCC should die of colorectal cancer, assuming that the patient at increased risk has been identified, has a knowledgeable physician, and has been referred to a gastroenterologist or surgeon who prescribes frequent (annual) screening colonoscopies initiated at age 25.

1.2.9.3 Familial Adenomatous Polyposis

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disease found in less than 1% of patients with colorectal cancer, but will lead to cancer almost 100% of the time. Incidence is estimated to be 1:8000 of the general population. It is caused by mutations in the adenomatous polyposis (APC) gene, a tumour suppressor gene, located on chromosome 5 and characterized by large numbers of adenomatous polyps (hundreds to thousands) throughout the colon. Classical FAP is defined clinically by 100 or more adenomatous colon and rectal polyps, and typically occurs in patients younger than age 40. A variant of FAP called attenuated FAP (AFAP) is characterized by less than 100 colon

polyps and the onset of polyposis and cancer occurs later than in FAP. AFAP is generally defined in individuals with 10-99 colonic adenomatous polyps, or those with 100 or more colonic polyps occurring at an older age, or those with a history of colorectal cancer before age 60 and a family history of multiple adenomatous polyps. The latter group of patients will usually have rectal sparing, have right-sided colonic adenomas, and lack extra colonic manifestations. People with FAP should undergo regular surveillance and, ultimately, prophylactic colectomy. Despite this, the association with duodenal polyps and extracolonic malignancies including pancreatic mucinous adenocarcinoma, hepatoblastoma and desmoid tumours means that a significant number of patients with FAP still die from malignant disease (Belchetz et al., 1996).

1.2.9.4 Hamartomatous polyposis syndromes

A number of different syndromes have been described whereby patients have a propensity to develop multiple hamartomatous polyps in the gastrointestinal tract. The majority of these syndromes are inherited in an autosomal dominant fashion and include Juvenile Polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), hereditary mixed polyposis syndrome (HMPS) and the PTEN hamartoma tumour syndromes (Cowden disease). Although the clinical features of these syndromes are variable, all give patients an increased risk of developing colorectal cancer. The lifetime risk of colorectal cancer in JPS is 60% and in PJS it is 30%. Colon cancer develops in up to 10% of people with PTEN. In addition, patients with multiple hamartomatous polyps are prone to malignancies of the stomach, pancreas and small bowel. The progression of hamartomatous polyps to cancer is poorly understood.

1.3 Presentation and Diagnosis of Colorectal Cancer

The presentation of colorectal cancer varies depending on the site of the tumour and the stage of disease. Early tumours may be asymptomatic and detected via population screening. Patients with proximal colonic tumours may present with iron deficiency anaemia secondary to occult blood loss, a right-sided abdominal mass or abdominal pain. In contrast, patients presenting with left-sided colonic tumours may suffer from a change in bowel habit, large bowel obstructive symptoms, colicky left-sided lower abdominal pain, intermittent distension and bloating, or dark blood mixed with stool. Patients with distal or rectal tumours may complain of urgency and frequency of stools, sensation of incomplete evacuation, pelvic pain or tenesmus, and fresh bleeding. Occasionally, presentation is as an emergency with intestinal obstruction, fistulation or perforation.

The Scottish Bowel Screening Programme was first introduced in Scotland in 2007. It is a biennial programme which involves both men and women between the ages of 50 and 74 years. Recently it has been extended to allow those over the age of 74 to opt into the programme. Individuals are invited to participate in screening for colorectal cancer using at home faecal occult blood testing kits. Screening for colorectal cancer increases the number of early stage cancers diagnosed and consequently reduces cancer specific mortality, and may also reduce the incidence of colorectal cancer by removing pre-cancerous polyps.

In the elective setting, a histological diagnosis should be made and the disease fully staged before treatment is commenced. Flexible sigmoidoscopy or colonoscopy and biopsy chosen according to symptoms have the highest diagnostic sensitivity and specificity. If colorectal cancer is diagnosed at sigmoidoscopy, a full colonoscopy is indicated to check for synchronous bowel lesions (present in 4-5%). Colonoscopy is the gold standard

investigation of the colon and rectum allowing direct visualisation of the mucosal surface and offering the capacity to obtain tissue for histological diagnosis. CT colonography is a less invasive technique increasingly used as an alternative for frail or elderly patients.

It has long been recognised that emergency presentation is associated with a high postoperative mortality rate (McArdle and Hole, 2004). Furthermore, compared to those who undergo elective resection, there is also a reduction in overall and cancer specific survival, independent of other clinicopathological factors including tumour stage (McArdle et al., 2006). Indeed, it is of interest that the presence of a systemic inflammatory response prior to surgery, as evidenced by an elevated C-reactive protein concentration, predicts overall and cancer specific survival, independent of tumour stage, in patients undergoing potentially curative resection for colorectal cancer (McMillan et al., 2003b).

The main determinant of colorectal cancer survival is stage at presentation (Dukes and Bussey, 1958). Older age, stage at diagnosis, deprivation and emergency presentation are associated with increased mortality following resection.

1.4 Staging of Colorectal Cancer

Colorectal cancer staging quantifies the extent of the disease and provides a framework for selecting the appropriate treatment. Staging is usually by the Tumour, Node, Metastases (TNM) classification system, produced by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) (Brierley, 2006, UICC, 2015, AJCC, 2015). Scores are given based on the extent of the primary tumour (T), the number of regional lymph nodes involved (N), and the presence of metastatic disease (M), which are combined to form stage groupings. In the UK, the alternative Dukes' classification is often still quoted (Dukes, 1937). Pathological reporting following surgery should include staging, tumour differentiation, margins, and extramural vascular invasion. The prognosis of colorectal cancer is often summarised according to tumour stage at diagnosis. Five year survival rates in the UK vary from over 90% for patients with tumours confined to the mucosa, to less than 10% for those with metastatic disease (CRUK, 2014).

Pre-treatment staging relies on a combination of visualisation of the colon, radiological imaging of the chest, abdomen and pelvis, and histopathology from biopsies where possible. A CT scan of the chest, abdomen and pelvis is the radiological investigation of choice to define the extent of local tumour invasion and establish the presence or absence of regional lymphatic spread and metastatic disease. In rectal cancers further investigation with MRI of the pelvis to assess local extension of rectal cancer or transrectal ultrasound to assess depth of invasion of particularly early rectal cancer may also be required. If there is diagnostic uncertainty regarding the presence of distant metastatic disease, additional modalities such as MRI or PET scans may also be used. Pre-treatment staging helps to guide selection of the most appropriate management strategy as well as planning the

operative approach, and decision making regarding the provision of neo-adjuvant therapy, if surgery is indicated.

1.5 Pathology and tumour characteristics

The pathological stage of the tumour is widely regarded as the single biggest determinant of outcome in colorectal cancer. The staging systems most commonly employed in the UK are the Dukes and TNM classifications. In addition, a number of other pathological characteristics have been reported to affect prognosis and may help to stratify patients with node negative disease for the allocation of adjuvant treatment. Assessment of these characteristics depends almost exclusively on accurate pathological processing and reporting.

Histological subtypes of colorectal cancer are as follows: 98% adenocarcinomas, 2% adenosquamous carcinoma or adenocarcinoid carcinoma. Spread may be directly into adjacent organs (e.g. duodenum, bladder, uterus), haematogenous (liver and lungs preferential sites), lymphatic (pericolic and mesenteric nodes) or transcoelomic. In terms of distribution, left sided tumours are more common with 30% arising in the sigmoid or descending colon and 40% in the rectum.

1.5.1 Tumour grade

Tumour grade describes how well the tumour is differentiated and is reported subjectively by the pathologist. Colorectal tumours are generally categorised as low grade (well or moderately differentiated) or high grade (poorly differentiated). Reduced 5 year survival and increased risk of local recurrence have been reported in high grade tumours.

1.5.2 Venous invasion

The microscopic diagnosis of venous invasion is made when tumour cells are identified within an endothelium lined space surrounded by a rim of smooth muscle and / or containing red blood cells (Sternberg et al., 2002). Venous invasion is an established predictor of poor prognosis in colorectal cancer and its presence is associated with an increased incidence of disease recurrence and reduced survival (Roxburgh et al., 2009a). The presence of venous invasion is associated with an increased risk of developing distant metastases (particularly hepatic) in the future and cancer related death. Clinical application is hampered by variations in reporting rates and techniques of assessment (Roxburgh and Foulis, 2011).

1.5.3 Perineural invasion

Perineural invasion is a pathological process whereby tumour cells invade nervous tissues and spread along nerve sheaths. It is recognised to represent an aggressive tumour phenotype and its presence in colorectal tumours is reported to be a poor prognostic sign associated with local recurrence and reduced survival (Ueno et al., 2001).

1.5.4 Peritoneal involvement

Peritoneal involvement is said to be present if tumour cells are visible either on the peritoneal surface or free in the peritoneal cavity. It is regarded as a poor prognostic sign in both colon and rectal cancer and is associated with disease recurrence and metastatic spread (Petersen et al., 2002).

1.5.5 Tumour perforation

Tumour perforation is defined as a visible defect through the tumour such that the bowel lumen is in communication with the external surface of the resected specimen. It is widely recognised as a high risk pathological characteristic and has been associated with increased risk of disease recurrence and reduced survival, independent of tumour stage, in patients with colorectal cancer (Petersen et al., 2002).

1.5.6 Margin involvement

Tumour cells present at or within 1mm of the surgical margin indicate inadequate tumour excision and are an exceedingly poor prognostic indicator (Petersen et al., 2002).

1.5.7 Petersen index

Petersen and coworkers set out to identify objective and easily determined pathological features that could help identify which patients with Dukes B colon cancer may benefit from chemotherapy. After a meticulous pathological review of 268 consecutive cases the authors concluded that four factors – venous invasion, peritoneal involvement, tumour perforation and margin involvement – were independent prognostic markers on multivariate analysis (Petersen et al., 2002). Combining these factors into a cumulative scoring system stratified patients effectively into low risk (score 0-2) or high risk (score 3-5) categories. The prognostic value of the Petersen Index was subsequently confirmed in a large validation cohort of patients with Dukes B disease (Morris et al., 2007).

1.5.8 Tumour necrosis

Tumour necrosis has been reported to be associated with decreased local inflammatory infiltrate and with elevated markers of systemic inflammation in colorectal cancer and is related to poorer prognosis. The extent of tumour necrosis is assessed semi-quantitatively and graded as ‘absent’ (none), ‘focal’ (less than 10% of tumour area), ‘moderate’ (10-30% of tumour area), or ‘extensive’ (>30% of tumour area) (Pollheimer et al., 2010).

In conclusion, a number of prognostic criteria in addition to the widely used TNM classification have been validated, however their subjective nature leads to difficulties in reproducibility and hence many are not widely reported or utilised. Therefore, an objective prognostic indicator would be beneficial.

1.6 Management of Colorectal Cancer

1.6.1 Surgery

Approximately 80% of colorectal cancers are localised to the bowel wall and can be surgically resected with curative intent. These operations involve complete removal of the tumour, the vascular pedicle and the lymphatic drainage of the affected colonic segment. The aim is to remove all macroscopic disease with an adequate margin of normal tissue. The nature of the resection is dependent on tumour site and blood supply. Primary anastomosis is usual unless there is acute obstruction, significant peritonitis, a severely ill or grossly malnourished patient. Low rectal anastomoses are often protected by a temporary loop ileostomy. Surgery may be undertaken as an open procedure or laparoscopic-assisted. Small rectal cancers confined to the mucosa may be effectively managed by local excision using transanal endoscopic microsurgery (TEMS) while larger tumours require more radical resection. For low-lying rectal cancers with confirmed sphincter invasion or where a clear distal resection margin cannot be guaranteed, the operation of choice is an abdominoperineal resection (APR). Resection of the primary tumour remains the principle element of treatment and potential cure for patients diagnosed with colorectal cancer. However, surgery itself is only one component of a series of assessments and investigations that make up the patients management. All patients with a diagnosis of colorectal cancer should be discussed with a multi-disciplinary team, including surgeons, oncologists, radiologists, pathologists and colorectal nurse specialists.

1.6.2 Enhanced Recovery After Surgery

Over the past decade, there has been a revolution in the nature of perioperative care with the introduction of enhanced recovery after surgery (ERAS) protocols (Kehlet, 1997). More recently, this has been proposed for cancer surgery, particularly colorectal cancer resection. Enhanced recovery programmes aim to attenuate the stress response to surgery, accelerate recovery, reduce the length of hospital stay and have been proposed to be associated with reduced hospital morbidity and mortality (Teeuwen et al., 2010). For example, patients undergoing colorectal resection within an enhanced recovery programme have been reported to stay in hospital half as long as those receiving conventional care (King et al., 2006).

1.6.3 Neo-adjuvant therapy

For patients with large or low-lying rectal tumours initially precluding sphincter sparing surgery, neo-adjuvant treatment may reduce tumour bulk and enhance the prospect of resection with curative intent but its provision is unlikely to avoid the need for abdominoperineal resection. The indications for neo-adjuvant chemoradiotherapy include T3/4 tumours, positive mesorectal nodes on preoperative imaging and tumours threatening or involving the mesorectal fascia. Radiotherapy acts to downsize the tumour and reduce the chance of positive margins remaining after surgery.

1.6.4 Adjuvant chemotherapy

In patients with colorectal cancer who have undergone potentially curative surgery, disease recurrence is thought to be the result of clinically occult metastases that are present at the

time of resection. The goal of adjuvant chemotherapy is to eliminate these tumour cells and thereby increase the likelihood of cure. A 5 year survival advantage after adjuvant chemotherapy has been clearly demonstrated in node positive colon cancer but its benefit in node negative disease has yet to be confirmed and only those with high risk pathological features are usually considered. There is uncertainty as to whether adjuvant chemotherapy offers a survival advantage to patients who have previously undergone preoperative treatment. Radiotherapy for rectal cancer is usually only given for unexpected positive surgical margins where neo-adjuvant treatment has not been given.

1.6.5 Metastatic disease

Approximately 15% of patients presenting with colorectal cancer will have advanced disease (CRUK, 2014). Resection of isolated liver or lung metastases can offer significant survival advantage. If potentially curative resection is not an option, these patients are managed with palliative treatments. For example, stenting, local resection or creation of a defunctioning stoma may be considered for symptom palliation. Alternatively, oncological palliation with chemotherapy or radiotherapy may be administered via the oncologist.

1.7 Inflammation and cancer

It is now recognised that disease progression in colorectal cancer is not only influenced by tumour characteristics but that patient characteristics also play an important role in cancer progression and survival. The tumour characteristics alone, while providing a degree of prognostic information, cannot fully explain the survival differences observed in patients with cancers of the same pathological stage. It is increasingly apparent that patient characteristics such as chronological age as well as potentially modifiable traits such as exercise tolerance relate to survival in colorectal cancer. In addition, the presence or absence of local or systemic inflammatory responses have received particular attention in relation to cancer outcomes and may represent the intrinsic ability of a person to generate an anti-tumour response.

Links between inflammation and cancer are already established. For example, chronic inflammatory bowel disease is known to increase the risk of developing colorectal cancer, and the administration of anti-inflammatory drugs has been shown to reduce the risk of colorectal malignancy. These links between inflammation and cancer are further strengthened by the fact that immune cells and inflammatory mediators are often observed in tumour tissue and the cellular processes usually associated with chronic inflammation are also active in the tumour microenvironment (Mantovani et al., 2008). Inflammation is now recognised as a key component of the biological capabilities that are acquired during tumour development (Colotta et al., 2009). These capabilities, described as the “hallmarks” of cancer, enable tumour cells to survive, proliferate and disseminate (Hanahan and Weinberg, 2011).

1.7.1 The host immune response

The human immune system works to protect the body from foreign pathogens and is broadly categorised into innate (non-specific) and adaptive (acquired) immunity. The immune system can also recognise cancer-specific antigens, allowing the identification and destruction of tumour cells in a process known as immunosurveillance. Paradoxically, some non-specific processes associated with inflammation can promote tumour progression and it is therefore the balance of pro- and anti-tumour factors that many believe to be of primary importance in determining cancer outcomes.

1.7.1.1 Innate immunity

In addition to epithelialised barriers such as skin and mucosa, the innate immune system, comprising phagocytic cells (neutrophils and macrophages), degranulating cells (basophils, eosinophils and mast cells) and natural killer (NK) cells as well as humoral (complement) components, provides a crucial (non-specific) first line of defence against pathogens. Bacteria that successfully penetrate the epithelial surfaces of the body attract macrophages, are bound by cell surface receptors and engulfed in a process known as phagocytosis. This is followed by the release of biologically active molecules, known as chemokines and cytokines, which generate an inflammatory response. Although most pathogens and/or tissue damage initially induce this non-specific response, the innate system may subsequently activate an adaptive immune response (Janeway and Medzhitov, 2002, Medzhitov, 2007).

1.7.1.2 Adaptive immunity

The adaptive immune system is composed primarily of lymphocytes and is a specific antibody response in recognition of “non-self” antigens, enabling a stronger, more focussed response to eliminate specific pathogens and produce and develop immunological memory. Adaptive immunity can be divided into humoral and cell-mediated immunity although many of the processes and cell types are inter-dependent. Activation of adaptive immunity is usually triggered by the presentation of antigens by specialised cells associated with the innate immune system known as antigen-presenting cells. B cells are the major cell types in humoral immunity and produce antibodies, known as immunoglobulins, which recognise and bind to specific antigens, making them easy targets for phagocytes and triggering the complement cascade (Janeway, 2001). T lymphocytes are responsible for coordinating cell-mediated immunity and can be categorised into a number of subsets; helper T cells (CD4+), cytotoxic T cells (CD8+), memory T cells (CD45R0+) and regulatory T cells (FOXP3+). Each subset plays a specific role in the identification and destruction of antigens. CD8+ T cells are the effector cells of adaptive immunity, inducing cell death through the release of cytotoxins (Janeway, 2001).

1.7.1.3 Cancer immunosurveillance

Cancer immunosurveillance is the process whereby tumour-specific antigens provoke an effective immunological reaction and remove transformed cells thereby preventing the development of otherwise inevitable malignancy (Burnet, 1957). The concept is not new but advances in genetic understanding have now validated the hypothesis and expanded it to include contributions from both the innate and adaptive immune systems (Dunn et al., 2004). This is thought to be further evidenced by the fact that the immunocompromised

state is associated with increased development of malignancy (Dunn et al., 2002). However, there is growing recognition that the relationship between cancer and the immune response is yet more complex still and may involve the promotion as well as prevention of tumourigenesis. The immune response in cancer is thus now recognised as a complex relationship between pro- and anti-tumour factors with the potential to impact outcome in either a positive or negative manner.

1.7.2 The tumour microenvironment

The tumour microenvironment can be defined as the tissue medium in which tumour cells grow and develop. It is a complex and unique environment comprised of the invasive margin, proliferating tumour cells, tumour stroma, blood vessels, tissue cells and inflammatory cells. The tumour microenvironment represents a dynamic interface between tumour and host and it is postulated that the molecular events which occur here dictate whether a tumour is successfully eliminated by the host or a tumour progresses (Whiteside, 2008). The local inflammatory response can be considered an attempt to destroy tumour cells but it is often attenuated. Pro-inflammatory cytokines are produced which alter the microenvironment to benefit the tumour. This cascade of cytokines influences a variety of key events including angiogenesis, cellular proliferation and matrix re-modelling, ultimately resulting in tumour growth and progression (Balkwill and Coussens, 2004). Indeed, the nature, function, density and localization of immune cells within the tumour microenvironment have all been reported to influence tumour progression and clinical outcomes in colorectal cancer (Pages et al., 2008).

1.7.3 The local inflammatory response

A strong inflammatory response at a local level has been consistently associated with improved clinical outcomes in patients with colorectal cancer. Over the past 40 years, a number of studies, often using different methodologies, have examined the prognostic implications of the local inflammatory response in colorectal cancer. Several studies have shown an inverse relationship between the degree of local inflammatory infiltrate and survival in patients with colorectal tumours.

In 1987 Jass described a prognostic classification based on four characterisitics: the presence or absence of lymphocytic infiltration, the tumour margin characterisitics (infiltrating or expanding), tumour growth beyond the bowel wall and increasing nodal involvement (Jass et al., 1987). A pronounced peritumoural infiltrate, which describes the stromal / inflammatory response at the tumour's invasive edge, was associated with good outcome. The subjective nature of these assessments led to problems with reproducibility, particularly with the assessment of lymphocytic infiltrate, and as a result the classification has not been adopted widely.

Using a semi-quantitative assessment of peritumoural inflammatory infiltrate on haematoxylin and eosin (H&E) stained sections, Klintrup and Makinen reported high-grade inflammation at the invasive margin to be an important prognostic indicator in patients with node negative colorectal cancer (Klintrup et al., 2005). These findings were subsequently validated in an external cohort of patients with node-negative disease (Roxburgh et al., 2009c). Overall, there is consistent evidence that a generalised increase in

inflammatory cell infiltrate is associated with improved prognosis in patients with colorectal cancer.

The Galon immune score, an immunohistochemistry based score grading specific T cell subtypes at both the invasive margin and the centre of the tumour has also been proposed more recently. A high density of these cytotoxic and memory T cells in the centre and the invasive margin of the primary tumour is associated with long disease free and overall survival and low risk of recurrence and metastasis (Mlecnik et al., 2011, Galon et al., 2012).

Increased levels of faecal calprotectin, a calcium and zinc binding protein of the S-100 family with antimicrobial and apoptotic properties, and thought to be a marker of local inflammation, have been described for patients with colorectal cancer as well as for patients with colonic inflammation (Kristinsson et al., 1998).

1.7.4 The systemic inflammatory response

It is now widely recognised that outcomes in patients with cancer are not determined by tumour characteristics alone, and that patient related factors are also key to outcome. In the last decade, it has become increasingly apparent that cancer associated inflammation is a key determinant of disease progression and survival in most cancers (Hanahan and Weinberg, 2000, Hanahan and Weinberg, 2011). In particular, the host response in the form of systemic inflammation has been shown to independently predict outcome. Inflammation is a normal and usually beneficial physiological response to injury. Problems for the host can arise however if the normal tight controls of the inflammatory response are lost. Loss of these controls results in an exaggerated inflammatory response. With a failure

of normal homeostasis there is a flood of inflammatory mediators and the predominant effects of cytokines start to become destructive rather than protective.

The presence of a systemic inflammatory response in patients with cancer is almost universally considered an indicator of poor prognosis. There is evidence that systemic inflammation is associated with the cachexia and functional decline of patients with advanced disease (McMillan et al., 1994) and measures of the systemic inflammatory response have been reported as prognostic markers in a variety of tumour types including lung (Forrest et al., 2004), breast (Al Murri et al., 2006) and pancreatic cancer (Glen et al., 2006).

Biochemical and haematological tests are carried out routinely for patients with cancer in a variety of clinical scenarios, and as such represent an easily measurable objective parameter to enable assessment of the severity of the systemic inflammatory response.

Inflammation can be detected by measuring serum concentrations of acute phase proteins; a class of proteins synthesised in the liver whose concentrations change in the presence of inflammation. Positive acute phase proteins including C-reactive protein increase during an inflammatory response while negative acute phase proteins such as albumin decrease (Gruys et al., 2005). The measurement of changes in acute phase proteins is important clinically in indicating the presence and severity of inflammation. The level of this inflammatory response is usually best seen by measuring C-reactive protein because of large changes from its initial concentration in the presence of inflammation (Thompson et al., 1992).

1.7.4.1 C-reactive protein

C-reactive protein was first described in 1930 and was named due to its ability to bind to the C-polysaccharide in the pneumococcal cell wall. It is a non-specific positive acute phase protein which is secreted by the liver in response to a variety of inflammatory cytokines, mainly interleukin-6 (IL-6), interleukin-1 (IL-1) and tumour necrosis factor (TNF) (Du Clos and Mold, 2004). C-reactive protein is widely used to monitor the systemic inflammatory response and therefore the extent, activity and prognosis of various diseases.

The function of C-reactive protein is felt to be related to its role in the innate immune system. It activates complement, binds to Fc receptors and acts as an opsonin for various pathogens. Interaction of C-reactive protein with Fc receptors leads to the generation of pro-inflammatory cytokines that enhance the inflammatory response. It is thought to act as a surveillance molecule for altered self and certain pathogens. This recognition provides early defence and leads to a pro-inflammatory signal and activation of the humoral, adaptive immune system (Pepys and Hirschfield, 2003).

Specifically in colorectal cancer, the presence of a systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, is associated with increased recurrence and poor survival, independent of tumour stage, in patients undergoing potentially curative surgery for colorectal cancer (McMillan et al., 1995, McMillan et al., 2003b).

1.7.4.2 Albumin

Albumin is a major negative acute phase protein. It appears to be primarily mediated in the acute phase response by the altered protein and energy metabolism that occurs. In the acute phase response there is an increased demand for specific amino acids for mediator and acute phase protein synthesis and immune and antioxidant defences. This promotes the progressive loss of the available protein components including albumin. As the albumin pool size is modest in relation to body cell mass its loss is noticeable at an earlier stage (McMillan et al., 2001).

It has long been recognised that there is an association between reduced serum albumin and elevated C-reactive protein concentrations with severity of illness and poor outcome.

1.7.4.3 Interleukin-6

Interleukin-6 is a multifunctional pro-inflammatory cytokine which plays a major role in regulating the immune and inflammatory responses via the synthesis of most acute phase proteins including C-reactive protein. Elevated interleukin-6 production is seen in infectious disease, inflammatory diseases and malignant disease (Gabay and Kushner, 1999). In patients with colorectal cancer increased concentrations of circulating interleukin-6 have been shown to reflect disease status and correlates with cancer stage, C-reactive protein concentrations, tumour necrosis and survival.

1.7.4.4 White cell count

Measuring the numbers of inflammatory cells present in the bloodstream represents an alternative technique for quantifying the presence of an inflammatory response in patients.

Total white cell count, neutrophils, lymphocytes and platelets can all be detected using standard laboratory tests.

In an effort to standardise the measurement of the systemic inflammatory response in patients with cancer, a number of inflammatory scores have been described whose values have been shown to correlate directly with clinical outcomes. The modified Glasgow Prognostic Score (mGPS) combines circulating CRP and albumin concentrations (McMillan et al., 2007). Alternative inflammatory scores include the neutrophil lymphocyte ratio (NLR), which measures the relative values of neutrophil and lymphocyte counts (Walsh et al., 2005), and the platelet to lymphocyte ratio (PLR) (Smith et al., 2008).

1.7.4.5 Modified Glasgow prognostic score

Indeed, the last decade has seen the evolution of a prognostic scoring system, the Glasgow prognostic score (mGPS) based on the combination of acute phase proteins albumin and C-reactive protein that provides objective, reliable prognostic information for both operable and inoperable cancers. This scoring system is the most extensively validated systemic inflammation based prognostic score. It has been validated in a variety of clinical scenarios, in over 60 studies (>30,000 patients) and is now recognised to have prognostic value, independent of tumour based factors.

1.7.4.6 Neutrophil-Lymphocyte ratio

It is also well established that the systemic inflammatory response is associated with alterations in circulating white blood cells, specifically the presence of neutrophilia with a relative lymphocytopenia (Gabay and Kushner, 1999). One routinely available marker of

the systemic inflammatory response is the neutrophil-lymphocyte ratio (NLR), which is derived from the absolute neutrophil and absolute lymphocyte counts of a full blood count. To date, over 60 studies (>37,000 patients) have examined the clinical utility of the NLR to predict patient outcomes in a variety of cancers. Studies have shown that NLR is elevated in patients with advanced or aggressive disease evidenced by increased tumour stage, nodal stage, number of metastatic lesions and as such these patients may represent a particularly high risk population. Furthermore, NLR may be of prognostic value in those patients who require adjuvant therapy.

1.8 The postoperative systemic inflammatory response

Operative injury to the body from all procedures causes a stereotypical cascade of neuroendocrine, cytokine, myeloid and acute phase responses. Surgery, both elective and emergency, produces local trauma and results in a response with pro- and anti-inflammatory components. This response may be of an appropriate magnitude, and appropriately down-regulated. An explanation of how these processes work together and, paradoxically, how they can cause a systemic inflammatory response leading to immunological dissonance was first described by Bone in five stages, as summarised below (Bone, 1996) (Figure 1).

1.8.1 Local response to surgery

An insult such as trauma from a surgical incision prompts release of pro-inflammatory mediators in the microenvironment. These mediators limit new damage and ameliorate whatever damage has already occurred. They destroy damaged tissue, promote new tissue growth, and combat pathogenic organisms, neoplastic cells and foreign antigens. To ensure that the effects of pro-inflammatory mediators do not become destructive, an anti-inflammatory response ensues. Anti-inflammatory agents are known to alter monocyte function, impair antigen-presenting activity, and reduce the ability of cells to produce pro-inflammatory cytokines. Some of them have been shown to downregulate their own production.

1.8.2 Initial systemic response

If the original insult is sufficiently severe, pro-inflammatory, and, later, anti-inflammatory mediators appear in the systemic circulation. At this stage, the presence of these mediators in the circulation is seen as part of the normal response to injury. These agents signal that the microenvironment cannot control the initiating insult and that more help is needed. Pro-inflammatory mediators recruit neutrophils, lymphocytes, platelets, and coagulation factors to the local site. There is a compensatory systemic anti-inflammatory response to downregulate the pro-inflammatory reaction and, if all goes well, few (if any) clinical signs and symptoms are produced.

1.8.3 Pro-inflammatory state

In some patients, regulation of the inflammatory response is lost resulting in a massive systemic reaction. Activation of the sympathetic nervous system results in a neuroendocrine response of increased secretion of catecholamines (adrenaline and noradrenaline) into the circulation. In most cases, this reaction is initially pro-inflammatory and produces clinical findings such as hypotension, pyrexia and tachycardia, known as the systemic inflammatory response syndrome. At the same time there is also increased secretion of pituitary hormones such as corticotrophin, growth hormone, and arginine vasopressin. Corticotrophin acts on the adrenal cortex to stimulate cortisol secretion. There is often a subsequent increase in the production of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α) and interleukins, in particular interleukin 6 (IL-6). These cytokines are produced by many cells throughout the body in response to injury and form a complex signalling system for subsequent production of acute phase proteins from the liver and increased stimulation of myeloid tissue (Gabay and Kushner, 1999). There are

increases in circulating white cells, particularly neutrophils, as well as myeloid derived suppressor cells and platelets. Plasma concentrations of actue phase proteins change, in particular C-reactive protein, which peaks at 48-72 hours following injury. The net effect of the evolution of the systemic inflammatory response is increased catabolism of skeletal muscle to provide energy and substrates for the liver, to maintain fluid and cardiovascular homeostasis and for healing. Therefore, although this response to injury has been referred to as the operative stress or acute phase response, it is more informatively known as the systemic inflammatory response because of its effects on all organs and tissues of the body (Gabay and Kushner, 1999). Various pathophysiologic changes underlie these effects, the net result of which can be severe shock. Unless homeostasis is restored, organ dysfunction and, ultimately, failure can develop.

1.8.3.1 Systemic inflammatory response syndrome criteria

≥ 2 of the following:

- Temperature >38◦C or <36◦C
- Heart rate >90 beats per minute
- Respiratory rate >20 breaths per minute or $PaCO_2$ <32 mmHg
- White cell count >12 or <4 ($x10^9$/L)

1.8.4 Over production of anti-inflammatory mediators

In those patients with persistent or overwhelming inflammation who survive, anti-inflammatory mechanisms may be able to control inflammation. Once healing is established, anti-inflammatory components of the systemic inflammatory response become

prominent, causing it to return to the normal non-inflammatory state. In some patients, however, the compensatory reaction may be as excessive as the pro-inflammatory response, and immunosuppression ensues. Patients without an overwhelming pro-inflammatory response may also develop immunosuppression if release of anti-inflammatory mediators is excessive or if the balance between pro- and anti-inflammatory mediators is lost. This may lead to increased susceptibility to infection.

1.8.5 Immunologic dissonance

At this stage the balance of pro- and anti-inflammatory mediators has been lost. Some patients may have persistent, massive inflammation, others may have continuing immunosuppression and secondary infections. Furthermore, some may oscillate between periods of inflammation and immunosuppression. The pro-inflammatory and anti-inflammatory forces may ultimately reinforce each other, creating a state of increasingly destructive immunological dissonance (Bone, 1996). It has been suggested that inflammatory reactions may result in anti-tumour activity. Alternatively, an inflammatory / immunocompromised state may promote and maintain tumour growth (Colotta et al., 2009). Surgery for the treatment of cancer in this context may be seen as a double-edged sword.

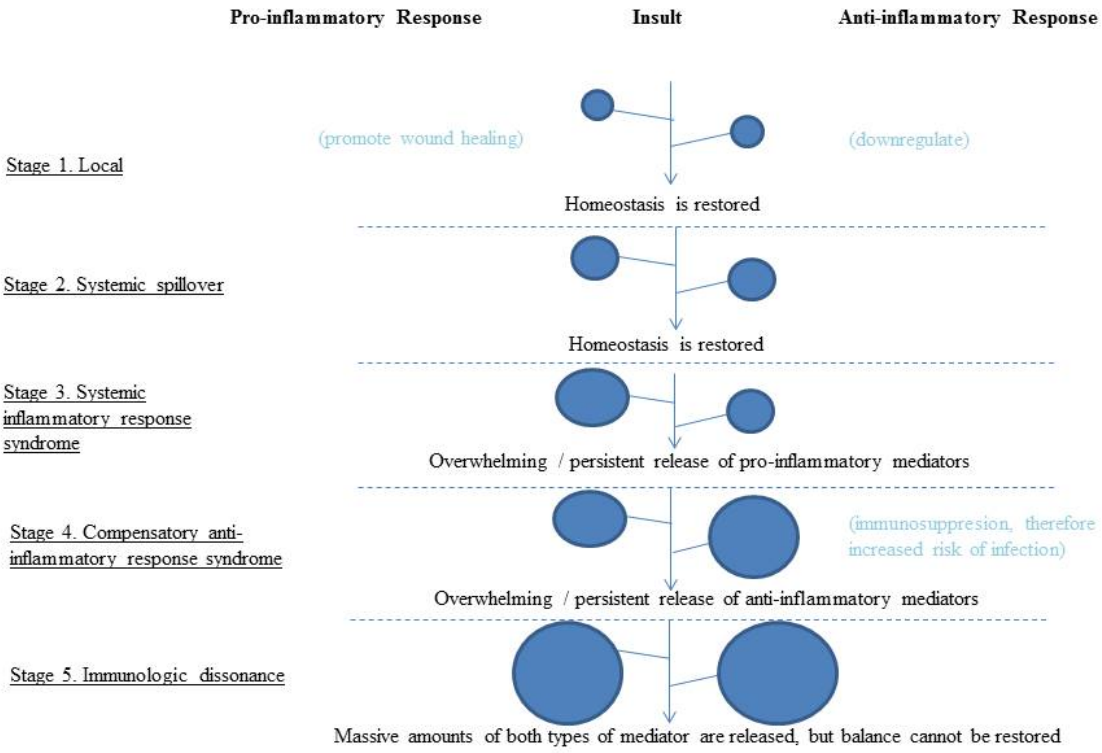


Figure 1-1 - Stages of the inflammatory response, leading to immunological dissonance (adapted from Bone, 1996)

1.9 The role of the systemic inflammatory response in predicting infective complications following colorectal cancer resection

In patients with colorectal cancer, the presence of both systemic and/ or local inflammatory responses are predictors of survival independent of tumour stage (Roxburgh and McMillan, 2010, Richards et al., 2010, Roxburgh et al., 2011, Hanahan and Weinberg, 2011, Colotta et al., 2009, Roxburgh et al., 2009b). The relationship between a raised perioperative systemic inflammatory response and the development of postoperative infective complications is also well established (MacKay et al., 2011, Dutta et al., 2011). Furthermore, it has become clear that the development of infective complications, in particular anastomotic leak, is also associated with increased recurrence and poorer cancer specific survival (McArdle et al., 2005, Jung et al., 2008, Marra et al., 2009). Therefore, infective complications can be catastrophic for the patient, in both short and long term outcomes.

Resection for colorectal cancer is associated with relatively high rates of postoperative infective complications. Of these cancer patients, 20-40% (Velasco et al., 1996) are at risk of complications such as; respiratory, wound, or urinary tract infection, anastomotic leakage, intra-abdominal abscess and septicaemia of unknown origin. During the early postoperative period, sepsis can be difficult to distinguish from the normal postoperative systemic inflammatory response related to surgical trauma. Recognition during this period is challenging and lacks sensitivity at a stage when early diagnosis may significantly improve outcome (Welsch et al., 2007).

C-reactive protein is an acute phase protein found in the blood in response to inflammation. It is thought to play an important role in innate immunity as an early defence against infection, assisting complement binding to foreign and damaged cells and enhancing phagocytosis by macrophages (Gabay and Kushner, 1999). Its short half-life of 19 hours makes it a valuable marker to detect disease activity, inflammatory response and post-operative recovery (Pepys and Hirschfield, 2003).

A number of studies have investigated the association of the systemic inflammatory response with postoperative complications, with previous studies suggesting that an abnormally elevated C-reactive protein level or persistent elevation may be a useful predictor of infective complications (Welsch et al., 2007, Bianchi et al., 2004, Matthiessen et al., 2008, Welsch et al., 2008).

One Glasgow based study has investigated the sensitivity and specificity of C-reactive protein as an early marker for postoperative infective complications in patients undergoing elective colorectal resection for cancer. Looking at data from 150 patients, this study concluded that a C-reactive protein concentration greater than 145mg/l on postoperative day 4 has a high specificity and sensitivity for infective complications following elective colorectal resection, and could therefore be used to aid clinical decision making (MacKay et al., 2011). Multiple similar studies have been carried out internationally, dating as far back as 1979.

A further prospective study of elective colorectal cancer resection patients in France concluded that C-reactive protein was a good early predictor of infective complications, with a concentration >125mg/l on day 4 detecting 80% of infective complications. They also suggest that patients with values >125mg/l on the fourth postoperative day should not

be discharged (Ortega-Deballon et al., 2010). This pre-clinical warning is of particular importance in an era of enhanced recovery and early discharge.

A study of 231 patients in a university teaching hospital in Norway also confirmed that increased C-reactive protein concentrations on day 3 strongly indicate a high risk of developing an anastomotic leak after colorectal resection (Korner et al., 2009). This supported previous findings that a raised C-reactive protein is an early indicator of anastomotic leakage in colorectal surgery (Matthiessen et al., 2008, Woeste et al., 2010). Their results also agreed with others that white cell count had low sensitivity and specificity at an early stage.

In Germany, a study of 688 consecutive pancreatic resection patients concluded that persistence of C-reactive protein elevation above 140mg/l on postoperative day 4 is predictive of inflammatory postoperative complications and should prompt an intense clinical search for major septic processes (Welsch et al., 2008). They also demonstrated that a rise in white cell count and temperature develop several days later, along with clinical symptoms.

Another Glasgow based study, looking at oesophagogastric cancer resections, also concluded that postoperative C-reactive protein measurements on days 3 and 4 were predictive of infective complications, particularly anastomotic leaks (Dutta et al., 2011). Again this was more useful than white cell count.

If it is possible to predict an infective complication at a pre-clinical stage, it may also be possible to intervene pre-emptively (Rivers et al., 2001, Chromik et al., 2006). C-reactive protein could be used to identify a group at high risk of infective complications, or as a

discharge criteria. It is possible that by administering pre-emptive antibiotics directed by C-reactive protein thresholds that potential postoperative morbidity could be attenuated or even avoided (MacKay et al., 2011).

An appropriately sized, prospective, multi-centre trial is indicated to establish whether early prediction of infective complications can be treated empirically to improve the short term morbidity and mortality for patients undergoing potentially curative colorectal cancer resection.

2 Summary and Aims

Colorectal cancer remains the second most common cause of cancer death in western Europe (CRUK, 2014). Despite advances in surgical techniques, perioperative care and adjuvant chemoradiotherapy, overall survival remains poor with only 50% of patients surviving to 5 years after potentially curative resection (McArdle and Hole, 2002). It is now recognised that postoperative complications contribute to poor cancer specific survival (Rizk et al., 2004, Khuri et al., 2005, McArdle et al., 2005, Law et al., 2007). In particular, anastomotic leak following potentially curative colorectal cancer resection is associated with poorer cancer specific survival, independent of tumour stage (Law et al., 2007, McArdle et al., 2005). The basis of this observation is not clear. One hypothesis is that the presence of a raised postoperative systemic inflammatory response may be responsible (McArdle et al., 2005), as the postoperative systemic inflammatory response has been reported to be associated with increased postoperative complications, including anastomotic leak.

Furthermore, preoperative systemic inflammation, as evidenced by the mGPS, has been shown to predict postoperative infective complications (Moyes et al., 2009). Moreover, C-reactive protein concentrations postoperatively predict the development of infective complications and anastomotic leak (Welsch et al., 2007, Matthiessen et al., 2008). It has been suggested that early postoperative infective complications might lead to a rise in C-reactive protein prior to the development of clinical symptoms, and that this infection leads to decreased long term survival. However it has been reported that preoperative, but not postoperative, elevated C-reactive protein concentrations are associated with cancer specific survival (Crozier et al., 2007).

Resection for colorectal cancer is associated with high rates of postoperative infective complications. Of these cancer patients, 20-40% (Velasco et al., 1996) are at risk of complications such as; respiratory, wound, or urinary tract infection, anastomotic leakage, intra-abdominal abscess and septicaemia of unknown origin. During the early postoperative period, sepsis can be difficult to distinguish from the normal postoperative systemic inflammatory response related to surgical trauma. Recognition during this period is challenging and lacks sensitivity at a stage when early diagnosis may significantly improve outcome (Welsch et al., 2007).

It is increasingly appreciated that the "immunological hit" caused by surgery may compromise the antitumour immune defences of the host. Furthermore, this "hit" can be compounded by various perioperative factors and the development of postoperative infective complications further augments the recurrence risk (Richards et al., 2011). It is considered that these conditions result in a further immunological insult and lead to a compromised immune response to residual disease as well as prolonged recovery. It is apparent that a range of patient related factors influence disease outcome in the perioperative period, such as age, emergency presentation, comorbidity and postoperative complications. Therefore, the perioperative period represents an opportunity for clinicians to intervene early, acting to suppress high-grade non-specific systemic inflammation and maintaining effective immunological competence of the host.

However, whether a raised postoperative systemic inflammatory response is the result of an early underlying infection, or whether a raised inflammatory response leads to increased susceptibility to subsequent infection is not clear. If the former is the case, it is possible that intervention targeting at risk patients may reduce infective complications, or the extent

of them, and therefore improve patient outcomes. If the later is the case, perioperative intervention to reduce the postoperative inflammatory response may in turn reduce postoperative complications and hence improve outcomes, both short and long term, for patients undergoing surgery for colorectal cancer.

The present thesis aims to further examine the nature of the postoperative systemic inflammatory response and its relationship with infective complications following potentially curative resection for colorectal cancer, specifically:

1. To determine whether the perioperative systemic inflammatory response is the result of an impaired anti-inflammatory response by assessing adrenocortical function.

2. To examine the diagnostic accuracy of serial postoperative white cell count, albumin and C-reactive protein in detecting infective complications.

3. To compare the value of daily C-reactive protein concentrations in the prediction of infective complications following open versus laparoscopic colorectal cancer resection.

4. To examine the impact of an enhanced recovery programme on the systemic inflammatory response and rate of infective complications postoperatively.

5. To assess the impact of the peak systemic inflammatory response, as evidenced by postoperative day 2 C-reactive protein, on C-reactive protein thresholds predictive of infective complications.

6. To examine the determinants of the peak systemic inflammatory response in patients following resection for colorectal cancer.

7. To examine the relationships between postoperative predictive C-reactive protein thresholds and infective complications in the context of pre-emptive antibiotic therapy.

3 Is perioperative systemic inflammation the result of insufficient cortisol production in patients with colorectal cancer?

3.1 Introduction

Colorectal cancer is the second most common cause of cancer death in the UK, accounting for 16,000 deaths annually (CRUK, 2014). Even with modern treatments, of those deemed suitable candidates for curative resections approximately 50% suffer disease recurrence and die at 5 years.

It is increasingly recognised that disease progression and cancer specific survival in colorectal cancer patients is not solely determined by the intrinsic characteristics of the tumour but also by host characteristics and responses to the tumour. In terms of the host, age, comorbidity and the presence of both systemic and/ or local inflammatory responses are stage independent predictors of survival (Roxburgh and McMillan, 2010, Richards et al., 2010, Roxburgh et al., 2009b, Roxburgh and McMillan, 2012, Colotta et al., 2009, Hanahan and Weinberg, 2011). The presence of a systemic inflammatory response in particular has been consistently demonstrated to predict poorer survival independent of stage in all gastrointestinal cancers with most published reports in colorectal cancer (Roxburgh and McMillan, 2010, Roxburgh et al., 2009b). Furthermore, following apparently curative resection, persistent postoperative evidence of systemic inflammation has previously been associated with earlier recurrence and reduced survival (McMillan et al., 1995, Moyes et al., 2009). It may be that the systemic inflammatory response is initially a defence mechanism, which, when exacerbated beyond a certain point, becomes

harmful. Such observations have given rise to development of prognostic scores for systemic inflammation in cancer patients, namely the Glasgow Prognostic Score, based on C-reactive protein (CRP) and albumin and the neutrophil lymphocyte ratio, both of which are widely validated in different cohorts of colorectal cancer as well as other cancer types (Roxburgh et al., 2009a, Roxburgh and McMillan, 2010, Walsh et al., 2005).

The information provided by such prognostic scores provides clinicians with information to more accurately determine speed of disease progression and survival. However, it remains to be seen whether such information may also guide allocation of further treatment. One possibility is that therapies could be developed that reduce cancer-associated systemic inflammation, in particular, in the perioperative period. In order to take this next step, further work is required to determine the underlying stimulus or basis of the systemic inflammatory response.

One possibility is that the presence of a systemic inflammatory response represents failure of innate anti-inflammatory mechanisms. The glucocorticoids are an important group of endogenous anti-inflammatory agents. These hormones have many effects including down regulation of pro-inflammatory cytokines and promotion of anti-inflammatory cytokines produced by monocytes and macrophages, in addition to the induction of apoptosis of cells recruited by inflammatory responses (Tuckermann et al., 2005). Their release from the adrenal cortex is stimulated by adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, which is in turn regulated by corticotrophin releasing hormone from the hypothalamus forming the Hypothalamic-Pituitary-Adrenal (HPA) axis (Tuckermann et al., 2005) (Figure 3). It is well recognised that the HPA axis plays an important role in moderating the systemic inflammatory response to tissue injury and hypoxia (Gabay and Kushner, 1999).

Previous work in cancer patients has suggested that poorer outcomes can be expected when there is a failure of normal HPA axis function. Flattening of the diurnal rhythm of cortisol release was reported to be associated with advanced stage of disease and elevation of pro-inflammatory cytokines (Rich et al., 2005, Mussi et al., 2006). Indeed, the systemic inflammatory response in cancer has been previously reported to be associated with normal or slightly raised serum cortisol levels (Scott et al., 1996). Of interest, previous work by Jenkins et al reported a significant increase in size of adrenal glands in patients with cancer (Jenkins et al., 1999) a feature associated with low ACTH levels and resistance to dexamethasone suppression. Normal / raised serum cortisol with low ACTH levels implies a secondary over-riding stimulus to cortisol production. Indeed, it would appear that pro-inflammatory cytokines such as IL-2, IL-6 and IL-1 can exert stimulatory effects on the adrenal cortex production of glucocorticoids (Roh et al., 1987, Salas et al., 1990, Tominaga et al., 1991). Subversion of innate anti-inflammatory feedback mechanisms may occur as a result of desensitization of receptors due to high cytokine/ chemokine levels (Coussens and Werb, 2002). One hypothesis, therefore, is that the presence of a systemic inflammatory response is representative of a state in which endogenous anti-inflammatory feedback mechanisms are lost resulting in pro-inflammatory cytokine mediated cortisol release from the adrenal cortex. In such a state inflammation could persist unchecked. Diurnal cortisol rhythmicity would be lost and cortisol response to synthetic ACTH (synacthen) may be lost.

The aim of the present prospective study was to examine whether patients undergoing potentially curative surgery for colorectal cancer exhibit evidence of impairment of endogenous cortisol release and feedback mechanisms (measured using the short synacthen test and diurnal salivary cortisols). Furthermore, whether an impaired cortisol response

was associated with the presence of a perioperative systemic inflammatory response was examined.

3.2 Patients and Methods

Patients with a histologically proven diagnosis of colorectal cancer who, on the basis of pre-operative staging, underwent elective resection with curative intent between February 2008 and December 2011 in a single surgical unit at Glasgow Royal Infirmary were prospectively included in the study. The assessment of adrenocortical function using synthetic ACTH, a short synacthen test, was carried out as part of the pre-operative assessment of patients. Short synacthen tests were performed on the morning of surgery, at approximately 06:00am.

Patients were excluded if they had inoperable or metastatic disease where a curative operation was not possible, neo-adjuvant treatment, emergency presentation, concurrent steroid use and conditions associated with impaired HPA axis function (e.g. Addison's Disease, Cushing's Disease, pituitary tumours), or significant chronic inflammatory diseases requiring long term medication (e.g. rheumatoid arthritis, inflammatory bowel disease, connective tissue disease), or if they were using oral contraceptives or hormone replacement therapy (as oestrogen induces cortisol binding globulin and leads to elevation in measured serum cortisol). Consecutive patients admitted for potentially curative colorectal cancer resection were approached and given written information prior to the day of surgery. Eighty patients agreed to participate within the time period, no formal power calculation was undertaken. Written informed consent was obtained from all patients. The study was approved by the Research Ethics Committee, Glasgow Royal Infirmary.

Patient co-morbidity was classified using the American Society of Anaesthesiologists (ASA) grading system, where ‘1’ represents a normal healthy patient, ‘2’ a patient with mild systemic disease, ‘3’ a patient with severe systemic disease and ‘4’ a patient with

severe systemic disease that is a constant threat to life. This assessment was carried out by the anaesthetist preoperatively.

The tumours were staged according to conventional tumour, node, metastases (TNM) classification. Assessment of tumour necrosis, a stage-independent prognostic marker in colorectal cancer, was undertaken using methodology previously described. The sections were examined at magnification x40 for evidence of tumour necrosis. The extent of tumour necrosis was assessed semi-quantitatively and graded as 'absent' (none), 'focal' (less than 10% of tumour area), 'moderate' (10-30% of tumour area), or 'extensive' (>30% of tumour area) in each section before an assessment was made of the overall extent of necrosis (Richards et al., 2012, Pollheimer et al., 2010).

The inflammatory reaction at the invasive margin, another prognostic indicator in colorectal cancer, was analysed using the Klintrup-Makinen criteria as previously described (Klintrup et al., 2005). Briefly, tumours were scored according to a 4-point score. Scores were allocated based on appearances at the deepest area of tumour invasion. A score of '0' was given where there was no increase in inflammatory cells at the deepest point of the invasive margin; '1' denoting a mild and patchy increase in inflammatory cell; '2' denoting a prominent inflammatory reaction forming a band at the invasive margin with some evidence of destruction of cancer cell islands; and '3' denoting a florid cup-like inflammatory infiltrate at the invasive edge with frequent destruction of cancer cell islands.

To test consistency of scoring, sections for 30 patients were examined independently by 2 observers (MLR and GG, intra-class correlation coefficient [ICC] 0.59 for tumour necrosis and ICC 0.57 for Klintrup-Makinen criteria, demonstrating moderate agreement of a

subjective score). One observer (MLR) then scored all sections and this data was used in the analysis.

Preoperative systemic inflammatory response was assessed using the modified Glasgow Prognostic Score (mGPS) and the neutrophil lymphocyte ratio (NLR). Briefly, to determine mGPS patients with both an elevated C-reactive protein (>10mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of '2'. Patients in whom neither of these abnormalities was present were allocated a score of '0'. Patients with an elevated C-reactive protein alone were scored as '1' while those with hypoalbuminaemia alone were scored as '0'. The NLR was calculated from the differential white cell count by dividing the neutrophil count by the lymphocyte count (Table 3.1). All measurements of C-reactive protein (CRP), albumin and differential white cell count were taken on admission, prior to surgery. The perioperative systemic inflammatory response was assessed using pre and postoperative CRP concentrations, either until the patient was discharged or up to day 7.

The use of the short Synacthen test in the diagnosis of cortisol insufficiency has been validated and widely used (Grinspoon and Biller, 1994, Dickstein and Shechner, 1997). Fasting blood samples were taken for baseline cortisol, then a 250mcg dose of synacthen (tetracosactide acetate Ph. Eur., Alliance Pharmaceuticals), an analogue of corticotropin (ACTH), was administered intravenously. After 30 minutes a further blood sample was taken to measure post-synacthen peak cortisol levels. Biochemical criteria defining a "normal" serum cortisol or an adequate cortisol response to ACTH have been variously proposed. In the recent National UK audit of the short synacthen test 69% of laboratories stated that a baseline cortisol of more than 200 nmol/L would be considered a "normal" response; 73% of laboratories consider a peak cortisol of at least 450 nmol/L a "normal" response and 89% of laboratories consider an incremental increase of 200 nmol/L a

"normal" response to a short Synacthen test (Chatha et al., 2010). Cut-off values for cortisol insufficiency were recommended by our hospital expert (Dr Dinesh Talwar, Biochemistry Consultant) in keeping with the National UK audit and local laboratory policy (baseline cortisol <200 nmol/L, peak cortisol <450 nmol/L, and a change in cortisol <200 nmol/L). As a surrogate measure of plasma free cortisol, patients were also asked for salivary samples, only 30 patients returned these as many forgot to bring an evening sample from the night before.

Statistics

Grouping of variables was carried out using standard or previously published thresholds. Associations between categorical and continuous variables were examined using X^2 tests for linear trend and non-parametric tests. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 19.0 (IBM SPSS, Chicago, IL, USA).

3.3 Results

The clinicopathological characteristics of patients undergoing potentially curative resection for colorectal cancer are shown in Table 2. A total of 80 patients underwent short Synacthen testing. All patients had macroscopically curative resections. Eleven patients also underwent synchronous resection of liver metastases. The majority of patients were under 75 years old (79%), were male (59%), had an ASA of 1 or 2 (60%), had colon cancer (75%) and had TNM stage I/II disease (54%) (Table 3.2). Approximately 40% had an elevated mGPS or NLR.

In terms of adrenal insufficiency, there were no patients in whom this was clearly diagnosed on discussion with our hospital expert (Dr Dinesh Talwar, Biochemistry Consultant). 11 patients had a baseline serum cortisol of <200 nmol/L. There is no clearly established upper limit for a baseline cortisol, but 3 patients had a seemingly high result of >650 nmol/L (675, 701 and 850 nmol/L). At 30 minutes, a different 3 patients had a peak cortisol <450 nmol/L (398, 411 and 433 nmol/L) and an absolute change <200 nmol/L (69, 29 and 191 nmol/L), but these all had a baseline >200 nmol/L. There were 24 patients with an absolute change in cortisol <200 nmol/L, however all but 3 (as above) of these had a baseline >200 nmol/L and a peak >450 nmol/L.

The relationship between patient and tumour related factors and standard thresholds (Chatha et al., 2010) for cortisol (a baseline cortisol <200 nmol/L, a change in cortisol <200 nmol/L and a 30 minute cortisol <450 nmol/L) are shown in Table 3.3. There were no significant associations between these thresholds and patient related factors such as age (all $p>0.10$), sex (all $p>0.10$), ASA grade (all $p>0.10$), white cell count (all $p>0.10$), CRP (all $p>0.10$), albumin (all $p>0.10$), mGPS (all $p>0.05$), or NLR (all $p>0.10$). There were

no significant associations between these thresholds and tumour related factors such as TNM stage (all p>0.10), venous invasion (all p>0.10), tumour site (all p>0.10), Klintrup-Makinen criteria (all p>0.10), or tumour necrosis (all p>0.10, Table 3.3). The relationship between the perioperative systemic inflammatory response, as demonstrated by CRP concentrations, and cortisol is also shown in Table 3.3. There were no significant associations between the above thresholds and the CRP concentrations pre and postoperatively on days 1 to 7 (all p>0.05).

The relationship between patient and tumour related factors and salivary free cortisol was examined in 30 patients. In terms of patient related factors, there were no significant associations between the late night, morning, or change in salivary cortisol and age (all p>0.10), sex (all p>0.05), ASA grade (all p>0.10), white cell count (all p>0.10), CRP (all p>0.10), albumin (all p>0.10), mGPS (all p>0.10), or NLR (all p>0.05). In terms of tumour related factors, there were no significant associations between the late night, morning, or change in salivary cortisol and TNM stage (all p>0.05), venous invasion (all p>0.10), tumour site (all p>0.10), Klintrup-Makinen criteria (all p>0.05), or tumour necrosis (all p>0.10, Table 3.4). Only 57 patients (approximately 70%) had pathology slides available for review and the assessment of the tumour inflammatory cell infiltrate and tumour necrosis. This may have influenced the results obtained.

3.4 Discussion

The results of the present study demonstrate, for the first time, that impaired cortisol production, as evidenced by the short Synacthen test, was uncommon in patients with potentially curable colorectal cancer. Moreover, they indicate that neither tumour related factors or the presence of a systemic inflammatory response in the perioperative period were associated with impaired cortisol production in these patients.

In the present study, it was of interest that in the group of 11 patients with liver metastases there was an elevated baseline plasma cortisol. This may suggest that there was disregulated cortisol production in these patients, however no other measures of cortisol response to Synacthen were different. Therefore, it would appear that there was not consistent evidence of impaired cortisol production in patients with operable colorectal cancer.

The presence of a systemic inflammatory response in patients with colorectal cancer has been shown to be common and associated with poorer clinical outcome in both localized and advanced disease (McMillan et al., 1995, Nozoe et al., 1998, Nielsen et al., 2000, Moyes et al., 2009), independent of tumour stage (McMillan et al., 1995, Moyes et al., 2009, Roxburgh and McMillan, 2010). Indeed, the presence of a systemic inflammatory response is thought to be beneficial to the tumour in creating an environment where tumour growth and spread are promoted (Coussens and Werb, 2002). It has also been suggested that the tumour itself may act in suppressing the immune response, by driving the recruitment of regulatory T cells, as a strategy of immunoevasion (Sellitto et al., 2011). At present, the underlying stimulus of the systemic inflammatory response in cancer patients remains to be elucidated. Systemic inflammation persists in cancer patients following

apparently curative resection (Galizia et al., 2002, Moyes et al., 2009, Ramsey et al., 2006). Thus, the basis or stimulus for the ongoing systemic inflammatory response appears to be independent of the tumour and more likely due to host responses. Pre-existing immune or physiological abnormalities may even pre-date development of malignancy, possibly an immune dissonance leading to impaired cytokine responses.

Indeed, it is of interest that results from prospective nested case controlled studies report pre-diagnostic raised levels of C-reactive protein are associated with the subsequent development of colorectal cancer (Erlinger et al., 2004, Otani et al., 2006, Gunter et al., 2006), and that chronic administration of aspirin and other non-steroidal anti-inflammatory drugs confers a protection against subsequent development of colorectal cancer. Clearly, if the basis of this response could be identified it would aid attempts to moderate the systemic inflammatory response and tumour progression in primary operable colorectal cancer.

Furthermore, it has been suggested that preoperative administration of corticosteroids is associated with a decrease in postoperative morbidity in patients undergoing surgery for oesophageal cancer (Sato et al., 2002), and may attenuate the inflammatory response to surgery following oesophageal (Sato et al., 2002) and liver (Schmidt et al., 2007) resection. However, there remains a lack of consensus on the utility of perioperative steroids in alleviating surgical stress and further study is required.

In summary, the presence of a systemic inflammatory response would appear not to be due the lack of an anti-inflammatory response in patients with colorectal cancer. In contrast to impaired cortisol production, elevated pro-inflammatory cytokine release has been consistently reported (Kantola et al., 2012). The present results therefore suggest that the

systemic inflammatory response is mainly a result of a pro-inflammatory stimulus rather than an impaired anti-inflammatory response. Further work examining the nature of such pro-inflammatory cytokine release is warranted.

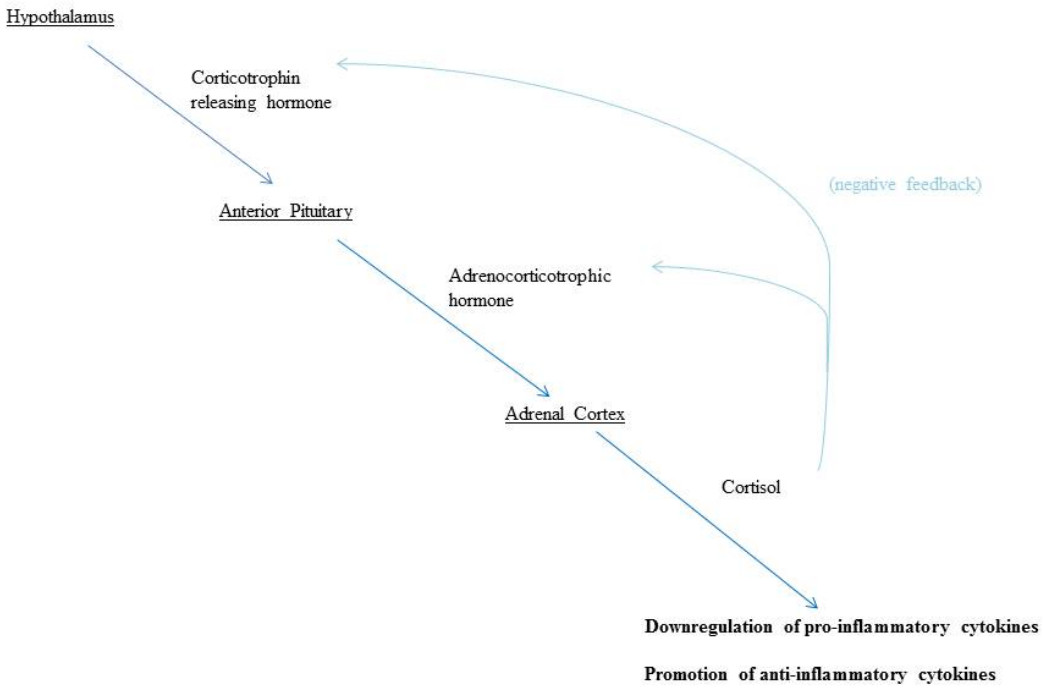


Figure 3-1 - The HPA axis and negative feedback

Table 3-1 Calculation of the modified Glasgow Prognostic Score (mGPS) and the Neutrophil to Lymphocyte ratio (NLR)

The modified Glasgow Prognostic Score	**Score**
C-reactive protein ≤10 mg/l and albumin ≥35 g/l	0
C-reactive protein ≤10 mg/l and albumin <35 g/l	0
C-reactive protein > 10 mg/l	1
C-reactive protein >10 mg/l and albumin <35 g/l	2
Neutrophil Lymphocyte Ratio	
Neutrophil count : lymphocyte count <5:1	0
Neutrophil count : lymphocyte count ≥5:1	1

Table 3-2 The clinicopathological characteristics of patients undergoing potentially curative resection for colorectal cancer (n=80)

Variable	Patients (n=80)
Age <65 / 65-74 / ≥75 years	34 / 29 / 17
Sex - Male / Female	47 / 33
ASA (1+2 / 3+4) [a]	48 / 30
White Cell Count ≤11 / >11 ($x10^9$/L)	75 / 5
C-reactive protein <10 / ≥10 (mg/L)	55 / 25
Albumin ≥35 / <35 (g/L)	23 / 57
mGPS 0 / 1 / 2	55 / 14 / 11
NLR <5 / ≥5	67 / 13
T stage (I / II / III)	17 / 46 / 17
TNM stage (I / II / III / IV)	16 / 27 / 26 / 11
Venous Invasion [b] (No / Yes)	37 / 40
Tumour Site - Colon / Rectum	60 / 20
Klintrup [c] (0-1 / 2- 3)	18 / 39
Tumour Necrosis [c] (absent - focal / moderate - extensive)	39 / 18

[a]n=78, [b]n=77, [c]n=57

Table 3-3 Relationship between patient and tumour related variables and standard thresholds for baseline, 30 minute and change in cortisol (n=80)

Patient Related Variable (number of patients)		**Baseline Cortisol (nmol/L)**		**p value**	**Peak Cortisol (nmol/L)**		**p value**	**Change in Cortisol (nmol/L)**		**p value**
		>200	**<200**		**>450**	**<450**		**>200**	**<200**	
Age (years)	<65	27	7		33	1		22	12	
	65 – 74	26	3		29	0		22	7	
	≥ 75	16	1	0.125	15	2	0.213	12	5	0.549
Sex	Female	39	8		32	1		31	16	
	Male	30	3	0.314	45	2	0.778	25	8	0.349
ASA [a]	1 + 2	39	9		47	1		33	15	
	3 + 4	28	2	0.136	29	1	0.734	22	8	0.666
White Cell Count ($x10^9$/L)	≤ 11	65	10		72	3		51	24	
	> 11	4	1	0.677	5	0	0.651	5	0	0.133
C-reactive Protein (mg/L)	<10	45	10		54	1		39	16	
	≥10	24	1	0.090	23	2	0.180	17	8	0.794
Albumin (g/L)	≥35	47	10		55	2		40	17	
	<35	22	1	0.123	22	1	0.859	16	7	0.957
Modified Glasgow Prognostic	0	45	10		54	1		39	16	
Score (mGPS)	1	13	1		13	1		10	4	
	2	11	0	0.078	10	1	0.182	7	4	0.687
Neutrophil:Lymphocyte Ratio	<5	56	11		64	3		45	22	
(NLR)	≥5	13	0	0.118	13	0	0.440	11	2	0.212

Tumour Related Variable (number of patients)		**Baseline Cortisol (nmol/L)**		**p value**	**Peak Cortisol (nmol/L)**		**p value**	**Change in Cortisol (nmol/L)**		**p value**
		>200	**<200**		**>450**	**<450**		**>200**	**<200**	
T stage	I	14	3		15	2		12	5	
	II	41	5		46	0		31	15	
	III	14	3	0.685	16	1	0.081	13	4	0.782
TNM stage	I	14	2		14	2		11	5	
	II	23	4		26	1		21	6	
	III	22	4		26	0		19	7	
	IV	10	1	0.959	11	0	0.184	5	6	0.255
Venous Invasion [b]	No	33	4		35	2		25	12	
	Yes	33	7	0.405	39	1	0.513	29	11	0.639
Tumour Site	Colon	52	8		57	3		40	20	
	Rectum	17	3	0.852	20	0	0.311	16	4	0.263
Klintrup [c]	0-1	32	7		37	2		31	8	
	2-3	16	2	0.514	18	0	0.332	11	7	0.147
Tumour Necrosis [c]	Absent – Focal	32	7		37	2		28	11	
	Moderate – Extensive	16	2	0.510	18	0	0.328	14	4	0.633

Perioperative Inflammatory Response	**Baseline Cortisol (nmol/L)**		**p**	**Peak Cortisol (nmol/L)**		**p**	**Change in Cortisol (nmol/L)**		**p value**
(median, range) mg/L	**>200**	**<200**	**value**	**>450**	**<450**	**value**	**>200**	**<200**	
Preoperative C-reactive Protein	7 (1-86)	3 (1-17)	0.077	6 (1-86)	18 (2-56)	0.403	6 (1-86)	7 (1-59)	0.781
Day 1 C-reactive Protein	111 (1-247)	94 (70-198)	0.531	111 (1-247)	86 (43-128)	0.607	117 (1-247)	79 (43-140)	0.022
Day 2 C-reactive Protein	167 (60-373)	200 (36-295)	0.858	169 (36-373)	177 (135-219)	0.932	180 (36-373)	158 (75-305)	0.322
Day 3 C-reactive Protein	164 (49-357)	164 (66-224)	0.674	164 (49-357)	177 (127-226)	0.850	166 (49-357)	161 (62-304)	0.956
Day 4 C-reactive Protein	137 (30-330)	122 (49-253)	0.803	135 (30-330)	182 (76-288)	0.778	127 (37-330)	147 (30-291)	0.343
Day 5 C-reactive Protein [a]	118 (17-333)	89 (28-216)	0.464	117 (17-333)	86 (48-298)	0.974	116 (17-333)	115 (48-298)	0.412
Day 6 C-reactive Protein [b]	93 (8-352)	69 (17-131)	0.457	80 (8-352)	131 (76-186)	0.422	75 (8-352)	106 (35-186)	0.425
Day 7 C-reactive Protein [b]	89 (9-310)	63 (12-128)	0.340	79 (9-310)	91 (15-167)	0.795	75 (9-310)	111 (15-173)	0.618

[a]n=68, [b]n=57

Table 3-4 Relationship between patient and tumour related variable and salivary free cortisol (n=30)

Patient Related Variable		10pm Salivary Cortisol (nmol/L)	p value	8am Salivary Cortisol (nmol/L)	p value	Absolute change in Salivary Cortisol (nmol/L)	p value
Age (years)	<65	8.2 (1.7-61.0)		17.4 (7.5-225.0)		10.7 (0.8-164.0)	
	65 – 74	8.3 (1.0-16.5)		13.4 (10.7-28.3)		6.2 (1.0-11.8)	
	≥ 75	10.7 (8.2-13.2)	0.329	32.1 (32.0-32.2)	0.410	21.4 (19.0-23.8)	0.498
Sex	Female	8.2 (1.0-44.0)		15.5 (7.5-58.0)		10.7 (0.8-23.8)	
	Male	11.4 (2.3-61.0)	0.139	20.2 (10.6-225.0)	0.081	12.5 (0.2-164.0)	0.096
ASA	1 + 2	7.6 (1.0-44.0)		16.9 (8.4-62.5)		11.4 (0.8-22.3)	
	3 + 4	8.6 (4.2-61.0)	0.290	19.8 (7.5-225.0)	0.935	11.6 (0.5-164.0)	1.000
White Cell Count ($x10^9$/L)	≤ 11	8.6 (1.0-61.0)		17.9 (7.5-225.0)		11.6 (0.8-164.0)	
	> 11	6.9 (4.4-9.3)	0.352	15.9 (12.5-19.3)	0.861	9.1 (8.1-10.0)	0.837
C-reactive Protein (mg/L)	<10	8.3 (1.3-61.0)		15.0 (7.5-225.0)		8.3 (0.8-164.0)	
	≥10	8.2 (1.0-44.0)	0.752	21.5 (7.5-62.5)	0.896	12.5 (2.9-22.3)	0.733
Albumin (g/L)	≥35	9.0 (1.0-61.0)		16.3 (7.5-225.0)		10.7 (0.8-164.0)	
	<35	8.2 (4.4-44.0)	0.232	29.2 (7.5-62.5)	0.789	14.0 (2.9-23.8)	0.881
Modified Glasgow Prognostic Score	0	8.6 (2.3-61.0)		13.8 (7.5-225.0)		8.2 (0.8-164.0)	
(mGPS)	1	7.1 (1.0-13.2)		22.0 (11.7-32.2)		14.9 (10.7-19.0)	
	2	8.8 (7.7-44.0)	0.330	24.7 (19.3-58.0)	0.923	13.3 (10.0-21.0)	0.735
Neutrophil:Lymphocyte Ratio (NLR)	<5	8.2 (1.0-61.0)		15.3 (7.5-225.0)		11.0 (0.8-164.0)	
	≥5	11.3 (4.4-44.0)	0.048	25.8 (12.5-58.0)	0.563	12.0 (8.1-19.0)	0.879

Tumour Related Variable		10pm Salivary Cortisol (nmol/L)	p value	8am Salivary Cortisol (nmol/L)	p value	Absolute change in Salivary Cortisol (nmol/L)	p value
T stage	I	8.2 (2.7-12.1)		25.3 (11.6-34.4)		19.9 (0.2-23.8)	
	II	9.1 (2.3-61.0)		14.8 (7.5-225.0)		8.2 (0.8-164.0)	
	III	6.4 (1.0-42.0)	0.288	18.4 (9.1-62.5)	0.279	11.6 (7.4-20.6)	0.095
TNM stage	I	8.2 (2.7-12.1)		29.2 (15.5-34.4)		21.0 (11.3-23.8)	
	II	9.2 (3.6-61.0)		20.3 (7.5-225.0)		12.0 (0.8-164.0)	
	III	6.8 (1.0-42.0)		14.6 (10.6-62.5)		8.3 (0.2-20.5)	
	IV	4.6 (1.7-9.1)	0.316	9.1 (7.5-21.5)	0.112	7.4 (2.9-12.4)	0.072
Venous Invasion	No	8.6 (1.0-61.0)		13.6 (7.5-225.0)		10.4 (0.8-164.0)	
	Yes	10.5 (2.3-44.0)	0.834	20.8 (10.6-58.0)	0.473	12.2 (1.0-19.0)	0.369
Tumour Site	Colon	8.2 (1.0-44.0)		15.3 (7.5-58.0)		10.4 (0.2-23.8)	
	Rectum	12.9 (7.7-61.0)	0.301	24.3 (8.4-225.0)	0.227	12.2 (0.8-164.0)	0.214
Klintrup	0-1	7.7 (1.0-16.5)		15.5 (10.6-32.0)		10.7 (0.2-23.8)	
	2-3	9.2 (7.0-61.0)	0.026	29.2 (7.5-225.0)	0.090	14.0 (0.8-164.0)	0.188
Tumour Necrosis	Absent – Focal	8.2 (1.0-61.0)		20.3 (7.5-225.0)		12.2 (0.2-164.0)	
	Moderate - Extensive	9.3 (2.3-44.0)	0.297	15.5 (8.4-58.0)	0.462	10.0 (0.8-14.0)	0.114

median (range)

4 The systemic inflammatory response as a predictor of postoperative infective complications following curative resection in patients with colorectal cancer

4.1 Introduction

Despite improvements in surgery and perioperative care, in particular infection control measures and the use of preoperative antibiotic prophylaxis, infective complications still represent a major cause of morbidity and mortality following colorectal cancer resection (Fujita et al., 2007, Rovera et al., 2007, Tornqvist et al., 1981). Overall complication rates have been reported to be approximately 30% and the perioperative mortality rate approximately 3-4% (Alves et al., 2005, Sjo et al., 2009).

Postoperative infections have traditionally been classified into surgical site infection (SSI) and remote site infection (RSI) (Edwards, 1976, Miki et al., 2006, Matsuda et al., 2009, Mangram et al., 1999). SSI can be further divided into incisional (wound infection) and organ/space (anastomotic leak and intra-abdominal collection). RSI includes pneumonia, urinary tract infection (UTI), septicaemia, antibiotic enterocolitis and central line infection.

Anastomotic leak is the most serious infective complication with an associated increase in postoperative mortality (Alves et al., 1999, Petersen et al., 1998, Buchs et al., 2008). Anastomotic leak can be clinically silent in the early stages and may only become clinically evident as late as post-operative days 8 to 12 when the patient is critically ill (Buchs et al., 2008, Hyman et al., 2007). Furthermore, it has become clear that the development of anastomotic leak is also associated with poorer long term survival

(McArdle et al., 2005, Jung et al., 2008, Marra et al., 2009). Therefore, infective complications, in particular an anastomotic leak, can be catastrophic for the patient, both in short and long term outcomes.

Whilst subsequent leak of faeces into the peritoneal cavity may not be avoided, if these patients could be identified earlier, resultant sepsis and intervention may be reduced. There is increasing evidence that the magnitude of the systemic inflammatory response using the acute phase proteins, in particular C-reactive protein, during the perioperative period might usefully identify those patients at risk of developing a postoperative infective complication. Welsch and co-workers reported that, in 48 patients with infective complications matched with 48 patients with no infective complications undergoing surgery for rectal cancer, increased C-reactive protein concentrations on postoperative day 3 were associated with infective complications with an optimal predictive threshold value of 140mg/l (Welsch et al., 2007). Matthiessen and co-workers reported that, in 32 patients undergoing anterior resection for rectal cancer, an early rise in serum CRP is a strong indicator of anastomotic leakage (Matthiessen et al., 2008). Korner and co-workers reported that, in 231 patients undergoing surgery for colorectal cancer, increased C-reactive protein concentrations on postoperative day 3 were associated with intra-abdominal infections with an optimal predictive threshold value of 190mg/l (Korner et al., 2009).

More recently Woeste and colleagues reported that, in 342 patients undergoing surgery for colorectal cancer, a prolonged elevation in the C-reactive protein concentration with no subsequent decrease precedes the development of anastomotic leakage (Woeste et al., 2010). Ortega-Deballon et al demonstrated that, in 133 patients undergoing elective colorectal surgery, elevated C-reactive protein levels on postoperative day 4 were

associated with anastomotic leakage with an optimal predictive threshold value of 125mg/l (Ortega-Deballon et al., 2010). Mackay and co-workers demonstrated that, in 160 patients undergoing surgery for colorectal cancer, increased C-reactive protein concentrations on postoperative day 4 were associated with infective complications with an optimal predictive threshold value of 145mg/l (MacKay et al., 2011). In a larger study, Warschkow and co-workers demonstrated that, in 1,187 patients undergoing open resection of colorectal cancer, that C-reactive protein concentrations above 123mg/l on postoperative day 4 should raise suspicion of inflammatory complications (Warschkow et al., 2012b).

Therefore, it is not clear what threshold concentration of C-reactive protein or what post-operative day of measurement is most predictive of infective complications following colorectal cancer resection. Establishing this is vital before instigating a change in clinical practice. Therefore, the aim of the present study was to examine the value of serial daily markers of the systemic inflammatory response including white cell count, albumin and C-reactive protein in the prediction of post-operative infective complications in patients undergoing potentially curative resection of colorectal cancer.

4.2 Patients and methods

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and pre-operative abdominal computed tomography, were considered to have undergone potentially curative resection between January 1997 and February 2007 in a single surgical unit at Glasgow Royal Infirmary, were included in the study. Patient characteristics were collected in a prospective surgical database. This was approved by the Research Ethics Committee, Royal Infirmary, Glasgow as part of surgical audit and at this time consent was part of the surgical procedure. All patient data was de-identified.

The tumours were staged using conventional TNM classification. All resections were performed by open surgery and involved either hand sewn or stapled anastomosis. Low pelvic anastomoses were performed using a transanal circular stapling device. The majority of operations involved an anastomosis (95%) with the remaining being either a Hartmann`s procedure or an abdominoperineal resection of rectum. Emergency presentation was defined as a patient who had an unplanned admission to hospital resulting in having their surgery during the same admission.

Pre-operatively every patient received DVT and antibiotic prophylaxis as per local protocol. Serial daily blood samples were taken for routine laboratory analysis of white cell count, albumin and C-reactive protein in the pre- and post-operative period (days 1-7). Postoperatively, all patients had a daily clinical assessment and additional investigations were carried out as indicated clinically.

Patients were assessed for the following complications: infective and non-infective (cardiac events encompassing acute coronary syndrome and acute myocardial infarction,

and pulmonary embolism). Infective complications were separated into surgical site infection (SSI) and remote site infection (RSI). Surgical site infections were further classified into incisional (wound) and organ/space (anastomotic leak). A remote site infection such as pneumonia is often exogenous and occurs at sites not directly associated with the surgical procedure. The criteria used to define infective complications were the same as that previously described (Ytting et al., 2005). (1) Wound infection was defined as the presence of pus, either discharged spontaneously or requiring drainage. Wound infection included a subgroup of patients with perineal infection following abdomino-perineal resection of the rectum. (2) Intra-abdominal abscess was verified by either surgical drainage or by image guided aspiration of pus. (3) Anastomotic leakage was defined as radiologically verified fistula to bowel anastomosis or diagnosed by relaparatomy. (4) Pneumonia was defined by fever above 38.5°C and a positive X-ray, requiring antibiotic treatment. (5) Septicaemia was defined by clinical symptoms combined with a positive blood culture. Non-symptomatic or minor urinary tract infection was not recorded, and therefore only included if complicated by septicaemia.

The extent of deprivation was defined using the Carstairs deprivation index (Carstairs and Morris, 1990). This is an area-based measure derived from the 1991 census, using the postcode of residence at diagnosis, which divides the score into a seven-point index. For illustrative purposes, the results are presented by amalgamating the seven categories into three groups: affluent (categories 1 and 2), intermediate (categories 3–5) and deprived (categories 6 and 7). The Carstairs deprivation index has been extensively utilised in cancer patients and is particularly appropriate for use in the central belt of Scotland (Hole and McArdle, 2002).

Methods

The white cell count (reference range 4–10×10^9/L) was analysed using a haematological blood analyser (Advia 120, Bayer, or CellDyn, Abbott). Serum concentrations of albumin (normal range 35-50 g/L) and C-reactive protein (normal range 0-10 mg/L) were measured by a BCG dye-binding method and turbidometric assay, respectively, using an auto-analyser (Abbott Diagnostics, Abbott Park, IL). The limit of detection of the assay was a C-reactive protein concentration lower than 6 mg/L prior to 2007, and 1mg/L thereafter. The coefficient of variation for these methods, over the range of measurement, was less than 10% as established by routine quality control procedures.

Statistical analysis

Data are presented as median (range). Data from different time periods were tested for statistical significance using the Friedman test and where appropriate comparisons of data from different time periods were carried out using the Wilcoxon signed rank test. The diagnostic accuracy of white cell count, albumin and C-reactive protein was assessed by ROC (Receiver Operator Curve) analysis (Robertson and Zweig, 1981, Zweig and Campbell, 1993, Soreide, 2009). The area under the ROC curve (AUC) is a direct measure of the diagnostic accuracy of the test. An AUC value greater than >50% indicates the ability of a test to significantly discriminate between positive and negative cases with regard to the classification variable (e.g., presence or absence of disease). A test with an AUC greater than 0.75 was considered as having a high diagnostic accuracy and indicates that at least 75% of the patients with the disease were classified correctly. A p-value <0.05 (two-sided tests) was considered significant. Statistical analysis was performed using SPSS version 15.0 for Windows (SPSS Inc, Chicago, IL).

4.3 Results

Baseline characteristics of the 454 patients who underwent curative surgery for colorectal cancer are shown in Table 4.1. The majority of patients were 65 or older (67%), male (55%), had colonic tumours (66%) and node negative disease (57%). Most patients underwent elective resection (87%) and were from a deprived area (55%). The majority of patients had pre-operative white cell count (89%), albumin (80%), and C-reactive protein (55%) in the normal range.

During follow up 120 (26%) patients developed a postoperative complication; 104 (86%) of which were infective complications. The 104 patients with infective complications included 53 RSIs (pneumonia n=36, septicaemia n=5, urinary tract infection n=4, central line tip infection n=3, peripheral cellulitis n=3 and antibiotic entercolitis n=2), 25 wound infections and 26 anastomotic leaks. Of those with an infective complication, 9 patients developed a second infective complication and 6 patients developed an additional non-infective complication. The 16 non-infective complications were pulmonary embolism (n=2), atrial fibrillation (n=4), acute coronary syndrome (n=3), myocardial infarction (n=5), acute urinary retention (n=1) and ischaemic stoma (n=1). Only infective complications were associated with emergency presentation ($p<0.001$), a deprived background ($p<0.05$), an elevated preoperative white cell count ($p<0.001$) and an elevated preoperative C-reactive protein ($p<0.001$).

The relationship between circulating white cell count, albumin and C-reactive protein concentrations and infective and non-infective complications in the perioperative period are shown in Table 4.2 and Figures 4.1-4.4. Compared with those patients who did not develop complications, the white cell count and C-reactive protein were significantly

higher, and albumin lower preoperatively ($p<0.001$, $p<0.001$, $p=0.028$ respectively) and on post-operative days 2 ($p<0.001$, $p<0.001$, $p<0.001$ respectively), 3 ($p<0.001$, $p=0.001$, $p<0.001$ respectively), 4 ($p=0.002$, $p<0.001$, $p<0.001$ respectively), 5 ($p=0.002$, $p<0.001$, $p<0.001$ respectively), 6 ($p<0.001$, $p<0.001$, $p<0.001$ respectively) and 7 ($p<0.001$, $p<0.001$, $p<0.001$ respectively) in those patients who developed infective complications (Table 4.2 and Figures 4.1-4.3). Compared with those patients who did not develop complications white cell count, albumin and C-reactive protein were not significantly different on any perioperative day in those patients who developed non-infective complications (Table 4.2). When those patients who presented as an emergency were removed from the analysis leaving elective patients only (n=396), white cell count was only significantly associated on post-operative day 7 ($p=0.003$) whereas C-reactive protein was significantly higher, and albumin lower on post-operative days 2 to 7 (all $p<0.001$ and all $p<0.001$ respectively) in those who developed infective complications. Compared with those patients who did not develop complications white cell count, albumin and C-reactive protein were not significantly different on any perioperative day in elective patients who developed non-infective complications.

In all patients, compared with those patients who did not develop an anastomotic leak (n=334), C-reactive protein was significantly higher from post-operative day 3 onwards (all $p<0.001$) in those patients who developed an anastomotic leak (n=26, Figure 4.4).

The relationship between circulating white cell count, albumin and C reactive protein concentrations and surgical site and remote site infective complications in the perioperative period are shown in Table 4.3. Compared with those patients who did not develop complications, the white cell count and C-reactive protein were significantly higher, and albumin lower preoperatively ($p=0.041$, $p=0.003$, $p=0.018$ respectively) and on

postoperative days 3 ($p=0.009$, $p<0.001$, $p<0.001$ respectively), 4 ($p=0.013$, $p<0.001$, $p<0.001$ respectively), 5 ($p=0.024$, $p<0.001$, $p<0.001$ respectively), 6 ($p=0.001$, $p<0.001$, $p<0.001$ respectively) and 7 ($p<0.001$, $p<0.001$, $p<0.001$ respectively) in those patients who developed surgical site infective complications (Table 4.3). Compared with those patients who did not develop complications, the white cell count and C-reactive protein were significantly higher, and albumin lower on postoperative days 2 ($p<0.001$, $p<0.001$, $p<0.001$ respectively), 3 ($p<0.001$, $p<0.001$, $p<0.001$ respectively), 4 ($p=0.021$, $p<0.001$, $p<0.001$ respectively), 5 ($p=0.015$, $p<0.001$, $p<0.001$ respectively), 6 ($p=0.002$, $p<0.001$, $p<0.001$ respectively) and 7 ($p=0.005$, $p<0.001$, $p<0.001$ respectively) in those patients who developed remote site infective complications (Table 4.3).

In order to establish a threshold for the relationship between the white cell count, albumin and C-reactive protein in predicting an infective complication following surgery for colorectal cancer Receiver Operator Curves were plotted for postoperative days 3 and 4 (Figures 4.5-4.7). The AUC day 3 and day 4 graphs for prediction of infective complications using white cell count, albumin and C-reactive protein were similar and therefore the day 3 thresholds for white cell count, albumin and C-reactive protein were examined further. For white cell count, the AUC on day 3 was 0.64 with an optimal threshold of $8.6x10^9/l$, sensitivity 69% and specificity 52% (OR 1.09, 95% CI 0.57-0.71, $p<0.05$) (Figure 4.5). For albumin, AUC on postoperative day 3 was 0.68 with an optimal threshold of 25g/l, sensitivity 59%, specificity 67% (OR 0.87, 95% CI 0.62-0.74, $p<0.001$) (Figure 4.6). For C-reactive protein, the AUC was 0.80 with an optimal threshold of 170mg/l, sensitivity 74%, specificity 75% (OR 1.02, 95% CI 0.74-0.85, $p<0.001$) (Figure 4.7). When the threshold values of day 3 white cell count, albumin and C-reactive protein in predicting infective complications were compared in binary logistic regression analysis, both albumin (OR 0.21, 95% CI 0.12-0.39, $p<0.001$) and C-reactive protein (OR 0.10, 95%

CI 0.06-0.19, p<0.001) retained independent predictive value. Therefore, the value of a composite score on day 3 was considered. Receiver Operator Curves were plotted using the thresholds of albumin <25g/l and C-reactive protein >170mg/l on postoperative day 3, AUC was 0.79 (OR 1.44, 95% CI 0.73-0.85, p<0.001) which was similar to the predictive value of day 3 C-reactive protein alone. Therefore, only C-reactive protein at the threshold of >170mg/l was considered in further analysis.

The threshold for the relationship between C-reactive protein and the development of an anastomotic leak following surgery for colorectal cancer Receiver Operator Curves were plotted for post-operative days 3 and 4 (Figure 4.8). This demonstrated that the increased levels on postoperative day 3 were the earliest to be predictive of a postoperative anastomotic leak, with the AUC being 0.84 (p<0.001). The optimal cut off value was 190mg/l, sensitivity 77%, specificity 80% (Figure 4.8). On post-operative day 4, for an anastomotic leak, the AUC was 0.83 (p<0.001). The optimal cut off value was 125mg/l, sensitivity 77%, specificity 76% (Figure 4.8).

The median length of hospital stay was 11 days. Of those patients who had a day 3 C-reactive protein >170mg/l, the median length of hospital stay was 13 days compared with a median of 10 days in those who had a day 3 C-reactive protein <170mg/l (p<0.001). On follow up there were 13 deaths (3% of all patients) after day 3 and within the 30 days following surgery. Of those patients who had a day 3 C-reactive protein >170mg/l, 8 patients died within 30 days (6%), compared with 5 deaths (2%) in those who had a day 3 C-reactive protein <170mg/l (p=0.046). Between 30 days and 1 year, there were a further 23 deaths, 20 of whom had a day 3 C-reactive protein. Of those patients who had a day 3 C-reactive protein >170mg/l, 11 patients died with 30 days and 1 year (8%), compared with 9 deaths (3%) in those who had a day 3 C-reactive protein <170mg/l (p=0.061).

4.4 Discussion

The results of the present study show that the magnitude of the post-operative systemic inflammatory response, in particular C-reactive protein, is associated with the development of postoperative infective complications. Furthermore, a C-reactive protein threshold can be used to predict the likelihood of an infective complication with very good diagnostic accuracy at an early pre-clinical stage prior to the development of clinical signs and symptoms.

The results of the present study are consistent with previous studies and the C-reactive protein thresholds are remarkably similar, although not identical. The present study also examines other routine markers of inflammation in the context of predicting a postoperative infective complication, and investigates the use of a combined predictive score. The differences in C-reactive protein thresholds are likely to be accounted for by different patient numbers and varying thresholds for diagnosis of an infective complication, which is difficult to standardize. When the threshold values from different studies were applied to the data gained in the present study (Table 4.4), on POD 3 there was variation in the sensitivity between 74% and 82% and specificity between 50% and 80% in predicting infections. Also, on post-operative day 4 there was variation in the sensitivity between 54% and 77% and specificity between 75% and 84% in predicting infections. Therefore, depending on the threshold value of C-reactive protein used and the post-operative day chosen there is a considerable variation in the predictive value of the C-reactive protein concentration. Nevertheless, monitoring of C-reactive protein concentrations in the post-operative period has considerable clinical potential.

Clearly, when considering clinical application of post-operative monitoring of C-reactive

protein concentrations it will be used in the context of other existing clinical and biochemical parameters. For example, it may be possible to predict the development of an infective complication at a pre-clinical stage (day 3 rather than approximately day 7) and therefore institute early investigation of a potential infective complication. One approach to investigate this further would be to carry out a prospective randomized trial to study the impact of early diagnosis / intervention based on postoperative monitoring of C-reactive protein concentration. However, in light of the evidence from the present and previous studies this may be considered unethical since clinicians, certainly in our centre, might expect to have access to these C-reactive protein concentrations. Furthermore, the pre-emptive investigations and treatments that might result from early identification (C-reactive protein approximately 170 mg/l, on post-operative day 3) of a potential infective complication are as yet unclear. One approach, if an infective complication was suspected from postoperative C-reactive protein monitoring at day 3, would be to carry out a clinical review including respiratory and abdominal examination, together with appropriate blood, urine and sputum cultures and radiological investigation. If these clinical investigations confirmed or heightened suspicion of an infective complication then it might be reasonable to institute pre-emptive antibiotic use or surgical intervention. If this clinical protocol was proven to allow earlier treatment of infective complications, in particular an anastomotic leak, and therefore reduce post-operative morbidity and mortality this would be a significant contribution to improved care of patients undergoing resection for colorectal cancer.

It is of interest that a small randomised trial investigating the effect of pre-emptive antibiotic treatment on infective complications following colorectal surgery, that used procalcitonin to identify high risk patients, reported a significant reduction in the rate of infective complications (Chromik et al., 2006). However, it remains to be determined

whether such early identification of infective complications can improve the short term morbidity and mortality for patients undergoing potentially curative colorectal cancer resection.

Usually, a rise in circulating C-reactive protein concentration is considered to be a result, rather than a cause, of an infective complication. However, it may be that C-reactive protein is more than just a sensitive measure of the presence of infection. Indeed, C-reactive protein has an important role in innate immunity as an early defense against infection, assisting complement-binding to foreign and damaged cells and enhancing phagocytosis by macrophages. For example, through activation of complement and interaction with Fc gamma receptors, C-reactive protein has been shown to provide a link between the innate and adaptive immune systems (Peisajovich et al., 2008, Coventry et al., 2009, Du Clos and Mold, 2004, Sander et al., 2010). Furthermore, with increasing concentrations of C-reactive protein there is a depression of T-lymphocyte function (Sander et al., 2010, Fietta et al., 2009) and an increase in the stress response and the degree of hyperglycaemia (Wichmann et al., 2005). Also, postoperative hyperglycemia has recently been shown to be an important factor associated with the promotion of bacterial growth and the development of postoperative infective complications (Motoyama et al., 2010, Ramos et al., 2008, Ambiru et al., 2008). Therefore, in addition to giving advance notice of a clinical infection, it may also play an important direct role in modulating the postoperative immune function of patients with colorectal cancer.

In summary, early identification of postoperative infective complications in patients undergoing colorectal cancer resection is crucial to the implementation of adequate therapeutic interventions. The present study shows that C-reactive protein measurements on postoperative day 3 can accurately predict infective complications, including

anastomotic leak, after resection for colorectal cancer. Given that the average time for the development of an infective complication, including an anastomotic leak, is between post-operative day 6 and day 8, daily monitoring of C-reactive protein concentrations may improve their early detection and subsequent management, thereby reducing post-operative morbidity and mortality. Clearly, if this is proven to be the case in prospective trials, then this would be a major contribution to the post-operative management of patients undergoing surgery for colorectal cancer.

Table 4-1 Clinical characteristics of 454 colorectal cancer patients with and without postoperative complications

	No complications n=334	Infective complications n=104	[a]P-value	Non-infective complications n=16	[a]P-value
Age (<65/ 65-74/ ≥ 75)	120/ 99/ 115	30/ 41/ 33	0.637	1/ 8/ 7	0.068
Sex (Male/ Female)	181/ 153	62/ 42	0.367	8/ 8	0.801
Emergency (No/ Yes)	306/ 28	75/ 29	<0.001	15/ 1	0.763
Tumour site (Colon/ Rectum)	216/ 118	72/ 32	0.393	12/ 4	0.398
TNM stage (I/ II/ III)	45/ 142/ 147	17/ 47/ 40	0.283	0/ 7/ 9	0.145
Deprivation (1-2/ 3-5/ 6-7)	13/ 127/ 156	0/ 32/ 55	0.030	0/ 6/ 9	0.445
Pre-operative white cell count (median, range)	7.5 (3.0-23.5)	8.9 (3.4-23.8)	<0.001	8.7 (5.2-15.5)	0.069
Pre-operative white cell count (<11/ >11 x 10^9/l)	242/ 37	69/ 25	0.003	13/ 2	0.994
Pre-operative albumin (median, range)	40 (16-52)	38 (18-47)	0.028	41 (29-47)	0.164
Pre-operative albumin (≥35/ <35 g/l)	269/ 63	79/ 25	0.262	15/ 1	0.200
Pre-operative C-reactive protein (median, range)	8 (1-222)	14 (1-317)	<0.001	6.5 (1-120)	0.567
Pre-operative C-reactive protein (≤10/ >10 mg/l)	194/ 138	42/ 61	0.002	10/ 6	0.747
Length of hospital stay (days)	10 (3-108)	16 (6-187)	<0.001	12 (6-25)	0.603
Mortality at 30 days (No/ Yes)	334/ 0	93/ 11	<0.001	15/ 3	0.459
Mortality at 1 year (No/ Yes)	317/ 16	85/ 7	0.370	13/ 0	0.346

[a]compared with no complications

(30 day mortality excluded for 1 year analysis)

Table 4-2 The relationship between serial postoperative values of white cell count, albumin and C-reactive protein and the development of infective and non-infective complications

	No complications n=334	Infective Complications n=104	P-value[a]	Non-infective complications n=16	P-value[a]
Pre-op WCC	7.5 (3.0-23.5)	8.9 (3.4-23.8)	<0.001	8.7 (5.2-15.5)	0.069
WCC day 1	11.0 (3.2-33.8)	11.4 (3.3-22.5)	0.359	11.4 (8.1-22.0)	0.442
WCC day 2	10.3 (4.0-52.6)	11.7 (3.0-45.7)	<0.001	10.1 (6.6-21.3)	0.681
WCC day 3	8.4 (2.5-45.6)	9.9 (1.4-34.5)	<0.001	9.6 (4.4-20.2)	0.230
WCC day 4	7.4 (2.2-38.3)	8.7 (2.7-35.9)	0.002	8.2 (3.3-15.0)	0.301
WCC day 5	7.3 (2.2-44.4)	9.1 (3.9-29.4)	0.002	8.3 (4.4-14.2)	0.332
WCC day 6	8.0 (3.0-40.5)	9.6 (3.9-23.8)	<0.001	8.7 (5-19.4)	0.374
WCC day 7	8.5 (3.0-41.3)	10.4 (4.7-25.1)	<0.001	9.3 (6.4-16.7)	0.190
Pre-op Alb	40 (16-52)	38 (18-47)	0.028	41 (29-47)	0.164
Alb day 1	28 (11-42)	24 (9-41)	<0.001	28 (19-32)	0.640
Alb day 2	27 (13-44)	23 (9-39)	<0.001	28 (15-32)	0.916
Alb day 3	28 (14-41)	24 (11-37)	<0.001	28 (20-33)	0.736
Alb day 4	29 (13-41)	24 (8-38)	<0.001	30 (20-36)	0.753
Alb day 5	30 (11-42)	26 (10-41)	<0.001	31 (20-35)	0.904
Alb day 6	31 (11-44)	26 (12-46)	<0.001	32 (18-36)	0.582
Alb day 7	32 (14-47)	26 (11-42)	<0.001	32 (21-37)	0.959
Pre-op CRP	8 (1-222)	14 (1-317)	<0.001	6.5 (1-120)	0.567
CRP day 1	108 (5-348)	125 (14-343)	0.064	130 (66-162)	0.116
CRP day 2	163 (17-356)	215 (82-358)	<0.001	180 (74-289)	0.753
CRP day 3	132 (6-319)	208 (38-352)	0.001	150 (42-217)	0.604
CRP day 4	90 (6-306)	149 (23-317)	<0.001	101 (19-215)	0.339
CRP day 5	59 (5-265)	108 (17-283)	<0.001	65 (16-162)	0.665
CRP day6	47 (5-285)	103 (13-354)	<0.001	47 (12-106)	0.915
CRP day 7	38 (5-347)	105 (6-329)	<0.001	36 (9-91)	0.743

Median (range), WCC white cell count, Alb albumin, CRP C-reactive protein

[a] compared with no complications

Table 4-3 The relationship between serial postoperative values of white blood cell count, albumin and C-reactive protein and the development of surgical site and remote site infective complications

	No complications n=334	Surgical site infection n=51	P-value[a]	Remote infection n=53	P-value[a]
Pre-op WCC	7.5 (3.0-23.5)	9 (3.4-19.2)	0.041	8.5 (5.1-23.8)	0.001
WCC day 1	10.9 (3.2-33.8)	11.7 (5.4-22.5)	0.495	11 (3.3-22.3)	0.481
WCC day 2	10.3 (4.0-52.9)	11.1 (4.7-45.7)	0.081	12.5 (3.0-32.1)	<0.001
WCC day 3	8.4 (2.5-45.6)	9.7 (1.4-21.4)	0.009	10.6 (4.9-34.5)	<0.001
WCC day 4	7.4 (2.2-38.3)	9.0 (2.8-24.3)	0.013	8.2 (2.7-35.9)	0.021
WCC day 5	7.3 (2.2-44.4)	8.4 (4.0-17.2)	0.024	9.4 (3.8-29.4)	0.015
WCC day 6	8.0 (3.0-40.5)	10 (3.9-23.8)	0.001	9.2 (5.3-22.6)	0.002
WCC day 7	8.5 (3.0-41.3)	10.4 (4.8-25.1)	<0.001	9.9 (4.7-22.4)	0.005
Pre-op Alb	40 (16-52)	37 (21-47)	0.018	39 (18-44)	0.334
Alb day 1	28 (11-42)	24 (13-41)	0.001	24 (9-34)	<0.001
Alb day 2	27 (13-44)	23 (12-39)	<0.001	23 (9-34)	<0.001
Alb day 3	28 (14-41)	25 (12-37)	<0.001	24 (11-32)	<0.001
Alb day 4	29 (13-41)	25 (12-38)	<0.001	24 (8-34)	<0.001
Alb day 5	30 (11-42)	27 (12-41)	<0.001	24 (10-38)	<0.001
Alb day 6	31 (11-44)	26 (12-46)	<0.001	25 (12-37)	<0.001
Alb day 7	32 (14-47)	26 (11-42)	<0.001	27 (13-40)	<0.001
Pre-op CRP	8 (1-222)	14 (1-306)	0.003	12 (2-317)	0.009
CRP day 1	108 (5-348)	127 (14-264)	0.171	123 (25-343)	0.154
CRP day 2	163 (17-356)	217 (82-317)	<0.001	214 (82-358)	<0.001
CRP day 3	132 (6-319)	221 (38-308)	<0.001	202 (80-352)	<0.001
CRP day 4	90 (6-306)	168 (23-317)	<0.001	144 (49-295)	<0.001
CRP day 5	59 (5-265)	109 (17-283)	<0.001	103 (24-277)	<0.001
CRP day6	47 (5-285)	125 (21-354)	<0.001	80 (13-242)	<0.001
CRP day 7	38 (5-347)	122 (6-329)	<0.001	69 (13-249)	<0.001

Median (range), WCC white cell count, Alb albumin, CRP C-reactive protein

[a]compared with no complications

Table 4-4 Comparison of reported threshold values of C-reactive protein in predicting infective complications

						Analysis of CRP threshold in the present study (n=454)			
Study	C/R	Patients (n)	POD	CRP Threshold	Complication	Infective Complications		Anastomotic leak	
						Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Welsch et al	R	48	3	140	All infections	82	50		
Kørner et al	CR	231	3	190	AL			77	80
This study	**CR**	**454**	**3**	**170**	**All infections**	**74**	**75**		
Ortega-Deballon et al	CR	133	4	125	AL			77	76
MacKay et al	CR	160	4	145	All infections	54	84		
Warschow et al	CR	1187	4	123	All infections	69	78		

C/R colon or rectum, POD postoperative day, CRP C-reactive protein, AL anastomotic leak

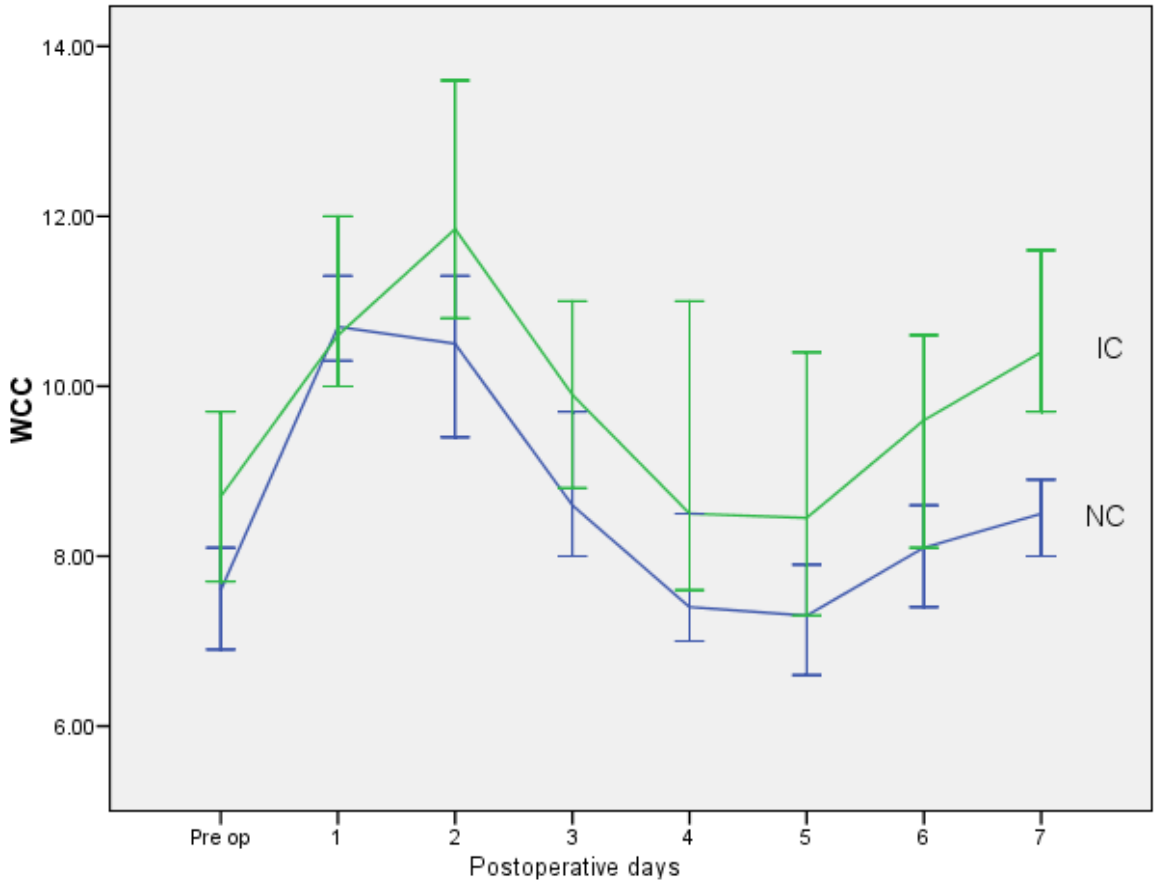


Figure 4-1 The perioperative changes in white cell count in patients with infective complications (IC) and no complications (NC)

White cell count (10^9/l). (median, IQR)

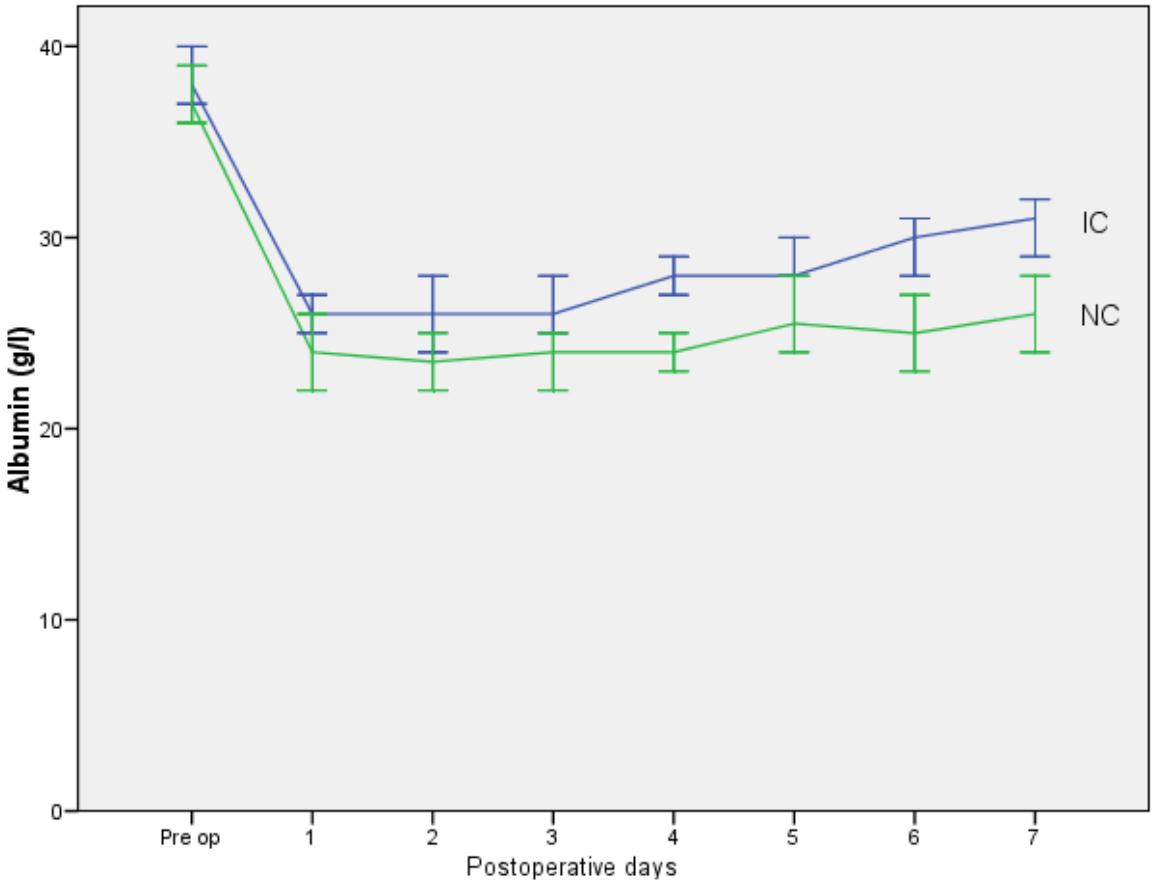


Figure 4-2 The perioperative changes in albumin in patients with infective complications (IC) and no complications (NC)

(median, IQR)

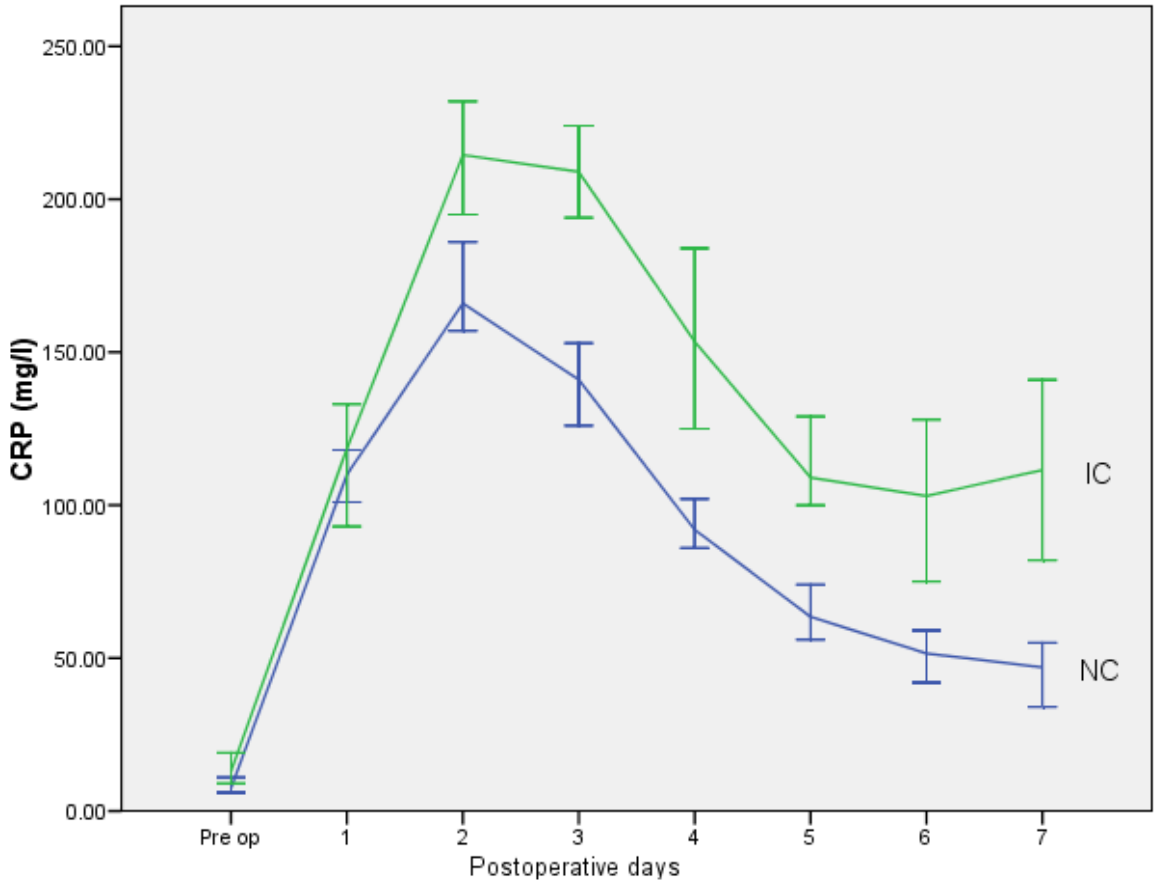


Figure 4-3 The perioperative changes in C-reactive protein in patients with infective complications (IC) and no complications (NC)

(median, IQR)

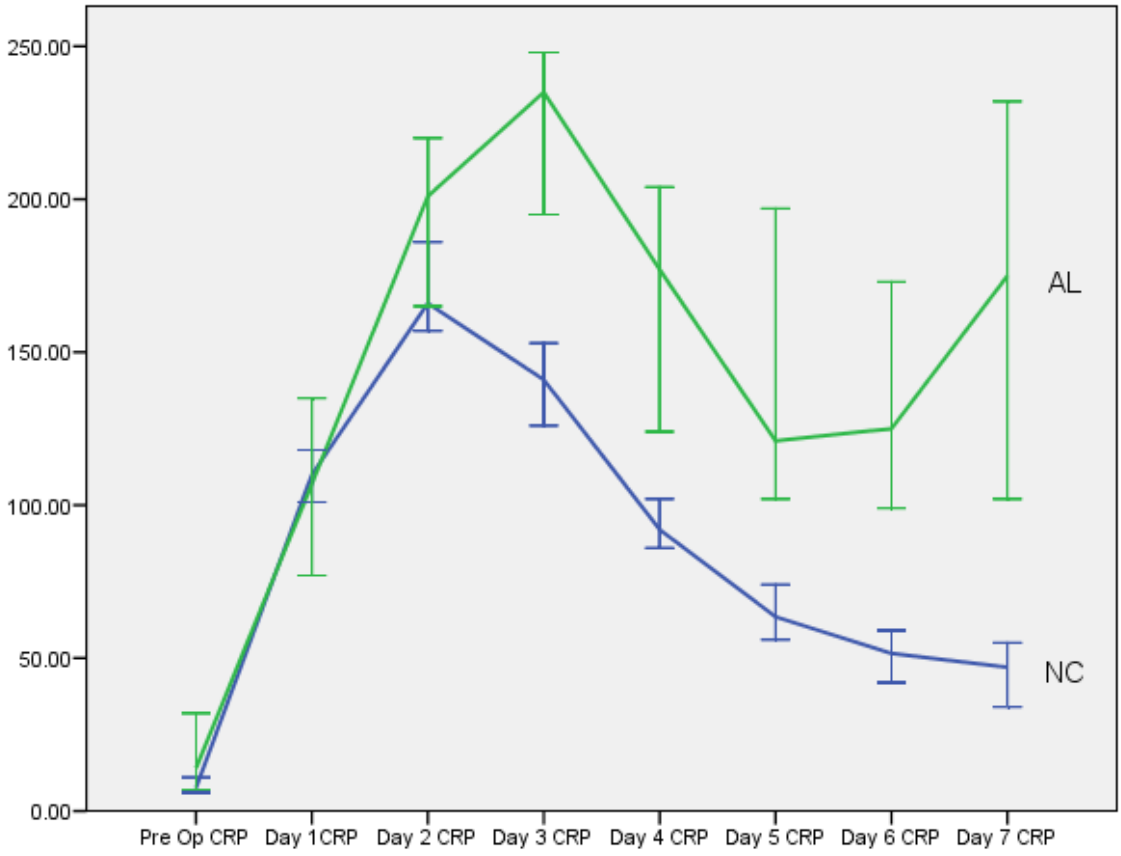
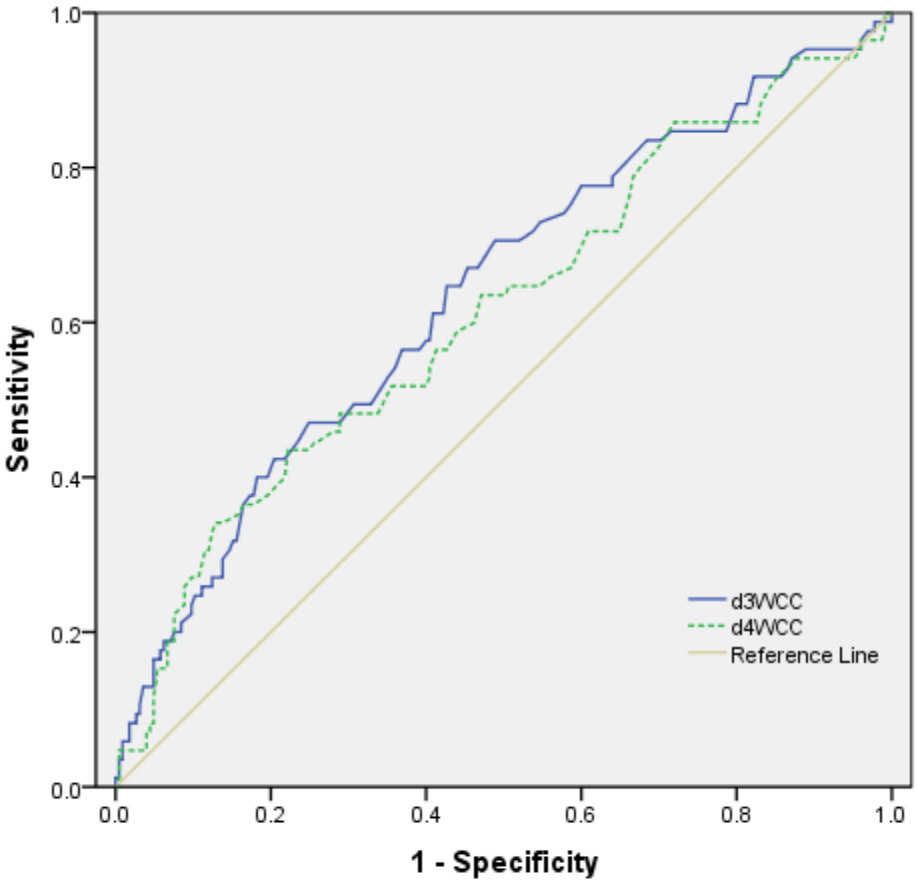


Figure 4-4 The perioperative changes in C-reactive protein in patients with anastomotic leak (AL) and no complications (NC)

(median, IQR)



[redacted]

infective complications following surgery for colorectal cancer

The AUC values were 0.64 ($p<0.001$) and 0.62 ($p=0.002$) for postoperative days (POD) 3 and 4 respectively.

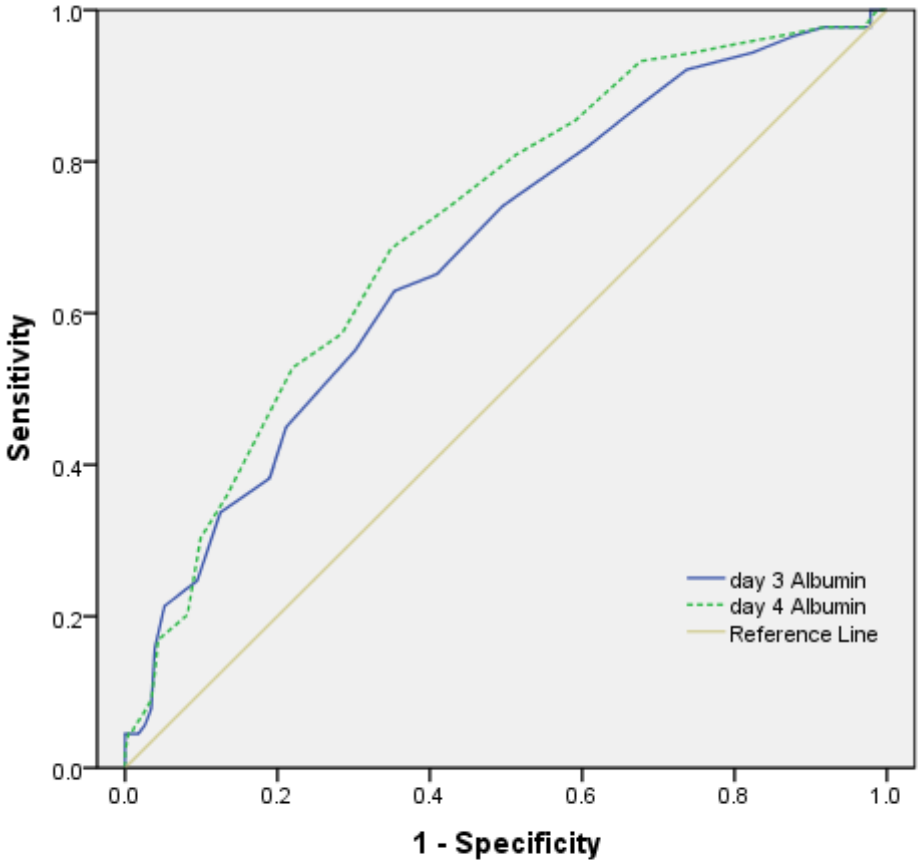


Figure 4-6 Diagnostic accuracy of albumin with regard to the development of infective complications following surgery for colorectal cancer

The AUC values were 0.68 ($p<0.001$) and 0.72 ($p<0.001$) for postoperative days (POD) 3 and 4 respectively.

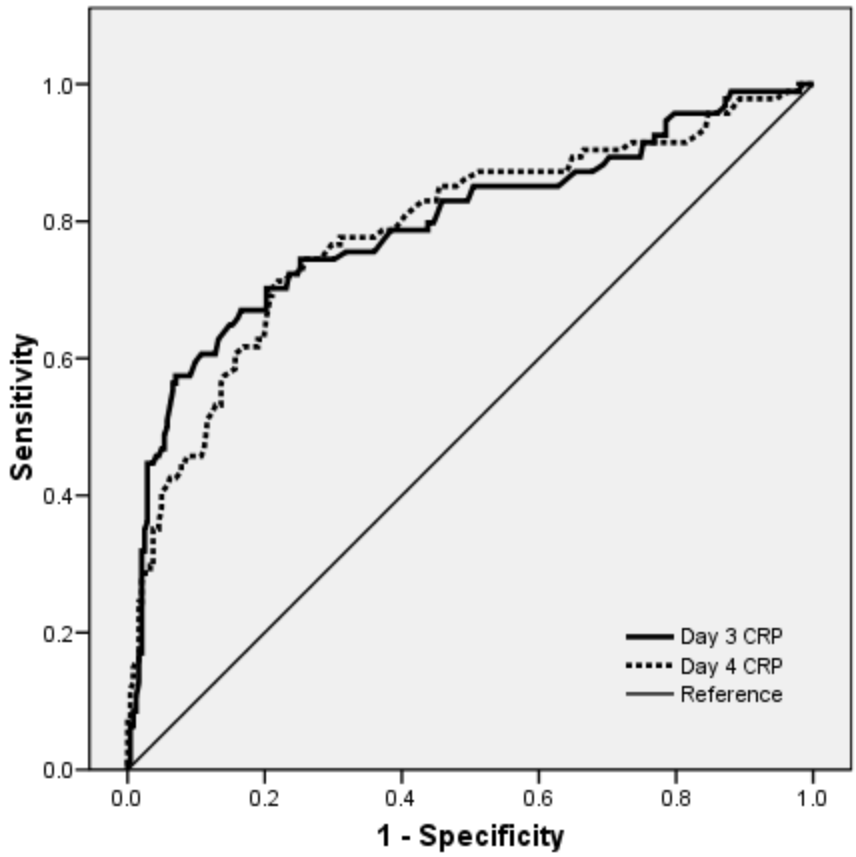


Figure 4-7 Diagnostic accuracy of C-reactive protein with regard to the development of infective complications following surgery for colorectal cancer

The AUC values were 0.80 ($p<0.001$) and 0.79 ($p<0.001$) for postoperative days (POD) 3 and 4 respectively.

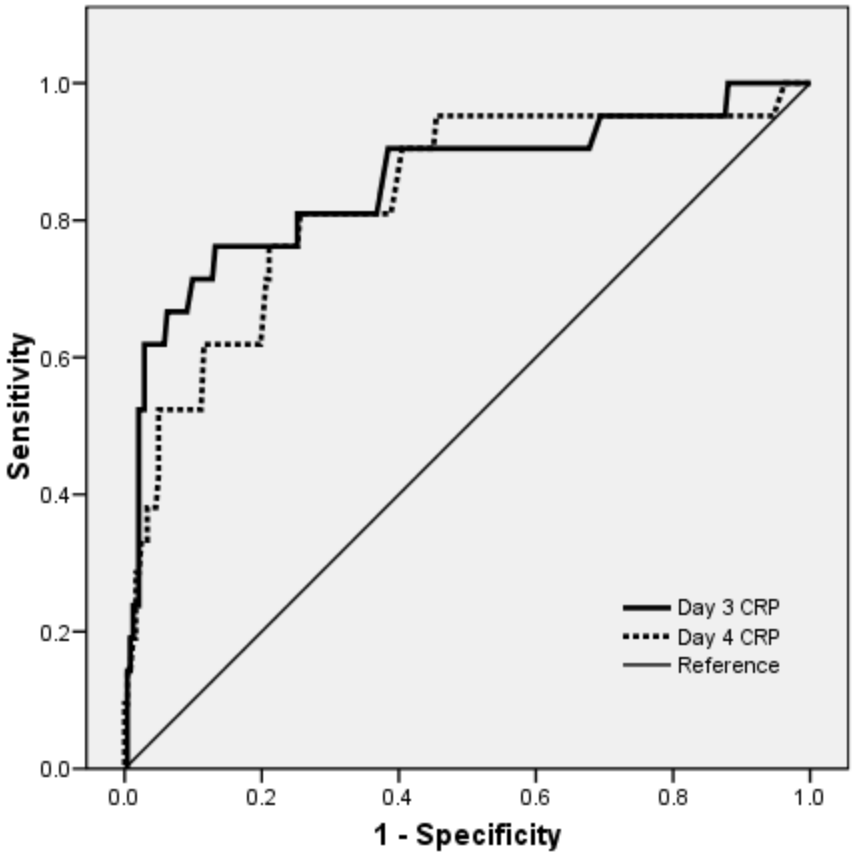


Figure 4-8 Diagnostic accuracy of C-reactive protein with regard to the development of anastomotic leak following surgery for colorectal cancer

The AUC values were 0.84 and 0.83 for postoperative day (POD) 3 and 4 respectively.

5 The impact of open vs laparoscopic resection for colon cancer on C-reactive protein concentrations as a predictor of postoperative infective complications

5.1 Introduction

Despite improvements in surgery and perioperative care, in particular infection control measures and the use of preoperative antibiotic prophylaxis, infective complications still represent a major cause of morbidity, resulting in prolonged hospital stay and increased health care costs, and mortality following colorectal cancer resection (Fujita et al., 2007, Rovera et al., 2007, Tornqvist et al., 1981). Postoperative complication rates remain high, approximately 30%, and the perioperative mortality rate approximately 3-4% (Sjo et al., 2009, Alves et al., 2005). Infective complications and anastomotic leak can be difficult to detect in the early postoperative stage and may only become clinically evident as late as postoperative day 8-12, when the patient is critically ill (Alves et al., 1999, Hyman et al., 2007). Furthermore, it has been shown that the development of an anastomotic leak is also associated with poorer long term survival (McArdle et al., 2005, Jung et al., 2008, Marra et al., 2009). Therefore, infective complications can be serious for the patient both in the short term and in the long term.

Recently, it has become clear that C-reactive protein, an acute phase protein almost exclusively synthesised in the liver and a reliable routinely available measure of the systemic inflammatory response, is a positive predictor of infective complications (Warschkow et al., 2012a) and an anastomotic leak (Singh et al., 2014). In particular, C-reactive protein concentrations on postoperative day 3 (< ~170 mg/l) and day 4 (< ~130mg/l) have been proposed to be of clinical utility since they aid safe and early

discharge of selected patients following colorectal surgery. However, since the majority of studies have examined such C-reactive protein thresholds in open colorectal surgery it is not clear whether such thresholds are applicable in laparoscopic surgery specifically.

It is plausible that predictive C-reactive protein thresholds may be altered in laparoscopic surgery since postoperative C-reactive protein concentrations have been reported to be reduced in laparoscopic compared with open colorectal surgery in some studies (He et al., 2009, Schwenk et al., 2000, Veenhof et al., 2012) but not all (Dunker et al., 2003). Due to the increasing utilisation of the laparoscopic approach, whether similar thresholds apply is important for clinical practice. If there were differences in these thresholds then this would have implications for the use of C-reactive protein as a negative predictor of infective complications in patients undergoing resection for colorectal cancer. Alternatively, if there were no differences in the thresholds then this would confirm the immunological rationale for measuring C-reactive protein. Therefore, the aim of the present study was to compare the value of C-reactive protein concentration as a predictor of postoperative infective complications in patients undergoing open versus laparoscopic resection for colon cancer.

5.2 Patients and Methods

Patients with histologically proven colon cancer who, on the basis of intraoperative findings and preoperative abdominal computed tomography, were considered to have undergone potentially curative resection in one of two university teaching hospitals in Glasgow over a three year period were included in the study (n=344). Patient characteristics were collected in a prospective surgical database. All patient data was de-identified.

The tumours were staged using conventional TNM classification. All resections were elective cases and were performed using either open (n=191) or laparoscopic surgery (n=153). All operations involved an anastomosis. In order to reduce possible confounding factors patients undergoing emergency surgery, surgery for rectal cancer, or laparoscopic surgery converted to open were excluded from the analysis.

Pre-operatively all patients received thromboembolism and antibiotic prophylaxis according to the local protocol (the same in both units). Blood samples were taken for routine laboratory analysis of C-reactive protein in the pre- and postoperative period (days 1-4). Postoperatively, all patients had a daily clinical assessment and additional investigations were carried out as clinically indicated.

Patients were assessed for the following complications: infective and non-infective (persistent ileus, cardiac events encompassing acute coronary syndrome and acute myocardial infarction, and pulmonary embolism)**.** Infective complications can be described as surgical site infections (SSI) and remote site infections (RSI). Surgical site infections can be further classified into incisional (wound) and organ/space (intra-

abdominal abscess / anastomotic leak). A remote site infection such as pneumonia is often exogenous and occurs at sites not directly associated with the surgical procedure. The criteria used to define infective complications were the same as previously described (Ytting et al., 2005). Casenotes, clinic letters and the hospital computer system containing lab results were reviewed at 30 days postoperatively.

Statistical analysis

Data are presented as median (range). Comparison between unpaired data was carried out using a Mann-Whitney U test. A p-value <0.05 was considered significant. The diagnostic accuracy of C-reactive protein was assessed by receiver operating characteristic (ROC) analysis. Statistical analysis was performed using SPSS version 21.0 for Windows (SPSS Inc, Chicago, IL).

5.3 Results

Baseline characteristics of the 344 patients who underwent surgery for colon cancer are shown in Table 5-1. The majority of patients were age 65 or older (75%), male (52%), had left sided tumours (54%), node negative disease (77%), and did not undergo neo-adjuvant treatment (94%). Patients undergoing open and laparoscopic resection were similar in terms of age, sex, tumour site, TNM stage, comorbidity and infective complications. In contrast, pre-operative and postoperative days 1 to 3 C-reactive protein concentrations were lower following laparoscopic compared with open resection in the whole cohort (n=344; all $p<0.001$) and in those who did not develop infective complications (n=251; Table 5-2; all $p<0.001$). The median length of hospital stay was shorter in the laparoscopic resection ($p<0.001$).

During follow up 127 (37%) patients developed a postoperative complication; 93 (73%) of which were infective complications. The 93 patients with infective complications included 43 remote site infections (pneumonia n=35, urinary tract infection n=4, peripheral cellulitis n=2, sepsis due to a central line tip infection n=2, and clostridium difficile n=1), 36 wound infections, 5 intra-abdominal abscess and 18 anastomotic leaks. Of those with an infective complication, 10 patients developed a second infective complication and 17 patients developed an additional non-infective complication. The 51 non-infective complications were persistent ileus (n=10), atrial fibrillation (n=8), myocardial infarction (n=6), acute urinary retention (n=5), haematoma or bleeding (n=3), acute renal failure (n=3), wound dehiscence (n=7), small bowel obstruction (n=4), pulmonary or deep vein thrombosis (n=3), port site hernia (n=1) and multi-organ failure (n=1). Both infective and non-infective complications were associated with an increased length of hospital stay (both $p<0.001$).

The relationship between open and laparoscopic resections, daily C-reactive protein concentrations and infective complications in the postoperative period are shown in Figure 5-1 and Table 5-2. In those who developed an infective complication there was no significant difference in the C-reactive protein concentrations between open and laparoscopic resections on postoperative days 1 to 4 (Figure 5-1, Table 5-2).

In order to establish a threshold for the relationship between C-reactive protein concentrations in predicting an infective complication following surgery for colon cancer Receiver Operator Curves were plotted for postoperative days 3 and 4 (Figures 5-2 and 5-3). Following open surgery, the AUC for postoperative day 3 was 0.75 with an optimal threshold of 180mg/l, sensitivity 71%, specificity 61% ($p<0.001$). For postoperative day 4 the AUC was 0.78 with an optimal threshold of 140mg/l, sensitivity 75%, specificity 74% ($p<0.001$) (Figure 5-2). Following laparoscopic surgery, the AUC for postoperative day 3 was 0.74 with an optimal threshold of 180mg/l, sensitivity 71%, specificity 79% ($p=0.001$). For postoperative day 4 the AUC was 0.72 with an optimal threshold of 140mg/l, sensitivity 71%, specificity 72% ($p=0.001$) (Figure 5-3).

5.4 Discussion

The results of the present study show, for the first time, that although the magnitude of the systemic inflammatory response, as evidenced by C-reactive protein, following surgery was greater in open compared with laparoscopic resection, the threshold concentrations of C-reactive protein for the development of postoperative infective complications were remarkably similar on days 3 and 4. Taken together these results would suggest a mechanistic association between over-elaboration of the systemic inflammatory response and the development of infective complications.

The results of the present study are consistent with and, in particular, the C-reactive protein thresholds remarkably similar to previous studies (Singh et al., 2014). These present results appear to confirm the lesser magnitude of the systemic inflammatory response associated with laparoscopic resection in those patients who did not develop an infective complication. Clearly, one possibility for this is that the systemic inflammatory insult is reduced with the use of laparoscopic surgery. However, it was of interest that, in the present study, those patients who underwent a laparoscopic resection had a lower pre-operative C-reactive protein concentration. Therefore it might be that patients who undergo a laparoscopic resection for colon cancer have less of a baseline systemic inflammatory response, perhaps secondary to lesser comorbidity such as obesity and smoking, or have a lower inflammatory insult postoperatively, or both.

One limitation of the present study is that it examines contemporaneous cohorts at two different hospitals, which may lead to slight differences in patient factors such as deprivation (data not collected) and perioperative care other than surgical approach, a randomised control trial would reduce such confounding factors. Furthermore, the C-reactive protein threshold predictive of

infective complications in this chapter is similar, although not identical, to the threshold determined in chapter 4. This may be accounted for due to different patient numbers and the exclusion of emergency presentation.

C-reactive protein thresholds predictive of infective complications following both open and laparoscopic surgery for colorectal cancer have important potential clinical application, for instance in raising suspicion of the development of complications and guiding investigations at an early stage prior to clinical symptoms evolving, or indeed aiding safe early discharge from hospital. Moreover, these thresholds may be useful in identifying interventions targeted at reducing infective complications. The basis of the relationship between a threshold concentration of C-reactive protein in the post-operative period and the development of infective complications is not clear. However, given that this observation has been made across surgical procedures (Singh et al., 2014, Warschkow et al., 2012d, Warschkow et al., 2012c, Noble et al., 2013), and now across surgical techniques, with varying magnitudes of surgical injury would suggest an immunological process. In particular, the elaboration of a systemic inflammatory response is associated with an up regulation of the innate immune response and a consequent down regulation of the adaptive immune response. If this was indeed the case this would suggest a number of approaches (selective and unselective) to reduce the infective complication rate. For example, in order to investigate the temporal relationship between the inflammatory response and infective complications, one approach would be to target the magnitude of the systemic inflammatory response using anti-inflammatory agents. Another approach, given that post-operative hyperglycaemia promotes bacterial growth, would be to institute tight glycaemic control. Furthermore, based on high C-reactive protein concentrations post-operatively, another approach would be to provide additional antibiotic therapy. It remains to be determined what approach will best bring about a reduction of infective

complications in patients undergoing surgery for colon cancer.

In summary, the results of the present study show that the magnitude of the postoperative systemic inflammatory response, as evidenced by C-reactive protein concentrations, was similar in patients who developed postoperative infective complications irrespective of whether they underwent open or laparoscopic surgery for colon cancer.

Table 5-1 Clinicopathological characteristics of patients undergoing elective resection for colon cancer (n=344)

	Open surgery (n=191)	Laparoscopic surgery (n=153)	p-value
Age (<65/ 65-74/ ≥ 75)	47/ 58/ 86 (25/ 30/ 45)	37/ 59/ 57 (24/ 39/ 37)	0.338
Sex (Male/ Female)	95/ 96 (50/ 50)	85/ 68 (56/ 44)	0.284
Neo-adjuvant (No/ Yes)	176/ 15 (92/ 8)	146/ 7 (95/ 5)	0.218
ASA (1/ 2/ 3/ 4)	11/ 53/ 41/ 9 (6/ 28/ 21/ 5)	14/ 62/ 40/ 2 (9/ 41/ 26/ 1)	0.120
TNM stage (0/ I/ II/ III)	8/ 30/ 84/ 63 (4/ 16/ 44/ 33)	3/ 39/ 58/ 48 (2/ 25/ 38/ 31)	0.344
Tumour Site (Right / Left)	76/ 76 (40/ 40)	64/ 89 (42/ 58)	0.153
Pre-operative C-reactive protein	9 (1-223)	5 (1-236)	<0.001
Day 1 C-reactive protein	110 (2-313)	79 (5-236)	<0.001
Day 2 C-reactive protein	175 (20-358)	128 (12-392)	<0.001
Day 3 C-reactive protein	169 (6-443)	122 (11-339)	<0.001
Day 4 C-reactive protein	124 (6-415)	94 (21-346)	0.182
Any complication	77 (40)	50 (33)	0.145
Non-infective complication	32 (17)	19 (12)	0.261
Infective complication	54 (28)	39 (25)	0.564
Surgical site infection	32 (17)	26 (17)	0.953
Remote site infection	29 (15)	14 (9)	0.093
Anastomotic leak	11 (6)	7 (5)	0.625
Wound infection	20 (10)	16 (10)	0.997
Pneumonia	23 (12)	12 (8)	0.201
Length of hospital stay (days)	8 (3-78)	6 (2-27)	<0.001

Results shown as number (percentage) or median (range), C-reactive protein (mg/l)

Table 5-2 The relationship between serial postoperative values of C-reactive protein and the development of infective complications following open versus laparoscopic surgery for colon cancer (n=344)

	No infective complication (n=251)			**Infective Complication** (n=93)		
	Open surgery (n=137)	**Laparoscopic** (n=114)	**p-value**	**Open surgery** (n=54)	**Laparoscopic** (n=39)	**p-value**
Pre-op CRP	9 (1-209)	4 (1-45)	<0.001	9 (1-101)	5 (2-28)	0.019
CRP day 1	103 (4-229)	60 (18-173)	<0.001	117 (2-240)	112 (43-236)	0.092
CRP day 2	169 (20-320)	116 (32-317)	<0.001	201 (82-358)	188 (60-392)	0.193
CRP day 3	160 (6-352)	129 (44-316)	<0.001	220 (78-430)	226 (40-339)	0.635
CRP day 4	110 (6-388)	87 (28-346)	0.196	187 (23-415)	233 (27-314)	0.923

Median (range), CRP C-reactive protein (mg/l)

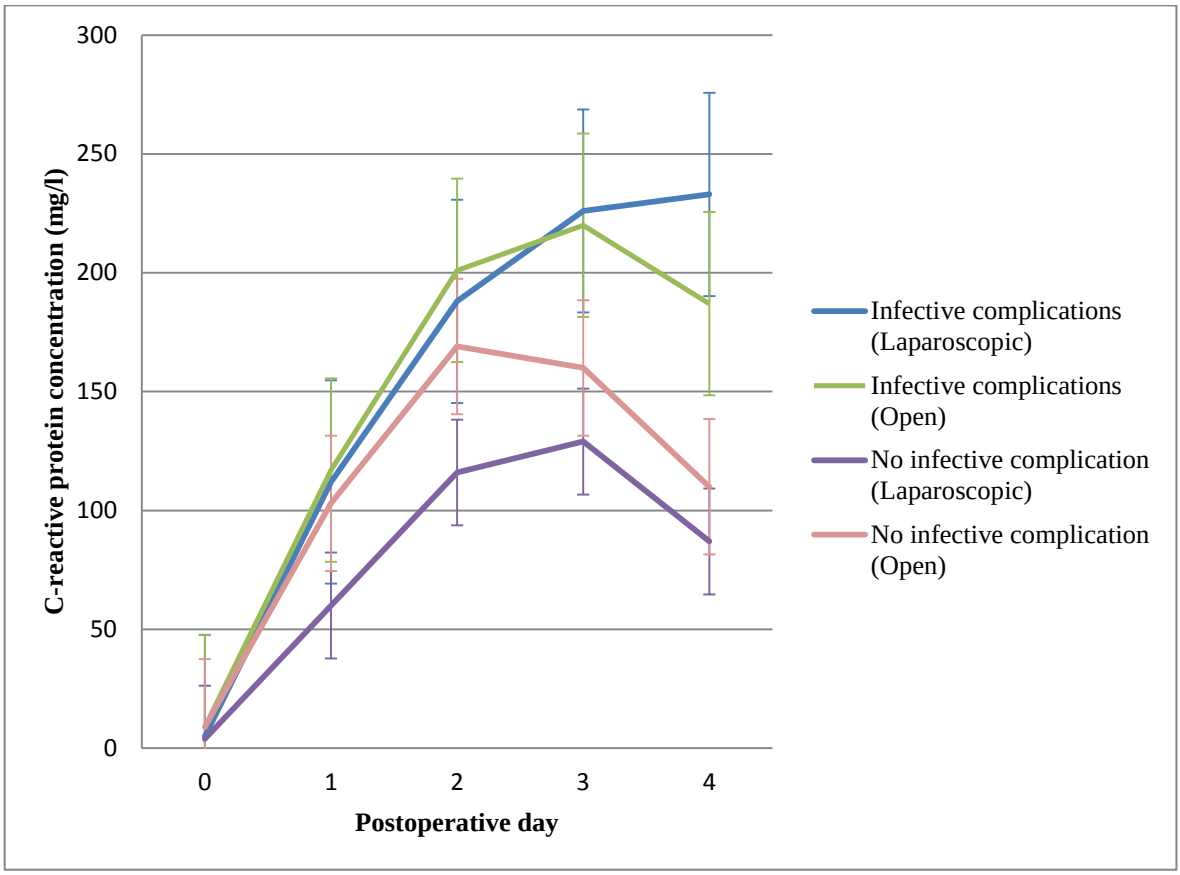


Figure 5-1 The perioperative changes in C-reactive protein in patients with infective complications following open and laparoscopic surgery for colorectal cancer

Median, 95% Confidence Interval.

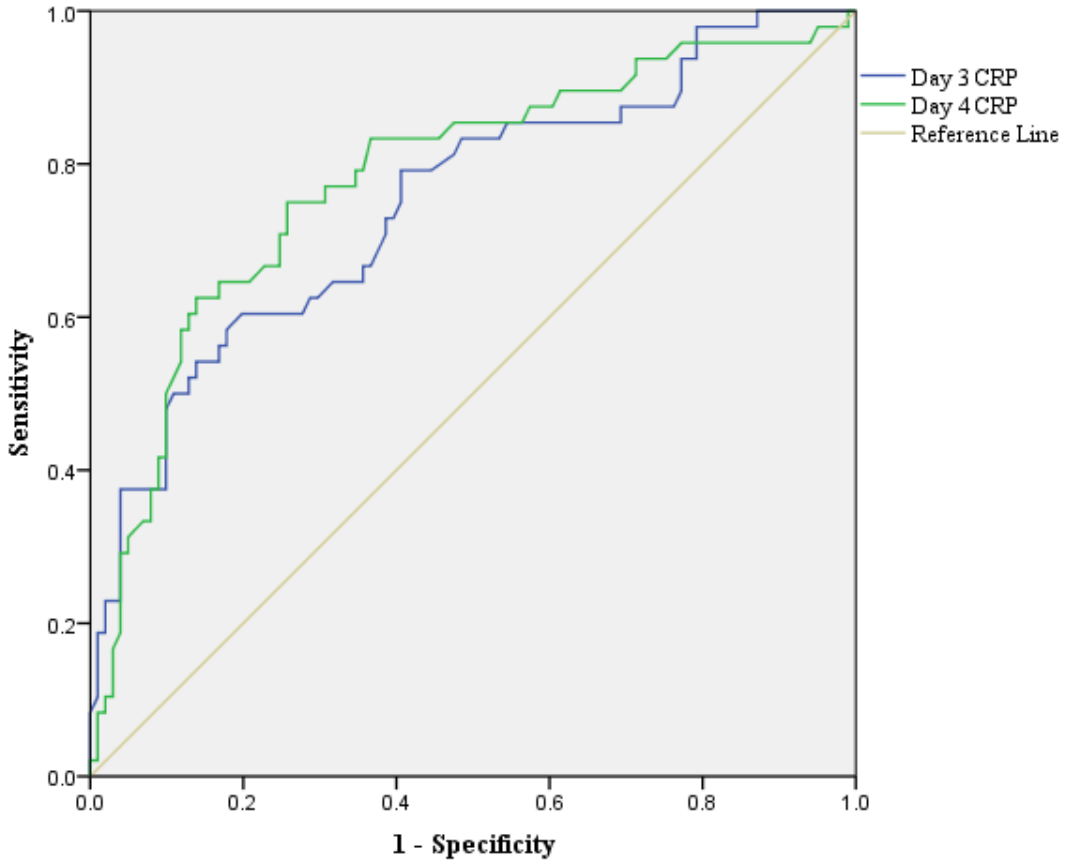


Figure 5-2 Diagnostic accuracy of C-reactive protein with regards to the development of infective complications following open surgery for colorectal cancer

The AUC values were 0.75 ($p<0.001$) and 0.78 ($p<0.001$) for postoperative days 3 and 4 respectively.

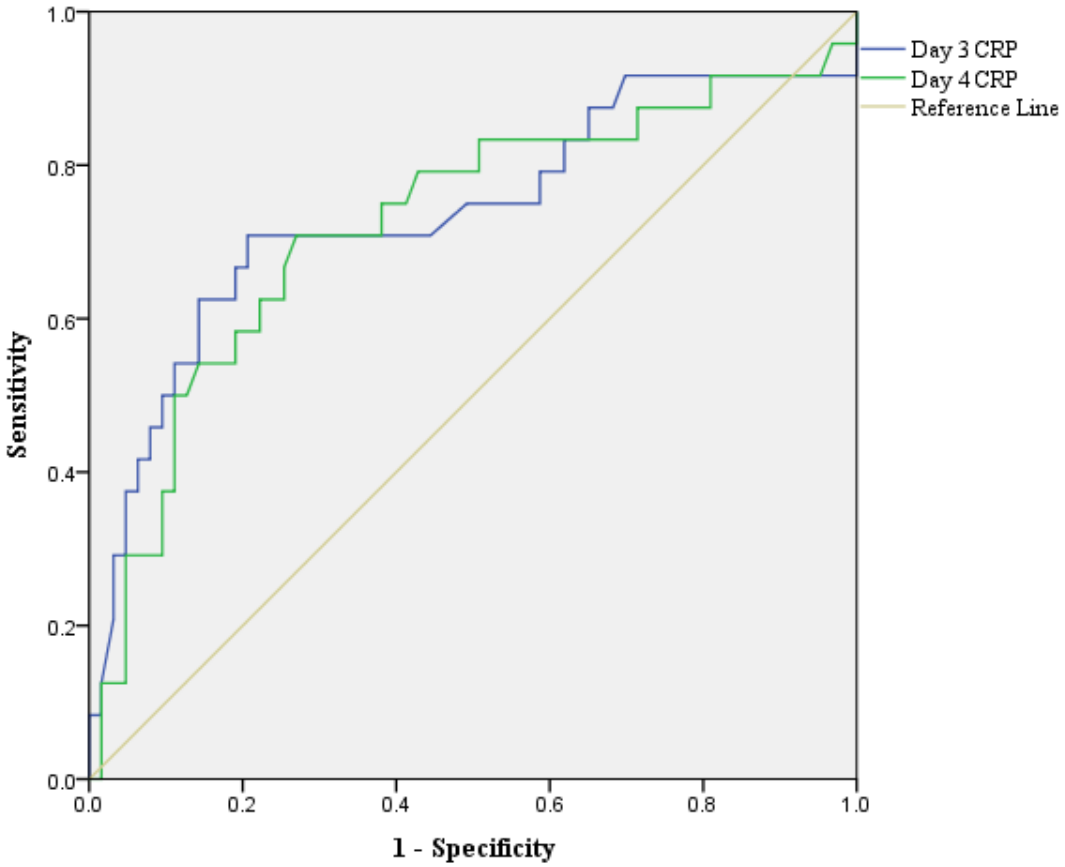


Figure 5-3 Diagnostic accuracy of C-reactive protein with regard to the development of infective complications following laparoscopic surgery for colorectal cancer

The AUC values were 0.74 (p=0.001) and 0.72 (p=0.001) for postoperative days 3 and 4 respectively.

6 The impact of the day 2 C-reactive protein on day 3 and 4 thresholds associated with infective complications following curative surgery in colorectal cancer

6.1 Introduction

Colorectal cancer is the second most common cause of cancer death in the UK, accounting for 16,000 deaths annually (CRUK, 2014). Outcomes are primarily dependent on stage at presentation but even with modern treatments, of those deemed suitable candidates for curative resections approximately 50% will suffer disease recurrence and die of their disease. It is now recognised that postoperative complications contribute to poor cancer specific survival (Rizk et al., 2004, Khuri et al., 2005). In particular, anastomotic leak following colorectal cancer resection has been negatively associated with survival, independent of tumour stage (McArdle et al., 2005, Law et al., 2007).

It is of interest, therefore, that the postoperative systemic inflammatory response, as evidenced by C-reactive protein concentrations on day 3 and day 4, has been consistently reported to be associated with the development of infective complications and anastomotic leak (Welsch et al., 2007, Korner et al., 2009, Ortega-Deballon et al., 2010, MacKay et al., 2011, Warschkow et al., 2012b). However, patients in enhanced recovery after surgery programmes require earlier assessment as they are likely to be discharged from hospital earlier, around day 3. Therefore, assessment of the peak C-reactive protein response to surgery at day 2 would prove useful. As a result, it would be important to determine

whether the systemic inflammatory response, as measured by C-reactive protein, on postoperative day 3 and day 4 is influenced by the peak systemic inflammatory response to surgery, as measured by C-reactive protein on day 2 (Gabay and Kushner, 1999, Lane et al., 2013).

The aim of the present study was to assess the impact of the peak systemic inflammatory response, as evidenced by day 2 C-reactive protein, on C-reactive protein concentrations and their thresholds on day 3 and day 4, and therefore its potential impact on the development of infective complications and anastomotic leak.

6.2 Patients and Methods

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and preoperative investigations, were considered to have undergone potentially curative resection in one of two university teaching hospitals, with C-reactive protein measurement carried out on postoperative days 1 to 4, were included in the study (n=357). Patient characteristics and postoperative complications within 30 days were recorded in a prospective database. An enhanced recovery programme was utilised in one hospital (n=92). Patients with incomplete blood results or those who had other pre-existing inflammatory conditions, such as rheumatoid arthritis and inflammatory bowel disease, metastatic disease, or who were admitted as an emergency were excluded from the study (n=157). This was approved by the research ethics committee, as part of surgical audit. All patient data was anonymised.

All tumours were staged according to the conventional tumour, node, metastasis (TNM, 5th Edition) staging system classification. Daily blood samples were taken, as per hospital routine, for analysis of C-reactive protein in the pre- and postoperative period. Prior to surgery, all patients received thromboprophylaxis and antibiotic prophylaxis as per hospital protocol. Postoperatively, all patients were clinically assessed and additional investigations carried out as indicated.

Patients were assessed for infective complications whilst inpatients and at their first routine outpatient follow up. Infective complications were defined as described previously (Ytting et al., 2005). Briefly, superficial wound infection was defined as the presence of pus, either discharging spontaneously or requiring drainage. Intra-abdominal abscess was verified by either surgical or image guided drainage of pus. An anastomotic leak was

diagnosed radiologically or at re-laparotomy. Pneumonia was defined as a fever >38.5°C plus a positive chest X-ray or CT scan, requiring treatment with antibiotics. Septicaemia was defined by clinical symptoms and a positive blood culture. Urinary tract infection was only included if complicated by septicaemia.

Statistics

Data was compared using X^2, Mann-Whitney U and Kruskal Wallis tests. In order to assess the correlation between day 2 C-reactive protein and other perioperative days, scatter plots were drawn with a best fit line, and r^2 calculated as a measure of correlation (Figures 6-1 and 6-2). r^2 is the fraction of the total variance of day 3 or 4 C-reactive protein that is associated with variation in day 2 C-reactive protein concentrations. The corresponding postoperative day 2 C-reactive protein concentrations for various day 3 and 4 thresholds from previous studies were calculated using the regression equation derived from the plot of data. The diagnostic accuracy of day 2 C-reactive protein thresholds was assessed by receiver operating characteristic (ROC) analysis. A p-value of less than 0.05 was considered significant. Statistical analysis was performed using SPSS version 19 for Windows (SPSS, Chicago, IL).

6.3 Results

Baseline characteristics of the 357 patients who underwent curative surgery for colorectal cancer are shown in Table 6-1. The majority of patients were 65 or older (72%), male (53%), underwent right or left hemicolectomy (63%) and had node negative disease (61%). Most patients had a preoperative CRP less than 10 mg/L (80%).

During follow-up 84 of 357 patients developed postoperative infective complications (pneumonia n=28, septicaemia n=8, urinary tract infection with septicaemia n=7, central line tip infection n=3, peripheral cellulitis n=1, and antibiotic enterocolitis n=1). Twenty two patients developed a superficial wound infection, 6 developed an intra-abdominal abscess or collection and 14 patients developed an anastomotic leak. Of those with an infective complication, 6 patients developed a second infection and 7 developed a non-infective complication. In total, 26 patients developed a non-infective complication (acute coronary syndrome / myocardial infarction n=12, atrial fibrillation n=6, ileus n=3, pulmonary embolism n=2, acute urinary retention n=1, pulmonary oedema n=1, and renal failure n=1).

The relationship between postoperative days 1 and 2 C-reactive protein and C-reactive protein concentrations on postoperative days 3 and 4 was examined. Day 1 C-reactive protein was associated with day 3 (r^2=0.153, p<0.001) and day 4 (r^2=0.081, p<0.001) concentrations. Day 2 C-reactive protein was not associated with age (p=0.204), sex (p=0.621), operation type (p=0.913), TNM stage (p=0.840), open surgery (p=0.692) or enhanced recovery (p=0.714). Using scatter plots with a line of best fit drawn (Figures 6-1 and 6-2) day 2 C-reactive protein was directly associated with day 3 (r^2=0.601, p<0.001) and day 4 (r^2=0.270, p<0.001) concentrations.

The relationship between day 2 C-reactive protein concentrations in the present study and previously published postoperative day 3 and 4 thresholds for the prediction of infective complications and anastomotic leak following colorectal surgery is shown in Table 6-2 (Welsch et al., 2007, Korner et al., 2009, Ortega-Deballon et al., 2010, MacKay et al., 2011, Warschkow et al., 2012b, Almeida et al., 2012). For infective complications, the median day 2 C-reactive protein concentrations associated with the previously published optimum day 3 and 4 thresholds was approximately 190mg/L. For the development of an anastomotic leak, the median day 2 C-reactive protein concentrations associated with the previously published optimum day 3 and 4 thresholds was approximately 200mg/L.

Regarding patients who developed an anastomotic leak, 8 out of 14 had a postoperative day 2 C-reactive protein concentration greater than 200 mg/L, 10 out of 14 had a concentration greater than 190 mg/L. Using the C-reactive protein threshold of >200 mg/L the area under the curve (AUC) for the prediction of infective complications was 0.63 ($p<0.001$) and 0.52 ($p=0.072$) for the prediction of anastomotic leaks. Similarly, using the threshold of >190 mg/L the area under the curve (AUC) for the prediction of infective complications was 0.62 ($p<0.001$) and 0.52 ($p=0.020$) for the prediction of anastomotic leaks.

In terms of length of hospital stay, four patients were discharged on postoperative day 3, none of whom developed infective complications at 30 days. Overall, the median length of hospital stay was 10 days (range 3 – 187 days).

The relationship between clinicopathological characteristics and the day 2 C-reactive protein concentration threshold of >190 mg/L is shown in Table 6-3. This day 2 threshold

was not significantly associated with sex, co-morbidity, operation type, TNM stage, open surgery, enhanced recovery or length of hospital stay. However, it was associated with infective complications ($p<0.001$).

6.4 Discussion

The results of the present study show that the magnitude of the peak systemic inflammatory response, as evidenced by C-reactive protein on postoperative day 2, has a significant impact on subsequent C-reactive protein concentrations on day 3 (approximately 60% of the variation) and day 4 (approximately 30% of the variation) in patients following potentially elective curative resection of colorectal cancer. The implications of this association are that a C-reactive protein concentration of more than 190 mg/L on day 2 contributes substantially to a concentration above established thresholds on day 3. Therefore, the peak systemic inflammatory response, as evidenced by day 2 C-reactive protein >190mg, contributes to increasing concentrations above the thresholds on day 3 and day 4 associated with the development of infective complications (Chapters 4 and 5). Previous chapters (Chapters 4 and 5) have also demonstrated that day 3 C-reactive protein thresholds of 170-180 mg/l are the most sensitive and specific in predicting infective complications. For the first time, we introduce the notion that postoperative infective complications may result from an increased inflammatory insult and suggest that day 2 C-reactive protein might be a useful measure of this “inflammatory hit” and could be used to assess interventions to reduce this.

It is also of interest that a number of perioperative interventions have been shown to be associated with a reduced day 2 C-reactive protein concentration and the reduced development of infective complications. Indeed, enhanced recovery programmes and laparoscopic surgery have been shown to attenuate the patient’s systemic inflammatory response to surgery (Wang et al., 2012, Kehlet, 2011, Lane et al., 2013). However, objective data on this association remains scarce. Clearly, the use of a well defined

objective measure of the systemic inflammatory response such as C-reactive protein has the potential to identify therapeutic interventions likely to improve patient outcomes.

As the use of enhanced recovery after surgery programmes increases, particularly in colorectal surgery, patients are being discharged from hospital earlier than previously. An early warning, prior to discharge, of the likelihood that complications might develop, perhaps leading to readmission, would prove useful. In a recent study of 533 patients undergoing colorectal surgery within an enhanced recovery programme, Lane and co-workers demonstrated that the measurement of C-reactive protein after elective surgery can identify those at risk of adverse events and prolonged hospital stay. Furthermore, they highlight that patients within an enhanced recovery programme are often suitable for discharge on postoperative days 3 or 4, and that assessing C-reactive protein beyond these time points is becoming potentially redundant. Earlier prediction would therefore be beneficial. Equally, a low or falling C-reactive protein may be reassuring in an era of early discharge. They note a C-reactive protein concentration of >150 mg/L on postoperative day 2 as well as a rising C-reactive protein on day 3 were independent predictors of adverse events, and should alert the surgeon at an early phase to an increased likelihood of such events (Lane et al., 2013). When the threshold of 150 mg/L was applied to the present study the AUC for the prediction of infective complications was 0.57 ($p=0.002$) and 0.52 ($p=0.044$) for the prediction of anastomotic leaks, compared to the C-reactive protein threshold of >190 mg/L with an AUC of 0.62 ($p<0.001$) for the prediction of infective complications and 0.52 ($p=0.020$) for the prediction of anastomotic leaks. For both thresholds of 150 and 190 mg/L, the negative predictive value was 87%. A day 2 C-reactive protein threshold of 100 mg/L determined a negative predictive value of 90%.

The present study has a number of limitations. It was conducted over a seven year period and included patients from two teaching hospitals hence some variation in care, anaesthesia and surgeon may be a confounding factor. However, in the present cohort, day 3 and 4 C-reactive protein thresholds for predicting the development of infective complications were similar to previous studies. Furthermore, trends in C-reactive protein, as demonstrated in Table 6-1, show a peak at postoperative day 2, in keeping with previous literature.

The role of C-reactive protein as a reliable early predictor of postoperative infective complications (and anastomotic leak), prior to the traditional rise in white cell count and temperature and subsequent development of symptoms, is now increasingly recognised (Dutta et al., 2011, Korner et al., 2009, Ortega-Deballon et al., 2010, MacKay et al., 2011, Lane et al., 2013). Furthermore, the development of an anastomotic leak post-operatively is now recognised to not only compromise short term but also longer term outcomes in patients undergoing surgery for colorectal cancer (Mirnezami et al., 2011). However, the question of whether a high peak systemic inflammatory response following surgery is associated with an increased risk of developing infective complications has not been previously addressed directly. Whether complications result from the peak systemic inflammatory response or whether the peak systemic inflammatory response is already raised due to the presence of an underlying infective complication remains uncertain. If the former is the case then it might be expected that intervention to reduce this peak systemic inflammatory response might well improve outcomes, both short and long term. Conversely, if the later proves true, perhaps pre-emptive treatment such as antibiotic therapy would be beneficial. In any case, measurement of the peak systemic inflammatory response, using day 2 C-reactive protein concentrations, and the establishment of clinically important thresholds is a step forward in such investigations.

Table 6-1 The clinicopathological characteristics of patients undergoing elective resection for colorectal cancer (n=357)

Characteristic	Number of Patients (%)
Age (<65 / 65-74 / ≥75)	99 / 113 / 145 (28 / 32 / 40)
Sex (male / female)	188 / 169 (53 / 47)
Operation (RH or LH / AR or APR)	224 / 133 (63 / 37)
TNM Stage (I / II / III)	75 / 142 / 132 (21 / 40 / 37)
Laparoscopic Surgery (no / yes)	330 / 27 (92 / 8)
Enhanced Recovery (no / yes)	265 / 92 (74 / 26)
Infective Complication (no / yes)	274 / 83 (77 / 23)
Anastomotic Leak (no / yes)	343 / 14 (96 / 4)
Preoperative CRP (median, range)	8 (1-209)
Day 1 CRP (median, range)	112 (5-245)
Day 2 CRP (median, range)	172 (20-377)
Day 3 CRP (median, range)	148 (6-443)
Day 4 CRP (median, range)	99 (6-452)
Length of hospital stay (median, range) days	10 (3-187)

RH right hemicolectomy, *LH* left hemicolectomy

AR anterior resection, *APR* abdominoperineal resection

TNM tumour, node, metastasis staging system, 5th edition

CRP C-reactive protein, mg/L

Table 6-2 Corresponding day 2 C-reactive protein concentration in present cohort with previously reported threshold values of C-reactive protein in predicting infective complications

Study	Number of Patients	Post- operative day	CRP (mg/L)	Complication	Corresponding day 2 CRP in present cohort (mg/L)
Welsch et al[(Welsch et al., 2007)]	48	3	140	All infections	170
MacKay et al[(MacKay et al., 2011)]	160	4	145	All infections	197
Warschkow et al[(Warschkow et al., 2012b)]	1187	4	123	All infections	186
Chapter 4	454	3	170	All infections	192
					Median 189
Kørner et al[(Korner et al., 2009)]	231	3	190	Anastomotic Leak	207
Ortega-Deballon et al[(Ortega-Deballon et al., 2010)]	133	4	125	Anastomotic Leak	187
Almeida et al[(Almeida et al., 2012)]	173	3	140	Anastomotic Leak	170
Chapter 4	454	3	190	Anastomotic Leak	207
					Median 202

Table 6-3 The clinicopathological characteristics of patients with a day 2 C-reactive protein concentration (CRP) ≤190 / >190 mg/l (n=357)

Characteristic	Number of Patients		p value
	Day 2 CRP ≤190 mg/L	Day 2 CRP >190 mg/L	
Age (<65 / 65-74 / ≥75)	54 / 57 / 98	45 / 56 / 47	0.014
Sex (male / female)	117 / 92	71 / 77	0.135
Operation (RH or LH / AR or APR)	128 / 81	96 / 52	0.486
TNM stage (I / II / III)	44 / 82 / 79	31 / 61 / 56	0.974
Laparoscopic Surgery (no / yes)	191 / 18	139 / 9	0.373
Enhanced Recovery (no / yes)	155 / 54	110 / 38	0.973
Infective Complication (no / yes)	181 / 28	93 / 55	<0.001
Anastomotic Leak (no / yes)	205 / 4	138 / 10	0.020
Length of hospital stay (median, range)	10 (3-108)	10 (3-187)	0.489

RH right hemicolectomy, *LH* left hemicolectomy

AR anterior resection, *APR* abdominoperineal resection

TNM tumour, node, metastasis staging system

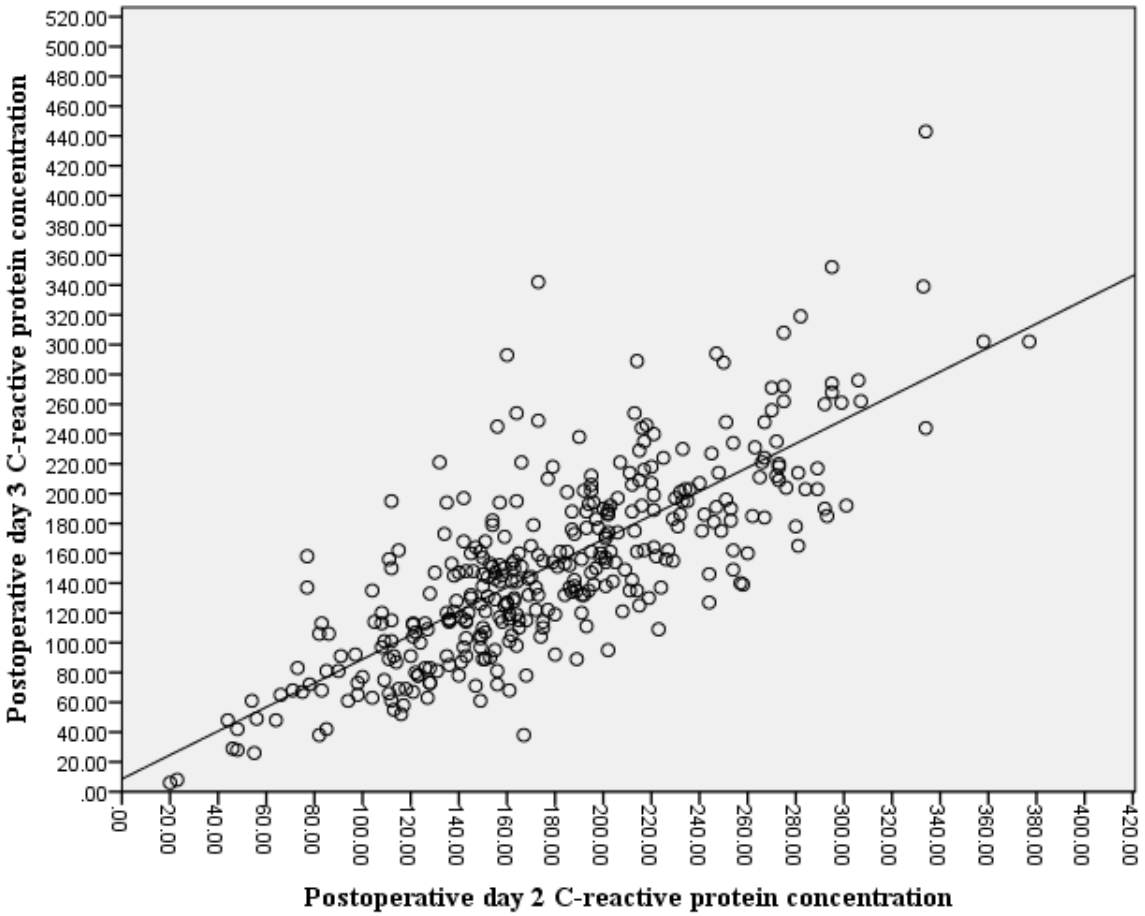


Figure 6-1 The relationship between postoperative day 2 C-reactive protein and postoperative day 3 C-reactive protein concentrations (mg/l)

r^2=0.601 ($p<0.001$)

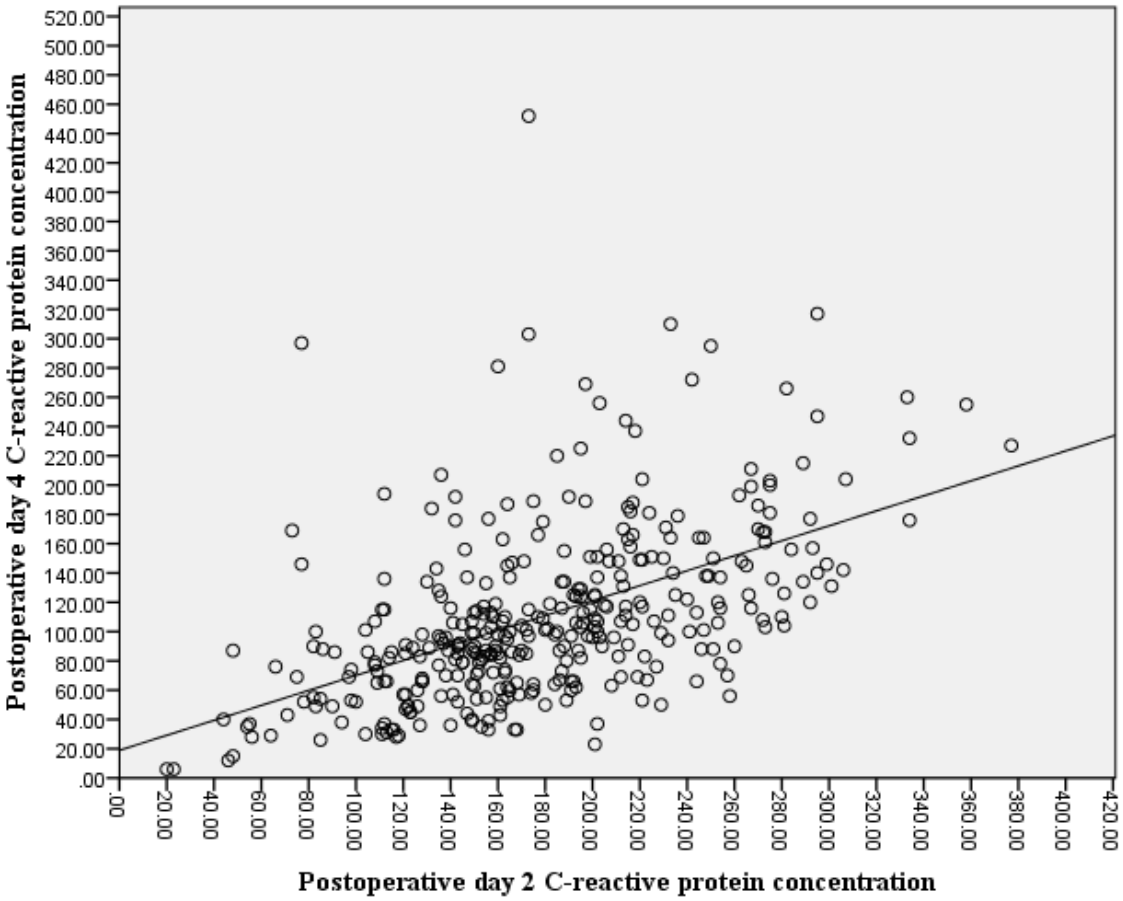


Figure 6-2 The relationship between postoperative day 2 C-reactive protein and postoperative day 4 C-reactive protein concentrations (mg/l)

r^2=0.270 ($p<0.001$)

7 Clinicopathological determinants of the magnitude of the systemic inflammatory response following colorectal cancer resection

7.1 Introduction

Colorectal cancer is the second most common cause of cancer death in the United Kingdom, accounting for 16,000 deaths annually (CRUK, 2014). It is now recognised that the preoperative systemic inflammatory response is related to outcome, both short and long term, in patients following potentially curative surgery for colorectal cancer (Mohri et al., 2014, Roxburgh and McMillan, 2010, Moyes et al., 2009). It has also been shown that postoperative C-reactive protein concentrations above thresholds of approximately 180mg/l and 140mg/l on days 3 and 4 respectively can be a useful early predictor of the development of postoperative infective complications and anastomotic leak following colorectal cancer resection , independent of surgical approach (i.e. open or laparoscopic) (Chapter 5). Furthermore, such complications, particularly anastomotic leak, are in turn associated with poorer long term survival (Trencheva et al., 2013). Moreover, the systemic inflammatory response, as demonstrated by day 2 postoperative C-reactive protein concentration (Crozier et al., 2007) and threshold above 190 mg/l, has a significant influence on the likelihood of having a C-reactive protein above these previously noted predictive thresholds on days 3 and 4 (Chapter 6). Clinicopathological factors associated with the day 2, day 3 and day 4 C-reactive protein concentrations, and thresholds predictive of infective complications, are of considerable interest since they may be modifiable and could therefore potentially be considered as objective future therapeutic targets.

The aim of the present study was to examine the clinicopathological determinants of the systemic inflammatory response, as evidenced by C-reactive protein thresholds on postoperative days 2, 3 and 4, in patients following resection of colorectal cancer.

7.2 Patients and Methods

Patients with a histologically proven diagnosis of colorectal cancer who, based on preoperative investigations and operative findings, were thought to have undergone potentially curative resection during a period from 1999 to 2013, and in whom C-reactive protein concentrations were measured on postoperative days 2, 3 and 4 were included in the study (n=536). Patient characteristics, including perioperative C-reactive protein concentrations, were recorded routinely in a prospective departmental audit database. All patient data were anonymised.

All tumours were staged according to the conventional tumour, node, metastasis (TNM, 5th edition) classification. Daily blood samples were obtained, as per hospital routine, for analysis of C-reactive protein during the perioperative period. Prior to surgery, all patients received thromboprophylaxis and antibiotic prophylaxis as per local protocol. Lesions from the caecum to the sigmoid colon were classified as colon cancers, lesions of the rectosigmoid junction and rectum were classified as rectal cancers. An enhanced recovery programme was introduced during the data collection (period 2011 to 2013), the features of which are shown in Table 7-1.

Emergency presentation was determined if the patient presented via an unplanned hospital admission and underwent surgery during the same admission. Surgeons were identified as a specialist if they had a major commitment to colorectal cancer surgery within the NHS, were regarded as a colorectal surgeon by their peers and colleagues, had access to a dedicated colonoscopy session, and were part of a colorectal cancer multi-disciplinary team (Oliphant et al., 2013a). All other surgeons were classified as non-specialists in

colorectal cancer. Surgeon volume was determined by the total number of cases per surgeon recorded in the database, divided by number of years they were included.

Patient co-morbidity was classified using the American Society of Anaesthesiologists (ASA) grading system, where '1' represents a normal healthy patient, '2' a patient with mild systemic disease, '3' a patient with severe systemic disease and '4' a patient with severe systemic disease that is a constant threat to life. This assessment was carried out by an anaesthetist preoperatively. Body Mass Index (BMI) was categorised as underweight (<20), normal weight (20-25), overweight (>25-30), and obese (>30).

The extent of deprivation was defined by the Carstairs deprivation index (Carstairs and Morris, 1990), an area based measure derived from the 1991 census, using the postal code of residence at diagnosis, which divides the score into a seven-point index. For illustrative purposes, the results are presented by amalgamating the seven categories into three groups: affluent (categories 1 and 2), intermediate (categories 3-5) and deprived (categories 6 and 7). The Carstairs deprivation index has been extensively utilized in cancer patients and is particularly appropriate for use in the central belt of Scotland (Carstairs and Morris, 1990).

Data were compared using the χ^2 and Mann-Whitney U tests. Variables statistically significant on univariate analysis were subsequently entered into a multivariate model using a backwards conditional method. A p-value of <0.05 was considered significant. Statistical analysis was performed using SPSS version 21.0 for Windows (IBM Corporation, Armonk, NY, USA).

7.3 Results

Baseline characteristics of the 536 patients who underwent surgery for colorectal cancer are shown in Table 7-2. The majority of patients were 65 or older (68%), male (57%), were overweight or obese (59%), were from a deprived area (55%), had colonic tumours (67%), were not inflamed (60%) and had node negative disease (58%). Most patients underwent elective resection (83%) and had an open resection (89%). The peak systemic inflammatory response, as evidenced by C-reactive protein concentrations, was on postoperative day 2 (median 175mg/l, range 17-454mg/l).

The relationships between clinicopathological characteristics and peak postoperative C-reactive protein concentrations are shown in Table 7-3. Postoperative day 2 C-reactive protein thresholds of ≥190 mg/l were significantly associated with deprivation ($p=0.006$), emergency presentation ($p<0.001$), preoperative systemic inflammation ($p<0.001$), and open surgery ($p=0.001$). Postoperative day 3 C-reactive protein thresholds of ≥180 mg/l were significantly associated with BMI ($p=0.008$), deprivation ($p=0.024$), emergency presentation ($p=0.002$), and an enhanced recovery programme ($p<0.001$). Postoperative day 4 C-reactive protein thresholds of ≥140 mg/l were significantly associated with BMI ($p=0.004$), emergency presentation ($p=0.004$), preoperative systemic inflammation ($p=0.020$), neoadjuvant treatment ($p=0.004$), tumour site ($p=0.010$), and an enhanced recovery programme ($p<0.001$).

The relationships between clinicopathological characteristics and peak postoperative C-reactive protein concentrations in elective cases only are shown in Table 7-4. In elective cases, postoperative day 2 C-reactive protein thresholds of ≥190 mg/l were significantly associated with deprivation ($p=0.026$), preoperative systemic inflammation ($p=0.006$), and

open surgery (p=0.002). On multivariate analysis of significant variables, only deprivation (OR 1.43, 95% CI 0.99-2.08, p=0.059) and preoperative systemic inflammation (OR 1.37, 95% CI 1.01-1.85, p=0.043) were independently associated with a postoperative day 2 C-reactive protein concentration above the threshold.

In elective cases, postoperative day 3 C-reactive protein thresholds of ≥180 mg/l were significantly associated with BMI (p=0.018) and an enhanced recovery programme (p<0.001). On multivariate analysis of significant variables only BMI (OR 1.46, 95% CI 1.07-1.99, p=0.018) was independently associated with a postoperative day 3 C-reactive protein concentration above the predictive threshold.

In elective cases, postoperative day 4 C-reactive protein thresholds of ≥140 mg/l were significantly associated with BMI (p=0.007), neoadjuvant treatment (p=0.005), tumour site (p=0.024), and an enhanced recovery programme (p<0.001). On multivariate analysis of significant variables, BMI (OR 1.42, 95% CI 1.02-1.98, p=0.039), enhanced recovery (OR 2.86, 95% CI 1.40-5.84, p=0.004), and neoadjuvant treatment (OR 0.19, 95% CI 0.06-0.59, p=0.004) were all independently associated with a postoperative day 4 C-reactive protein above the predictive threshold.

7.4 Discussion

The results of the present study show that several clinical factors were independently associated with the postoperative systemic inflammatory response, as evidenced by day 2, day 3 and day 4 C-reactive protein concentrations, following resection of colorectal cancer. In particular, emergency presentation, preoperative systemic inflammation, socioeconomic deprivation, BMI and an enhanced recovery programme were independently associated with a C-reactive protein concentration above predictive thresholds for the development of postoperative infective complications. In contrast, laparoscopic surgery and neoadjuvant treatment were associated with C-reactive protein concentrations below these thresholds.

Emergency presentation has been repeatedly reported to be associated with high post-operative complication and mortality rates (McArdle and Hole, 2004, Anderson et al., 1992, Crozier et al., 2009). Moreover, emergency presentation predicts poorer cancer specific survival independent of other clinicopathological factors, including tumour stage (McArdle et al., 2006). Indeed, in the present study, it was of interest that patients undergoing emergency surgery had a higher preoperative C-reactive protein concentration and were twice as likely to breach the day 2 C-reactive protein threshold of 190 mg/l than those presenting electively. Patients presenting as emergencies are also more likely to undergo surgery by a non-specialist surgeon, although only a small number of resections were performed by non-specialists in the present study. Therefore, it may be that the impact of emergency presentation on both short and long term outcomes is, in part, determined by the magnitude of the systemic inflammatory response following surgery for colorectal cancer. Tumour stenting may be advocated as a bridge to surgery in the emergency setting.

It has long been recognised that socioeconomic deprivation is independently associated with increased postoperative morbidity and mortality following colorectal cancer surgery, as well as with a raised systemic inflammatory response (Oliphant et al., 2013b, McMillan et al., 2003a). It has been proposed that deprivation is associated with an aggregation of features that result in a low grade background systemic inflammation not fully explained by smoking or increased weight (O'Reilly et al., 2006). Indeed, the presence of a raised systemic inflammatory response prior to surgery, as evidenced by an elevated C-reactive protein concentration, has been shown to predict overall and cancer specific survival, independent of tumour stage, in patients undergoing colorectal cancer resection (McMillan et al., 2003b, Oliphant et al., 2014, Crozier et al., 2009). In the present study, in patients who presented electively, it was of interest that those patients who were deprived were twice as likely to breach the day 2 C-reactive protein threshold of 190 mg/l than those who were affluent. Also, those patients who were deprived, presenting electively and who were not systemically inflamed (mGPS 0) were twice as likely to breach the day 2 C-reactive protein threshold of 190 mg/l than those who were affluent. Therefore, the present results are consistent with the concept that deprivation impacts on poor outcomes through the magnitude of the post-operative systemic inflammatory response.

In the present study, BMI was only available in approximately 50% of patients. Therefore conclusions regarding the influence of BMI on the peak systemic inflammatory response should be limited. However, increased BMI is an established risk factor for the development of colorectal cancer, and has been shown to influence C-reactive protein concentrations (O'Reilly et al., 2006). Patients who are overweight or obese are more likely to have other pre-existing co-morbidities and perhaps longer operating times. Indeed, it is of interest that BMI and deprivation were directly associated and both have been associated with an increased inflammatory response (O'Reilly et al., 2006). The exact

mechanism relating increased BMI and the postoperative systemic inflammatory response has yet to be explained. However, it may be that surgery involving trauma to an increased amount of subcutaneous fat leads to a more profounding systemic inflammatory response.

Surgery under the care of a specialist surgeon has been repeatedly reported to be independently associated with lower postoperative and long term mortality rates (Oliphant et al., 2013a). A Cochrane review in 2012 reported that hospital volume, surgeon volume, and treatment by specialist surgeons to be important in determining 5 year survival (Archampong et al., 2012), whereas findings of a recent study of 6432 patients reported that hospital volume and specialist surgeons, but not surgeon volume, impacted on survival rates (Etzioni et al., 2014). In the present study there was no significant association between surgical specialisation or volume and the magnitude of the post-operative systemic inflammatory response. However, the number of non-specialists was small and the median peak C-reactive protein concentration was only slightly higher (188 mg/l vs 173 mg/l) and therefore further work is required to examine whether association of specialisation and improved short term and long term outcomes is mediated in part by the postoperative systemic inflammatory response.

Consistent with the results of the present study laparoscopic surgery has been repeatedly shown to attenuate the systemic inflammatory response to surgery (Lane et al., 2013, Srinivasa et al., 2011, Wang et al., 2012). However, patients who undergo a laparoscopic resection for colon cancer are more likely to be fit enough to tolerate a pneumoperitoneum and longer anaesthetic times. They therefore may be younger, and have fewer co-morbidities. Furthermore, it is less likely that those patients with emergency presentation will undergo laparoscopic resection. Nevertheless, the results of the present study provide

objective evidence to support the laparoscopic approach, where possible, in patients undergoing surgery for colorectal cancer.

It is accepted that neoadjuvant therapy may lead to immunocompromise in some patients. Therefore, it may be that the systemic inflammatory response is reduced as a result of this effect. Further investigation into the pathophysiological relationship between neoadjuvant treatment and the systemic inflammatory response in larger cohorts would be of interest.

A number of advances in clinical care have taken place in the last decade or so that may also have had an impact on the magnitude of the postoperative systemic inflammatory response, such as enhanced recovery programmes. It is of particular interest that in this study enhanced recovery appears to be associated with an increased likelihood of developing a C-reactive protein concentration above predictive thresholds, and retains significance on multivariate analysis. This contradicts expectations and therefore merits further investigation. The multifactorial nature of an enhanced recovery programme leads to the effect of individual elements being notoriously difficult to study.

A particular strength of this study is in establishing clinicopathological determinants of the magnitude of the postoperative systemic inflammatory response, and highlights C-reactive protein concentration as a potential tool to enable an objective assessment of such factors. Furthermore, C-reactive protein thresholds may prove a useful benchmark of the impact of future innovations of clinical care, which may influence the postoperative systemic inflammatory response.

Table 7-1 Features of the enhanced recovery protocol used in the present study

Preoperative preparation	Written preoperative information. Free fluids and high calorie drinks up to 4 hours before surgery. No bowel preparation, except for those having left-sided surgery, who received a phosphate enema the night before and on the morning of surgery.
Anaesthesia	A standard protocol was used. Normothermia was maintained throughout. No nasogastric tubes or intra-abdominal drains were used.
Analgesia	PCA morphine for 48 hours. Regular paracetamol with tramadol for breakthrough pain. Use of non-steroidal anti-inflammatory drugs was with-held until the morphine PCA had been discontinued.
Diet and Fluids	Oral fluids and protein drinks encouraged immediately after surgery. Normal food introduced on postoperative day 1.
Mobilisation	All patients received chest physiotherapy and commenced active mobilisation with a physiotherapist from postoperative day 1.

Table 7-2 Clinicopathological characteristics of patients undergoing colorectal cancer resection (n=536)

Characteristic	Number of Patients (%)
Age (<65/ 65-74/ ≥75 years)	174 / 176 / 186 (33 / 33 / 35)
Sex (male/ female)	307 / 229 (57 / 43)
ASA (1 / 2 / 3 / 4)	37 / 158 / 173 / 25 (9 / 40 / 44 / 6)
BMI (underweight/ normal/ overweight/ obese)	18 / 82 / 88 / 56 (7 / 34 / 36 / 23)
Deprivation (affluent / intermediate / deprived)	21 / 164 / 230 (5 / 40 / 55)
Emergency (no / yes)	461 / 75 (83 / 17)
Pre-op CRP >10 mg/l (no / yes)	311 / 207 (60 / 40)
Neoadjuvant treatment (no / yes)	447 / 89 (83 / 17)
Tumour site (colon / rectum)	359 / 177 (67 / 33)
T stage (T0 / T1 / T2 / T3 / T4)	6 / 34 / 72 / 266 / 151 (1 / 6 / 14 / 50 / 29)
N stage (N0 / N1 / N2)	308 / 152 / 69 (58 / 29 / 13)
Surgery (open / laparoscopic)	454 / 55 (89 / 11)
Enhanced recovery (no / yes)	342 / 194 (64 / 36)
Colorectal specialist (no / yes)	8 / 477 (2 / 98)
Surgeon volume (<10 / 10-20 / >20)	23 / 310 / 152 (5 / 64 / 31)
Day 2 CRP ≥190 mg/l (no / yes)	309 / 227 (58 / 42)
Day 3 CRP≥180 mg/l (no / yes)	328 / 208 (61 / 39)
Day 4 CRP≥140 mg/l (no / yes)	350 / 186 (65 / 35)

Results are given as the number and percentage or as the median and range.
ASA n=393, *BMI* n=244, *Deprivation Category* n=415
TNM tumour, node, metastasis staging system, 5th edition
CRP C-reactive protein concentration

Table 7-3 The relationship between clinicopathological characteristics and postoperative C-reactive protein concentration thresholds in patients undergoing surgery for colorectal cancer (n=536)

Characteristic		Day 2 CRP (<190 / ≥190 mg/l)	p value	Day 3 CRP (<180 / ≥180 mg/l)	p value	Day 4 CRP (<140 / ≥140 mg/l)	p value
Age (years)	**<65**	99 / 75		105 / 69		112 / 62	
	65-74	91 / 85		103 / 73		109 / 67	
	≥75	119 / 67	0.163	120 / 66	0.408	129 / 57	0.311
Sex	**Male**	178 / 129		181 / 126		190 / 117	
	Female	131 / 98	0.857	147 / 82	0.219	160 / 69	0.055
ASA	**1**	24 / 13		25 / 12		25 / 12	
	2	99 / 59		107 / 51		116 / 42	
	3	105 / 68		110 / 63		118 / 55	
	4	12 / 13	0.238	16 / 9	0.471	17 / 8	0.595
BMI	**Underweight**	11 / 7		14 / 4		14 / 4	
	Normal	53 / 29		55 / 27		58 / 24	
	Overweight	57 / 31		59 / 29		62 / 26	
	Obese	28 / 28	0.180	26 / 30	0.008	26 / 30	0.004
Deprivation	**Affluent**	16 / 5		15 / 6		14 / 7	
	Intermediate	107 / 57		118 / 46		123 / 41	
	Deprived	124 / 106	0.006	139 / 91	0.024	153 / 77	0.204
Emergency	**No**	280 / 181		294 / 167		312 / 149	
	Yes	29 / 46	<0.001	34 / 41	0.002	38 / 37	0.004
Pre-op CRP	**≤10mg/l**	198 / 113		201 / 110		217 / 94	
	>10mg/l	98 / 109	<0.001	117 / 90	0.064	124 / 83	0.020

Neoadjuvant treatment	**No**	258 / 189		266 / 181		280 / 167	
	Yes	51 / 38	0.942	62 / 27	0.073	70 / 19	0.004
Tumour site	**Colon**	200 / 159		210 / 149		221 / 138	
	Rectum	109 / 68	0.196	118 / 59	0.068	129 / 48	0.010
T stage	**T0**	4 / 2		4 / 2		6 / 0	
	T1	19 / 15		19 / 15		20 / 14	
	T2	48 / 24		54 / 18		53 / 19	
	T3	151 / 115		155 / 111		165 / 101	
	T4	84 / 67	0.322	92 / 59	0.436	102 / 49	0.648
N stage	**N0**	182 / 126		194 / 114		205 / 103	
	N1	93 / 59		97 / 55		105 / 47	
	N2	31 / 38	0.116	33 / 36	0.068	36 / 33	0.104
Surgery	**Open**	254 / 200		280 / 174		304 / 150	
	Laparoscopic	44 / 11	0.001	39 / 16	0.181	36 / 19	0.823
Enhanced recovery	**No**	193 / 149		230 / 112		252 / 90	
	Yes	116 / 78	0.449	98 / 96	<0.001	98 / 96	<0.001
Colorectal specialist	**No**	4 / 4		4 / 4		4 / 4	
	Yes	276 / 201	0.655	293 / 184	0.511	307 / 170	0.401
Surgeon volume	**<10**	14 / 9		14 / 9		13 / 10	
	10-20	173 / 137		190 / 120		199 / 111	
	>20	93 / 59	0.440	93 / 59	0.999	99 / 53	0.565

Table 7-4 The relationship between clinicopathological characteristics and postoperative C-reactive protein concentration thresholds in patients undergoing elective surgery for colorectal cancer (n=461)

Characteristic		Day 2 CRP (<190 / ≥190 mg/l)	p value	Day 3 CRP (<180 / ≥180 mg/l)	p value	Day 4 CRP (<140 / ≥140 mg/l)	p value
Age (years)	**<65**	91 / 63		96 / 58		103 / 51	
	65-74	81 / 68		90 / 59		94 / 55	
	≥75	108 / 50	0.092	108 / 50	0.266	115 / 43	0.261
Sex	**Male**	159 / 102		162 / 99		169 / 92	
	Female	121 / 79	0.927	132 / 68	0.384	143 / 57	0.125
ASA	**1**	24 / 12		25 / 11		25 / 11	
	2	94 / 51		100 / 45		107 / 38	
	3	96 / 61		99 / 58		105 / 52	
	4	9 / 9	0.218	12 / 6	0.359	12 / 6	0.366
BMI	**Underweight**	10 / 6		13 / 3		12 / 4	
	Normal	51 / 27		52 / 26		56 / 22	
	Overweight	55 / 28		57 / 26		60 / 23	
	Obese	28 / 25	0.262	26 / 27	0.018	25 / 28	0.007
Deprivation	**Affluent**	15 / 5		14 / 6		13 / 7	
	Intermediate	100 / 50		108 / 42		113 / 37	
	Deprived	111 / 84	0.026	125 / 70	0.166	134 / 61	0.481

Pre-op CRP	**≤10mg/l**	191 / 104		194 / 101		207 / 88	
	>10mg/l	77 / 73	0.006	90 / 60	0.232	96 / 54	0.187
Neoadjuvant treatment	**No**	231 / 147		235 / 143		245 / 133	
	Yes	49 / 34	0.726	59 / 24	0.126	67 / 16	0.005
Tumour site	**Colon**	173 / 118		179 / 112		186 / 105	
	Rectum	107 / 63	0.459	115 / 55	0.186	124 / 44	0.024
T stage	**T0**	4 / 2		4 / 2		6 / 0	
	T1	19 / 14		18 / 15		19 / 14	
	T2	47 / 24		53 / 18		52 / 19	
	T3	142 / 95		145 / 92		153 / 84	
	T4	65 / 43	0.715	71 / 37	0.995	79 / 29	0.724
N stage	**N0**	167 / 107		177 / 97		187 / 87	
	N1	85 / 42		85 / 42		91 / 36	
	N2	25 / 29	0.267	29 / 25	0.308	31 / 23	0.343
Surgery	**Open**	227 / 160		247 / 140		267 / 120	
	Laparoscopic	44 / 11	0.002	39 / 16	0.304	36 / 19	0.597
Enhanced recovery	**No**	173 / 119		205 / 87		222 / 70	
	Yes	107 / 62	0.389	89 / 80	<0.001	90 / 79	<0.001
Colorectal specialist	**No**	2 / 4		3 / 3		3 / 3	
	Yes	254 / 167	0.180	267 / 154	0.498	281 / 140	0.388
Surgeon volume	**<10**	12 / 9		13 / 8		12 / 9	
	10-20	159 / 108		170 / 97		179 / 88	
	>20	85 / 54	0.684	87 / 52	0.910	93 / 46	0.637

8 The impact of enhanced recovery on the systemic inflammatory response and the infective complication rate following elective surgery for colorectal cancer

8.1 Introduction

In the 1990s, Kehlet introduced the idea of a multimodal approach to modifying the surgical stress response and subsequent increased demands on organ function. While no single technique or drug regimen has been shown to eliminate postoperative morbidity and mortality, multimodal interventions led to a reduction in the undesirable sequelae of surgical injury with improved recovery and reduction in postoperative morbidity and overall costs (Kehlet, 1997). Over the past decade, there has been a revolution in the nature of perioperative care with the introduction of enhanced recovery after surgery (ERAS) protocols (Fearon et al., 2013). More recently, this has been proposed for cancer surgery, particularly colorectal cancer resection. Enhanced recovery programmes aim to attenuate the stress response to surgery, accelerate recovery, reduce the length of hospital stay and have been proposed to be associated with reduced hospital morbidity and mortality (Teeuwen et al., 2010). For example, patients undergoing colorectal resection within an enhanced recovery programme have been reported to stay in hospital half as long as those receiving conventional care (King et al., 2006).

Rates of infective complications following colorectal surgery may range from 15-30%. Despite improvements in surgery and care, in particular infection control measures and the use of pre-operative antibiotic prophylaxis, infective complications remain a major cause of morbidity and mortality following colorectal cancer resection (Velasco et al., 1996). Patients are at risk of surgical site infections, such as wound infections and intra-abdominal

abscess, as well as anastomotic leak, and remote site infections, such as urinary tract infections, respiratory tract infections and line sepsis. These complications may require further surgery and can lead to prolonged hospital stay or, in an era of early discharge, readmission. It has more recently been recognised that postoperative infective complications, particularly anastomotic leak, may also compromise long term outcomes (McArdle et al., 2006). Recent studies have shown that the magnitude of the systemic inflammatory response following surgery can predict the development of infective complications (Welsch et al., 2007, MacKay et al., 2011, Welsch et al., 2008, Warschkow et al., 2012c, Dutta et al., 2011). However, data on the effect of enhanced recovery on the postoperative systemic inflammatory response and infective complications remains limited.

The aim of the present study was to examine the impact of enhanced recovery on the length of hospital stay, the systemic inflammatory response and the rate of infective complications following elective surgery for colorectal cancer.

8.2 Patients and Methods

All patients had a histological diagnosis of colorectal cancer and on the basis of pre-operative imaging and operative findings, were thought to have undergone potentially curative resection. All operating surgeons had a specialist interest in colorectal surgery. In total, 310 consecutive patients undergoing elective resection were included in the study. The patients were admitted to one of two university teaching hospitals within the same city, between September 2003 and October 2006. One unit employed ERAS procedures, and admitted 150 patients; the other was using conventional care methods and admitted 164 patients (4 of these patients were excluded from the study due to incomplete data collection). Patients undergoing emergency or palliative surgery were excluded from the data collection, as were patients who received neo-adjuvant chemotherapy.

An enhanced recovery programme is multifactorial (Kehlet, 1997). Features of the enhanced recovery protocols used in this study, as previously described in Chapter 7 (Table 7.1), included pre-operative patient education, standardised anaesthetic technique, maintenance of normothermia intra-operatively, an opioid-sparing analgesic regime and early post-operative mobilisation and nutrition, as well as minimally invasive or laparoscopic surgery carried out by an experienced surgeon, as previously described (MacKay et al., 2007). The decision on suitability for laparoscopic surgery was made on a case by case basis by the operating surgeon. Examples of conservative care include prolonged preoperative fasting, placement of surgical drains, and soft diet until first bowel movement. All patients received pre-operative antibiotics and thomboprophylaxis.

Data was extracted from a prospectively maintained database, including baseline patient characteristics, C-reactive protein concentrations on postoperative days 2, 3 and 4,

postoperative infective and non-infective complications, and length of hospital stay. Postoperative infective complications included surgical site infections (wound infection, anastomotic leak, intra-abdominal collection) as well as remote site infections (e.g. urinary tract infection, respiratory tract infection, line sepsis) as previously described (Chapter 4).

Statistics

Grouping of variables was carried out using standard or previously published thresholds. Associations between categorical and continuous variables were examined using X^2 tests for linear trend and non-parametric tests. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 19.0 (IBM SPSS, Chicago, IL, USA).

8.3 Results

Baseline characteristics of the 310 patients who underwent potentially curative surgery for colorectal cancer are shown in Table 8-1. Age and sex were similar in both the enhanced recovery and conventional care groups. Co-morbidity, as demonstrated by ASA scores, was significantly less in the enhanced recovery group, and there were also fewer rectal cancers as well as earlier tumour stage ($p<0.01$, $p<0.05$, and $p<0.01$ respectively). In the enhanced recovery group length of stay was significantly shorter and laparoscopic surgery was used in approximately 30% of cases (both $p<0.001$, Table 8-1).

During follow-up 75 of 310 patients developed postoperative infective complications (pneumonia n=21, septicaemia n=7, urinary tract infection n=5, central line tip infection n=2, peripheral cellulitis n=2, and antibiotic enterocolitis n=2). Twenty patients developed a wound infection and 16 patients developed an anastomotic leak. Of those with an infective complication, 3 patients developed a second infection. In total, 23 patients developed a non-infective complication (acute coronary syndrome / myocardial infarction n=6, atrial fibrillation n=6, ileus n=5, acute urinary retention n=2, pulmonary oedema n=2, haematoma n=1, and renal failure n=1).

The relationship between the method of perioperative care and the postoperative systemic inflammatory response following elective colorectal cancer resection is shown in Table 8-1. There was no significant association between the method of perioperative care and the magnitude of the systemic inflammatory response on postoperative days 2, 3 or 4, nor the rate of postoperative infective complications following elective resection for colorectal cancer.

Laparoscopic surgery was performed in the enhanced recovery group exclusively and so these cases were excluded from the analysis of enhanced recovery and conventional care groups. Baseline characteristics of the 263 patients who underwent open surgery for colorectal cancer are shown in Table 8-2. Patients in the enhanced recovery group were more likely to be elderly than those in the conventional care group ($p<0.05$). Sex, co-morbidity, tumour site and tumour stage were similar in both groups (Table 8-2).

The relationship between the method of perioperative care and postoperative systemic inflammatory response following open resection for colorectal cancer is shown in Table 8-2. There was no significant association between the method of perioperative care and the magnitude of the systemic inflammatory response on postoperative days 2, 3 and 4. Enhanced recovery was significantly associated with a reduction in the development of pneumonia following elective surgery for colorectal cancer ($p<0.05$) and the length of hospital stay ($p<0.001$, Table 8-2).

In the enhanced recovery group alone, baseline characteristics of the patients who received laparoscopic surgery compared to open surgery are shown in Table 8-3. Age, sex, co-morbidity, tumour site and tumour stage were similar in both groups.

In the enhanced recovery group, the relationship between the method of surgery and post-operative systemic inflammatory response following resection for colorectal cancer is shown in Table 8-3. The method of surgery was not significantly associated with the magnitude of the systemic inflammatory response on postoperative days 2, 3 or 4, nor the rate of postoperative complications following elective surgery for colorectal cancer. The length of hospital stay was also similar in those patients who received laparoscopic surgery compared with open surgery (Table 8-3).

8.4 Discussion

In the present study, enhanced recovery was associated with a significant reduction in the development of pneumonia and the length of hospital stay following surgery for colorectal cancer. These findings are in keeping with previous work demonstrating a reduction in respiratory complications but no difference in other complications (Basse et al., 2004, Teeuwen et al., 2011, Keane et al., 2012). In contrast, the conventional care and enhanced recovery groups were similar in their postoperative systemic inflammatory response and overall complication rates. Therefore, although enhanced recovery is associated with shorter length of hospital stay, it does not appear to be associated with a reduction in the postoperative systemic inflammatory response or a difference in overall infective complications. Results in this study differ from results in Chapter 7, where enhanced recovery was unexpectedly associated with an increased systemic inflammatory response on day 4 postoperatively. It is possible this difference is a result of comparing data from two hospital sites in this study, as opposed to a single site in the previous chapter. Whilst the main guidance in each study with regards to enhanced recovery protocol was the same (Table 7.1), enhanced recovery programmes are multifactorial, with varying compliance, and therefore difficult to study. This limitation could be overcome using a randomised control trial however, as each element of an enhanced recovery programme should be the best clinical practice based on current evidence, excluding elements in a control group may be viewed as unethical. Furthermore, as several elements are involved and there is no clear definition on how many are required (i.e. this varies between units), several large cohorts would be required. Hence, comparison in observational studies, whilst limited, is likely the most appropriate method to examine the differences between conventional care and enhanced recovery.

The implications of the present results are several. Firstly, it would appear that the reduction in length of hospital stay in the enhanced recovery group may merely reflect a culture change in terms of time to hospital discharge rather than an actual reduction in post-operative stress and reduced complications in patients undergoing elective surgery for colorectal cancer. Despite considerable advocacy (King et al., 2006, Basse et al., 2004, Fearon et al., 2013), whether enhanced recovery protocols equate to optimal treatment remains controversial. Indeed, a recent randomized control trial attributed a reduction in the stress response to laparoscopic surgery as opposed to the approach to postoperative care and there were no significant differences in postoperative infective complications (Veenhof et al., 2012, Watt et al., 2015). These results might also be interpreted as somewhat reassuring, in that despite earlier discharge, enhanced recovery was not associated with increased morbidity.

Secondly, if there is indeed no apparent benefit to enhanced recovery over conventional care on the magnitude of the stress response or patient morbidity, then the decision on whether to pursue enhanced recovery protocols may come down to economic evaluation. However, it is not clear whether the costs of implementing an enhanced recovery protocol are outweighed by the difference in length of hospital stay.

Finally, the results of the present study point to an insufficient understanding of the determinants of the postoperative systemic inflammatory response and infective complications following elective surgery for colorectal cancer. Therefore, further work is required to identify the components of perioperative care that most significantly impact on these postoperative outcomes. This will provide a rational basis for the incorporation of treatment modalities into enhanced recovery protocols.

For example, it has been reported that laparoscopic surgery, compared with open surgery, is associated with a reduction in the magnitude of post-operative C-reactive protein concentrations (Chapter 5) (Veenhof et al., 2012). Furthermore, laparoscopic surgery, compared with open surgery, has been associated with less suppression of cell-mediated immune response postoperatively (Whelan et al., 2003). However, with reference to C-reactive protein, this was not the case in the present study and may reflect the relatively small effect size of laparoscopic compared with open surgery in this study.

Current enhanced recovery protocols are multimodal with little consensus on the relative contribution of each component that should constitute an optimal protocol. The evolution of enhanced recovery guidelines should be dynamic, allowing modifications of certain aspects of the protocol as new data on postoperative outcomes becomes available (Lyon et al., 2012). Further studies examining the effect of individual elements of the enhanced recovery protocol will prove challenging but are of the utmost importance in determining effective protocols. The recent and present work would suggest that monitoring the postoperative C-reactive protein concentrations could provide an objective measure of the efficacy of each element in reducing the patient's stress response and the risk of developing infective complications following surgery. For instance, the introduction of preoperative administration of anti-inflammatory medication could be assessed by measuring the magnitude of the postoperative systemic inflammatory response, as demonstrated by C-reactive protein concentrations and thresholds predictive of infective complications. An intervention successfully reducing postoperative inflammation, and the likelihood of developing complications, could improve patient outcomes.

The present observational study has a number of limitations inherent to its design, such as the use of contemporaneous cohorts with potentially confounding factors, such as different

surgeons and anaesthetists, and in addition, that laparoscopic surgery was used in the enhanced recovery cohort exclusively. The extent of deprivation was not assessed. Higher co-morbidity in the conventional care cohort may be due to a more deprived population, which may have an effect on the results (Oliphant et al., 2013b).

In summary, enhanced recovery was associated with a significant reduction in length of hospital stay. In contrast, the postoperative systemic inflammatory response and overall complication rates, both non-infective and infective, were similar to that of conventional care. Therefore, enhanced recovery does not appear to be associated with a reduction in the postoperative systemic inflammatory response or a difference in overall infective complications.

Table 8-1 The relationship between the method of perioperative care, patient characteristics, the systemic inflammatory response and postoperative complications following elective resection for colorectal cancer (n=310)

Characteristics	**Conventional Care Group n=160**	**Enhanced Recovery Group n=150**	**p value**
Age (<65 / 65-74 / ≥75)	49 / 61 / 50	37 / 48 / 65	0.088
Sex (Male / Female)	86 / 74	74 / 76	0.437
ASA score (1 / 2 / 3 / 4)	13 / 57 / 60 / 7[a]	12 / 93 / 42 / 3	0.005
Site (Colon / Rectum)	100 / 60	111 / 39	0.030
TNM Stage (I / II / III)	28 / 66 / 66	38 / 53 / 44[b]	0.008
Operation (Open / Lap)	160 / 0	103 / 47	<0.001
Systemic Inflammatory Response	**Conventional Care Group n=160**	**Enhanced Recovery Group n=150**	**p value**
Day 2 CRP <190 / ≥190 (mg/L)	90 / 65	85 / 48	0.311
Day 3 CRP <180 / ≥180 (mg/L)	105 / 45	87 / 45	0.462
Day 4 CRP <140 / ≥140 (mg/L)	103 / 32	89 / 37	0.300
Complications	**Conventional Care Group n=160**	**Enhanced Recovery Group n=150**	**p value**
All Complications	52 (33%)	46 (31%)	0.729
Non-infective Complications	10 (6%)	13 (9%)	0.417
Infective Complications	42 (26%)	33 (22%)	0.383
Surgical Site Infections	20 (13%)	17 (11%)	0.752
Remote Site Infections	22 (14%)	16 (11%)	0.408
Anastomotic Leak	9 (6%)	7 (5%)	0.703
Wound Infection	11 (7%)	9 (6%)	0.754
Pneumonia	15 (9%)	6 (4%)	0.060
Length of hospital stay	**Conventional Care Group median (range)**	**Enhanced Recovery Group median (range)**	**p value**
Days	11 (3-351)	6 (3-78)	<0.001

aASA not defined for 23 patients
bTNM not defined for 15 patients
CRP C-reactive protein

Table 8-2 The relationship between the method of perioperative care, patient characteristics, the systemic inflammatory response and postoperative complications following open surgery for colorectal cancer (n=263)

Characteristics	**Conventional Care Group n=160**	**Enhanced Recovery Group n=103**	**p value**
Age (<65 / 65-74 / ≥75)	49 / 61 / 50	27 / 28 / 48	0.037
Sex (Male / Female)	86 / 74	54 / 49	0.834
ASA score (1 / 2 / 3 / 4)	13 / 57 / 60 / 7[a]	8 / 60 / 32 / 3	0.082
Site (Colon / Rectum)	100 / 60	74 / 29	0.118
TNM Stage (I / II / III)	28 / 66 / 66	26 / 37 / 28[b]	0.082
Systemic Inflammatory Response	**Conventional Care Group n=160**	**Enhanced Recovery Group n=103**	**p value**
Day 2 CRP <190 / ≥190 (mg/L)	90 / 65	59 / 34	0.403
Day 3 CRP <180 / ≥180 (mg/L)	105 / 45	59 / 33	0.343
Day 4 CRP <140 / ≥140 (mg/L)	103 / 32	62 / 27	0.270
Complications	**Conventional Care Group n=160**	**Enhanced Recovery Group n=103**	**p value**
All Complications	52 (31%)	34 (33%)	0.931
Non-infective Complications	10 (6%)	11 (11%)	0.196
Infective Complications	42 (26%)	23 (22%)	0.472
Surgical Site Infections	20 (13%)	12 (12%)	0.837
Remote Site Infections	22 (14%)	11 (11%)	0.463
Anastomotic Leak	9 (6%)	5 (5%)	0.786
Wound Infection	11 (7%)	6 (6%)	0.735
Pneumonia	15 (9%)	2 (2%)	0.017
Length of hospital stay	**Conventional Care Group median (range)**	**Enhanced Recovery Group median (range)**	**p value**
Days	11 (3-86)	6 (3-78)	<0.001

aASA not defined for 23 patients
bTNM not defined for 12 patients
CRP C-reactive protein

Table 8-3 The relationship between the method of surgery, patient characteristics, the systemic inflammatory response and postoperative complications following elective surgery for colorectal cancer within an enhanced recovery programme (n=150)

Characteristics	**Open Surgery (n=103)**	**Laparoscopic Surgery (n=47)**	**p value**
Age (<65 / 65-74 / ≥75)	27 / 28 / 48	10 / 20 / 17	0.173
Sex (Male / Female)	54 / 49	20 / 27	0.262
ASA score (1 / 2 / 3 / 4)	8 / 60 / 32 / 3	4 / 33 / 10 / 0	0.356
Site (Colon / Rectum)	74 / 29	37 / 10	0.373
TNM Stage (I / II / III)	7 / 19 / 37 / 28[a]	1 / 11 / 16 / 16[b]	0.548
Systemic Inflammatory Response	**Open Surgery (n=103)**	**Laparoscopic Surgery (n=47)**	**p value**
Day 2 CRP <190 / ≥190 (mg/L)	59 / 34	26 / 14	0.864
Day 3 CRP <180 / ≥180 (mg/L)	59 / 33	28 / 12	0.513
Day 4 CRP <140 / ≥140 (mg/L)	62 / 27	27 / 10	0.710
Complications	**Open Surgery (n=103)**	**Laparoscopic Surgery (n=47)**	**p value**
All complications	34 (33%)	12 (26%)	0.357
Non-infective Complications	11 (11%)	2 (4%)	0.195
Infective Complications	23 (22%)	10 (21%)	0.885
Surgical Site Infections	12 (12%)	5 (11%)	0.856
Remote Site Infections	11 (11%)	5 (11%)	0.994
Anastomotic Leak	5 (5%)	2 (4%)	0.872
Wound Infection	6 (6%)	3 (6%)	0.894
Pneumonia	2 (2%)	4 (9%)	0.057
Length of hospital stay	**Open Surgery median (range)**	**Laparoscopic Surgery median (range)**	**p value**
Days	6 (3-78)	6 (3-27)	0.317

aTNM not defined for 12 patients
bTNM not defined for 3 patients
CRP C-reactive protein

9 Daily C-reactive protein concentration thresholds and infective complications following colorectal cancer resection: Effect of pre-emptive antibiotic therapy

9.1 Introduction

Colorectal resection is associated with relatively high rates of postoperative infective complications. Approximately 20-40% are at risk of complications such as respiratory, wound or urinary tract infection, anastomotic leak, intra-abdominal abscess and septicaemia of unknown origin (Velasco et al., 1996). Many of these infections can be treated with antibiotics alone, whilst others, such as anastomotic leak, may require further intervention. Whether the administration of antibiotics prior to a clinically evident anastomotic leak may reduce the need for surgical intervention is not known. During the early postoperative period, sepsis can be difficult to distinguish from the normal postoperative systemic inflammatory response related to surgical trauma. Recognition during this period is challenging and lacks sensitivity at a stage when early diagnosis may significantly improve outcome (Welsch et al., 2007). Patients who encounter postoperative infective complications, in particular anastomotic leak, have been shown not only to have poorer short term outcomes, but also an increased recurrence rate in the long term (McArdle et al., 2005, Mirnezami et al., 2011).

A number of studies have investigated the association of the systemic inflammatory response and postoperative complications, with previous studies suggesting that an abnormally elevated C-reactive protein concentration or persistent elevation may be a useful predictor of infective complications (Table 9-1) (Welsch et al., 2007, Bianchi et al.,

2004, Matthiessen et al., 2008, Welsch et al., 2008, MacKay et al., 2011, Ortega-Deballon et al., 2010, Korner et al., 2009, Woeste et al., 2010) (Chapters 4-6). A recent systematic review and meta-analysis included 7 studies (2483 patients) and concluded that C-reactive protein was a useful predictive test for the development of anastomotic leak following colorectal resection, with derived thresholds of approximately 170 mg/l on postoperative day 3 and approximately 145 mg/l on day 4, prior to the development of clinical symptoms (Singh et al., 2014). Similar findings exist in studies looking at patients with pancreatic and oesophagogastric cancer (Welsch et al., 2008, Dutta et al., 2011). In contrast, white blood cell count contributes little to the early detection of complications (Warschkow et al., 2012b). This pre-clinical warning is of particular importance in an era of enhanced recovery and early discharge.

Only one previous study has addressed the utility of pre-emptive antibiotics used in conjunction with a biochemical predictor of infective complications following elective colorectal surgery. This study examined serum procalcitonin as a predictive marker for postoperative complications and the effect of administration of pre-emptive antibiotics in ten patients with elevated procalcitonin compared with standard treatment. They concluded that a significant reduction in the rate of postoperative infective complications in patients with an elevated procalcitonin was achieved by means of pre-emptive antibiotic treatment (Chromik et al., 2006).

Moreover, in patients undergoing resection for colorectal cancer, it has previously been demonstrated that a raised peak postoperative C-reactive protein concentration on day 2 above 190 mg/l leads to patients being more likely to meet thresholds predictive of infective complications on days 3 and 4, prior to the traditional rise in white cell count or clinical symptoms developing. Whether intervention to attenuate this post-operative

systemic inflammatory response will improve postoperative complication rates has yet to be determined and whether a raised systemic inflammatory response, as evidenced by C-reactive protein concentrations, is potentially the cause or consequence of the development of infective complications remains unclear.

Therefore, the aim of the present study was to examine the relationships between postoperative predictive thresholds of C-reactive protein and infective complications, in the context of the administration of pre-emptive antibiotic therapy, in patients undergoing resection for colorectal cancer.

9.2 Patients and Methods

Consecutive patients with histologically proven colorectal cancer who, on the basis of intraoperative findings and preoperative abdominal computed tomography, were considered to have undergone potentially curative resection in one of two university teaching hospitals in Glasgow between May 2011 and January 2013 were included in the study (n=223). Patient characteristics were collected in a prospective surgical database. All patient data was de-identified. Emergency admissions were excluded from the study, along with those who had a penicillin allergy, were on immunosuppressant medications, or had neo-adjuvant chemotherapy. Two patients were admitted to intensive care postoperatively and commenced on antibiotics prior to postoperative day 3, they were also excluded.

The tumours were staged using conventional TNM classification. All resections were elective cases and were performed using either open (n=121) or laparoscopic surgery (n=102). All operations involved an anastomosis. Pre-operatively all patients received thromboembolism and antibiotic prophylaxis according to the local protocol. Blood samples were taken for routine laboratory analysis of C-reactive protein in the pre- and postoperative period (days 1-7). Postoperatively, all patients had a daily clinical assessment by the operating team and additional investigations were carried out as clinically indicated.

On the basis of previous observations of antibiotic prescribing and clinical suspicion secondary to high postoperative C-reactive protein concentrations in each unit, it was considered that using C-reactive protein thresholds shown to be predictive of infective complications to guide the administration of antibiotics may help to rationalise antibiotic

prescribing and short term outcomes could be audited prospectively. Published evidence was presented (MLR) at a minimum of two departmental meetings in each unit. Guidance on prescribing pre-emptive antibiotics based on C-reactive protein thresholds was proposed (MLR and GM) and agreed by the consultant colorectal teams. Guidance was then disseminated to staff via meetings, phone calls and posters. Patients who underwent elective colorectal cancer resection had daily monitoring of postoperative C-reactive protein concentrations, as per standard practice in both units. Patients underwent clinical review and appropriate investigations based on clinical findings. Thresholds where derived from previous studies in both units (MacKay et al., 2011) (Chapters 4 and 5). Those who had a C-reactive protein >180mg/l on postoperative day 3 or >125mg/l on day 4 were considered at high risk of developing infective complications and the operating team was then prompted to prescribe pre-emptive antibiotics. Co-amoxiclav was recommended by our lead microbiologist due to its broad spectrum of activity and its availability in oral and intravenous forms. A course of 5 days was prescribed via the most appropriate route of administration. Antibiotics were changed accordingly if and when positive culture results were obtained. Patients were monitored until discharge and then reviewed at approximately 30 days following that in routine outpatient clinics. Outcome measures were the systemic inflammatory response, as evidenced by C-reactive protein concentrations, infective complications and length of hospital stay (Figure 9-1). Adverse effects of antibiotics were also to be monitored, however none were apparent. Data was recorded in a prospective database and audited at 18 months. This intervention was intended as an audit to rationalise antibiotic prescribing based on published evidence regarding C-reactive protein thresholds predictive of infective complications from both units (Chapters 4 and 5) (MacKay et al., 2011) therefore ethical approval was not sought and this study was not formally powered.

Patients were assessed for the following complications: infective and non-infective (persistent ileus, cardiac events encompassing acute coronary syndrome and acute myocardial infarction, and pulmonary embolism). Infective complications can be described as surgical site infections (SSI) and remote site infections (RSI). Surgical site infections can be further classified into incisional (wound) and organ/space (intra-abdominal abscess / anastomotic leak). A remote site infection such as pneumonia is often exogenous and occurs at sites not directly associated with the surgical procedure. The criteria used to define infective complications were the same as previously described (Ytting et al., 2005).

Initially, comparison was planned between patients who received antibiotics based on C-reactive protein concentrations above predictive thresholds and a historical control group. Due to compliance issues with the agreed guidance, not all patients meeting criteria for pre-emptive antibiotics received them, hence two contemporaneous groups were generated: those who received antibiotics and those who did not, all of whom met day 3 or 4 C-reactive protein thresholds predictive of postoperative infective complications.

Statistical analysis

Data are presented as median (range) or number (percentage). Comparison between data was carried out using a Chi-square or Mann-Whitney U test. A p-value <0.05 was considered significant. Statistical analysis was performed using SPSS version 21.0 for Windows (IBM Corporation, Armonk, NY, USA).

9.3 Results

Baseline characteristics of the 223 patients who underwent surgery for colorectal cancer are shown in Table 9-2. The majority of patients were age 65 or older (70%), male (62%), had colonic tumours (87%), node negative disease (64%), and underwent open resection (54%).

During follow up, of the 223 patients, 117 (52%) patients developed a postoperative complication; 89 (40%) of which were infective complications. The 89 patients with infective complications included 38 remote site infections (pneumonia n=28, urinary tract infection n=6, peripheral cellulitis n=2, and clostridium difficile n=2), 42 wound infections, 11 intra-abdominal abscesses and 13 anastomotic leaks. Clostridium difficile was diagnosed in two patients, neither of whom had received pre-emptive antibiotics. Of those with an infective complication, 15 patients developed a second infective complication and 27 patients developed an additional non-infective complication. The 55 patients with non-infective complications suffered from persistent ileus (n=16), atrial fibrillation (n=10), myocardial infarction (n=4), acute urinary retention (n=4), haematoma or bleeding (n=6), acute renal failure (n=6), wound dehiscence (n=8), small bowel obstruction (n=6), deep vein thrombosis (n=1), and multi-organ failure (n=2).

Of those patients who met the C-reactive protein thresholds predictive of infective complications, patients who did not receive antibiotics (n=55) and patients who did (n=64) were similar in terms of sex, tumour site, TNM stage, comorbidity and preoperative C-reactive protein concentration. Patients who received antibiotics as per protocol tended to be older (p=0.018) and had a longer median length of hospital stay (p=0.015) than those who did not receive antibiotics (Table 9-2).

Of those patients who had C-reactive protein concentrations above the threshold for pre-emptive antibiotics on days 3 or 4 postoperatively, patients who were prescribed antibiotics had significantly more infective complications ($p<0.001$). In particular, the incidence of postoperative pneumonia was higher in those who received antibiotics ($p=0.005$) (Table 9-2).

Of those patients who had C-reactive protein concentrations above the threshold for pre-emptive antibiotics on days 3 or 4 postoperatively, patients who were prescribed antibiotics had significantly higher C-reactive protein concentrations on postoperative days 5, 6 and 7 ($p=0.004$, $p=0.001$, $p=0.041$ respectively) than those who did not receive antibiotics (Table 9-3).

9.4 Discussion

The results of this prospective non-randomised observational study suggest that, in those patients who received antibiotics, infective complication rates and C-reactive protein concentrations were subsequently higher than those who were not prescribed antibiotics. Therefore, the administration of pre-emptive antibiotics guided by C-reactive protein thresholds predictive of infective complications did not appear to reduce infective complication rates or the magnitude of the postoperative inflammatory response following elective resection for colorectal cancer.

The present study has a number of limitations. In particular, deviation from the agreed protocol. The reasons for such deviation from the protocol are likely to be several. Initially, a randomised controlled trial had been proposed to study the impact of pre-emptive antibiotics guided by C-reactive protein concentrations. However, in light of the evidence from previous studies, some clinicians considered this unethical, and certainly expected access to post-operative C-reactive protein concentration results. Conversely, some may have favoured clinical judgement alone and been less influenced by C-reactive protein concentrations or wished to wait for a trend on subsequent days. Whilst used as a guide, it seems likely that perhaps pre-emptive antibiotics were only utilized in patients who clinically appeared to be at higher risk of developing an infective complication. This may have been due to a longer or more difficult operation, to these patients being older, or having persistently elevated C-reactive protein concentrations on postoperative day 4. Perhaps they simply appeared more clinically unwell. Furthermore, this protocol was intended as an exploratory pilot study, and therefore not formally powered. Both units followed the pre-emptive antibiotic guidance to a similar extent. Throughout the period observed there was changeover of junior staff at regular intervals, therefore it is possible

that some staff were not aware of the pre-emptive antibiotic guidance. One approach to eliminate these factors would be to carry out a formally powered prospective randomized trial with a strict protocol that was ethically approved. This may prove difficult due to issues of blinding clinicians, for instance, to blood results.

Irrespective, almost one third of patients receive antibiotic therapy following elective colorectal cancer resection. This is currently under close scrutiny due to the prevalence of opportunistic infections such as Clostridium difficile and MRSA, plus concerns regarding increasing antibiotic resistance. A strategy of pre-emptive antibiotics guided by postoperative day 3 or 4 CRP may rationalise antibiotic prescribing by flagging up patients at high risk of developing infective complications and lead to earlier treatment.

Conventionally, a rise in circulating C-reactive protein concentration has been interpreted as a consequence, rather than a cause, of an infective complication. However, it may be that C-reactive protein is more than just a sensitive measure of the presence of infection. Indeed, C-reactive protein has an important role in innate immunity as an early defense against infection, assisting complement-binding to foreign and damaged cells and enhancing phagocytosis by macrophages. For example, through activation of complement and interaction with Fc gamma receptors, C-reactive protein has been shown to provide a link between the innate and adaptive immune systems (Peisajovich et al., 2008, Coventry et al., 2009, Du Clos and Mold, 2004, Sander et al., 2010). Furthermore, with increasing concentrations of C-reactive protein there is a depression of T-lymphocyte function (Sander et al., 2010, Fietta et al., 2009) and an increase in the stress response and the degree of hyperglycaemia (Wichmann et al., 2005). Also, postoperative hyperglycemia has been shown to be an important factor associated with the promotion of bacterial growth and the development of postoperative infective complications (Motoyama et al., 2010,

Ramos et al., 2008).

Whether infective complications result from a raised systemic inflammatory response, as evidenced by C-reactive protein concentrations, or whether the systemic inflammatory response is already raised due to the presence of an underlying infective complication remains uncertain. The results of the present study demonstrate that the administration of early antibiotic therapy did not normalise C-reactive protein concentrations and helps to provide unique insight into the underlying mechanism between postoperative C-reactive protein concentrations and infective complications following resection for colorectal cancer. Hence, the persistence of an elevated C-reactive protein concentration may suggest that the systemic inflammatory response is a cause, rather than a consequence, of infective complications following colorectal cancer resection. In addition to giving advance notice of a clinical infection, C-reactive protein may also play an important direct role in modulating the postoperative immune function of patients with colorectal cancer. If this is indeed the case then it might be expected that investigation into the influence of perioperative factors and intervention to reduce this systemic inflammatory response might well improve outcomes, both short and long term. The nature of this relationship warrants further investigation.

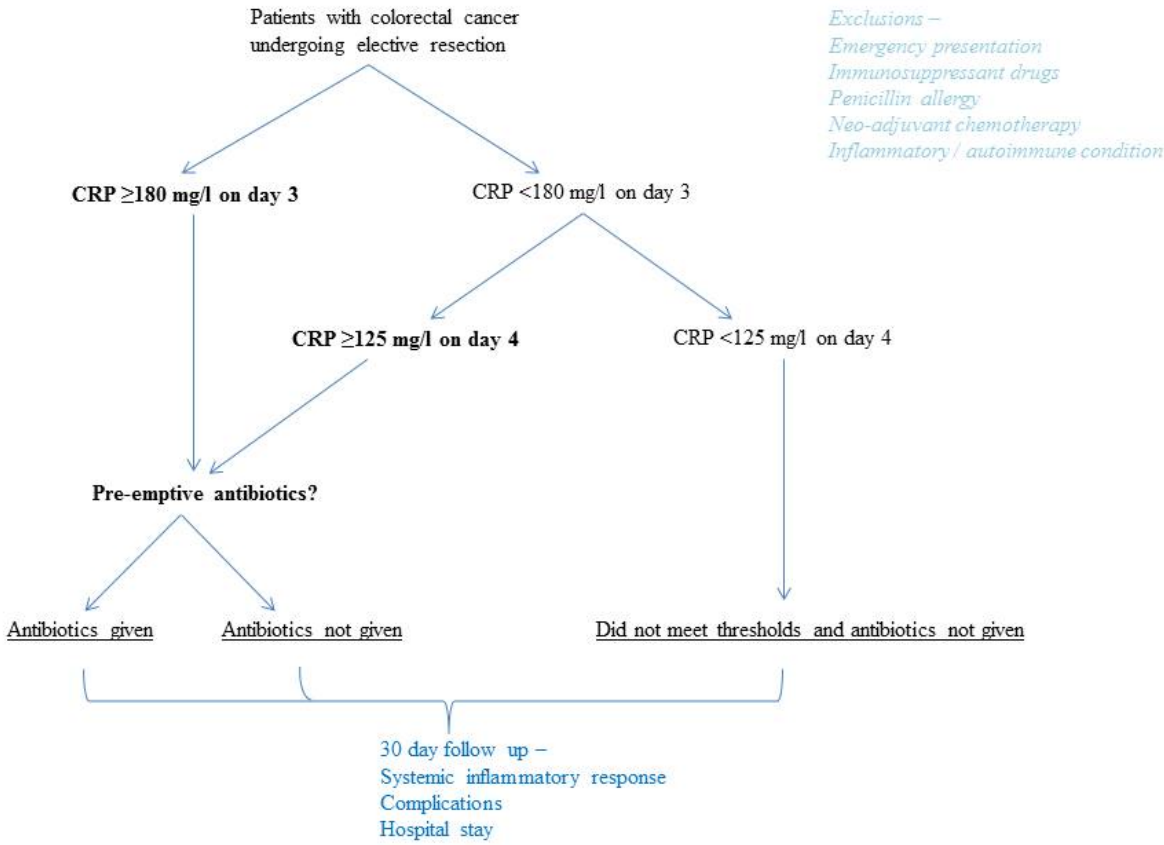


Figure 9-1 Scheme of an 18 month audit of C-reactive protein guided pre-emptive antibiotics in patients undergoing resection for colorectal cancer

Table 9-1 Previous studies examining C-reactive protein as a predictor of infective complications and anastomotic leak following colorectal surgery

Study	Patients	C-reactive protein threshold (sensitivity)
Welsch 2007 (Welsch et al., 2007)	383	>140mg/l on day 3 (80%)
Korner 2009 (Korner et al., 2009)	231	>190mg/l on day 3 (82%)
Ortega-Deballon 2010 (Ortega-Deballon et al., 2010)	133	>125mg/l on day 4 (82%)
Mackay 2010 (MacKay et al., 2011)	160	>145mg/l on day 4 (85%)
Chapter 4	454	>170mg/l on day 3 (78%)
Warschkow 2011 (Warschkow et al., 2012b)	1,187	>123mg/l on day 4 (66%)
Almeida 2012 (Almeida et al., 2012)	173	>140mg/l on day 3 (78%)
Lagoutte 2012 (Lagoutte et al., 2012)	100	>130mg/l on day 4 (80%)
Garcia-Granero 2013 (Garcia-Granero et al., 2013)	205	>147mg/l on day 3 (91%)
Chapter 5	344	>180mg/l on day 3 (71%) >140mg/l on day 4 (71%)

Table 9-2 Clinical characteristics of patients undergoing elective resection for colorectal cancer (n=223)

	Day 3 and 4 CRP below threshold for antibiotics (n=104)	Day 3 or 4 CRP above threshold, no antibiotics prescribed (n=55)	Day 3 or 4 CRP above threshold, antibiotics given (n=64)	p-value[a]
Age (<65/ 65-74/ ≥ 75)	29/ 31/ 44	21/ 10/ 24	18/ 27/ 19	0.018
Sex (Male/ Female)	63/ 41	38/ 17	37/ 27	0.206
ASA (1/ 2/ 3/ 4)	12/ 33/ 25/ 2	3/ 9/ 16/ 2	1/ 24/ 16/ 2	0.128
Hospital (a/ b)	67/ 37	37/ 18	38/ 26	0.376
TNM stage (I/ II/ III/ IV)	29/ 44/ 28/ 3	7/ 26/ 17/ 5	17/ 20/ 23/ 3	0.120
Tumour Site (Colon/ Rectum)	93/ 11	48/ 7	53/ 11	0.500
Surgical approach (Open/ Lap)	39/ 65	43/ 12	39/ 25	0.044
Preoperative CRP	3 (1-236)	10 (1-249)	9 (1-65)	0.933
Day 2 CRP ≤190/ >190	85/ 9	23/ 30	24/ 37	0.663
Length of hospital stay (days)	6 (2-70)	8 (3-72)	10 (6-63)	0.015
Any complication	33 (32)	31 (56)	53 (83)	0.002
Non-infective complication	19 (18)	16 (29)	20 (31)	0.799
Infective complication	21 (20)	21 (38)	47 (73)	<0.001
Surgical site infection	13 (13)	18 (33)	32 (50)	0.058
Remote site infection	8 (8)	8 (15)	22 (34)	0.013
Anastomotic leak	1 (1)	6 (11)	6 (9)	0.783
Wound infection	12 (12)	10 (18)	20 (31)	0.103
Pneumonia	4 (4)	5 (9)	19 (30)	0.005

Results shown as number (percentage) or median (range), *CRP* C-reactive protein (mg/l)

[a]compared with those who had day 3 or 4 CRP above thresholds for antibiotics, but no antibiotics prescribed

Table 9-3 Trends in C-reactive protein in patients undergoing elective resection for colorectal cancer (n=223)

C-reactive protein	Day 3 and 4 CRP below threshold for antibiotics (n=104)	Day 3 or 4 CRP above threshold, no antibiotics prescribed (n=55)	Day 3 or 4 CRP above threshold, antibiotics given (n=64)	p-value[a]
Preoperative	3 (1-236)	10 (1-249)	9 (1-65)	0.933
Day 1	70 (2-203)	91 (4-309)	111 (7-313)	0.456
Day 2	98 (1-224)	214 (48-337)	224 (39-454)	0.946
Day 3	100 (2-175)	217 (89-426)	264 (110-601)	0.012
Day 4	75 (16-125)	181 (119-403)	241 (97-528)	0.006
Day 5	62 (11-213)	147 (53-351)	218 (64-397)	0.004
Day 6	55 (15-304)	110 (40-399)	176 (59-406)	0.001
Day 7	52 (12-265)	107 (21-348)	157 (33-393)	0.041

Results shown as median (range)

CRP C-reactive protein (mg/l)

[a]compared with those who had day 3 or 4 CRP above thresholds for antibiotics, but no antibiotics prescribed

10 Conclusions

It has previously been demonstrated that patients with a raised systemic inflammatory response prior to surgery for colorectal cancer have poorer short and longer term outcomes than those who are not inflamed preoperatively. This has been thought to be due to inflammation promoting tumour growth and spread. Patients undergoing colorectal surgery are at a relatively high risk of developing postoperative infective complications and anastomotic leak. It is known that patients who develop these complications, particularly an anastomotic leak, have poorer cancer specific survival. The aims of this thesis were to further examine the nature of the postoperative systemic inflammatory response and its relationship with infective complications following resection for colorectal cancer.

Some patients have an increased systemic inflammatory response preoperatively. We hypothesised that this might be due to impaired cortisol production. Chapter 3 examines the relationship between the perioperative systemic inflammatory response and cortisol production, i.e. to determine whether this results from an impaired anti-inflammatory response rather than a pro-inflammatory response. The opening chapter was a prospective study assessing the preoperative adrenocortical function, using short Synacthen testing, in 80 patients undergoing colorectal cancer resection. This study showed that the perioperative systemic inflammatory response was not significantly associated with impaired cortisol production. This suggests that the systemic inflammatory response is likely a result of a pro-inflammatory stimulus rather than an impaired anti-inflammatory response in patients with colon cancer.

As previously mentioned, infective complications particularly anastomotic leak represent serious morbidity after colorectal cancer surgery. They can be difficult to detect in the early postoperative period due to the systemic inflammatory response to surgery. Some blood tests help to provide clues but their relative predictive value was unclear. Chapter 4 was a retrospective observational study to examine the diagnostic accuracy of serial postoperative white cell counts, albumin and C-reactive protein concentrations in predicting infective complications in 454 patients undergoing resection for colorectal cancer. C-reactive protein was the most sensitive test in detecting the development of an infective complication, with an optimal predictive threshold of 170 mg/L on postoperative day 3. Indeed, in a review of 7 studies (n=2483), Singh et al concluded that C-reactive protein was a useful negative predictive test for the development of anastomotic leak following colorectal resection, furthermore the pooled C-reactive protein thresholds were remarkably similar (Singh et al., 2014). In conclusion, C-reactive protein measurements on postoperative day 3 can accurately predict infective complications, including anastomotic leak, following colorectal cancer resection, prior to the development of clinical signs and symptoms.

It was not clear whether the same predictive thresholds would apply in patients undergoing laparoscopic surgery. Chapter 5 was a retrospective observational study, comparing the value of daily C-reactive protein concentrations in the prediction of postoperative infective complications in 334 patients undergoing open versus laparoscopic resection for colon cancer. C-reactive protein thresholds predictive of infective complications were the same on postoperative day 3 (180 mg/L) and day 4 (140 mg/L) following both open and laparoscopic resection for colorectal cancer. In patients who develop postoperative

infective complications, the magnitude of the postoperative systemic inflammatory response, as evidenced by C-reactive protein concentration, was similar regardless of surgical approach. Although there is considerable variation in the C-reactive protein response following open versus laparoscopic surgery in patients who did not develop infective complications, the basis for this variation is not clear and worthy of further investigation.

Whether the C-reactive protein thresholds predictive of infective complications are high because the patient already has an underlying infective complication earlier than expected, or whether a raised systemic inflammatory response means they are then more likely to develop a subsequent infective complication is not known. The peak systemic inflammatory response, as evidenced by C-reactive protein, has been shown to occur on postoperative day 2. Chapter 6 examined the impact of the peak inflammatory response on the C-reactive protein thresholds predictive of infective complications on days 3 and 4 in 357 patients undergoing resection for colorectal cancer. This study demonstrated that a postoperative day 2 C-reactive protein of ≥190 mg/L corresponded to previously determined day 3 and 4 thresholds predictive of infective complications. If background inflammation makes patients at higher risk of developing complications, this would suggest that the magnitude of the peak inflammatory response may influence who will meet day 3 and 4 thresholds. To test this hypothesis, intervention to lower the peak systemic inflammatory response should be investigated in future work to determine whether this is beneficial in the care of patients with colorectal cancer.

Postoperative C-reactive protein concentration on days 3 and 4 can be a useful early predictor of the development of postoperative infective complications and anastomotic leak

following colorectal cancer resection. Moreover, the systemic inflammatory response as demonstrated by day 2 postoperative C-reactive protein concentration >190mg/L has a significant influence on the likelihood of having a C-reactive protein above predictive thresholds on days 3 and 4. Therefore, chapter 7 examined the clinicopathological determinants of the postoperative systemic inflammatory response, as evidenced by day 2, day 3 and day 4 C-reactive protein concentrations, in 686 patients following resection of colorectal cancer. Emergency presentation was associated with a higher systemic inflammatory response on days 2-4 postoperatively. In elective cases, preoperative systemic inflammation, BMI, socioeconomic deprivation and an enhanced recovery programme were associated with a higher systemic inflammatory response. In contrast, laparoscopic surgery was associated with a lower systemic inflammatory response.

Chapter 8 examined further the impact of an enhanced recovery programme on the systemic inflammatory response and the rate of infective complications in 310 patients following elective surgery for colorectal cancer. There were no significant differences in the magnitude of the systemic inflammatory response or rates of infective complications in those who underwent colorectal cancer resection within an enhanced recovery programme compared to conventional care. However, the multifactorial and variable nature of enhanced recovery programmes makes them notoriously difficult to study. It may be that only some elements used in an enhanced recovery programme, such as laparoscopic surgery, actually modify the systemic inflammatory response. Therefore, the use of markers such as C-reactive protein could objectively determine which components reduce the magnitude of the systemic inflammatory response after surgery.

To try to answer the question as to whether a raised C-reactive protein on days 3 and 4 was cause or consequence of the development of an infective complication, Chapter 9 examined the effect of giving antibiotics based on previously determined thresholds. Whether infective complications result from a raised systemic inflammatory response or whether the systemic inflammatory response is already raised due to the presence of an underlying infective complication remains uncertain. This pilot study would provide insight into the underlying mechanism between postoperative C-reactive protein concentrations and infective complications following resection for colorectal cancer.

Chapter 9 examined the relationship between postoperative predictive thresholds of C-reactive protein and infective complications, in the context of pre-emptive antibiotic therapy, in 223 patients undergoing surgery for colorectal cancer. Patients who were prescribed antibiotics had more infective complications than those patients with similar C-reactive protein concentrations who were not given antibiotics. Administration of pre-emptive antibiotics guided by C-reactive protein thresholds did not reduce rates of infective complications or the magnitude of the systemic inflammatory response.

Hence a raised systemic inflammatory response may be a cause, rather than a consequence, of infective complications following colorectal cancer resection. C-reactive protein may play a role in modulating the postoperative immune function of patients with colorectal cancer. Further investigation into the influence of perioperative factors in order to reduce the systemic inflammatory response to surgery is required. Therefore, future randomised studies that examine the effect of a reduced systemic inflammatory response on postoperative complications are of particular interest.

In conclusion, the work presented herein demonstrated that the systemic inflammatory response in patients with colorectal cancer is likely an innate pro-inflammatory process. This may in fact create a pro-tumorigenic environment and lead to increased rates of cancer recurrence and reduced cancer specific survival, therefore these outcomes should also be examined in future work. Postoperative C-reactive protein concentrations on days 3 and 4 following colorectal cancer resection are useful early predictors of the development of infective complications, prior to clinical signs and symptoms being apparent. These predictive thresholds are useful in patients undergoing open or laparoscopic colorectal resection, and in those treated within an enhanced recovery programme or with conventional care. The postoperative peak systemic inflammatory response, as evidenced by day 2 C-reactive protein, influences the systemic inflammatory response on subsequent days and therefore may determine patients at high risk of developing complications. The peak systemic inflammatory response has a number of clinicopathological associations. A raised peak systemic inflammatory response was associated with emergency presentation, deprivation, high BMI, and enhanced recovery. In contrast, laparoscopic surgery was associated with a reduced peak systemic inflammatory response. C-reactive protein threshold guided pre-emptive antibiotics did not act to reduce the postoperative systemic inflammatory response or the rate of infective complications following colorectal cancer resection.

Therefore, the development of postoperative infective complications following resection for colorectal cancer may be the consequence of a raised systemic inflammatory response and relative immunocompromise of the patient. This insult may also influence tumorigenesis and lead to increased recurrence rates and poorer cancer specific survival.

The implications of this are profound. Firstly, the utility of postoperative C-reactive protein thresholds as a prognostic tool, particularly with regards to the development of infective complications following colorectal cancer resection is demonstrated. It has recently been reported that C-reactive protein concentrations can also be used to predict both the type and severity of postoperative complications in a small study (n=127) of patients undergoing surgery for colorectal cancer (Selby and Prabhudesai, 2014). Secondly, postoperative C-reactive protein concentrations may be a useful objective therapeutic target by which the efficacy of future interventions to reduce the systemic inflammatory response, and therefore risk of postoperative infective complications, could be assessed. For instance, the effect of therapeutic interventions to reduce the perioperative systemic inflammatory response, such as the use of steroids or NSAIDs, could be assessed using postoperative C-reactive protein concentrations and the likelihood a patient is to breech the described thresholds. If an elevated postoperative inflammatory response is a pro-inflammatory process, then NSAIDs may work to reduce this response and hence reduce susceptibility to postoperative infection. Randomised trials are required to confirm this hypothesis.

In summary, the objective measurement of the postoperative systemic inflammatory response and its relationship with postoperative outcomes has profound implications for assessment and treatment of the surgical stress response in patients with colorectal cancer.

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