AN INVESTIGATION INTO THE ROLE OF THE INNATE IMMUNE SYSTEM IN PATIENTS UNDERGOING SURGERY FOR COLORECTAL CANCER

BY

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Abstract

Colorectal cancer is the 4th most common cancer in the UK and the second commonest cause of cancer death. Whilst mortality rates from colorectal cancer haven fallen over the last 2 decades, around 40% of those diagnosed with colorectal cancer will die from their disease. Surgery currently remains the only chance of cure. Around 10% of patients present as an emergency with perforation, obstruction or bleeding. Outcomes from these emergency operations are substantially worse than from elective procedures.

The presence of a systemic inflammatory response pre-operatively is now widely recognised as a predictor of disease progression and poor outcomes, both long and short term, regardless of tumour stage in those with colorectal cancer. Numerous scoring systems that measure various components of the systemic inflammatory response have been documented, the most commonly used are the modified Glasgow Prognostic Score (mGPS) and the Neutrophil-Lymphocyte Ratio (NLR). The NLR has the advantage of using 2 components of the differential white cell count, which is routinely measured in surgical and oncological practice, whereas CRP is less commonly routinely measured. However, studies utilising the NLR have used a variety of thresholds, making comparison of the results from study to study difficult. Whether one of the components of the NLR is more important than the other remains to be seen and indeed whether there is a more optimal score that utilises the white cell count is not clear. To date no work has examined similar scoring systems in the post-operative period.

The present thesis aims to examine the impact of the innate immune response, through such systemic inflammation based scoring systems, on patients undergoing surgery for colorectal cancer. Furthermore, it analyses the nature of the inflammatory response in the post-operative period in order to ascertain whether similar scoring systems may be of clinical utility.

Chapter 1 provides an overview of colorectal cancer, its presentation and treatment and its known determinants of outcomes. Furthermore, the immune response to injury and post-operative inflammatory response are discussed. Chapter 2 documents a survey of clinicians who have an interest in systemic inflammation. The survey asks the participants whether they routinely measure systemic inflammation, to what purpose and which scoring system they prefer. Unsurprisingly, the majority of participants use these scoring systems for research purposes only with an even split in terms of which scoring system they prefer to use. Their

use in clinical practice remains small but their use in some oncological studies may signify a step towards their incorporation into clinical practice in the future.

Chapter 3 presents data from a cohort of patients whom have undergone surgery for colorectal cancer with pre-operative differential white cell counts in order to determine whether any of the white cell count components are important in determining long term outcomes. Only the neutrophil count was independently associated with poor long term survival in patients undergoing surgery for colorectal cancer. These results highlight the importance of both the neutrophil count and the innate immune system in outcomes in patients with colorectal cancer.

In chapter 4, a cohort of colorectal cancer patients and a cohort of patients with cancer were utilised in order to determine whether a pre-operative systemic inflammation based score using the neutrophil and platelet count was capable of predicting survival in these patients. This was based on the fact that recent in-vitro work had suggested that a critical checkpoint early in the inflammatory process involved the interaction between neutrophils and activated platelets. The subsequent score – the neutrophil platelet score (NPS)- was shown to be capable of predicting survival, independent of TNM stage, in patients with colorectal cancer and had prognostic value in patients with a variety of other tumours.

Chapter 5 describes a systematic review of studies analysing the effect of various surgical procedures on markers of the systemic inflammatory response. Only CRP and IL-6 were found to represent the degree of surgical trauma and invasiveness of the procedure. This work provides a framework for analysing the post-operative SIR and how it is affected by surgery and peri-operative programmes such as ERAS that are reported to improve length of stay and sort term outcomes following surgery for colorectal cancer.

It was of interest in the previous chapter that white cell count did not reflect the degree of surgical trauma. Whether individual white cell components act differently and represent the degree of surgical trauma was unclear. Chapter 6 sought to clarify this by analysing, in a cohort of patients undergoing surgery for colorectal cancer, the differential white cell count and whether it reflected the magnitude of injury and short term outcomes. Only the neutrophil count reflected the magnitude of trauma and development of infective complications. However, it remains inferior to other well established markers such as CRP.

Whilst the pre-operative systemic inflammatory response is a well-recognised determinant of both long term outcomes and short term outcomes such as infective complications, little work has focussed on the post-operative systemic inflammatory response. In chapter 7, the possibility of the post-operative systemic inflammatory response also being capable of predicting both short and long term outcomes was explored in a cohort of patients whom had undergone surgery for colorectal cancer. A score using the combination of post-operative CRP and albumin was created and called the post-operative Glasgow Prognostic Score (poGPS). In this cohort of patients, this score predicted the development of infective complications and also long term survival. Given that these results would indicate that a reduction in the post-operative systemic inflammatory response would improve outcomes, the clinicopathological factors that may alter this post-operative systemic inflammatory response should be investigated as some of these may be modifiable and may therefore improve outcomes following surgery for colorectal cancer.

ERAS programmes have changed perioperative management and are reported to be beneficial in reducing length of hospital stay and post-operative complications. It is purposed that this is due to the reduction on the surgical stress response. However it is unclear which of the components of an ERAS programme are responsible for this reduction in the systemic inflammatory response. Chapter 8 describes a systematic review analysing studies of the various ERAS components and whether there is objective evidence of a reduction in the SIR, evidenced by a reduction in either CRP or IL-6. Only laparoscopic surgery was reported to reduce the SIR in these studies, all the remaining components had either little or no evidence of a reduction in the SIR. Further work is required to ascertain whether any of the other components also reduce the SIR. This will hopefully allow streamlining of the ERAS process in order to improve outcomes.

Specific clinicopathological factors that may alter the post-operative systemic inflammatory response are examined in chapter 9. Common clinicopathological factors were examined using the poGPS to ascertain which factors resulted in increased poGPS scores. In those patients undergoing elective surgery, year of operation, ASA grade, pre-operative systemic inflammation, and tumour site were associated with increased poGPS scores. These findings may have important clinical consequences as whilst factors such as ASA grade and BMI are not readily modifiable in the short time frame between diagnosis and surgery, pre-operative

inflammation could potentially be targeted with anti-inflammatory medication. However, more work is required to identify the specific agent and the timing of its delivery.

In chapter 10, a cohort of patients undergoing surgery for colorectal cancer in whom there was prescription information available. Patients prescribed aspirin or statin were identified and their post-operative inflammatory response and short term outcomes were compared to those not prescribed aspirin or statins. In 446 patients, neither aspirin nor statin prescription was associated with a reduction in the post-operative systemic inflammatory response. Therefore, it would appear that these medications will not be useful in moderating the systemic inflammatory response following surgery. However, further work is required to identify which medications will be of benefit and should take the format of a randomised controlled trial.

Chapter 11 provides a summary of the main findings of this thesis, discussed their implications and provides some discussion surrounding future work in this field.

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Acknowledgement

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Author's Declaration

The work presented in this thesis was undertaken during a period of research between 2013 and 2015 in the Academic Unit of Surgery at Glasgow Royal Infirmary. The work has been completed whilst working as a Specialty Registrar in General Surgery in the West of Scotland.

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

- Creation of survey questions and survey concept by Mr Campbell Roxburgh (Chapter 2)

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- Analysis of data from database of patients with a range of common cancers and their pre-operative blood test results by Mr Michael Proctor

- Collection of aspirin and statin usage data by Shoukee Ng (Chapter 10)

Publications

The work presented in this thesis has resulted in following publications:

1. A survey of attitudes towards the clinical application of systemic inflammation based prognostic scores in cancer.
   Watt DG, Roxburgh CS, White M, Chan JZ, Horgan PG, McMillan DC.
   Med of Inflamm 2015; 2015:842070.

2. Neutrophil count is the most important prognostic component of the differential white cell count in patients undergoing elective surgery for colorectal cancer.
   Watt DG, Martin JC, Park JH, Horgan PG, McMillan DC.
   Am J Surg 2015; 210 (1): 24-30.

3. The neutrophil-platelet score (NPS) predicts survival in primary operable colorectal cancer and a variety of common cancers.
   Watt DG, Proctor MJ, Park JH, Horgan PG, McMillan DC.
   PLOS One 2015; 10 (11): e0142159.

4. Routine clinical markers of the systemic inflammatory response after elective operation: a systematic review.
   Watt DG, Horgan PG, McMillan DC.
   Surgery 2015; 157 (2): 362-380.

5. A Postoperative Systemic Inflammation Score Predicts Short- and Long-Term Outcomes in Patients Undergoing Surgery for Colorectal Cancer.
   Watt DG, McSorley ST, Park JH, Horgan PG and McMillan DC.
   Ann Surg Oncol 2017; 24(4): 1100-1109

6. Enhanced Recovery After Surgery: Which components, if any, impact on the systemic inflammatory response following colorectal surgery? A systematic review
   Watt DG, McSorley ST, Horgan PG, McMillan DC.
   Medicine (Baltimore) 2015; 94 (36): e1286.

7. Clinicopathological Determinants of an Elevated Systemic Inflammatory Response Following Elective Potentially Curative Resection for Colorectal Cancer.
   Watt DG, Ramanathan ML, McSorley ST, Walley K, Park JH, Horgan PG, McMillan DC.
   Ann Surg Oncol 2017; 24 (9): 2588-2594.

8. Comment on "Prognostic performance of inflammation-based prognostic indices in patients with resectable colorectal liver metastases."
   Watt DG, Horgan PG, McMillan DC.
   Med Oncol 2015; 32 (5): 1-2.

9. Re: Meta-analysis of the predictive value of C-reactive protein for infectious complications in abdominal surgery.
   Watt DG, McSorley ST, Horgan PG, McMillan DC.
   BJS – Your views 2015: 102 (6)

Presentations

The work presented in this thesis has resulted in the following presentations:

1. Neutrophil count is the most important prognostic component of the differential white cell count in patients undergoing elective surgery for colorectal cancer.
   National Cancer Research Institute Conference, Liverpool, UK. 2014 (Poster Presentation)

2. Pre- and post-operative inflammatory response to predict survival in patients undergoing potentially curative resection for colorectal cancer.
   American Society of Clinical Oncology GI Symposium, San Francisco, USA. 2015 (Poster Presentation)

3. The relationship between red cell distribution width (RDW), markers of systemic inflammation and survival in patients undergoing curative surgery for colorectal cancer.
   American Society of Clinical Oncology GI Symposium, San Francisco, USA. 2015 (Poster Presentation)

4. The validation of C-reactive protein and albumin as predictors of post-operative infective complications and their clinical utility in patients with colorectal cancer.
   Digestive Diseases Week, Washington D.C., USA. 2015 (Oral Presentation)

5. Neutrophil count, but not other components of a white cell count, reflects the impact of the systemic inflammatory response and long term survival in patients following elective surgery for colorectal cancer.
   Digestive Diseases Week, Washington D.C., USA. 2015 (Poster Presentation)

6. The post-operative systemic inflammatory response predicts development of infective complications following colorectal cancer surgery.
   West of Scotland Surgical Association AGM, Glasgow, UK. 2015 (Poster Presentation)

7. Post-operative C-reactive protein concentration: a potential therapeutic target following surgery for colorectal cancer?
   American Society of Clinical Oncology GI Symposium, San Francisco, USA. 2016 (Poster Presentation)

8. Does pre-operative aspirin and statin prescription modulate the post-operative systemic inflammatory response following surgery for colorectal cancer?
   American Society of Clinical Oncology GI Symposium, San Francisco, USA. 2016 (Poster Presentation)

Dedication

To my wife, Katie and my two daughters Lily and Kaitlyn who have supported my throughout this process. Their continual encouragement ensured my onward progress and motivated me to finish this thesis.

Definitions/Abbreviations

APC: Adenomatous Polyposis Coli

APR: Abdomino-perineal Resection

ASA: American Society of Anaesthesiologists

BMI: Body Mass Index

CRM: Circumferential Resection Margin

CRP: C-reactive Protein

CT: Computed Tomography

DNA: Deoxyribonucleic Acid

EGFR: Epidermal Growth Factor Receptor

ERAS: Enhanced Recovery After Surgery

FAP: Familial Adenomatous Polyposis

FIT: Faecal Immunochemical Test

gFOBt: Guaiac Based Faecal Occult Blood Test

HNPCC: Hereditary Nonpolyposis Colorectal Cancer Syndrome

IL-6: Interleukin-6

LNR: Lymph Node Ratio

MMP-9: Matrix Metallopeptidase 9

MDSC: Myeloid Derived Suppressor Cells

MDT: Multi-disciplinary Team

mGPS: Modified Glasgow Prognostic Score

MMR: Mismatch Repair Gene

MRI: Magnetic Resonance Imaging

MSI: Microsatellite Instability

NLR: Neutrophil Lymphocyte Ratio

NPS: Neutrophil-Platelet Score

NSAID: Non-Steroidal Anti-Inflammatory Drugs

PET: Positron Emission Tomography

PLR: Platelet Lymphocyte Ratio

PNI: Perineural Invasion

poGPS: Post-operative Glasgow Prognostic Score

ROC: Receiver Operating Curves

SIR: Systemic Inflammatory Response

SSI: Surgical Site Infection

TME: Total Mesorectal Excision

TNF: Tumour Necrosis Factor

TNM: Tumour, Node, Metastases

VEGF: Vascular Endothelial Growth Factor

WCC: White Cell Count

1 Introduction

1.1 Epidemiology of Colorectal Cancer

Colorectal cancer is the third most common cancer diagnosed worldwide in men and the second most common in women with 1,360,000 cases diagnosed in total in 2012. The majority of cases are found in developing countries with the highest rates found in Australia and New Zealand and lowest rates in Western Africa (WHO-Globocan, 2012). More deaths are recorded in the less developed parts of the world, resulting in poorer survival statistics for these regions (WHO-Globocan, 2012).

In the United Kingdom, colorectal cancer is now the fourth most common cancer *(Figure 1.1)*. Its incidence has been increasing over the last 4 decades, with the largest increases seen in the elderly population (CRUK, 2014b). In total greater than 41,000 people were diagnosed with colorectal cancer in 2011, with 95% of these occurring in people aged greater than 50 years old and 43% in those aged over 75 years old. Colorectal cancer is the second most common cause of cancer death in the United Kingdom, after lung cancer, with approximately 16,200 deaths in 2012 (CRUK, 2014b).

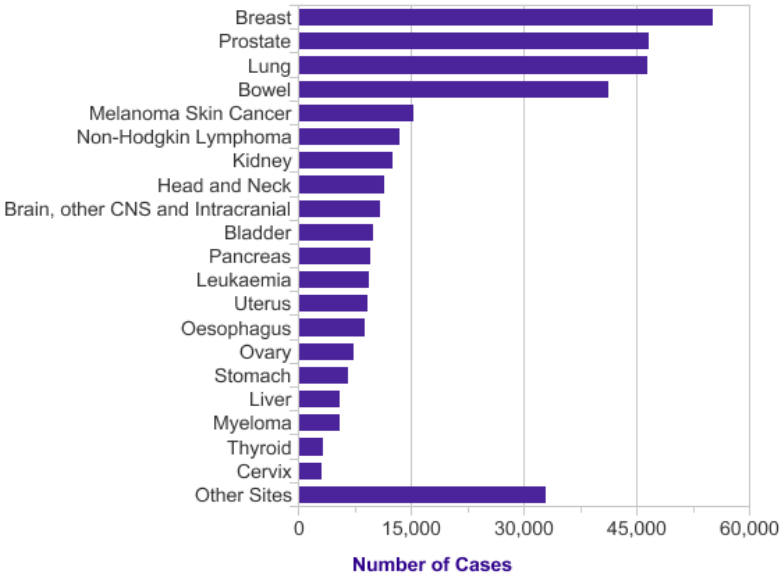


Figure 1.1 **The 20 Most Common Cancers in the UK 2014 (Reproduced Cancer Research UK)**

In Scotland, colorectal cancer is the fourth most common cancer diagnosed and the third most common cause of cancer death. The lifetime risk of developing colorectal cancer is 1 in 15 for men and 1 in 19 for women. In 2013, approximately 2,100 males and 1,712 females were diagnosed with colorectal cancer (SPHO, 2014).

Despite increases in incidence, there has been a trend for a reduction in the mortality from colorectal cancer over the last 30 years with approximately 60% of patients surviving at 5 years (Oliphant et al., 2013, SPHO, 2014). This may be due to improved treatment modalities, earlier presentation, the advent of national bowel screening programmes and increased surgical specialisation (Oliphant et al., 2013, Oliphant et al., 2014b). Despite this, mortality has been noted to be higher in areas of socioeconomic deprivation, a problem affecting large parts of Scotland in particular, with around 1,578 deaths occurring in Scotland in 2013 (SPHO, 2014).

1.2 Colorectal Cancer Carcinogenesis Pathways

Colorectal cancer arises from one or a combination of three mechanisms: chromosomal instability (adenoma-carcinoma sequence), microsatellite instability and hypermethylation.

1.2.1 Adenoma-Carcinoma Sequence

Greater than 90% of colorectal cancers are said to arise in colorectal adenomas. This forms the classical adenoma-carcinoma sequence (Arends, 2013) and involves a stepwise transformation from normal mucosal epithelium to adenomas and through eventually to carcinomas (Walther et al., 2009). This stepwise transformation would appear to be associated with accumulating genetic mutations within the tumour cell itself *(Figure 1.2)*. The most frequently documented of these are the APC, KRAS, MMR, MLH1 and MSH2 (Arends, 2013). As these mutations and alterations occur over a period of time, there is potential to intervene during this process, ideally at the adenoma stage. By removing these adenomas then this process can be effectively halted and the long term effect of this would be overall reduction in the number of colorectal cancers. This ideology forms the basis of the aims of the National Bowel Screening Programme.

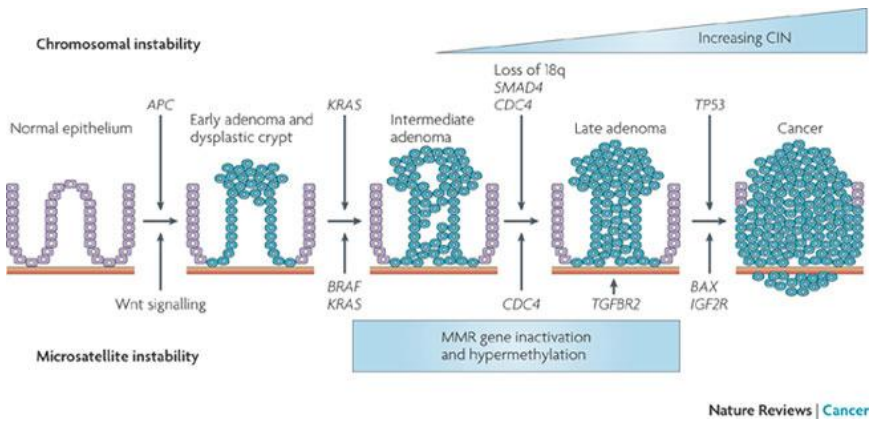


Figure 1.2 **Adenoma–carcinoma sequence model for chromosomal instability in colorectal cancer**
(Reproduced from Walther et al. 2009)

1.2.2 Microsatellite Instability

Microsatellite instability (MSI) is present in around 15% of colorectal cancer cases. MSI occurs when there are mutations in the DNA mismatch repair genes (MMR). These are the genes that correct DNA replication errors. Mutations in these genes cause these mismatch repair genes to become inactive, resulting in base pair mismatches during DNA replication (Armaghany et al., 2012).

Lynch Syndrome or Hereditary Nonpolyposis Colorectal Cancer Syndrome (HNPCC) is due to autosomal dominant inheritance of a mutant and wild-type DNA mismatch repair gene. Only about 3% of patients with MSI will develop Lynch syndrome. Patients with Lynch syndrome develop a few colorectal polyps which are thought to progress (due to the MMR mutations) at a faster rate to carcinomas. This has implications in terms of more frequent endoscopic surveillance (Arends, 2013).

1.2.3 Hypermethylation

Hypermethylation in the gene promoter region results in transcriptional inactivation of genes that are involved in tumour suppression or the cell cycle (Armaghany et al., 2012). Colorectal cancers which are formed via this pathway account for 10-20% of all colorectal cancers and are often associated with BRAF mutations (Arends, 2013). The presence of BRAF mutations has implications on the types of adjuvant chemotherapy given to patients, based on their efficacy in randomized clinical trials. For example, anti-epidermal growth factor receptor (EGFR) therapies have been shown to be less effective in patients with BRAF mutation.

1.3 Aetiology of Colorectal Cancer

The aetiology of colorectal cancer is not fully understood and thought to be multi-factorial. The majority are said to be sporadic and result from random genetic mutations or interactions between the host and various environmental factors. The remainder are hereditary, typically caused by certain medical conditions or inherited through well documented genetic alterations that predispose the patient to developing colorectal cancer.

1.3.1 Sporadic Colorectal Cancer

A vast number of factors have been implicated in the development of sporadic colorectal cancer. The evidence for most of these in terms of definite causative agents is lacking. These factors include certain lifestyle elements such as diet, obesity and smoking status as well as certain medications that are believed to increase the risk of developing colorectal cancer.

1.3.1.1 Age

Age remains the single biggest risk factor for colorectal cancer. 95% of colorectal cancers diagnosed are in people aged over 50 years old (CRUK, 2014b). Therefore as people get older, their risk of developing colorectal cancer increases. This is perhaps due to increased exposure to other environmental risk factors and also age related degradation of the genetic code which can lead to genetic mutations. Improved health and social care has meant that people are living longer, resulting in a natural increase in the incidence of cancer in an increasingly elderly population.

1.3.1.2 Diet & Lifestyle

The highest rates of colorectal cancer are found in the Western world. Migrants who move to another country have been found to adopt the incidence rates of their host country within a generation (Muir and Parkin, 1985). Therefore many people believe that a Western lifestyle is responsible for the development of many cases of colorectal cancer.

There has been much research into dietary components that may increase the risk of developing colorectal cancer. A diet that is high in fibre is said to be protective against the development of colorectal cancer (CRUK, 2014b, Aune et al., 2011a). High fibre diets enable smoother passage of faeces through the colon. Why a diet high in fibre reduces the risk of developing colorectal cancer is not entirely clear. People who eat lot of fibre may

eat less of the food groups that increase the risk of colorectal cancer such as red meat or processed meat or it may be that the colonic mucosa is exposed to toxins for a shorter period of time, reducing the risk of developing colorectal cancer.

Eating moderate amounts of fruit and vegetables may reduce the risk of colorectal cancer. One meta-analysis suggested the reduction could be by as much as 10% in people who ate a moderate amount of fruit or vegetables a day (Aune et al., 2011b). It can be postulated that antioxidants and vitamins that are present in many fruits and vegetables may prevent the cell damage that may lead to the development of colorectal cancer (CRUK, 2014b).

Red meat and processed meats are thought to increase the risk of developing colorectal cancer, as are diets high in fats and sugar. Conversely, fish, high levels of calcium and Vitamin D and dairy produce are thought to be protective against developing colorectal cancer (CRUK, 2014b).

1.3.1.3 Obesity

It has been long established that obesity is a risk factor for developing colorectal cancer. In those who are obese (Body Mass Index (BMI) >30 kg/m^2), the risk of developing colorectal cancer is 33% higher than those of a normal BMI. This association is stronger in men than in women (Ma et al., 2013). Furthermore, men with predominantly central (abdominal) obesity were at greater risk of developing colorectal cancer with a 2cm increase in waist circumference associated with a 4% increase in risk of developing colorectal cancer (Moghaddam et al., 2007).

1.3.1.4 Smoking and Alcohol

Smoking is a risk factor for developing colorectal cancer (Walter et al., 2014). People who smoke cigarettes have a significantly increased incidence of colorectal cancer. Furthermore, the duration of smoking, in numbers of pack years, was also significantly associated with the increased incidence of colorectal cancer (Liang et al., 2009).

There have been numerous reports that alcohol consumption is related to the risk of developing colorectal adenomas and colorectal cancers (Fedirko et al., 2011, Zhu et al., 2014). It remains unclear how excess alcohol consumption causes this increased risk of cancer development. Rather than being a carcinogen itself, it may act as a promoter of tumour growth and dissemination. Ethanol is metabolised into acetaldehyde and free

radicals, which are known to carcinogenic and therefore may promote cancer growth (Poschl and Seitz, 2004).

1.3.1.5 Medications

Several medications have been shown to have a protective effect with respect to the development of colorectal cancer.

1.3.1.5.1 Aspirin

Patients' prescribed non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin have been reported to have less aggressive tumours and be less likely to present with metastatic disease (Benedetti et al., 2003) or indeed develop metastatic disease (Rothwell et al., 2012). Exactly how these drugs cause this effect remains unclear, however NSAIDs have been reported to have effects directly on the tumour as well as beneficial effects on both the local and systemic inflammatory response, both of which have been associated with tumour progression (Park et al., 2014).

1.3.1.5.2 Statin

Statins, a group of drugs commonly prescribed for the treatment of hypercholesterolaemia, have been reported to reduce the risk of developing colorectal cancer (Lytras et al., 2014) as well as a reduction in mortality associated with colorectal cancer (Liu et al., 2014). Statins work primarily by inhibiting 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase, potentially preventing production of non-steroidal isoprenoids and also exhibit pro-apoptotic, anti-angiogenic and immunomodulatory effects which likely prevent the growth of tumours (Vallianou et al., 2014).

1.3.1.5.3 Metformin

Metformin is a drug commonly prescribed in type 2 diabetes mellitus. Its method of action is to lower circulating levels of both glucose and insulin in patients with insulin resistance by reducing hepatic glucose output. In addition to this, the use of metformin appears to be associated with a reduced risk of colorectal cancer (Singh et al., 2013, Franciosi et al., 2013) as well as lowering risks of mortality associated with cancer (Noto et al., 2012, Franciosi et al., 2013). Its exact mechanism of action is unclear but it is thought that metformin induces cell cycle arrest, apoptosis, activation of the immune system and possibly removes cancer stem cells (Franciosi et al., 2013).

1.3.1.6 Systemic Inflammatory Response

Inflammation is now established as a main factor that is involved in the development of cancer throughout the body (Roxburgh and McMillan, 2010, Diakos et al., 2014). Indeed, one study of 22,887 patients, reported elevated concentrations of CRP in those who eventually developed colorectal cancer (Erlinger et al., 2004). Inflammation is now recognised as a 'hallmark of cancer' and a key proponent of tumour proliferation and dissemination (Hanahan and Weinberg, 2011). Inflammation also has links with several other risk factors for cancer development and through a complex network of processes appears to be detrimental to the outcomes of patients with a range of tumours. It remains to be seen whether modulating this inflammatory response either empirically i.e. prior to any cancer diagnosis or following a cancer diagnosis, has any impact on improvement of outcomes in patients with cancer (Diakos et al., 2014).

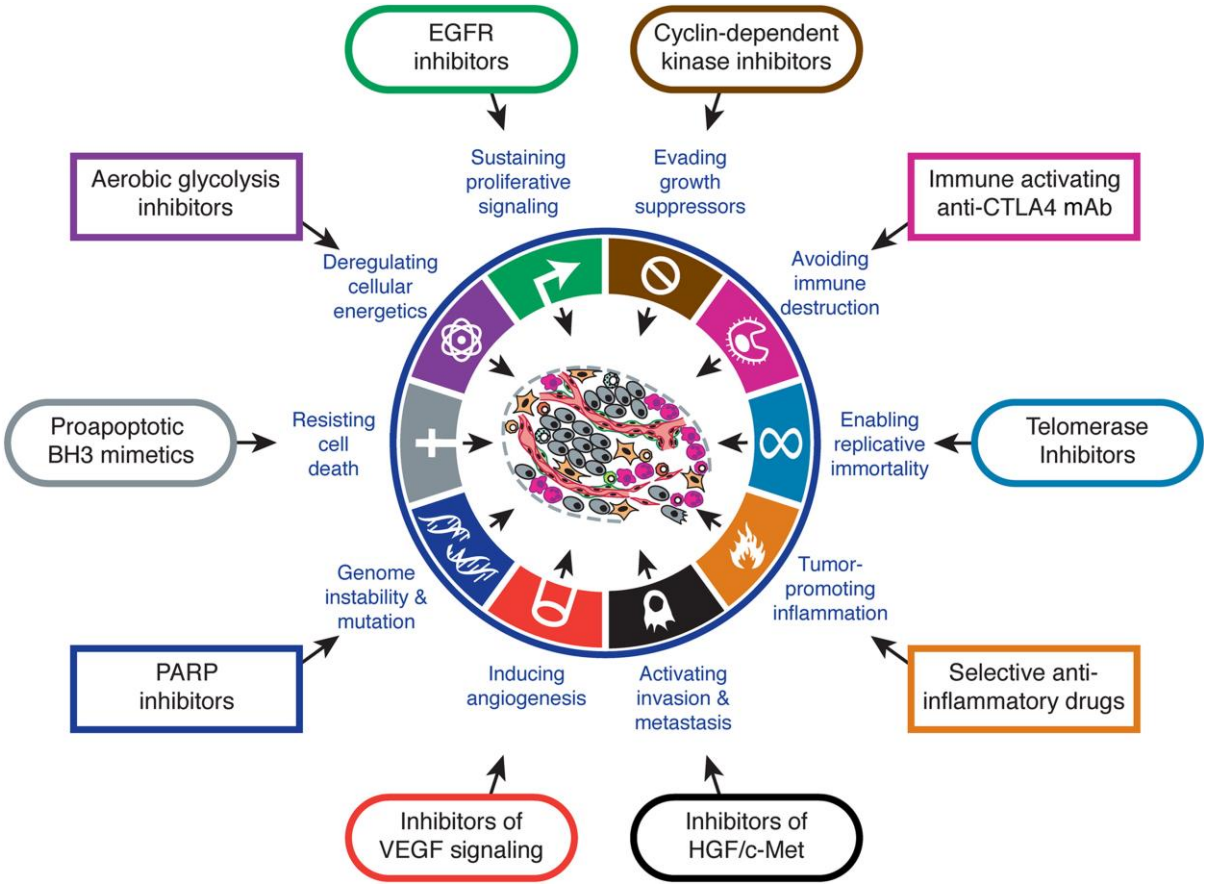


Figure 1.3 Therapeutic Targeting of the Hallmarks of Cancer (Reproduced from Hanahan and Weinberg 2011)

1.3.2 Hereditary Colorectal Cancer

In certain colorectal cancer cases the specific cause is more obvious. This includes either specific diseases or medical conditions that predispose patients to the development of colorectal cancer or inherited forms of the disease.

1.3.2.1 Inflammatory Bowel Disease

Inflammatory bowel disease such as ulcerative colitis or Crohn's disease is associated with an increased risk of developing colorectal cancer. Ulcerative colitis and colorectal cancer increases with time, with the risk being approx. 2% at 10 years, 12% at 20 years and 18% at 30 years (Eaden et al., 2001). The link between colorectal cancer and Crohn's disease is not as well documented; however it is thought to be around 3% at 10 years duration (Canavan et al., 2006). This increase in risk related to inflammatory bowel disease is likely due to a chronic inflammatory state where continual production of inflammatory mediators such as tumour necrosis factor (TNF) and IL-6 promote signalling and prevent apoptosis of tumour cells (Canavan et al., 2006).

1.3.2.2 Familial Adenomatous Polyposis

Familial Adenomatous Polyposis (FAP) is an inherited condition that is characterised by hundreds or thousands of tiny polyps (adenomas) in the colon. If untreated, the risk of developing colorectal cancer is almost 100%. FAP is an autosomal dominant condition caused by a germline mutation in the adenomatous polyposis coli (APC) gene on chromosome 5. Patients usually develop cancer around the age of 40. Patients should initially undergo regular endoscopic surveillance but most will eventually have a prophylactic procto-colectomy (Galiatsatos and Foulkes, 2006).

Patients with FAP can also develop benign tumours called desmoids, which often recur after they have been removed. Furthermore, patients with FAP can develop polyps (benign or malignant) in other areas of the body, most commonly the duodenum and stomach (Belchetz et al., 1996).

1.3.2.3 Hereditary Non-polyposis Colorectal Cancer

Hereditary Non-polyposis Colorectal Cancer (HNPCC) or Lynch Syndrome is a common hereditary cause of colorectal cancer caused by an autosomal dominant genetic mutation of

the DNA mismatch repair genes. It accounts for approximately 3% of all colorectal cancers (Schlussel et al., 2014).

Patients with HNPCC tend to be young, have a predominance to develop right sided colonic tumours (70% proximal to splenic flexure) and often present with synchronous or metachronous tumours (Lynch et al., 1997). Patients with Lynch syndrome can also develop tumours in other organs such as the endometrium, ovary, small bowel and hepatobiliary tract. Colorectal tumours in Lynch Syndrome are more commonly poorly differentiated or mucinous and often have a strong local inflammatory infiltrate (Jass, 1998). This coupled with the young age of these patients confers a fairly good prognosis for these colorectal cancers.

1.4 Clinical Presentation

The presentation of colorectal cancer depends on the location of the tumour and the extent of the disease present. Some patients will present with symptoms related to the primary tumour. These symptoms vary dependent on the location of the tumour itself. Other patients will have no symptoms at all and will have their colorectal cancer diagnosed via the Bowel Screening Programme and some patients will present with symptoms from distant metastases rather than symptoms from primary disease.

1.4.1 Symptomatic Primary Disease

Common symptoms associated with colorectal cancer include abdominal pain, alteration in bowel habit, rectal bleeding, and weight loss (Thompson et al., 2007, Astin et al., 2011). These symptoms may be present together or in part and are dependent on the location of the tumour itself. Right sided lesions tend not to present with rectal bleeding as any blood present in the lumen of the bowel tends to be degraded during passage of stool around the colon. Right sided cancers therefore tend to cause occult bleeding and often patients present with iron deficiency anaemia, and abdominal mass or abdominal pain (Khanbhai et al., 2014). Conversely, distal or left sided lesions often present with change on bowel habit, rectal bleeding or lower abdominal pain (Cappell, 2005). Distal rectal lesions often cause a change in bowel habit, rectal bleeding, tenesmus and urgency (Cappell, 2005). Around 10-20% of patients will present as an emergency with obstruction or perforation having had little symptoms prior to this episode (Thompson et al., 2007).

1.4.2 Bowel Screening Programme

The Scottish National Bowel Screening Programme was introduced in pilot form in 2007 and by 2009 had been rolled out across all Scottish Health Boards (Mackay et al., 2014). Patients aged 50-74 are invited to participate in the screening programme on a biennial basis. Currently, the guaiac based faecal occult blood test (gFOBt) is used as the primary screening tool *(Figure 1.4)*. This measures the peroxidase activity of haematin in faeces (Mansouri et al., 2013). Patients are invited to participate and to send 2 samples from 3 different bowel motions to the national centre for processing. If the gFOBt is positive then they are subsequently invited for colonoscopy following a pre-assessment check. If the gFOBt is weakly positive (1-4 windows out of 6 positive) then they are re-tested with a Faecal Immunochemical Test (FIT) – targets human haemoglobin and is a quantitative test- which has a higher sensitivity and similar specificity to gFOBt. If the gFOBt is negative

then nothing further will happen and the patient will be invited to undertake another screening test in 2 years (Mansouri et al., 2013).

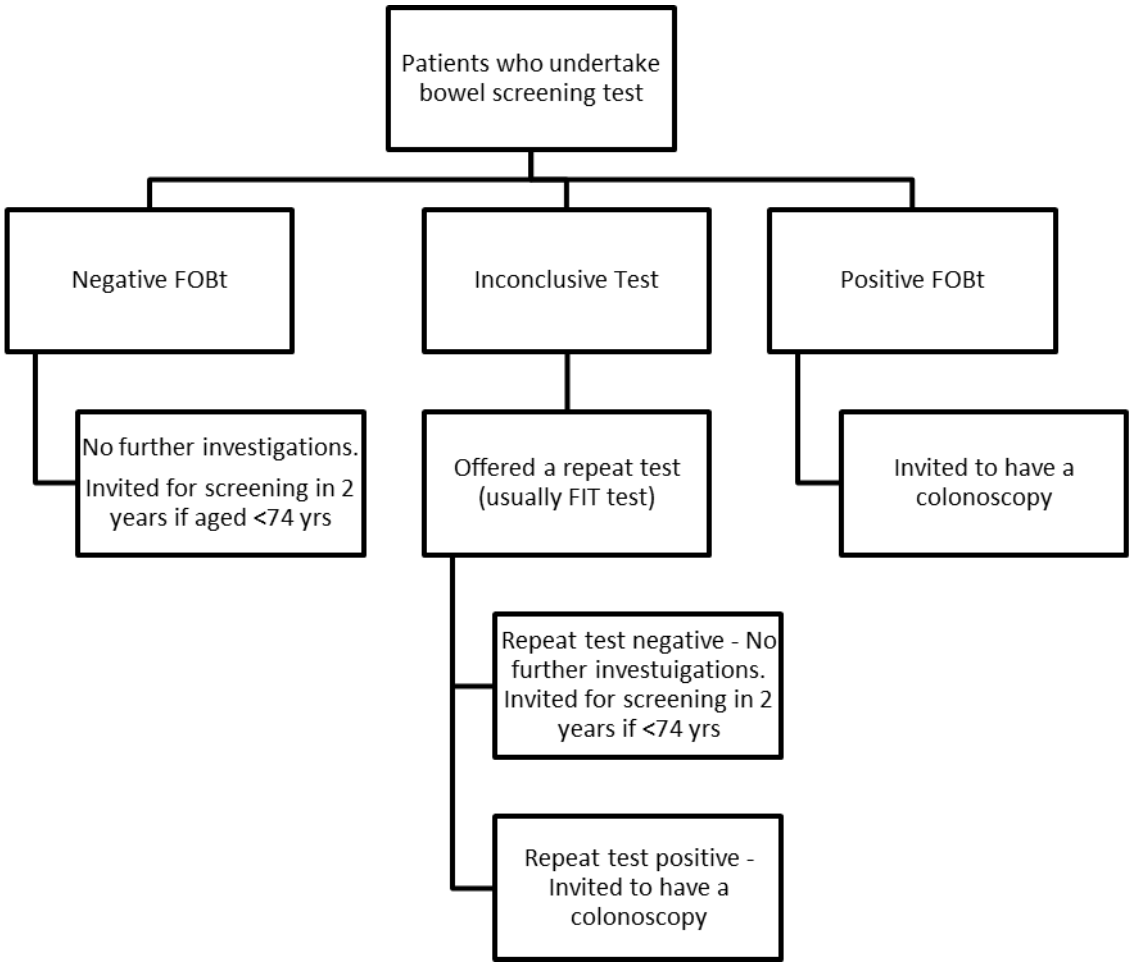


Figure 1.4 Overview of the Scottish National Bowel Screening Programme

Over the first 3 rounds of the pilot programme uptake was around 55% with the positivity rate of approximately 2% and cancer detection rate of approximately 1.5 per 1000 screened (Steele et al., 2009). It is believed that in the long term, an effective bowel screening programme will reduce the incidence of colorectal cancer (by removing pre-cancerous polyps at colonoscopy) and increase the detection of early stage cancers.

1.4.3 Metastatic Disease

Some patients will present not with symptoms of the primary tumour but with symptoms from metastatic disease. Colorectal cancer can metastasise via the lymphatics, blood vessels or transperitoneally. Drainage of the abdominal cavity is via the portal vein and so haematogenous spread tends to affect the liver first, followed by the lungs and bone.

However, in low rectal lesions, drainage is via inferior rectal vein which drains into the inferior vena cava rather than the portal system and so may metastasise to the lungs initially. Therefore symptoms can be related to multiple sites in the body and can include upper abdominal pain or jaundice from liver metastases; shortness of breath from lung metastases; confusion or focal neurology from cerebral metastases or bony pain or discomfort from bony metastases.

1.5 Investigations and Diagnosis

In those patients who are symptomatic, the diagnosis of colorectal cancer is made using a combination of history taking, clinical examination and diagnostic tests. Histopathological confirmation of the presence of malignancy is required prior to elective surgical intervention. In some situations, more than one diagnostic test may be required to obtain a definitive diagnosis. Furthermore, accurate staging of the disease is required to ensure appropriate management of the disease present, be that treatment with curative intent or palliative treatment.

1.5.1 Blood Tests

There is no specific blood test that is capable of detecting the presence of colorectal cancer. Routine blood tests such as a full blood count may show a microcytic anaemia that may give an indication that there is occult blood loss from the gastrointestinal tract and raise suspicions of a malignant process. Other blood tests such as measures of renal function, inflammatory status and liver function may be beneficial in assessing the general health of the patient, assessing any suspicions for the presence of metastatic disease and offering some prognostic information regarding outcome of any future treatment.

1.5.2 Endoscopic Evaluation

Endoscopic assessment of the large bowel is the gold standard investigation where colorectal cancer is suspected. Colonoscopy therefore should be offered to patients who have no significant co-morbidities and if a suspicious lesion is seen it should be biopsied in order to obtain a histological diagnosis (NICE, 2011). Colonoscopy is a relatively safe procedure with a high sensitivity for detecting colorectal cancer. It allows biopsies of suspicious lesions and the opportunity to perform polypectomy (removal of pre-cancerous polyps endoscopically). It does not involve any ionising radiation but may require some intravenous sedation to tolerate the examination. The risks associated with colonoscopy include bleeding, perforation (0.01%), failure to examine the entirety of the large bowel and the possibility of missed lesions (Bowles et al., 2004, Arora et al., 2009). Patients who have a failed colonoscopy should either be offered a repeat colonoscopy or a CT Colonography (see below).

1.5.3 Computed Tomography

Computed tomography (CT) colonography can be used as an alternative to colonoscopy in diagnosing colorectal cancer. It is generally reserved for patients who are not suitable for colonoscopy or who have had a failed colonoscopy and is generally well tolerated (Koo et al., 2006, Rosman and Korsten, 2007, Ng et al., 2008). However, unlike with colonoscopy, histological diagnosis is not possible and if deemed necessary then the patient will require a colonoscopy.

CT also plays a role in the staging of patients with colorectal cancer. Contrast-enhanced CT of the chest, abdomen and pelvis is routinely performed in all patients who are diagnosed with colorectal cancer. It allows local staging of colonic tumours and also detection and assessment of distant metastases.

1.5.4 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is not routinely used to detect colonic lesions but can be useful in some circumstances (Zijta et al., 2010). It can also be used to further assess suspicious lesions identified from staging CT scans situated in the liver. MRI allows accurate assessment of liver metastases and suitability of them being amenable to resection or ablation (Blyth et al., 2008).

MRI is also utilised in local staging of rectal cancers as it has been shown to be superior to CT in this regard. It enables clinicians to accurately assess factors such as the circumferential resection margin, venous invasion, tumour and nodal staging. In doing so it permits selection of patients who would be suitable to go straight for surgery and those who would benefit from neoadjuvant therapy prior to surgical resection (Mercury Study Group, 2006, Purkayastha et al., 2007).

1.5.5 Staging of Colorectal Cancer

The staging of colorectal cancer allows quantification of the extent of disease and provides a framework for deciding on the appropriate treatment for the patient. The most commonly used staging system worldwide is the Tumour, Node, Metastases (TNM) system that was produced by the American Joint Committee on Cancer (AJCC) (AJCC, 1992). This system stages the cancer using three components, the tumour itself (T), regional lymph node involvement (N) and the presence of distant metastases (M). These are combined to form

stage groupings *(Table 1.1).* In the UK an alternative staging system called the Duke's Classification is also still used. This stages the cancer from stage A to D and is similar to the TNM system, using tumour grade, nodal involvement and the presence of distant metastases.

Table 1.1 TNM staging system for colorectal cancer

Primary Tumour (T)	
T_x	Tumour cannot be assessed
T_{is}	Carcinoma in situ
T_1	Tumour invades submucosa
T_2	Tumour invades muscularis propria
T_3	Tumour invades subserosa
T_4	Tumour directly invades into adjacent organs or structures or through the visceral peritoneum
Regional Lymph Nodes (N)	
N_x	Lymph nodes cannot be assessed
N_0	No lymph node metastases
N_1	Metastases in 1-3 lymph nodes
N_2	Metastases in ≥4 lymph nodes
Distant Metastases (M)	
M_x	Metastases cannot be assessed
M_0	No distant metastases
M_1	Distant metastases present

Final staging of colorectal cancer relies on pathological assessment of the resected specimen. This can only be performed following surgery. Prior to treatment, staging is reliant on clinical examination, direct visualisation of the tumour and radiological imaging of the rest of the body. A CT of chest, abdomen and pelvis is performed to assess the invasiveness of the tumour itself as well as to assess involvement of the regional lymph nodes and detect potential distant metastases. Patients with suspicious lesions that may represent distant metastases may require further imaging such as Positron Emission Tomography (PET) CT or MRI to further clarify whether metastatic disease is present prior to surgical intervention. In patients with rectal cancer, an MRI of the pelvis is also performed to assess the circumferential resection margin and extension of disease out with the mesorectum. Patients where the circumferential resection margin may be involved may benefit from neo-adjuvant chemoradiotherapy prior to surgical resection (Brown and Daniels, 2005).

Whilst complete radiological staging is preferable prior to surgery it may not always be possible as some patients will require an emergency operation for a complication related to a newly diagnosed cancer. In these patients radiological staging can be completed following surgery.

1.5.6 Multi-disciplinary Team Meetings

The multi-disciplinary team (MDT) has become an essential component of the investigation and treatment of patients with suspected colorectal cancer (Wood et al., 2008). It involves regular meetings of a group of health professionals from multiple disciplines including surgeons, radiologists, pathologists, oncologists and nurse specialists, each with specialised knowledge in their field that relates to the management of colorectal cancer (Fleissig et al., 2006). These regular meetings allow well-co-ordinated and high quality patient centred care, facilitating prompt and appropriate diagnostic techniques, evidence-based decision making and top quality treatment from a range of professions (Fleissig et al., 2006, Taylor et al., 2010). Their routine use has ensured that each patient receives the most appropriate treatment for their cancer. The use of MDT meetings has seen improvement in 5 year survival in colorectal (Morris et al., 2006a) and oesophageal cancer (Stephens et al., 2006), improved 2 year survival in head and neck cancer (Birchall et al., 2004) and improved survival in lung cancer (Coory et al., 2008). Furthermore, in rectal cancer they have been reported to reduce the rate of positive circumferential resection margins (Burton et al., 2006).

1.6 Management of Colorectal Cancer

The management of patients with colorectal cancer has significantly evolved over the last 2 decades. Treatment is multi-faceted involving surgical resection, adjuvant and/or neo-adjuvant therapy and palliative therapy (surgical or oncological). For the majority of patients, surgery will be the main treatment option and represents the best chance at cure.

1.6.1 Surgery

Surgery for patients with colorectal cancer should only be performed by appropriately trained surgeons who are part of a multi-disciplinary team (ACPGBI, 2007). Approximately 90% of surgery performed will be planned or elective surgery. The remainder of those who undergo surgery will have an emergency operation. Not everyone diagnosed with a colorectal cancer will undergo operative intervention. For some, their disease is not curable at diagnosis and for others the risks of surgery (usually due to medical co-morbidities) will outweigh potential benefits of surgical resection. In these patients, non-operative strategies to palliate symptoms and the disease should be adopted (see below).

1.6.1.1 Elective Surgery

The majority of patients will undergo elective surgery for their colorectal cancer. These patients will have been diagnosed by attending the outpatient department with symptoms requiring investigation or through the National Bowel Screening Programme.

The goals of surgical resection are to remove the tumour along with a section of bowel and its feeding blood vessels and lymph nodes. This ‘oncological resection’ is determined by the location of the tumour itself and its blood supply. Once the surgical specimen is removed, intestinal continuity is usually restored by anastomosing the remaining two ends of bowel together either with sutures or stapling devices. Where this is not possible or desirable the distal end of bowel is exteriorised onto the abdominal wall with a stoma.

In those patients with rectal cancers, the surgeon must decide whether the anal sphincter complex can be preserved whilst ensuring the bowel can be divided distal to the tumour without compromising oncological clearance. If this is not possible then an abdomino-perineal resection (involving removing the anus and sphincter complex) with a permanent end colostomy should be undertaken. More recently, a more radical resection involving

removing the levator muscles has been described, the so-called cylindrical or extra-levator APR with greater oncological clearance and reduced risk of local recurrence (Dalton et al., 2012).

In patients with tumours situated below the peritoneal reflection, the tumour should be excised following the principles of TME (Total Mesorectal Excision). First described by Heald and colleagues it entails removing the specimen with the mesorectum intact. The mesorectum envelopes the bowel and by removing it intact has been shown to reduce the rates of local recurrence (Heald et al., 1982).

1.6.1.2 Emergency Surgery

In some patients, the presence of tumour perforation, bowel obstruction or bleeding will necessitate emergency surgery. Whilst the principles of surgical resection remain the same as those undergoing elective surgery, restoring intestinal continuity is sometimes not attempted due to the patients general condition and comorbidities and the higher risk of anastomotic dehiscence. Patients undergoing emergency surgery have been reported to have higher complication rates, poorer cancer-specific survival and increased mortality rates compared to elective surgery (McArdle and Hole, 2004, Crozier et al., 2009). Increased access to diagnostic investigations and the introduction of bowel screening have meant that the number of emergency procedures has decreased over the last few decades but a substantial number of patients still present as an emergency.

1.6.1.3 Surgery Performed by Specialists

Prior to subspecialisation of surgeons, surgery for colorectal cancer was performed by a variety of surgeons with varying outcomes. McArdle and Hole reported a wide range in the rates of mortality, anastomotic leak, curative resection and survival in patients having surgery for colorectal cancer between 1974 and 1979 (McArdle and Hole, 1991).

Reorganisation of cancer services has meant that in the main, elective colorectal cancer surgery is performed by specialist colorectal surgeons. In practice, this means fewer surgeons perform colorectal cancer surgery but each surgeon performs more specialist procedures on an annual basis. This has resulted in improved long term survival for patients having elective colorectal cancer surgery (Oliphant et al., 2013).

1.6.2 Enhanced Recovery after Surgery (ERAS)

Enhanced Recovery after Surgery (ERAS) protocols have become a routine part of post-operative care. ERAS is a method of peri-operative care involving multimodal, protocol driven strategies designed to improve morbidity and shorten hospital stay following surgery (Wilmore and Kehlet, 2001).

The number of components used in each ERAS protocol varies from centre to centre. Furthermore, there is evidence to suggest that only certain components such as laparoscopic surgery, early enteral nutrition and early mobilisation play a role in improving outcomes following surgery (Vlug et al., 2012). Regardless of how they are constructed, ERAS protocols have been shown to reduce morbidity and shorten length of stay following elective colorectal surgery (Rawlinson et al., 2011).

1.6.3 Neoadjuvant Therapy

In patients with rectal cancer there is a role for pre-operative radiotherapy to downstage the tumour. It has become standard practice to utilise pre-operative chemo-radiotherapy for patients with T3/4 tumours, nodal disease or where the circumferential resection margin is threatened on pre-operative staging MRI scans (Engstrom et al., 2009).

Pre-operative radiotherapy can be delivered in conventional fractionation (45-50 Gy in 25 fractions over a 5 weeks period) or short course therapy (25 Gy in 5 fractions daily for 1 week). Conventional fractionation is usually combined with synchronous 5-fluorouracil (5-FU) chemotherapy, known as chemo-radiotherapy, in order to improve its efficacy. Surgery is usually performed 5-8 weeks following this in order to allow for maximal tumour shrinkage. With short course radiotherapy, surgery happens within 10 days of it being complete, as such it is not useful for downsizing tumour bulk but has been shown to reduce local recurrence rates (Colorectal Cancer Collaborative, 2001). Which of these methods of delivering pre-operative radiotherapy are optimal remains a controversial topic.

1.6.4 Adjuvant Therapy

Adjuvant therapy is treatment given after surgery in order to reduce the risk of cancer recurrence. The mainstay of this treatment is chemotherapy. Adjuvant chemotherapy is offered to patients deemed to have a high risk of recurrence. These high risk features include: node positive disease, serosal involvement (T4), perforated or obstructed tumours,

poorly differentiated tumours and tumours with extra-mural venous invasion (ACPGBI, 2007). Chemotherapies are toxic chemicals that damage normal cells as well as tumour cells. As such, patients often experience side effects including fatigue, nausea and vomiting, diarrhoea, neutropenia, myelosuppression and peripheral neuropathy.

Which specific chemotherapeutic agents to use is a vast topic that is dependent on a variety of tumour biological markers. Potential agents include 5-FU, FOLFOX (folinic acid, 5-FU and oxaliplatin), capecitabine or Xelox (capecitabine and oxalipatin). The duration of therapy varies but typically lasts from 6-8 months. In node positive disease, adjuvant chemotherapy can improve overall survival by 13% (NICE, 2006).

In rectal cancers, where there is a positive resection margin, adjuvant radiotherapy can be used as a salvage technique, as long as they did not receive pre-operative radiotherapy. However, the benefit on prevention of local recurrence is smaller compared with pre-operative radiotherapy (Colorectal Cancer Collaborative, 2001).

1.6.5 Palliative Therapy

In patients with inoperable disease, metastatic disease or who are not medically fit for resection then palliative chemotherapy can be used to limit disease progression and treat symptoms. Patient selection is important as the side effects from chemotherapy can be detrimental to the patient's quality of life and performance status has been reported to be an important factor in outcomes with palliative therapies (Thirion et al., 1999, Simmonds, 2000). Some studies have shown that compared to best supportive care, palliative chemotherapy can improve life expectancy by approximately 3-6 months (Simmonds, 2000).

Ultimately patients may be left with no therapeutic options and in these situations quality of life and symptom control are of the upmost importance. Good communication between the surgical team, oncology team and palliative care team are important in ensuring the patient has adequate control of their symptoms.

1.7 Prognostic Factors

There are a number of tumour related factors that have been reported to have prognostic value in patients with colorectal cancer. These range from the stage and grade of tumour to various tumour characteristics that have been described as conveying high risk of recurrence and poor long term outcomes. These prognostic factors are often used in determining whether patients' should receive adjuvant therapy (see above) following surgical resection.

1.7.1 Duke's Classification

Originally described by Cuthbert Dukes in 1937 (Dukes, 1937), his classification of rectal cancers has undergone multiple modifications since its original description to attempt to improve stratification, include colon cancers and those with metastatic disease. Dukes system stratifies cancers dependent on their depth of invasion into the bowel wall, the presence (or absence) of lymph node metastasis and presence of distal metastases. Dukes A describes a tumour involving the mucosa or submucosa only; Dukes B1 tumours extend to the muscularis propria with Dukes B2 tumours penetrating through the muscularis propria. Dukes B tumours by definition have no lymph node involvement. In contrast, Dukes C tumours have lymph node involvement. Dukes C1 tumours extend into the muscularis propria but not through it and Dukes C2 tumours extend through the muscularis propria. Dukes D tumours involve distant metastases. Whilst its use has been superseded by the TNM staging system (see below) most pathological specimens in the UK are still described in terms of Dukes staging as well as TNM staging.

Table 1.2 Dukes stage and 5-year survival in patients with colorectal cancer (Adapted from National Cancer Intelligence Network 2009)

Stage	5-year Survival
Dukes A	93%
Dukes B	77%
Dukes C	48%
Dukes D	7%

1.7.2 TNM Classification

The tumour, node, metastases (TNM) classification was developed by the American Joint Committee on Cancer (AJCC) and International Union against Cancer (UICC). It is

derived based on the invasiveness of the tumour itself (T-stage), the degree of regional lymph node involvement (n-stage) and the presence of distant metastases. It is updated on a regular basis, currently there are 7 editions but, in the UK, the current recommendation from the Royal College of Pathologists is to use version 5 (RCPath, 2014). Both TNM and Dukes staging are reliant on a good quality surgical specimen and also a satisfactory lymph node harvest in order to adequately stage the tumour (Johnson et al., 2006). A specimen should contain at least 12 lymph nodes (RCPath, 2014) and a specimen containing fewer lymph nodes than this can result in a tumour being under-staged. A comparison of TNM staging and Dukes staging is shown in *Table 1.3*.

Table 1.3 Comparison of TNM Staging and Dukes Classification (Adapted from John Hopkins Colon Cancer Website – Staging of Colorectal Cancer)

TNM Classification				**Dukes' Classification**
Stages	**T**	**N**	**M**	**Stages**
Stage 0	Tis	N0	M0	
Stage I	T1	N0	M0	A
	T2	N0	M0	B1
Stage II	T3	N0	M0	B2
	T4	N0	M0	B2
Stage III	T1, T2	N1 or N2	M0	C1
	T3, T4	N1 or N2	M0	C2
Stage IV	Any T	Any N	M1	D

1.7.3 Grade of Tumour

Tumour differentiation grade is a subjective measure of how well the tumour is differentiated. Colorectal tumours can be characterised as low grade (well or moderately differentiated) and high grade (poorly differentiated). A high tumour differentiation grade has been described as an adverse prognostic indicator (Compton, 2003). Furthermore, tumour grade differentiation has been shown to be associated with TNM stage, T stage and lymph node metastasis (Derwinger et al., 2010). A higher grade tumour is significantly more likely to be associated with a higher positive lymph node rate in stage III (Dukes' C) disease.

1.7.4 Venous Invasion

Venous invasion is diagnosed pathologically when there are tumour cells seen within an endothelium lined space surrounded by smooth muscle and/or containing red blood cells

(Sternberg et al., 2002). The presence of venous invasion is now well recognised as an adverse prognostic indicator (Roxburgh et al., 2010a, Roxburgh et al., 2010b). The use of elastic staining improves the detection rates of venous invasion and has been reported to improve its use a prognostic marker in colorectal cancer and forms part of the routine pathological dataset (RCPath, 2014).

1.7.5 Perineural Invasion

Perineural invasion (PNI) occurs when tumour cells invade the nerves and spread along the nerve sheaths (Liebig et al., 2009). In colorectal cancer, the presence in the resected specimen of perineural invasion is said to be a high risk factor for recurrence and poorer survival with some studies reporting a 4-fold increase in survival for patients with PNI-negative tumours (Liebig et al., 2009). Its presence is under-reported in colorectal specimens and it may explain the difference in outcomes seen in those with node-negative disease.

1.7.6 Peritoneal Involvement

Peritoneal involvement is present if there are tumour cells visible on the peritoneum or free in the peritoneal cavity. Again its presence is a poor prognostic indicator and has been reported to be associated with disease recurrence and metastases (Morris et al., 2006b, Stewart et al., 2007).

1.7.7 Tumour Perforation

Tumour perforation occurs when a visible defect is identified during pathological analysis in the bowel wall through to the lumen of the bowel. This is a poor prognostic indicator and has been associated with tumour recurrence and metastases, independent of tumour stage. (Petersen et al., 2002)

1.7.8 Margin Involvement

A positive longitudinal or circumferential resection margin is one that has tumour cells at the margin or within 1mm of the margin. It indicates that the tumour has not been fully excised and as such is a well-documented poor prognostic marker for local recurrence and metastatic disease. Particularly in rectal cancer, a positive circumferential resection margin (CRM) is associated with the development of metastatic disease and poor survival (Nagtegaal and Quirke, 2008).

1.7.9 Petersen Index

Petersen and colleagues attempted to clarify why there were such differences in outcomes in patients with stage II (Dukes' B) disease. The aim was to identify patients who would benefit from adjuvant therapy. They assessed 268 colonic cancers, staged as Dukes' B tumours and evaluated all pathological factors that could be of prognostic benefit. On multivariate analysis, 4 factors were independently prognostic – peritoneal involvement, venous invasion, margin involvement and tumour perforation (Petersen et al., 2002). The presence of each was awarded 1 point with the exception of tumour perforation which was awarded 2 points. Patients were then grouped in to those at low risk (score 0-2) and those at high risk (score 3-5). The Petersen index was subsequently validated another cohort of patients with Dukes' B disease by identifying those at higher risk of disease recurrence and poorer survival (Morris et al., 2007).

1.7.10 Lymph Node Ratio

The lymph node ratio (LNR) is calculated by dividing the number of positive lymph nodes (lymph nodes with tumour deposits present) by the total number of lymph nodes harvested. In a study of 26,181 patients a ratio of 0.4 was used as a cut off to divide the patients into 2 groups and reported that the LNR was a strong independent indicator for cancer-specific survival (De Ridder et al., 2006). The use of a ratio has some advantages over the total lymph node yield as there is less chance of under-staging and under-treating patients as may happen if the lymph node yield was poor. This is perhaps why it is seen as a useful prognostic marker. Despite this, the LNR is not commonly used possibly because there is no consensus on the optimal cut off for dividing patient groups, hence limiting its usefulness as a prognostic marker.

1.7.11 Tumour Necrosis

Tumour necrosis is a feature of solid tumours. It is believed that it occurs because the tumour becomes ischaemic due to rapid growth and expansion. Therefore it has been associated with aggressive, more advanced tumours and is associated with poor prognosis. In a study of 343 patients undergoing surgery for colorectal cancer, tumour necrosis was associated with a systemic inflammatory response, TNM stage, and Petersen index. Furthermore, it was also associated with reduced cancer-specific survival (Richards et al., 2012b).

1.8 Inflammation and Cancer

It is now recognised that disease recurrence and long term survival in all tumour types is not solely influenced by the characteristics and prognostic factors related solely to the tumour (see above). Whilst these factors undoubtedly play an important role in providing vital prognostic information they do not fully explain the differences in outcomes seen in patients with cancers of the same pathological stage e.g. Dukes' B colorectal cancer.

Patient or host characteristics which can be modifiable such as exercise tolerance, BMI and smoking status as well as non-modifiable characteristics such as age and sex are also related to outcomes in patients with cancer.

Furthermore, the presence or absence of an inflammatory response either locally at the tumour site or in the patient generally (systemically) affects the ability of the body to generate an adequate anti-tumour response.

A link between inflammation and cancer has long been established. In 1863 Rudolph Virchow noted the presence of leucocytes in tumours and made the connection between inflammation and cancer (Balkwill and Mantovani, 2001). Indeed, chronic inflammatory conditions such as Inflammatory Bowel are known to increase the risk of developing colorectal cancer. So much so that inflammation is now considered one of the 'Hallmarks of Cancer', its presence enabling tumour cells to survive, proliferate and disseminate (Hanahan and Weinberg, 2011).

1.8.1 Host Response

The body's immune system is responsible for protecting the body against foreign pathogens. It is composed of a non-specific component (innate) and a specific component (adaptive). Indeed some of the body's own defence mechanisms can promote tumour progression as well as others its destruction. Rather than one specific component of the body's immune system being key, a homeostatic balance between these pro and anti-tumour effects appears the most important determinant affecting cancer outcomes (Roxburgh et al., 2013).

1.8.1.1 The Innate Immune System

The innate immune system does not confer long lasting immunity but serves as an immediate defence mechanism to pathogens and includes physical, chemical and microbiological barriers as well as elements of the immune system (Parkin and Cohen, 2001). It involves recruitment of myeloid cells such as neutrophils, macrophages/monocytes, eosinophils, basophils, mast cells, dendritic cells and natural killer cells. Furthermore, antigen presenting cells from the innate immune system such as dendritic cells provide a basis for activation of the adaptive immune system (Turvey and Broide, 2010). To enhance these cellular defence mechanisms, a humoral component of the innate immune system includes complement proteins and acute phase proteins such as CRP. These proteins are involved in both sensing pathogens and facilitating clearance of pathogens (Turvey and Broide, 2010). Moreover, innate immune cells need to be recruited to the site of inflammation and activated appropriately. This involves interaction of cellular receptors such as adhesion molecules and external molecules such as cytokines. Cytokines are produced by cells all over the body and have a variety of different functions including cell activation, division, apoptosis or cell movement (Parkin and Cohen, 2001). Cytokines that are produced by leucocytes and mainly affect white cells are referred to as interleukins and those which have chemoattractant activity as known as chemokines.

CRP is a pentameric protein found in blood and produced by the liver. It is a key component of the innate immune system. It primarily acts as an opsonin and acts to enhance clearance of pathogens (Gabay and Kushner, 1999). It has both pro and anti-inflammatory properties and has been reported to begin rising several hours after injury, peaking at 48 hours post injiury. CRP concentrations following surgery vary depending on the type of procedure. Whether CRP could be used as a marker of the degree of trauma in the body remains to be seen.

Interleukin-6 (IL-6) is a pro-inflammatory cytokine that is produced throughout the body. It is produced in response to injury and stimulates both CRP and neutrophils to migrate to the site of injury. It also peaks and falls following injury but at an earlier stage compared to CRP.

Neutrophils are the most abundant cell that constitutes the white cell count. They are a key part of the innate immune system and act as first responders, migrating to the site of injury and beginning to destroy invading microbes either by phagocytosis, secretion of anti-

microbial substances or formation of neutrophil extracellular traps (NETs). Neutrophils can exhibit both pro and anti-tumour properties but elevated circulating concentrations have been shown to be associated with poorer outcomes in a variety of cancers (Atzpodien and Reitz, 2008).

1.8.1.2 The Adaptive Immune System

Unlike the innate immune system, the adaptive immune system provides a specific response against a pathogen. It is mainly composed of lymphocytes and responds to recognition of 'non-self' antigens. It provides a stronger response to these antigens and also enables the body to create an immunological memory of these antigens, should they be encountered again. Adaptive immunity involves both humoral (B cells) and cell-mediated immunity (T-cells) although they often work together. Adaptive immunity begins when antigen-presenting cells recognise the presence of foreign antigens. B cells produce antibodies (immunoglobulins) in response to specific antigens. These antibodies bind to these antigens, triggering the complement cascade and alerting phagocytes of their presence in order for them to be destroyed (Janeway, 2001).

There are several different types of T-cells that make up the cell mediated response: helper T cells (CD4+), cytotoxic T cells (CD8+), memory T cells (C45R0+) and regulatory T cells (FOXP3+). Each has its own role to play in detection, and removal of foreign antigens from the body (Janeway, 2001). Cytotoxic T cells are responsible for cell death, releasing cytotoxins that destroy the specific antigen.

1.8.2 The Tumour Microenvironment

Solid tumours consist of the tumour cells themselves, which are contained within a complex system of mesenchymal and inflammatory cells. This is known as the tumour microenvironment and as well as tumour cells, contains the invasive margin, stroma, blood vessels and lymphatics (Balkwill et al., 2012, McAllister and Weinberg, 2014). It has recently become apparent that interactions between the tumour cells and the tumour microenvironment are responsible for regulating tumour growth and progression (McAllister and Weinberg, 2014). These interactions are controlled by a complex system of cytokines, chemokines, growth factors and inflammatory enzymes. Indeed, the structure and functions of the tumour microenvironment are said to be similar to the processes of wound healing or inflammation (Balkwill et al., 2012). Whilst there has been particular interest towards the immune cells within the tumour microenvironment, which have been

reported to influence tumour progression and outcomes, targeting other non-tumour cells or mediators of communication between tumour microenvironment components may yet be of therapeutic benefit (Balkwill et al., 2012).

1.8.3 The Local Inflammatory Response

It has been well documented that the infiltration of inflammatory cells locally around the tumour is associated with improved cancer specific outcomes in patients with colorectal cancer, regardless of tumour stage (Roxburgh and McMillan, 2012, Richards et al., 2014). It has been assumed that this is a direct response from the patient and represents an effective immune response (Richards et al., 2014). There have been numerous studies that have attempted to clarify the exact components of this inflammatory infiltrate. What appears to be clear however is that an increasing density or number of these inflammatory cells, such as infiltrating T lymphocytes and macrophages, are associated with improved clinical outcomes. A number of scoring systems have been devised in an attempt to predict outcomes based on this local inflammatory response; however none have become part of routine clinical practice.

In the late 1980's Jass and colleagues devised a scoring system based on 4 pathological characteristics: the presence of peritumoural lymphocytic infiltrate, the invasive margin (expanding/infiltrating), limitation of growth to bowel wall and lymph node involvement (Jass et al., 1987). The presence of a peritumoural infiltrate was associated with improved outcomes. Despite being validated on multiple occasions as a stage independent determinant of cancer-specific survival (Roxburgh and McMillan, 2012), the subjective nature of its measurements have limited its widespread clinical use.

Klintrup and colleagues used semi-quantitative analysis of the peritumoural inflammatory infiltrate using standard Haematoxylin and eosin (H&E) stained sections. They reported that high grade inflammatory cell infiltrate at the invasive margin was associated with improved survival in patients with node negative colorectal cancer. Conversely, those with low grade inflammatory cell infiltrate had poor prognosis with a 5 year survival of 47%, despite these patients having node negative disease (Klintrup et al., 2005). This work was subsequently externally validated in a cohort of node negative colorectal cancer patients (Roxburgh et al., 2009).

The Galon Immune Score is based on quantitative immunohistochemistry on specific T-cell subtypes at the invasive margin and centre of the tumour (Galon et al., 2012). It has been shown to be associated with tumour recurrence (Mlecnik et al., 2011). However, the complexity of immunohistochemistry and the difficulty in reliably repeating this process has limited its widespread use.

Overall, while there is clear evidence that an increased inflammatory cell infiltrate, is a good prognostic marker. The presence of several different scoring systems utilising different methods of scoring and different cell types means their reproducibility and reliability, in terms of widespread clinical use, is limited.

1.8.4 The Systemic Inflammatory Response

As previously mentioned, the importance of inflammation in the growth and progression of cancer has become more apparent in the last decade. Inflammation itself is a normal physiological process that occurs in response to injury and indeed to surgical trauma. Whilst in these circumstances its response is measured and enables healing of the injured site; in cancer, the normally tight physiological controls are lost or weakened to such an extent that an exaggerated inflammatory response is often present. Rather than being beneficial, this exaggerated inflammatory response then results in massive release of pro-inflammatory cytokines, many of which are used by the tumour to aid tumour growth and progression.

In patients with cancer, whilst the presence of increased inflammatory infiltrate at a local level around the tumour itself has been shown to be beneficial, the presence of a systemic inflammatory response has been reported to be associated with disease progression, recurrence and poor long term survival, independent of tumour stage and tumour type (Roxburgh and McMillan, 2010, Proctor et al., 2011b, Guthrie et al., 2013a, McMillan, 2013b). Moreover, a systemic inflammatory response has been reported to be associated with functional decline, weight loss, cachexia and low levels of skeletal muscle (Scott et al., 2002, McMillan, 2009, Richards et al., 2012c, Douglas and McMillan, 2014) as well as increased development of post-operative complications following surgery for colorectal cancer (Moyes et al., 2009).

There are various ways of measuring the systemic inflammatory response. The stereotypical marker of the systemic inflammatory response is CRP, but other readily

available and easy to obtain biochemical markers such as albumin, white cell count and interleukin-6 (IL-6) are also good measures of the systemic inflammatory response. Given that patients with cancer often require multiple routine blood sampling, this has become an accepted method of monitoring these biochemical markers.

CRP is an acute phase protein synthesised in the liver and responds to a variety of inflammatory cytokines, the main one being IL-6 (Gabay and Kushner, 1999). It is used in a variety of settings clinically to monitor the response to illness and response to medical therapy. CRP acts by activating complement and binding with Fc receptors as well as acting as an opsonin for foreign pathogens. Several systematic reviews, including 105 studies involving 21,733 patients, have reported the prognostic value of CRP in patients with breast, gastric, hepatocellular, urological, colorectal, pancreatic and oesophageal cancer (Han et al., 2011, Yu et al., 2013, Zheng et al., 2013, Dai et al., 2014, Pathak et al., 2014, Stevens et al., 2014). Raised CRP concentrations were reported to be associated with poorer survival. Therefore, a raised CRP appears to be associated with poorer survival in patients with cancer, regardless of tumour type.

Albumin is a negative acute phase protein, synthesised by the liver. Its serum concentrations can be used to estimate synthetic liver function and are known to decrease in the presence of inflammation. This is the result of an increased demand of amino acids for acute phase protein synthesis which results in progressive loss of available protein such as albumin. It has also been reported as a prognostic marker in patients with cancer. A recent systematic review, including 59 studies involving a total of 16, 666 patients, reported the prognostic value of albumin in patients with cancer (Gupta and Lis, 2010). In these studies, lower serum albumin concentrations were associated with poorer survival in patients with cancer, regardless of tumour type.

IL-6 is a pro-inflammatory cytokine that is produced by many cells throughout the body in response to injury. IL-6 levels have been reported to rise from approximately 2 hours following stimulus and peak at approximately 12-24 hours. IL-6 production is primarily regulated by a negative feedback mechanism through suppressors of cytokine signaling (Socs) molecules, coded by genes of the JAK-STAT pathway (Kishimoto, 2010). IL-6 is thought to be the main inducer of acute phase proteins such as CRP from hepatocytes as well as causing differentiation, proliferation and maturation of haemopoietic progenitors.

The white cell count (WCC) is a group of inflammatory cells that can also be used as a marker of the systemic inflammatory response. The total white cell count consists of: neutrophils, lymphocytes, monocytes, basophils and eosinophils. Neutrophils are the major component of the WCC. They are 'first responders' present at the site of injury within 1 hour and peak at 24 hours. Levels begin to wane after 48 hours with neutrophils cleared from circulation via the liver, spleen and bone marrow (Kolaczkowska and Kubes, 2013). There is little evidence that a raised WCC has prognostic value in patients with cancer.

1.8.5 Systemic Inflammation Based Prognostic Scores

A number of prognostic scores based on various different combinations of these inflammatory markers have been derived. This has been driven by the need to standardise the measurement of the systemic inflammatory response and also provide a measure capable of predicting outcomes.

The most extensively validated of these scores is the modified Glasgow Prognostic Score (mGPS). This cumulative, prognostic score is based on thresholds of both CRP and albumin, It is scored as follows: CRP<10 mg/L scores 0, CRP >10 mg/L and albumin ≥35 g/L scores 1 and CRP >10 mg/L and albumin <35 g/L scores 2 (Roxburgh and McMillan, 2010). The mGPS has been shown to predict outcomes in patients with a range of solid tumours (Proctor et al., 2011b). A recent review of >50 studies involving >30,000 patients reported that a raised mGPS was independently associated with poor survival in patients with cancer, regardless of tumour type (McMillan, 2013b).

The neutrophil-lymphocyte ratio (NLR) is another prognostic score that has been extensively reported to predict outcomes in patients with cancer. The NLR takes the absolute neutrophil count and divides it by the absolute lymphocyte count, with a threshold of 5 originally used to distinguish between those with a high and those with a low NLR score (Walsh et al., 2005). Despite different centres utilising different thresholds to differentiate low and high scores the NLR has been extensively validated. In greater than 60 studies evaluating >37,000 patients, and an elevated NLR was reported to predict poor outcomes in patient with a variety of cancers (Guthrie et al., 2013a).

The platelet-lymphocyte ratio (PLR) is constructed from the absolute platelet count and lymphocyte count in a similar way to the NLR. An elevated PLR has been reported to be associated with reduced cancer-specific survival (Proctor et al., 2011a).

Despite these scores being extensively validated, their clinical use remains limited and mainly in clinical research.

1.9 The Physiological Response to Surgery

Following surgical intervention, normal physiological processes occur in the body to aid with tissue healing and recovery, known as the surgical stress response. Serum markers that can measure the degree of surgical trauma may be of clinical benefit to monitor patient recovery following surgery as well as objectively assessing whether operative techniques or multi-modality peri-operative care protocols such as Enhanced Recovery after Surgery (ERAS) modify this stress response or whether the patient is likely to develop post-operative complications that may impact on quality of life and long term survival.

1.9.1 The Surgical Stress Response

Injury to the body causes a stereotypical cascade of neuroendocrine, cytokine, acute phase and metabolic responses (Cuthbertson, 1979). Within minutes there is activation of the sympathetic nervous system resulting in a neuroendocrine response of increased secretion of catecholamines (adrenaline and noradrenaline) into the circulation leading to tachycardia, hypertension, fever and tachypnoea (Desborough, 2000). Simultaneously, there is increased secretion of the pituitary hormones such as corticotrophin, growth hormone and arginine vasopressin. Production and release of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), IL-8, IL-12, IL-18 and in particular interleukin 6 (IL-6) then follows (Marik and Flemmer, 2012, Dinarello et al., 2013). There are also changes in circulating myeloid cells, in particular towards increased numbers of white cells (WCC) dominated by neutrophils as well as increased numbers of myeloid derived suppressor cells and platelets as well as changes in plasma concentrations of a number of acute phase proteins, in particular CRP (Pepys and Hirschfield, 2003, Kao et al., 2006, Cole et al., 2008).

It has been hypothesised that the degree of elevation of these markers may reflect the degree of surgical trauma following surgery. However, to date no studies have compared these different markers of the systemic inflammatory response to try and ascertain which markers are the most reliable and clinically useful. If these could indeed be identified then these markers could be utilised to ascertain whether the benefit of laparoscopic surgery and multi-modal post-operative recovery programmes such as ERAS are due to a reduced post-operative systemic inflammatory response and reduced surgical trauma.

1.9.2 The Role of Enhanced Recovery after Surgery (ERAS)

Enhanced Recovery after Surgery (ERAS) involves multimodal, protocol driven perioperative care which proponents have asserted reduce the stress response to surgery (Wilmore and Kehlet, 2001). ERAS is now widely used in many different surgical specialties and procedures in surgical units across the UK and Europe. However, no standard protocol exists for such programmes and as such the number of components used varies between units, making comparison of different ERAS studies challenging (Neville et al., 2014). Despite this variability, ERAS has been widely reported to reduce hospital length of stay and complications following a range of surgical procedures (Greco et al., 2014).

The trauma of surgery leads to well understood metabolic, neuroendocrine and immune responses (see above), the aims of which are to promote physiological stability and wound healing (Cuthbertson, 1979). Although it is recognised that laparoscopic surgery generates a reduced post-operative systemic inflammatory response, evidenced by reduced post-operative concentrations of CRP, the impact of individual components of ERAS protocols on the systemic inflammatory response is unclear. Given that ERAS programmes have been developed to reduce patients' hospital stay and improve recovery, presumably by reducing the stress/systemic inflammatory response, it would be important to know which of the components reduced the systemic inflammatory response in order to create a more streamlined, sustainable and manageable ERAS programme. This would require a reliable and readily available biomarker of the systemic inflammatory response to be identified to determine which components affect the systemic inflammatory response and then to continue monitoring ERAS programmes as they continue to develop.

1.9.3 Complications Following Surgery for Colorectal Cancer

Despite advances in oncological treatment, the main method of treatment of colorectal cancer remains surgery. Surgery is not risk free and is often associated with a significant rate of post-operative complications. This complication rate ranges from 20% to 40 % in the published literature (Velasco et al., 1996). A post-operative complication can be considered as anything that causes the patient to deviate from what is considered a normal post-operative course (Dindo et al., 2004). These complications can be classified by type of complication (infective or non-infective) or by severity of complication. The Clavien-Dindo classification attempted to classify complications by their severity based on the action required to treat them. In greater than 6000 patients, this scoring system was found to be reliable, reproducible and comprehensive in classifying post-operative complications by severity (Dindo et al., 2004). This had the advantage over other severity scoring systems of being more objective and easier to replicate across a variety of surgical procedures and centres.

Regardless of how complications are classified, what has become clear is that they are detrimental to the patient in multiple ways. Complications not only result in short term problems such as increased length of stay, but have also been reported to adversely affect oncological outcome (Law et al., 2007a, Law et al., 2007b), quality of life (Brown et al., 2014) and long term survival following surgery (Khuri et al., 2005, McArdle et al., 2005). Therefore, much attention has been directed to try and reduce the risk of developing complications such as the use of pre-operative antibiotics, specific skin preparation and specialist surgeons. Despite this, complication rates remain significant. The systemic inflammatory response both pre-operatively and post-operatively have been reported to be associated with increased risk of development of complications (Moyes et al., 2009, Platt et al., 2012, Ramanathan et al., 2013). The pre-operative scoring system, the modified Glasgow prognostic score (mGPS) has been reported to be associated with post-operative complications but no such scoring system has been described in the post-operative period. If such a score could be devised then it may be of clinical utility in identifying, early in the post-operative phase, patients at increased risk of developing complications, or conversely, identify those at low risk and facilitate early discharge from hospital. Furthermore, if the post-operative systemic inflammatory response is associated with the development of complications then it could be hypothesised that modification of the post-operative systemic inflammatory response, either by operative technique or anti-inflammatory

medication may reduce the risk of developing complications and hence improve outcomes following cancer surgery.

1.10 Aims of Thesis

This thesis aims to:

- Determine the attitudes towards systemic inflammation based scoring systems and their use in routine clinical practice

- Examine other pre-operative markers of the innate immune system and determine whether they accurately reflect systemic inflammation and are capable of predicting outcomes

- Determine whether common markers of systemic inflammation can reflect surgical trauma and in turn use these markers to analyse the post-operative systemic inflammatory response and its effects on outcomes

- Using these markers analyse whether individual components of ERAS reduce the systemic inflammatory response

- Ascertain whether the systemic inflammatory response can be modulated pharmacologically in order to improve outcomes following surgery for colorectal cancer.

2 A survey of attitudes towards the clinical application of systemic inflammation based prognostic scores in cancer

2.1 Introduction

Allocation of patients to the correct form of treatment, be that surgical, oncological or palliative, remains a difficult decision. However, if patients were allocated to the most appropriate treatment then outcomes for all patients would improve, irrespective of new, more effective treatments. Traditionally, in those with early stage operable disease the treatment decision has been made largely based on staging of the cancer itself e.g. the Tumour, Node, Metastasis (TNM) staging system whereas in advanced stage inoperable disease the treatment decision has been made largely based on the general health and fitness and whether the patient had lost weight (cachexia).

In the last decade or so it has become apparent that a host inflammatory response, in particular the systemic inflammatory response, plays a key role in determining cachexia and the survival of patients with cancer (McMillan, 2009, Roxburgh and McMillan, 2010). With this new knowledge, a number of prognostic scoring systems that provide an objective measurement of the systemic inflammatory response have been developed and have been shown to have prognostic value in patients with cancer. These include the Glasgow Prognostic score/modified Glasgow Prognostic Score (GPS/mGPS), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), white cell-lymphocyte ratio (WLR) and others (Proctor et al., 2011a, East et al., 2014). The mGPS (combination of the values of pre-operative serum albumin and C-reactive protein) and the NLR (ratio of neutrophil and lymphocyte counts) are the most widely reported prognostic scores worldwide and both have been shown to have prognostic value in a variety of common solid tumours (McMillan, 2013b, Guthrie et al., 2013a, Li et al., 2014a). For example, by the end of 2012, the GPS/mGPS had been shown to have independent prognostic value in cancer patients in 51 studies involving 28,500 patients (McMillan, 2013b). Furthermore, the NLR has been shown to have independent prognostic value in 100 studies involving greater than 40 000 patients, with greater than 50% of these studies published since the start of 2012 (Templeton et al., 2014).

Despite the plethora of reported studies for these prognostic scores, their value in routine clinical practice either as tools to stratify patients in terms of outcomes or for consideration

for therapies such as adjuvant chemotherapy or in clinical trials is not clear. With this in mind, the aim of the present survey, in an international cohort, was to examine the range of opinions on the routine use of systemic inflammation based prognostic scoring systems and their potential incorporation into clinical guidelines.

2.2 Methods

A worldwide survey designed to establish opinions on the use of systemic inflammation based prognostic scoring systems was created. This was a web-based survey that included 10 questions on 'systemic inflammation based prognostic scores in cancer' *(Table 2.1)*. The survey was generated through the SurveyMonkey website (www.surveymonkey.com, SurveyMonkey, Paulo Alto, USA) and the access link emailed to the target group. The target group was selected primarily from two recent reviews (Mcmillan, 2013b, Guthrie et al., 2013a) and by performing a more recent literature search for articles using the keywords cancer, inflammation, recurrence, survival, mGPS and NLR. This literature search was performed at the end of January 2014. Once a comprehensive list of articles was obtained, the email addresses of corresponding authors from each article formed the basis of a mailing list for distribution. The email sent out clearly stated that the aim of the survey was to establish whether there was a role for the application of systemic inflammation based prognostic scores in routine clinical practice and research and that participation was voluntary. Software on the website ensured duplication of responses from the same individual was not recorded. No incentives were used to promote or encourage participation.

The survey was first sent out on 26th February 2014 with a reminder sent out one week later. The survey remained open for 4 weeks and was closed on the 26th March 2014. Data was analysed and graphs of results compiled using Microsoft Excel 2007 (Redmond, WA, USA).

2.3 Results

In February 2014, the survey was emailed to 238 individuals worldwide who had published articles related to systemic inflammation in patients with cancer. 43% were from Asia, 42% from Europe, 12% from America and 3% from Australia. The response to survey question 1 is shown in *Figure 2.1 (a & b).* In total, 60 people completed the survey (25%). 26 respondents (43%) were surgeons, 15 (25%) oncologists and 19 (32%) from other medical specialties. The proportion of respondents is shown in *Figure 2.1 (b)* with 55% of respondents being from Europe, 29% from Asia, 13% from Americas and 3% from Australia.

In response to question 2, 39 (65%) of the respondents answered yes that they routinely measured the systemic inflammatory response in patients with cancer. The median number of patients each participant assessed per year was 100 and the median number of patients each participant assessed in total was 330.

The response to question 3 is shown in *Figure 2.2.* Of the respondents, 11 (27%) reported its use for the purpose of prognostication and research, 11 (27%) reported its use for research purposes alone, 5 (12%) for the purpose of prognostication alone, 4 (10%) reported its use for audit purposes, and 3 (8%) for the purpose of treatment allocation.

The response to question 4 is shown in *Figure 2.*3. Of those who responded, 16 (40%) answered the measure of the systemic inflammatory response they used was the GPS, 8 (20%) answered the GPS/NLR and 6 (15%) answered the NLR alone.

The response to question 5 is shown in *Figure 2.4 (a).* Of the respondents, 31 (56%) answered yes they would use a measure of the systemic inflammatory response to stratify patients entering clinical trials.

The response to question 6 is shown in *Figure 2.4 (b).* Of the respondents, 20 (57%) answered that they would use the GPS, 4 (11%) answered the NLR and 4 (11%) answered the GPS/NLR for stratifying patients entering clinical trials.

The response to question 7 is shown in *Figure 2.*5. Of the respondents, 12 (25%) reported that the clinical scenarios where a measure of the systemic inflammatory response offers most benefit were making decisions on palliative chemotherapy, 10 (21%) reported making decisions on allocation of adjuvant therapy, 6 (12%) reported on decisions about either

adjuvant therapy or palliative chemotherapy and 5 (10%) reported on all 4 categories. Only 2 (4%) reported in making decisions on allocation of surgical treatment.

The response to question 8 is shown in *Figure 2*.6 *(a)*. Of the respondents, 46 (81%) answered yes to whether a measure of the systemic inflammatory response should be adopted into clinical guidelines.

The response to question 9 is shown in *Figure 2*.6 *(b)*. Of those who responded, 30 (60%) answered that the measure of the systemic inflammatory response they would prefer to use in clinical guidelines was the GPS, 7 (14%) answered GPS/NLR and 5 (10%) answered NLR.

2.4 Discussion

The results of the present study showed that the majority of respondents routinely measured the systemic inflammatory response, used the GPS/mGPS, mainly for research and prognostication purposes, and that the majority of respondents reported that a measure of the systemic inflammatory response should be adopted into clinical guidelines.

A small number of people responded to our survey (25%) although this rate falls within the average response rate of between 20 and 30% (SurveyMonkey, 2014). Factors that are known to improve the survey response rate include incentives, reduced survey length, reduced complexity of questions, and reminder emails (SurveyMonkey, 2014). In the present study the questions were intentionally simple and limited to 10 in total and sent a reminder email to encourage respondents but did not employ any incentive for completing the survey.

The survey was sent to potential participants worldwide with the majority to Asia and Europe. The majority of respondents of this survey were surgeons (43%) with oncologists making up a quarter of respondents. The location of the respondents did not closely match the locations of the potential survey participants. Those invited to participate were mainly from Asia and Europe however, only 29% of respondents were from Asia while 55% were from Europe. Perhaps this lack of response from Asia is due to cultural differences which were not present in those from Europe or due to greater language barriers. Whatever the reason, the poor response rate from Asia was disappointing given that the majority of work using these prognostic scores has been carried out in Europe and Asia. In the present study, respondents were asked to estimate how many patients with cancer they had assessed using these systemic inflammation based scores in each year. The response was approximately 100 per year. With this volume of work it could be considered that those who responded were specialists and had an interest in systemic inflammation based scores.

It has been widely reported that markers of the systemic inflammatory response are good prognostic markers in patients with cancer. The majority of survey respondents reported that they routinely assessed the systemic inflammatory response in patients with cancer and the majority used this assessment for research or prognostication purposes. This is not unexpected since the majority of studies examining these scoring systems were performed for research purposes or were performed retrospectively to aid prognostication of patients into high and low risk groups. Whilst CRP has been shown to have prognostic value in a

number of tumours, the mGPS, which utilises a combination of CRP and albumin at standard thresholds, has been shown to have superior prognostic value and obviates the problem of different CRP threshold values being used within and across different tumour types. In the present study, the majority of respondents reported that they would use GPS/mGPS as their method of assessing the systemic inflammatory response. This would appear to be consistent with the literature and whilst the participants of this survey have an interest in this field, it was not clear, prior to this survey, what views they had on the clinical application of systemic inflammation based prognostic scores, in particular which, if any, score that they would prefer to use clinically.

Interestingly, only a small number of respondents reported that they used assessment of the systemic inflammatory response to determine treatment allocation and this is an area where proponents of these scoring systems would hope to expand their use in order to better stratify patients to appropriate treatment modalities (McMillan, 2013a). Of the survey respondents, 56% reported that they would use a measure of the systemic inflammatory response to stratify patients entering into clinical trials and 57% said they would choose mGPS/GPS for this. Moreover, of the survey respondents, 25% reported that these scores were used in making decisions about palliative chemotherapy, 21% in making decisions about allocation of adjuvant therapy and 12% in making decisions either about adjuvant therapy or palliative chemotherapy. Only 4% reported that a measure of the systemic inflammatory response would be of benefit in making decisions about allocation of surgical treatment. This is of interest as the majority of respondents were surgeons, with the majority of research in these scoring systems having been undertaken by surgeons, yet the consensus was that it would not be of benefit to allocate surgical treatment based on these scoring systems. The basis of this approach is not clear. However it may be that surgeons wish to operate on all patients with potentially curable disease. It remains to be seen whether this approach will be maintained in the long term, particularly in aggressive cancers such as pancreatic cancer where neoadjuvant therapy is increasingly used as first line therapy.

Furthermore, recent work has suggested that markers of the systemic inflammatory response may be useful as a therapeutic target. The recent addition of an anti-angiogenic monoclonal antibody to VEGF therapy, such as Bevacizumab to standard chemotherapy regimens has resulted in improved efficacy of these regimens. However, recent studies have reported that patients with a raised neutrophil count, high NLR or mGPS 1 or 2 received no significant survival benefit from these regimens (Botta et al., 2011, Botta et al.,

2013, Maillet et al., 2014). In addition, Botta and colleagues reported in their study that preoperative systemic inflammatory status was a marker of resistance to bevacizumab therapy (Botta et al., 2013). Also, recent work has suggested that the mGPS may be useful in stratifying oncological treatment. Hurwitz et al. recently reported that Ruxolitinib (a Janus Kinase 1 (JAK1)/Janus Kinase 2 (JAK2) inhibitor) along with capecitabine improved overall survival and progression free survival in patients with metastatic pancreatic cancer with inflammation characterised by mGPS 1 or 2 (Hurwitz, 2014).

Of the survey respondents, 80% reported that they felt a measure of the systemic inflammatory response should be adopted into clinical guidelines and 60% reported that GPS/mGPS would be their preference. For example, cancer cachexia affects greater than 50% of patients with advanced disease and its clinical definition and symptoms have been intensively discussed in recent years (Aapro et al., 2014, Douglas and McMillan, 2014). Recently, the European School of Oncology Task Force conducted a review the literature on cancer cachexia. They concluded that cachexia is a complex process but that along with anorexia, the presence of a systemic inflammatory response results in the features of the disease (Aapro et al., 2014). Furthermore, Douglas and McMillan (2014) recently proposed that the mGPS be used as the basis for formation of an objective and clinically relevant definition of cachexia (Douglas and McMillan, 2014). The findings of the present study would appear to confirm that the mGPS is the most commonly used systemic inflammation based score and therefore appropriate for forming the basis of an objective definition of cancer cachexia.

The present study has a number of possible limitations. Firstly, respondents did not have to enter their location in order to complete the questionnaire, meaning the location for all the respondents was not obtained. In all surveys there is a tension between making the sample size as large as possible in order to eliminate bias and asking questions appropriate to those surveyed. In the present survey, we targeted those with a known interest in systemic inflammation based prognostic scores (those who had already published in this field) in order to maximise the number of appropriate and meaningful responses. The mGPS and NLR are the most popular scores as they have the largest evidence base. Although other systemic inflammation based prognostic scores such as the derived NLR (dNLR), lymphocyte monocyte ratio (LMR) and platelet-lymphocyte ratio (PLR) have been reported they have not established a sufficient body of evidence in the literature. Moreover, where they have been directly compared, the mGPS had the greatest prognostic value in patients with cancer, independent of age, sex, deprivation and tumour stage (Guthrie et al.,

2013b, Proctor et al., 2011a). Therefore, it is likely that the results of this survey reflect the reality of attitudes towards the application of these scores in those individuals with an interest in the field. It was of interest that 43% of the respondents were surgeons. This may reflect the activity of surgeons in this field. Indeed, it is recognised that surgeons are key members of the multi-disciplinary team that decides treatment allocation. Irrespectively, this would confirm that the survey was directed at clinicians in routine clinical practice.

In summary, the present study has shown that in those who responded, the majority routinely measured the systemic inflammatory response in patients with cancer, with the majority using the GPS/mGPS, mainly for research and prognostication purposes. The majority reported that these scoring systems were of most clinical benefit in making decisions on adjuvant therapy and palliative chemotherapy and that the systemic inflammatory response, as evidenced by the GPS/mGPS, should be adopted into clinical guidelines, such as a new, objective and clinically relevant definition of cancer cachexia.

Table 2.1 Survey of systemic inflammation based prognostic scores in cancer

Survey Question
1. What is your discipline (surgeon/oncologist/pathologist etc.) and in which country are you based?
2. Do you or your colleagues routinely assess the systemic inflammatory response as part of the clinical assessment of patients with cancer? *Since 2008, could you estimate how many patients have been assessed (a) in total and (b)per year?*
3. If you answered yes to question 2, for what purpose? Audit Prognostication Treatment allocation Research
4. If you answered yes to question 2, what measure of the systemic inflammatory response do you use? GPS NLR Other
5. Would you use a measure of the systemic inflammatory response to stratify patients entering into clinical trials?
6. If you answered yes to question 5, which would you prefer to use? GPS NLR Other
7. In which clinical scenario do you think a measure of the systemic inflammatory response offers most benefit to patients? In making decisions about allocation of surgical treatment for primary operable disease In making decisions on allocation of neoadjuvant treatment In making decisions on allocation of adjuvant treatment In making decisions on palliative chemotherapy
8. Do you think that a measure of the systemic inflammatory response should be adopted into clinical guidelines?
9. If yes, which would you prefer to use? GPS NLR Other
10. If you do not think that a measure of the systemic inflammatory response is useful in the routine clinical assessment of cancer patients, please comment.

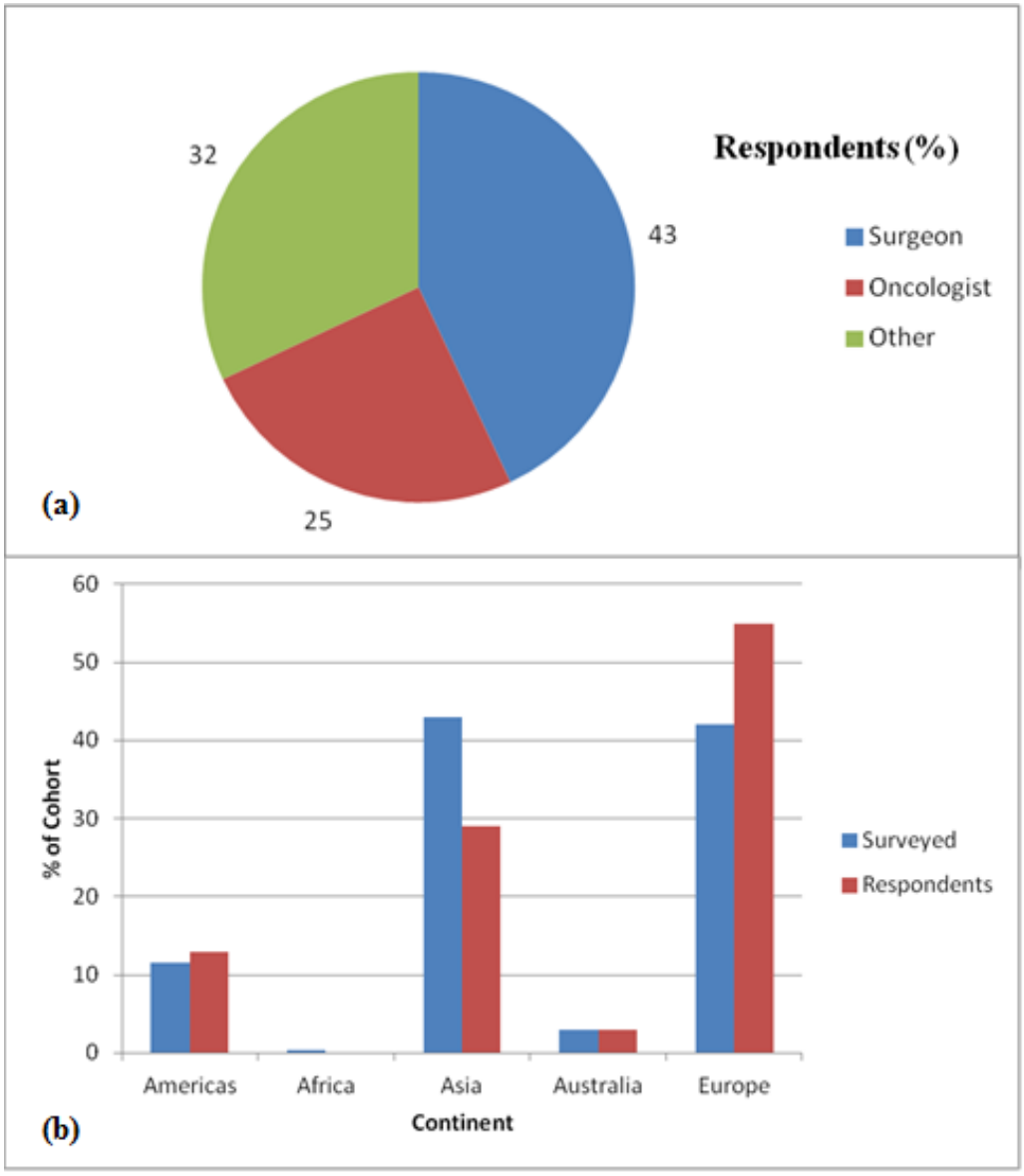


Figure 2.1 **Responses to survey question 1.**
(a) What is your discipline? Respondents (n = 60) and (b) In which country are you based? Respondents (n = 31)

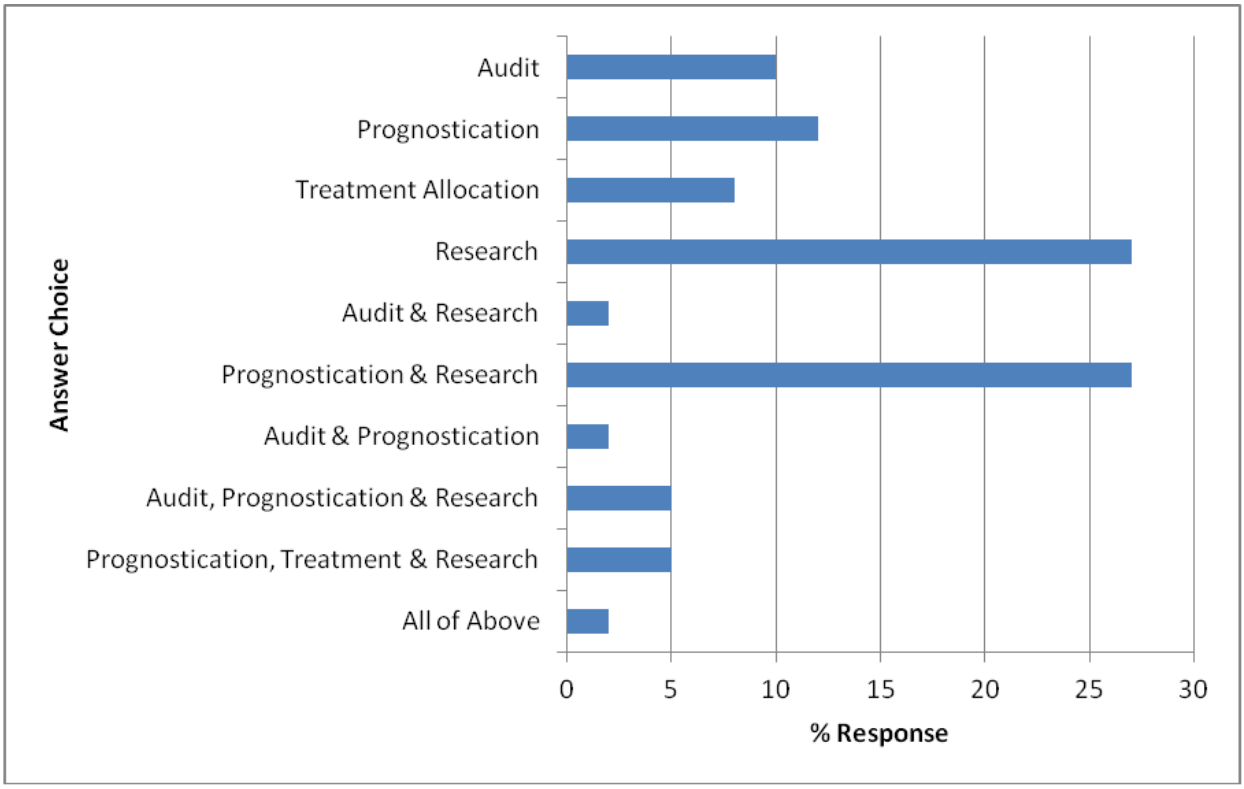


Figure 2.2 **Responses to survey question 3.**
For what purpose do you measure the systemic inflammatory response?
Respondents (n = 41).

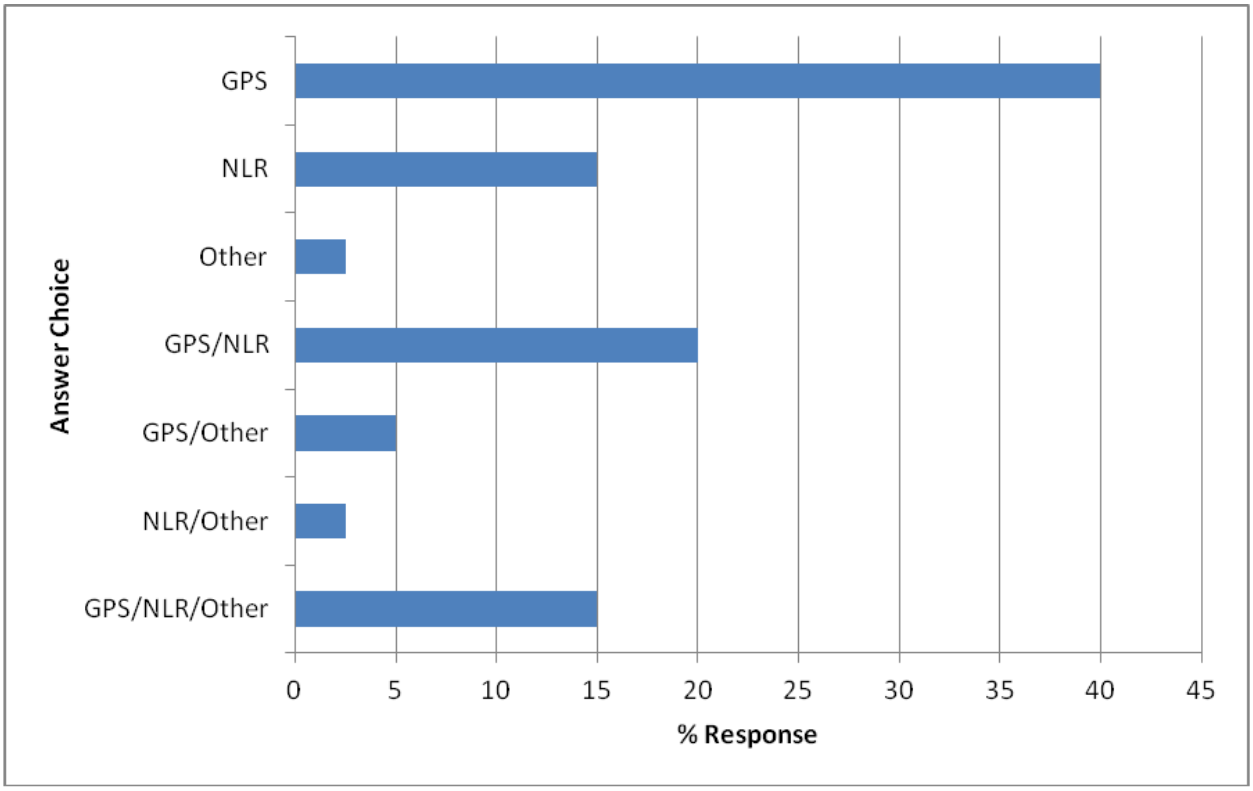


Figure 2.3 **Responses to survey question 4.**
What measure of the systemic inflammatory response do you use?
Respondents (n = 40).

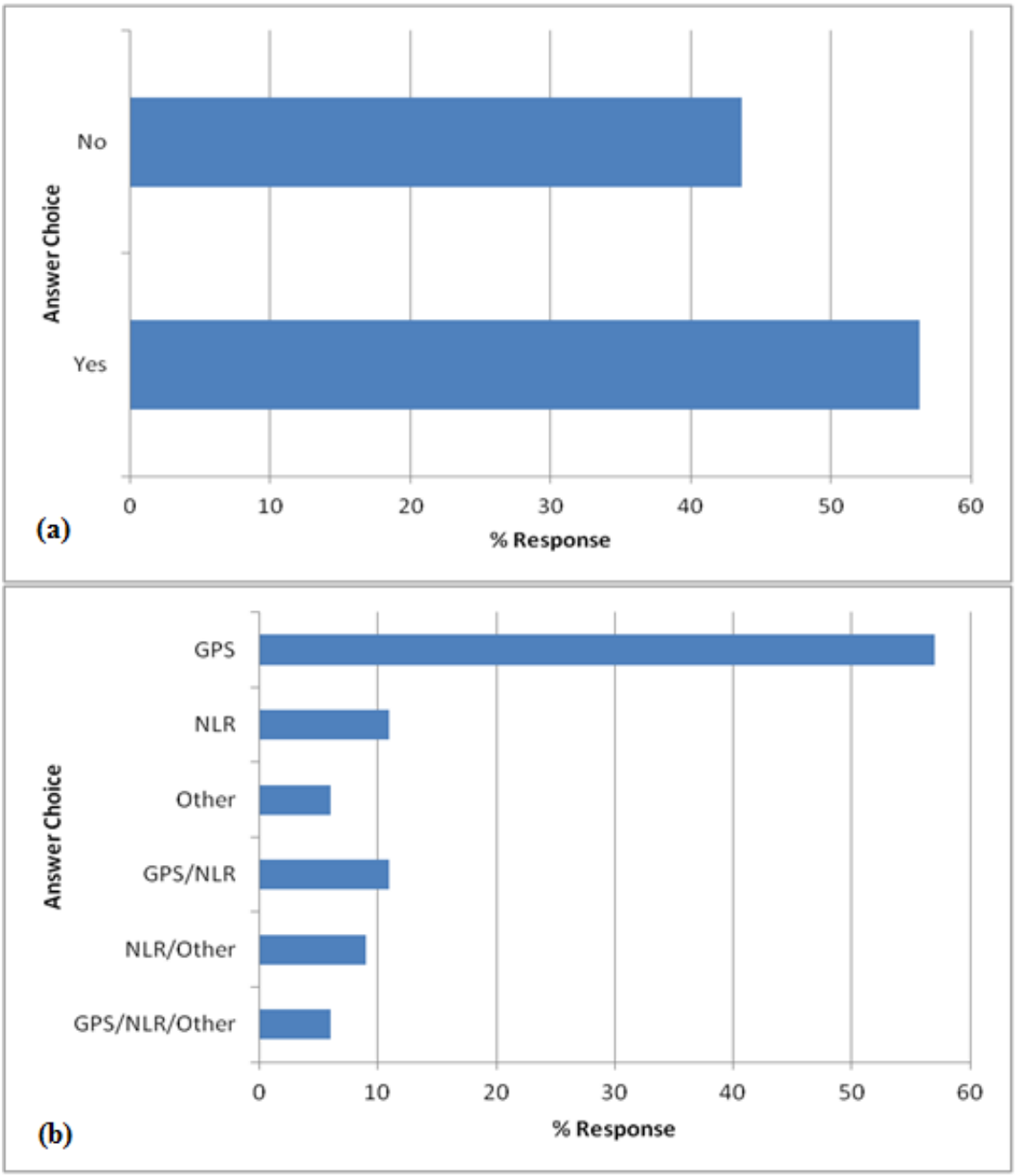


Figure 2.4 **Responses to survey question 5.**
(a) Would you use a measure of the systemic inflammatory response to stratify patients entering into clinical trials? Respondent (n = 55) and (b) Which measure of the systemic inflammatory response would you use to stratify patients entering into clinical trials? Respondents (n = 35).

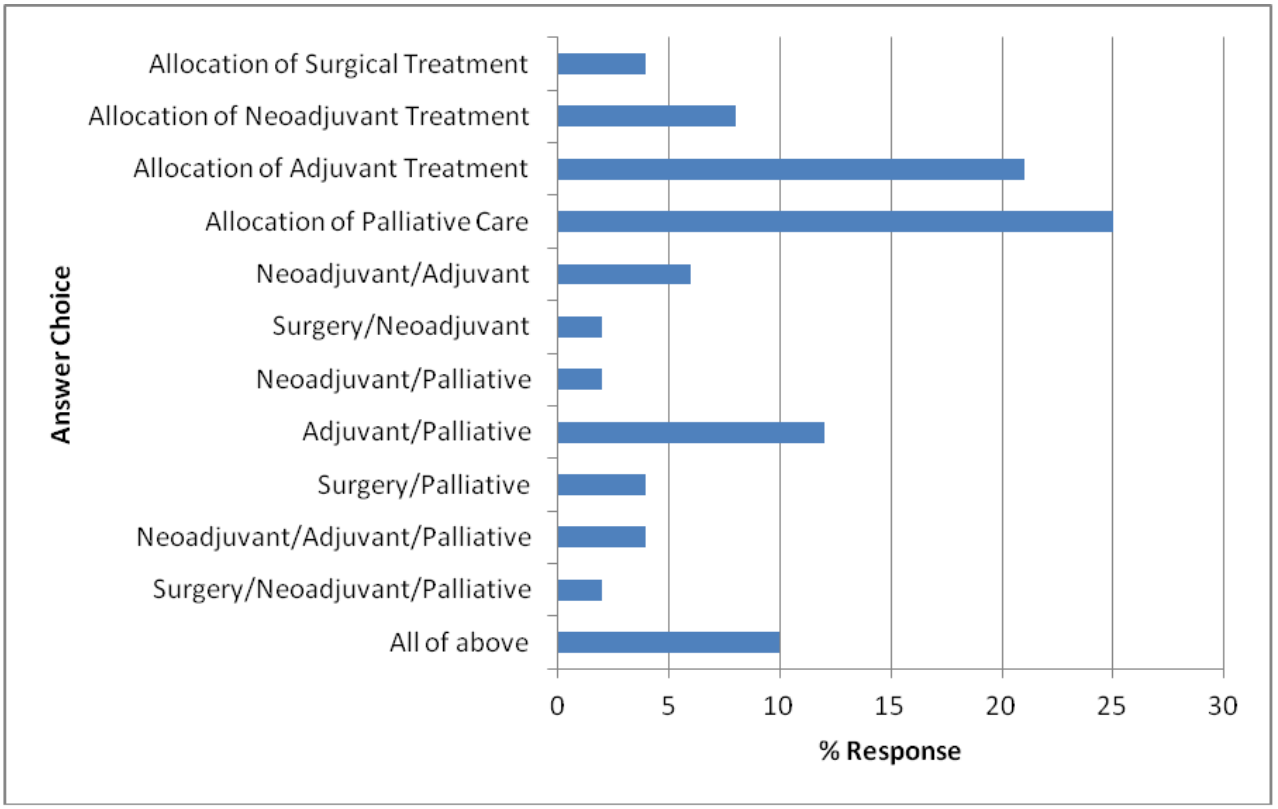


Figure 2.5 **Responses to survey question 7.**
In which clinical scenario do you think a measure of the systemic inflammatory response offers most benefit to patients? Respondents (n=49).

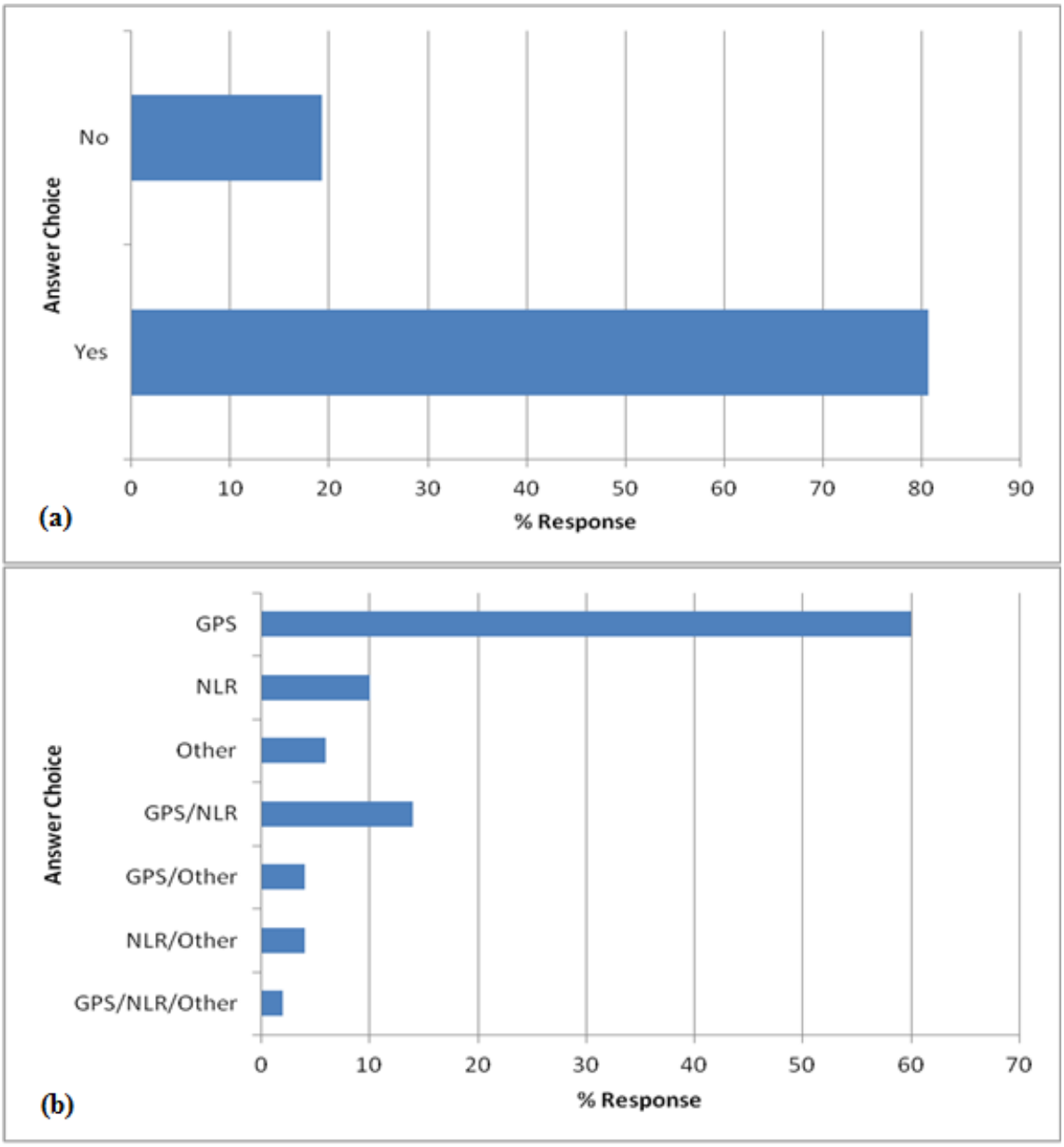


Figure 2.6 **Responses to survey questions 8 and 9.**
(a) Do you think that a measure of the systemic inflammatory response should be adopted into clinical guidelines? Respondents (n = 57) and (b) Which measure of the systemic inflammatory response should be included? Respondent (n = 50).

3 Neutrophil count is the most important prognostic component of the differential white cell count in patients undergoing elective surgery for colorectal cancer

3.1 Introduction

Although there have been improvements in the treatment and management of colorectal cancer, outcomes remain poor with approximately 40% of those who undergo curative surgery dying from their disease (Oliphant et al., 2013). Traditionally, likely outcomes following surgery and treatment options offered to patients have been determined by prognostic stratification systems based on pathological tumour characteristics such as the Tumour, Nodal, Metastasis (TNM) system (Ajcc, 2010). However, clinical outcomes vary considerably amongst patients within the same TNM stage (Horgan and McMillan, 2010).

It is increasingly recognised that patient factors such as nutritional decline, functional decline and the presence of a systemic inflammatory response are associated with progression of cancer and poorer outcomes (McMillan, 2009, Roxburgh and McMillan, 2010). With reference to the systemic inflammatory response, prognostic scoring systems such as the modified Glasgow Prognostic Score (mGPS), a combination of C-reactive protein and albumin,(McMillan, 2013b) and various combinations of components of a differential white cell count (WCC) have been reported to be of value in many solid organ cancers including colorectal cancer (East et al., 2014, Guthrie et al., 2013a, Li et al., 2014a, Kwon et al., 2012). Although apparently inferior to the mGPS and a number of different neutrophil-lymphocyte ratio (NLR) thresholds being reported, many recent publications have documented its prognostic value in patients with colorectal cancer (Clarke et al., 2011, Guthrie et al., 2013b, Li et al., 2014a). However, it is not clear whether both components of the NLR contribute equally to the prognostic value since NLR reflects the activity of both the innate (neutrophil) and adaptive (lymphocyte) aspects of the immune system. Indeed, it may be hypothesised that the neutrophil count has the dominant prognostic value. The aim of the present study was to examine, in detail, the relationships between the components of the differential white cell count and survival in patients undergoing elective surgery for colorectal cancer.

3.2 Methods

3.2.1 Patients

Patients were identified from a prospectively maintained database of elective and emergency colorectal resections performed in a single surgical unit at Glasgow Royal Infirmary. Patients who, on the basis of pre-operative abdominal computed tomography and operative findings, were considered to have undergone potentially curative resection between 1997 and 2008 were included. Exclusion criteria were neoadjuvant therapy, emergency presentation and presence of metastatic disease. The study was approved by the West of Scotland Research Ethics Committee, Glasgow.

Patient demographics and pre-operative laboratory measurements including the differential white cell count: total white cell count, neutrophil, lymphocyte, monocyte, eosinophil and basophil and platelet counts were analysed. Data was also available for the mGPS. Tumours were staged according to the 5th edition of the tumour, node, metastases (TNM) classification (AJCC, 2010) and any additional pathological data obtained from the pathology reports issued following resection. Patients were routinely followed up for 5 years following resection as per national guidelines. Date and cause of death were crosschecked with the cancer registration system and Registrar General (Scotland). Cancer specific survival was measured from date of surgery until date of death.

3.2.2 Statistical Analysis

Kaplan Meier analysis of clinicopathological factors that are, a priori, known to be important predictors of outcome and of components of the differential white cell count (total white cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count and platelet count) was performed and plots included to confirm the linearity of different variables *(Figure 3.1)*. Statistically significant components of the differential white cell count were then included in a multivariate model along with the clinicopathological variables. Multivariate analysis was performed using backwards conditional Cox regression. A p-value of <0.05 was considered statistically significant. Analyses were carried out using SPSS version 21.0 (IBM, SPSS, IL, USA).

3.3 Results

A total of 508 patients who underwent resection were included in the analysis. The clinicopathological characteristics are summarised in *Table 3.1*. The majority were over the age of 65 (67%) with similar numbers of males and females. The majority had TNM stage II (50%) or III disease (39%) with the majority of tumours being colonic in origin (69%) and 21% had adjuvant chemotherapy following resection. Median follow up of survivors was 109 months (range 51-194).

The relationship between the clinicopathological characteristics and both cancer-specific and overall survival in the whole cohort (n=508) is shown in *Tables 3.1 & 3.2*. There were 172 cancer deaths and 120 non-cancer deaths. Using Kaplan Meier analysis, age, TNM stage, venous invasion, margin involvement, peritoneal involvement and tumour perforation (all $p < 0.05$) as well as white cell count and neutrophil count (both $p < 0.05$) were significantly associated with cancer-specific survival *(Table 3.1)*. Similarly, age, TNM stage, venous invasion, margin involvement, peritoneal involvement and tumour perforation (all $p < 0.05$) as well as white cell count and neutrophil count (both $p < 0.005$) were associated with overall survival *(Table 3.1)*. In the whole cohort, on multivariate survival analysis *(Table 3.2)*, age (HR 1.29 CI 1.07-1.56, $p = 0.007$); site (HR 1.40, CI 1.01-1.94, $p = 0.041$); TNM stage (HR 2.19, CI 1.64-2.91, $p < 0.001$); margin involvement (HR 2.97, CI 1.93-4.59, $p < 0.001$); peritoneal involvement (HR 1.48, CI 1.07-2.05, $p = 0.019$) and mGPS (HR 1.54, CI 1.25-1.90, $p < 0.001$) were independently associated with cancer-specific survival. On multivariate analysis *(Table 3.2)*, age (HR 1.60, CI 1.36-1.87, $p < 0.001$); sex (HR 1.40, CI 1.10-1.79, $p = 0.007$); TNM stage (HR 1.44 CI 1.15-1.80, $p = 0.001$); venous invasion (HR 1.50, CI 1.17-1.94, $p = 0.002$); margin involvement (HR 2.49, CI 1.69-3.68, $p < 0.001$); tumour perforation (HR 2.48, CI 1.23-4.99, $p = 0.011$); and the mGPS (HR 1.32, CI 1.12-1.56, $p = 0.001$) were independently associated with overall survival.

The relationship between the clinicopathological characteristics and both cancer specific and overall survival in node negative colon cancer (n=226) is shown in *Tables 3.2 & 3.3*. There were 49 cancer deaths and 69 non-cancer deaths. Using Kaplan Meier analysis, TNM stage, venous invasion and margin involvement (all $p < 0.05$) as well as white cell count, neutrophil count, basophil count, platelet count and NLR ($p < 0.05$) were associated with cancer-specific survival *(Table 3.3)*. Furthermore, age, venous invasion and tumour perforation (all $p < 0.05$) as well as white cell count and neutrophil count (all $p < 0.05$)

were associated with overall survival *(Table 3.3)*. In the node negative colon cohort, on multivariate survival analysis *(Table 3.2)*, age (HR 1.53, CI 1.05-2.23, p = 0.028); venous invasion (HR 2.23, CI 1.19-4.16, p = 0.012); mGPS (HR 1.52, CI 1.03-2.24, p = 0.035) and neutrophil count (HR 2.31, CI 1.13-4.71, p = 0.022) were independently associated with cancer-specific survival. On multivariate analysis *(Table 3.2)*, age (HR 2.05, CI 1.59-2.64, p < 0.001); sex (HR 1.57, CI 1.06-2.31, p = 0.023); venous invasion (HR 2.24, CI 1.47-3.41, p <0.001); margin involvement (HR 3.19, CI 1.36-7.49, p = 0.008); mGPS (HR 1.36, CI 1.06-1.75, p = 0.017) and neutrophil count (HR 1.74, CI 1.06-2.88, p = 0.030) were independently associated with overall survival.

The neutrophil count was added to the mGPS in the same way as previously described (Proctor et al., 2013) to create the optimised Glasgow Prognostic Score (oGPS). In short, C-reactive protein (CRP) <10 mg/L scored 0; CRP >10 mg/L and albumin ≥ 35g/L scored 1; CRP >10 mg/L and albumin < 35 g/L scored 2 and CRP >10 mg/L, albumin < 35 g/L and neutrophil count > 7.5 $x10^9$/L scored 3. Kaplan Meier curve of the oGPS and cancer-specific survival in the whole cohort is shown in *Figure 3.4.* As the oGPS score increase from 0 to 3 cancer-specific survival worsens (p < 0.001). Furthermore, on Cox regression analysis, the oGPS appears to be associated with both cancer-specific survival and overall survival in the whole cohort (CSS - HR 1.44, CI 1.21-1.70, p < 0.001; OS - HR 1.40, CI 1.22-1.60, p < 0.001).

3.4 Discussion

The results of the present study show that of the components of a white cell count, only the neutrophil count was independently associated with survival. In particular, a high neutrophil count was associated with poorer cancer-specific survival, especially in node negative colon cancer. Therefore, it would appear that the prognostic value of the NLR derives mainly from the neutrophil count in patients with colorectal cancer. It remains to be determined whether this is the case in all solid tumours in which the NLR has been reported to have prognostic value.

The results of the present study are consistent with the recent report of Jankova and colleagues (Jankova et al., 2013) who, in 322 patients with node positive colorectal cancer, concluded that NLR was independently and weakly associated with poorer overall survival but not cancer-specific survival. They also examined, using receiver operating characteristic (ROC) curves, optimal thresholds for the NLR but found that no single cut off could be recommended due to the weak association between NLR and survival (Jankova et al., 2013). Furthermore, a recent longitudinal study reported that whilst preoperative NLR and mGPS were independently associated with cancer-specific survival only post-operative mGPS was independently associated with cancer-specific survival (Guthrie et al., 2013b).

The present results may shed some light into the prognostic variability of the NLR, irrespective of the threshold used, since it is clear that the lymphocyte count, although it contributes approximately equally to the NLR *(Figures 3.2 and 3.3)*, has relatively weak prognostic value compared with the neutrophil count alone. A plausible explanation of why the neutrophil count has superior prognostic value is that it better reflects the basis of the systemic inflammatory response, which is primarily an upregulation of the innate immune system (Roxburgh et al., 2013).

The results of the present study are consistent with the work of Proctor and colleagues (Proctor et al., 2013), who reported in a large unselected cohort of patients with cancer that, compared with other markers of the systemic inflammatory response, an elevated neutrophil count had independent prognostic value whereas the lymphocyte count did not. Moreover, they proposed that the neutrophil count was added to the mGPS and termed the score the Optimised GPS (oGPS). It is therefore of interest that the neutrophil count along with mGPS, has been shown to be independently prognostic in patients with advanced

gastric cancer (Li et al., 2014b). Therefore, the use of a neutrophil count, may add prognostic value to the mGPS in patients colorectal cancer. Indeed, in the present study, the use of the oGPS was useful in further stratifying cancer-specific survival in patients undergoing elective surgery for colorectal cancer.

The recent addition of an anti-angiogenic monoclonal antibody to vascular endothelial growth factor (VEGF) therapy, such as Bevacizumab to standard chemotherapy regimens has resulted in improved efficacy of these regimens. Recent studies have reported that a reduced neutrophil count and low NLR are associated with improved survival in advanced non-small cell lung cancer (Botta et al., 2013) and metastatic colorectal cancer (Botta et al., 2011) treated with these therapies. Indeed, it has previously been reported that neutrophils are found in increased number at tumour sites and that they are able to promote angiogenesis by secreting factors such as VEGF, interleukin-8 (IL-8) and matrix metallopeptidase 9 (MMP-9). In these studies, despite blockade of VEGF activity with Bevacizumab, patients with high circulating levels of neutrophils or high NLR received no significant survival benefit. This recent observation is consistent with the findings from the present study and suggests that it is neutrophils that drive tumour progression and dissemination.

The results of the present study have implications not only for the prognostic value of scores other than the NLR that include components of the innate and adaptive immune systems, usually lymphocyte counts, for example, the platelet-lymphocyte ratio (PLR) (Kwon et al., 2012), white cell-lymphocyte ratio (WLR) (East et al., 2014), lymphocyte-monocyte ratio (LMR) (Hu et al., 2014) and may explain why the derived NLR (dNLR), based solely on neutrophil and white cell count had similar or better prognostic value compared with the NLR (Proctor et al., 2012).

The present study is limited by its retrospective cross-sectional nature. Nonetheless, this is the first time that detailed analysis of the components of a white cell count has been examined in patients undergoing potentially curative surgery for colorectal cancer.

In summary, the results of the present study clearly show the independent prognostic value of the neutrophil count and that it is the basis of the prognostic value of the NLR in patients with primary operable colorectal cancer. These results have implications for prognostic scores based on the components of a differential white cell counts.

Table 3.1 **Clinicopathological characteristics, components of the differential white cell count and survival in patient undergoing elective resection for colorectal cancer**

Characteristics	**n (%) or Median (range)**	**Cancer-specific Survival**	**Overall Survival**
		p-value[a]	***p-value***[a]
Age (<65/65-74/>74)	165 (33)/ 163 (32)/ 180 (35)	0.010	<0.001
Sex (Female/Male)	233 (46)/ 275 (54)	0.399	0.199
Site (Colon/Rectum)	350 (69)/ 158 (31)	0.125	0.535
TNM Stage (I/II/III)	55 (11)/ 254 (50)/ 199 (39)	<0.001	<0.001
Differentiation (Mod-well/Poor)	450 (89)/ 56 (11)	0.018	0.005
Venous Invasion (No/Yes)	298 (59)/ 210 (41)	<0.001	<0.001
Margin involvement (No/Yes)	467 (92)/ 41 (8)	<0.001	<0.001
Peritoneal involvement (No/Yes)	384 (76)/ 124 (24)	<0.001	0.020
Tumour perforation (No/Yes)	498 (98)/ 10 (2)	0.002	<0.001
Adjuvant therapy (No/Yes)	400 (79)/ 108 (21)	0.747	0.086
Time dependent variable (1997-2002/2003-2008)	214 (42)/ 294 (58)	0.367	0.109
WCC (<8.5/8.5-11/>11)	320 (63)/ 118 (23)/ 60 (12)	0.029	<0.001
Neutrophil (<7.5/>7.5)	434 (85)/ 64 (13)	0.007	0.001
Lymphocytes (<1/1-3/>3)	49 (10)/ 427 (84)/ 22 (4)	0.717	0.940
Monocytes (<0.9/>0.9)	413 (81)/ 70 (14)	0.053	0.019
Eosinophils (<0.04/0.04-0.4/>0.4)	54 (11)/ 392 (77)/ 37 (7)	0.131	0.040
Basophils (<0.01/0.01-0.1/>0.10)	276 (54)/ 201 (40)/ 6 (1)	0.102	0.001
Platelets (<400/>400)	406 (80)/ 83 (16)	0.199	0.314
NLR (<5/>=5)	405 (80)/ 93 (18)	0.533	0.125
Alive/ cancer death/non-cancer death	216 (43)/172(34)/120(23)		

NLR = neutrophil-lymphocyte ratio; TNM = Tumour, Nodal, Metastasis; WCC = white cell count

[a] Log rank p-value

Table 3.2 Multivariate analysis of clinicopathological factors and components of differential white cell count following elective, potentially curative surgery for colorectal cancer

	Cancer-specific Survival		Overall Survival	
	HR (95% CI)	*p-value*	HR (95% CI)	*p-value*
Whole Cohort (n = 508)				
Clinicopathological Factors				
Age (<65/65-74/>74)	1.29 (1.07-1.56)	0.007	1.60 (1.36-1.87)	<0.001
Sex (Female/Male)	-	0.109	1.40 (1.10-1.79)	0.007
Site (Colon/Rectum)	1.40 (1.01-1.94)	0.041	-	0.182
TNM Stage (I/II/III)	2.19 (1.64-2.91)	<0.001	1.44 (1.15-1.80)	0.001
Differentiation (Mod-well/Poor)	-	0.275	1.41 (1.00-2.00)	0.053
Venous Invasion (No/Yes)	-	0.113	1.50 (1.17-1.94)	0.002
Margin involvement (No/Yes)	2.97 (1.93-4.59)	<0.001	2.49 (1.69-3.68)	<0.001
Peritoneal involvement (No/Yes)	1.48 (1.07-2.05)	0.019	-	0.600
Tumour perforation (No/Yes)	-	0.175	2.48 (1.23-4.99)	0.011
Adjuvant therapy (No/Yes)	-	0.230	0.75 (0.54-1.04)	0.080
Time dependent variable (1997-2002/ 2003-2008)	-	0.883	-	0.617
Neutrophil (Threshold) (<7.5/>7.5)	-	0.424	-	0.145
mGPS (0/1/2)	1.54 (1.25-1.90)	<0.001	1.32 (1.12-1.56)	0.001
Node negative colon cohort (n = 226)				
Clinicopathological Factors				
Age (<65/65-74/>74)	1.53 (1.05-2.23)	0.028	2.05 (1.59-2.64)	<0.001
Sex (Female/Male)	-	0.129	1.57 (1.06-2.31)	0.023
TNM Stage (I/II)	4.14 (0.55-30.98)	0.167	-	0.774
Differentiation (Mod-well/Poor)	-	0.147	-	0.826
Venous Invasion (No/Yes)	2.23 (1.19-4.16)	0.012	2.24 (1.47-3.41)	<0.001
Margin involvement (No/Yes)	-	0.115	3.19 (1.36-7.49)	0.008
Peritoneal involvement (No/Yes)	-	0.665	-	0.152
Tumour perforation (No/Yes)	-	0.743	-	0.264
Adjuvant therapy (No/Yes)	-	0.416	-	0.389
Time dependent variable (1997-2002/ 2003-2008)	-	0.484	-	0.202
Neutrophil (Threshold) (<7.5/>7.5)	2.31 (1.13-4.71)	0.022	1.74 (1.06-2.88)	0.030
mGPS (0/1/2)	1.52 (1.03-2.24)	0.035	1.36 (1.06-1.75)	0.017

CI = confidence interval; HR = hazard ratio; mGPS = modified Glasgow Prognostic Score; TNM = Tumour, Nodal, Metastasis; NLR = neutrophil-lymphocyte ratio; WCC = white cell count

Table 3.3 **Clinicopathological characteristics, components of the differential white cell count and survival in patients undergoing elective resection for node negative colon cancer**

Characteristics	**n (%) or Median (range)**	**Cancer-specific Survival**	**Overall Survival**
		p-value[a]	***p-value***[a]
Age (<65/65-74/>74)	70 (31)/ 76 (34)/ 80 (35)	0.140	<0.001
Sex (Female/Male)	106 (47)/ 120 (53)	0.840	0.323
TNM Stage (I/II)	33 (15)/ 193 (85)	0.014	0.204
Differentiation (Mod-well/Poor)	204 (91)/ 21 (9)	0.519	0.332
Venous Invasion (No/Yes)	153 (68)/ 73 (32)	0.002	0.001
Margin involvement (No/Yes)	218 (97)/ 8 (3)	0.032	0.064
Peritoneal involvement (No/Yes)	181 (80)/ 45 (20)	0.274	0.651
Tumour perforation (No/Yes)	221 (98)/ 5 (2)	0.631	0.011
Adjuvant therapy (No/Yes)	202 (89)/ 24 (11)	0.519	0.140
Time dependent variable (1997-2002/2003-2008)	94 (42)/ 132 (58)	0.653	0.089
WCC (<8.5/8.5-11/>11)	148 (67)/ 41 (19)/ 31 (14)	0.010	0.023
Neutrophil (<7.5/>7.5)	190 (87)/ 29 (13)	0.004	0.034
Lymphocytes (<1/1-3/>3)	23 (11)/ 187 (85)/ 9 (4)	0.859	0.575
Monocytes (<0.9/>0.9)	178 (84)/ 35 (16)	0.094	0.535
Eosinophils (<0.04/0.04-0.4/>0.4)	28 (13)/ 169 (79)/ 16 (8)	0.760	0.690
Basophils (<0.01/0.01-0.1/>0.10)	114 (53)/ 97 (46)/ 2 (1)	0.009	0.144
Platelets (<400/>400)	172 (80)/ 43 (20)	0.036	0.495
NLR (<5/>=5)	170 (78)/ 49 (22)	0.007	0.056
Alive/ cancer death/non-cancer death	108 (48)/ 49 (22)/ 69 (30)		

CI = confidence interval; HR = hazard ratio; mGPS = modified Glasgow Prognostic Score; TNM = Tumour, Nodal, Metastasis; NLR = neutrophil-lymphocyte ratio; WCC = white cell count

[a] Log rank p-value

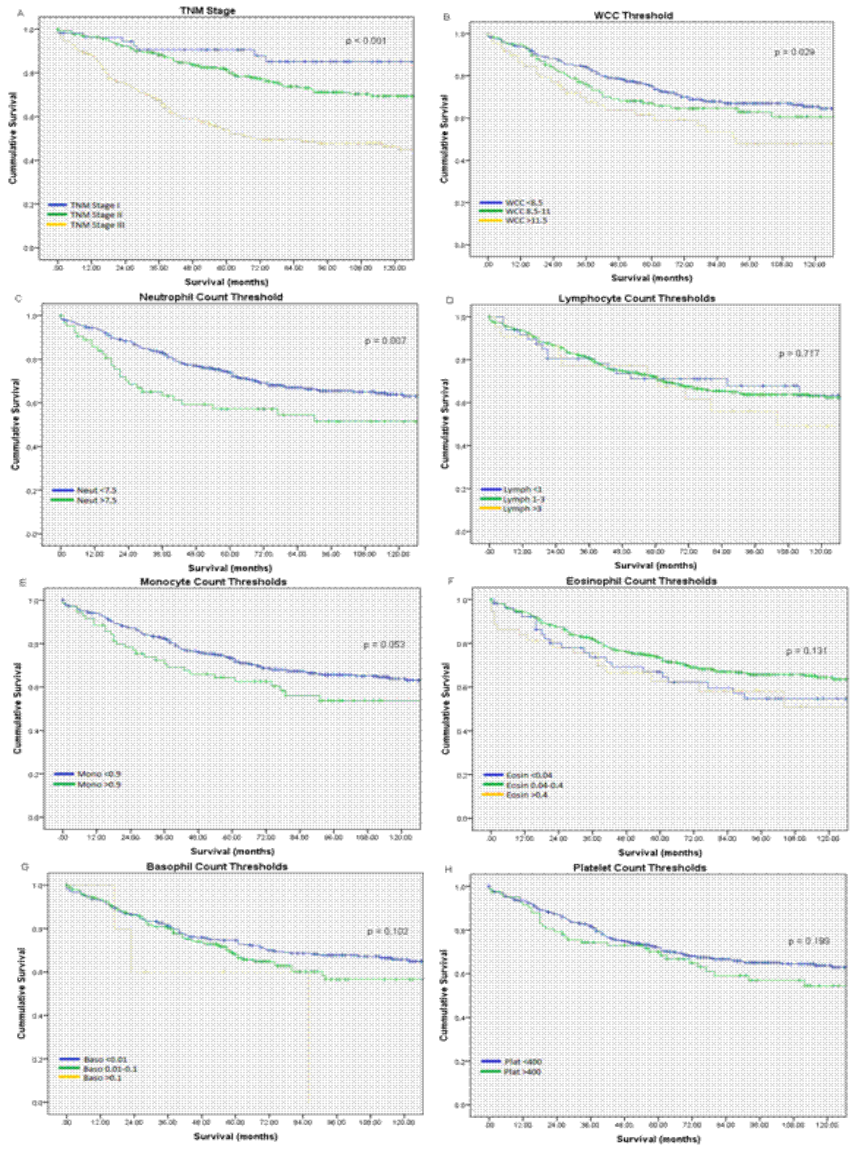


Figure 3.1 **Kaplan Meier plots of TNM stage and components of the differential WCC and cancer-specific survival in total cohort**

(A) TNM stage, (B) white cell count, (C) neutrophil count, (D) lymphocyte count, (E) monocyte count, (F) eosinophil count, (G) basophil count, (H) platelet count

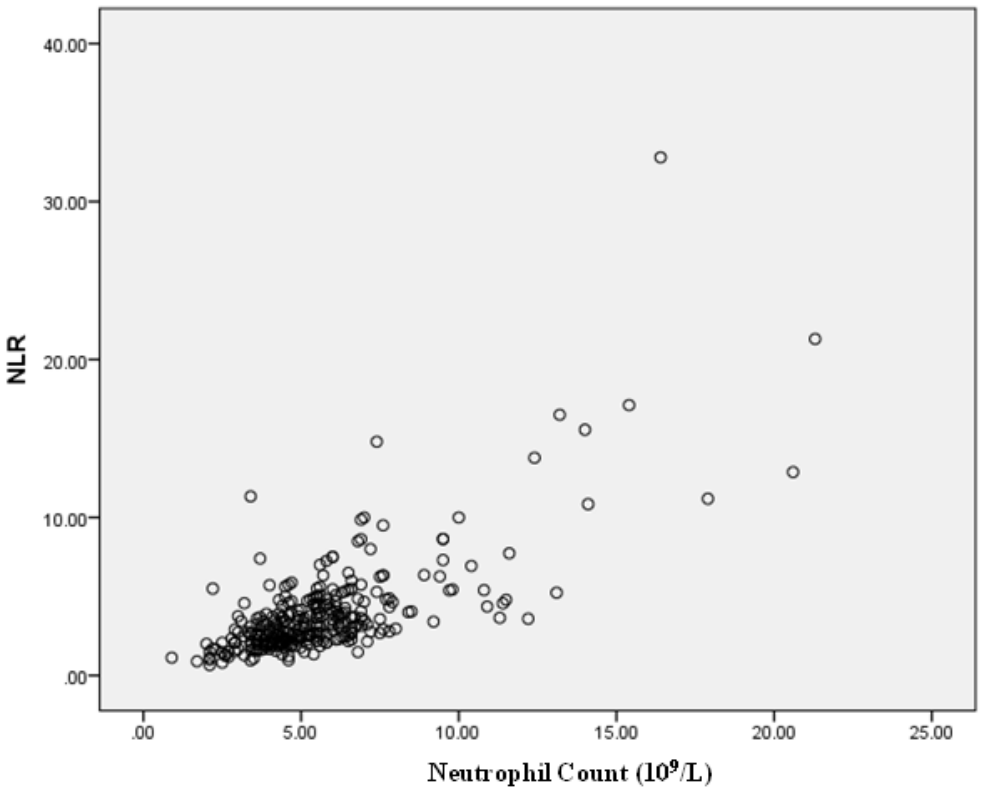


Figure 3.2 **The relationship between the neutrophil count and the NLR (r_s = 0.633, p < 0.001) in patients undergoing elective surgery for colorectal cancer with node negative disease**

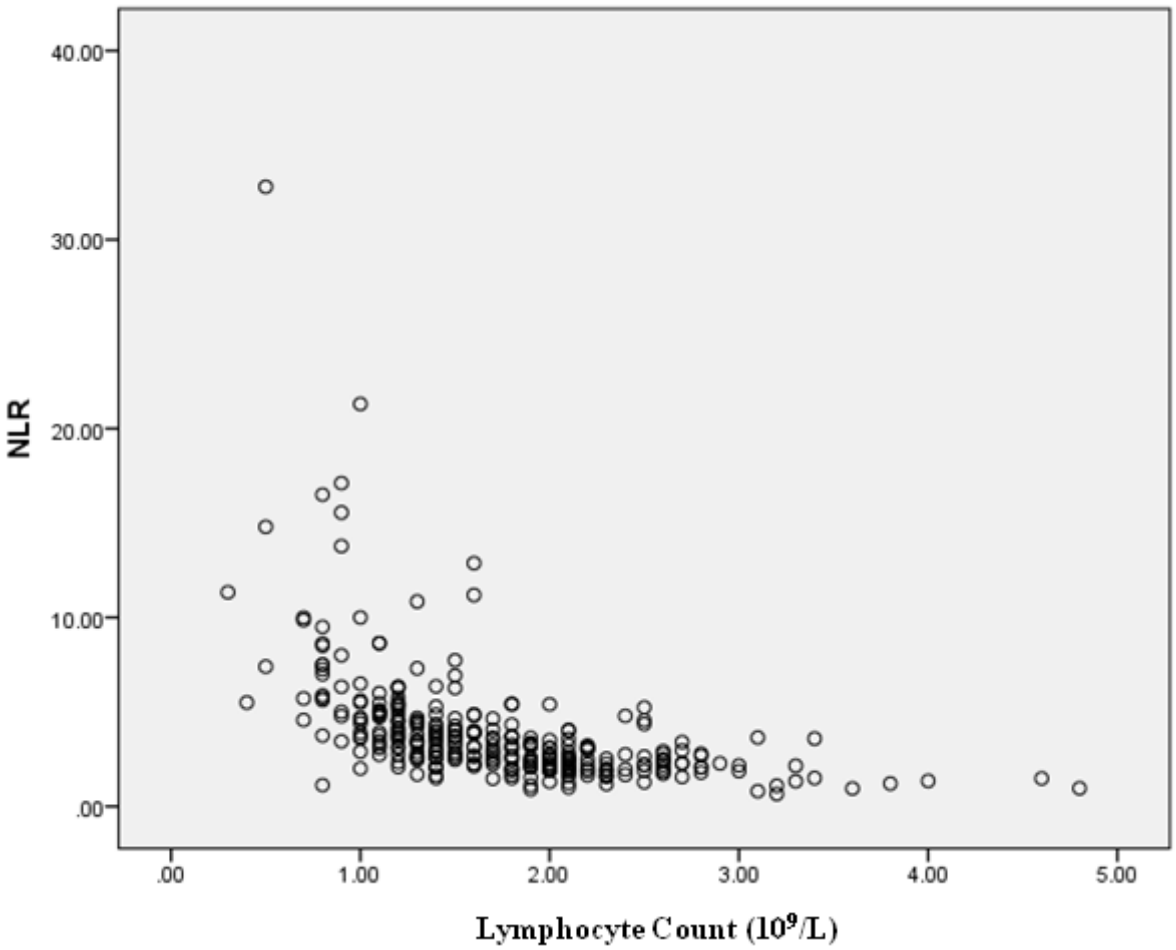


Figure 3.3 **The relationship between the lymphocyte count and the NLR (r_s = 0.689, p<0.001) in patients undergoing elective surgery for colorectal cancer with node negative disease**

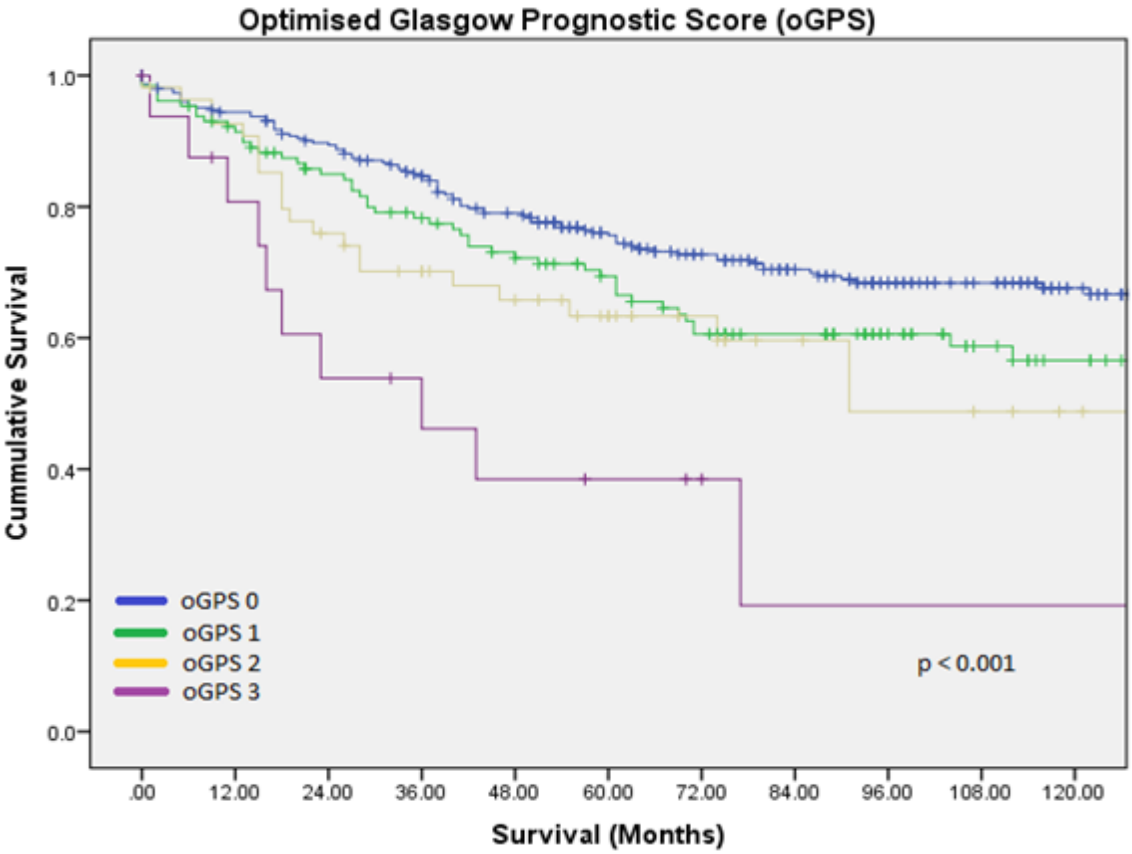


Figure 3.4 **Kaplan Meier plot of optimised Glasgow Prognostic Score (oGPS) and cancer specific survival in total cohort**

4 The neutrophil-platelet score (NPS) predicts survival in primary operable colorectal cancer and a variety of common cancers

4.1 Introduction

It is now established that the presence of a pre-operative systemic inflammatory response is predictive of disease progression and poorer outcome, regardless of tumour stage, in patients with colorectal cancer (Roxburgh and McMillan, 2010). Indeed, systemic inflammation based scoring systems such as the modified Glasgow Prognostic Score (mGPS) and the Neutrophil-Lymphocyte ratio (NLR) have prognostic value in a range of common solid tumours (Guthrie et al., 2013a, McMillan, 2013b, Li et al., 2014a, Templeton et al., 2014, Park et al., 2015, Wang et al., 2015). However, with reference to the NLR, multiple thresholds have been used to define high and low NLR values and some have suggested that its prognostic value is mainly derived from the neutrophil count and that the lymphocyte count makes little contribution (Chapter 3).

Therefore, it is of interest that recent in-vitro studies have suggested that a critical checkpoint early in the inflammatory process involves the interaction between neutrophils and platelets (Sreeramkumar et al., 2014). During this process, neutrophils that are recruited to injured tissues/vessels, scan for activated platelets and when detected neutrophils undergo intravascular migration, further elaborating the inflammatory process. This in-vitro research highlights the importance of the innate immune system, in particular neutrophils, in the elaboration of the systemic inflammatory response. If the interaction between neutrophils and platelets were of clinical relevance then it might be expected that an elevated neutrophil count in the presence of an elevated platelet count would result in an enhanced systemic inflammatory response. Indeed the combination of a platelet count and the NLR (COP-NLR) has recently been reported as a cumulative predictor of survival in patients with colorectal (Ishizuka et al., 2013), gastric (Ishizuka et al., 2014) and oesophageal cancer (Feng et al., 2014). Therefore, the aim of the present study was to examine whether a combination of the neutrophil count and the platelet count was predictive of survival in patients undergoing potentially curative surgery for colorectal cancer and in patients with a variety of common cancers.

4.2 Methods

4.2.1 Patients

For the colorectal cancer cohort, patients with histologically proven colorectal cancer who, on the basis of intra-operative findings and pre-operative computed tomography, were considered to have undergone potentially curative resection at a single centre between March 1999 and May 2013 (n = 813) were initially selected for analysis. Patients in whom a pre-operative neutrophil or platelet count were not available were excluded from analysis (n = 6) as were those patients with TNM stage 0 disease (n = 11). Patient characteristics were collected in a prospectively maintained database and all patient data was anonymised. All tumours were staged according to conventional tumour, node, metastasis classification and additional pathological data obtained from the pathology reports issued at the time of the resection.

Pre-operatively, all patients received thromboembolism prophylaxis and antibiotic prophylaxis as per local protocols and blood samples were taken for routine laboratory analysis. Cut-off values for both neutrophil and platelet count were based on previously reported values (Leitch et al., 2007). The neutrophil-platelet score (NPS) was calculated as follows *(Table 4.1)*: patients with a neutrophil count $\leq 7.5 \times 10^9$/L and platelets $\leq 400 \times 10^9$/L scored 0, patients with neutrophils $>7.5 \times 10^9$/L or platelets $>400 \times 10^9$/L scored 1 and patients with both neutrophils $>7.5 \times 10^9$/L and platelets $>400 \times 10^9$/L scored 2.

Patients were routinely followed up for 5 years following resection as per national guidelines. Date and cause of death were crosschecked with the cancer registration system and Registrar General (Scotland). Cancer specific survival was measured from date of surgery until date of death. The study was approved by the West of Scotland Research Ethics Committee, Glasgow.

For the larger, common cancer cohort, data was taken from the Glasgow Inflammation Outcome Study (Proctor et al., 2015). Patients with routine laboratory measurements of C-reactive protein, albumin and a differential white cell count sampled between January 2000 and December 2007, including neutrophil and platelet counts were obtained by systematically searching the North Glasgow biochemical and haematological database systems. Of the 160,481 patients identified, through linkage with the Scottish Cancer Registry using exact matches of the patient's forename, surname and date of birth, 27 465 were found to have an associated diagnosis of cancer. Of those that had common cancers

previously studied in the GIOS cohort, 9649 had been sampled within two years of their cancer diagnosis and were included in the analysis. Cancers were coded according to the International Classification of Disease 10 (ICD-10) and broadly grouped according to the tumour site. Tumours were listed in order of the magnitude of their inflammatory status as previously demonstrated (Proctor et al., 2010). Patient mortality was established through linkage with the Information Service Division for Scotland (ISD). Patients were excluded if they did not have a blood sample within 2 years of their cancer diagnosis, had incomplete cancer registry follow up, under 16 years old, did not have a complete set of blood results available, had multiple tumours or metastatic disease or had a primary tumour of unknown origin.

4.2.2 Statistical Analysis

The comparison of clinicopathological variables across different NPS scores was performed using a Chi square test. The relationship between the NPS and 5-year survival was examined using log-rank survival analysis. Kaplan-Meier analysis was used to examine the relationship between patients characteristics, NPS, tumour site and cancer-specific and overall survival. Cox proportional hazards multivariate regression models (stratified by tumour site) were used to correct for age and sex and examine the relationship between patient characteristics, NPS and survival. A two-sided p-value of < 0.05 was considered statistically significant. Analyses were performed using SPSS 22.0 (IBM, SPSS, IL, USA).

4.3 Results

A total of 796 patients were included in the analysis of patients undergoing potentially curative surgery for colorectal cancer. The majority were over the age of 65 (66%), male (55%), underwent elective surgery (90%), had an open procedure (87%) and had node negative disease (61%). Median follow up of survivors was 49 (10-180) months with 173 cancer deaths and 135 non-cancer deaths. *Table 4.2* shows the distribution of the clinicopathological characteristics based on the NPS score. Mode of presentation, tumour site, T-stage, TNM stage, margin involvement, peritoneal involvement, tumour perforation and mGPS were significantly different between the 3 NPS groups. On multivariate analysis, adjusting for age and sex and stratified by TNM stage, incremental increase in the NPS was associated with poorer cancer-specific (NPS 1 – HR 1.37, p = 0.091; NPS 2 – HR 1.61, p 0.082) and overall survival (NPS 1 – HR 1.48, p = 0.005; NPS 2 – HR 1.51, p 0.056).

Tables 4.3 & 4.4 show the relationship between pre-operative NPS, TNM stage and 5 year cancer-specific (CSS) and overall survival (OS). CSS in the whole cohort at 5 years varied from 97% in patients with stage I colorectal cancer to 62% in those with stage III colorectal cancer ($p < 0.001$) and from 79% in patients with NPS = 0 to 65% in patients with NPS = 2 ($p = 0.001$). When combined, 5 year CSS varied from 97% in patients with stage I disease and NPS = 0, to 60% in patients with stage III disease and NPS = 2 ($p = 0.026$). OS at 5 years ranged from 86% in patients with stage I disease to 52% in patients with stage III disease ($p < 0.001$) and from 68% in patients with NPS = 0 to 48% in patients with NPS = 2 ($p < 0.001$). When combined, OS at 5 years ranged from 89% in patients with stage I disease and NPS = 0 to 49% in patients with stage III disease and NPS = 2 ($p = 0.001$).

The combination of the platelet count and NLR (COP-NLR) was calculated (using an NLR threshold of 5) in order to determine its effect on survival in patients with operable colorectal cancer. CSS in the whole cohort at 5 years ranged from 78% in patients with COP-NLR = 0 to 67% in patients with COP-NLR = 2 ($p = 0.010$). Furthermore, on multivariate analysis, adjusting for age and sex and stratified by TNM stage, incremental increase in the COP-NLR was not independently associated with cancer-specific survival (COP-NLR 1 – HR 1.31, p = 0.112; COP-NLR 2 – HR 1.41, p 0.268). Therefore, in comparison to COP-NLR, the NPS was superior in predicting survival in patients with operable colorectal cancer.

As emergency surgery, presence of a colonic tumour and nodal status were associated with the NPS, to control for confounding of these, 5 year survival (both CSS and OS) was examined in patients undergoing elective surgery and then in patients with node negative disease and node negative colonic disease *(Tables 4.3 & 4.4).*

In patients undergoing elective surgery, CSS at 5 years ranged from 97% in stage I disease to 63% in stage III disease ($p < 0.001$) and from 80% in patients with NPS = 0 to 62% in patients with NPS = 2 ($p = 0.001$). When combined, CSS at 5 years ranged from 97% in patients with stage I disease and NPS = 0 to 57% in patients with stage III disease and NPS = 2 ($p = 0.019$). A similar relationship was observed in OS at 5 years as survival ranged from 86% to 52% ($p < 0.001$) and from 69% to 41% ($p < 0.001$) with TNM stage and NPS alone, the combination of TMN stage and NPS stratified OS from 89% (TNM I, NPS = 0) to 42% (TNM III, NPS = 2) ($p < 0.001$).

In patients undergoing elective surgery for node negative disease, CSS at 5 years ranged from 97% in stage I disease to 83% in stage II disease ($p = 0.003$) and from 89% in patients with NPS= 0 to 71% in patients with NPS = 2 ($p = 0.002$). When combined CSS ranged from 97% (TNM stage I, NPS = 0) to 68% (TNM stage II, NPS = 2) ($p = 0.018$). Similarly, OS at 5 years ranged from 86% to 71% ($p = 0.012$) and from 78% to 42% ($p < 0.001$) with TNM stage and NPS alone and the combination of TNM stage and NPS stratified OS from 89% (TNM I, NPS = 0) to 37% (TNM II, NPS = 0) ($p < 0.001$).

In patients undergoing elective surgery for node negative colonic disease CSS at 5 years ranged from 99% in stage I disease to 77% in stage II disease ($p = 0.003$) and from 91% in patients with NPS = 0 to 65% in patients with NPS = 2 ($p < 0.001$). When combined, CSS ranged from 98% (TNM stage I, NPS = 0) to 65% (TNM stage II, NPS = 2) ($p = 0.004$). Similarly, OS at 5 years ranged from 83% to 72% ($p = 0.039$) and from 78% to 46% ($p < 0.001$) with TNM stage and NPS alone, the combination of TNM stage and NPS stratified OS from 56% (TNM stage I, NPS = 0) to 46% (TNM stage II, NPS = 2) ($p = 0.002$).

The relationship between the clinicopathological characteristics and the NPS score in patients with a range of common cancers are in shown in *Table 4.5.* Age, sex, mode of presentation, tumour site, mGPS, survival status and survival length was significantly different between NPS groups. On multivariate analysis, adjusting for age and sex and stratified by tumour site, incremental increase in the NPS was significantly associated with

poorer cancer-specific (NPS 1 – HR 1.60, $p < 0.001$; NPS 2 – HR 2.14, $p < 0.001$) and overall survival (NPS 1 – HR 1.61, $p < 0.001$; NPS 2 – HR 2.19, $p < 0.001$).

On Kaplan Meier survival analysis, a greater neutrophil-platelet score is associated with poorer cancer-specific survival in all patients ($p < 0.001$) *(Figure 4.1)*. On Kaplan Meier survival analysis, based on individual tumour types is shown in *Figure 5.2*. Increasing NPS was significantly associated with poorer cancer-specific survival in patients with breast ($p < 0.001$), bladder ($p < 0.001$), colorectal ($p < 0.001$), gastroeosophageal ($p < 0.001$), gynaecological ($p < 0.001$), head and neck ($p < 0.001$), Hepaticopancreaticobiliary (HPB) ($p = 0.009$), prostatic ($p < 0.001$), pulmonary ($p < 0.001$) and renal cancers ($p < 0.001$).

4.4 Discussion

The results of the present study show that the combination of neutrophils and platelets in a clinical scoring system, the neutrophil-platelet score (NPS), can be used to predict survival, independent of TNM stage, in patients undergoing potentially curative surgery for colorectal cancer. Furthermore, the results of the present study provide evidence that this simple, novel, objective score has prognostic value in a variety of common cancers. These results confirm the importance of activation of the innate immune response in predicting outcome in patients with cancer.

The results of the present study are consistent with those of Ishizuka and colleagues who reported that the combination of platelets and the NLR was a predictor of post-operative survival in both colorectal and gastric cancer (Ishizuka et al., 2013, Ishizuka et al., 2014). However, recent evidence would appear to suggest that when using the differential white cell count to predict outcomes, the neutrophil count is the dominant component and as a result the lymphocyte count adds little to its prognostic effect (Chapter 3). Furthermore, recent work (Ishizuka et al., 2013, Ishizuka et al., 2014) has suggested that the combination of a platelet count to the NLR (COP-NLR) improves the prediction of outcome. In the present study when the prognostic value of the COP-NLR was examined, the NPS had superior prognostic value. Due to the differences in the formation of the COP-NLR and the NPS the basis of the difference in prognostic value is not clear. Nevertheless, taken together these results would suggest that neutrophils and platelets were the main factors determining the prognostic value of the COP-NLR.

There are other systemic inflammation based scores that have prognostic value in patients with primary operable colorectal cancer and a variety of common solid tumours. The most validated of these is the GPS/mGPS (McMillan., 2013b, Park et al., 2015, Proctor et al., 2015). Indeed, it was of interest in the present study that as the NPS increased from 0 to 2 the median concentration of CRP increased from 6 to 55 mg/L and median concentration of albumin decreased from 38 to 36 g/L *(both $p < 0.001$)*. Therefore it would appear that both these scoring systems are related measures of the systemic inflammatory response. Nevertheless, the present results are of considerable interest since the GPS/mGPS requires the measurement of two acute phase proteins and in many centres they may not be routinely assessed. Together with previous results (Proctor et al., 2013) the present results show the complementary prognostic value of the NPS.

It is of interest that Kumar and colleagues recently reported that, in 1300 patients in phase I clinical cancer trials, the neutrophil-lymphocyte ratio (NLR) was an independent prognostic factor for overall survival (Kumar et al., 2015). Furthermore, they reported that the neutrophil count but not the lymphocyte count had prognostic value. This finding is consistent with our work in patients with primary operable colorectal cancer (Leitch et al., 2007) (Chapter 3). The results of the present study demonstrate that the combination of increased neutrophils and platelets (both components of the innate immune response) was associated with an elaboration of the systemic inflammatory response and significantly poorer survival in patient with a range of common cancers. Taken together, these findings suggest that activation of the innate immune response is a key step in disease progression and poor survival in patients with cancer.

The elaboration of this systemic inflammatory response and the presence of high numbers of neutrophils and platelets may result in an enhancement of cellular breakdown and proliferation (tissue remodelling). Specifically, neutrophils contain multiple enzymes such as, myeloperoxidase, interleukin-6 (IL-6), defensins lysozyme and collagenase which may directly promote cancer cell intravasation and extravasation (Ardi et al., 2007, Ten Kate et al., 2007). Moreover, activated platelets contain significant quantities of IL-6 and secrete factors such as vascular-endothelial growth factor (VEGF) and other factors that promote angiogenesis and may prevent recognition of cancer cells by the body's own immune system (Ferrara and Davis-Smyth, 1997, Segal, 2005, Jain et al., 2010). Furthermore, both neutrophils and platelets are stimulated by IL-6. This may tip the tumour microenvironment towards disease dissemination and promotion of the growth of metastatic disease.

The present study has a number of possible limitations. Detailed data on the use of pre-operative chemo/radiotherapy in the colorectal cancer cohort and its relation to the timing of the pre-operative blood samples was not available. In both cohorts, data relating to other factors that may have affected neutrophil or platelet levels such as drugs and other co-morbidities were not available.

In conclusion, the neutrophil-platelet score can predict survival in patients undergoing potentially curative surgery for colorectal cancer and in a variety of common cancers. This confirms the importance of activation of the innate immune system in patients with cancer.

Table 4.1 The neutrophil-platelet score (NPS)

Score	Thresholds
NPS 0	Neutrophils $\leq 7.5 \times 10^9$/L and Platelets $\leq 400 \times 10^9$/L
NPS 1	Neutrophils $>7.5 \times 10^9$/L or Platelets $>400 \times 10^9$/L
NPS 2	Neutrophils $>7.5 \times 10^9$/L and Platelets $>400 \times 10^9$/L

Table 4.2 The relationship between Neutrophil-Platelet Score (NPS) and clinicopathological characteristics in patients with colorectal cancer

Clinicopathological Characteristic		All n = 796 (%)	NPS = 0 n = 621 (%)	NPS = 1 n = 133 (%)	NPS = 2 n = 42 (%)	*p-value*
Age						0.318
	<65	266 (34)	210 (34)	42 (32)	14 (33)	
	65-74	272 (34)	221 (36)	39 (29)	12 (29)	
	>75	258 (32)	190 (30)	52 (39)	16 (38)	
Sex						0.553
	Female	361 (45)	276 (44)	66 (50)	27 (64)	
	Male	435 (55)	345 (56)	67 (50)	23 (55)	
Presentation						<0.001
	Elective	718 (90)	582 (94)	109 (82)	27 (64)	
	Emergency	78 (10)	39 (6)	24 (18)	15 (36)	
Adjuvant Therapy						0.241
	No	585 (74)	464 (75)	94 (71)	27 (64)	
	Yes	211 (26)	157 (25)	39 (29)	15 (36)	
Tumour Site						<0.001
	Colon	525 (66)	385 (62)	103 (77)	37 (88)	
	Rectum	271 (34)	236 (38)	30 (23)	5 (12)	
T-stage						<0.001
	1	58 (7)	54 (9)	4 (3)	0 (0)	
	2	102 (13)	94 (15)	7 (5)	1 (2)	
	3	432 (54)	338 (54)	74 (56)	20 (48)	
	4	204 (26)	135 (22)	48 (36)	21 (50)	
N-stage						0.068
	0	486 (61)	391 (63)	71 (53)	24 (57)	
	1	224 (28)	172 (28)	42 (32)	10 (24)	
	2	86 (11)	58 (9)	20 (15)	8 (19)	
TNM Stage						<0.001
	1	132 (17)	124 (20)	7 (5)	1 (2)	
	2	354 (44)	267 (43)	64 (48)	23 (55)	
	3	310 (39)	230 (37)	62 (47)	18 (43)	
Differentiation						0.225
	Mod/well	710 (90)	560 (91)	113 (86)	37 (88)	
	Poor	78 (10)	55 (9)	18 (14)	5 (12)	
Venous invasion						0.309
	No	354 (45)	285 (46)	53 (40)	16 (38)	
	Yes	442 (55)	336 (54)	80 (60)	26 (62)	
Margin Involvement						<0.001
	No	738 (93)	590 (95)	110 (83)	38 (91)	
	Yes	58 (7)	31 (5)	23 (17)	4 (9)	
Peritoneal Involvement						0.001
	No	617 (78)	499 (80)	93 (70)	25 (60)	
	Yes	179 (22)	122 (20)	40 (30)	17 (40)	
Tumour perforation						<0.001
	No	776 (98)	612 (99)	127 (96)	37 (88)	
	Yes	20 (2)	9 (1)	6 (4)	5 (12)	
Modified Glasgow Prognostic Score (mGPS)						<0.001
	0	505 (63)	450 (73)	46 (35)	9 (21)	
	1	164 (21)	102 (16)	45 (34)	17 (41)	
	2	127 (16)	69 (11)	42 (31)	16 (38)	
Survival Status						<0.001
	Alive	488 (61)	407 (66)	64 (48)	17 (40)	
	Cancer death	173 (22)	120 (19)	38 (29)	15 (36)	
	Non-cancer death	135 (17)	94 (15)	31 (23)	10 (24)	
Survival (Months)[$]		103	107	69	57	<0.001

[$] median overall survival

Table 4.3 The relationship between the NPS and 5 year cancer-specific survival in patients undergoing curative resection of colorectal cancer

	NPS = 0 (Neut ≤ 7.5 x10^9/L and Plat ≤400 x10^9/L)		NPS = 1 (Neut >7.5 x10^9/L or Plat >400 x10^9/L)		NPS = 2 (Neut >7.5 x10^9/L and Plat >400 x10^9/L)		All (NPS 0-2)	
All Patients	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	**n**	**5-yr CSS % (SE)**
Stage I	124	97 (2)	7	-	1	-	132	97 (2)
Stage II	267	85 (3)	64	79 (6)	23	68 (11)	354	82 (2)
Stage III	230	63 (4)	62	56 (7)	18	60 (12)	310	62 (3)
All (Stage 0-III)	621	79 (2)	133	69 (5)	42	65 (8)	796	76 (2)
Elective	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**
Stage I	124	97 (2)	7	-	1	-	132	97 (2)
Stage II	248	85 (3)	53	79 (6)	14	68 (13)	315	83 (2)
Stage III	210	65 (4)	49	58 (8)	12	57 (15)	271	63 (3)
All (Stage 0-III)	582	80 (2)	109	70 (5)	27	62 (10)	718	78 (2)
Elective, Node Negative	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**
Stage I	124	97 (2)	7	-	1	-	132	97 (2)
Stage II	248	85 (3)	53	79 (6)	14	68 (13)	315	83 (2)

All (Stage 0-II)	372	89 (2)	60	81 (6)	15	71 (12)	447	87 (2)
Elective, Node Negative Colon	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**	***n***	**5-yr CSS % (SE)**
Stage I	71	98 (2)	6	-	0	-	77	99 (1)
Stage II	161	89 (3)	40	82 (7)	12	65 (14)	213	77 (4)
All (Stage 0-II)	232	91 (2)	46	84 (6)	12	65 (14)	290	89 (2)

CSS - cancer-specific survival. Survival not calculated if $n<10$

Table 4.4 The relationship between NPS and 5 year overall survival in patients undergoing curative resection of colorectal cancer

	NPS = 0 (Neut ≤ 7.5 x10^9/L and Plat ≤400 x10^9/L)		NPS = 1 (Neut >7.5 x10^9/L or Plat >400 x10^9/L)		NPS = 2 (Neut >7.5 x10^9/L and Plat >400 x10^9/L)		All (NPS 0-2)	
All Patients	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	**n**	**5-yr OS % (SE)**
Stage I	124	89 (4)	7	-	1	-	132	86 (4)
Stage II	267	73 (3)	64	68 (6)	23	45 (11)	354	70 (3)
Stage III	230	54 (4)	62	45 (7)	18	49 (12)	310	52 (3)
All (Stage 0-III)	621	68 (2)	133	56 (5)	42	48 (8)	796	65 (2)
Elective	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**
Stage I	124	89 (4)	7	-	1	-	132	86 (4)
Stage II	248	74 (3)	53	68 (7)	14	37 (14)	315	71 (3)
Stage III	210	55 (4)	49	45 (8)	12	42 (14)	271	52 (3)
All (Stage 0-III)	582	69 (2)	109	56 (5)	27	41 (10)	718	66 (2)
Elective, Node Negative	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**
Stage I	124	89 (4)	7	-	1	-	132	86 (4)
Stage II	248	74 (3)	53	68 (7)	14	37 (14)	315	71 (3)
All (Stage 0-II)	372	78 (3)	60	65 (7)	15	42 (14)	447	75 (2)

Elective, Node Negative Colon	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**	***n***	**5-yr OS % (SE)**
Stage I	71	56 (12)	6	-	0	-	77	83 (5)
Stage II	161	75 (4)	40	25 (10)	12	46 (15)	213	72 (4)
All (Stage 0-II)	232	78 (3)	46	24 (9)	12	46 (15)	290	75 (3)

OS - overall survival. Survival not calculated if $n<10$

Table 4.5 The relationship between NPS and patient demographics in an incidentally sampled cohort of patients with cancer

		All	NPS = 0	NPS = 1	NPS = 2	*p-value*
		n = 9649	n = 5933 (%)	n = 2779 (%)	n = 937 (%)	
Age						<0.001
	<65	4631	3032 (65)	1170 (25)	429 (10)	
	65-74	2885	1696 (59)	886 (31)	303 (10)	
	>75	2133	1205 (56)	723 (34)	205 (10)	
Sex						<0.001
	Female	4584	2646 (58)	1468 (32)	470 (10)	
	Male	5065	3287 (65)	1311 (26)	467 (9)	
Presentation						<0001
	Non-Emergency	6098	4236 (69)	1398 (23)	464 (8)	
	Emergency	3551	1697 (48)	1381 (39)	473 (13)	
Tumour Site						<0.001
	Breast	1921	1611 (84)	268 (14)	42 (2)	
	Bladder	437	259 (59)	128 (29)	50 (11)	
	Gynaecological	507	298 (59)	142 (28)	67 (13)	
	Prostatic	509	322 (63)	159 (31)	28 (6)	
	Gastroesophageal	933	548 (59)	294 (32)	91 (9)	
	Haematological	914	678 (74)	188 (21)	48 (5)	
	Renal	459	288 (63)	134 (29)	37 (8)	
	Colorectal	1086	604 (56)	356 (33)	126 (11)	
	Head And Neck	633	365 (58)	204 (32)	64 (10)	
	Hepaticopancreaticobiliary	556	309 (56)	183 (33)	64 (11)	
	Pulmonary	1694	651 (38)	723 (43)	320 (19)	
mGPS						<0.001
	0	4013	3305 (82)	629 (16)	79 (2)	
	1	2757	1504 (55)	931 (34)	322 (11)	
	2	2879	1124 (39)	1219 (42)	536 (19)	
Survival Status						<0.001
	Alive	3502	2757 (79)	633 (18)	112 (3)	
	Cancer death	5218	2620 (50)	1849 (35)	749 (15)	
	Non-cancer death	929	556 (60)	297 (32)	76 (8)	
Survival (Months)[$]		21	55	7	3	<0.001

[$] median overall survival

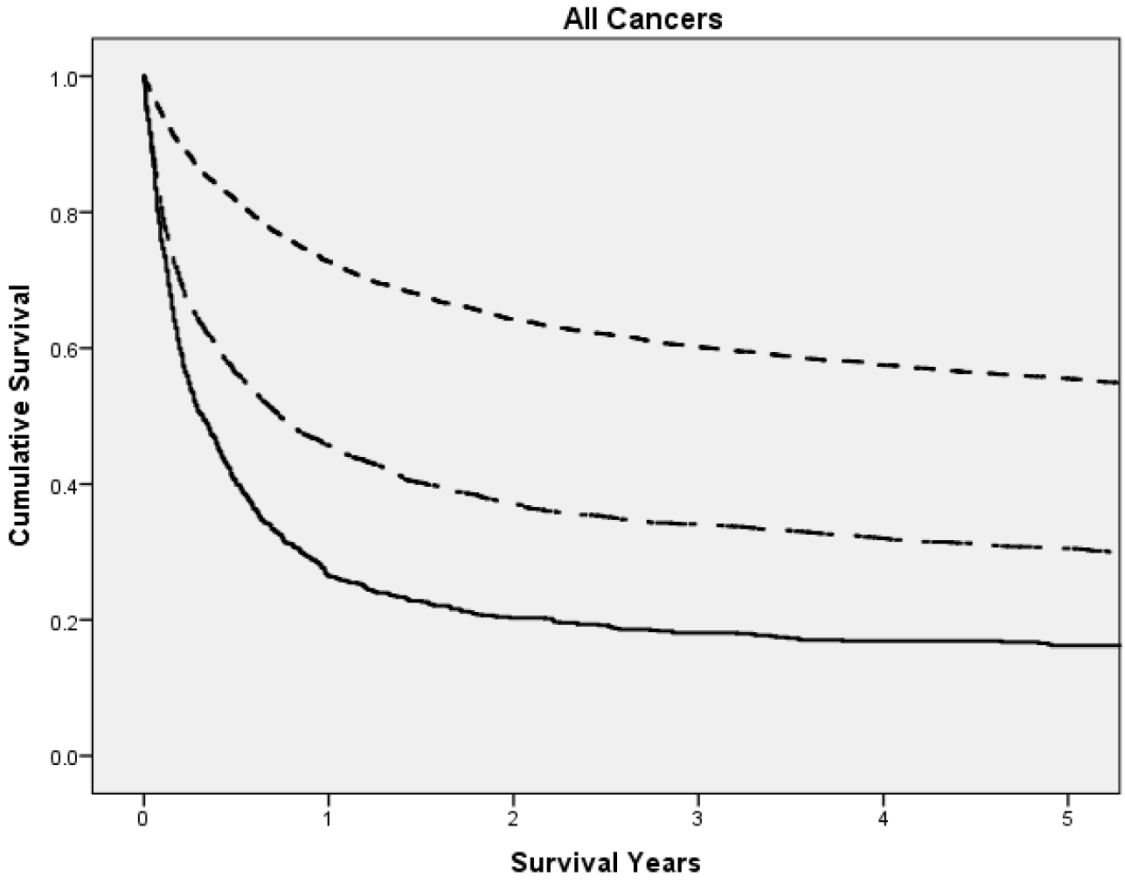


Figure 4.1 **The relationship between NPS and cancer-specific survival in all patients in the GIOS cohort.**
NPS 0 (top, small dash line); NPS 1 (middle, large dash line); NPS 2 (bottom, solid line); p<0.001

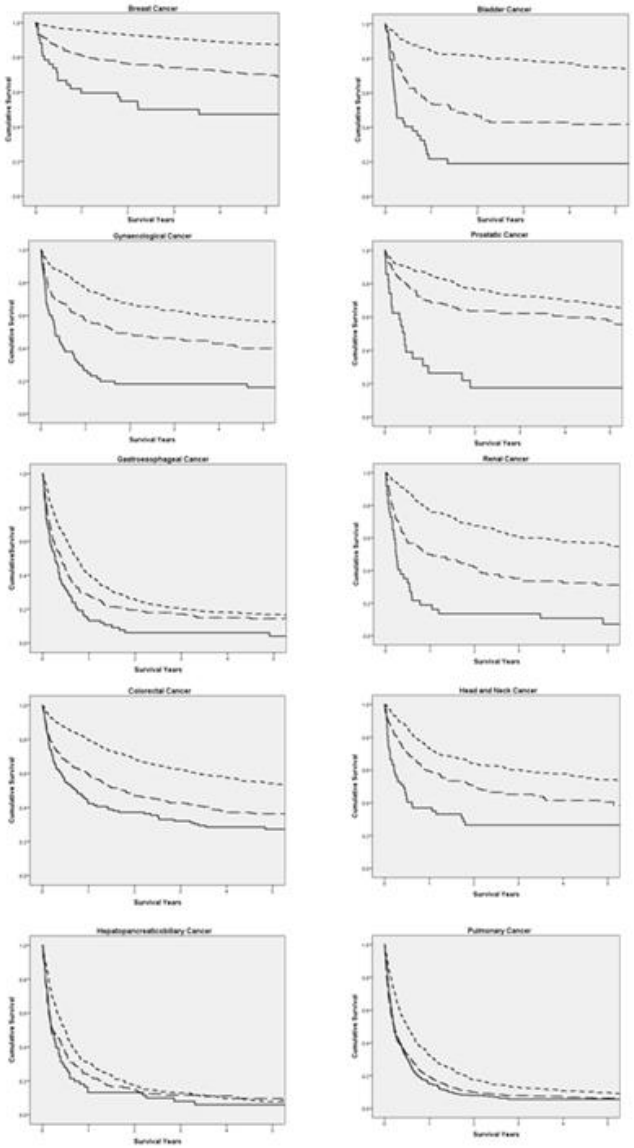


Figure 4.2 **The relationship between the NPS and cancer-specific survival in each tumour site.**
NPS 0 (top, small dash line); NPS 1 (middle, large dash line); NPS 2 (bottom, solid line)

Breast p<0.001; Bladder p<0.001; Gynaecological p<0.001; Prostatic p<0.001; Gastroesophageal p<0.001; Renal p<0.001; Colorectal p<0.001, Head and Neck p<0.001; HPB p=0.009 and pulmonary p<0.001.

5 Routine Clinical Markers of the Magnitude of the Systemic Inflammatory Response Following Elective Surgery: A Systematic Review

5.1 Introduction

It has long been recognised that injury to the body, either from trauma or from major and minor surgical procedures causes a stereotypical cascade of neuroendocrine, cytokine, acute phase and metabolic responses (Cuthbertson, 1979). For example, following uncomplicated surgical injury, within minutes there is activation of the sympathetic nervous system resulting in a neuroendocrine response of increased secretion of catecholamines (adrenaline and noradrenaline) into the circulation leading to tachycardia, hypertension, fever and tachypnoea (Desborough, 2000). At the same time there is also increased secretion of the pituitary hormones such as corticotrophin, growth hormone and arginine vasopressin. Corticotrophin acts on the adrenal cortex to stimulate cortisol secretion peaking at approximately 4-6 hours following surgical injury whilst vasopressin affects the kidney and fluid balance. There is then a subsequent increase in the production of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), IL-8, IL-12, IL-18 and in particular interleukin 6 (IL-6) (Marik and Flemmer, 2012, Dinarello et al., 2013). IL-6 peaks at approximately 18-24 hours following surgical injury. These cytokines are produced in response to injury by many cells throughout the body and form a complex signalling system for subsequent production of acute phase proteins from the liver and increased stimulation of myeloid tissue (Bauer and Herrmann, 1991, Baigrie et al., 1992, Gabay and Kushner, 1999). There are changes in circulating myeloid cells, in particular towards increased numbers of white cells (WCC) dominated by neutrophils as well as increased numbers of myeloid derived suppressor cells and platelets. There are changes in plasma concentrations of a number of acute phase proteins, in particular C-reactive protein (CRP) (Pepys and Hirschfield, 2003, Kao et al., 2006, Cole et al., 2008) which peaks at approximately 48-72 hours (Colley et al., 1983).

The net effect of the evolution of the systemic inflammatory response is increased catabolism of skeletal muscle in order to provide energy and substrates for the liver, to maintain fluid and cardiovascular homeostasis and for healing of the surgical wound (Desborough, 2000). Therefore, while this response to injury has been referred to as the surgical stress or acute phase response it is more informatively known as the systemic

inflammatory response (SIR) since it affects all organs and tissues of the body (Gabay and Kushner, 1999). It appears to be primarily the result of activation of the innate and humoral immune/inflammatory system. A number of the components of the systemic inflammatory response have both pro and anti-inflammatory properties. Once healing of the injured site is established, anti-inflammatory components of the systemic inflammatory response become prominent, causing it to wane and return to the normal non-inflammatory state.

Following uncomplicated surgery, the degree of elevation of these markers is considered to reflect the magnitude of the SIR (Desborough, 2000). Most commonly cortisol, IL-6, WCC and CRP have been used in clinical practice. Therefore, the aim of the present systematic review was to examine, across a range of surgical procedures, the magnitude of the SIR following elective surgery by using the markers cortisol, IL-6, WCC and CRP. Such information is a pre-requisite to objectively identifying those peri-operative interventions purported to have clinical value and those surgical procedures that may compromise immune function.

5.2 Methods

A literature search was performed using the keywords: surgery, endocrine response, systemic inflammatory response, acute phase response, cortisol, IL-6, WCC and CRP was made of the US National Library of Medicine (MEDLINE), the Excerpta Medica Database (EMBASE), PubMed and the Cochrane Database of Systemic Reviews (CDSR) for articles that reported cortisol, IL-6, WCC and CRP following surgery. From this, titles of studies and abstracts were analysed for relevance. Bibliographies of relevant studies were searched and cross referenced to identify any additional studies relevant for inclusion. Included studies had to assess the systemic inflammatory response using at least one objective marker (cortisol, IL-6, WCC or CRP). On review the studies were divided into those that analysed cortisol, IL-6, WCC and CRP and subsequently into different surgical specialties for each analyte. The number of post-operative sampling points was recorded and any study where less than 3 post-operative sampling points were reported was excluded as it was not possible to examine the peak of the response. The number of hours post-operatively at which the peak response occurred was documented. Where more than one study was reported for an analyte in a surgical specialty a weighted average peak value was calculated. Where the analyte was reported in different units across different studies they were converted to SI units. Included papers were examined by the authors and any conflicts that arose were discussed with the senior author.

Although there is a continuum between minor and major surgery, for comparison three arbitrary categories were made, based on the degree of clinical support. These were minor (requiring general clinical care), moderate (requiring specialist clinical care or short high dependency stay) and major surgical injury (requiring specialist clinical care, prolonged high dependency or intensive care stay). For each analyte, the included studies were sorted into one of these three categories and then classified according to the surgical procedure in order to investigate whether this continuum might well be delineated by the magnitude of cortisol, IL-6, WCC and CRP.

5.3 Results

5.3.1 Study Selection Process

The selection process is summarised in *Figure 5.1.* Using the above search criteria, abstracts were identified and the bibliographies of these articles were hand searched for additional articles. In total 296 abstracts were identified and full text obtained. Subsequent analysis of the full text of these articles resulted in 132 being excluded from analysis: 3 were unavailable in English language, 12 were review articles, 5 were animal studies, 14 articles involved emergency or trauma procedures, 1 study did not separate laparoscopic and open procedures and 84 studies had less than 3 post-operative sampling points. 164 studies were available for further analysis: 43 reported the response of cortisol (n = 1881), 103 the response of interleukin-6 (n = 4192), 35 the response of WCC (n = 2064) and 108 the response of CRP (n = 6531). It should be noted that some studies measured more than one analyte.

5.3.2 Markers of the Systemic Inflammatory Response

Cortisol Response Following Elective Surgery

43 studies reported the response of cortisol following elective surgery (n = 1881). These results are shown in *Table 5.1.* The time of peak response occurred from 0-4 hours post-operatively and a peak response was seen in both minor and major procedures.

In the minor surgical injury category there was a wide range of peak cortisol levels, ranging from 459 for rectopexy to 1009 nmol/L for inguinal hernia repair. In the moderate surgical injury group peak cortisol responses again varied from 450 nmol/L in a knee replacement to 621 nmol/L for colorectal cancer resection, 772 nmol/L in hip replacement, 910 nmol/L in spinal surgery and 1018 nmol/L in gastric bypass. In the major surgical injury group there was a range of peak responses ranging from 743 nmol/L for oesophagectomy to 1200 nmol/L for aneurysm repair.

Within minor to major surgery groupings there also appeared to be little difference when comparing minimally invasive or laparoscopic procedures with open procedures. This was best seen with laparoscopic and open cholecystectomy (600 vs 750 nmol/L), laparoscopic and open inguinal hernia repair (946 vs 1071 nmol/L) and laparoscopic and open gastric bypass (993 vs 1048 nmol/L). Therefore the magnitude of cortisol concentrations following elective surgery was variable and was not clearly associated with the magnitude

of surgical injury within the surgical category or within the invasiveness of the surgical procedure.

Interleukin-6 (IL-6) Response Following Surgery

103 studies reported the response of IL-6 following different surgical procedures (n = 4192). The results are shown in *Table 5.2.* The time of peak response occurs between 12 and 24 hours and a peak response was seen in both minor and major procedures.

In the minor surgical injury group there was a peak IL-6 response seen with different procedures including inguinal hernia repair and cholecystectomy (13 and 77 pg/ml). In the moderate surgical injury group, the response of IL-6 was generally greater than that seen in the minor injury group. Peak response ranged from 62 pg/ml with prostatectomy, to 140 pg/ml with total hip replacement, 161 pg/ml with colorectal cancer resection and 321 pg/ml with total knee replacement. The magnitude of response was greater again in the major surgical injury group, ranging from 248 pg/ml with AAA repair to 345 pg/ml with major liver resection and 428 pg/ml with open cardiac surgery.

Within minor to major surgery groupings there also appeared to be a difference between minimally invasive or laparoscopic procedures and open procedures. This difference was best seen with laparoscopic and open rectopexy (21 vs 111 pg/ml), laparoscopic and open cholecystectomy (62 vs 95 pg/ml), laparoscopic and open gastrectomy (44 vs 129 pg/ml), laparoscopic and open abdominal hysterectomy (19 vs 166 pg/ml), laparoscopic and open miscellaneous colorectal resection (140 vs 393 pg/ml), and endovascular and open aneurysm repair (116 vs 332 pg/ml). Therefore, the magnitude of IL-6 concentrations following elective surgery was associated with the magnitude of surgical injury and with the invasiveness of the surgical procedure.

White Cell Count (WCC) Response Following Surgery

35 studies reported the response of WCC following different surgical procedures (n = 2064). The results are shown in *Table 5.3.* The peak response occurred at approximately 24 hours post-operatively.

In the minor injury category there was a range of peak WCC levels, ranging from 10.3 $x10^9$/L with inguinal hernia repair to 11.8 $x10^9$/L with cholecystectomy. In the moderate surgical injury group peak WCC responses varied from 7.5 $x10^9$/L for breast reconstruction to 8.7 $x10^9$/L for colorectal cancer resection, 9.0 $x10^9$/L for hip replacement

and knee replacement, 11.7 $x10^9$/L for hysterectomy and 13.3 $x10^9$/L for gastric bypass. Similar was seen in the major surgical injury group with a range of responses from 9.9 $x10^9$/L in abdominal aortic aneurysm repair to 12.5 $x10^9$/L with oesophagectomy and 16.6 $x10^9$/L in endovascular thoracic aneurysm repair.

Within minor to major surgery groupings there also was little difference in the magnitude of response when comparing minimally invasive or laparoscopic with open procedures. This was best seen in laparoscopic and open inguinal hernia repair (10.1 vs 10.4 $x10^9$/L), laparoscopic and open colorectal cancer resection (9.9 vs 8.6 $x10^9$/L), laparoscopic and open gastrectomy (9.0 vs 10.8 $x10^9$/L), and endovascular and open repair of abdominal aortic aneurysm (9.7 vs 10.1 $x10^9$/L). Therefore, the magnitude of WCC concentrations following elective surgery was variable and was not clearly associated with the magnitude of surgical injury associated with the surgical category or with the invasiveness of the procedure.

C - reactive protein (CRP) Response Following Surgery

108 studies reported the response of CRP following different surgical procedures (n = 6531). The results are shown in *Table 5.4.* The timing of peak response occurred later than that of IL-6, between 24 and 72 hours post operatively and was seen in both minor and major procedures.

In the minor surgical injury group there was a peak CRP response in different procedures including inguinal hernia repair and cholecystectomy (40 and 52 mg/L). In the moderate surgical injury group the magnitude of CRP response was greater than in the minor injury group and ranged from 74 mg/L with neurosurgical procedures to 123 mg/L with colorectal cancer resection, 145 mg/L with hip replacement, 151 mg/L with spinal surgery and 153 mg/L with knee replacement. The magnitude of response of CRP was greater still in the major surgical injury group ranging from163 mg/L in those undergoing abdominal aortic aneurysm repair to 186 mg/L with oesophagectomy and 189 mg/L in open cardiac surgery.

Within minor to major surgery groupings there also was a difference between minimally invasive or laparoscopic and open procedures. This difference was best seen with laparoscopic and open inguinal hernia repair (30 vs 49 mg/L), laparoscopic and open cholecystectomy (27 vs 80 mg/L), laparoscopic and open abdominal hysterectomy (29 vs 99 mg/L), laparoscopic and open colorectal cancer resection (97 vs 133 mg/L), and

endovascular and open aneurysm repair (132 vs 180 mg/L). Therefore, the magnitude of CRP concentrations following elective surgery was associated with the magnitude of surgical injury, and with the invasiveness of the surgical procedure.

5.4 Discussion

The present review shows that cortisol, IL-6, WCC and CRP all peak following all types of elective surgery, minor and major; laparoscopic and open. The peak responses following surgery occur at approximately 0-4 hours for cortisol, 12-24 hours for IL-6, 24-48 hours for WCC and 24-72 hours for CRP. Only IL-6 and CRP were consistently associated with the magnitude of surgical injury. Therefore, IL-6 and CRP would appear to be useful markers for assessing the magnitude of the SIR following elective surgery. However, in contrast to IL-6, CRP is routinely measured in clinical laboratories worldwide and extensively used in clinical practice and therefore may be useful in the monitoring and modulation of the SIR following elective surgery. Indeed, using CRP in this way, from the present review of the literature it was identified that laparoscopic surgery was associated with a reduction of the SIR.

The present review has a number of possible limitations. The conclusions drawn from the present study are based on the premise that the peak values of IL-6 and CRP are mainly due to the degree of tissue damage and magnitude of the SIR during the surgical procedure. However, there are several potential confounding factors that need to be considered. Pre-operative factors such age, obesity, co-morbid disease, emergency presentation and inflammatory status may enhance the peak SIR. For example, increasing age has been reported to be associated with chronic innate immune activation and changes in monocyte function (Hearps et al., 2012, Kale and Yende, 2011, Michaud et al., 2013). Also, co-morbid disease is associated with increased markers of the SIR such as IL-6 and CRP (Esser et al., 2014).

The development of post-operative complications may also be considered as a potential confounding factor since the peak SIR may reflect, in part, the development of complications. However, with respect to CRP, day 2 concentrations were similar in both those who did and did not go on to develop post-operative complications (Warschkow et al., 2012b). Furthermore, with respect to post-operative complications including anastomotic leak, CRP concentrations on days 3-5 have been reported to be of most importance (Singh et al., 2014, McDermott et al., 2015). The selection of only studies in which surgery was carried out electively would help minimise such potential confounding factors.

Another limitation was that the data was from a variety of surgical procedures across a range of surgical specialties that span several decades. During this time period there may have been changes in the measurement of the analytes and clinical management of surgical procedures that might alter the magnitude of the peak values. However, it is unlikely to have altered the timing and relative magnitude of the peak SIR for the analytes considered. Also, the timing of blood samples was different across the range of studies included which may affect the peak values of the analytes considered. Also, in order to minimise the effect of sampling time on results, studies were only included if they included 3 or more measurements post operatively and at the time of the known analyte peak. It was of interest that the median peak IL-6 and CRP concentrations for the surgical procedures of cholecystectomy, colorectal cancer resection and abdominal aortic aneurysm repair were not lower in the more recent studies *(See Tables 5.2 & 5.4)*. Therefore it would appear that the magnitude of the post-operative inflammatory response was not associated with the more recent surgery and the introduction of perioperative care protocols. Other than laparoscopic surgery, it is not clear whether individual components of these protocols are associated with a reduction in the magnitude of IL-6 or CRP. Indeed, it is anticipated that the present review will stimulate more research in this area.

It remains unclear which aspect of the surgery was responsible for the magnitude of the SIR. However, the lower SIR associated with laparoscopic surgery would suggest that certain aspects of this technique, for example smaller wounds, are associated with a reduced degree of tissue trauma. Factors other than the degree of tissue trauma or injury may be involved. Specifically, perioperative hypoxia can result in raised inflammatory markers and in certain circumstances can be seen to promote inflammation (Eltzschig and Carmeliet, 2011). Perioperative pain and its management may also be involved. Peripherally active opioids can modulate the inflammatory process and wound healing (Stein and Kuchler, 2013), whilst improved overall survival has been reported in patients with breast cancer who received intra-operative non-steroidal anti-inflammatory agents and opiates (Forget et al., 2014). Despite this, it has been reported that there is no difference in the levels of post-operative CRP in patients who receive general or regional anaesthesia (Buyukkocak et al., 2006, Kahveci et al., 2014), in patients receiving general or combined general and regional anaesthesia (Papadima et al., 2009) and following the use of 2 different inhalation agents (Marana et al., 2013). Similarly, there appears to be no difference in CRP levels comparing those who receive epidural anaesthesia followed by epidural analgesia or spinal anaesthesia followed by morphine analgesia (Chloropoulou et al., 2013) and those who receive continuous opiate or intermittent non-opiate analgesia

(Avdagic et al., 2010). Therefore, it would appear that the magnitude of the SIR, as evidenced by CRP, is mainly determined by the magnitude of surgical insult.

The results of the present study would indicate that cortisol is rapidly and maximally stimulated since the peak values were similar across a variety of elective surgical procedures. Secretion of cortisol by the adrenal gland is stimulated by release of corticotrophin from the anterior pituitary gland and levels begin to increase minutes after surgery. In the normal state the body employs a negative feedback mechanism to limit the amount of corticotrophin released from the pituitary but following surgical injury this mechanism is perturbed such that cortisol concentrations are increased over a period of hours before returning to normal (Weissman, 1990). Therefore, the release of cortisol following surgical injury is a neuroendocrine response that appears to be an all or none phenomenon and appears to be consistent with the present results. In contrast, the peak IL-6 and CRP concentrations appear to be responsive to the magnitude of elective surgical injuries and procedures. Consistent with these observations, IL-6 is a pro-inflammatory cytokine that is produced by many cells throughout the body in response to injury. IL-6 levels have been reported to rise from approximately 2 hours following stimulus and peak at approximately 12-24 hours. IL-6 production is primarily regulated by a negative feedback mechanism through suppressors of cytokine signaling (Socs) molecules, coded by genes of the JAK-STAT pathway (Kishimoto, 2010). This acts to reduce overproduction of IL-6 concentrations following injury. Circulating IL-6 concentrations have been reported to be reduced dramatically by 48-72 hours in those with no post-operative complications (Baigrie et al., 1992). IL-6 is thought to be the main inducer of acute phase proteins such as CRP from hepatocytes as well as causing differentiation, proliferation and maturation of haemopoietic progenitors. CRP is considered to be an opsonin and activator of innate immune cells, in particular neutrophils, as well as having both anti and pro-inflammatory properties (Gabay and Kushner, 1999). Elevations of CRP levels begin at approximately 4-6 hours following surgical injury and typically peak at 48 hours. Following uncomplicated surgery, the levels of CRP begin to fall, typically normalising at 72 – 168 hours (Gabay and Kushner, 1999). Therefore, it is perhaps not surprising that, given their relationship and kinetics in plasma, both IL-6 and CRP appear to similarly reflect the magnitude of surgical injury.

Although there are more than 2 dozen interleukins, with IL-12 and IL-18 being uniquely inflammatory, these have been rarely assessed in elective surgery and subject to intervention studies. In contrast, the established pro-inflammatory cytokines have been

subject to such examination. There have been attempts to modify or block aspects of the systemic inflammatory response, namely via tumour necrosis factor (TNF) and IL-1. TNF binding proteins inactivate TNF and are presumed to provide some control of the SIR. Indeed, anti-TNF-α therapies (inflixamab and adalimumab) are widely used in patients with inflammatory bowel disease with good clinical benefit (Yang et al., 2010, Kopylov et al., 2012). Similarly, IL-1 receptor antagonists have been used in patients with sepsis and rheumatoid arthritis with only some clinical benefit seen in rheumatoid arthritis (Freeman and Buchman, 2001). Of the pro-inflammatory cytokines, IL-6 has attracted interest since IL-6 stimulation and production causes a cascade of effects that result in stimulation of acute phase proteins (in particular CRP) and activation of the innate immune system. Clinical trials involving IL-6 blockade have reported efficacy in several inflammatory conditions including rheumatoid arthritis and juvenile idiopathic arthritis, resulting in their use as a monotherapy or in combination with disease modifying antirheumatic drugs (Tanaka and Kishimoto, 2012).

In the present study, WCC did not appear to reflect the magnitude of the surgical injury. However, neutrophils are the major component of the WCC but not normally separately identified in the elective surgery literature. They are 'first responders' present at the site of injury within 1 hour and peak at 24 hours. Levels begin to wane after 48 hours with neutrophils cleared from circulation via the liver, spleen and bone marrow (Kolaczkowska and Kubes, 2013). Moreover, there are other white cells such as lymphocytes, monocytes, basophils, and eosinophils that may respond differently to a surgical injury. To date such changes in these white cells following elective surgical injury has been poorly documented.

Over the last decade, minimal access or laparoscopic surgery has become routinely applied and has been associated with quicker recovery following surgery, shorter hospital stays, better cosmetic results, reduced post-operative pain and quicker return to normal activities (Vittimberga et al., 1998, Buunen et al., 2004, Ng et al., 2005). It has been assumed that some of these benefits are due to a reduced SIR caused by reduced surgical injury. The present review, across the elective surgical literature, would appear to confirm the view that laparoscopic operations evoke less of an inflammatory response, as evidenced by IL-6 and CRP, than open operations.

Anaesthetic practice has also changed in recent times with the development of safer anaesthetic agents, improved pain management and better peri-operative monitoring. A

regimen of short acting anaesthetic agents, muscle relaxation if necessary, minimally invasive monitoring, multimodal peri-operative analgesia and aggressive anti-emetic therapy have been reported to allow quicker return of normal organ functions and fewer episodes of organ dysfunction (Kehlet and Dahl, 2003). Widespread use of peri-operative monitoring such as the oesophageal Doppler has been undertaken with the perceived benefit of allowing the patients cardiac output to be optimised and to guide subsequent fluid administration (Abbas and Hill, 2008). However a recent meta-analysis suggested that actually oesophageal Doppler guided fluid therapy did not influence length of stay or complication rates following colorectal surgery (Srinivasa et al., 2013). It remains to be determined which such anaesthetic practices do indeed modulate the SIR.

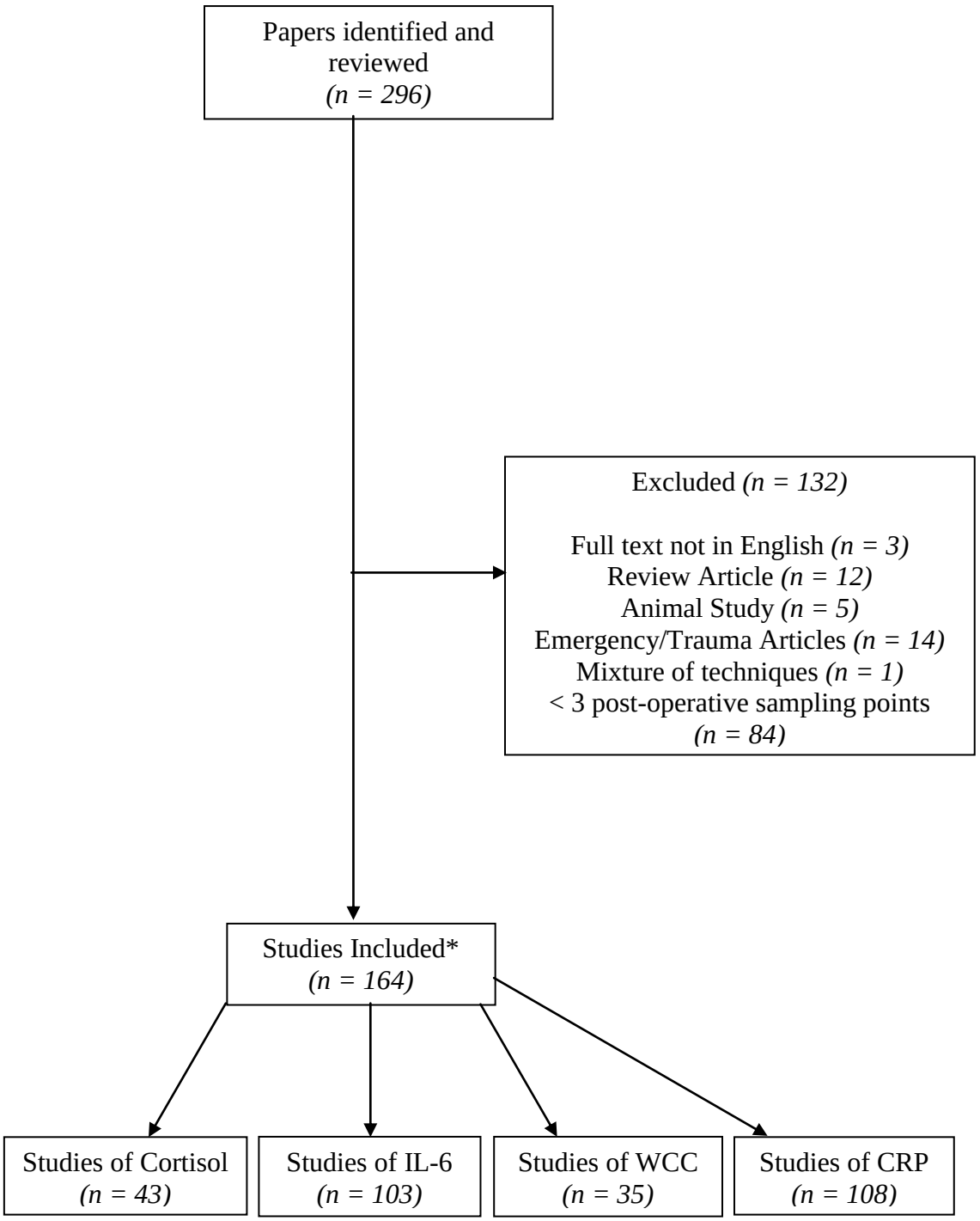
An extension of such work is Enhanced Recovery after Surgery (ERAS) programmes. It has been reported that they reduce the incidence of post-operative complications, resulting in reduced length of hospital stay and improved short term outcomes (Kehlet and Wilmore, 2005, Wind et al., 2006, Kehlet, 2008, Varadhan et al., 2010, Melnyk et al., 2011, Rawlinson et al., 2011) by purportedly reducing the SIR following surgery. Systematic reviews and meta-analyses of several randomized trials across multiple surgical specialties indicate that ERAS programmes reduce post-operative morbidity, reduce hospital stay and accelerate recovery (Eskicioglu et al., 2009, Varadhan et al., 2010, Rawlinson et al., 2011, Spanjersberg et al., 2011, Coolsen et al., 2013a, Coolsen et al., 2013b). However, with no standardised protocol and with no objective measure of response, in particular the SIR, the basis of the effect of ERAS is unclear. A recent systematic review and meta-analysis suggested that the quality of reporting in many ERAS studies was poor and that studies where fewer ERAS elements were employed showed greater reduction in mortality and complications than those with a greater number of ERAS elements (Nicholson et al., 2014). Indeed, it might have been expected that the greater number of ERAS elements employed, the greater then benefits seen. However, it may be that only some of the elements used in ERAS programmes, such as laparoscopic surgery, actually modify the SIR. It may be that, as in the present review, the use of markers such as CRP would objectively determine which components reduce the magnitude of the systemic inflammatory response following elective surgery.

It is therefore of interest that Neville and colleagues recently published a systematic review of outcomes used to evaluate enhanced recovery after surgery (Neville et al., 2014) and commented that one of the main challenges in evaluating outcomes in ERAS programmes is how to measure the effect of the ERAS programme on the systemic inflammatory

response. In many studies, objective markers of the SIR were not monitored. For example, they reported that only 11 out of the 38 included studies (29%) measured "immunological factors" such as CRP and interleukins and only 3 out of the 38 included studies (9%) used "markers of the stress response" such as cortisol, prolactin and growth hormone levels (Neville et al., 2014). Strategies that can be shown to reduce these peak levels of CRP or avoid excessive concentrations could therefore form the basis of an evidence based ERAS protocol.

Therefore, the finding that these markers are a marker for the complexity and severity of a given surgery is clear, but it is not clear whether the levels correlate with outcome for a particular procedure. The results of the present review may have a clinical impact out with its implication for components of the ERAS programme. Cancer surgery has long been proposed to produce an environment whereby micrometastatic disease is stimulated to progress due to the associated immunosuppressive state i.e. a loss of immune equilibrium and growth of so-called dormant micrometastases (Baum et al., 2005, Retsky et al., 2008). However, confirmatory data supporting this hypothesis has been lacking, in particular, the magnitude of the SIR associated with different surgical procedures and its impact on immune equilibrium has not been clear. From the results of the present study it is clear that only some surgical operations can significantly perturb the SIR such that immune function is compromised. Therefore, it would be of considerable interest to reinvestigate the tumour dormancy hypothesis in the context of this new information. In this context, it is of interest that it has recently been reported that following elective colorectal cancer resection, elevated CRP levels are associated with an increased risk of development of post-operative complications (Dutta et al., 2011, Welsch et al., 2007, Mackay et al., 2011, Matthiessen et al., 2008, Singh et al., 2014), specifically, that peak (day 2) CRP levels greater than 190 ml/L are associated with an increased risk of development of infective complications (Ramanathan et al., 2013). However, whether these elevated peak levels effect long term outcome has yet to be determined.

In summary, the results of the present review may allow monitoring of patient recovery following surgery and allow surgeons to identify those at increased risk of developing infective complications based on the magnitude of the SIR, allowing appropriate decisions to be made on their investigation and management. Moreover, these values may provide a therapeutic target that would allow prompt and pre-emptive treatment of potential post-operative complications, with the resultant benefit to recovery following elective surgery.



* Some studies assessed more than one marker of the stress response

Figure 5.1 Flow chart demonstrating the process used for study selection

Table 5.1 The timing and magnitude of cortisol concentrations following elective surgery

Surgical Procedure	Studies *(n)*	Patients *(n)*	Sampling Points *(n)*	Time of Peak (hrs)	Peak Value (nmol/L)	References
Minor Surgical Injury						
Rectopexy	1	39	3	4	459	(Solomon et al., 2002)
• *Laparoscopic*		*20*			*343*	
• *Open*		*19*			*582*	
Cholecystectomy	12	418	3-10	0-12	659	(Joris et al., 1992, McMahon et al., 1993, Deuss et al., 1994, Jakeways et al., 1994, Milheiro et al., 1994, Glaser et al., 1995, Ortega et al., 1996, Karayiannakis et al., 1997, Yamauchi et al., 1998, Kristiansson et al., 1999, Yoshida et al., 2000, Crema et al., 2005)
• *Laparoscopic*		*254*			*600*	
• *Open*		*164*			*750*	
Inguinal Hernia Repair	2	70	3-6	3-24	1009	(Akhtar et al., 1998, Uzunkoy et al., 2000)
• *Laparoscopic*		*35*			*946*	
• *Open*		*35*			*1071*	
Moderate Surgical Injury						
Primary Total Knee Replacement	1	12	3	6	450	(Smith et al., 2006)
Prostatectomy	3	94	3-4	0-0.5	484	(Bedalov et al., 2008, Hong et al., 2011, Fant et al., 2013)
• *TURP*		*24*			*515*	
• *Endoscopic Laser Ablation*		*24*			*435*	
• *Radical Retropubic*		*46*			*494*	
Hysterectomy	4	127	3-5	2-24	595	(Yuen et al., 1998, Rorarius et al., 2001, Maciejczyk-Pencula et al., 2004, Pirbudak et al., 2004)
• *Laparoscopic*		*20*			*300*	
• *Open*		*87*			*591*	
• *Laparoscopic Assisted Vaginal*		*20*			*909*	
Misc. Colorectal Resection	2	103	4-7	4-24	610	(Harmon et al., 1994, Braga et al., 2002)
• *Laparoscopic*		*52*			*610*	
• *Open*		*51*			*610*	
Colorectal Cancer Resection	4	449	4-7	4-24	621	(Ozawa et al., 2000, Delgado et al., 2001, Borgdorff et
• *Laparoscopic*		*47*			*851*	
• *Open*		*402*			*594*	

						al., 2004, Ren et al., 2012)
Exploratory Gyn. Surgery • *Laparoscopy* • *Mini-laparotomy* • *Laparotomy*	3	90 *60* *10* *20*	3-4	0-2	693 *539* *579* *1214*	(Muzii et al., 1996, Marana et al., 2003, Marana et al., 2013)
Primary Total Hip Replacement	4	152	4-6	0-8	772	(Hogevold et al., 2000, Hall et al., 2001, Bjornsson et al., 2007, Al Oweidi et al., 2010)
Spinal Surgery	1	85	3	0	910	(Ezhevskaya et al., 2013)
Open Gastrectomy	1	20	3	3	998	(Servis et al., 2008)
Gastric Bypass • *Laparoscopic* • *Open*	1	48 *26* *22*	4	1	1018 *993* *1048*	(Nguyen et al., 2002)
Mastectomy	1	5	3	0	1112	(Yamauchi et al., 1998)
Major Surgical Injury						
Oesophagectomy • *Minimally Invasive* • *Open*	2	34 *14* *20*	3-4	0-24	743 *714* *764*	(Yamauchi et al., 1998, Maas et al., 2013)
Coronary Artery Bypass	2	120	3	6-24	754	(Roth-Isigkeit et al., 1998, Velissaris et al., 2004)
Pulmonary Lobectomy	1	5	3	0	1007	(Yamauchi et al., 1998)
Open Abdominal Aortic Aneurysm Repair	1	10	4	12	1200	(Smeets et al., 1993)

Table 5.2 The timing and magnitude of interleukin-6 (IL-6) concentrations following elective surgery

Surgical Procedure	Studies *(n)*	Patients *(n)*	Sampling Points *(n)*	Time Peak (hrs)	Peak Value (pg/ml)	References
Minor Surgical Injury						
Inguinal Hernia Repair	5	104	3-8	4-24	13	(Baigrie et al., 1992, Takahara et al., 1995, Schrenk et al., 1996, Jess et al., 2000, Suter et al., 2002)
• *Laparoscopic*		*49*			*11*	
• *Open*		*55*			*15*	
Rectopexy	1	39	3	4-24	65	(Solomon et al., 2002)
• *Laparoscopic*		*20*			*21*	
• *Open*		*19*			*111*	
Cholecystectomy	15	593	3-9	4-24	77	(Cruickshank et al., 1990, Joris et al., 1992, McMahon et al., 1993, Cho et al., 1994, Jakeways et al., 1994, Glaser et al., 1995, Yamauchi et al., 1998, Kristiansson et al., 1999, Yoshida et al., 2000, Schietroma et al., 2001, Schietroma et al., 2004a, Schietroma et al., 2004b, Han et al., 2012, Sista et al., 2013a, Sista et al., 2013b)
• *Single Incision*		*31*			*54*	
• *Laparoscopic*		*291*			*62*	
• *Open*		*271*			*95*	
Moderate Surgical Injury						
Spinal Surgery	1	85	3	24	10	(Ezhevskaya et al., 2013)
Mastectomy	2	15	3-5	0-48	27	(Yamauchi et al., 1998, Khan et al., 1999)
Exploratory Gyn. Surgery	1	40	3	4	28	(Torres et al., 2007)
• *Laparoscopic*		*25*			*20*	
• *Open*		*15*			*40*	
Prostatectomy	2	429	3-6	6-24	62	(Jurczok et al., 2007, Fant et al., 2013)
• *Laparoscopic*		*163*			*70*	
• Open		*266*			*57*	
Nephrectomy	2	104	4	6-24	73	(Adler et al., 1998, Greco et al., 2012)
• *Laparoscopic Single Site*		*31*			*60*	
• *Laparoscopic*		*35*			*65*	
• *Open*		*38*			*90*	

Restrictive Gastric Surgery • *Laparoscopic* • *Open*	2	40 *15* *25*	4-5	6-24	94 *83* *100*	(Kragsbjerg et al., 1995, Zengin et al., 2002)
Gastrectomy • *Laparoscopic* • *Open*	3	149 *49* *100*	3-5	3-24	101 *44* *129*	(Adachi et al., 2000, Fujita and Yanaga, 2007, Servis et al., 2008)
Gastric Bypass • *Laparoscopic* • *Open*	1	48 *26* *22*	4	24	102 *72* *137*	(Nguyen et al., 2002)
Hysterectomy • *Laparoscopic* • *Open* • *Laparoscopic Vaginal* • *Open Vaginal*	7	295 *58* *121* *74* *42*	3-4	2-24	117 *19* *166* *163* *27*	(Yuen et al., 1998, Harkki-Siren et al., 2000, Malik et al., 2001, Rorarius et al., 2001, Ribeiro et al., 2003, Hou et al., 2011, Roy et al., 2012)
Lumbar Discectomy	2	26	5-6	12-24	124	(Huang et al., 2005, Kumbhare et al., 2009)
Total Hip Replacement • *Primary* • *Revision*	9	205 *201* *4*	3-7	4-24	140 *142* *40*	(Cruickshank et al., 1990, Hogevold et al., 1992, Wilson et al., 1993, Kragsbjerg et al., 1995, Hogevold et al., 2000, Wirtz et al., 2000, Hall et al., 2001, Minetto et al., 2006, Bjornsson et al., 2007)
Colorectal Cancer Resection • *Laparoscopic* • *Open*	20	1110 *347* *763*	4-7	3-24	161 *177* *154*	(Cruickshank et al., 1990, Schulze et al., 1992, Stage et al., 1997, Hewitt et al., 1998, Leung et al., 2000, Ozawa et al., 2000, Schwenk et al., 2000, Delgado et al., 2001, Mehigan et al., 2001, Nishiguchi et al., 2001, Ordemann et al., 2001,

						Wichmann et al., 2005, Catena et al., 2009, Evans et al., 2009, Persec et al., 2009, Vignali et al., 2009, Pascual et al., 2011, Ren et al., 2012, Wang et al., 2012, Kvarnstrom et al., 2013)
Open Minor Liver Resection	3	37	3-7	2-48	179	(Lan et al., 2003, Jansen et al., 2008, Strey et al., 2011)
Nissen Fundoplication • *Laparoscopic* • *Open*	2	69 *61* *8*	3-4	4-6	233 *240* *180*	(Zieren et al., 2000, Schietroma et al., 2013)
Misc. Colorectal Resection • *Laparoscopic* • *Open*	2	66 *33* *33*	3-6	6	267 *140* *393*	(Harmon et al., 1994, Hildebrandt et al., 2003)
Total Knee Replacement • *Primary Unilateral* • *Primary Bilateral*	3	35 *20* *15*	3-6	6-24	321 *261* *400*	(Kragsbjerg et al., 1995, Wirtz et al., 2000, Jules-Elysee et al., 2011)
Major Surgical Injury						
Thoracic Surgery • *Video-assisted (VATS)* • *Open*	7	180 *37* *143*	3-5	0-24	85 *51* *94*	(Yamauchi et al., 1998, Yim et al., 2000, Nagahiro et al., 2001, Franke et al., 2005, Wang et al., 2005, Friscia et al., 2007, Avdagic et al., 2010)
Oesophagectomy • *Minimally Invasive* • *Open*	2	34 *14* *20*	3-4	0-24	180 *116* *225*	(Yamauchi et al., 1998, Maas et al., 2013)
Abdominal Aortic Aneurysm Repair • *Endovascular (EVAR)* • *Open*	13	297 *116* *181*	3-9	4-24	248 *116* *332*	(Cruickshank et al., 1990, Baigrie et al., 1992, Parry-Billings et al., 1992, Swartbol et al., 1996, Norman and Fink, 1997, Syk et al., 1998, Holzheimer et al., 1999, Boyle et al.,

						2000, Elmarasy et al., 2000, Galle et al., 2000, Odegard et al., 2000, Bolke et al., 2001, Rowlands and Homer-Vanniasinkam, 2001)
Renal Transplant	1	46	3	2	268	(Hadimioglu et al., 2012)
Open Major Liver Resection	5	59	3-9	2-48	345	(Badia et al., 1998, Wiezer et al., 1999, Schmidt et al., 2007, Jansen et al., 2008, Strey et al., 2011)
Open Cardiac Surgery	4	112	3-6	0-8	428	(Kragsbjerg et al., 1995, Fransen et al., 1998, Mollhoff et al., 1999, Franke et al., 2005)
Endovascular Thoracic Aneurysm Repair	1	9	5	36	700	(Chang et al., 2009)

Table 5.3 The timing and magnitude of white cell count (WCC) concentrations following elective surgery

Surgical Procedure	Studies *(n)*	Patients *(n)*	Sampling Points *(n)*	Time of Peak (hrs)	Peak Value ($X10^9/L$)	References
Minor Surgical Injury						
Inguinal Hernia Repair • *Laparoscopic* • *Open*	4	129 *64* *65*	3	4-24	10.3 *10.1* *10.4*	(Takahara et al., 1995, Schrenk et al., 1996, Uzunkoy et al., 2000, Suter et al., 2002)
Cholecystectomy • *Laparoscopic* • *Open*	1	30 *15* *15*	3	4	11.8 *11.0* *12.5*	(Joris et al., 1992)
Moderate Surgical Injury						
Breast Reconstruction • *Lateral Thoracodorsal Flap* • *Latissimus Dorsi Flap* • *Transverse Rectus Abdominus Myocutaneous Flap*	1	51 *11* *21* *19*	3	24	7.5 *7.4* *6.9* *8.1*	(Blomqvist et al., 1998)
Prostatectomy • *TURP* • *Endoscopic Laser Ablation* • *Radical Retropubic*	2	74 *24* *24* *26*	3-4	24	8.0 *8.4* *6.9* *8.7*	(Bedalov et al., 2008, Fant et al., 2013)
Colorectal Cancer Resection • *Laparoscopic* • *Open*	4	397 47 350	3-6	6-24	8.7 *9.9* *8.6*	(Nishiguchi et al., 2001, Ordemann et al., 2001, Kvarnstrom et al., 2013, Warschkow et al., 2011)
Primary Total Hip Replacement	4	123	3-5	4-24	9.0	(Shih et al., 1987, Hogevold et al., 1992, Moreschini et al., 2001, Bjornsson et al., 2007)
Primary Unilateral Total Knee Replacement	1	18	3	24	9.0	(Moreschini et al., 2001)
Lumbar Discectomy	1	197	4	48	9.9	(Kraft et al., 2011)
Gastrectomy • *Laparoscopic* • *Open*	5	316 *145* *171*	3-4	3-24	10.0 *9.0* *10.8*	(Adachi et al., 2000, Usui et al., 2005, Servis et al., 2008, Kawamura et al., 2009, Sakuramoto et al., 2009)

Spinal Surgery	2	253	3-4	24	10.2	(Chung et al., 2011, Kraft et al., 2011)
Hysterectomy • *Laparoscopic* • *Open* • *Laparoscopic Assisted Vaginal*	2	84 *20* *44* *20*	3-4	2-24	11.7 *9.0* *11.5* *14.8*	(Yuen et al., 1998, Rorarius et al., 2001)
Nissen Fundoplication • *Laparoscopic* • *Open*	2	50 *34* *16*	3-4	1-2	12.9 *12.3* *14.1*	(Sietses et al., 1999, Zieren et al., 2000)
Gastric Bypass • *Laparoscopic* • *Open*	1	176 *59* *117*	3	24	13.3 *14.0* *13.0*	(Csendes et al., 2009)
Major Surgical Injury						
Abdominal Aortic Aneurysm Repair • *Endovascular (EVAR)* • *Open*	4	89 *47* *42*	5-7	6-48	9.9 *9.7* *10.1*	(Swartbol et al., 1996, Boyle et al., 2000, Galle et al., 2000, Odegard et al., 2000)
Open Major Liver Resection	1	12	8	24	11.3	(Wiezer et al., 1999)
Oesophagectomy • *Minimally Invasive* • *Open*	1	27 *14* *13*	4	24	12.5 *10.9* *14.3*	(Maas et al., 2013)
Endovascular Thoracic Aneurysm Repair	1	38	4	24	16.6	(Chang et al., 2009)

Table 5.4 The timing and magnitude of C-reactive protein (CRP) concentrations following elective surgery

Surgical Procedure	Studies (*n*)	Patients (*n*)	Sampling Points (*n*)	Time of Peak (hrs)	Peak Value (mg/L)	References
Minor Surgical Injury						
Inguinal Hernia Repair	5	149	3-6	24-48	40	(Takahara et al., 1995, Schrenk et al., 1996, Akhtar et al., 1998, Uzunkoy et al., 2000, Suter et al., 2002)
• *Laparoscopic*		*74*			*30*	
• *Open*		*75*			*49*	
Anterior Cruciate Ligament Reconstruction	1	25	4	48	45	(Orrego et al., 2005)
Cholecystectomy	13	459	3-10	24-48	52	(Cruickshank et al., 1990, Joris et al., 1992, Roumen et al., 1992, McMahon et al., 1993, Cho et al., 1994, Jakeways et al., 1994, Bruce et al., 1999, Kristiansson et al., 1999, Yoshida et al., 2000, Schietroma et al., 2004b, Schietroma et al., 2007, Sista et al., 2013a, Sista et al., 2013b)
• *Laparoscopic*		*241*			*27*	
• *Open*		*218*			*80*	
Rectopexy	1	39	3	48	90	(Solomon et al., 2002)
• *Laparoscopic*		*20*			*70*	
• *Open*		*19*			*112*	
Moderate Surgical Injury						
Nissen Fundoplication	2	51	3	6-24	58	(Sietses et al., 1999, Schietroma et al., 2013)
• *Laparoscopic*		*43*			*54*	
• *Open*		*8*			*82*	
Lumbar Discectomy	4	270	3-5	24-72	73	(Larsson et al., 1992, Huang et al., 2005, Orrego et al., 2005, Kraft et al., 2011)
Neurosurgical Procedures	3	103	5-6	24-48	74	(Bengzon et al., 2003, Mirzayan et al., 2007, Al-Jabi and El-Shawarby, 2010)
• *Craniotomy*		*46*			*32*	
• *Ventricular-Peritoneal Shunt*		*4*			*35*	
• *Cerebral Biopsy*		*14*			*60*	
• *Cerebral Tumour Debulking*		*39*			*133*	
Hysterectomy	6	200	3-5	36-48	75	(Yuen et al., 1998, Harkki-Siren et al., 2000, Malik et al., 2001, Rorarius et al., 2001, Aka et al., 2004, Maciejczyk-Pencula et al., 2004)
• *Laparoscopic*		*38*			*29*	
• *Open*		*107*			*99*	
• *Laparoscopic Assisted Vaginal*		*30*			*45*	
• *Open Vaginal*		*25*			*75*	

Breast Reconstruction Surgery • *Lateral Thoracodorsal Flap* • *Latissimus Dorsi Flap* • *Transverse Rectus Abdominus Myocutaneous Flap*	1	51 *11* *21* *19*	3	72	88 *44* *74* *130*	(Blomqvist et al., 1998)
Miscellaneous Colorectal Resection • *Laparoscopic* • *Open*	2	121 *61* *60*	3-6	48	110 *109* *112*	(Braga et al., 2002, Hildebrandt et al., 2003)
Gastrectomy • *Laparoscopic* • *Open*	6	343 *145* *198*	3-5	48-96	115 *98* *127*	(Adachi et al., 2000, Usui et al., 2005, Fujita and Yanaga, 2007, Servis et al., 2008, Kawamura et al., 2009, Sakuramoto et al., 2009)
Open Restrictive Gastric Surgery	1	10	6	96	121	(Kragsbjerg et al., 1995)
Colorectal Cancer Resection • *Laparoscopic* • *Open*	15	775 *248* *527*	3-7	24-72	123 *97* *133*	(Cruickshank et al., 1990, Schulze et al., 1992, Stage et al., 1997, Leung et al., 2000, Schwenk et al., 2000, Delgado et al., 2001, Mehigan et al., 2001, Nishiguchi et al., 2001, Wichmann et al., 2005, Catena et al., 2009, He et al., 2009, Vignali et al., 2009, Wang et al., 2012, Kvarnstrom et al., 2013, Warschkow et al., 2011)
Gastric Bypass • *Laparoscopic* • *Open*	3	614 *475* *139*	3-5	48-72	124 *123* *126*	(Nguyen et al., 2002, Csendes et al., 2009, Warschkow et al., 2012a)
Nephrectomy • *Laparoscopic Single Site* • *Laparoscopic* • *Open*	2	104 *31* *35* *38*	4	48-72	128 *140* *120* *125*	(Adler et al., 1998, Greco et al., 2012)
Prostatectomy • *TURP* • *Laparoscopic* • *Open*	3	453 *24* *163* *266*	3-6	48-72	133 *70* *120* *146*	(Nielsen et al., 1999, Jurczok et al., 2007, Fant et al., 2013)

Breast Surgery • *Mastectomy* • *Breast Implant Exchange* • *Breast Augmentation* • *Breast Reduction*	2	100 *10* *30* *30* *30*	5-6	24-120	141 *100* *136* *149* *153*	(Khan et al., 1999, Toman et al., 2008)
Total Hip Replacement • *Primary* • *Revision*	16	894 *885* *9*	3-7	24-72	145 *145* *136*	(Aalto et al., 1984, Shih et al., 1987, Cruickshank et al., 1990, Hogevold et al., 1992, Larsson et al., 1992, Kragsbjerg et al., 1995, Niskanen et al., 1996, Okafor and MacLellan, 1998, White et al., 1998, Wirtz et al., 2000, Bilgen et al., 2001, Hall et al., 2001, Moreschini et al., 2001, Orrego et al., 2005, Neumaier et al., 2006, Bjornsson et al., 2007)
Open Minor Liver Resection	2	25	3-7	48-72	146	(Lan et al., 2003, Jansen et al., 2008)
Spinal Surgery • *With Instrumentation* • *Without Instrumentation*	6	577 *438* *139*	3-6	48-72	151 114 267	(Takahashi et al., 2001, Munoz et al., 2004, Mok et al., 2008, Chung et al., 2011, Kraft et al., 2011, Kong et al., 2012)
Primary Total Knee Replacement	9	416	3-6	48-72	153	(Larsson et al., 1992, Kragsbjerg et al., 1995, Niskanen et al., 1996, White et al., 1998, Wirtz et al., 2000, Bilgen et al., 2001, Moreschini et al., 2001, Orrego et al., 2005, Smith et al., 2006)
Open Mixed Gastro-oesophageal Cancer Resection	1	31	10	48	171	(Warschkow et al., 2012b)
Abdominoplasty	1	30	6	120	196	(Toman et al., 2008)
Major Surgical Injury						
Thoracic Surgery • *Video-assisted (VATS)* • *Open*	3	143 *19* *124*	3-6	48-72	85 *15* *96*	(Craig et al., 2001, Franke et al., 2005, Avdagic et al., 2010)
Open Major Liver Resection	3	158	3-7	72	94	(Schmidt et al., 2007, Jansen et al., 2008, Rahman et al., 2008)

Cerebral Aneurysm Surgery • *Endovascular Repair* • *Clipping*	2	20 *8* *12*	5	48-72	112 *41* *160*	(Bengzon et al., 2003, Al-Jabi and El-Shawarby, 2010)
Abdominal Aortic Aneurysm Repair • *Endovascular (EVAR)* • *Open*	9	234 *81* *180*	5-8	24-72	163 *132* *180*	(Cruickshank et al., 1990, Baigrie et al., 1992, Swartbol et al., 1996, Syk et al., 1998, Galle et al., 2000, Morikage et al., 2000, Odegard et al., 2000, Bolke et al., 2001, Kucukakin et al., 2010)
Oesophagectomy • *Minimally Invasive* • *Open*	1	27 *14* *13*	4	72	186 *189* *183*	(Maas et al., 2013)
Open Cardiac Surgery	4	109	3-7	24-72	189	(Boralessa et al., 1986, Kragsbjerg et al., 1995, Mollhoff et al., 1999, Franke et al., 2005)

6 Comparison of the components of a white cell count and acute phase proteins as markers of the magnitude of injury and the development of infective complications following elective surgery for colorectal cancer

6.1 Introduction

Following surgical intervention, a cascade of different processes occurs throughout the body that results in the elaboration of the systemic inflammatory response (Chapter 5). It has long been proposed that cancer surgery, due to the profound immunosuppression it causes, may promote progression of dormant micrometastatic disease (Baum et al., 2005, Retsky et al., 2008, O'Leary et al., 2016). Indeed, post-operative CRP and the NLR have been reported to predict the development of post-operative complications (Cook et al., 2007, Forget et al., 2015, Singh et al., 2014). Therefore, it is of interest that, of the routine clinically available markers, the magnitude of the post-operative systemic inflammatory response was best assessed by circulating concentrations of CRP (Chapter 5). In contrast, WCC did not appear to accurately reflect the magnitude of the systemic inflammatory response following surgery. Whilst the majority of white cells are neutrophils, there are other cells that are included in the WCC such as lymphocytes, monocytes, basophils and eosinophils and these may compromise its value. Therefore, it is possible that these different cell types may each respond differently following surgical intervention. The aim of the present study was to compare white cells with acute phase proteins as markers of the magnitude of surgical injury and the development of infective complications following elective surgery for colorectal cancer.

6.2 Methods

6.2.1 Patients

This was a retrospective study of patients with histologically proven colorectal cancer (based on pre-operative endoscopic biopsies, pre-operative computed tomography and confirmed on laparotomy findings) who were considered to have undergone elective, potentially curative resection between December 2003 and June 2013 in one of two University teaching hospitals in Glasgow (n = 378). Patient characteristics were collected in a prospectively maintained database and all patient data was anonymised.

All tumours were staged according to conventional tumour, node, metastasis classification. All resections were performed electively and were performed using open (n = 291) or laparoscopic (n = 87) surgery. Patients with metastatic disease or who underwent emergency surgery were excluded from the analysis.

Pre-operatively all patients received thromboembolic prophylaxis and antibiotic prophylaxis as per the local protocol. Surgery was performed by specialist colorectal surgeons, experienced in treating patients with colorectal cancer, either by traditional open method or laparoscopic method utilising a small lower abdominal extraction wound. Blood samples were taken for routine laboratory analysis of C-reactive protein and white cell count (neutrophil, lymphocyte, monocyte, eosinophil and basophil count) during the pre and post-operative period (days 1-4). Post-operatively, patients had a daily clinical assessment and investigations carried out as clinically indicated. Patients were discharged after day 4 when the surgical team felt this was clinically indicated. Patients were assessed for both infective and non-infective complications. Infective complications can be classified as either surgical site infections (SSI) or remote site infections (RSI). The criteria used to define these infective complications were the same as has been previously described (Ramanathan et al., 2013) and is as follows: a wound infection included the presence of pus that discharged spontaneously or required drainage; an intra-abdominal abscess was confirmed by imaging such as computed tomography (CT) and required either conservative therapy with antibiotics or drainage; an anastomotic leak, defined as a fistula to the bowel anastomosis, was confirmed radiologically or diagnosed at re-laparotomy; pneumonia was diagnosed as the presence of x-ray changes and fever that required antibiotic therapy and urinary tract infection as positive urine culture in presence of

symptoms that required antibiotic therapy. The West of Scotland research ethics committee approved this study.

6.2.2 Statistical Analysis

Data is presented as the median (range). Comparison of post-operative values with pre-operative values was performed using Wilcoxon signed-rank test and comparison of continuous data was performed using a Mann Whitney U test. Receiver Operating Curves (ROC) were used to identify optimal thresholds for predicting the development of infective complications. A p value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 22.0 for Windows (IBM, SPSS, IL, USA).

6.3 Results

378 patients were included in this study and their baseline characteristics are shown in *Table 6.1.* The majority of patients were aged over 65 years old (71%) with similar numbers of males and females and the majority having TNM stage II disease (43%). 23% underwent a laparoscopic procedure and 28% developed an infective post-operative complication.

Of the components of a WCC, only the neutrophil and monocyte count significantly increased following surgery, both peaking on post-operative day 1 *(Table 6.2).* On the first post-operative day the neutrophil count increased by 89% and monocyte count by 17%. The neutrophil count and monocyte counts on post-operative day 1 were significantly different to the pre-operative value *($p < 0.001$)*. Lymphocytes showed a reduction of 42% on the first post-operative day, reducing further on post-operative day 3 and the counts were statistically different on all post-operative days compared to pre-operative values *($p < 0.001$)*. Similarly, the basophil and platelet counts all decreased following surgery and with no real discernible nadir demonstrated but with the counts on all post-operative days statistically different to pre-operative values *($p < 0.001$)*. Eosinophils decreased by 100% on post-operative day 1 before beginning to rise back to pre-operative levels. The eosinophil count on post-operative day 1 was significantly different to the pre-operative value *($p < 0.001$)*. In comparison, CRP increased profoundly following surgery, its peak value being 2800% greater than the pre-operative concentration *($p < 0.001$)* and albumin fell by 29% *($p < 0.001$)*.

Comparison of the components of the differential WCC in open and laparoscopic procedures is shown in *Table 6.2.* Neutrophil and lymphocyte counts evoked a smaller response in laparoscopic procedures, however this response was only statistically different for the neutrophil count pre-operatively *($p = 0.002$)* and on post-operative day 2 *($p = 0.006$)* and for lymphocyte count on all days *(all $p < 0.05$).* The monocyte count was greater in laparoscopic procedures and was significantly different on all post-operative days *(all $p < 0.01$)*. Eosinophil and platelet counts were not statistically different in laparoscopic or open groups. In comparison, CRP was lower in laparoscopic procedures both pre-operatively and on post-operative days 1-3 *($p < 0.001$)* and albumin higher in laparoscopic procedures on post-operative days 1-3 *($p < 0.001$)*. Both appeared superior to the neutrophil count in this regard *(Figure 6.1)*.

Comparison of the components of the WCC in patients who developed post-operative infective complications and no complications following both open and laparoscopic surgery is shown in *Table 6.3* In those patients undergoing open surgery, only the neutrophil count, on post-operative day 3, was significantly greater in those who developed an infective complication ($p < 0.05$). In comparison, CRP concentrations were significantly higher and albumin concentrations significantly lower from day 2 onwards ($p < 0.001$). In those patients undergoing laparoscopic surgery, the neutrophil count on days 2, 3 and 4 was significantly greater ($p < 0.05$) and the basophil count on day 3 significantly reduced ($p <0.05$) in those developing infective complications. In comparison, CRP concentrations were significantly greater on all post-operative days in those developing infective complications ($p < 0.05$) and appeared superior to the neutrophil count to this regard *(Figure 6.2).*

The correlation between the neutrophil count and CRP *(Table 6.4)* and between the neutrophil count and albumin *(Table 6.5)* was examined. There was a significant correlation between the neutrophil count and CRP which appeared to happen contemporaneously, with day 2 neutrophil count most closely correlated with day 2 CRP concentration and the same with day 3 and day 4. There was also a significant correlation between the neutrophil count and albumin, particularly on day 3 and day 4.

Receiver Operating Curves (ROC) were used to determine the optimal neutrophil threshold for the development of infective complications on day 3 and day 4 *(Figures 6.3 (a) & 6.4 (a))*. On day 3 the area under the curve was 0.617 (95% CI 0.55-0.69, $p = 0.001$) and the optimal threshold value was 7.5 $x10^9$/L. On day 4 the area under the curve was 0.608 (95% CI 0.53-0.68, $p = 0.005$) and the optimal threshold value was 6.5 $x10^9$/L. When compared to the ROC for CRP on days 3 and 4 *(Figures 6.3 (b) and 6.4 (b))* the neutrophil count is inferior with regards to the area under the curve (Day 3 – CRP AUC 0.748, $p < 0.001$; Day 4 – CRP AUC 0.749, p , 0.001). Using the established neutrophil threshold of 7.5 $x10^9$/L on day 3, the rate of infective complications ranges from 23% in those with a low neutrophil count to 42% in those with a high neutrophil count ($p = 0.001$). This results in a sensitivity of 44%, specificity of 66%, positive predictive value of 42% and negative predictive value of 77%. Using the neutrophil threshold of 6.5 $x10^9$/L on day 4, the rate of infective complications ranges from 26% on those with a low neutrophil count to 48% in those with a high neutrophil count ($p < 0.001$). This results in a sensitivity of 58%, specificity of 66%, positive predictive value of 48% and negative predictive value of 74%.

6.4 Discussion

The results of the present study show that of the components of a WCC, only the neutrophil count consistently reflected the impact of the magnitude of injury and development of infective complications. Therefore, if a WCC is to be used to monitor the course of the systemic inflammatory response following surgery, especially the development of infective complications, then particular attention should be paid to the neutrophil count.

It was of interest that the components of the WCC behaved in a similar fashion to other components of the systemic inflammatory response with regards to the method of surgery, be that open or laparoscopic. Laparoscopic surgery resulted in a relatively lower neutrophil count, which is in keeping with similar work looking at CRP in colorectal cancer surgery and a range of surgical procedures (Chapter 5) (Ramanathan et al., 2015). Indeed, there was a correlation between the neutrophil count and both CRP and albumin. This correlation (neutrophil and CRP) appears to happen contemporaneously, with day 2 neutrophil count most closely correlated with day 2 CRP concentration and the same with day 3 and day 4. This was also evident with the neutrophil count and albumin on day 3 and day 4. Moreover, similar to that of CRP and albumin, a neutrophil count above the established threshold on day 3 (>7.5 $x10^9$/L) was associated with the development of infective complications. Taken together this would suggest that they play a coordinated role in the initiation and maintenance of the systemic inflammatory response following surgery.

Infective complications, including anastomotic leak, have previously been reported to have a negative impact on long term outcomes and quality of life following surgery for colorectal cancer (Brown et al., 2014, Artinyan et al., 2015). Furthermore, CRP has been reported to be able to predict the development of these complications using defined thresholds on different post-operative days, (Platt et al., 2012, Singh et al., 2014) with concentrations on days 3 and 4 thought to be most clinically relevant. In the present study, of the components of a white cell count, only the neutrophil count was significantly increased in the group with infective complications and would appear to predict infective complications, particularly on post-operative days 3 and 4. However, compared with CRP and albumin, the neutrophil count was inferior in this regard. Clinical signs such as fever, abdominal pain and tachycardia can be absent early in complication development and therefore the use of CRP, albumin and neutrophil thresholds on day 3 may allow a more

objective assessment of the patient's condition and provide a 'tip-off' to the likely safe discharge of the patient (Singh et al., 2014).

The molecular basis of the observations of the present study is not clear. However, it is of interest that interleukin-6 (IL-6) is known to stimulate production of both neutrophils and CRP, which are both components of the innate immune response. Indeed, immature granulocytes, a subset of neutrophils that are released prematurely from the bone marrow during periods of infection and inflammation, may offer further insight into the exact role IL-6 plays. Such myeloid derived suppressor cells (MDSCs) are known to be involved in chronic inflammation induced immunosuppression both locally and systemically (Baniyash et al., 2014) and to invoke tumour-mediated adaptive immune suppression in patients with different tumour types (Diaz-Montero et al., 2009). Furthermore, levels of MDSCs have been reported to be correlated with tumour stage and metastatic tumour burden (Diaz-Montero et al., 2009). It has previously been reported that MDSC recruitment was associated with increased IL-6 levels and that the levels of both MDSCs and IL-6 predicted prognosis in patients with oesophageal cancer (Chen et al., 2014). Pharmacological methods of reducing MDSC levels therefore may be of benefit in the treatment of patients with cancer, perhaps by targeting the inhibition of IL-6/ JAK/ STAT pathway (Roxburgh and McMillan, 2016).

In the present study, it was of interest that the lymphocyte count fall following surgery, was less affected in laparoscopic surgery and was not associated with the development of infective complications. These results may have implications for the prognostic value of the neutrophil-lymphocyte ratio (NLR) in the post-operative period (Cook et al., 2007, Forget et al., 2015).

Laparoscopic surgery is being increasingly utilised as a method of curative surgery for colorectal cancer and has been reported to shorten hospital stay, reduce morbidity and reduce post-operative pain (Reza et al., 2006, Lourenco et al., 2008). More recently it has also been reported to evoke a reduced systemic inflammatory response as evidenced by CRP concentrations (Ramanathan et al., 2015) and IL-6 concentrations (Chapter 5) . In the present study, in addition to the post-operative CRP concentrations being significantly reduced in those undergoing laparoscopic procedures, the neutrophil count was also significantly reduced and therefore can be considered to reliably reflect the magnitude of surgical injury. Therefore, in addition to drug interventions, different operative techniques

may make it possible to reduce circulating IL-6, CRP and MDSCs, causing relative adaptive immunosuppression and thus potentially improve long term outcomes.

The present study has some potential limitations that should be considered. Plasma cytokine levels were not measured in these patients and as such no comment can be made on specific mediators of the systemic inflammatory response following surgery. Measures of pre-operative morbidity were also not carried out and therefore it is not clear whether co-morbidity would have had any bearing on the overall results. Nevertheless, comparative measures of the perioperative systemic inflammatory response were examined. Furthermore, Clavien-Dindo classification was not available for all included cases. However, it has recently been reported that post-operative CRP concentrations were associated with the severity of complications (McSorley et al., 2015). Therefore, it may be hypothesised that the neutrophil count may also be associated with the severity of complications but further research is required.

In summary, of the components of a WCC, only the neutrophil count reflected both the magnitude of injury and the impact on the development of infective complications. Although the neutrophil count would appear to be inferior to CRP and albumin in this regard, it confirms that the development of the systemic inflammatory response following surgery for colorectal cancer is principally due to an upregulation of the innate immune response.

Table 6.1 Clinicopathological characteristics of patients undergoing elective resection for colorectal cancer

Characteristic	No. of patients (%)
Age: <65/ 65-74/ >=75	109 (29)/ 127 (33)/ 142 (38)
Sex: Male/ Female	197 (52)/ 181 (48)
Operation: Colon/ Rectum	272 (72)/ 105 (28)
Laparoscopic: No/ Yes	291 (77)/ 87 (23)
TNM Stage: I/ II/ III	75 (20)/ 162 (43)/ 120 (32)
All Complications: No/ Yes	231 (61)/ 147 (39)
Infective Complication: No/ Yes	272 (72)/ 106 (28)

Results given as number (%)
CRP, C-reactive protein; TNM, tumour node metastasis

Table 6.2 Changes in the concentrations of components of the differential white cell count and acute phase proteins in patients undergoing elective surgery for colorectal cancer

Overall Cohort				Comparison of Open and Laparoscopic Surgery			
Characteristic	Median (range)	Percentage change from pre-operative value (%)	*p-value*[a]	Open *Median*	Laparoscopic *Median*	Percentage difference (%)	*p-value*
White Cell							
Pre-operative	7.5 (3.5 – 23.5)	0		7.8	7.2	-8	*0.106*
POD 1	10.8 (0.7 – 33.8)	44	<0.001	10.9	10.4	-5	*0.471*
POD 2	10.7 (1.4 – 52.9)	43	<0.001	10.9	9.9	-9	*0.217*
POD 3	9.1 (2.7 – 45.6)	21	<0.001	9.1	8.8	-3	*0.447*
POD 4	8.2 (1.6 – 38.3)	9	<0.001	8.3	8.2	-1	*0.800*
Neutrophil							
Pre-operative	4.7 (1.1 – 12.8)	0		5.0	4.1	-18	*0.002*
POD 1	8.9 (0.4 – 23.3)	89	<0.001	9.1	8.4	-8	*0.086*
POD 2	8.6 (0.8 – 25.4)	83	<0.001	9.0	7.6	-16	*0.006*
POD 3	7.2 (1.7 – 26.7)	53	<0.001	7.3	6.6	-10	*0.100*
POD 4	6.0 (1.4 – 22.8)	28	<0.001	6.3	5.7	-10	*0.743*
Lymphocytes							
Pre-operative	1.7 (0.3 – 19.1)	0		1.6	1.8	13	*0.003*
POD 1	1.0 (0.1 – 24.3)	-42	<0.001	1.0	1.1	10	*0.001*
POD 2	1.0 (0.2 – 38.4)	-42	<0.001	1.0	1.2	20	*<0.001*
POD 3	0.9 (0.2 – 36.7)	-47	<0.001	0.9	1.2	33	*0.001*
POD 4	0.9 (0.1 – 32.7)	-47	<0.001	0.9	1.2	33	*0.001*
Monocytes							
Pre-operative	0.6 (0.2 – 2.0)	0		0.6	0.6	0	*0.761*
POD 1	0.7 (0.1 – 2.0)	17	<0.001	0.7	0.9	29	*0.001*
POD 2	0.7 (0 – 2.5)	17	<0.001	0.7	0.8	14	*0.003*
POD 3	0.6 (0.1 – 1.8)	0	0.117	0.6	0.7	17	*<0.001*

POD 4	0.6 (0.1 – 1.6)	0	0.040	0.6	0.7	17	*0.007*
Eosinophils							
Pre-operative	0.19 (0 – 1.18)	0		0.20	0.15	-25	*0.906*
POD 1	0 (0 – 1.98)	-100	<0.001	0.01	0.00	-100	*0.180*
POD 2	0.09 (0 – 0.61)	-53	<0.001	0.10	0.07	-30	*0.796*
POD 3	0.19 (0 – 2.07)	0	0.982	0.20	0.14	-30	*0.198*
POD 4	0.20 (0 – 1.30)	5	0.018	0.20	0.20	0	*0.904*
Basophils							
Pre-operative	0.02 (0 – 0.20)	0		0.02	0.03	50	*0.001*
POD 1	0.01 (0 – 0.32)	-50	<0.001	0.01	0.01	0	*0.058*
POD 2	0.01 (0 – 0.10)	-50	<0.001	0.01	0.01	0	*<0.001*
POD 3	0.01 (0 – 0.10)	-50	<0.001	0.01	0.01	0	*<0.001*
POD 4	0.01 (0 – 0.10)	-50	<0.001	0.01	0.02	100	*<0.001*
Platelets							
Pre-operative	275 (94 – 811)	0		278	269	-3	*0.385*
POD 1	227 (71 – 732)	-17	<0.001	233	216	-7	*0.552*
POD 2	219 (78 – 649)	-20	<0.001	223	218	-2	*0.809*
POD 3	231 (73 – 761)	-16	<0.001	236	226	-4	*0.642*
POD 4	266 (59 – 861)	-3	<0.001	268	265	-1	*0.146*
C-reactive Protein							
Pre-operative	6 (0.4 – 249)	0		8.2	3.3	-60	*<0.001*
POD 1	98 (1.9 – 284)	1533	<0.001	104	71	-32	*<0.001*
POD 2	173 (17 – 358)	2783	<0.001	185	126	-32	*<0.001*
POD 3	161 (2.3 – 430)	2583	<0.001	173	131	-24	*<0.001*
POD 4	113 (6.0 – 425)	1783	<0.001	120	87	-28	*0.296*
Albumin							
Pre-operative	38 (17-49)	0		38	37	-3	*0.212*
POD 1	27(12-40)	-29	<0.001	26	29	12	*<0.001*
POD 2	27 (11-44)	-29	<0.001	26	29	12	*<0.001*
POD 3	27 (9-40)	-29	<0.001	26	29	12	*<0.001*

POD 4	27 (11-40)	-29	<0.001	26	28	8	*0.146*

[a] *Comparison of post-operative median value with pre-op median value. POD, post-operative day*

Table 6.3 Components of the differential white cell count and acute phase proteins in patients developing no complications and infective complications following open and laparoscopic surgery for colorectal cancer

	Open Surgery (n = 291)			Laparoscopic Surgery (n = 87)		
Characteristic	No Complications (n = 206)	Infective Complications (n = 85)	*p-value*	No Complications (n = 66)	Infective Complications (n = 21)	*p-value*
White Cell						
Pre-operative	7.4	7.7	*0.396*	7.1	7.5	*0.629*
POD 1	11.1	10.9	*0.635*	10.5	11.5	*0.036*
POD 2	10.6	10.7	*0.649*	8.9	11.2	*0.004*
POD 3	8.6	9.8	*0.040*	8.3	10.2	*0.022*
POD 4	8.0	8.6	*0.132*	8.0	9.2	*0.014*
Neutrophil						
Pre-operative	5.0	5.0	*0.728*	4.3	4.1	*0.612*
POD 1	9.1	9.1	*0.653*	8.0	9.2	*0.073*
POD 2	8.8	8.9	*0.698*	7.0	9.0	*0.007*
POD 3	6.7	7.7	*0.021*	5.9	7.7	*0.016*
POD 4	6.0	6.8	*0.065*	5.4	7.1	*0.006*
Lymphocytes						
Pre-operative	1.5	1.6	*0.294*	1.8	1.9	*0.363*
POD 1	1.0	1.0	*0.822*	1.2	1.3	*0.124*
POD 2	0.9	0.9	*0.812*	1.4	1.2	*0.820*
POD 3	0.9	0.9	*0.826*	1.2	1.0	*0.171*
POD 4	0.9	0.8	*0.270*	1.3	1.0	*0.619*
Monocytes						
Pre-operative	0.6	0.6	*0.070*	0.6	0.5	*0.563*
POD 1	0.7	0.7	*0.115*	0.9	0.9	*0.470*
POD 2	0.6	0.7	*0.406*	0.7	0.9	*0.378*
POD 3	0.5	0.6	*0.064*	0.7	0.8	*0.487*
POD 4	0.6	0.7	*0.092*	0.7	0.7	*0.520*
Eosinophils						
Pre-operative	0.17	0.20	*0.085*	0.15	0.28	*0.003*
POD 1	0.01	0.01	*0.794*	0.01	0.01	*0.689*
POD 2	0.10	0.08	*0.361*	0.08	0.11	*0.457*
POD 3	0.20	0.20	*0.658*	0.14	0.25	*0.094*
POD 4	0.20	0.20	*0.775*	0.19	0.21	*0.286*
Basophils						
Pre-operative	0.02	0.03	*0.071*	0.03	0.03	*0.511*
POD 1	0.00	0.01	*0.180*	0.01	0.01	*0.089*
POD 2	0.00	0.01	*0.192*	0.01	0.02	*0.274*
POD 3	0.01	0.01	*0.937*	0.02	0.01	*0.024*
POD 4	0.01	0.01	*0.793*	0.02	0.02	*0.936*
Platelets						
Pre-operative	280	273	*0.321*	278	283	*0.474*
POD 1	245	222	*0.393*	236	230	*0.533*
POD 2	238	214	*0.065*	232	219	*0.427*
POD 3	244	221	*0.142*	265	226	*0.242*
POD 4	288	258	*0.072*	270	266	*0.386*
C-reactive						

Protein						
Pre-operative	7	10	*0.222*	3	3	*0.309*
POD 1	102	113	*0.158*	55	96	*0.002*
POD 2	163	211	*<0.001*	87	151	*<0.001*
POD 3	143	213	*<0.001*	87	175	*0.003*
POD 4	94	179	*<0.001*	76	206	*0.016*
Albumin						
Pre-operative	39	37	*0.208*	37	38	*0.320*
POD 1	27	26	*0.038*	29	30	*0.190*
POD 2	27	25	*<0.001*	29	30	*0.850*
POD 3	27	24	*<0.001*	29	27	*0.122*
POD 4	28	24	*<0.001*	29	27	*0.122*

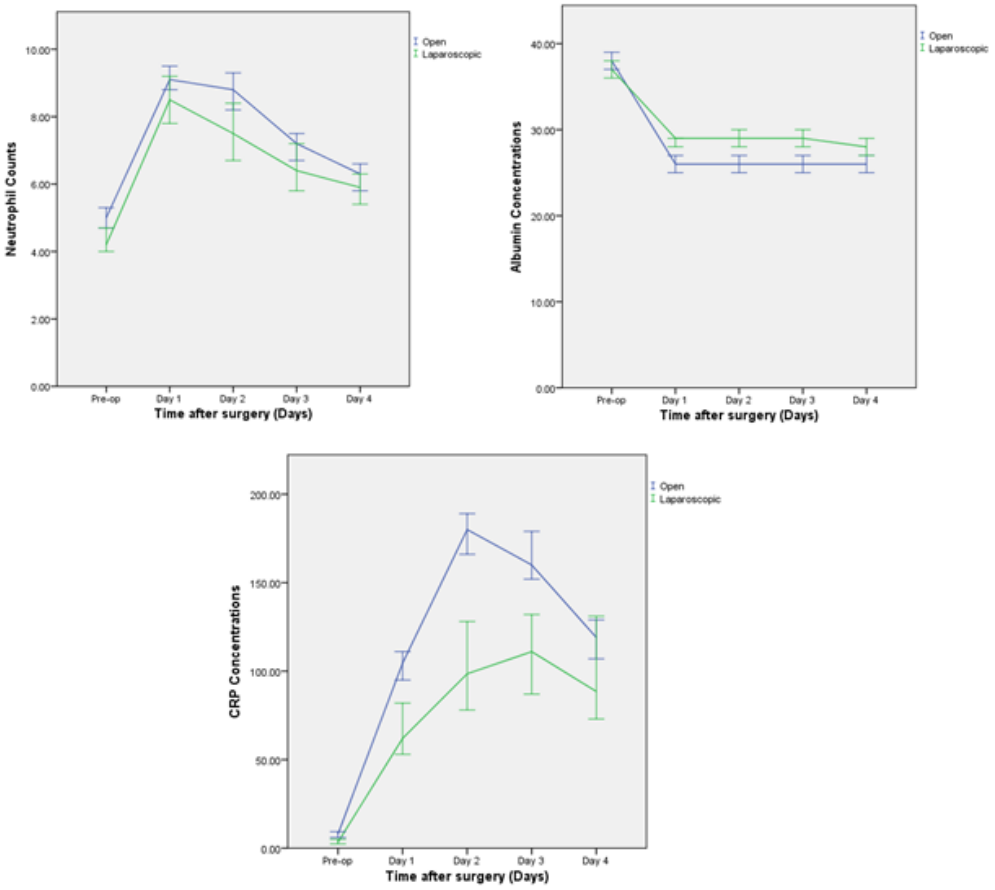
POD, post-operative day

Table 6.4 **Correlations between the neutrophil count and CRP concentrations on post-operative days 1-4**

	Day 1 CRP Concentration	Day 2 CRP Concentration	Day 3 CRP Concentration	Day 4 CRP Concentration
Day 1 Neutrophil Count	$r_s = 0.070$ $p = 0.198$	$r_s = 0.031$ $p = 0.569$	$r_s = 0.015$ $p = 0.781$	$r_s = -0.006$ $p = 0.911$
Day 2 Neutrophil Count	$r_s = 0.163$ $p = 0.003$	$r_s = 0.226$ $p < 0.001$	$r_s = 0.204$ $p < 0.001$	$r_s = 0.072$ $p = 0.210$
Day 3 Neutrophil Count	$r_s = 0.109$ $p = 0.053$	$r_s = 0.147$ $p = 0.008$	$r_s = 0.251$ $p < 0.001$	$r_s = 0.195$ $p = 0.001$
Day 4 Neutrophil Count	$r_s = 0.075$ $p = 0.213$	$r_s = 0.093$ $p = 0.122$	$r_s = 0.219$ $p < 0.001$	$r_s = 0.219$ $p < 0.001$

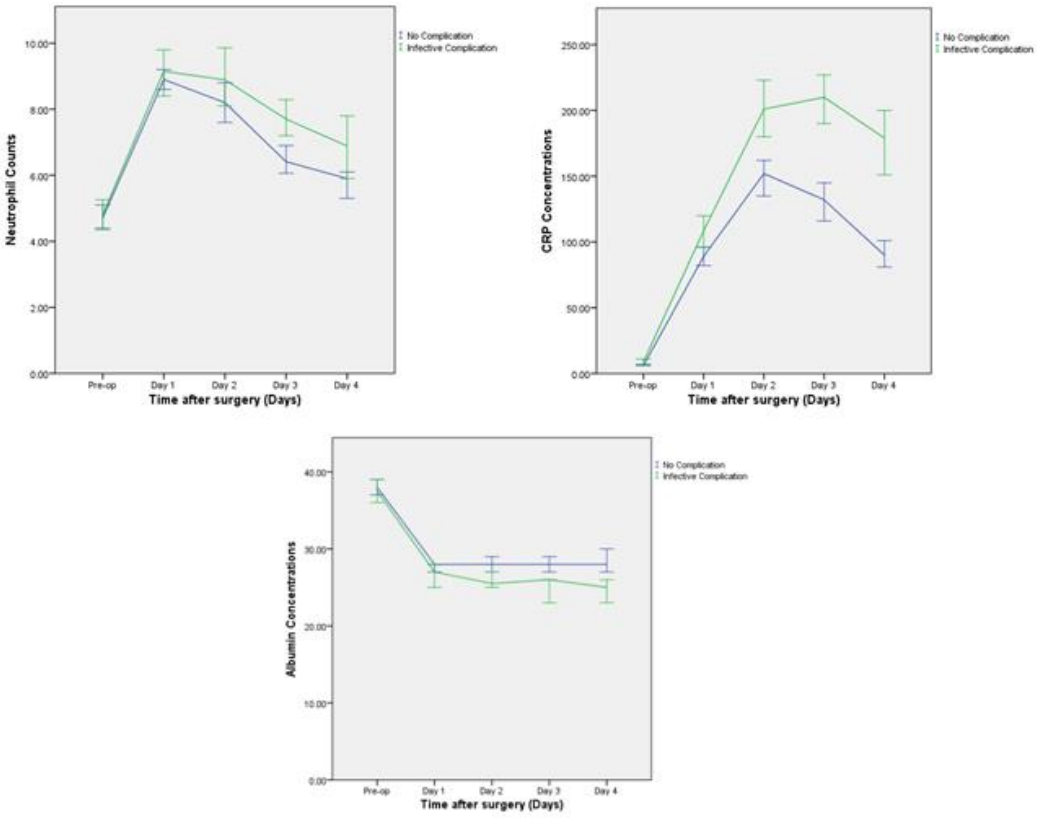
Table 6.5 **Correlations between neutrophil count and albumin concentrations on post-operative days 1-4**

	Day 1 Albumin Concentration	Day 2 Albumin Concentration	Day 3 Albumin Concentration	Day 4 Albumin Concentration
Day 1 Neutrophil Count	$r_s = 0.101$ $p = 0.084$	$r_s = 0.072$ $p = 0.215$	$r_s = -0.001$ $p = 0.983$	$r_s = 0.22$ $p = 0.723$
Day 2 Neutrophil Count	$r_s = -0.118$ $p = 0.045$	$r_s = -0.058$ $p = 0.321$	$r_s = -0.130$ $p = 0.030$	$r_s = -0.141$ $p = 0.024$
Day 3 Neutrophil Count	$r_s = -0.139$ $p = 0.024$	$r_s = -0.163$ $p = 0.008$	$r_s = -0.126$ $p = 0.038$	$r_s = -0.238$ $p = <0.001$
Day 4 Neutrophil Count	$r_s = -0.148$ $p = 0.022$	$r_s = -0.162$ $p = 0.012$	$r_s = -0.216$ $p = 0.001$	$r_s = -0.216$ $p = 0.001$



Time after surgery (Days)	Open (median)	Laparoscopic (median)	*p-value*
Neutrophil count (10^9/L)			
Pre-op	5.0	4.1	*0.002*
Day 1	9.1	8.4	*0.086*
Day 2	9.0	7.6	*0.006*
Day 3	7.3	6.6	*0.100*
Day 4	6.3	5.7	*0.743*
C-reactive Protein (CRP) (mg/L)			
Pre-op	8.2	3.3	*<0.001*
Day 1	104	71	*<0.001*
Day 2	185	126	*<0.001*
Day 3	173	131	*<0.001*
Day 4	120	87	*0.296*
Albumin (g/L)			
Pre-op	38	37	*0.212*
Day 1	26	29	*<0.001*
Day 2	26	29	*<0.001*
Day 3	26	29	*<0.001*
Day 4	26	28	*0.146*

Figure 6.1 **The magnitude of the neutrophil count, CRP and albumin in both open and laparoscopic elective colorectal cancer resection**



Time after surgery (Days)	No Complication (median)	Infective Complication (median)	*p-value*
Neutrophil Count (10^9/L)			
Pre-op	4.6	4.8	*0.755*
Day 1	8.9	9.0	*0.610*
Day 2	8.6	8.8	*0.112*
Day 3	6.7	7.8	*0.001*
Day 4	5.9	6.9	*0.005*
C-reactive Protein (CRP) (mg/L)			
Pre-op	6	8	*0.075*
Day 1	91	108	*0.006*
Day 2	161	201	*<0.001*
Day 3	143	209	*<0.001*
Day 4	93	174	*<0.001*
Albumin (g/L)			
Pre-op	38	38	*0.518*
Day 1	28	27	*0.105*
Day 2	28	26	*0.001*
Day 3	28	26	*0.001*
Day 4	28	25	*<0.001*

Figure 6.2 **The magnitude of the neutrophil count, CRP and albumin in patients developing infective complications and no complications following elective colorectal cancer resection**

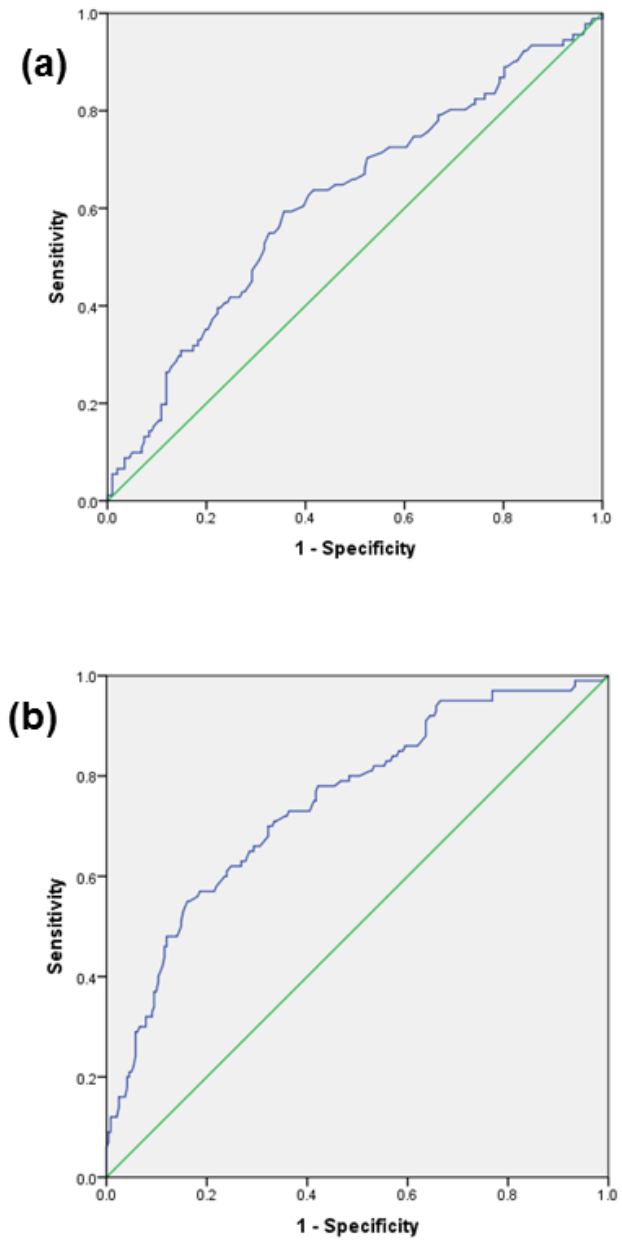


Figure 6.3 **(a) ROC curve of the day 3 neutrophil count and development of infective complications and (b) ROC curve of the day 3 CRP and the development of infective complications.**
(a)The area under the curve (AUC) is 0.617 (95% CI 0.55-0.69; p=0.001) and (b) the area under the curve (AUC) is 0.748 (95% CI 0.69-0.81; p<0.001).

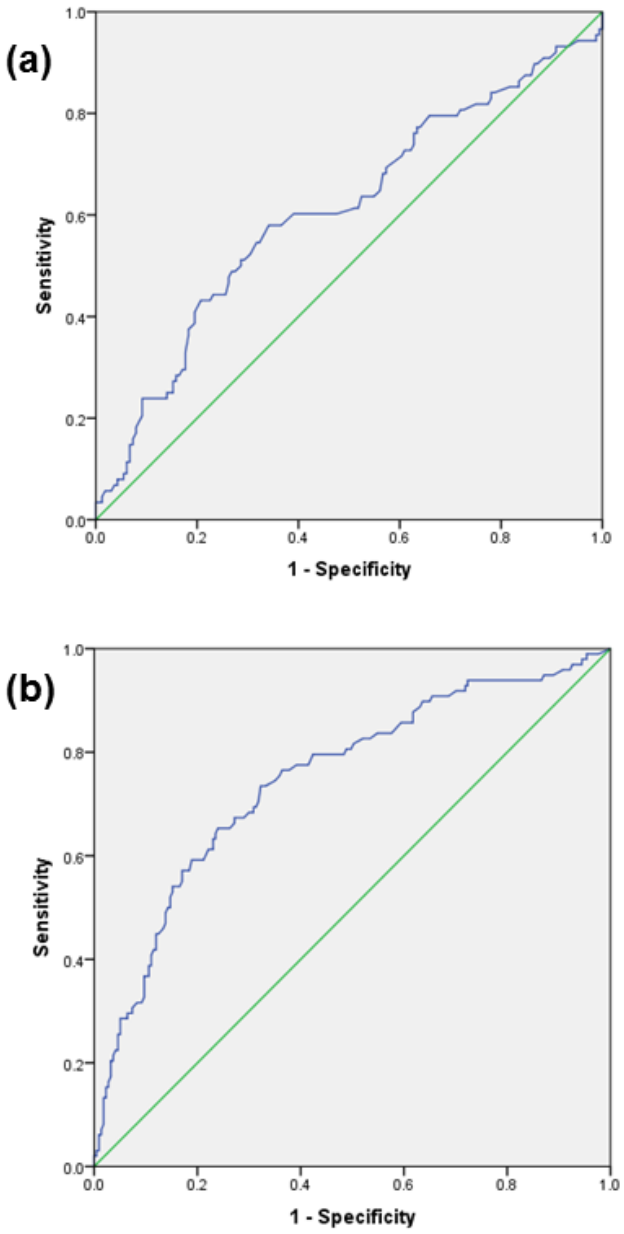


Figure 6.4 **(a) ROC curve of the day 4 neutrophil count and development of infective complications and (b) ROC curve of the day 4 CRP and the development of infective complications.**
(a) The area under the curve (AUC) is 0.6089 (95% CI 0.53-0.68; p=0.005) and (b) the area under the curve (AUC) is 0.749 (95% CI 0.69-0.81; p<0.001).

7 A post-operative systemic inflammation score predicts short and long term outcomes in patients undergoing surgery for colorectal cancer

7.1 Introduction

In those deemed to have non-metastatic disease, surgery is the primary modality of cure. However, there has been a longstanding concern that although surgery provides the means of a potential cure for patients with colorectal cancer, the injury associated with it may stimulate tumour growth and dissemination (Abramovitch et al., 1999, Mynster et al., 2000, McArdle et al., 2005, Retsky et al., 2008). Despite various infection control measures and the use of pre-operative antibiotic prophylaxis following surgery, a significant proportion of patients develop post-operative complications, with the majority of these being infective in nature. Recently, it has become apparent these complications, as well as having an adverse effect on patients quality of life (Brown et al., 2014) are associated with both increased risk of cancer recurrence and poorer long term survival (McArdle et al., 2005, Mirnezami et al., 2011, Richards et al., 2011, Pucher et al., 2014, Artinyan et al., 2015).

Therefore, there has been considerable interest in objectively identifying, early in the post-operative phase, which patients are at increased risk of developing infective complications in order to facilitate prompt investigation, treatment or alternatively facilitate safe discharge. In particular, the stereotypical marker of the systemic inflammatory response (SIR), C-reactive protein (CRP) has been extensively examined and concentrations greater than approximately 150mg/l on days 3-5 have been shown to be useful (Singh et al., 2014). In particular, it has been proposed that in patients undergoing resection for colorectal cancer, CRP concentrations of $\leq$150 mg/L on post-operative days 3-5 are unlikely to develop of infective complications facilitating safe early discharge (McDermott et al., 2015).

Recently, McSorley et al. reported in 377 patients that the post-operative systemic inflammatory response, evidenced by CRP concentrations greater than 150 mg/L, were associated with both complication severity and long term outcome (McSorley et al., 2016b). However, whether this observation provides the basis for a post-operative scoring system to predict both short term and long term outcomes is not clear.

Therefore, the aim of the present study was to examine whether the combination of post-operative markers of the SIR, namely CRP and albumin, are useful in predicting the development of post-operative infective complications and long term survival in a large cohort of patients undergoing potentially curative surgery for colorectal cancer.

7.2 Methods

7.2.1 Patients

Patients with histologically proven colorectal cancer who, on the basis of intra-operative findings and pre-operative computed tomography, were considered to have undergone potentially curative resection at a single centre between March 1999 and May 2013 were included in the analyses (n=813). All patient data was anonymised and all tumours were staged according to conventional tumour, node, metastasis (TNM5) classification, as per the Royal College of Pathologists guidelines (RCPath, 2014) and additional pathological data obtained from the pathology reports issued at the time of the resection. Patients were grouped into 2 cohorts, in both cohorts patient characteristics were collected in a prospective manner. In the test cohort (surgery from March 1999 to November 2007; n=402) post-operative complication data was collected retrospectively from electronic records. In the validation cohort (surgery from January 2008 to May 2013; n=411) post-operative complication data was collected prospectively from patient records following discharge. Due to the prospective method of data collection, Clavien-Dindo classification of complications was also recorded for this validation cohort.

Pre-operatively, all patients received thromboembolism prophylaxis and antibiotic prophylaxis as per local protocols. Blood samples were taken for routine laboratory analysis pre and post-operatively. The pre-operative SIR was assessed using the modified Glasgow Prognostic Score (McMillan, 2013b) (mGPS, Table 7.1).

The post-operative SIR was assessed using the post-operative Glasgow Prognostic Score (poGPS, Table 7.1). In essence, a post-operative CRP concentration below 150 mg/L, regardless of albumin concentration scored 0, a CRP concentration ≥150 mg/L and albumin >25 g/L scored 1 and CRP ≥150 mg/L and albumin <25 g/L scored 2. The creation of this score was initially performed in the retrospective test cohort and an attempt to subsequently validate this in the prospective validation cohort then performed.

Post-operatively all patients underwent daily clinical assessment. Clinicians were not blinded to these daily blood results and additional investigations and management were instigated at the surgical team's discretion based on the relevant clinical findings.

Patients were assessed for both non-infective (ileus, acute coronary syndrome, acute myocardial infarction, pulmonary embolism and arrhythmias) and infective complications

(wound, intra-abdominal abscess, anastomotic leak, urinary tract infection and pneumonia). The criteria used to define these complications were the same as has been previously described (Platt et al., 2012, Ramanathan et al., 2013). In short, a wound infection included the presence of pus that discharged spontaneously or required drainage; an intra-abdominal abscess was confirmed by imaging and required either conservative therapy with antibiotics or drainage; an anastomotic leak was defined as a fistula to the bowel anastomosis that was confirmed radiologically or diagnosed at re-laparotomy; pneumonia was diagnosed as the presence of x-ray changes and fever that required antibiotic therapy and urinary tract infection as positive urine culture in presence of symptoms that required antibiotic therapy. Patients were routinely followed up for 5 years following resection as per national guidelines. Date and cause of death were crosschecked with the cancer registration system and Registrar General (Scotland). The West of Scotland research ethics committee approved this study.

7.2.2 Statistical Analysis

The comparison of categorical variables was performed using Chi square test and of continuous variables using the Mann Whitney U test. Univariate survival analysis was performed using Cox proportional hazards regression in order to calculate hazard ratios (HR) and 95% confidence intervals (95% CI). A two sided *p value* of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 22.0 for Windows (IBM, SPSS, IL, USA).

7.3 Results

In both the test cohort (n=402) and the validation cohort (n=411), the majority of patients were aged >65 years old, were male, had a colonic tumour and had an elective operation. Comparison of the clinicopathological characteristics in both cohorts is shown in *Table 7.2*. There were significantly fewer emergency procedures ($p<0.05$), more T1 tumours and less T3 tumours ($p<0.005$), greater overall complication rate ($p<0.001$) and greater infective complication rate ($p<0.001$) in the validation cohort. Median follow up of survivors in the test cohort was 116 months (range 76-180) and in the validation cohort was 31 months (range 10-71 months).

7.3.1 Test Cohort

With regards to short term outcomes, 87 patients (22%) developed an infective complication. In this cohort, of the pre-operative factors, emergency presentation ($p<0.01$) and raised pre-operative CRP concentrations ($p<0.05$) were associated with the development of infective complications. Moreover, rectal surgery ($p<0.01$), exceeding the post-operative thresholds for CRP on days 3 and 4 (both $p<0.001$) and for albumin on days 3 and 4 (both $p<0.001$) and the day 3 and 4 poGPS (both $p<0.001$) were also associated with the development of infective complications *(Table 7.3)*.

In patients undergoing surgery for colon cancer (n=259), 46 patients (18%) of patients developed an infective complication. In these patients, emergency presentation ($p<0.001$), exceeding the post-operative day 3 and 4 thresholds of CRP ($p<0.001$) and albumin ($p<0.005$) and the day 3 and 4 poGPS (both $p<0.001$) were associated with the development of infective complications *(Table 7.4)*.

When using the post-operative scoring system *(Table 6.6)*, post-operative Glasgow Prognostic Score (poGPS), an incremental increase in the day 3 poGPS from 0 to 1 to 2 resulted in an increase in the infective complication rate from 10.3% to 31.0% to 56.6% ($p<0.001$) and using the day 4 poGPS from 17.0% to 50.0% to 73.9% ($p<0.001$).

7.3.2 Validation Cohort

In this cohort there were similar findings to those in the test cohort. With regards to short term outcomes, 106 patients (26%) developed an infective complication. Male sex ($p<0.005$), exceeding the post-operative thresholds for CRP on days 3 and 4 (both

p<0.001) and for albumin on days 3 and 4 (both p<0.005) and the day 3 and 4 poGPS (both p<0.001) were also associated with the development of infective complications *(Table 7.5)*.

When using the post-operative scoring system *(Table 7.6)* an incremental increase in the day 3 poGPS resulted in an increase in the infective complication rate from 14.4% to 28.9% to 41.9% (p<0.001) and using the day 4 poGPS from 17.9% to 32.2% to 53.7% (p<0.001). Increase in the day 3 poGPS score resulted in an increase in severity of Clavien-Dindo Scores (p<0.001) as did an increase in the day 4 poGPS (p<0.001).

7.3.3 Overall Cohort

With regards to long term outcomes, univariate survival analysis between the clinicopathological characteristics, the pre-operative and post-operative SIR is shown in *Table 7.7.* There were 175 cancer deaths and 139 non-cancer deaths. Age (p<0.001), TNM stage (p<0.001), venous invasion (p<0.005), margin involvement (p<0.001), peritoneal involvement (p<0.001), adjuvant therapy (p<0.05) and mGPS (p<0.001) as well as exceeding the post-operative day 3 and 4 threshold for CRP (both p<0.05) and for albumin (both p<0.05) and the day 3 and 4 poGPS (p<0.05) were associated with OS.

Furthermore, in patients undergoing resection of colon cancer *(Table 7.8)*, age, (p<0.01), emergency presentation (p<0.05), TNM stage (p<0.001), venous invasion (p<0.05), margin involvement (p<0.001), and adjuvant therapy (p<0.05) as well as the mGPS (p<0.001), exceeding day 3 and 4 threshold for CRP (p<0.05) and albumin (p<0.001) and the day 3 and 4 poGPS (p<0.005) were associated with overall survival.

7.4 Discussion

The results of the present study confirm that the development of post-operative complications, in particular infective complications, were associated with the post-operative SIR in patients undergoing surgery for colorectal cancer. Furthermore, an objective post-operative scoring approach (poGPS) was capable of stratifying the risk of developing post-operative infective complications, ranging from approximately 12% with a score of 0 to approximately 30% with a score of 1 and approximately 50% with a score of 2. These results have been demonstrated in a retrospective 'test cohort' and subsequently validated in the prospective 'validation' cohort. Moreover, the post-operative SIR, evidenced by the poGPS, was associated with survival in patients undergoing surgery for colorectal cancer.

This study appears to finally tie the SIR to outcomes following surgery for colorectal cancer. Specifically, the present study builds on work by McSorley et al. who reported in a cohort of 377 patients that the magnitude of the post-operative SIR was significantly associated with long term outcomes, independent of complications and tumour stage (McSorley et al., 2016b). These findings, along with the present study would appear to suggest that the mechanisms behind the development of post-operative complications and poor long term survival are linked by the SIR. Therefore a plausible hypothesis is that the cancer itself elicits an SIR in a significant proportion of patients while the added surgical injury produces an immunological hit in all patients that leads to homeostatic decompensation in some. The nature and the consequences of that decompensation may be the same as seen in patients with untreated or metastatic disease, perhaps even stimulating the growth of residual cancer cells (micrometastatic disease). Moreover, it would appear that the SIR involves both a dimension of magnitude and duration, both of which affect the prognosis. Consistent with this hypothesis, it has recently been reported that serum and peritoneal fluid samples from patients undergoing colorectal cancer surgery who had post-operative peritoneal infection increased the in-vitro invasiveness capacity of cancer cell lines, causing increased tumour dissemination and tumour cell survival (Salvans et al., 2014). If this were to prove to be the case then it may be that colorectal cancer surgery should be practiced in such a way to minimise the post-operative SIR. This study lays the foundation for further work in this field.

In the present study it was of interest that elevated poGPS scores were significantly associated with emergency presentation and the presence of a SIR pre-operatively. It has

now been established that patients who present as an emergency for surgery for colorectal cancer have poorer 5 year survival (McArdle and Hole, 2004, Oliphant et al., 2014a, Oliphant et al., 2014b). In the present study, when only patients who had elective surgery and had a mGPS 0 were examined the poGPS stratified the post-operative infective complication rate on both day 3 (poGPS0 rate was 13.7%, poGPS1 rate was 32.8% and poGPS2 rate was 50.7%; $p<0.001$) and day 4 (poGPS0 rate was 17.3, poGPS1 rate was 43.1% and poGPS2 rate was 71.4%; $p<0.001$). Also, the poGPS stratified the 5 year survival rates on day 3 (poGPS0 rate was 74%, poGPS1 rate was 67% and poGPS2 rate was 60%; $p = 0.039$). Therefore, the results of the present study would indicate a role for a post-operative SIR scoring system in predicting both short and long term outcomes in patients undergoing surgery for colorectal cancer.

Recently, it was reported that the depletion of skeletal muscle mass following surgery for colorectal cancer was greater with older age, female sex, open surgery and an elevated pre-operative SIR, as evidenced by the neutrophil lymphocyte ratio (Malietzis et al., 2016). Therefore, consistent with the present results, it may be hypothesised that this was related to a greater post-operative SIR. Given the above it would be of interest to examine whether approaches to minimise the poGPS, other than laparoscopic surgery, such as peri-operative steroids (McSorley et al., 2016a) would reduce the loss of skeletal muscle mass following surgery for colorectal cancer.

This study has several potential clinical benefits: In those with a low score, it may provide the clinician with reassurance regarding the development of infective complications and allow prompt discharge, particularly in an enhanced recovery setting. In contrast, in those with a high score it may provide an early warning to the clinician and prompt re-assessment and management of the patient. Also, by enabling objective comparison of the impact of different surgical approaches and techniques on the magnitude of the post-operative SIR following surgery it may be possible to identify individuals or techniques that minimise the poGPS score (Chapter 5) . Finally, by acting as a therapeutic target, the use of post-operative anti-inflammatory agents has the potential to improve short term outcomes. The use of anti-inflammatory agents in the post-operative period, particularly following colorectal surgery continues to be a subject of intense debate, with studies reporting conflicting outcomes (Klein et al., 2012, Bhangu et al., 2014, STARSURG, 2014). Cautious use in the post-operative period may provide benefit to patients with an exaggerated post-operative SIR but more work is required to test this.

The magnitude of surgical injury in different colorectal procedures may be different e.g. for colonic resections and rectal resections. This may also vary across surgical centres, with differences in patient cohorts and in operative expertise. Therefore, the present results require external validation. However, given the simplicity of the measurement of post-operative SIR developed such validation can be readily tested.

In the present study thresholds for CRP and albumin were examined using ROC analysis and post-operative infective complications as an end point. On day 3 they were 153mg/l and 26g/l respectively. On day 4 they were 125mg/l and 27g/l respectively. These were similar to that established from previous meta-analysis (CRP >150mg/l and albumin <25g/l) and therefore the latter thresholds were used in the analysis. A limitation of the present study was that there are intrinsic and extrinsic factors not accounted for that may potentially affect the relationship between the post-operative SIR and long and short term outcomes in patients undergoing surgery for colorectal cancer. For example, comorbidities, the quality and type of anaesthesia/surgery, blood loss and blood transfusion may all affect this relationship. Nevertheless, the poGPS provide an objective framework against which such factors to be investigated.

In summary, the magnitude of the post-operative SIR, as evidenced by the poGPS, was associated with an incremental increase in the post-operative infective complication rates and a reduction in survival. Elevated systemic inflammation whether prior to or following surgery is associated with poor outcome in patients with colorectal cancer.

Table 7.1 The pre-operative modified Glasgow Prognostic Score (mGPS) and the post-operative Glasgow Prognostic Score (poGPS)

The modified Glasgow Prognostic Score (mGPS)	***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 and Albumin ≥35 g/L	1
C-reactive protein >10 mg/L and Albumin <35 g/L	2
The post-operative Glasgow Prognostic Score (poGPS)	
C-reactive protein ≤150 mg/L and Albumin ≥25 g/L	0
C-reactive protein ≤150 mg/L and Albumin <25 g/L	0
C-reactive protein >150 mg/L and Albumin ≥25 g/L	1
C-reactive protein >150 mg/L and Albumin <25 g/L	2

Table 7.2 Comparison of clinicopathological characteristics in both the test and validation cohort

Characteristic		Test Cohort (n = 402)	Validation Cohort (n = 411)	
		n (%)	n (%)	*p-value*
Age				0.153
	<65	131 (33)	137 (33)	
	65-74	131 (33)	155 (38)	
	>74	140 (34)	119 (29)	
Sex				0.828
	Female	184 (46)	185 (45)	
	Male	218 (54)	226 (55)	
Emergency Presentation				0.047
	No	354 (88)	379 (92)	
	Yes	48 (12)	32 (8)	
Tumour Site				0.933
	Colon	261 (65)	268 (65)	
	Rectum	141 (35)	143 (35)	
TNM Stage				0.002
	0	1 (1)	10 (2)	
	I	52 (13)	80 (20)	
	II	179 (44)	177 (43)	
	III	170 (42)	144 (35)	
All complications				<0.001
	No	298 (74)	254 (62)	
	Yes	100 (25)	156 (38)	
Infective Complications				<0.001
	No	298 (74)	254 (62)	
	Yes	87 (22)	106 (26)	

mGPS, modified Glasgow Prognostic Score; TNM, Tumour, Node, Metastasis; CRP, C-reactive protein

Table 7.3 The clinical characteristics, pre-operative systemic inflammation and post-operative complications in the test cohort

Characteristic		No Complication (n = 298)	Infective Complications (n = 87)	
		n (%)	n (%)	*p-value*
Age				0.359
	<65 yrs	102 (79)	27 (21)	
	65-74 yrs	89 (73)	33 (21)	
	>74 yrs	107 (80)	27 (20)	
Sex				0.356
	Female	140 (79)	36 (21)	
	Male	158 (76)	51 (24)	
Emergency Presentation				0.006
	No	269 (80)	69 (20)	
	Yes	29 (62)	18 (38)	
Tumour Site				0.007
	Colon	204 (82)	46 (18)	
	Rectum	94 (70)	41 (30)	
TNM Stage				0.255
	0	0 (0)	1 (100)	
	I	39 (75)	13 (25)	
	II	131 (77)	40 (23)	
	III	128 (79)	33 (21)	
Pre-op CRP				0.040
	≤10 mg/L	174 (81)	40 (19)	
	>10 mg/L	124 (72)	47 (28)	
Pre-op Albumin				0.677
	≥35 g/L	239 (78)	68 (22)	
	<35 g/L	59 (76)	19 (24)	
Pre-op mGPS				0.119
	0	174 (81)	40 (19)	
	1	84 (73)	31 (27)	
	2	40 (71)	16 (29)	
Post-op Day 3				<0.001
	CRP ≤150 mg/L	165 (89)	20 (11)	
	CRP >150 mg/L	99 (60)	66 (40)	
Post-op Day 3				<0.001
	Alb ≥25 g/L	183 (82)	41 (18)	
	Alb <25 g/L	68 (60)	45 (40)	
D3 poGPS				<0.001
	0	165 (89)	20 (11)	
	1	74 (67)	36 (33)	
	2	22 (42)	30 (58)	
Post-op Day 4				<0.001
	CRP ≤150 mg/L	202 (82)	44 (18)	
	CRP >150 mg/L	23 (40)	35 (60)	
Post-op Day 4				<0.001
	Alb ≥ 25 g/L	176 (82)	38 (18)	
	Alb < 25 g/L	42 (52)	39 (48)	
D4 poGPS				<0.001

0	202 (82)	44 (18)
1	15 (45)	18 (55)
2	5 (23)	17 (77)

mGPS, modified Glasgow Prognostic Score; TNM, Tumour, Node, Metastasis; CRP, C-reactive protein

Table 7.4 Clinical characteristics and post-operative complications of patients undergoing resection for colon cancer in test cohort

Characteristic		No Complication (n = 204)	Infective Complications (n = 46)	
		n (%)	n (%)	*p-value*
Age				0.985
	<65 yrs	60 (81)	14 (19)	
	65-74 yrs	69 (82)	15 (18)	
	>74 yrs	75 (81)	17 (19)	
Sex				0.910
	Female	95 (82)	21 (18)	
	Male	109 (81)	25 (19)	
Emergency Presentation				<0.001
	No	176 (86)	29 (14)	
	Yes	28 (62)	17 (38)	
TNM Stage				0.393
	I	20 (74)	7 (26)	
	II	99 (85)	18 (15)	
	III	85 (80)	21 (20)	
Pre-op CRP				0.075
	≤10 mg/L	105 (86)	17 (14)	
	>10 mg/L	99 (77)	29 (23)	
Pre-op Albumin				0.083
	≥35 g/L	158 (84)	30 (16)	
	<35 g/L	46 (74)	16 (26)	
Pre-op mGPS				0.097
	0	105 (86)	17 (14)	
	1	66 (80)	16 (20)	
	2	33 (72)	13 (28)	
Post-op Day 3				<0.001
	CRP ≤150 mg/L	107 (90)	12 (10)	
	CRP >150 mg/L	71 (68)	34 (32)	
Post-op Day 3				0.001
	Alb ≥25 g/L	40 (89)	5 (11)	
	Alb <25 g/L	5 (45)	6 (6 (55)	
D3 poGPS				<0.001
	0	107 (90)	12 (10)	
	1	56 (78)	16 (22)	
	2	12 (40)	18 (60)	
Post-op Day 4				<0.001
	CRP ≤150 mg/L	136 (84)	26 (16)	
	CRP >150 mg/L	17 (55)	14 (45)	
Post-op Day 4				<0.001
	Alb ≥ 25 g/L	123 (88)	17 (12)	
	Alb < 25 g/L	24 (52)	22 (48)	
D4 poGPS				<0.001
	0	136 (84)	26 (16)	
	1	12 (63)	7 (37)	
	2	3 (30)	7 (70)	

mGPS, modified Glasgow Prognostic Score; TNM, Tumour, Node, Metastasis; CRP, C-reactive protein

Table 7.5 Clinical characteristics and post-operative complications of patients undergoing resection of colorectal cancer in the validation cohort

Characteristic		No Complication (n = 254)	Infective Complications (n = 106)	
		n (%)	n (%)	*p-value*
Age				0.853
	<65 yrs	88 (69)	39 (31)	
	65-74 yrs	99 (72)	38 (28)	
	>74 yrs	67 (70)	29 (30)	
Sex				0.003
	Female	132 (78)	37 (22)	
	Male	122 (64)	69 (36)	
Emergency Presentation				0.135
	No	239 (72)	95 (28)	
	Yes	15 (58)	11 (42)	
Tumour Site				0.055
	Colon	173 (74)	61 (26)	
	Rectum	81 (64)	45 (36)	
TNM Stage				0.221
	0	8 (80)	2 (20)	
	I	54 (76)	17 (24)	
	II	112 (73)	42 (27)	
	III	80 (64)	45 (36)	
Pre-op CRP				0.688
	≤10 mg/L	185 (71)	75 (29)	
	>10 mg/L	69 (69)	31 (31)	
Pre-op Albumin				0.594
	≥35 g/L	168 (71)	67 (29)	
	<35 g/L	86 (69)	39 (31)	
Pre-op mGPS				0.902
	0	185 (71)	75 (29)	
	1	26 (70)	11 (30)	
	2	43 (68)	20 (32)	
Post-op Day 3				<0.001
	CRP ≤150 mg/L	131 (83)	26 (17)	
	CRP >150 mg/L	108 (59)	75 (41)	
Post-op Day 3				0.003
	Alb ≥25 g/L	142 (77)	42 (23)	
	Alb <25 g/L	97 (62)	59 (38)	
D3 poGPS				<0.001
	0	131 (83)	26 (17)	
	1	54 (67)	26 (33)	
	2	53 (52)	49 (48)	
Post-op Day 4				<0.001
	CRP ≤150 mg/L	142 (79)	38 (21)	
	CRP >150 mg/L	63 (50)	64 (50)	
Post-op Day 4				<0.001
	Alb ≥ 25 g/L	130 (78)	37 (22)	
	Alb < 25 g/L	75 (54)	64 (46)	

D4 poGPS			<0.001
	0	142 (79)	38 (21)
	1	36 (65)	19 (35)
	2	26 (37)	44 (63)

mGPS, modified Glasgow Prognostic Score; TNM, Tumour, Node, Metastasis; CRP, C-reactive protein

Table 7.6 **The day 3 and day 4 poGPS and the development of post-operative complications in patients undergoing resection for colorectal cancer in both test and validation cohorts**

Post-operative Day	CRP (mg/L)	Albumin (g/L)	poGPS	No. of patients	No Complications/Infective Complications (n)	Infective Complication Rate (%)	Clavien-Dindo Grade (0/1-2/3-5)[#]
Test Cohort (n=402)							
3							
	≤150	<25 OR ≥25	0	194	165/20	10.3	-
	>150	≥25	1	116	74/36	31.0	-
	>150	<25	2	53	22/30	56.6	-
4							
	≤150	<25 OR ≥25	0	259	202/44	17.0	-
	>150	≥25	1	36	15/18	50	-
	>150	<25	2	23	5/17	73.9	-
Validation Cohort (n=411)							
3							
	≤150	<25 OR ≥25	0	180	131/26	14.4	72/22/6
	>150	≥25	1	90	54/26	28.9	61/30/9
	>150	<25	2	117	53/49	41.9	45/41/14
4							
	≤150	<25 OR ≥25	0	212	142/38	17.9	67/25/8
	>150	≥25	1	59	36/19	32.2	61/34/5
	>150	<25	2	82	26/44	53.7	32/47/21

CRP, C-reactive protein; poGPS, post-op Glasgow Prognostic Score

[#] only available in validation cohort

Table 7.7 The relationship between clinicopathological factors and the pre- and post-operative systemic inflammatory response in patients undergoing resection for colorectal cancer

Clinicopathological Characteristic	Cancer-specific Survival		Overall Survival	
	Univariate Analysis (95% CI)	*p-value*	Univariate Analysis (95% CI)	*p-value*
Age (<65/65-74/>74)	1.20 (1.00-1.44)	0.056	1.57 (1.36-1.81)	<0.001
Sex (Female/Male)	1.32 (0.98-1.79)	0.071	1.21 (0.96-1.51)	0.100
Site (Colon/Rectum)	1.06 (0.78-1.44)	0.712	0.89 (0.70-1.13)	0.332
Emergency (No/ Yes)	1.75 (1.16-2.63)	0.008	1.33 (0.95-1.86)	0.094
TNM Stage (0/I/II/III)	2.26 (1.77-2.89)	<0.001	1.53 (1.30-1.80)	<0.001
Venous Invasion (No/Yes)	1.75 (1.29-2.39)	<0.001	1.44 (1.15-1.80)	0.002
Margin involvement (No/Yes)	4.85 (3.34-7.03)	<0.001	3.13 (2.25-4.35)	<0.001
Peritoneal involvement (No/Yes)	2.22 (1.63-3.01)	<0.001	1.68 (1.32-2.14)	<0.001
Tumour perforation (No/Yes)	2.21 (1.09-4.50)	0.028	1.51 (0.80-2.83)	0.201
Adjuvant therapy (No/Yes)	1.07 (0.76-1.50)	0.695	0.75 (0.57-0.99)	0.045
Pre-operative Systemic Inflammation				
mGPS (0/1/2)	1.36 (1.13-1.65)	0.001	1.36 (1.18-1.57)	<0.001
Post-operative Systemic Inflammation				
Day 3 CRP > 150 mg/L (No/Yes)	1.31 (0.96-1.79)	0.088	1.41 (1.12-1.78)	0.004
Day 3 Albumin <25 g/L (No/Yes)	1.38 (1.00-1.90)	0.047	1.42 (1.11-1.81)	0.005
Day 3 poGPS (0/1/2)	1.20 (0.99-1.46)	0.059	1.27 (1.10-1.47)	0.001
Day 4 CRP > 150 mg/L (No/Yes)	1.31 (0.93-1.84)	0.129	1.33 (1.02-1.74)	0.035
Day 4 Albumin <25 g/L (No/Yes)	1.36 (0.98-1.90)	0.068	1.48 (1.15-1.91)	0.002
Day 4 poGPS (0/1/2)	1.22 (0.99-1.50)	0.065	1.21 (1.03-1.42)	0.024

mGPS, modified Glasgow Prognostic Score; poGPS, post-operative Glasgow Prognostic Score; TNM, Tumour, Node, Metastasis; CRP, C-reactive protein

Table 7.8 The relationship between clinicopathological factors and pre- and post-operative systemic inflammatory response in patients undergoing resection for colon cancer

Clinicopathological Characteristic	Cancer-specific Survival		Overall Survival	
	Univariate Analysis (95% CI)	*p-value*	Univariate Analysis (95% CI)	*p-value*
Age (<65/65-74/>74)	1.23 (0.97-1.56)	0.086	1.60 (1.34-1.91)	<0.001
Sex (Female/Male)	1.07 (0.74-1.55)	0.726	1.01 (0.77-1.32)	0.958
Emergency (No/ Yes)	2.13 (1.38-3.28)	0.001	1.47 (1.04-2.08)	0.031
TNM Stage (0/I/II/III)	2.78 (1.99-3.90)	<0.001	1.53 (1.24-1.90)	<0.001
Venous Invasion (No/Yes)	2.03 (1.36-3.02)	<0.001	1.40 (1.06-1.84)	0.018
Margin involvement (No/Yes)	4.51 (2.61-7.80)	<0.001	3.15 (1.96-5.06)	<0.001
Peritoneal involvement (No/Yes)	2.86 (1.97-4.15)	<0.001	1.79 (1.35-2.36)	<0.001
Tumour perforation (No/Yes)	2.86 (1.97-4.15)	<0.001	1.63 (0.83-3.18)	0.154
Adjuvant therapy (No/Yes)	1.09 (0.72-1.65)	0.679	0.64 (0.45-0.90)	0.011
Pre-operative Systemic Inflammation				
mGPS (0/1/2)	1.61 (1.29-2.02)	<0.001	1.54 (1.30-1.82)	<0.001
Post-operative Systemic Inflammation				
Day 3 CRP > 150 mg/L (No/Yes)	1.43 (0.96-2.12)	0.082	1.50 (1.12-2.01)	0.006
Day 3 Albumin <25 g/L (No/Yes)	2.22 (1.47-3.34)	<0.001	2.00 (1.47-2.72)	<0.001
Day 3 poGPS (0/1/2)	1.36 (1.06-1.75)	0.015	1.42 (1.18-1.72)	<0.001
Day 4 CRP > 150 mg/L (No/Yes)	1.38 (0.89-2.14)	0.149	1.55 (1.12-2.14)	0.009
Day 4 Albumin <25 g/L (No/Yes)	1.86 (1.22-2.83)	0.004	1.87 (1.37-2.56)	<0.001
Day 4 poGPS (0/1/2)	1.35 (1.03-1.77)	0.030	1.40 (1.14-1.71)	0.002

mGPS, modified Glasgow Prognostic Score; poGPS, post-operative Glasgow Prognostic Score; TNM, Tumour, Node, Metastasis; CRP, C-reactive protein

8 Enhanced Recovery after Surgery (ERAS); which components, if any, impact on the systemic inflammatory response following colorectal surgery? – A systematic review

8.1 Introduction

Surgery for colorectal disease is associated with variable short term outcomes. Recent advances in peri-operative care methods have attempted to improve these outcomes. The development and widespread application of enhanced recovery or fast track surgical protocols (ERAS), in combination with laparoscopic surgery, represents a paradigm shift in peri-operative care (Lassen et al., 2009). ERAS involves multimodal, protocol driven perioperative care which proponents have asserted reduce the SIR to surgery (Wilmore and Kehlet, 2001).

The trauma of surgery leads to well understood metabolic, neuroendocrine and immune responses, the aims of which are to promote physiological stability and wound healing (Cuthbertson, 1979). The cellular response to surgical injury is to activate neutrophils and macrophages of the innate immune system by the production of pro-inflammatory cytokines such as tumour necrosis factor (TNF) alpha, and the interleukins (IL) e.g. IL-1 and IL-6 (Baigrie et al., 1992, Marik and Flemmer, 2012). Pro-inflammatory cytokines alter the levels of circulating acute phase proteins, e.g. C-reactive protein (CRP), albumin, ferritin, transferrin and fibrinogen, through their action on hepatocytes (Gabay and Kushner, 1999). Indeed, it has been reported that concentrations of circulating acute phase proteins and cytokines are associated with the magnitude of the stress response i.e. the systemic inflammatory response to surgery (Chapter 5). Furthermore, CRP and IL-6 have been reported to have the strongest association with the magnitude of the surgical injury, although CRP is perhaps the most clinically useful of these (Chapter 5) . Moreover, this knowledge forms the basis of an objective examination of the evidence for the impact of ERAS protocols and their components.

Although it is recognised that laparoscopic surgery generates a reduced post-operative systemic inflammatory response following colorectal surgery, the impact of individual components of ERAS protocols, in terms of the systemic inflammatory response, have not been examined in a systematic manner. The aim of the present review was to examine the

evidence in relation to ERAS protocols and their components having an effect on objective markers of the post-operative systemic inflammatory response.

8.2 Methods

Recent separate guidelines on ERAS recommendations following elective colonic and elective rectal/pelvic surgery have been published (Gustafsson et al., 2012a, Nygren et al., 2012). The present systematic review focuses on the components reported in the ERAS Group consensus review in colorectal surgery (Lassen et al., 2009). These recommendations for patients undergoing colorectal surgery are summarised in *Table 8.1*.

A systematic literature search of the US National Library of Medicine (MEDLINE), the Excerpta Medica Database (EMBASE), PubMed and the Cochrane Database of Systemic Reviews (CDSR) was made using the following search criteria: 'ERAS component' AND (systemic inflammation OR systemic inflammatory response OR stress response OR C-reactive protein OR CRP OR IL-6) AND surgery. This was performed independently by the 2 lead authors and any conflicts that were encountered were discussed with the senior authors. From this search, abstracts of articles were analysed for relevance and the bibliographies of relevant studies as well as the bibliography of the consensus review of perioperative care following colorectal surgery (Lassen et al., 2009) were hand-searched for any additional studies. Included studies had to assess the impact of the selected ERAS component on the systemic inflammatory response using either CRP or interleukin-6 (IL-6). Both prospective clinical trials and observational trials were included.

The selection process is summarised in *Figure 8.*1. Using the aforementioned search strategy relevant abstracts were obtained for each ERAS component. Articles were excluded if they were animal studies, not in the English language, were review articles, were not related to colorectal surgery or did not use either CRP or IL-6 as the marker of the systemic inflammatory response. *Table 8.2* summarises the included studies for each ERAS component and the marker of the systemic inflammatory response analysed. Evidence relating to other outcomes of ERAS programmes such as length of stay or post-operative complications were obtained from the most recent Cochrane Reviews or meta-analyses on the specific topic. Meta-analysis of included studies was not performed due to significant heterogeneity amongst study methodology, populations and outcomes measured.

Subjective assessment of study validity was carried out by two authors independently (DW and SM) using the Cochrane Collaboration tool provided by Review Manager version 5.3 (RevMan 5.3, The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen,

Denmark). Any uncertainties were resolved by consensus following discussion with the senior authors (PG and DM). Both prospective clinical trials and observational trials were included.

8.3 Results

8.3.1 Assessment of included study validity

The validity of included studies is summarised in Figure 8.2. The present systematic review included 15 randomised controlled trials (RCT), 1 controlled clinical trial (CCT), and 3 observational studies. The included studies were of varying methodological quality. In particular those RCTs investigation the impact of laparoscopic vs. open surgery on postoperative IL-6 or CRP had issues relating to blinding to both patients and clinicians. All included studies examined the impact of ERAS components on patients undergoing elective colorectal surgery. No studies including emergency surgery or presentation.

8.3.2 Enhanced Recovery after Surgery (ERAS) protocols

Amongst the published literature surrounding ERAS there is considerable variation in the number and nature of the ERAS components applied and also in the outcomes measured (Ahmed et al., 2012, Neville et al., 2014, Nicholson et al., 2014). Indeed, a recent meta-analysis reported that using fewer ERAS components was associated with a greater reduction in mortality and complications compared to those with a greater number of components (Nicholson et al., 2014). Some evidence suggests that only the provision of laparoscopic surgery, early enteral nutrition and early mobilisation shorten length of stay following colorectal surgery within the ERAS framework (Vlug et al., 2012). Two studies including 249 patients examined the impact of ERAS protocols when compared to standard perioperative care and laparoscopic surgery when compared to open procedures, on postoperative CRP and IL-6 (Veenhof et al., 2012, Wang et al., 2012). Wang and colleagues reported that, in 170 patients, CRP and IL-6 on post-operative days 1 and 3 were lower in those cared for using an ERAS protocol, independent of the mode of surgery. Veenhof and coworkers, in their study of 79 patients, reported that no observed difference in postoperative CRP or IL-6 could be attributed solely to the use of an ERAS protocol. No studies examined the impact of ERAS protocols as a whole versus standard perioperative care on the post-operative systemic inflammatory response in colorectal surgery without the inclusion of a laparoscopic surgery vs. open surgery arm, making the interpretation of the impact of ERAS protocols alone difficult.

Laparoscopic Surgery

Laparoscopic colorectal surgery has been reported to shorten length of stay and reduce postoperative pain when compared to open surgery (Schwenk et al., 2005). Furthermore, it has been reported to produce equivalent oncological outcomes when compared to open surgery (Kuhry et al., 2008, Vennix et al., 2014). Ten studies including a total of 1,040 patients have demonstrated lower post-operative CRP following laparoscopic colorectal surgery when compared to open procedures (Schwenk et al., 2000, Delgado et al., 2001, Braga et al., 2002, Dunker et al., 2003, Ng et al., 2009, Gustafsson et al., 2012b, Tsimogiannis et al., 2012, Veenhof et al., 2012, Wang et al., 2012, Ramanathan et al., 2015). Furthermore, a recent review has also reported similar findings in both benign and malignant colorectal disease (Chapter 5). Four studies, including 206 patients, reported no difference in postoperative serum CRP when those undergoing laparoscopic colorectal surgery were compare to those undergoing open procedures (Han et al., 2010, Tsamis et al., 2012, Wu et al., 2003, Veenhof et al., 2011). Therefore, laparoscopic surgery appears to be associated with a reduction in the post-operative systemic inflammatory response, as evidenced by circulating concentrations of CRP, following colorectal surgery and is likely to reduce the SIR as part of an ERAS protocol.

Post-operative Analgesia

Systemic opioids provide effective analgesia but are associated with side effects, including nausea, vomiting, gut dysfunction, respiratory depression and drowsiness, which are likely to prolong hospital stay (Kehlet, 2005, Marret et al., 2007). Methods of analgesia which minimise the amount of opioids used are therefore key components of ERAS programmes.

Epidural analgesia is an effective method of analgesia that can be used with local anaesthetic and low dose opioids. However, they are associated with a failure rate of approximately 30%, epidural haematoma and hypotension (Hermanides et al., 2012). In a recent meta-analysis comparing epidural analgesia (EA) versus opioid analgesia in patients undergoing colorectal surgery, the use of EA decreased the duration of postoperative ileus, allowed more intensive post-operative physiotherapy and mobilisation and resulted in a reduction in pain scores without a significant reduction in length of stay (Marret et al., 2007). EA have been reported to result in a decrease in CRP levels following colorectal surgery (Chen et al., 2015).

Local anaesthesia techniques such as the Transversus Abdominis Plane block (TAP) have been reported to be successful in multiple surgical specialties and operations (Johns et al., 2012). Subsequent meta-analysis of the use of TAP blocks showed a reduction in morphine use 24 hours postoperatively and reduced postoperative nausea and vomiting (Johns et al., 2012). A continuous infusion of local anaesthetic (LA) delivered directly into the surgical wound via a catheter allows longer benefit from the local anaesthetic, is easy to insert and associated with few complications and a low failure rate (Thornton and Buggy, 2011). When compared to EA, wound catheters were reported to be of equal efficacy in terms of pain scores at 48 hours postoperatively with a lower rate of urinary retention (Ventham et al., 2013). However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery.

Non-steroidal anti-inflammatory drugs (NSAIDs) can be used to good effect in the post-operative period. Some studies have suggested that combining NSAIDs and opioid medications results in decreased opioid consumption over a 24 hour period (Maund et al., 2011). If an EA has been used, the combination of paracetamol and an NSAID provides good analgesia during the period around EA removal. However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. In summary, there is some evidence that epidural anaesthesia but not local anaesthesia or NSAIDs can reduce the SIR as part of an ERAS protocol.

Pre-operative Fasting & Carbohydrate Loading

Surgery performed in a fasted state is thought to worsen the catabolic state and delay patient recovery. A recent Cochrane review reported that pre-operative carbohydrate loading was only associated with a small reduction in length of stay and had no effect on complication rates (Smith et al., 2014). One study reported no effect on systemic inflammation, as evidenced by CRP and IL-6, in patients undergoing major abdominal surgery (Mathur et al., 2010). Therefore, there is no evidence that carbohydrate loading can reduce the SIR as part of an ERAS protocol.

Mechanical Bowel Preparation

A recent Cochrane review has reported that there is no statistically significant evidence that the use of mechanical bowel preparation (MBP) alone prevents post-operative complications such as anastomotic leak in patients undergoing colorectal surgery (Guenaga et al., 2011).

The benefits of MBP in rectal surgery remains unclear with some studies reporting no difference in anastomotic leak rates (Van't Sant et al., 2010) and others finding that MBP reduced infective complication rates (Bretagnol et al., 2010). However, recent work from the United States has reported that MBP in combination with oral antibiotics was associated with significantly reduced surgical site infections, anastomotic leak, hospital readmission and ileus (Kiran et al., 2015, Scarborough et al., 2015, Klinger et al., 2017). Despite these reported benefits, no studies analysed whether MBP had an impact on the systemic inflammatory response. Therefore, there is no evidence that MBP can reduce the SIR as part of an ERAS protocol.

Goal directed fluid therapy

Goal directed therapy is the use of intravenous fluids and vasoactive drugs to meet defined targets for blood flow to achieve optimal oxygen delivery (Abbas and Hill, 2008). A recent Cochrane Review of goal directed therapy, including 5,291 patients from 31 studies across several surgical specialities, demonstrated a modest reduction in postoperative complications and hospital stay when compared to conventional fluid regimens (Grocott et al., 2013). Two studies have demonstrated a reduction in postoperative complications following the use of goal directed therapy in colorectal surgery (Wakeling et al., 2005, Noblett et al., 2006). One study demonstrated a significant reduction in postoperative serum interleukin-6 when goal directed therapy was compared to conventional fluid management in colorectal surgery (Noblett et al., 2006), however this has not been reproduced in other studies (Wakeling et al., 2005). No study has specifically examined the impact of goal directed therapy on CRP following colorectal surgery. Therefore, there is no evidence that goal directed fluid therapy can reduce the SIR as part of an ERAS protocol.

Prevention of Post-operative Ileus

Post-operative ileus is a common problem following colorectal surgery and often results in the patient feeling nauseous and bloated and can delay discharge from hospital (Fitzgerald and Ahmed, 2009). The exact cause of an ileus is unknown but it is thought to be multi-factorial (Luckey et al., 2003). The use of thoracic epidural analgesia instead of opioids has been shown to improve gut motility and reduce the length of postoperative ileus (Jorgensen et al., 2000, Miedema and Johnson, 2003, Marret et al., 2007). Avoidance of gut oedema

due to fluid overloading during can improve gut function postoperatively can be improved (Lobo et al., 2002) as can the use of laparoscopic surgery (Tjandra and Chan, 2006).

Other strategies that have been reported to reduce post-operative ileus include the use of chewing gum and the use of intravenous lignocaine intra-operatively. Some studies report that the use of chewing gum in the postoperative period reduced postoperative ileus and inpatient stay (Fitzgerald and Ahmed, 2009, Li et al., 2013) whilst others reported only a mild reduction in time to flatus with no difference in length of stay or complication rate (Su'a et al., 2015). In one study analysing the effect of intravenous lignocaine given intra-operatively, they reported a reduction in post-operative analgesic requirements as well as improved time to flatus and reduced post-operative nausea and vomiting (Sridhar et al., 2014). As previously stated, there is little reported evidence on the effects of different analgesic methods on the systemic inflammatory response following colorectal surgery. One study reported that those receiving intravenous lignocaine had lower post-operative levels of both CRP and IL-6 following major abdominal surgery (Sridhar et al., 2014). Therefore, there is some evidence that strategies to reduce post-operative ileus such as intravenous lidocaine and epidural anaesthesia but not chewing gum can reduce the SIR as part of an ERAS protocol.

Early post-operative enteral nutrition

A Cochrane Review of 14 trials including 1,224 patients undergoing colorectal surgery (Andersen et al., 2006) reported a non-significant trend toward fewer complications, in particular infections, in patients allowed enteral nutrition within 24 hours of surgery. However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that early post-operative enteral nutrition can reduce the SIR as part of an ERAS protocol.

Avoidance of nasogastric tubes

A Cochrane Review of nasogastric tube decompression, including 5,240 patients from 33 studies undergoing abdominal surgery, reported earlier return of bowel function and fewer pulmonary complications in those without routine nasogastric tube (Nelson et al., 2007). There was no significant increase in other complications. A recent meta-analysis, including 1,416 patients from 7 trials, in patients undergoing elective colorectal surgery reported similar results (Rao et al., 2011). However, there would appear to be no literature

examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that avoidance of nasogastric tubes can reduce the SIR as part of an ERAS protocol.

Avoidance of Peritoneal Drains

There is no evidence to support the routine use of peritoneal drains following colorectal surgery to either reduce the incidence or severity of anastomotic leaks nor to reduce the development of intra-abdominal collections (Jesus et al., 2004, Karliczek et al., 2006, Nygren et al., 2012, Puleo et al., 2013). Moreover, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that avoidance of peritoneal drains can reduce the SIR as part of an ERAS protocol.

Early removal of urinary catheter

A meta-analysis of patients undergoing abdominal surgery reported reduced rates of bacteriuria and discomfort when suprapubic catheters were compared to transurethral catheters (Mcphail et al., 2006). A systematic review of urinary catheter management following colorectal surgery suggests removal of urinary catheters on the first postoperative day in colonic resections and from postoperative day 3 to 6 in rectal surgeries (Hendren, 2013). This is associated with a lower incidence of urosepsis and a non-significant increase in urinary retention and re-catheterisation, however this is based on a single small RCT and several observational studies (Benoist et al., 1999, Basse et al., 2000, Kahokehr et al., 2010, Zmora et al., 2010). However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that early removal of urinary catheter can reduce the SIR as part of an ERAS protocol.

Surgical Incisions

A Cochrane Review, including 3,464 patients from 19 trials comparing transverse and vertical midline abdominal incisions across several surgical specialities, reported reduced rates of incisional hernia, analgesia requirement and improved pulmonary function when transverse incisions were employed (Brown and Goodfellow, 2005). Similar results were reported in a more recent meta-analysis of 24 trials which also included para-median incisions, however, neither study reported a significant reduction in pulmonary

complications or recovery time (Bickenbach et al., 2013). Two small randomised controlled trials included in the above review articles focused on colorectal surgery, describe conflicting results with regards to the impact of transverse or midline incisions on postoperative pulmonary function and pain (Lindgren et al., 2001, Brown et al., 2004). However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that different surgical incisions can reduce the SIR as part of an ERAS protocol.

Early mobilisation

Within an enhanced recovery programme following colorectal surgery, mobilisation on postoperative days 1 to 3 was associated with a reduced length of hospital stay (Maessen et al., 2007, Vlug et al., 2012). There is no specific evidence for early mobilisation following colorectal surgery out with the context of enhanced recovery programmes. However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that early mobilisation can reduce the SIR as part of an ERAS protocol.

Thromboprophylaxis

Patient with cancer have a 4-6 fold higher risk than the general population of developing venous thromboembolism (Imberti et al., 2008). Pharmacological methods (low-molecular weight heparin (LMWH) or unfractionated heparin) and mechanical methods (intermittent pneumatic compression; graduated compression stockings) are used either solely (Levine et al., 2001, Borly et al., 2005) or in combination with each other (Wille-Jorgensen, 1991). No studies have analysed thromboprophylaxis in an ERAS setting, although conclusions regarding the benefits can be drawn from non-ERAS patient groups. Furthermore, no studies reported whether the use of thromboprophylaxis had any impact on the systemic inflammatory response. Therefore, whilst the use of LMWH is effective in reducing VTE rates, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that thromboprophylaxis can reduce the SIR as part of an ERAS protocol.

Antibiotic Prophylaxis

A recent Cochrane Review (Nelson et al., 2014) of antimicrobial prophylaxis in colorectal surgery, including 43,451 patients from 260 trials, has demonstrated a significant reduction in postoperative wound infections when prophylactic antibiotics are compared to placebo

(RR 0.34, 95% CI 0.28-0.41). Furthermore, additional benefit in terms of wound infection reduction was reported when combination oral and intravenous antibiotics were used and when antibiotics with anaerobic cover were used (Nelson et al., 2014). Although it is understood that an exaggerated postoperative systemic inflammatory response, determined by measuring serum CRP, is associated with the development of infective complications (Platt et al., 2012, Singh et al., 2014), there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that antibiotic prophylaxis can reduce the SIR as part of an ERAS protocol.

Maintenance of intraoperative normothermia

The development of hypothermia in the perioperative period is multifactorial, and can be attributed to general anaesthesia, surgical technique and the theatre environment (Sessler, 2000). In the context of colorectal surgery, warming with forced air blankets and intravenous fluids to maintain normothermia has been shown to reduce wound infection rates (Kurz et al., 1996), blood transfusion requirements and complication rates (Wong et al., 2007). However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that maintenance of intra-operative normothermia can reduce the SIR as part of an ERAS protocol.

Pre-operative Counselling

Preoperative patient counselling has been reported to reduce a patient's anxiety (Kiyohara et al., 2004) and allow quicker recovery and discharge (Halaszynski et al., 2004, Lassen et al., 2009) following surgery. If patients are aware of what is likely to happen during their hospital stay and are given targets postoperatively then their recovery and hospital discharge are perceived to be quicker (Fearon et al., 2005). However, there would appear to be no literature examining the impact of this strategy on the systemic inflammatory response following colorectal surgery. Therefore, there is no evidence that pre-operative counselling can reduce the SIR as part of an ERAS protocol.

8.4 Discussion

The results of the present review shows that although there is evidence of the benefits of ERAS protocols in terms of reducing length of hospital stay and the reduction of post-operative complications following colorectal surgery, evidence of an effect on the SIR is surprisingly limited. Moreover, with the exception of laparoscopic surgery, evidence of an effect of individual components of ERAS protocols on the SIR is also limited. Without such information, establishment of an optimal ERAS protocol will be based on subjective evidence rather than evidence of a beneficial effect on the SIR.

In the present review only laparoscopic surgery was shown to have substantial evidence demonstrating its beneficial effect on the reduction of markers of the post-operative systemic inflammatory response. It is of interest therefore that it has recently been reported that of the recommended ERAS components, only laparoscopic surgery, early oral intake, and early mobilisation were identified as independent determinants of early recovery (Vlug et al., 2012). It could be considered from a clinical point of view that ERAS protocols were developed to reduce pain and hospital stay and to reduce the time to return to work. Implicit in this is the assumption that the SIR would also be reduced. From our review it is clear, with respect to laparoscopic surgery, there is evidence that these aims have been achieved. However, it would appear that there is no evidence that other components of ERAS protocols fulfil the above criteria. Future studies could utilise an objective marker of the post-operative systemic inflammatory response, such as CRP, which has been reported to be reflective of the magnitude of surgical trauma (Chapter 5), to assess which individual components of ERAS programmes modulated the systemic inflammatory response. These elements could then form future ERAS protocols which would have the proven ability to reduce the SIR. On the basis of the present review, these new protocols would likely be more streamlined and would perhaps allow easier implementation of the ERAS approach in clinical practice.

Initial studies into enhanced recovery were mainly performed in patients undergoing colorectal surgery but ERAS is now used in many different surgical specialties and procedures. Indeed there are now consensus guidelines from the ERAS society for patients undergoing upper gastrointestinal surgery (Mortensen et al., 2014), urological surgery (Cerantola et al., 2013), colorectal surgery (Lassen et al., 2009, Nygren et al., 2012, Gustafsson et al., 2012a), hepato-biliary and pancreatic surgery (Lassen et al., 2012). This guidance has resulted in the widespread acceptance of the ERAS principles and adoption

of these programmes in surgical units across the UK and Europe. However, no standard protocol exists for such programmes and as such the number of components used varies between units, making comparison of different ERAS studies problematic (Neville et al., 2014).

Furthermore, it has previously been presumed that the greater the number of elements in an ERAS programme the better the outcomes. However, often studies make no distinction between the number of elements intended to be included versus the number that were actually successfully implemented (Vlug and Bemelman, 2015). Indeed, it has recently been reported that increasing compliance with ERAS protocols improves outcomes in patients undergoing elective colorectal cancer resection (ECG, 2015). It has also been reported that studies with fewer ERAS components were associated with greater reduction in mortality and complications (Nicholson et al., 2014). This finding raises the possibility that some of the components of ERAS programmes offer little additional benefit to the overall outcomes.

The recently reported LAFA-study was designed to identify whether laparoscopic or open surgery, in combination with ERAS or standard care, was the optimal approach for colorectal surgery (Vlug et al., 2011). Interestingly, the authors reported that length of stay was shortest in the laparoscopic/ERAS group (5 days), followed by the laparoscopic/standard group (6 days) and then by both open groups (7 days). These findings would appear to imply that while ERAS and laparoscopic surgery work synergistically, perhaps the majority of the benefit seen in these programmes is due to the use of laparoscopic surgery (Vlug and Bemelman, 2015). Additional evidence for this hypothesis, in the context of the post-operative systemic inflammatory response, is provided by the finding that the two groups undergoing laparoscopic surgery had a lower postoperative CRP than either open surgery group regardless of perioperative care methods (Veenhof et al., 2012).

Despite laparoscopic surgery appearing to be the key component in ERAS protocols, the majority of patients undergoing resection for colorectal cancer are still likely to receive an open procedure. Recent data from the National Bowel Cancer Audit 2014 has reported that, of the 19 4533 patients who underwent resection of colorectal cancer, less than half (45%) had laparoscopic surgery (HQUIP, 2014). Therefore, rather than increased efforts to deliver ERAS protocols it may be more appropriate to increase the number of laparoscopic procedures carried out.

In the present study a potential confounding factor would be the duration of operation since this may vary with surgical approach. However, few of the included studies that examined the impact of laparoscopic vs. open colorectal surgery on the postoperative systemic inflammatory response reported operation duration. In a recent audit of our centre there was no association between operation duration and the postoperative systemic inflammatory response following either laparoscopic or open surgery for colorectal cancer.

In conclusion, the present systematic review shows that, with the exception of laparoscopic surgery, objective evidence of the effect of individual components of ERAS protocols in reducing the SIR following colorectal surgery is limited. This review, examining the literature pertaining to a known indicator of surgical trauma and predictor of post-operative complication severity, may be the first step in stimulating further research in this area.

Table 8.1 Components of Enhanced Recovery after Surgery – ERAS Group Recommendations

ERAS Component	Recommendation
Pre-operative counselling	Should receive oral and written information about admission, what to expect and their role in recovery
Pre-operative fasting and carbohydrate loading	Fasting - 2 hours for liquids and 6 hours for solids Patients should receive carbohydrate loading pre-operatively
Mechanical bowel preparation	Patients should not routinely receive mechanical bowel preparation
Thromboprophylaxis	Patients should wear compression stockings, have intermittent pneumatic compression and pharmacological prophylaxis with LMWH
Antibiotic prophylaxis	Single-dose antibiotic prophylaxis 30-60 minutes prior to surgery
Maintenance of intra-operative normothermia	Intra-operative maintenance of normothermia with an upper body forced air warmer should be used routinely
Goal directed fluid therapy	Balanced crystalloids preferred. Goal-directed fluid therapy should be considered on an individual basis
Surgical incisions	Midline or transverse laparotomy incision of minimal length should be used
Laparoscopic surgery	Laparoscopic surgery recommended if the appropriate expertise is available
Avoidance of nasogastric tubes	Should not be used routinely post-operatively
Post-operative analgesia	Thoracic epidural analgesia or spinal analgesia with local anaesthetic and opioids Paracetamol and NSAIDs used following epidural withdrawal
Prevention of post-operative ileus	Mid thoracic epidural analgesia Avoidance of fluid overload Laparoscopic surgery (if available)
Avoidance of peritoneal drains	Not indicated routinely for resections above peritoneal reflection Short term (<24 hrs) may be appropriate

	after low rectal resections
Early removal of urinary catheter	For colonic surgery, both suprapubic and urethral techniques appropriate. Suprapubic catheter should be used for pelvic surgery
Early post-operative enteral nutrition	Patients should be encouraged to commence oral diet as early as possible after surgery Oral nutrition supplements should be given until normal diet has been resumed
Early mobilisation	Patients should be nursed in environment that encourages mobilisation

LMWH, Low-molecular weight heparin; NSAID, non-steroidal anti-inflammatories

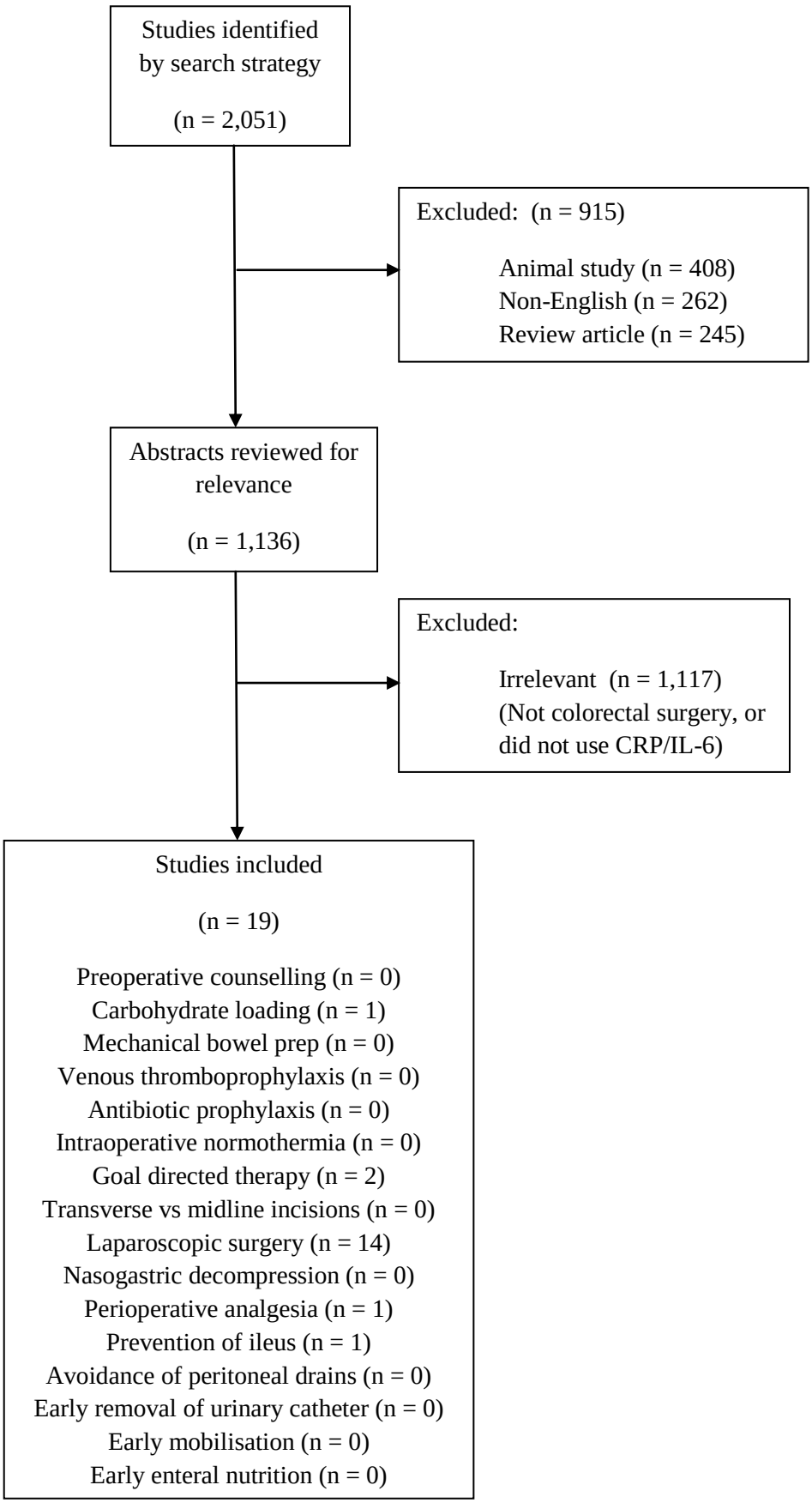


Figure 8.1 PRISMA flowchart demonstrating study selection

Table 8.2 Summary of included studies for each ERAS component and the marker of the systemic inflammatory response analysed

ERAS Component	Included studies	Author(s)	Year of publication	Journal	Country	Study Type	No. of patients	Marker of SIR analysed
Pre-operative counselling	0	-	-	-	-		-	-
Pre-operative fasting and carbohydrate loading	1	Mathur, S *et al.*	2010	*British Journal of Surgery*	New Zealand	RCT	142	CRP
Mechanical bowel preparation	0	-	-	-	-		-	-
Thromboprophylaxis	0	-	-	-	-		-	-
Antibiotic prophylaxis	0	-	-	-	-		-	-
Intra-operative normothermia	0	-	-	-	-		-	-
Goal directed fluid therapy	2	Noblett, SE *et al.*	2006	*British Journal of Surgery*	UK	RCT	108	IL-6
		Wakeling, HG *et al.*	2005	*British Journal of Anaesthesia*	UK	RCT	128	IL-6
Surgical incision	0	-	-	-	-		-	-
Laparoscopic surgery	13	Schwenk, W *et al.*	2000	*Langenbecks Archives of Surgery*	Germany	RCT	60	CRP & IL-6
		Delgado, S *et al.*	2001	*Diseases of the Colon & Rectum*	Spain	RCT	97	CRP & IL-6
		Braga, M *et al.*	2002	*Diseases of the Colon & Rectum*	Italy	RCT	79	CRP
		Dunker, MS *et al.*	2003	*Diseases of the*	The	RCT	34	CRP & IL-6

		Wu, FP *et al.*	2003	*Colon & Rectum Diseases of the Colon & Rectum*	Netherlands The Netherlands	RCT	26	IL-6
		Ng, SS *et al.*	2009	*Diseases of the Colon & Rectum*	Hong Kong	RCT	153	CRP
		Han, SA *et al.*	2010	*International Journal of Colorectal Disease*	Korea	CCT	74	CRP
		Veenhof, AA *et al.*	2011	*International Journal of Colorectal Disease*	The Netherlands	RCT	40	CRP & IL-6
		Gustafsson, UO *et al.*	2012	*World Journal of Surgery*	Sweden	Obs	114	CRP
		Tsamis, D *et al.*	2012	*Surgical Endoscopy*	Greece	MCC	30	CRP & IL-6
		Tsimogiannis, KE *et al.*	2012	*Surgical Endoscopy*	Greece	RCT	40	CRP
		Veenhof, AA *et al.*	2012	*Annals of Surgery*	The Netherlands	RCT	79	CRP
		Wang, G *et al.*	2012	*Journal of Gastrointestinal Surgery*	China	RCT	163	CRP & IL-6
		Ramanathan, ML *et al.*	2015	*Annals of Surgical Oncology*	UK	Obs	344	CRP
Avoidance of nasogastric tubes	0	-	-	-	-		-	-
Post-operative analgesia	1	Chen, WK *et al.*	2015	*International Journal of*	China	RCT	53	CRP

Prevention of post-operative ileus	1	Sridhar, P *et al.*	2014	*Colorectal Disease ANZ Journal of Surgery*	India	RCT	134	CRP & IL-6
Avoidance of peritoneal drains	0	-	-	-	-		-	-
Early removal of urinary catheter	0	-	-	-	-		-	-
Early post-operative enteral nutrition	0	-	-	-	-		-	-
Early mobilisation	0	-	-	-	-		-	-

SIR, systemic inflammatory response; RCT, randomized controlled trial; CCT, controlled clinical trial; MCC, matched case control study; Obs, observational study

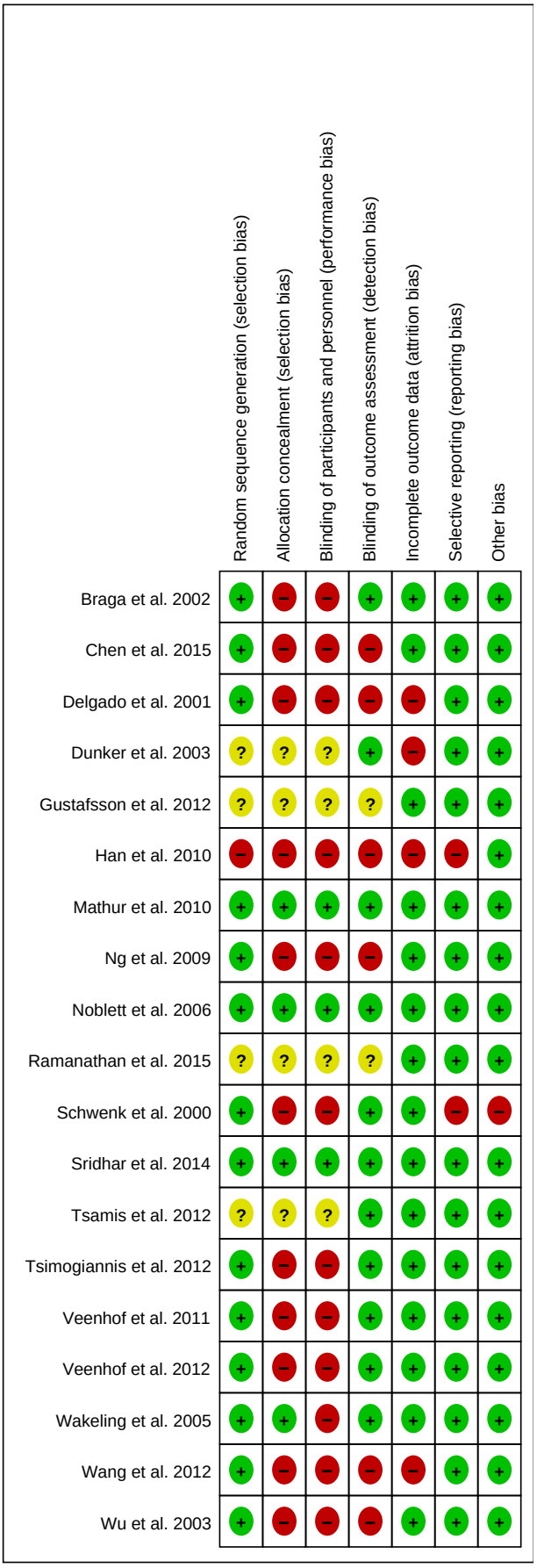


Figure 8.2 **Cochrane risk of bias summary**
Green = low risk of bias; red = high risk of bias; yellow = unclear risk of bias

9 Clinicopathological determinants of an elevated systemic inflammatory response following elective potentially curative resection for colorectal cancer

9.1 Introduction

Colorectal cancer is the second most common cause of cancer death in the United Kingdom, accounting for 16,000 deaths annually (CRUK, 2014a). It has been recognised that the pre-operative systemic inflammatory response is related to long term outcome in patients following potentially curative surgery for colorectal cancer (Moyes et al., 2009, Roxburgh and McMillan, 2010, Mohri et al., 2014).

Moreover, it has also been reported that the post-operative systemic inflammatory response can be a useful early predictor of the development of post-operative infective complications and anastomotic leak following colorectal cancer resection (Platt et al., 2012, Ramanathan et al., 2013, Singh et al., 2014), regardless of surgical approach (Ramanathan et al., 2015). Indeed, C-reactive protein (CRP) concentrations >150mg/L on post-operative days 3-5 have been consistently reported to be associated with post-operative complications (McDermott et al., 2015). With this in mind, a post-operative systemic inflammation score, based on the combination of CRP and albumin was developed and termed the post-operative Glasgow Prognostic Score (poGPS). This score has recently been reported to predict the development and severity of post-operative infective complications as well as long term survival (Chapter 6).

With the expansion in the use of enhanced recovery after surgery (ERAS) programmes to improve short term outcomes following surgery and promote timely discharge, ERAS programmes have been proposed to reduce the surgical stress response. However, in a recent systematic review, it was concluded that of the components of an ERAS programme, only the use of laparoscopic surgery was consistently associated with a lower post-operative systemic inflammatory response (Chapter 8) . Therefore, clinicopathological factors that influence the post-operative systemic inflammatory response, as evidenced by the poGPS, are of considerable interest as they may be modifiable and could therefore potentially be considered as future therapeutic targets. The aim of the present study was to examine the clinicopathological determinants of the post-operative systemic inflammatory

response, as evidenced by the poGPS on postoperative days 3 and 4, in patients following resection of colorectal cancer.

9.2 Methods

9.2.1 Patients

Patients with a histologically proven diagnosis of colorectal cancer who, based on preoperative investigations and operative findings, were considered to have undergone potentially curative resection at a single centre during a period from 1999 to 2013 were initially included in the study (n=834). All procedures were performed at Glasgow Royal Infirmary, a University teaching hospital where procedures were performed by consultant surgeons with a subspecialist interest in colorectal surgery or by trainees who were supervised by these consultants. 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. Patients with metastatic disease were excluded from analysis. Daily blood samples were obtained, as per hospital routine, during the perioperative period and were standard care for all patients. Prior to surgery, all patients received thromboprophylaxis and antibiotic prophylaxis as per hospital policy at the time. Lesions from the caecum to the sigmoid colon were classified as colon cancers, lesions of the recto-sigmoid junction and rectum were classified as rectal cancers.

The pre-operative systemic inflammatory response was assessed using the modified Glasgow Prognostic Score (mGPS), an extensively validated and independently prognostic systemic inflammation based score. Briefly, mGPS patients with a normal C-reactive protein (<10mg/l) were allocated a score of '0', those with an elevated C-reactive protein (>10mg/l) allocated a score '1' and those with an elevated C-reactive protein (>10mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of '2'.

The poGPS was calculated as previously described (Chapter 7) and is as follows: a postoperative CRP concentration below 150 mg/L, regardless of albumin concentration, scored 0, a CRP concentration > 150 mg/L and albumin > 25 g/L scored 1, and CRP > 150 mg/L and albumin < 25 g/L scored 2.

Emergency presentation was determined if the patient presented via an unplanned hospital admission and underwent surgery during the same admission.

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 normal weight (<25), overweight (≥25-30), and obese (>30). BMI was obtained from the patients' electronic pre-operative assessment record and ASA grade from the Opera Theatre Management system (OPERA v4.0, CHCA, Canada). The study was approved by the West of Scotland Research Ethics Committee, Glasgow.

9.2.2 Statistical Analysis

The relationship between clinicopathological variables and the day 3 and 4 poGPS were examined using the χ^2 test for categorical variables. Binary logistic regression was used to examine the relationship between clinicopathological factors and the presence of a post-operative systemic inflammatory response, indicated by a poGPS score of >1 on both days 3 and 4 and calculate an OR and 95% CI. Clinicopathological factors that on univariate analysis had a p value <0.10 were taken into a multivariate model using a backward conditional model to identify independently significant factors. A *p* value of <0.05 was considered significant. Statistical analysis was performed using SPSS version 22.0 for Windows (IBM Corporation, Armonk, NY, USA).

9.3 Results

Baseline characteristics of the 752 patients who underwent elective surgery for colorectal cancer are shown in *Table 7.1*. The majority of patients were 65 or older (64%), male (54%), were overweight or obese (58%), had colonic tumours (62%), were not systemically inflamed (68% mGPS 0; 52% NLR ≤3) and had node negative disease (63%). Most patients underwent open resection (85%). Overall, 23% of patients developed an infective complication.

The relationships between day 3 and day 4 poGPS and clinicopathological characteristics are shown in *Table 7.2*. The day 3 poGPS was significantly associated with male sex ($p<0.05$), later year of operation ($p<0.001$), ASA grade ($p<0.01$), BMI ($p<0.001$), mGPS ($p<0.001$), pre-op NLR (<0.001), rectal cancer ($p<0.01$), laparoscopic surgery ($p<0.01$) and T stage ($p<0.01$). The day 4 poGPS was significantly associated with later year of operation ($p<0.001$), BMI ($p<0.05$), mGPS ($p<0.01$), NLR ($p<0.005$) and laparoscopic surgery ($p<0.005$). Moreover, when year of operation was further divided into 3 groups (1999-2003/ 2004-2008/ 2009-2013) the poGPS of both day 3 and day 4 remained significantly associated with year of operation (both $p<0.001$). The day 3 poGPS was significantly associated with an increase in infective complications from 12% to 31% to 45% and the day 4 poGPS was significantly associated with an increase in infective complication rates from 16% to 39% to 58%.

Binary logistic regression of the factors significantly associated with low poGPS (poGPS 0) versus a high poGPS (poGPS 2) on day 3 and day 4 are shown in *Tables 7.3 and 7.4* respectively. A high day 3 poGPS was independently associated with year of operation (HR 4.63; CI 2.28-9.42; $p<0.001$), ASA (HR 2.02; CI 1.25-3.26; $p=0.004$), mGPS (HR 1.97; CI 1.29-3.01; $p=0.002$), tumour site (HR 2.62; CI 1.32-5.17; $p=0.006$) and laparoscopic surgery (HR 0.20; CI 0.05-0.73; $p = 0.015$). When year of operation was removed from the binary logistic regression analysis only ASA grade (HR 1.96; CI 1.25-3.09; $p=0.003$), BMI (HR 1.60; CI 1.07-2.38; $p=0.001$), mGPS (HR 2.03; CI 1.35-3.03; $p=0.001$) and tumour site (HR 2.99; CI 1.56-5.71; $p<0.001$) were independently associated with a high day 3 poGPS.

A high day 4 poGPS was independently associated with year of operation (HR 5.32; CI 2.49-11.34; $p<0.001$), ASA (HR 1.61; CI 1.02-2.55; $p=0.043$), mGPS (HR 1.98; CI 1.32-2.96; $p=0.001$), NLR (HR 0.47; CI 0.24-0.93; $p=0.031$) and tumour site (HR 2.97; CI 1.49-

5.91; p=0.002). When year of operation was removed from the binary logistic regression analysis only ASA grade (HR 1.65; CI 1.06-2.57; p=0.028), mGPS (HR 1.81; CI 1.22-2.68; p=0.003), NLR (HR 0.50; CI 0.26-0.95; p=0.034) and tumour site (HR 2.90; CI 1.49-5.65; p=0.002) were independently associated with a high day 4 poGPS.

9.4 Discussion

The results of the present study showed that, in elective surgery, year of operation, ASA grade, the pre-operative modified Glasgow Prognostic Score (mGPS) and tumour site were independently associated with the post-operative systemic inflammatory response, as evidenced by the poGPS on both post-operative day 3 and 4. Therefore, in a large cohort, the present results establish the main clinicopathological factors determining an elevated systemic inflammatory response following elective potentially curative resection for colorectal cancer.

In the present study it was of interest that the more recent time period (2007-2013) was significantly associated with a greater magnitude of the post-operative systemic inflammatory response, as evidenced by the poGPS. This was unexpected since it might have been anticipated that with more recent surgical and anaesthetic techniques fewer patients would exceed poGPS thresholds. On further analysis, patients in the more recent time period had higher BMI ($p<0.001$) and mGPS ($p<0.001$) values. Indeed, the median BMI in the cohort operated on in 1999-2006 was 25.4 and on those operated on in 2007-2013 was 27.5. The median BMI of the general population has been increasing over the last two decades and this would also appear to be represented in patients attending for colorectal surgery. Various lifestyle factors may account for this such as poor diet, increased consumption of processed foods and lack of exercise. This increase in average BMI may also explain why those in the more recent cohort are more likely to be systemically inflamed. Regardless of the causative factors, these findings would suggest that elective surgery for colorectal cancer is increasingly being carried out in obese and systemically inflamed patients. However, these factors were adjusted for in the present analysis and it may be that other factors, related or unrelated, account for the greater magnitude of the post-operative systemic inflammatory response in the more recent time period.

The present results may have implications for future clinical care, particularly with respect to minimising the magnitude of the post-operative systemic inflammatory response. For example, ASA and BMI are problematic therapeutic targets in that it would be difficult to treat these directly in the relatively short time between diagnosis and elective surgery. However, the post-operative systemic inflammatory response could be targeted. For example, patients who have a high BMI or ASA may selectively receive laparoscopic surgery and peri-operative care that minimises the post-operative systemic inflammatory

response. Included in such care would be the use of anti-inflammatory agents such as corticosteroids (McSorley et al., 2016a, McSorley et al., 2017). However, such targeted peri-operative care is in its infancy and the optimal timings, the agents and the doses to minimise the magnitude of the post-operative systemic inflammatory response that will result in improved post-operative outcomes remains to be determined (Roxburgh et al., 2013).

In the present study, an elevated BMI was associated with an increased poGPS. To our knowledge this relationship has not been previously reported. However, a direct relationship between BMI and C-reactive protein concentrations has been widely reported (Choi et al., 2013) and both are established risk factors for the development of colorectal cancer. The mechanism underlying the relationship between BMI and the magnitude of the post-operative systemic inflammatory response is not clear. However, it may be that surgical injury to an increased amount of subcutaneous fat leads to a more profound systemic inflammatory response. This relationship is worthy of further study.

Multiple NLR Thresholds have been used to define high and low NLR states. The majority of these have been shown to be of prognostic benefit (Guthrie et al. 2013a). In chapter 3 of this thesis, an NLR threshold of greater than 5 was used to define a high NLR value. This was associated with poorer survival in patients with node negative colorectal cancer. In the present chapter a threshold of greater than 3 was used to define a high NLR value, in keeping with recent work in the literature (Enriquez et al. 2017, Feliciano et al. 2017) and this was also significantly associated with an elevated poGPS. Regardless of the threshold value used, an elevated NLR appears to be prognostic in determining poor short and long term outcomes in patients with colorectal cancer.

The laparoscopic surgical approach, compared with the open approach, has been repeatedly shown to attenuate the systemic inflammatory response (Lane et al., 2013, Srinivasa et al., 2011, Wang et al., 2012). In the present study, laparoscopic surgery was independently associated with the post-operative SIR on day 3 but not day 4. The basis of this differential response to laparoscopic surgery is not clear. However, it may relate to the transient effect of this surgical approach or the interaction between laparoscopic surgery, ASA grade and BMI. For example, patients who undergo a laparoscopic resection for colon cancer may have less comorbidity and be less obese. Indeed, it would therefore appear that the benefit of laparoscopic surgery is more pronounced in the immediate post-operative period. The significance of this is not clear but perhaps highlights the important

role the systemic inflammatory response plays early in the post-operative period. Given the increasing use of the laparoscopic approach (approximately 45% of colorectal cancer resections, (HQUIP, 2014)) these relationships warrant further study.

The present study has some potential limitations that should be considered. Many pre-operative factors associated with a greater post-operative systemic inflammatory response such as obesity, diabetes and other comorbidities are inter-related and therefore potential confounding is an important issue in the analysis of the present study. Nevertheless, an objective measurement of the post-operative systemic inflammatory response, such as the poGPS will, in future studies, facilitate the dissection of factors that have independent influence.

In conclusion, the mGPS, ASA grade, BMI and tumour site were independently associated with a greater magnitude of the post-operative systemic inflammatory response in patients undergoing elective surgery for colorectal cancer.

Table 9.1 Clinicopathological characteristics of patients undergoing elective, potentially curative resection for colorectal cancer

Characteristic	No. of patients (%)
Age (<65/ 65-74/ ≥75)	245 (33)/ 272 (33)/ 235 (31)
Sex (female/ male)	344 (46)/ 408 (54)
Year of Operation (1999-2006/2007-2013)	311 (41)/ 408 (54)
ASA (1/ 2/ 3/ 4)[a]	58 (11)/ 214 (42)/ 219 (42)/ 24 (5)
BMI (<25/ >25-30/ >30)[b]	186 (34)/ 223 (41)/ 131 (24)
TNM Stage (0/ I/ II/ III)	12 (2)/ 141 (19)/ 321 (43)/ 278 (37)
T Stage (I/II/III/IV)	62 (8)/ 106 (14)/ 411 (55)/ 161 (21)
N Stage (0/I/II)	474 (63)/ 206 (27)/ 72 (10)
Tumour site (colon/ rectum)	467 (62)/ 285 (38)
Laparoscopic (No/ Yes)	640 (85)/ 112 (15)
Pre-op mGPS	498 (68)/ 134 (18)/ 100 (14)
Pre-op NLR (≤3/ >3)	391 (52)/ 358 (48)
Day 3 poGPS (0/1/2)	361 (53)/ 186 (27)/ 135 (20)
Day 4 poGPS (0/1/2)	439 (72)/ 87 (14)/ 86 (14)
Infective Complications (No/ Yes)	580 (77)/ 169 (23)

ASA, American Society of Anaesthesiology Grading System; BMI, Body Mass Index; mGPS, modified Glasgow Prognostic Score; TNM, Tumour Node Metastases; CRP, C-reactive protein

[a] n = 516

[b] n = 540

Table 9.2 **Relationship between clinicopathological characteristics and day 3 & 4 poGPS in patients undergoing elective, potentially curative surgery for colorectal cancer**

Characteristic		n	Day 3 poGPS (0/1/2)	*p-value*	Day 4 poGPS (0/1/2)	*p-value*
Age				0.247		0.662
	<65	245	117 (53)/ 62 (28)/ 41 (19)		135 (70)/ 28 (14)/ 31 (16)	
	65-74	272	120 (49)/ 76 (31)/ 47 (19)		155 (70)/ 35 (16)/ 30 (14)	
	≥75	235	124 (57)/ 48 (22)/ 47 (21)		149 (75)/ 24 (12)/ 25 (13)	
Sex				0.012		0.109
	Female	344	181 (57)/ 69 (22)/ 66 (21)		200 (75)/ 29 (11)/ 38 (14)	
	Male	408	180 (49)/ 117 (32)/ 69 (19)		239 (69)/ 58 (17)/ 48 (14)	
Year of Operation				<0.001		<0.001
	1999-2006	311	160 (58)/ 87 (32)/ 28 (10)		196 (82)/ 28 (12)/ 14 (6)	
	2007-2013	441	201 (49)/ 99 (24)/ 107 (26)		243 (65)/ 59 (16)/ 72 (19)	
Year of Operation				<0.001		<0.001
	1999-2003	172	89 (61)/ 49 (33)/ 9 (6)		102 (84)/ 15 (12)/ 5 (4)	
	2004-2008	228	113 (52)/ 60 (28)/ 43 (20)		148 (76)/ 22 (11)/ 24 (12)	
	2009-2013	352	159 (50)/ 77 (24)/ 83 (26)		189 (64)/ 50 (17)/ 57 (19)	
ASA				0.008		0.446
	1	58	37 (72)/ 8 (16)/ 6 (12)		30 (79)/ 5 (13)/ 3 (8)	
	2	214	109 (56)/ 64 (33)/ 22 (11)		132 (75)/ 28 (16)/ 15 (9)	
	3	219	107 (51)/ 57 (27)/ 44 (21)		137 (71)/ 30 (16)/ 25 (13)	
	4	24	7 (33)/ 7 (33)/ 7 (33)		15 (68)/ 2 (9)/ 5 (23)	
BMI				<0.001		0.003
	<25	186	103 (60)/ 33 (19)/ 35 (21)		97 (70)/ 15 (11)/ 26 (19)	
	>25-30	223	107 (53)/ 70 (35)/ 23 (12)		138 (73)/ 35 (19)/ 15 (8)	
	>30	131	45 (40)/ 39 (34)/ 29 (26)		62 (59)/ 25 (24)/ 18 (17)	
mGPS				<0.001		0.007

	0	498	260 (56)/ 128 (28)/ 75 (16)		302 (74)/ 54 (13)/ 50 (12)	
	1	134	50 (41)/ 45 (37)/ 26 (22)		71 (68)/ 21 (20)/ 13 (12)	
	2	100	43 (48)/ 13 (14)/ 34 (38)		55 (61)/ 12 (13)/ 23 (26)	
NLR				<0.001		0.002
	≤3	391	177 (49)/ 122 (33)/ 66 (18)		216 (66)/ 60 (18)/ 51 (16)	
	>3	358	184 (59)/ 62 (20)/ 68 (21)		222 (78)/ 27 (10)/ 34 (12)	
Tumour site				0.009		0.076
	Colon	467	225 (54)/ 126 (30)/ 69 (16)		264 (72)/ 58 (16)/ 43 (12)	
	Rectum	285	136 (52)/ 60 (23)/ 66 (25)		175 (71)/ 29 (12)/ 43 (17)	
Laparoscopic				0.006		0.002
	No	640	299 (51)/ 158 (27)/ 127 (22)		390 (73)/ 66 (12)/ 79 (15)	
	Yes	112	62 (63)/ 28 (29)/ 8 (8)		49 (64)/ 21 (27)/ 7 (9)	
T Stage				0.007		0.578
	I	62	23 (42)/ 26 (47)/ 6 (11)		31 (63)/ 11 (22)/ 7 (14)	
	II	106	61 (62)/ 23 (24)/ 14 (14)		62 (71)/ 16 (16)/ 11 (13)	
	III	411	186 (50)/ 102 (27)/ 85 (23)		241 (71)/ 48 (14)/ 49 (15)	
	IV	161	84 (58)/ 34 (23)/ 27 (19)		95 (75)/ 14 (11)/ 18 (14)	
N Stage				0.346		0.065
	0	474	229 (53)/ 109 (25)/ 94 (22)		275 (72)/ 49 (13)/ 59 (15)	
	I	206	100 (54)/ 58 (31)/ 29 (15)		129 (75)/ 27 (16)/ 15 (9)	
	II	72	32 (51)/ 19 (30)/ 12 (19)		35 (60)/ 11 (19)/ 12 (21)	
Infective Complications				<0.001		<0.001
	No	580	316 (61)/ 129 (25)/ 74 (14)		364 (80)/ 53 (12)/ 36 (8)	
	Yes	169	42 (26)/ 57 (36)/ 61 (38)		72 (46)/ 34 (22)/ 50 (32)	

Table 9.3 **Binary logistic regression of clinicopathological factors associated with low poGPS (poGPS 0) versus high poGPS (poGPS 2) on post-operative day 3**

Characteristic	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	***p-value***	**HR (95% CI)**	***p-value***
Age (<65/ 65-74/ ≥75)	1.04 (0.81-1.32)	0.760	-	-
Sex (Female/ Male)	1.05 (0.71-1.56)	0.804	-	-
Year of Operation (99-06/ 07-13)	3.04 (1.91-4.84)	<0.001	4.63 (2.28-9.42)	<0.001
ASA (1/ 2/ 3/ 4)	1.87 (1.31-2.69)	0.001	2.02 (1.25-3.26)	0.004
BMI (<25/ 25-30/>30)	1.36 (1.02-1.80)	0.033	1.44 (0.94-2.20)	0.090
mGPS (0/ 1/ 2)	1.67 (1.30-2.15)	<0.001	1.97 (1.29-3.01)	0.002
NLR (≤3/ >3)	0.99 (0.67-1.47)	0.965	-	-
Tumour site (Colon/ Rectum)	1.58 (1.06-2.36)	0.024	2.62 (1.32-5.17)	0.006
Laparoscopic (No/ Yes)	0.30 (0.14-0.65)	0.002	0.20 (0.05-0.73)	0.015
T Stage (I/ II/ III/ IV)	1.09 (0.86-1.36)	0.482	-	-
N Stage (0/ I/ II)	0.86 (0.63-1.18)	0.348	-	-

Table 9.4 **Binary logistic regression of clinicopathological factors associated with low poGPS (poGPS 0) versus high poGPS (poGPS 2) on post-operative day 4**

Characteristic	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	***p-value***	**HR (95% CI)**	***p-value***
Age (<65/ 65-74/ ≥75)	0.85 (0.64-1.14)	0.285	-	-
Sex (Female/ Male)	1.06 (0.66-1.68)	0.815	-	-
Year of Operation (99-06/ 07-13)	4.15 (2.27-7.58)	<0.001	5.32 (2.49-11.34)	<0.001
ASA (1/ 2/ 3/ 4)	1.55 (1.01-2.38)	0.045	1.61 (1.02-2.55)	0.043
BMI (<25/ 25-30/>30)	1.03 (0.75-1.43)	0.838	-	-
mGPS (0/ 1/ 2)	1.54 (1.16-2.04)	0.003	1.98 (1.32-2.96)	0.001
NLR (≤3/ >3)	0.65 (0.40-1.04)	0.073	0.47 (0.24-0.93)	0.031
Tumour site (Colon/ Rectum)	1.51 (0.95-2.40)	0.082	2.97 (1.49-5.91)	0.002
Laparoscopic (No/ Yes)	0.71 (0.31-1.61)	0.408	-	-
T Stage (I/ II/ III/ IV)	1.02 (0.79-1.32)	0.865	-	-
N Stage (0/ I/ II)	1.00 (0.70-1.43)	0.998	-	-

10 Pre-operative aspirin and statin prescription and the post-operative systemic inflammatory response following surgery for colorectal cancer

10.1 Introduction

It has been well established that following surgical intervention, the development of a systemic inflammatory response (SIR) is considered part of the normal homeostatic mechanisms and is an essential component of repair and healing. Furthermore, the magnitude of this systemic inflammatory response can be measured using stereotypical markers of the systemic inflammatory response such as C-reactive protein (CRP) or interleukin-6 (Chapter 5) .

Despite the use of different skin preparations, the use of prophylactic antibiotics and the increased use of laparoscopic surgery and enhanced recovery after surgery (ERAS) protocols, infective complications following surgery remains a significant problem. Subsequently, attention has focused on early identification of patients who are likely to develop or at risk of developing post-operative complications. Recent work has reported that a CRP of greater than 150 mg/L on post-operative days 3-5 was associated with the development of complications (McDermott et al., 2015). Furthermore, an exaggerated SIR, evidenced by the post-operative Glasgow Prognostic Score (poGPS), has been reported to be associated not only with the development of infective complications and their severity, but also with poorer long term outcomes (McSorley et al., 2016b) (Chapter 7).

Drugs such as aspirin and statins are commonly prescribed for their anti-platelet function to patients with ischaemic heart disease and to treat patients with hypercholesterolaemia. Corticosteroids such as dexamethasone are also commonly given around the peri-operative period for their anti-emetic properties and have become a staple component of modern peri-operative practices. However, these drugs also have anti-inflammatory properties. Recently, it has been reported that the use of corticosteroids in the peri-operative period causes a reduction in the post-operative systemic inflammatory response and a reduction in complications following surgery for gastrointestinal cancer (McSorley et al., 2016a). It could therefore be hypothesised that pre-operative use of aspirin and statins may be

capable of modulating the post-operative SIR, with the possible benefit of reducing post-operative complication rates and improving outcomes following surgery for colorectal cancer but there is little evidence currently to support this.

Therefore, the aim of the present study was to determine whether pre-operative use of aspirin and statins were associated with a reduced post-operative systemic inflammatory response in patients undergoing surgery for colorectal cancer.

10.2 Methods

10.2.1 Patients

Patients with histologically proven colorectal cancer who, on the basis of intra-operative findings and pre-operative computed tomography, were considered to have undergone potentially curative resection at a single centre between January 2010 and June 2014 were included in the analyses (n = 446). Patient characteristics were collected in a prospectively maintained database and all patient data was anonymised. All tumours were staged according to conventional tumour, node, metastasis classification and additional pathological data obtained from the pathology reports issued at the time of the resection.

Pre-operatively, all patients received thromboembolism prophylaxis and antibiotic prophylaxis as per local protocols. Blood samples were taken as part of routine care of patients both pre-operatively and post-operatively. The pre-operative systemic inflammatory response was assessed using the modified Glasgow Prognostic Score (mGPS) and the post-operative systemic inflammatory response assessed using the post-operative Glasgow Prognostic Score (poGPS) (Chapter 6).

Electronic patient case notes were reviewed for pre-operative use of aspirin and statin use. Primary care referral letters were the primary source of prescribing data, and pre-operative anaesthetic assessment documents and medical clerk-in documents completed on admission to hospital were also used if referral letters did not include the appropriate information. For the purposes of the present study, patients who were prescribed aspirin or statins at the time of surgery were considered as aspirin or statin users. Patient co-morbidity using ASA Physical Status grade, smoking status and body mass index (BMI) were all obtained from pre-operative anaesthetic assessments. The West of Scotland research ethics committee approved this study.

10.2.2 Statistical Analysis

The comparison of categorical variables was performed using Chi square test and of continuous variables using the Mann Whitney U test. A two sided *p value* of <0.05 was considered statistically significant. Binary logistic regression was used to examine the relationship between aspirin and statin use, clinicopathological characteristics and the presence of a systemic inflammatory response, as characterised by poGPS$\geq$1, by calculating ORs and 95% CIs. Variables with *P*- value $\leq$0.1 on univariate analysis were

entered into a multivariate model using a backwards conditional method. Statistical analysis was performed using SPSS version 22.0 for Windows (IBM, SPSS, IL, USA).

10.3 Results

The clinicopathological characteristics of the 446 included patients are shown in *Table 10.1*. The majority of patients were over the age of 65 years old (64%), male (57%), had elective surgery (91%), had colon cancer (59%) and had node negative disease (63%). The majority of patients had a BMI >25 (74%) and had open surgery (63%). The majority of patients were not systemically inflamed pre-operatively (mGPS = 0, 75%). 120 patients were prescribed aspirin and 187 patients prescribed a statin with 100 patients prescribed both aspirin and a statin.

The relationship between aspirin and statin use and clinicopathological characteristics is shown in *Table 10.1*. Aspirin prescription was significantly associated with increasing age ($p<0.001$), male sex ($p<0.05$), less pre-operative systemic inflammation ($p<0.05$) and increasing ASA grade ($p<0.05$). Statin prescription was significantly associated with increasing age ($p<0.001$) and increasing ASA grade ($p<0.001$).

The relationship between aspirin and statin prescription, the post-operative systemic inflammatory response and post-operative complications is shown in *Table 10.2*. On post-operative day 3, 50% had a poGPS 0, 23% a poGPS 1 and 27% a poGPS 2. Neither aspirin nor statin prescription was significantly associated with the day 3 poGPS. On post-operative day 4, 61% had a poGPS 0, 17% a poGPS 1 and 22% a poGPS 2. Neither aspirin nor statin prescription was significantly associated with the day 4 poGPS. Aspirin prescription was associated with the development of more complications of any type as was statin prescription ($p\leq0.05$). Statin prescription was also associated with the development of more non-infective complications ($p<0.05$).

The relationship between aspirin and statin prescription, the post-operative systemic inflammatory response and post-operative complications in patients who were systemically inflamed preoperatively (mGPS≥1) is shown in *Table 10.3*. On day 3, 36% had a poGPS 0, 17% a poGPS 1 and 47% poGPS 2. Neither aspirin prescription nor statin prescription was significantly associated with the day 3 poGPS . On day 4, 48% had a poGPS 0, 18% a poGPS 1 and 33% a poGPS 2. Neither aspirin prescription nor statin prescription was significantly associated with the day 4 poGPS. In this cohort of systemically inflamed patients, neither aspirin nor statin prescription was associated with the development of complications.

The relationship between aspirin and statin prescription and an elevated day 3 poGPS is shown in *Table 10.4*. On binary logistic regression, emergency presentation and open surgery, were significantly associated with an elevated poGPS. On multivariate analysis, open surgery, emergency presentation and male sex were independently associated with an elevated poGPS. Neither aspirin nor statin prescription was associated with an elevated day 3 poGPS.

The relationship between aspirin and statin prescription and an elevated day 4 poGPS is shown in *Table 10.5*. On binary logistic regression, none of the factors examined were associated with an elevated day 4 poGPS.

10.4 Discussion

The results of the present study showed that the prescription of aspirin, but not statin, prior to surgery was associated with a lower pre-operative systemic inflammatory response in patients undergoing surgery for colorectal cancer. However, the prescription of aspirin and statin was not associated with moderation of the post-operative systemic inflammatory response even when adjusted for the presence of systemic inflammation prior to surgery. Therefore, it would appear that at therapeutic doses given in patients with colorectal cancer, aspirin and statin are unlikely to be useful in the moderation of the post-operative systemic inflammatory response.

Although this is the first study, to our knowledge, to examine the effect of aspirin and statins on post-operative systemic inflammation and outcomes, there has been a long recognised relationship between the administration of aspirin and improved survival in patients with colorectal cancer (Frouws et al., 2017b). The basis of the latter relationship is not clear. However, inflammatory mechanisms have been implicated in the beneficial effect. From the results of the present study it is clear that aspirin has a modest or little effect on the systemic inflammatory response. This would therefore implicate a direct effect of aspirin on the tumour or its microenvironment as a plausible mechanism. For example, aspirin not only down regulates the cyclo-oxygenase enzyme in the tumour and stromal cells but also in platelets, preventing production of thromboxane A2 and hence preventing normal activation of the platelet (Sanchez et al., 2012). Indeed, consistent with this concept, recent work suggests that aspirin, given prior to or following diagnosis, improves survival in a number of gastrointestinal malignancies (Frouws et al., 2017a). Nevertheless, compared with other anti-inflammatory agents such as dexamethasone (McSorley et al., 2017) the effect of aspirin and statins on post-operative systemic inflammation and outcomes is much less.

It was of interest in the present study that patients on both aspirin and statin had an even greater reduction in the post-operative systemic inflammatory response. It has been previously reported that aspirin and statins appear to act synergistically, resulting in reduced concentrations of CRP (Fisher et al., 2008) and reduced rates of severe sepsis (O'Neal et al., 2011). To date there have been no studies examining the effect of aspitrin and statins on post-operative systemic inflammation or on outcomes following elective surgery. It would be of interest to see whether the reduction in systemic inflammation in

the present study in patients prescribed aspirin and statins resulted in improved short term outcomes.

Aspirin and statin use was significantly associated with the development of any type of post-operative complication. Statins were significantly associated with development of non-infective complications. This may represent the fact that patients who developed complications were also those patients with existing medical co-morbidities that put them at increased risk of developing complications. In particular with non-infective complications, many of which tend to be cardiovascular in nature, the fact that patients on statins were more likely to develop these types of complications highlighted the importance that existing medical co-morbidities plays on the nature of the post-operative course and may not be directly related to usage of the drug at all.

One potential limitation of the present study was the small sample size. Whilst the study included 446 patients, only 100 patients were on both aspirin and statins with 120 patients on aspirin alone. It may be that the results of this chapter are due to the relatively small sample size and that a larger group of patients on aspirin and statins may yield different results. It would be of interest to conduct similar work in the future on the back of a larger group of patients to see whether the results remained the same or whether aspirin and statins did indeed moderate the post-operative systemic inflammatory response.

In summary, the prescription of aspirin and statin was not associated with moderation of the post-operative systemic inflammatory response even when adjusted for the presence of systemic inflammation prior to surgery.

Table 10.1 Clinicopathological characteristics of patients undergoing surgery for colorectal cancer

Clinical characteristics		All (*n*=446) (%)	Aspirin use			Statin use		
			No aspirin (*n*=326) (%)	Aspirin (*n*=120) (%)	*P-value*	No statin (*n*=259) (%)	Statin (*n*=187) (%)	*P-value*
Age					<0.001			<0.001
	<65	159 (36)	136 (42)	23 (19)		114 (44)	45 (24)	
	65-74	170 (38)	110 (34)	60 (50)		92 (35)	78 (42)	
	>74	117 (26)	80 (24)	37 (31)		53 (21)	64 (34)	
Sex					0.014			0.117
	Female	191 (43)	151 (46)	40 (33)		119 (46)	72 (38)	
	Male	255 (57)	175 (54)	80 (67)		140 (54)	115 (62)	
Presentation					0.409			0.600
	Elective	402 (91)	292 (90)	110 (92)		232 (90)	170 (91)	
	Emergency	42 (9)	33 (10)	9 (8)		26 (10)	16 (9)	
mGPS					0.037			0.782
	0	323 (75)	223 (71)	99 (83)		183 (73)	139 (76)	
	1	42 (10)	33 (11)	9 (8)		25 (10)	17 (9)	
	2	67 (15)	56 (18)	11 (9)		41 (17)	26 (14)	
ASA grade					0.042			<0.001
	I	21 (16)	19 (20)	2 (5)		20 (25)	1 (2)	
	II	53 (39)	41 (42)	12 (32)		34 (42)	19 (34)	
	III	54 (40)	33 (34)	21 (55)		22 (28)	32 (58)	
	IV	7 (5)	4 (4)	3 (7)		4 (5)	3 (6)	
BMI					0.803			0.083
	<25	87 (26)	65 (27)	22 (24)		58 (31)	29 (20)	

	25-30	141 (43)	100 (42)	41 (46)		78 (41)	63 (44)	
	>30	102 (31)	75 (31)	27 (30)		52 (28)	50 (35)	
Tumour Site					0.431			0.194
	Colon	268 (64)	197 (65)	71 (61)		149 (61)	119 (67)	
	Rectum	153 (36)	107 (35)	46 (39)		95 (39)	58 (33)	
Tumour Location					0.366			0.391
	Right	162 (36)	115 (35)	47 (39)		88 (34)	74 (40)	
	Left	84 (19)	64 (20)	20 (17)		48 (19)	36 (19)	
	Rectum	182 (41)	131 (40)	51 (42)		114 (44)	68 (36)	
	Other	18 (4)	16 (5)	2 (2)		9 (3)	9 (5)	
TNM Stage					0.655			0.219
	I	101 (25)	71 (23)	30 (26)		54 (22)	47 (26)	
	II	170 (39)	124 (39)	46 (39)		99 (39)	71 (39)	
	III	158 (36)	117 (37)	41 (35)		93 (37)	65 (35)	
Laparoscopic					0.256			0.805
	No	282 (63)	201 (62)	81 (67)		165 (64)	117 (63)	
	Yes	164 (37)	125 (38)	39 (33)		94 (36)	70 (37)	
Corticosteroid Use					0.618			0.771
	No	146 (43)	105 (42)	41 (46)		81 (43)	65 (44)	
	Yes	191 (57)	142 (58)	49 (54)		109 (57)	82 (56)	

Table 10.2 **The relationship between aspirin and statin use, the post-operative systemic inflammatory response and post-operative complications in patients undergoing potentially curative resection of stage I-III colorectal cancer**

	All	Aspirin use			Statin use		
Clinical characteristics	**(*n*=446) (%)**	**No aspirin (*n*=326) (%)**	**Aspirin (*n*=120) (%)**	***P*-value**	**No statin (*n*=259) (%)**	**Statin (*n*=187) (%)**	***P*-value**
Day 3 poGPS				0.623			0.338
0	206 (50)	145 (49)	61 (53)		111 (47)	95 (54)	
1	95 (23)	72 (24)	23 (20)		59 (25)	36 (20)	
2	112 (27)	81 (27)	31 (27)		67 (28)	45 (26)	
Day 4 poGPS				0.303			0.213
0	220 (61)	151 (58)	69 (67)		117 (57)	103 (66)	
1	61 (17)	47 (18)	14 (14)		36 (18)	25 (16)	
2	81 (22)	61 (24)	20 (19)		52 (25)	29 (18)	
Any Complication				0.040			0.050
No	243 (55)	187 (58)	56 (47)		151 (59)	92 (50)	
Yes	195 (45)	133 (42)	62 (53)		103 (41)	92 (50)	
Infective Complications				0.159			0.613
No	308 (70)	231 (72)	77 (65)		181 (71)	127 (69)	
Yes	130 (30)	89 (28)	41 (35)		73 (29)	57 (31)	
Non-infective Complications				0.608			0.034
No	345 (79)	254 (79)	91 (77)		209 (82)	136 (74)	
Yes	93 (21)	66 (21)	27 (23)		45 (18)	48 (26)	

Table 10.3 **The relationship between aspirin and statin use, the post-operative systemic inflammatory response and post-operative complications in patients undergoing curative resection of stage I-III colorectal cancer with an mGPS ≥1**

	All	Aspirin use			Statin use		
Clinical characteristics	**(*n*= 109) (%)**	**No aspirin (*n*=89) (%)**	**Aspirin (*n*=20) (%)**	***P*-value**	**No statin (*n*=66) (%)**	**Statin (*n*=43) (%)**	***P*-value**
Day 3 poGPS				0.142			0.670
0	36 (36)	30 (37)	6 (30)		19 (32)	17 (40)	
1	17 (17)	16 (20)	1 (5)		10 (17)	7 (17)	
2	48 (47)	35 (43)	13 (65)		30 (51)	18 (43)	
Day 4 poGPS				0.163			0.833
0	45 (48)	36 (49)	9 (47)		26 (46)	19 (51)	
1	17 (18)	16 (22)	1 (5)		10 (18)	7 (19)	
2	31 (33)	22 (30)	9 (47)		20 (36)	11 (30)	
Any Complication				0.964			0.906
No	54 (49)	44 (50)	10 (50)		33 (50)	21 (49)	
Yes	55 (51)	45 (50)	10 (50)		33 (50)	22 (51)	
Infective Complications				0.594			0.997
No	71 (65)	59 (66)	12 (60)		43 (65)	28 (65)	
Yes	38 (35)	30 (34)	8 (40)		23 (35)	15 (35)	
Non-infective Complications				0.108			0.733
No	83 (76)	65 (73)	18 (90)		51 (77)	32 (74)	
Yes	26 (24)	24 (27)	2 (10)		15 (23)	11 (26)	

Table 10.4 The relationship between aspirin and statin use, clinicopathological characteristics and the day 3 poGPS in patients undergoing potentially curative resection of stage I-III colorectal cancer

	poGPS=0	poGPS≥1	Univariate OR (95% CI)	*P-value*	Multivariate OR (95% CI)	*P-value*
Age (<65/ 65-74/ >74)	76/86/44	68/73/66	1.27 (0.99-1.63)	0.058	1.26 (0.97-1.64)	0.083
Sex (Female/ Male)	96/110	78/129	1.44 (0.98-2.14)	0.067	1.63 (1.07-2.47)	0.022
ASA grade (I/ II/ III/ IV)	11/26/21/3	9/24/32/4	1.34 (0.87-2.07)	0.188	-	-
BMI (<25/ 25-30/ >30)	40/76/47	40/54/52	1.07 (0.80-1.44)	0.650	-	-
Presentation (Elective/ Emergency)	196/8	177/30	4.15 (1.86-9.30)	0.001	3.36 (1.46-7.72)	0.004
Laparoscopic (No/ Yes)	110/96	159/48	0.35 (0.23-0.53)	<0.001	0.39 (0.25-0.61)	<0.001
Tumour site (Colon/ Rectum)	122/77	127/65	0.81 (0.54-1.23)	0.320	-	-
TNM Stage (I/ II/ III)	37/49/49	34/56/54	1.18 (0.89-1.57)	0.257	-	-
Aspirin (No/ Yes)	145/61	153/54	0.84 (0.55-1.29)	0.425	-	-
Statin (No/ Yes)	111/95	126/81	0.75 (0.51-1.11)	0.152	-	-
Aspirin & Statin (No/ Yes)	102/104	116/91	0.77 (0.52-1.13)	0.185	-	-

Table 10.5 **The relationship between aspirin and statin use, clinicopathological characteristics and the day 4 poGPS in patients undergoing potentially curative resection of stage I-III colorectal cancer**

	poGPS=0	poGPS≥1	Univariate OR (95% CI)	*P-value*	Multivariate OR (95% CI)	*P-value*
Age (<65/ 65-74/ >74)	70/89/61	52/48/42	0.95 (0.73-1.25)	0.727	-	-
Sex (Female/ Male)	97/123	52/90	1.37 (0.89-2.10)	0.159	-	-
ASA grade (I/ II/ III/ IV)	10/30/30/4	6/15/22/3	1.17 (0.74-1.88)	0.502	-	-
BMI (<25/ 25-30/ >30)	32/84/46	27/34/38	1.05 (0.74-1.47)	0.792	-	-
Presentation (Elective/ Emergency)	204/15	122/20	2.23 (1.10-4.52)	0.026	-	-
Laparoscopic (No/ Yes)	147/73	106/36	0.68 (0.43-1.10)	0.114	-	-
Tumour site (Colon/ Rectum)	125/89	84/46	0.77 (0.49-1.21)	0.254	-	-
TNM Stage (I/ II/ III)	38/53/53	23/41/39	1.18 (0.86-1.61)	0.303	-	-
Aspirin (No/ Yes)	151/69	108/34	0.69 (0.43-1.11)	0.128	-	-
Statin (No/ Yes)	117/103	88/54	0.70 (0.45-1.07)	0.100	-	-
Aspirin & Statin (No/ Yes)	107/113	80/62	0.73 (0.48-1.12)	0.153	-	-

11 Conclusions

Although perhaps underappreciated, the systemic inflammatory response has now been established as a key determinant of cancer progression and outcome (Shinko et al., 2017). Patients with systemic inflammation have a 50% reduced survival compared to those who don't and make up approximately 30-50% of patients with advanced cancer (Shinko et al., 2017). Its presence, in a range of common tumour types, prior to treatment whether that be surgical or oncological, has been shown to adversely affect outcomes, resulting in increased rates of recurrence and poorer long term survival. This thesis sought to gain a greater understanding of the role that the systemic inflammatory response plays in the development and progression of cancer, specifically colorectal cancer. Work performed over the last two decades has demonstrated the importance that pre-operative systemic inflammation plays in outcomes for patients with a range of cancers as well as colorectal cancer (Guthrie et al., 2013a, McMillan, 2013b). The biology of not only the tumour itself, but of the host as well and the complex interaction between the two is thought to drive cancer progression (Hanahan and Weinberg, 2011). Whilst the systemic inflammatory response has been associated with poorer long term survival, the presence of a local inflammatory response has been reported to have beneficial effects on survival (Roxburgh and McMillan, 2012, Richards et al., 2012a). These inflammatory changes are generated by the innate, adaptive and humoral immune systems. Whilst the majority of previous work focussed on the changes relating to the adaptive immune response, more recently it has become apparent that components of the innate immune system (neutrophils, monocytes/macrophages and myeloid derived suppressor cells) and the humoral response (complement, collectins and pentraxins) have complex interactions that can result in an exaggerated systemic inflammatory response and are detrimental to the host.

Chapter 1 describes an overview of colorectal cancer and its current management. Chapter 2 details the results of a survey of clinicians who have recently published articles related to systemic inflammation and cancer. The survey aimed to establish the different opinions of worldwide experts on the use of systemic inflammation based scoring systems including the 2 most reported ones – the mGPS and the NLR- what their main use was and whether they should be incorporated into more into current clinical practice. Despite relatively low response rate (25%) we found that the majority of respondents used the GPS/mGPS as their score of preference and mainly for research and prognostication purposes. The fact that few respondents used these scores in clinical practice was disappointing but perhaps not surprising. It is probably unlikely that surgical treatment would be determined by these

scores, especially in potentially curable disease. However, the emergence of these scores into some clinical trials, in particular oncological trials in patients with advanced disease perhaps signals a step in the right direction (e.g. Hurwitz et al., 2015). Further evidence of their clinical utility in the context of randomised controlled trials will strengthen the argument for incorporation into routine clinical practice.

Chapter 3 aimed to determine whether any of the components of the pre-operative white cell count were associated with survival following surgery for colorectal cancer. It was thought important to ascertain whether both components of the NLR were equally weighted in terms of their effect on survival. The neutrophil count was the one component that was associated with cancer-specific survival. Therefore, the prognostic effect seen with the NLR was thought to be mainly driven by the neutrophil count and that further prognostic scores utilising the white cell count components should incorporate the neutrophil count but not necessarily the lymphocyte count. Indeed, many scores now incorporate the neutrophil count such as the optimised Glasgow Prognostic Score (oGPS) (Chapter 3) and the systemic immune-inflammation index (Geng et al., 2016).

In an attempt to utilise the neutrophil count in an optimal innate scoring system, and with recent *in-vitro* work suggesting that both neutrophils and platelets played a key role early in the development of the inflammatory process, a new scoring system based on both the pre-operative neutrophil and platelet counts was created using existing thresholds. The aim of chapter 4 was to determine whether this score, termed the Neutrophil-Platelet score (NPS) was capable of predicting long term outcomes in patients with cancer. In patients with colorectal cancer, the NPS was associated with cancer-specific survival, particularly in those with node negative disease. Moreover, in those patients with a range of common cancers, an incremental increase in the NPS was significantly associated with poorer cancer-specific survival. These findings further impress the importance of activation of the innate immune system in determining long term outcomes in patients with cancer. However, validation of these neutrophil-based prognostic scores is required in the context of randomised controlled trials in order to confirm their clinical utility.

Neutrophils are the most common leucocyte found in in blood. They are primarily responsible for protecting the host against inflammation and infection and are often referred to as the ‘first line of defence’ (Uribe-Querol and Rosales, 2015). Once they reach the site of injury or inflammation they destroy micro-organisms by either phagocytosis, secretion of anti-microbial substances or formation of neutrophil extracellular traps

(NETs). In patients with cancer, neutrophils are often found in abundance in the bloodstream. They can display both anti-tumour behaviour or can stimulate cancer progression (Coffelt et al., 2016). Which behaviour these neutrophils follow depends on a variety of signals received from the tumour itself or from stromal cells within the tumour microenvironment. It is possible that tumour production of granulocyte-macrophage colony stimulating growth factor (GM-CSF) and other cytokines such as IL-1 and IL-6 may contribute to increased circulating neutrophil numbers (Uribe-Querol and Rosales, 2015). Elevated circulating levels of neutrophils are associated with poorer outcomes in patients with lung cancer (Atzpodien and Reitz, 2008), melanoma (Schmidt et al., 2005), renal cancer (Atzpodien and Reitz, 2008) and in this thesis was shown to be associated with poorer survival in patients with colorectal cancer (Chapter 3).

Neutrophils have therefore emerged as potential therapeutic target in patients with cancer. Preventing activation of the neutrophil has been suggested as a possible intervention that may improve outcomes. Tumours can secrete chemokines such as IL-8 which attract neutrophils. Blockade of this pathway using an IL-8 antagonist has been shown to reduce tumour growth and metastasis in lung cancer (Huang et al., 2002). Similarly, blockade of chemokine receptors such as CXCR-1 and CXCR-2 would not only block IL-8 but also other chemokines responsible for neutrophil recruitment. Trials using a CXCR-2 antagonist in patients with chronic obstructive pulmonary disease have reported that treatment resulted in reduced neutrophil counts, inflammatory markers and symptoms (Rennard et al., 2015). Currently clinical trials analysing the effect of and CXCR-1and CXCR-2 antagonist are ongoing in breast cancer (USNLM, 2015) and pancreatic cancer (USNLM, 2015). The results of these trials are eagerly anticipated and may provide the basis for new therapeutic regimens for a variety of cancers.

Surgery itself is responsible for generating a significant systemic inflammatory response, including upregulation of the innate immune response and subsequent downregulation of cell mediated immunity (Marik and Flemmer, 2012). This places a great strain on the hosts' anti-tumour defences and can be further compounded by factors that exaggerate the systemic inflammatory response such as emergency surgery and the development of infective complications (Roxburgh et al., 2013). Surgery for colorectal cancer in the presence of both of these factors is associated with very poor outcomes, irrespective of tumour stage (Roxburgh and McMillan, 2010). Therefore, modification of the hosts' immune response, by way of downregulating or moderating the innate and humoral response may be beneficial in preventing cancer progression and dissemination (Roxburgh

and McMillan, 2016). How this is achieved is still unclear, however this thesis sought to clarify whether any particular components of the innate immune system are more important, how best to monitor the innate immune response and whether modification of this response using routine anti-inflammatory medications is of benefit.

It has long been established that following surgical insult, a stereotypical cascade of mainly acute phase and metabolic responses occurs. This is often referred to as the surgical stress response but is perhaps better known as the systemic inflammatory response. Whilst it has anecdotally been reported that certain markers of the SIR can reflect a degree of surgical injury, however there has never been any formal review to analyse this concept in more detail. Chapter 5 aimed to identify from 4 common markers of the systemic inflammatory response (cortisol, IL-6, CRP and white cell count) whether any of these were indicative of the degree of surgical trauma and if so which ones were the most representative of this trauma. A systematic review of the literature was performed and for each analyte, surgical procedures grouped into minor, moderate and severe. From 164 studies, analysing 14,362 patients IL-6 and CRP appear to be associated with the magnitude of surgical injury; cortisol and white cell count were not. CRP is more readily available in clinical practice and perhaps the best marker to use in clinical practice. Furthermore, CRP would appear the most useful marker for observing the magnitude of the post-operative SIR. This highly cited observation has many potential uses. In particular, it could be used to analyse the effect of individual ERAS components and anaesthesia techniques on the systemic inflammatory response in order to create the optimal ERAS programme that minimises the systemic inflammatory response.

Given that the WCC itself did not appear to reflect the degree of surgical trauma, Chapter 6 sought to clarify whether different components of the white cell count responded differently to surgical trauma and whether any of these individual components were helpful in predicting the development of post-operative complications. In this chapter, the neutrophil and monocyte counts were significantly raised following surgery compared to pre-operative values whilst eosinophils and basophils fell. When comparing laparoscopic and open procedures, laparoscopic surgery was associated with lower neutrophil counts but higher lymphocyte and monocyte counts. This would appear to indicate that neutrophils and lymphocytes reflect the magnitude of surgical trauma, with this being an inverse response in the case of lymphocytes. Regardless of method of surgery, neutrophils were associated with the development of infective complications. Therefore, the neutrophil count, which composes the majority of the white cell count itself, can be used to monitor

both surgical trauma and the development of infective complications. However, when compared to markers of the systemic inflammatory response that are already widely used such as CRP, it is inferior. Nonetheless, considering that neutrophils are a key component of the innate immune system, it provides further evidence of it importance in determining short term outcomes following surgery for colorectal cancer.

As well as having elevated concentrations of neutrophils, patients with cancer often have an increased number of immature myeloid cells at earlier stages of differentiation (Uribe-Querol and Rosales, 2015), collectively referred to as myeloid-derived suppressor cells (MDSC). These cells increase in number in pathological conditions such as cancer, infection and inflammation and can supress both the innate and adaptive immune responses and may contribute to cancer progression (Coffelt et al., 2016, Motallebnezhad et al., 2016). Multiple studies have found that MDSCs are involved both directly and indirectly in tumour progression by stimulating angiogenesis (Murdoch et al., 2008) and metastatic potential of cancer cells (Alizadeh et al., 2014). Therefore, by directly targeting these cells in order to destroy them or limit their function, it may be possible to improve treatment efficacy in patients with cancer (Motallebnezhad et al., 2016).

This can be achieved in a number of ways such as inhibition of MDSC recruitment (currently being tested using various antibodies, tyrosine kinase inhibitors and antagonists of CXCR2 and CXCR4); by induction of MDSC differentiation (using vitamin D3 and All trans-retinoic acid); by inhibiting MDSC suppressive activity (using COX-2 inhibitors or phosphodiesterase-5 inhibitors) and by inducing apoptosis in MDSCs (using different chemotherapeutic agents such as gemcitabine, doxorubicin and 5-fluoroucil) (Wesolowski et al., 2013, Pan et al., 2015, Motallebnezhad et al., 2016). All of these approaches are under investigation in animal models and some in early clinical trials. Whilst some have shown promise in animal models, MDSCs methods of action are multiple and complex and may require multi-targeted therapies to overcome their detrimental effect (Pan et al., 2015). However, these trials using animal models have limited clinical utility due to the varied functions seen in human MDSCs. As such large, further prospective clinical trials are required to establish the effectiveness of MDSCs as therapeutic targets and subsequently refine the therapeutic options in an attempt to improve cancer outcomes (Wesolowski et al., 2013, Pan et al., 2015).

It has been well established that the presence of pre-operative systemic inflammation is associated with increased rates of post-operative complications and poorer long term

survival. Therefore, it is perhaps surprising that investigation of the post-operative systemic inflammatory response and its association with both short term and long term outcomes following surgery for colorectal cancer would appear not to have been examined. In chapter 7, a prognostic scoring system utilising 2 key components of the systemic inflammatory response (CRP and albumin) was constructed in a similar way to the mGPS in order to determine whether it was capable of predicting outcomes. In a retrospectively collected test cohort and a prospectively collected validation cohort, raised CRP and reduced albumin concentrations were associated with infective complications. Furthermore, this new prognostic scoring system, the poGPS, on post-operative days 3 and 4 was associated with incremental increases in infective complication rates and complication severity. It was also associated with overall survival. This highlights the fact that the presence of systemic inflammation, either pre- or post-operatively, was associated with poor outcomes in patients undergoing surgery for colorectal cancer. Given that many interventions that occur in the peri-operative period may affect the degree of post-operative inflammation these findings raise the possibility of the poGPS being used as a therapeutic target for medications or peri-operative protocols designed to dampen this inflammatory response and potentially improve outcomes following colorectal cancer surgery. Further work is required with regards to this.

Enhanced Recovery after Surgery (ERAS) programmes have revolutionised peri-operative care and are promoted as reducing the surgical stress response or systemic inflammatory response. However the objective evidence of this reduced SIR was very limited and it was also not clear which specific components of these ERAS programmes were most valuable in terms of reducing the SIR. Therefore, Chapter 8 sought to establish which of the ERAS components reduced the systemic inflammatory response using objective markers of this response such as CRP or IL-6. A systematic review incorporating 19 studies and 1,898 patients established that only laparoscopic surgery objectively reduced the systemic inflammatory response. Further research into this area is warranted and may identify other components that also reduce the systemic inflammatory response, thus creating a more streamlined ERAS programme that provides maximal benefit to patients in improving outcomes following surgery.

Given the importance of the post-operative systemic inflammatory response, Chapter 9 aimed to determine which of the common clinicopathological factors are associated with the magnitude of this post-operative response. This has potential future benefit as some of these factors may be modifiable and therefore could act as possible therapeutic targets. Of

the clinicopathological factors assessed, the day 3 poGPS was significantly associated with males, ASA grade, BMI, pre-operative systemic inflammation, laparoscopic surgery, rectal tumours and T stage whilst the day 4 poGPS was significantly associated with BMI, pre-operative systemic inflammation and laparoscopic surgery. In binary logistic regression, after adjustment for year of surgery, only mGPS, ASA and tumour site were independently associated with a greater magnitude of the post-operative systemic inflammatory response. These results further strengthen the argument for the majority of colorectal cancer surgery to be performed laparoscopically and highlight the importance of pre-operative inflammation in outcomes following cancer surgery. It remains to be seen whether targeting those patients who are inflamed prior to surgery with anti-inflammatory medication will improve outcomes.

For example, whether pre-operative usage of aspirin and statin have any effect on the systemic inflammatory response following surgery for colorectal cancer is not clear. In Chapter 10, aspirin and statin prescription was collated retrospectively on patients whom had undergone surgery for colorectal cancer. Aspirin prescription was associated with increasing age, male sex and comorbidity. Statin prescription was also associated with male sex and comorbidity. Patients on either aspirin or a statin had reduced post-operative CRP concentrations compared to those on neither. Patients on both an aspirin and a statin had even lower post-operative CRP concentrations, suggesting a synergistic effect of these drugs. Aspirin use but not statin use was inversely associated with systemic inflammation pre-operatively whilst neither aspirin nor statin use was associated with the post-operative systemic inflammatory response, as evidenced by the poGPS on either day 3 or 4. Aspirin and statin use was also associated with an increase in complications, specifically non-infective complications. Overall it was concluded that aspirin and statin usage is not associated with the post-operative systemic inflammatory response and had no effect on infective complications following surgery but may have an effect on the rate of infective complications. Randomised controlled trials in larger cohorts of patients may be required to establish whether these drugs are of benefit in patients undergoing surgery for colorectal cancer.

The use of pre-operative aspirin and statins results in reduced post-operative CRP concentrations but it remains unclear whether it has a beneficial effect on outcomes following surgery (Chapter 8). Corticosteroids given at induction of anaesthesia are reportedly associated with reduced nausea and vomiting (Karanicolas et al., 2008) with dexamethasone now a key component in many ERAS programmes. As well as its anti-

emetic properties, recent systematic reviews suggest that administration of corticosteroids peri-operatively results in reduced length of stay, reduced post-operative complications and a reduced post-operative inflammatory response (Srinivasa et al., 2011, McSorley et al., 2016a). A recent retrospective study reported in 556 patients undergoing surgery for colorectal cancer, the effect of pre-operative dexamethasone on the post-operative systemic inflammatory response and development of complications. The authors report that dexamethasone use was associated with significantly reduced post-operative systemic inflammatory response and reduced complications (McSorley et al., 2017). These findings raise the unique possibility of a simple pharmacological strategy capable of reducing post-operative complications, presumably via its effect of dampening the post-operative inflammatory response. Importantly, despite long standing concerns regarding corticosteroids and their effect on wound healing and anastomotic leak, the authors report no increase in either adverse event (McSorley et al., 2017). However, this observation would be best tested in a prospective randomised trial.

11.1 Future Research

This thesis highlights the importance of systemic inflammation based prognostic scores, in particular those that reflect the innate immune system in patients with colorectal cancer. However their use in routine clinical practice remains small, prospective studies utilising these scores that demonstrate their prognostic significance may enhance their usage. The use of medications in particular corticosteroids has been reported retrospectively to reduce the post-operative systemic inflammatory response without increasing complications. A prospective RCT analysing their effect on both short term and long term effects would be a major step forward in perioperative care as if it demonstrated improved outcomes by reducing the post-operative SIR then it would signify a new standard of care in patients under-going surgery for colorectal cancer.

In summary, both the pre-operative systemic inflammatory response (e.g. the mGPS) and the post-operative systemic inflammatory response (e.g. the poGPS) are associated with poorer short term and long term outcomes in patients with colorectal cancer. As a result the systemic inflammatory response has become a therapeutic reference point to which pre- and post-operative anti-inflammatory treatments may be compared. In particular, from the present work, the magnitude of the post-operative systemic inflammatory response may be used to examine the value of different components of ERAS protocols. Such an approach will help to rationalise post-operative care in patients undergoing surgery for colorectal cancer.

References

Aalto, K., Osterman, K., Peltola, H. & Rasanen, J. 1984. Changes in Erythrocyte Sedimentation-Rate and C-Reactive Protein after Total Hip-Arthroplasty. *Clinical Orthopaedics and Related Research***,** 118-120.

Aapro, M., Arends, J., Bozzetti, F., Fearon, K., Grunberg, S. M., Herrstedt, J., Hopkinson, J., Jacquelin-Ravel, N., Jatoi, A., Kaasa, S. & Strasser, F. 2014. Early recognition of malnutrition and cachexia in the cancer patient: a position paper of a European School of Oncology Task Force. *Ann Oncol*.

Abbas, S. M. & Hill, A. G. 2008. Systematic review of the literature for the use of oesophageal Doppler monitor for fluid replacement in major abdominal surgery. *Anaesthesia,* 63**,** 44-51.

Abramovitch, R., Marikovsky, M., Meir, G. & Neeman, M. 1999. Stimulation of tumour growth by wound-derived growth factors. *Br J Cancer,* 79**,** 1392-8.

ACPGBI. 2007. *Guidelines for the Management of Colorectal Cancer* [Online]. Available: http://www.acpgbi.org.uk/content/uploads/2007-CC-Management-Guidelines.pdf.

Adachi, Y., Shiraishi, N., Shiromizu, A., Bandoh, T., Aramaki, M. & Kitano, S. 2000. Laparoscopy-assisted Billroth I gastrectomy compared with conventional open gastrectomy. *Archives of Surgery,* 135**,** 806-810.

Adler, G., Eichman, W., Szczepanski, M., Targonska, I. & Jasinska, A. 1998. Postoperative plasma interleukin-6 in patients with renal cancer correlates with C-reactive protein but not with total fibrinogen or with high molecular weight fibrinogen fraction. *Thrombosis Research,* 89**,** 243-8.

Ahmed, J., Khan, S., Lim, M., Chandrasekaran, T. V. & MacFie, J. 2012. Enhanced recovery after surgery protocols - compliance and variations in practice during routine colorectal surgery. *Colorectal Dis,* 14**,** 1045-51.

AJCC 1992. AJCC Cancer Staging Manual, 5th Edition.

AJCC 2010. *Colon and Rectum Cancer Staging. AJCC Cancer Staging Manual, 7th Edition,* New York, Springer.

Aka, N., Kose, G., Gonenc, I. & Api, M. 2004. Tissue trauma after vaginal hysterectomy and colporrhaphy versus abdominal hysterectomy: a randomised controlled study. *Aust N Z J Obstet Gynaecol,* 44**,** 328-31.

Akhtar, K., Walton, L., Kamalky-Asl, I. D., Pearson, R. C., Lamb, W. R., Parrott, N. R. & Laing, I. 1998. Metabolic and inflammatory responses after laparoscopic and open inguinal hernia repair. *Annals of the Royal College of Surgeons of England,* 80**,** 125-130.

Al-Jabi, Y. & El-Shawarby, A. 2010. Value of C-reactive protein after neurosurgery: a prospective study. *British Journal of Neurosurgery,* 24**,** 653-659.

Al Oweidi, A. S., Klasen, J., Al-Mustafa, M. M., Abu-Halaweh, S. A., Al-Zaben, K. R., Massad, I. M. & Qudaisat, I. Y. 2010. The impact of long-lasting preemptive epidural analgesia before total hip replacement on the hormonal stress response. A prospective, randomized, double-blind study. *Middle East J Anesthesiol,* 20**,** 679-84.

Alizadeh, D., Katsanis, E. & Larmonier, N. 2014. Chemotherapeutic targeting of myeloid-derived suppressor cells. *Oncoimmunology,* 3**,** e27359.

Andersen, H. K., Lewis, S. J. & Thomas, S. 2006. Early enteral nutrition within 24h of colorectal surgery versus later commencement of feeding for postoperative complications. *Cochrane Database Syst Rev***,** CD004080.

Ardi, V. C., Kupriyanova, T. A., Deryugina, E. I. & Quigley, J. P. 2007. Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis. *Proc Natl Acad Sci U S A,* 104**,** 20262-7.

Arends, M. J. 2013. Pathways of colorectal carcinogenesis. *Appl Immunohistochem Mol Morphol,* 21**,** 97-102.

Armaghany, T., Wilson, J. D., Chu, Q. & Mills, G. 2012. Genetic alterations in colorectal cancer. *Gastrointest Cancer Res,* 5**,** 19-27.

Arora, G., Mannalithara, A., Singh, G., Gerson, L. B. & Triadafilopoulos, G. 2009. Risk of perforation from a colonoscopy in adults: a large population-based study. *Gastrointest Endosc,* 69**,** 654-64.

Artinyan, A., Orcutt, S. T., Anaya, D. A., Richardson, P., Chen, G. J. & Berger, D. H. 2015. Infectious postoperative complications decrease long-term survival in patients undergoing curative surgery for colorectal cancer: a study of 12,075 patients. *Ann Surg,* 261**,** 497-505.

Astin, M., Griffin, T., Neal, R. D., Rose, P. & Hamilton, W. 2011. The diagnostic value of symptoms for colorectal cancer in primary care: a systematic review. *Br J Gen Pract,* 61**,** e231-43.

Atzpodien, J. & Reitz, M. 2008. Peripheral blood neutrophils as independent immunologic predictor of response and long-term survival upon immunotherapy in metastatic renal-cell carcinoma. *Cancer Biother Radiopharm,* 23**,** 129-34.

Aune, D., Chan, D. S., Lau, R., Vieira, R., Greenwood, D. C., Kampman, E. & Norat, T. 2011a. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ,* 343**,** d6617.

Aune, D., Lau, R., Chan, D. S., Vieira, R., Greenwood, D. C., Kampman, E. & Norat, T. 2011b. Nonlinear reduction in risk for colorectal cancer by fruit and vegetable intake based on meta-analysis of prospective studies. *Gastroenterology,* 141**,** 106-18.

Avdagic, S. S., Krdzalic, G., Avdagic, H., Uljic, V. & Piric, M. 2010. Effects of postoperative analgesia on acute phase response in thoracic surgery. *Med Arh,* 64**,** 113-5.

Badia, J. M., Ayton, C. L., Evans, T. J., Carpenter, A. J., Nawfal, G., Kinderman, H., Zografos, G., Uemoto, S., Cohen, J. & Habib, N. A. 1998. Systemic cytokine response to hepatic resections under total vascular exclusion. *European Journal of Surgery,* 164**,** 185-190.

Baigrie, R. J., Lamont, P. M., Kwiatkowski, D., Dallman, M. J. & Morris, P. J. 1992. Systemic Cytokine Response after Major Surgery. *British Journal of Surgery,* 79**,** 757-760.

Balkwill, F. & Mantovani, A. 2001. Inflammation and cancer: back to Virchow? *Lancet,* 357**,** 539-45.

Balkwill, F. R., Capasso, M. & Hagemann, T. 2012. The tumor microenvironment at a glance. *J Cell Sci,* 125**,** 5591-6.

Baniyash, M., Sade-Feldman, M. & Kanterman, J. 2014. Chronic inflammation and cancer: suppressing the suppressors. *Cancer Immunol Immunother,* 63**,** 11-20.

Basse, L., Hjort Jakobsen, D., Billesbolle, P., Werner, M. & Kehlet, H. 2000. A clinical pathway to accelerate recovery after colonic resection. *Ann Surg,* 232**,** 51-7.

Bauer, J. & Herrmann, F. 1991. Interleukin-6 in Clinical Medicine. *Annals of Hematology,* 62**,** 203-210.

Baum, M., Demicheli, R., Hrushesky, W. & Retsky, M. 2005. Does surgery unfavourably perturb the "natural history" of early breast cancer by accelerating the appearance of distant metastases? *Eur J Cancer,* 41**,** 508-15.

Bedalov, G., Bartolin, Z., Puskar, D., Savic, I., Bedalov, I., Rudolf, M. & Batinic, D. 2008. Differences in stress response between patients undergoing transurethral resection versus endoscopic laser ablation of the prostate for benign prostatic hyperplasia. *Lasers Med Sci,* 23**,** 65-70.

Belchetz, L. A., Berk, T., Bapat, B. V., Cohen, Z. & Gallinger, S. 1996. Changing causes of mortality in patients with familial adenomatous polyposis. *Dis Colon Rectum,* 39**,** 384-7.

Benedetti, A. L., Collet, J. P., Boivin, J. F. & Hanley, J. A. 2003. Effect of nonsteroidal anti-inflammatory drugs on stage of colon cancer at diagnosis. *J Clin Epidemiol,* 56**,** 782-7.

Bengzon, J., Grubb, A., Bune, A., Hellstrom, K., Lindstrom, V. & Brandt, L. 2003. C-reactive protein levels following standard neurosurgical procedures. *Acta Neurochirurgica,* 145**,** 667-671.

Benoist, S., Panis, Y., Denet, C., Mauvais, F., Mariani, P. & Valleur, P. 1999. Optimal duration of urinary drainage after rectal resection: a randomized controlled trial. *Surgery,* 125**,** 135-41.

Bhangu, A., Singh, P., Fitzgerald, J. E., Slesser, A. & Tekkis, P. 2014. Postoperative nonsteroidal anti-inflammatory drugs and risk of anastomotic leak: meta-analysis of clinical and experimental studies. *World J Surg,* 38**,** 2247-57.

Bickenbach, K. A., Karanicolas, P. J., Ammori, J. B., Jayaraman, S., Winter, J. M., Fields, R. C., Govindarajan, A., Nir, I., Rocha, F. G. & Brennan, M. F. 2013. Up and down or side to side? A systematic review and meta-analysis examining the impact of incision on outcomes after abdominal surgery. *Am J Surg,* 206**,** 400-9.

Bilgen, O. F., Atici, T., Durak, K., Karaeminogullari, O. & Bilgen, M. S. 2001. C-reactive protein values and erythrocyte sedimentation rates after total hip and total knee arthroplasty. *Journal of International Medical Research,* 29**,** 7-12.

Birchall, M., Bailey, D., King, P., South West Cancer Intelligence Service, H. & Neck Tumour, P. 2004. Effect of process standards on survival of patients with head and neck cancer in the south and west of England. *Br J Cancer,* 91**,** 1477-81.

Bjornsson, G. L., Thorsteinsson, L., Gudmundsson, K. O., Jonsson, H., Gudmundsson, S. & Gudbjornsson, B. 2007. Inflammatory cytokines in relation to adrenal response following total hip replacement. *Scandinavian Journal of Immunology,* 65**,** 99-105.

Blomqvist, L., Malm, M., Berg, A., Svelander, L. & Kleinau, S. 1998. The inflammatory reaction in elective flap surgery. *Plastic and Reconstructive Surgery,* 101**,** 1524-1528.

Blyth, S., Blakeborough, A., Peterson, M., Cameron, I. C. & Majeed, A. W. 2008. Sensitivity of magnetic resonance imaging in the detection of colorectal liver metastases. *Ann R Coll Surg Engl,* 90**,** 25-8.

Bolke, E., Jehle, P. M., Storck, M., Braun, C., Schams, S., Steinbach, G., Orth, K., Gorich, J., Scharrer-Pamler, R. & Sunder-Plassmann, L. 2001. Endovascular stent-graft placement versus conventional open surgery in infrarenal aortic aneurysm: a prospective study on acute phase response and clinical outcome. *Clin Chim Acta,* 314**,** 203-7.

Boralessa, H., Debeer, F. C., Manchie, A., Whitwam, J. G. & Pepys, M. B. 1986. C-Reactive Protein in Patients Undergoing Cardiac-Surgery. *Anaesthesia,* 41**,** 11-15.

Borgdorff, P. J., Ionescu, T. I., Houweling, P. L. & Knape, J. T. 2004. Large-dose intrathecal sufentanil prevents the hormonal stress response during major abdominal surgery: a comparison with intravenous sufentanil in a prospective randomized trial. *Anesthesia and Analgesia,* 99**,** 1114-20, table of contents.

Borly, L., Wille-Jorgensen, P. & Rasmussen, M. S. 2005. Systematic review of thromboprophylaxis in colorectal surgery -- an update. *Colorectal Dis,* 7**,** 122-7.

Botta, C., Barbieri, V., Ciliberto, D., Rossi, A., Rocco, D., Addeo, R., Staropoli, N., Pastina, P., Marvaso, G., Martellucci, I., Guglielmo, A., Pirtoli, L., Sperlongano, P., Gridelli, C., Caraglia, M., Tassone, P., Tagliaferri, P. & Correale, P. 2013. Systemic inflammatory status at baseline predicts bevacizumab benefit in advanced non-small cell lung cancer patients. *Cancer Biol Ther,* 14**,** 469-75.

Botta, C., Mazzanti, R., Cusi, M. G., Vincenzi, B., Mantovani, G., Licchetta, A., Tonini, G., Tassone, P., Tagliaferri, P. & Correale, P. 2011. 1418 POSTER Baseline Inflammatory Status Defined by Neutrophil to Lymphocyte Cell Count Ratio (NLR) Predicts Progression Free Survival (PFS) in Metastatic Colorectal Cancer Patients (mCRC) Undergoing Bevacizumab Based Biochemotherapy. *European Journal of Cancer,* 47, Supplement 1**,** S174-S175.

Bowles, C. J., Leicester, R., Romaya, C., Swarbrick, E., Williams, C. B. & Epstein, O. 2004. A prospective study of colonoscopy practice in the UK today: are we adequately prepared for national colorectal cancer screening tomorrow? *Gut,* 53**,** 277-83.

Boyle, J. R., Goodall, S., Thompson, J. P., Bell, P. R. & Thompson, M. M. 2000. Endovascular AAA repair attenuates the inflammatory and renal responses associated with conventional surgery. *J Endovasc Ther,* 7**,** 359-71.

Braga, M., Vignali, A., Zuliani, W., Radaelli, G., Gianotti, L., Martani, C., Toussoun, G. & Di Carlo, V. 2002. Metabolic and functional results after laparoscopic colorectal surgery: a randomized, controlled trial. *Diseases of the Colon & Rectum,* 45**,** 1070-7.

Bretagnol, F., Panis, Y., Rullier, E., Rouanet, P., Berdah, S., Dousset, B., Portier, G., Benoist, S., Chipponi, J., Vicaut, E. & Gr, F. R. G. R. C. S. 2010. Rectal Cancer Surgery With or Without Bowel Preparation The French Greccar III Multicenter Single-Blinded Randomized Trial. *Ann Surg,* 252**,** 863-867.

Brown, G. & Daniels, I. R. 2005. Preoperative staging of rectal cancer: the MERCURY research project. *Recent Results Cancer Res,* 165**,** 58-74.

Brown, S. R. & Goodfellow, P. B. 2005. Transverse verses midline incisions for abdominal surgery. *Cochrane Database Syst Rev***,** CD005199.

Brown, S. R., Goodfellow, P. J., Adam, I. J. & Shorthouse, A. J. 2004. A randomised controlled trial of transverse skin crease vs. vertical midline incision for right hemicolectomy. *Tech Coloproctol,* 8**,** 15-8.

Brown, S. R., Mathew, R., Keding, A., Marshall, H. C., Brown, J. M. & Jayne, D. G. 2014. The impact of postoperative complications on long-term quality of life after curative colorectal cancer surgery. *Ann Surg,* 259**,** 916-23.

Bruce, D. M., Smith, M., Walker, C. B., Heys, S. D., Binnie, N. R., Gough, D. B., Broom, J. & Eremin, O. 1999. Minimal access surgery for cholelithiasis induces an attenuated acute phase response. *American Journal of Surgery,* 178**,** 232-4.

Burton, S., Brown, G., Daniels, I. R., Norman, A. R., Mason, B., Cunningham, D. & Royal Marsden Hospital, C. C. N. 2006. MRI directed multidisciplinary team preoperative treatment strategy: the way to eliminate positive circumferential margins? *Br J Cancer,* 94**,** 351-7.

Buunen, M., Gholghesaei, M., Veldkamp, R., Meijer, D. W., Bonjer, H. J. & Bouvy, N. D. 2004. Stress response to laparoscopic surgery - A review. *Surgical Endoscopy and Other Interventional Techniques,* 18**,** 1022-1028.

Buyukkocak, U., Caglayan, O., Daphan, C., Aydinuraz, K., Saygun, O. & Agalar, F. 2006. Similar effects of general and spinal anaesthesia on perioperative stress response in patients undergoing haemorrhoidectomy. *Mediators Inflamm,* 2006**,** 97257.

Canavan, C., Abrams, K. R. & Mayberry, J. 2006. Meta-analysis: colorectal and small bowel cancer risk in patients with Crohn's disease. *Aliment Pharmacol Ther,* 23**,** 1097-104.

Cappell, M. S. 2005. The pathophysiology, clinical presentation, and diagnosis of colon cancer and adenomatous polyps. *Med Clin North Am,* 89**,** 1-42, vii.

Catena, F., Ansaloni, L., Avanzolini, A., Di Saverio, S., D'alessandro, L., Maldini Casadei, M. & Pinna, A. 2009. Systemic cytokine response after emergency and elective surgery for colorectal carcinoma. *International Journal of Colorectal Disease,* 24**,** 803-8.

Cerantola, Y., Valerio, M., Persson, B., Jichlinski, P., Ljungqvist, O., Hubner, M., Kassouf, W., Muller, S., Baldini, G., Carli, F., Naesheimh, T., Ytrebo, L., Revhaug, A., Lassen, K., Knutsen, T., Aarsether, E., Wiklund, P. & Patel, H. R. 2013. Guidelines for perioperative care after radical cystectomy for bladder cancer: Enhanced Recovery After Surgery (ERAS((R))) society recommendations. *Clin Nutr,* 32**,** 879-87.

Chang, C. K., Chuter, T. A., Niemann, C. U., Shlipak, M. G., Cohen, M. J., Reilly, L. M. & Hiramoto, J. S. 2009. Systemic inflammation, coagulopathy, and acute renal insufficiency following endovascular thoracoabdominal aortic aneurysm repair. *Journal of Vascular Surgery,* 49**,** 1140-6.

Chen, M. F., Kuan, F. C., Yen, T. C., Lu, M. S., Lin, P. Y., Chung, Y. H., Chen, W. C. & Lee, K. D. 2014. IL-6-stimulated CD11b+ CD14+ HLA-DR- myeloid-derived suppressor cells, are associated with progression and poor prognosis in squamous cell carcinoma of the esophagus. *Oncotarget*.

Chen, W. K., Ren, L., Wei, Y., Zhu, D. X., Miao, C. H. & Xu, J. M. 2015. General anesthesia combined with epidural anesthesia ameliorates the effect of fast-track surgery by mitigating immunosuppression and facilitating intestinal functional recovery in colon cancer patients. *Int J Colorectal Dis*.

Chloropoulou, P., Iatrou, C., Vogiatzaki, T., Kotsianidis, I., Trypsianis, G., Tsigalou, C., Paschalidou, E., Kazakos, K., Touloupidis, S. & Simopoulos, K. 2013. Epidural anesthesia followed by epidural analgesia produces less inflammatory response than spinal anesthesia followed by intravenous morphine analgesia in patients with total knee arthroplasty. *Med Sci Monit,* 19**,** 73-80.

Cho, J. M., Laporta, A. J., Clark, J. R., Schofield, M. J., Hammond, S. L. & Mallory, P. L. 1994. Response of Serum Cytokines in Patients Undergoing Laparoscopic Cholecystectomy. *Surgical Endoscopy-Ultrasound and Interventional Techniques,* 8**,** 1380-1384.

Choi, J., Joseph, L. & Pilote, L. 2013. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obes Rev,* 14**,** 232-44.

Chung, Y. G., Won, Y. S., Kwon, Y. J., Shin, H. C., Choi, C. S. & Yeom, J. S. 2011. Comparison of Serum CRP and Procalcitonin in Patients after Spine Surgery. *J Korean Neurosurg Soc,* 49**,** 43-8.

Clarke, S. J., Chua, W., Moore, M., Kao, S., Phan, V., Tan, C., Charles, K. & McMillan, D. C. 2011. Use of inflammatory markers to guide cancer treatment. *Clin Pharmacol Ther,* 90**,** 475-8.

Coffelt, S. B., Wellenstein, M. D. & De Visser, K. E. 2016. Neutrophils in cancer: neutral no more. *Nat Rev Cancer,* 16**,** 431-46.

Cole, D. S., Watts, A., Scott-Coombes, D. & Avades, T. 2008. Clinical utility or peri-operative C-reactive protein testing in general surgery. *Annals of the Royal College of Surgeons of England,* 90**,** 317-321.

Colley, C. M., Fleck, A., Goode, A. W., Muller, B. R. & Myers, M. A. 1983. Early Time Course of the Acute Phase Protein Response in Man. *Journal of Clinical Pathology,* 36**,** 203-207.

Colorectal Cancer Collaborative, G. 2001. Adjuvant radiotherapy for rectal cancer: a systematic overview of 8,507 patients from 22 randomised trials. *Lancet,* 358**,** 1291-304.

Compton, C. C. 2003. Colorectal carcinoma: diagnostic, prognostic, and molecular features. *Mod Pathol,* 16**,** 376-88.

Cook, E. J., Walsh, S. R., Farooq, N., Alberts, J. C., Justin, T. A. & Keeling, N. J. 2007. Post-operative neutrophil-lymphocyte ratio predicts complications following colorectal surgery. *Int J Surg,* 5**,** 27-30.

Coolsen, M., Van Dam, R. M., Van Der Wilt, A. A., Slim, K., Lassen, K. & Dejong, C. 2013a. Systematic Review and Meta-analysis of Enhanced Recovery After

Pancreatic Surgery with Particular Emphasis on Pancreaticoduodenectomies. *World Journal of Surgery,* 37**,** 1909-1918.

Coolsen, M. M. E., Wong-Lun-Hing, E. M., Van Dam, R. M., Van Der Wilt, A. A., Slim, K., Lassen, K. & Dejong, C. H. C. 2013b. A systematic review of outcomes in patients undergoing liver surgery in an enhanced recovery after surgery pathways. *Hpb,* 15**,** 245-251.

Coory, M., Gkolia, P., Yang, I. A., Bowman, R. V. & Fong, K. M. 2008. Systematic review of multidisciplinary teams in the management of lung cancer. *Lung Cancer,* 60**,** 14-21.

Craig, S. R., Leaver, H. A., Yap, P. L., Pugh, G. C. & Walker, W. S. 2001. Acute phase responses following minimal access and conventional thoracic surgery. *Eur J Cardiothorac Surg,* 20**,** 455-63.

Crema, E., Ribeiro, E. N., Hial, A. M., Alves Junior, J. T., Pastore, R. & Silva, A. A. 2005. Evaluation of the response of cortisol, corticotropin and blood platelets kinetics after laparoscopic and open cholecystectomy. *Acta Cir Bras,* 20**,** 364-7.

Crozier, J. E., Leitch, E. F., Mckee, R. F., Anderson, J. H., Horgan, P. G. & McMillan, D. C. 2009. Relationship between emergency presentation, systemic inflammatory response, and cancer-specific survival in patients undergoing potentially curative surgery for colon cancer. *Am J Surg,* 197**,** 544-9.

Cruickshank, A. M., Fraser, W. D., Burns, H. J. G., Vandamme, J. & Shenkin, A. 1990. Response of Serum Interleukin-6 in Patients Undergoing Elective Surgery of Varying Severity. *Clinical Science,* 79**,** 161-165.

CRUK 2014a. *http://www.cancerresearchuk.org/about-cancer/type/bowel-cancer/treatment/statistics-and-outlook-for-bowel-cancer*.

CRUK 2014b. *Cancer Research UK - Key Facts* [Online]. Available: http://www.cancerresearchuk.org/cancer-info/cancerstats/keyfacts/bowel-cancer/ 2014].

Csendes, A., Burgos, A. M., Roizblatt, D., Garay, C. & Bezama, P. 2009. Inflammatory response measured by body temperature, C-reactive protein and white blood cell count 1, 3, and 5 days after laparotomic or laparoscopic gastric bypass surgery. *Obesity Surgery,* 19**,** 890-3.

Cuthbertson, D. P. 1979. Second annual Jonathan E. Rhoads Lecture. The metabolic response to injury and its nutritional implications: retrospect and prospect. *JPEN J Parenter Enteral Nutr,* 3**,** 108-29.

Dai, J., Tang, K., Xiao, W., Yu, G., Zeng, J., Li, W., Zhang, Y. Q., Xu, H., Chen, Z. Q. & Ye, Z. Q. 2014. Prognostic significance of C-reactive protein in urological cancers: a systematic review and meta-analysis. *Asian Pac J Cancer Prev,* 15**,** 3369-75.

Dalton, R. S., Smart, N. J., Edwards, T. J., Chandler, I. & Daniels, I. R. 2012. Short-term outcomes of the prone perineal approach for extra-levator abdomino-perineal excision (elAPE). *Surgeon,* 10**,** 342-6.

De Ridder, M., Vinh-Hung, V., Van Nieuwenhove, Y., Hoorens, A., Sermeus, A. & Storme, G. 2006. Prognostic value of the lymph node ratio in node positive colon cancer. *Gut,* 55**,** 1681.

Delgado, S., Lacy, A. M., Filella, X., Castells, A., Garcia-Valdecasas, J. C., Pique, J. M., Momblan, D. & Visa, J. 2001. Acute phase response in laparoscopic and open colectomy in colon cancer: randomized study. *Diseases of the Colon & Rectum,* 44**,** 638-46.

Derwinger, K., Kodeda, K., Bexe-Lindskog, E. & Taflin, H. 2010. Tumour differentiation grade is associated with TNM staging and the risk of node metastasis in colorectal cancer. *Acta Oncol,* 49**,** 57-62.

Desborough, J. P. 2000. The stress response to trauma and surgery. *Br J Anaesth,* 85**,** 109-117.

Deuss, U., Dietrich, J., Kaulen, D., Frey, K., Spangenberger, W., Allolio, B., Matuszczak, M., Troidl, H. & Winkelmann, W. 1994. The stress response to laparoscopic cholecystectomy: investigation of endocrine parameters. *Endoscopy,* 26**,** 235-8.

Diakos, C. I., Charles, K. A., McMillan, D. C. & Clarke, S. J. 2014. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol,* 15**,** e493-503.

Diaz-Montero, C. M., Salem, M. L., Nishimura, M. I., Garrett-Mayer, E., Cole, D. J. & Montero, A. J. 2009. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol Immunother,* 58**,** 49-59.

Dinarello, C. A., Novick, D., Kim, S. & Kaplanski, G. 2013. Interleukin-18 and IL-18 Binding Protein. *Front Immunol,* 4**,** 289.

Dindo, D., Demartines, N. & Clavien, P. A. 2004. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg,* 240**,** 205-13.

Douglas, E. & McMillan, D. C. 2014. Towards a simple objective framework for the investigation and treatment of cancer cachexia: the Glasgow Prognostic Score. *Cancer Treat Rev,* 40**,** 685-91.

Dukes, C. 1937. Histological Grading of Rectal Cancer: (Section of Pathology). *Proc R Soc Med,* 30**,** 371-6.

Dunker, M. S., Ten Hove, T., Bemelman, W. A., Slors, J. F., Gouma, D. J. & Van Deventer, S. J. 2003. Interleukin-6, C-reactive protein, and expression of human leukocyte antigen-DR on peripheral blood mononuclear cells in patients after laparoscopic vs. conventional bowel resection: a randomized study. *Dis Colon Rectum,* 46**,** 1238-44.

Dutta, S., Fullarton, G. M., Forshaw, M. J., Horgan, P. G. & McMillan, D. C. 2011. Persistent elevation of C-reactive protein following esophagogastric cancer resection as a predictor of postoperative surgical site infectious complications. *World Journal of Surgery,* 35**,** 1017-25.

Eaden, J. A., Abrams, K. R. & Mayberry, J. F. 2001. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut,* 48**,** 526-35.

East, J. M., Hogan, J., Samaha, G., Medani, M., Mackerricher, W., Polinkevych, S., Walsh, S. & Coffey, J. C. 2014. Ratios derived from an array of standard haematologic indices predict the oncologic outcome in colon cancer. *Colorectal Dis*.

ECG. 2015. The Impact of Enhanced Recovery Protocol Compliance on Elective Colorectal Cancer Resection: Results From an International Registry. *Ann Surg,* 261 (6): 1153-9.

Elmarasy, N. M., Soong, C. V., Walker, S. R., Macierewicz, J. A., Yusuf, S. W., Wenham, P. W. & Hopkinson, B. R. 2000. Sigmoid ischemia and the inflammatory response following endovascular abdominal aortic aneurysm repair. *J Endovasc Ther,* 7**,** 21-30.

Eltzschig, H. K. & Carmeliet, P. 2011. Hypoxia and inflammation. *N Engl J Med,* 364**,** 656-65.

Engstrom, P. F., Arnoletti, J. P., Benson, A. B., 3rd, Chen, Y. J., Choti, M. A., Cooper, H. S., Covey, A., Dilawari, R. A., Early, D. S., Enzinger, P. C., Fakih, M. G., Fleshman, J., Jr., Fuchs, C., Grem, J. L., Kiel, K., Knol, J. A., Leong, L. A., Lin, E., Mulcahy, M. F., Rao, S., Ryan, D. P., Saltz, L., Shibata, D., Skibber, J. M., Sofocleous, C., Thomas, J., Venook, A. P., Willett, C. & National Comprehensive Cancer, N. 2009. NCCN Clinical Practice Guidelines in Oncology: rectal cancer. *J Natl Compr Canc Netw,* 7**,** 838-81.

Enriquez, D., De la Cruz Ku, G. A., Fernandez, M., Eyzaguirre, E., Luque, R. & Paitan, D. 2017. Predictive value of neutrophil-to-lymphocyte ratio on pathological complete response in triple negative breast cancer. *Journal of Clinical Oncology,* 35; suppl.e12012 *(published online before print).*

Erlinger, T. P., Platz, E. A., Rifai, N. & Helzlsouer, K. J. 2004. C-reactive protein and the risk of incident colorectal cancer. *JAMA,* 291**,** 585-90.

Eskicioglu, C., Forbes, S. S., Aarts, M. A., Okrainec, A. & Mcleod, R. S. 2009. Enhanced recovery after surgery (ERAS) programs for patients having colorectal surgery: a meta-analysis of randomized trials. *J Gastrointest Surg,* 13**,** 2321-9.

Esser, N., Legrand-Poels, S., Piette, J., Scheen, A. J. & Paquot, N. 2014. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract.*

Evans, C., Galustian, C., Kumar, D., Hagger, R., Melville, D. M., Bodman-Smith, M., Jourdan, I., Gudgeon, A. M. & Dalgleish, A. G. 2009. Impact of surgery on immunologic function: comparison between minimally invasive techniques and conventional laparotomy for surgical resection of colorectal tumors. *American Journal of Surgery,* 197**,** 238-45.

Ezhevskaya, A. A., Mlyavykh, S. G. & Anderson, D. G. 2013. Effects of continuous epidural anesthesia and postoperative epidural analgesia on pain management and stress response in patients undergoing major spinal surgery. *Spine (Phila Pa 1976),* 38**,** 1324-30.

Fant, F., Tina, E., Sandblom, D., Andersson, S. O., Magnuson, A., Hultgren-Hornkvist, E., Axelsson, K. & Gupta, A. 2013. Thoracic epidural analgesia inhibits the neuro-hormonal but not the acute inflammatory stress response after radical retropubic prostatectomy. *Br J Anaesth,* 110**,** 747-57.

Fearon, K. C., Ljungqvist, O., Von Meyenfeldt, M., Revhaug, A., Dejong, C. H., Lassen, K., Nygren, J., Hausel, J., Soop, M., Andersen, J. & Kehlet, H. 2005. Enhanced recovery after surgery: a consensus review of clinical care for patients undergoing colonic resection. *Clin Nutr,* 24**,** 466-77.

Fedirko, V., Tramacere, I., Bagnardi, V., Rota, M., Scotti, L., Islami, F., Negri, E., Straif, K., Romieu, I., La Vecchia, C., Boffetta, P. & Jenab, M. 2011. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. *Ann Oncol,* 22**,** 1958-72.

Feliciano, E. M. C., Kroenke, C. H., Meyerhardt, J., Prado, C. M., Bradshaw, P. T., Kwan, M., Xiao, J., Alexeef, S., Weltzien, E. K., Castillo, A. L. & Caan, B. J. 2017. Systemic inflammation and sarcopenia predict colorectal cancer survival [abstract]. AACR, *Cancer Res* 2017; 77 (13 Suppl, Abstract nr 2250).

Feng, J. F., Huang, Y. & Chen, Q. X. 2014. The combination of platelet count and neutrophil lymphocyte ratio is a predictive factor in patients with esophageal squamous cell carcinoma. *Transl Oncol,* 7**,** 632-7.

Ferrara, N. & Davis-Smyth, T. 1997. The biology of vascular endothelial growth factor. *Endocr Rev,* 18**,** 4-25.

Fitzgerald, J. E. F. & Ahmed, I. 2009. Systematic Review and Meta-Analysis of Chewing-Gum Therapy in the Reduction of Postoperative Paralytic Ileus Following Gastrointestinal Surgery. *World J Surg,* 33**,** 2557-2566.

Fleissig, A., Jenkins, V., Catt, S. & Fallowfield, L. 2006. Multidisciplinary teams in cancer care: are they effective in the UK? *Lancet Oncol,* 7**,** 935-43.

Forget, P., Bentin, C., Machiels, J. P., Berliere, M., Coulie, P. G. & De Kock, M. 2014. Intraoperative use of ketorolac or diclofenac is associated with improved disease-free survival and overall survival in conservative breast cancer surgery. *Br J Anaesth*.

Forget, P., Dinant, V. & De Kock, M. 2015. Is the Neutrophil-to-Lymphocyte Ratio more correlated than C-reactive protein with postoperative complications after major abdominal surgery? *PeerJ,* 3**,** e713.

Franciosi, M., Lucisano, G., Lapice, E., Strippoli, G. F., Pellegrini, F. & Nicolucci, A. 2013. Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. *PLoS One,* 8**,** e71583.

Franke, A., Lante, W., Fackeldey, V., Becker, H. P., Kurig, E., Zoller, L. G., Weinhold, C. & Markewitz, A. 2005. Pro-inflammatory cytokines after different kinds of cardio-thoracic surgical procedures: is what we see what we know? *Eur J Cardiothorac Surg,* 28**,** 569-75.

Fransen, E., Maessen, J., Dentener, M., Senden, N., Geskes, G. & Buurman, W. 1998. Systemic inflammation present in patients undergoing CABG without extracorporeal circulation. *Chest,* 113**,** 1290-5.

Freeman, B. D. & Buchman, T. G. 2001. Interleukin-1 receptor antagonist as therapy for inflammatory disorders. *Expert Opin Biol Ther,* 1**,** 301-8.

Friscia, M. E., Zhu, J., Kolff, J. W., Chen, Z., Kaiser, L. R., Deutschman, C. S. & Shrager, J. B. 2007. Cytokine response is lower after lung volume reduction through bilateral thoracoscopy versus sternotomy. *Annals of Thoracic Surgery,* 83**,** 252-6.

Frouws, M. A., Bastiaannet, E., Langley, R. E., Chia, W. K., Van Herk-Sukel, M. P., Lemmens, V. E., Putter, H., Hartgrink, H. H., Bonsing, B. A., Van De Velde, C. J., Portielje, J. E. & Liefers, G. J. 2017a. Effect of low-dose aspirin use on survival of patients with gastrointestinal malignancies; an observational study. *Br J Cancer,* 116**,** 405-413.

Frouws, M. A., Van Herk-Sukel, M. P. P., Maas, H. A., Van De Velde, C. J. H., Portielje, J. E. A., Liefers, G. J. & Bastiaannet, E. 2017b. The mortality reducing effect of aspirin in colorectal cancer patients: Interpreting the evidence. *Cancer Treat Rev,* 55**,** 120-127.

Fujita, T. & Yanaga, K. 2007. Clinical influence of vagotomy on postoperative acute phase response. *Langenbecks Arch Surg,* 392**,** 127-30.

Gabay, C. & Kushner, I. 1999. Mechanisms of disease: Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine,* 340**,** 448-454.

Galiatsatos, P. & Foulkes, W. D. 2006. Familial adenomatous polyposis. *Am J Gastroenterol,* 101**,** 385-98.

Galle, C., De Maertelaer, V., Motte, S., Zhou, L., Stordeur, P., Delville, J. P., Li, R., Ferreira, J., Goldman, M., Capel, P., Wautrecht, J. C., Pradier, O. & Dereume, J. P. 2000. Early inflammatory response after elective abdominal aortic aneurysm repair: a comparison between endovascular procedure and conventional surgery. *Journal of Vascular Surgery,* 32**,** 234-46.

Galon, J., Pages, F., Marincola, F. M., Thurin, M., Trinchieri, G., Fox, B. A., Gajewski, T. F. & Ascierto, P. A. 2012. The immune score as a new possible approach for the classification of cancer. *J Transl Med,* 10**,** 1.

Geng, Y., Shao, Y., Zhu, D., Zheng, X., Zhou, Q., Zhou, W., Ni, X., Wu, C. &Jiang, J. 2016. Systemic immune-inflammation index predicts prognosis of patients with esophageal squamous cell carcinoma: a propensity score-matched analysis. *Sci Rep,* 21; 6: 39482.

Glaser, F., Sannwald, G. A., Buhr, H. J., Kuntz, C., Mayer, H., Klee, F. & Herfarth, C. 1995. General Stress-Response to Conventional and Laparoscopic Cholecystectomy. *Annals of Surgery,* 221**,** 372-380.

Greco, F., Hoda, M. R., Mohammed, N., Springer, C., Fischer, K. & Fornara, P. 2012. Laparoendoscopic single-site and conventional laparoscopic radical nephrectomy result in equivalent surgical trauma: preliminary results of a single-centre retrospective controlled study. *European Urology,* 61**,** 1048-53.

Greco, M., Capretti, G., Beretta, L., Gemma, M., Pecorelli, N. & Braga, M. 2014. Enhanced recovery program in colorectal surgery: a meta-analysis of randomized controlled trials. *World J Surg,* 38**,** 1531-41.

Grocott, M. P., Dushianthan, A., Hamilton, M. A., Mythen, M. G., Harrison, D., Rowan, K. & Optimisation Systematic Review Steering, G. 2013. Perioperative increase in

global blood flow to explicit defined goals and outcomes after surgery: a Cochrane Systematic Review. *Br J Anaesth,* 111**,** 535-48.

Guenaga, K. F., Matos, D. & Wille-Jorgensen, P. 2011. Mechanical bowel preparation for elective colorectal surgery. *Cochrane Database Syst Rev***,** CD001544.

Gupta, D. & Lis, C. G. 2010. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. *Nutr J,* 9**,** 69.

Gustafsson, U. O., Scott, M. J., Schwenk, W., Demartines, N., Roulin, D., Francis, N., Mcnaught, C. E., Macfie, J., Liberman, A. S., Soop, M., Hill, A., Kennedy, R. H., Lobo, D. N., Fearon, K., Ljungqvist, O. & Enhanced Recovery after Surgery, S. 2012a. Guidelines for perioperative care in elective colonic surgery: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *Clin Nutr,* 31**,** 783-800.

Gustafsson, U. O., Tiefenthal, M., Thorell, A., Ljungqvist, O. & Nygrens, J. 2012b. Laparoscopic-assisted and open high anterior resection within an ERAS protocol. *World J Surg,* 36**,** 1154-61.

Guthrie, G. J., Charles, K. A., Roxburgh, C. S., Horgan, P. G., McMillan, D. C. & Clarke, S. J. 2013a. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. *Crit Rev Oncol Hematol,* 88**,** 218-30.

Guthrie, G. J., Roxburgh, C. S., Farhan-Alanie, O. M., Horgan, P. G. & McMillan, D. C. 2013b. Comparison of the prognostic value of longitudinal measurements of systemic inflammation in patients undergoing curative resection of colorectal cancer. *Br J Cancer,* 109**,** 24-8.

Hadimioglu, N., Ulugol, H., Akbas, H., Coskunfirat, N., Ertug, Z. & Dinckan, A. 2012. Combination of epidural anesthesia and general anesthesia attenuates stress response to renal transplantation surgery. *Transplant Proc,* 44**,** 2949-54.

Halaszynski, T. M., Juda, R. & Silverman, D. G. 2004. Optimizing postoperative outcomes with efficient preoperative assessment and management. *Crit Care Med,* 32**,** S76-86.

Hall, G. M., Peerbhoy, D., Shenkin, A., Parker, C. J. & Salmon, P. 2001. Relationship of the functional recovery after hip arthroplasty to the neuroendocrine and inflammatory responses. *Br J Anaesth,* 87**,** 537-42.

Han, H. J., Choi, S. B., Kim, W. B., Lee, J. S., Boo, Y. J., Song, T. J., Suh, S. O. & Choi, S. Y. 2012. Surgical stress response and clinical outcomes of single port laparoscopic cholecystectomy: prospective nonrandomized study. *Am Surg,* 78**,** 485-91.

Han, S. A., Lee, W. Y., Park, C. M., Yun, S. H. & Chun, H. K. 2010. Comparison of immunologic outcomes of laparoscopic vs open approaches in clinical stage III colorectal cancer. *Int J Colorectal Dis,* 25**,** 631-8.

Han, Y., Mao, F., Wu, Y., Fu, X., Zhu, X., Zhou, S., Zhang, W., Sun, Q. & Zhao, Y. 2011. Prognostic role of C-reactive protein in breast cancer: a systematic review and meta-analysis. *Int J Biol Markers,* 26**,** 209-15.

Hanahan, D. & Weinberg, R. A. 2011. Hallmarks of cancer: the next generation. *Cell,* 144**,** 646-74.

Harkki-Siren, P., Sjoberg, J., Toivonen, J. & Tiitinen, A. 2000. Clinical outcome and tissue trauma after laparoscopic and abdominal hysterectomy: a randomized controlled study. *Acta Obstetricia Et Gynecologica Scandinavica,* 79**,** 866-871.

Harmon, G. D., Senagore, A. J., Kilbride, M. J. & Warzynski, M. J. 1994. Interleukin-6 Response to Laparoscopic and Open Colectomy. *Diseases of the Colon & Rectum,* 37**,** 754-759.

He, W., Deng, H. J., Yu, J., Zhang, C., Wang, Y. N., Cheng, X. & Li, G. X. 2009. [Effect of laparoscopic-assisted resection of rectal carcinoma on C-reactive protein and humoral immunity]. *Zhonghua Wei Chang Wai Ke Za Zhi,* 12**,** 357-60.

Heald, R. J., Husband, E. M. & Ryall, R. D. 1982. The mesorectum in rectal cancer surgery--the clue to pelvic recurrence? *Br J Surg,* 69**,** 613-6.

Hearps, A. C., Martin, G. E., Angelovich, T. A., Cheng, W. J., Maisa, A., Landay, A. L., Jaworowski, A. & Crowe, S. M. 2012. Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. *Aging Cell,* 11**,** 867-75.

Hendren, S. 2013. Urinary catheter management. *Clin Colon Rectal Surg,* 26**,** 178-81.

Hermanides, J., Hollmann, M. W., Stevens, M. F. & Lirk, P. 2012. Failed epidural: causes and management. *Br J Anaesth,* 109**,** 144-154.

Hewitt, P. M., Ip, S. M., Kwok, S. P., Somers, S. S., Li, K., Leung, K. L., Lau, W. Y. & Li, A. K. 1998. Laparoscopic-assisted vs. open surgery for colorectal cancer: comparative study of immune effects. *Diseases of the Colon & Rectum,* 41**,** 901-9.

Hildebrandt, U., Kessler, K., Plusczyk, T., Pistorius, G., Vollmar, B. & Menger, M. D. 2003. Comparison of surgical stress between laparoscopic and open colonic resections. *Surgical Endoscopy and Other Interventional Techniques,* 17**,** 242-246.

Hogevold, H. E., Kierulf, P., Ovstebo, R. & Reikeras, O. 1992. Acute phase reactants and interleukin 6 after total hip replacement. Effects of high dose corticosteroids. *European Journal of Surgery,* 158**,** 339-45.

Hogevold, H. E., Lyberg, T., Kahler, H., Haug, E. & Reikeras, O. 2000. Changes in plasma IL-1beta, TNF-alpha and IL-6 after total hip replacement surgery in general or regional anaesthesia. *Cytokine,* 12**,** 1156-9.

Holzheimer, R. G., Gross, J. & Schein, M. 1999. Pro- and anti-inflammatory cytokine-response in abdominal aortic aneurysm repair: a clinical model of ischemia-reperfusion. *Shock,* 11**,** 305-10.

Hong, J. Y., Yang, S. C., Yi, J. & Kil, H. K. 2011. Epidural ropivacaine and sufentanil and the perioperative stress response after a radical retropubic prostatectomy. *Acta Anaesthesiologica Scandinavica,* 55**,** 282-289.

Horgan, P. G. & McMillan, D. C. 2010. Surgeons and selection of adjuvant therapy for node-negative colonic cancer. *Br J Surg,* 97**,** 1459-60.

Hou, C. Y., Li, X. L., Jiang, F., Gong, R. J., Guo, X. Y. & Yao, Y. Q. 2011. Comparative evaluation of surgical stress of laparoscopically assisted vaginal radical hysterectomy and lymphadenectomy and laparotomy for early-stage cervical cancer. *Oncol Lett,* 2**,** 747-752.

HQUIP. 2014. *National Bowel Cancer Audit Report* [Online]. Available: http://www.hscic.gov.uk/catalogue/PUB16021/nati-clin-audi-supp-prog-bowe-canc-2014-rep1.pdf [Accessed 16/03/2015].

Hu, P., Shen, H., Wang, G., Zhang, P., Liu, Q. & Du, J. 2014. Prognostic significance of systemic inflammation-based lymphocyte- monocyte ratio in patients with lung cancer: based on a large cohort study. *PLoS One,* 9**,** e108062.

Huang, S., Mills, L., Mian, B., Tellez, C., McCarty, M., Yang, X. D., Gudas, J. M. & Bar-Eli, M. 2002. Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. *Am J Pathol,* 161**,** 125-34.

Huang, T. J., Hsu, R. W. W., Li, Y. Y. & Cheng, C. C. 2005. Less systemic cytokine response in patients following microendoscopic versus open lumbar discectomy. *Journal of Orthopaedic Research,* 23**,** 406-411.

Hurwitz, H., Uppal, N., Wagner, Sa., Bendell, Jc., Beck, Jt., Wade, S., Nemunaitis, Jj., Stella, Pj., Pipas, Jm., Wainberg, Za., Manges, R., Garrett, Wm., Hunter, Ds., Clark, J., Leopold, L., Levy, Rs., Sandor, V. 2014. A randomized double-blind phase 2 study of ruxolitinib (RUX) or placebo (PBO) with capcitibine (CAPE) as second-line therapy in patients (pts) with metastatic pancreatic cancer (mPC). *J Clin Oncol,* 32**,** (suppl; abstr 4000)

Hurwitz, H. I., Uppal, N., Wagner, S. A., Bendell, J. C., Beck, J. T., Wade, S. M., 3rd, Nemunaitis, J. J., Stella, P. J., Pipas, J. M., Wainberg, Z. A., Manges, R., Garrett, W. M., Hunter, D. S., Clark, J., Leopold, L., Sandor, V. & Levy, R. S. 2015. Randomized, Double-Blind, Phase II Study of Ruxolitinib or Placebo in Combination With Capecitabine in Patients With Metastatic Pancreatic Cancer for Whom Therapy With Gemcitabine Has Failed. *J Clin Oncol,* 33**,** 4039-47.

Imberti, D., Agnelli, G., Ageno, W., Moia, M., Palareti, G., Pistelli, R., Rossi, R. & Verso, M. 2008. Clinical characteristics and management of cancer-associated acute venous thromboembolism: findings from the MASTER Registry. *Haematologica,* 93**,** 273-8.

Ishizuka, M., Nagata, H., Takagi, K., Iwasaki, Y. & Kubota, K. 2013. Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients with colorectal cancer. *Br J Cancer,* 109**,** 401-7.

Ishizuka, M., Oyama, Y., Abe, A. & Kubota, K. 2014. Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients undergoing surgery for gastric cancer. *J Surg Oncol,* 110**,** 935-41.

Jain, S., Harris, J. & Ware, J. 2010. Platelets: linking hemostasis and cancer. *Arterioscler Thromb Vasc Biol,* 30**,** 2362-7.

Jakeways, M. S. R., Mitchell, V., Hashim, I. A., Chadwick, S. J. D., Shenkin, A., Green, C. J. & Carli, F. 1994. Metabolic and Inflammatory Responses after Open or Laparoscopic Cholecystectomy. *British Journal of Surgery,* 81**,** 127-131.

Janeway, C. A., Jr. 2001. How the immune system protects the host from infection. *Microbes Infect,* 3**,** 1167-71.

Jankova, L., Dent, O. F., Chan, C., Chapuis, P. & Clarke, S. J. 2013. Preoperative neutrophil/lymphocyte ratio predicts overall survival but does not predict recurrence or cancer-specific survival after curative resection of node-positive colorectal cancer. *BMC Cancer,* 13**,** 442.

Jansen, M. C., Van Wanrooy, S., Van Hillegersberg, R., Rijken, A. M., Van Coevorden, E., Prevoo, W. & Van Gulik, T. M. 2008. Assessment of systemic inflammatory response (SIR) in patients undergoing radiofrequency ablation or partial liver resection for liver tumors. *Ejso,* 34**,** 662-667.

Jass, J. R. 1998. Diagnosis of hereditary non-polyposis colorectal cancer. *Histopathology,* 32**,** 491-7.

Jass, J. R., Love, S. B. & Northover, J. M. 1987. A new prognostic classification of rectal cancer. *Lancet,* 1**,** 1303-6.

Jess, P., Schultz, K., Bendtzen, K. & Nielsen, O. H. 2000. Systemic inflammatory responses during laparoscopic and open inguinal hernia repair: a randomised prospective study. *European Journal of Surgery,* 166**,** 540-4.

Jesus, E. C., Karliczek, A., Matos, D., Castro, A. A. & Atallah, A. N. 2004. Prophylactic anastomotic drainage for colorectal surgery. *Cochrane Database Syst Rev***,** CD002100.

Johns, N., O'Neill, S., Ventham, N. T., Barron, F., Brady, R. R. & Daniel, T. 2012. Clinical effectiveness of transversus abdominis plane (TAP) block in abdominal surgery: a systematic review and meta-analysis. *Colorectal Disease,* 14**,** E635-E642.

Johnson, P. M., Porter, G. A., Ricciardi, R. & Baxter, N. N. 2006. Increasing negative lymph node count is independently associated with improved long-term survival in stage IIIB and IIIC colon cancer. *J Clin Oncol,* 24**,** 3570-5.

Jorgensen, H., Wetterslev, J., Moiniche, S. & Dahl, J. B. 2000. Epidural local anaesthetics versus opioid-based analgesic regimens on postoperative gastrointestinal paralysis, PONV and pain after abdominal surgery. *Cochrane Database Syst Rev***,** CD001893.

Joris, J., Cigarini, I., Legrand, M., Jacquet, N., De Groote, D., Franchimont, P. & Lamy, M. 1992. Metabolic and respiratory changes after cholecystectomy performed via laparotomy or laparoscopy. *Br J Anaesth,* 69**,** 341-5.

Jules-Elysee, K. M., Lipnitsky, J. Y., Patel, N., Anastasian, G., Wilfred, S. E., Urban, M. K. & Sculco, T. P. 2011. Use of low-dose steroids in decreasing cytokine release during bilateral total knee replacement. *Reg Anesth Pain Med,* 36**,** 36-40.

Jurczok, A., Zacharias, M., Wagner, S., Hamza, A. & Fornara, P. 2007. Prospective non-randomized evaluation of four mediators of the systemic response after extraperitoneal laparoscopic and open retropubic radical prostatectomy. *BJU Int,* 99**,** 1461-6.

Kahokehr, A., Sammour, T., Zargar-Shoshtari, K., Srinivasa, S. & Hill, A. G. 2010. Recovery after open and laparoscopic right hemicolectomy: a comparison. *J Surg Res,* 162**,** 11-6.

Kahveci, K., Ornek, D., Doger, C., Aydin, G. B., Aksoy, M., Emre, C., Deveci, A., Bozkurt, M. & Ozgun, G. 2014. The effect of anesthesia type on stress hormone response: Comparison of general versus epidural anesthesia. *Niger J Clin Pract,* 17**,** 523-7.

Kale, S. S. & Yende, S. 2011. Effects of Aging on Inflammation and Hemostasis through the Continuum of Critical Illness. *Aging Dis,* 2**,** 501-11.

Kao, P. C., Shiesh, S. C. & Wu, T. J. 2006. Serum C-reactive protein as a marker for Wellness assessment. *Annals of Clinical and Laboratory Science,* 36**,** 163-169.

Karanicolas, P. J., Smith, S. E., Kanbur, B., Davies, E. & Guyatt, G. H. 2008. The impact of prophylactic dexamethasone on nausea and vomiting after laparoscopic cholecystectomy: a systematic review and meta-analysis. *Ann Surg,* 248**,** 751-62.

Karayiannakis, A. J., Makri, G. G., Mantzioka, A., Karousos, D. & Karatzas, G. 1997. Systemic stress response after laparoscopic or open cholecystectomy: A randomized trial. *British Journal of Surgery,* 84**,** 467-471.

Karliczek, A., Jesus, E. C., Matos, D., Castro, A. A., Atallah, A. N. & Wiggers, T. 2006. Drainage or nondrainage in elective colorectal anastomosis: a systematic review and meta-analysis. *Colorectal Dis,* 8**,** 259-65.

Kawamura, H., Yokota, R., Homma, S. & Kondo, Y. 2009. Comparison of invasiveness between laparoscopy-assisted total gastrectomy and open total gastrectomy. *World Journal of Surgery,* 33**,** 2389-95.

Kehlet, H. 2005. Postoperative opioid sparing to hasten recovery: what are the issues? *Anesthesiology,* 102**,** 1083-5.

Kehlet, H. 2008. Fast-track colorectal surgery. *Lancet,* 371**,** 791-3.

Kehlet, H. & Dahl, J. B. 2003. Anaesthesia, surgery, and challenges in postoperative recovery. *Lancet,* 362**,** 1921-8.

Kehlet, H. & Wilmore, D. W. 2005. Fast-track surgery. *Br J Surg,* 92**,** 3-4.

Khan, A. L., Larsen, F., Heys, S. D. & Eremin, O. 1999. Peri-operative acute phase response and cytokine releasein women with breast cancer: modulation bypolyadenylic-polyuridylic acid. *Eur J Surg Oncol,* 25**,** 574-9.

Khanbhai, M., Shah, M., Cantanhede, G., Ilyas, S. & Richards, T. 2014. The problem of anaemia in patients with colorectal cancer. *ISRN Hematol,* 2014**,** 547914.

Khuri, S. F., Henderson, W. G., Depalma, R. G., Mosca, C., Healey, N. A., Kumbhani, D. J. & Participants in The, V. a. N. S. Q. I. P. 2005. Determinants of long-term survival after major surgery and the adverse effect of postoperative complications. *Ann Surg,* 242**,** 326-41; discussion 341-3.

Kiran, P. K., Murray, A. C. A., Chiuzan, C., Estrada, D., Forde, K. 2015. Combined preoperative mechaniocal bowel preparation with oral antibiotics significantly reduces surgical site infection, anastomotic leak and ileus after colorectal surgery. *Ann Surg,* 262: 416-425.

Kishimoto, T. 2010. IL-6: from its discovery to clinical applications. *International Immunology,* 22**,** 347-352.

Kiyohara, L. Y., Kayano, L. K., Oliveira, L. M., Yamamoto, M. U., Inagaki, M. M., Ogawa, N. Y., Gonzales, P. E., Mandelbaum, R., Okubo, S. T., Watanuki, T. &

Vieira, J. E. 2004. Surgery information reduces anxiety in the pre-operative period. *Rev Hosp Clin Fac Med Sao Paulo,* 59**,** 51-6.

Klein, M., Gogenur, I. & Rosenberg, J. 2012. Postoperative use of non-steroidal anti-inflammatory drugs in patients with anastomotic leakage requiring reoperation after colorectal resection: cohort study based on prospective data. *BMJ,* 345**,** e6166.

Klinger, A. L., Green, H., Monlezun, D. J., Beck, D., Kann, B., Vargas, H., D., Whitlow, C., Margolin, D. 2017. The role of bowel preparation in colorectal surgery. *Ann Surg*; DOI 10.1097/SLA [epub ahead of print].

Klintrup, K., Makinen, J. M., Kauppila, S., Vare, P. O., Melkko, J., Tuominen, H., Tuppurainen, K., Makela, J., Karttunen, T. J. & Makinen, M. J. 2005. Inflammation and prognosis in colorectal cancer. *Eur J Cancer,* 41**,** 2645-54.

Kolaczkowska, E. & Kubes, P. 2013. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol,* 13**,** 159-75.

Kong, C. G., Kim, Y. Y. & Park, J. B. 2012. Postoperative changes of early-phase inflammatory indices after uncomplicated anterior cervical discectomy and fusion using allograft and demineralised bone matrix. *International Orthopaedics,* 36**,** 2293-7.

Koo, B. C., Ng, C. S., J, U. K.-I., Prevost, A. T. & Freeman, A. H. 2006. Minimal preparation CT for the diagnosis of suspected colorectal cancer in the frail and elderly patient. *Clin Radiol,* 61**,** 127-39.

Kopylov, U., Ben-Horin, S., Zmora, O., Eliakim, R. & Katz, L. H. 2012. Anti-tumor necrosis factor and postoperative complications in Crohn's disease: systematic review and meta-analysis. *Inflammatory Bowel Diseases,* 18**,** 2404-13.

Kraft, C. N., Kruger, T., Westhoff, J., Luring, C., Weber, O., Wirtz, D. C. & Pennekamp, P. H. 2011. CRP and leukocyte-count after lumbar spine surgery: fusion vs. nucleotomy. *Acta Orthopaedica,* 82**,** 489-493.

Kragsbjerg, P., Holmberg, H. & Vikerfors, T. 1995. Serum Concentrations of Interleukin-6, Tumor-Necrosis-Factor-Alpha, and C-Reactive Protein in Patients Undergoing Major Operations. *European Journal of Surgery,* 161**,** 17-22.

Kristiansson, M., Saraste, L., Soop, M., Sundqvist, K. G. & Thorne, A. 1999. Diminished interleukin-6 and C-reactive protein responses to laparoscopic versus open cholecystectomy. *Acta Anaesthesiol Scand,* 43**,** 146-52.

Kucukakin, B., Wilhelmsen, M., Lykkesfeldt, J., Reiter, R. J., Rosenberg, J. & Gogenur, I. 2010. No effect of melatonin to modify surgical-stress response after major vascular surgery: a randomised placebo-controlled trial. *Eur J Vasc Endovasc Surg,* 40**,** 461-7.

Kuhry, E., Schwenk, W., Gaupset, R., Romild, U. & Bonjer, J. 2008. Long-term outcome of laparoscopic surgery for colorectal cancer: a cochrane systematic review of randomised controlled trials. *Cancer Treat Rev,* 34**,** 498-504.

Kumar, R., Geuna, E., Michalarea, V., Guardascione, M., Naumann, U., Lorente, D., Kaye, S. B. & De Bono, J. S. 2015. The neutrophil-lymphocyte ratio and its utilisation for the management of cancer patients in early clinical trials. *Br J Cancer,* 112**,** 1157-65.

Kumbhare, D., Parkinson, W., Dunlop, B., Richards, C., Kerr, C., Buckley, N. & Adachi, J. 2009. Injury measurement properties of serum interleukin-6 following lumbar decompression surgery. *Journal of Surgical Research,* 157**,** 161-7.

Kurz, A., Sessler, D. I. & Lenhardt, R. 1996. Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization. Study of Wound Infection and Temperature Group. *N Engl J Med,* 334**,** 1209-15.

Kvarnstrom, A., Swartling, T., Kurlberg, G., Bengtson, J. P. & Bengtsson, A. 2013. Pro-inflammatory Cytokine Release in Rectal Surgery: Comparison Between Laparoscopic and Open Surgical Techniques. *Archivum Immunologiae Et Therapiae Experimentalis,* 61**,** 407-411.

Kwon, H. C., Kim, S. H., Oh, S. Y., Lee, S., Lee, J. H., Choi, H. J., Park, K. J., Roh, M. S., Kim, S. G. & Kim, H. J. 2012. Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer. *Biomarkers,* 17**,** 216-22.

Lan, A. K., Luk, H. N., Goto, S., Chen, S. M., Eng, H. L., Chen, Y. S., De Villa, V. H., Wang, C. C., Cheng, Y. F., Chen, C. L., Lee, J. H. & Jawan, B. 2003. Stress response to hepatectomy in patients with a healthy or a diseased liver. *World Journal of Surgery,* 27**,** 761-4.

Lane, J. C., Wright, S., Burch, J., Kennedy, R. H. & Jenkins, J. T. 2013. Early prediction of adverse events in enhanced recovery based upon the host systemic inflammatory response. *Colorectal Dis,* 15**,** 224-30.

Larsson, S., Thelander, U. & Friberg, S. 1992. C-Reactive Protein (CRP) Levels after Elective Orthopedic-Surgery. *Clinical Orthopaedics and Related Research***,** 237-242.

Lassen, K., Coolsen, M. M., Slim, K., Carli, F., De Aguilar-Nascimento, J. E., Schafer, M., Parks, R. W., Fearon, K. C., Lobo, D. N., Demartines, N., Braga, M., Ljungqvist, O., Dejong, C. H., Society, E., European Society for Clinical, N., Metabolism, International Association for Surgical, M. & Nutrition 2012. Guidelines for perioperative care for pancreaticoduodenectomy: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *Clin Nutr,* 31**,** 817-30.

Lassen, K., Soop, M., Nygren, J., Cox, P. B., Hendry, P. O., Spies, C., Von Meyenfeldt, M. F., Fearon, K. C., Revhaug, A., Norderval, S., Ljungqvist, O., Lobo, D. N. & Dejong, C. H. 2009. Consensus review of optimal perioperative care in colorectal surgery: Enhanced Recovery After Surgery (ERAS) Group recommendations. *Arch Surg,* 144**,** 961-9.

Law, W. L., Choi, H. K., Lee, Y. M. & Ho, J. W. 2007a. The impact of postoperative complications on long-term outcomes following curative resection for colorectal cancer. *Ann Surg Oncol,* 14**,** 2559-66.

Law, W. L., Choi, H. K., Lee, Y. M., Ho, J. W. & Seto, C. L. 2007b. Anastomotic leakage is associated with poor long-term outcome in patients after curative colorectal resection for malignancy. *J Gastrointest Surg,* 11**,** 8-15.

Leitch, E. F., Chakrabarti, M., Crozier, J. E., Mckee, R. F., Anderson, J. H., Horgan, P. G. & McMillan, D. C. 2007. Comparison of the prognostic value of selected markers of the systemic inflammatory response in patients with colorectal cancer. *Br J Cancer,* 97**,** 1266-70.

Leung, K. L., Lai, P. B., Ho, R. L., Meng, W. C., Yiu, R. Y., Lee, J. F. & Lau, W. Y. 2000. Systemic cytokine response after laparoscopic-assisted resection of rectosigmoid carcinoma: A prospective randomized trial. *Annals of Surgery,* 231**,** 506-11.

Levine, M. N., Raskob, G., Landefeld, S. & Kearon, C. 2001. Hemorrhagic complications of anticoagulant treatment. *Chest,* 119**,** 108S-121S.

Li, M. X., Liu, X. M., Zhang, X. F., Zhang, J. F., Wang, W. L., Zhu, Y., Dong, J., Cheng, J. W., Liu, Z. W., Ma, L. & Lv, Y. 2014a. Prognostic role of neutrophil-to-lymphocyte ratio in colorectal cancer: a systematic review and meta-analysis. *Int J Cancer,* 134**,** 2403-13.

Li, Q. Q., Lu, Z. H., Yang, L., Lu, M., Zhang, X. T., Li, J., Zhou, J., Wang, X. C., Gong, J. F., Gao, J., Li, Y. & Shen, L. 2014b. Neutrophil Count and the Inflammation-based Glasgow Prognostic Score Predict Survival in Patients with Advanced Gastric Cancer Receiving First-line Chemotherapy. *Asian Pac J Cancer Prev,* 15**,** 945-50.

Li, S., Liu, Y., Peng, Q., Xie, L., Wang, J. & Qin, X. 2013. Chewing gum reduces postoperative ileus following abdominal surgery: a meta-analysis of 17 randomized controlled trials. *J Gastroenterol Hepatol,* 28**,** 1122-32.

Liang, P. S., Chen, T. Y. & Giovannucci, E. 2009. Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. *Int J Cancer,* 124**,** 2406-15.

Liebig, C., Ayala, G., Wilks, J., Verstovsek, G., Liu, H., Agarwal, N., Berger, D. H. & Albo, D. 2009. Perineural invasion is an independent predictor of outcome in colorectal cancer. *J Clin Oncol,* 27**,** 5131-7.

Lindgren, P. G., Nordgren, S. R., Oresland, T. & Hulten, L. 2001. Midline or transverse abdominal incision for right-sided colon cancer-a randomized trial. *Colorectal Dis,* 3**,** 46-50.

Liu, Y., Tang, W., Wang, J., Xie, L., Li, T., He, Y., Deng, Y., Peng, Q., Li, S. & Qin, X. 2014. Association between statin use and colorectal cancer risk: a meta-analysis of 42 studies. *Cancer Causes Control,* 25**,** 237-49.

Lobo, D. N., Bostock, K. A., Neal, K. R., Perkins, A. C., Rowlands, B. J. & Allison, S. P. 2002. Effect of salt and water balance on recovery of gastrointestinal function after elective colonic resection: a randomised controlled trial. *Lancet,* 359**,** 1812-8.

Lourenco, T., Murray, A., Grant, A., McKinley, A., Krukowski, Z. & Vale, L. 2008. Laparoscopic surgery for colorectal cancer: safe and effective? - A systematic review. *Surg Endosc,* 22**,** 1146-60.

Luckey, A., Livingston, E. & Tache, Y. 2003. Mechanisms and treatment of postoperative ileus. *Archives of Surgery,* 138**,** 206-214.

Lynch, H. T., Smyrk, T. & Lynch, J. 1997. An update of HNPCC (Lynch syndrome). *Cancer Genet Cytogenet,* 93**,** 84-99.

Lytras, T., Nikolopoulos, G. & Bonovas, S. 2014. Statins and the risk of colorectal cancer: an updated systematic review and meta-analysis of 40 studies. *World J Gastroenterol,* 20**,** 1858-70.

Ma, Y., Yang, Y., Wang, F., Zhang, P., Shi, C., Zou, Y. & Qin, H. 2013. Obesity and risk of colorectal cancer: a systematic review of prospective studies. *PLoS One,* 8**,** e53916.

Maas, K. W., Biere, S. S., Van Hoogstraten, I. M., Van Der Peet, D. L. & Cuesta, M. A. 2013. Immunological Changes After Minimally Invasive or Conventional Esophageal Resection for Cancer: A Randomized Trial. *World Journal of Surgery*.

Maciejczyk-Pencula, M., Polak, G. & Kotarski, J. 2004. Serum cortisol and acute phase protein concentrations after hysterectomy with and without peritoneal sutures. *European Journal of Obstetrics Gynecology and Reproductive Biology,* 113**,** 240-244.

Mackay, C., Ramsay, G., Rafferty, A. & Loudon, M. 2014. Impact of the Scottish Bowel Cancer Screening Programme on patient and tumour characteristics at a single centre. *J Eval Clin Pract,* 20**,** 7-11.

Mackay, G. J., Molloy, R. G. & O'Dwyer, P. J. 2011. C-reactive protein as a predictor of postoperative infective complications following elective colorectal resection. *Colorectal Disease,* 13**,** 583-7.

Maessen, J., Dejong, C. H., Hausel, J., Nygren, J., Lassen, K., Andersen, J., Kessels, A. G., Revhaug, A., Kehlet, H., Ljungqvist, O., Fearon, K. C. & Von Meyenfeldt, M. F. 2007. A protocol is not enough to implement an enhanced recovery programme for colorectal resection. *Br J Surg,* 94**,** 224-31.

Maillet, M., Dreanic, J., Dhooge, M., Mir, O., Brezault, C., Goldwasser, F., Chaussade, S. & Coriat, R. 2014. The predictive and prognostic value of the Glasgow Prognostic Score in metastatic colorectal carcinoma patients receiving bevacizumab. *Anticancer Drugs*.

Malietzis, G., Currie, A. C., Johns, N., Fearon, K. C., Darzi, A., Kennedy, R. H., Athanasiou, T. & Jenkins, J. T. 2016. Skeletal Muscle Changes After Elective Colorectal Cancer Resection: A Longitudinal Study. *Ann Surg Oncol,* 23**,** 2539-47.

Malik, E., Buchweitz, O., Muller-Steinhardt, M., Kressin, P., Meyhofer-Malik, A. & Diedrich, K. 2001. Prospective evaluation of the systemic immune response following abdominal, vaginal, and laparoscopically assisted vaginal hysterectomy. *Surgical Endoscopy and Other Interventional Techniques,* 15**,** 463-6.

Mansouri, D., McMillan, D. C., Crighton, E. M. & Horgan, P. G. 2013. Screening for colorectal cancer: what is the impact on the determinants of outcome? *Crit Rev Oncol Hematol,* 85**,** 342-9.

Marana, E., Russo, A., Colicci, S., Polidori, L., Bevilacqua, F., Viviani, D. & Di Stasio, E. 2013. Desflurane versus sevoflurane: a comparison on stress response. *Minerva Anestesiol,* 79**,** 7-14.

Marana, E., Scambia, G., Maussier, M. L., Parpaglioni, R., Ferrandina, G., Meo, F., Sciarra, M. & Marana, R. 2003. Neuroendocrine stress response in patients undergoing benign ovarian cyst surgery by laparoscopy, minilaparotomy, and laparotomy. *J Am Assoc Gynecol Laparosc,* 10**,** 159-65.

Marik, P. E. & Flemmer, M. 2012. The immune response to surgery and trauma: Implications for treatment. *J Trauma Acute Care Surg,* 73**,** 801-8.

Marret, E., Remy, C. & Bonnet, F. 2007. Meta-analysis of epidural analgesia versus parenteral opioid analgesia after colorectal surgery. *Br J Surg,* 94**,** 665-73.

Mathur, S., Plank, L. D., McCall, J. L., Shapkov, P., Mcilroy, K., Gillanders, L. K., Merrie, A. E. H., Torrie, J. J., Pugh, F., Koea, J. B., Bissett, I. P. & Parry, B. R. 2010. Randomized controlled trial of preoperative oral carbohydrate treatment in major abdominal surgery. *British Journal of Surgery,* 97**,** 485-494.

Matthiessen, P., Henriksson, M., Hallbook, O., Grunditz, E., Noren, B. & Arbman, G. 2008. Increase of serum C-reactive protein is an early indicator of subsequent symptomatic anastomotic leakage after anterior resection. *Colorectal Disease,* 10**,** 75-80.

Maund, E., Mcdaid, C., Rice, S., Wright, K., Jenkins, B. & Woolacott, N. 2011. Paracetamol and selective and non-selective non-steroidal anti-inflammatory drugs for the reduction in morphine-related side-effects after major surgery: a systematic review. *Br J Anaesth,* 106**,** 292-7.

McAllister, S. S. & Weinberg, R. A. 2014. The tumour-induced systemic environment as a critical regulator of cancer progression and metastasis. *Nat Cell Biol,* 16**,** 717-27.

McArdle, C. S. & Hole, D. 1991. Impact of variability among surgeons on postoperative morbidity and mortality and ultimate survival. *BMJ,* 302**,** 1501-5.

McArdle, C. S. & Hole, D. J. 2004. Emergency presentation of colorectal cancer is associated with poor 5-year survival. *Br J Surg,* 91**,** 605-9.

McArdle, C. S., McMillan, D. C. & Hole, D. J. 2005. Impact of anastomotic leakage on long-term survival of patients undergoing curative resection for colorectal cancer. *Br J Surg,* 92**,** 1150-4.

McDermott, F. D., Heeney, A., Kelly, M. E., Steele, R. J., Carlson, G. L. & Winter, D. C. 2015. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. *Br J Surg,* 102**,** 462-79.

McMahon, A. J., O'Dwyer, P. J., Cruikshank, A. M., McMillan, D. C., O'Reilly, D. S. J., Lowe, G. D. O., Rumley, A., Logan, R. W. & Baxter, J. N. 1993. Comparison of Metabolic Responses to Laparoscopic and Minilaparotomy Cholecystectomy. *British Journal of Surgery,* 80**,** 1255-1258.

McMillan, D. C. 2009. Systemic inflammation, nutritional status and survival in patients with cancer. *Curr Opin Clin Nutr Metab Care,* 12**,** 223-6.

McMillan, D. C. 2013a. Cancer and systemic inflammation: stage the tumour and stage the host. *Br J Cancer,* 109**,** 529.

McMillan, D. C. 2013b. The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. *Cancer Treat Rev,* 39**,** 534-40.

McPhail, M. J., Abu-Hilal, M. & Johnson, C. D. 2006. A meta-analysis comparing suprapubic and transurethral catheterization for bladder drainage after abdominal surgery. *Br J Surg,* 93**,** 1038-44.

McSorley, S. T., Horgan, P. G. & McMillan, D. C. 2016a. The impact of preoperative corticosteroids on the systemic inflammatory response and postoperative complications following surgery for gastrointestinal cancer: A systematic review and meta-analysis. *Crit Rev Oncol Hematol,* 101**,** 139-50.

McSorley, S. T., Ramanathan, M. L., Horgan, P. G. & McMillan, D. C. 2015. Postoperative C-reactive protein measurement predicts the severity of complications following surgery for colorectal cancer. *Int J Colorectal Dis,* 30**,** 913-7.

McSorley, S. T., Roxburgh, C. S., Horgan, P. G. & McMillan, D. C. 2017. The Impact of Preoperative Dexamethasone on the Magnitude of the Postoperative Systemic Inflammatory Response and Complications Following Surgery for Colorectal Cancer. *Ann Surg Oncol*.

McSorley, S. T., Watt, D. G., Horgan, P. G. & McMillan, D. C. 2016b. Postoperative Systemic Inflammatory Response, Complication Severity, and Survival Following Surgery for Colorectal Cancer. *Ann Surg Oncol,* 23**,** 2832-40.

Mehigan, B. J., Hartley, J. E., Drew, P. J., Saleh, A., Dore, P. C., Lee, P. W. & Monson, J. R. 2001. Changes in T cell subsets, interleukin-6 and C-reactive protein after laparoscopic and open colorectal resection for malignancy. *Surgical Endoscopy and Other Interventional Techniques,* 15**,** 1289-93.

Melnyk, M., Casey, R. G., Black, P. & Koupparis, A. J. 2011. Enhanced recovery after surgery (ERAS) protocols: Time to change practice? *Can Urol Assoc J,* 5**,** 342-8.

Mercury Study Group 2006. Diagnostic accuracy of preoperative magnetic resonance imaging in predicting curative resection of rectal cancer: prospective observational study. *BMJ,* 333**,** 779.

Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., Cesari, M. & Nourhashemi, F. 2013. Proinflammatory cytokines, aging, and age-related diseases. *J Am Med Dir Assoc,* 14**,** 877-82.

Miedema, B. W. & Johnson, J. O. 2003. Methods for decreasing postoperative gut dysmotility. *Lancet Oncol,* 4**,** 365-72.

Milheiro, A., Sousa, F. C., Manso, E. C. & Leitao, F. 1994. Metabolic Responses to Cholecystectomy - Open Vs Laparoscopic Approach. *J Laparoendosc Surg,* 4**,** 311-317.

Minetto, M. A., Oprandi, G., Saba, L., Mussino, S., Aprato, A., Masse, A., Angeli, A. & Gallinaro, P. 2006. Serum interleukin-6 response to elective total hip replacement surgery. *International Orthopaedics,* 30**,** 172-176.

Mirnezami, A., Mirnezami, R., Chandrakumaran, K., Sasapu, K., Sagar, P. & Finan, P. 2011. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. *Ann Surg,* 253**,** 890-9.

Mirzayan, M. J., Gharabaghi, A., Samii, M., Tatagiba, M., Krauss, J. K. & Rosahl, S. K. 2007. Response of C-reactive protein after craniotomy for microsurgery of intracranial tumors. *Neurosurgery,* 60**,** 621-625.

Mlecnik, B., Tosolini, M., Kirilovsky, A., Berger, A., Bindea, G., Meatchi, T., Bruneval, P., Trajanoski, Z., Fridman, W. H., Pages, F. & Galon, J. 2011. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol,* 29**,** 610-8.

Moghaddam, A. A., Woodward, M. & Huxley, R. 2007. Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events. *Cancer Epidemiol Biomarkers Prev,* 16**,** 2533-47.

Mohri, Y., Miki, C., Kobayashi, M., Okita, Y., Inoue, M., Uchida, K., Tanaka, K., Inoue, Y. & Kusunoki, M. 2014. Correlation between preoperative systemic inflammation and postoperative infection in patients with gastrointestinal cancer: a multicenter study. *Surg Today,* 44**,** 859-67.

Mok, J. M., Pekmezci, M., Piper, S. L., Boyd, E., Berven, S. H., Burch, S., Deviren, V., Tay, B. & Hu, S. S. 2008. Use of C-reactive protein after spinal surgery - Comparison with erythrocyte sedimentation rate as predictor of early postoperative infectious complications. *Spine,* 33**,** 415-421.

Mollhoff, T., Loick, H. M., Van Aken, H., Schmidt, C., Rolf, N., Tjan, T. D., Asfour, B. & Berendes, E. 1999. Milrinone modulates endotoxemia, systemic inflammation, and subsequent acute phase response after cardiopulmonary bypass (CPB). *Anesthesiology,* 90**,** 72-80.

Moreschini, O., Greggi, G., Giordano, M. C., Nocente, M. & Margheritini, F. 2001. Postoperative physiopathological analysis of inflammatory parameters in patients undergoing hip or knee arthroplasty. *Int J Tissue React,* 23**,** 151-4.

Morikage, N., Esato, K., Zenpo, N., Fujioka, K. & Takenaka, H. 2000. Is endovascular treatment of abdominal aortic aneurysms less invasive regarding the biological responses? *Surgery Today-the Japanese Journal of Surgery,* 30**,** 142-146.

Morris, E., Haward, R. A., Gilthorpe, M. S., Craigs, C. & Forman, D. 2006a. The impact of the Calman-Hine report on the processes and outcomes of care for Yorkshire's colorectal cancer patients. *Br J Cancer,* 95**,** 979-85.

Morris, E. J., Maughan, N. J., Forman, D. & Quirke, P. 2007. Who to treat with adjuvant therapy in Dukes B/stage II colorectal cancer? The need for high quality pathology. *Gut,* 56**,** 1419-25.

Morris, M., Platell, C., De Boer, B., McCaul, K. & Iacopetta, B. 2006b. Population-based study of prognostic factors in stage II colonic cancer. *Br J Surg,* 93**,** 866-71.

Mortensen, K., Nilsson, M., Slim, K., Schafer, M., Mariette, C., Braga, M., Carli, F., Demartines, N., Griffin, S. M., Lassen, K. & Enhanced Recovery after Surgery, G. 2014. Consensus guidelines for enhanced recovery after gastrectomy: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *Br J Surg,* 101**,** 1209-29.

Motallebnezhad, M., Jadidi-Niaragh, F., Qamsari, E. S., Bagheri, S., Gharibi, T. & Yousefi, M. 2016. The immunobiology of myeloid-derived suppressor cells in cancer. *Tumour Biol,* 37**,** 1387-406.

Moyes, L. H., Leitch, E. F., Mckee, R. F., Anderson, J. H., Horgan, P. G. & McMillan, D. C. 2009. Preoperative systemic inflammation predicts postoperative infectious complications in patients undergoing curative resection for colorectal cancer. *Br J Cancer,* 100**,** 1236-9.

Muir, C. S. & Parkin, D. M. 1985. The world cancer burden: prevent or perish. *Br Med J (Clin Res Ed),* 290**,** 5-6.

Munoz, M., Garcia-Vallejo, J. J., Sempere, J. M., Romero, R., Olalla, E. & Sebastian, C. 2004. Acute phase response in patients undergoing lumbar spinal surgery: modulation by perioperative treatment with naproxen and famotidine. *European Spine Journal,* 13**,** 367-373.

Murdoch, C., Muthana, M., Coffelt, S. B. & Lewis, C. E. 2008. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer,* 8**,** 618-31.

Muzii, L., Marana, R., Marana, E., Paielli, F. V., Meo, F., Maussier, M. L., Sciarra, M. & Mancuso, S. 1996. Evaluation of stress-related hormones after surgery by laparoscopy or laparotomy. *J Am Assoc Gynecol Laparosc,* 3**,** 229-34.

Mynster, T., Christensen, I. J., Moesgaard, F. & Nielsen, H. J. 2000. Effects of the combination of blood transfusion and postoperative infectious complications on prognosis after surgery for colorectal cancer. Danish RANX05 Colorectal Cancer Study Group. *Br J Surg,* 87**,** 1553-62.

Nagahiro, I., Andou, A., Aoe, M., Sano, Y., Date, H. & Shimizu, N. 2001. Pulmonary function, postoperative pain, and serum cytokine level after lobectomy: a comparison of VATS and conventional procedure. *Annals of Thoracic Surgery,* 72**,** 362-5.

Nagtegaal, I. D. & Quirke, P. 2008. What is the role for the circumferential margin in the modern treatment of rectal cancer? *J Clin Oncol,* 26**,** 303-12.

Nelson, R., Edwards, S. & Tse, B. 2007. Prophylactic nasogastric decompression after abdominal surgery. *Cochrane Database Syst Rev***,** CD004929.

Nelson, R. L., Gladman, E. & Barbateskovic, M. 2014. Antimicrobial prophylaxis for colorectal surgery. *Cochrane Database Syst Rev,* 5**,** CD001181.

Neumaier, M., Metak, G. & Scherer, M. A. 2006. C-reactive protein as a parameter of surgical trauma - CRP response after different types of surgery in 349 hip fractures. *Acta Orthopaedica,* 77**,** 788-790.

Neville, A., Lee, L., Antonescu, I., Mayo, N. E., Vassiliou, M. C., Fried, G. M. & Feldman, L. S. 2014. Systematic review of outcomes used to evaluate enhanced recovery after surgery. *Br J Surg,* 101**,** 159-70.

Ng, C. S., Wei, W., Doyle, T. C., Courtney, H. M., Dixon, A. K. & Freeman, A. H. 2008. Minimal-preparation abdomino-pelvic CT in frail and elderly patients: prognostic value of colonic and extracolonic findings. *Clin Radiol,* 63**,** 424-32.

Ng, C. S. H., Whelan, R. L., Lacy, A. M. & Yim, A. P. C. 2005. Is minimal access surgery for cancer associated with immunologic benefits? *World Journal of Surgery,* 29**,** 975-981.

Ng, S. S., Leung, K. L., Lee, J. F., Yiu, R. Y., Li, J. C. & Hon, S. S. 2009. Long-term morbidity and oncologic outcomes of laparoscopic-assisted anterior resection for upper rectal cancer: ten-year results of a prospective, randomized trial. *Dis Colon Rectum,* 52**,** 558-66.

Nguyen, N. T., Goldman, C. D., Ho, H. S., Gosselin, R. C., Singh, A. & Wolfe, B. M. 2002. Systemic stress response after laparoscopic and open gastric bypass. *Journal of the American College of Surgeons,* 194**,** 557-566.

NICE. 2011. *131 - Colorectal Cancer: and management* [Online]. Available: https://www.nice.org.uk/Guidance/CG131.

NICE. 2006. *Capecitabine and oxaliplatin in the adjuvant treatment of stage III (Dukes' C) colon cancer* [Online]. Available: https://www.nice.org.uk/guidance/ta100/chapter/2-Clinical-need-and-practice.

Nicholson, A., Lowe, M. C., Parker, J., Lewis, S. R., Alderson, P. & Smith, A. F. 2014. Systematic review and meta-analysis of enhanced recovery programmes in surgical patients. *Br J Surg,* 101**,** 172-88.

Nielsen, J. D., Gram, J., Holm-Nielsen, A. & Jespersen, J. 1999. Bleeding and activation of coagulation during and after transurethral prostatectomy: importance of the acute-phase response and prostate specific antigen? *BJU Int,* 83**,** 990-5.

Nishiguchi, K., Okuda, J., Toyoda, M., Tanaka, K. & Tanigawa, N. 2001. Comparative evaluation of surgical stress of laparoscopic and open surgeries for colorectal carcinoma. *Diseases of the Colon & Rectum,* 44**,** 223-30.

Niskanen, R. O., Korkala, O. & Pammo, H. 1996. Serum C-reactive protein levels after total hip and knee arthroplasty. *J Bone Joint Surg Br,* 78**,** 431-3.

Noblett, S. E., Snowden, C. P., Shenton, B. K. & Horgan, A. F. 2006. Randomized clinical trial assessing the effect of Doppler-optimized fluid management on outcome after elective colorectal resection. *Br J Surg,* 93**,** 1069-76.

Norman, J. G. & Fink, G. W. 1997. The effects of epidural anesthesia on the neuroendocrine response to major surgical stress: a randomized prospective trial. *Am Surg,* 63**,** 75-80.

Noto, H., Goto, A., Tsujimoto, T. & Noda, M. 2012. Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. *PLoS One,* 7**,** e33411.

Nygren, J., Thacker, J., Carli, F., Fearon, K. C., Norderval, S., Lobo, D. N., Ljungqvist, O., Soop, M., Ramirez, J. & Enhanced Recovery after Surgery, S. 2012. Guidelines for perioperative care in elective rectal/pelvic surgery: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations. *Clin Nutr,* 31**,** 801-16.

O'Leary, D. P., O'leary, E., Foley, N., Cotter, T. G., Wang, J. H. & Redmond, H. P. 2016. Effects of surgery on the cancer stem cell niche. *Eur J Surg Oncol*.

Odegard, A., Lundbom, J., Myhre, H. O., Hatlinghus, S., Bergh, K., Waage, A., Bjerve, K. S., Mollnes, T. E., Aadahl, P., Lie, T. A. & Videm, V. 2000. The inflammatory response following treatment of abdominal aortic aneurysms: a comparison between open surgery and endovascular repair. *European Journal of Vascular and Endovascular Surgery,* 19**,** 536-544.

Okafor, B. & MacLellan, G. 1998. Postoperative changes of erythrocyte sedimentation rate, plasma viscosity and C-reactive protein levels after hip surgery. *Acta Orthop Belg,* 64**,** 52-6.

Oliphant, R., Horgan, P. G., Morrison, D. S., McMillan, D. C. & In Collaboration with the West of Scotland Colorectal Cancer Managed Clinical, N. 2014a. Validation of a modified clinical risk score to predict cancer-specific survival for stage II colon cancer. *Cancer Med*.

Oliphant, R., Mansouri, D., Nicholson, G. A., McMillan, D. C., Horgan, P. G., Morrison, D. S. & West of Scotland Colorectal Cancer Managed Clinical, N. 2014b. Emergency presentation of node-negative colorectal cancer treated with curative surgery is associated with poorer short and longer-term survival. *Int J Colorectal Dis,* 29**,** 591-8.

Oliphant, R., Nicholson, G. A., Horgan, P. G., Molloy, R. G., McMillan, D. C., Morrison, D. S. & West of Scotland Colorectal Cancer Managed Clinical, N. 2013. Contribution of surgical specialization to improved colorectal cancer survival. *Br J Surg,* 100**,** 1388-95.

Ordemann, J., Jacobi, C. A., Schwenk, W., Stosslein, R. & Muller, J. M. 2001. Cellular and humoral inflammatory response after laparoscopic and conventional colorectal resections. *Surgical Endoscopy and Other Interventional Techniques,* 15**,** 600-8.

Orrego, L. M., Perez, C. M., Perez, Y. M., Cheyre, E. J. & Mardones, P. R. 2005. [Plasma C reactive protein in elective orthopedic surgery]. *Rev Med Chil,* 133**,** 1341-8.

Ortega, A. E., Peters, J. H., Incarbone, R., Estrada, L., Ehsan, A., Kwan, Y., Spencer, C. J., Moore-Jeffries, E., Kuchta, K. & Nicoloff, J. T. 1996. A prospective randomized comparison of the metabolic and stress hormonal responses of laparoscopic and open cholecystectomy. *J Am Coll Surg,* 183**,** 249-56.

Ozawa, A., Konishi, F., Nagai, H., Okada, M. & Kanazawa, K. 2000. Cytokine and hormonal responses in laparoscopic-assisted colectomy and conventional open colectomy. *Surgery Today-the Japanese Journal of Surgery,* 30**,** 107-11.

Pan, W., Sun, Q., Wang, Y., Wang, J., Cao, S. & Ren, X. 2015. Highlights on mechanisms of drugs targeting MDSCs: providing a novel perspective on cancer treatment. *Tumour Biol,* 36**,** 3159-69.

Papadima, A., Boutsikou, M., Lagoudianakis, E. E., Kataki, A., Konstadoulakis, M., Georgiou, L., Katergiannakis, V. & Manouras, A. 2009. Lymphocyte apoptosis after major abdominal surgery is not influenced by anesthetic technique: a comparative study of general anesthesia versus combined general and epidural analgesia. *Journal of Clinical Anesthesia,* 21**,** 414-21.

Park, J. H., McMillan, D. C., Horgan, P. G. & Roxburgh, C. S. 2014. The impact of anti-inflammatory agents on the outcome of patients with colorectal cancer. *Cancer Treat Rev,* 40**,** 68-77.

Park, J. H., Watt, D. G., Roxburgh, C. S., Horgan, P. G. & McMillan, D. C. 2015. Colorectal Cancer, Systemic Inflammation, and Outcome: Staging the Tumor and Staging the Host. *Ann Surg*.

Parkin, J. & Cohen, B. 2001. An overview of the immune system. *Lancet,* 357**,** 1777-89.

Parry-Billings, M., Baigrie, R. J., Lamont, P. M., Morris, P. J. & Newsholme, E. A. 1992. Effects of major and minor surgery on plasma glutamine and cytokine levels. *Arch Surg,* 127**,** 1237-40.

Pascual, M., Alonso, S., Pares, D., Courtier, R., Gil, M. J., Grande, L. & Pera, M. 2011. Randomized clinical trial comparing inflammatory and angiogenic response after open versus laparoscopic curative resection for colonic cancer. *British Journal of Surgery,* 98**,** 50-59.

Pathak, S., Nunes, Q. M., Daniels, I. R. & Smart, N. J. 2014. Is C-reactive protein useful in prognostication for colorectal cancer? A systematic review. *Colorectal Dis,* 16**,** 769-76.

Pepys, M. B. & Hirschfield, G. M. 2003. C-reactive protein: a critical update. *Journal of Clinical Investigation,* 111**,** 1805-1812.

Persec, J., Persec, Z. & Husedzinovic, I. 2009. Postoperative pain and systemic inflammatory stress response after preoperative analgesia with clonidine or levobupivacaine: a randomized controlled trial. *Wien Klin Wochenschr,* 121**,** 558-63.

Petersen, V. C., Baxter, K. J., Love, S. B. & Shepherd, N. A. 2002. Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer. *Gut,* 51**,** 65-9.

Pirbudak, L., Balat, O., Cekmen, M., Ugur, M. G., Aygun, S. & Oner, U. 2004. Effect of ascorbic acid on surgical stress response in gynecologic surgery. *International Journal of Clinical Practice,* 58**,** 928-31.

Platt, J. J., Ramanathan, M. L., Crosbie, R. A., Anderson, J. H., McKee, R. F., Horgan, P. G. & McMillan, D. C. 2012. C-reactive protein as a predictor of postoperative infective complications after curative resection in patients with colorectal cancer. *Ann Surg Oncol,* 19**,** 4168-77.

Poschl, G. & Seitz, H. K. 2004. Alcohol and cancer. *Alcohol Alcohol,* 39**,** 155-65.

Proctor, M. J., Horgan, P. G., Talwar, D., Fletcher, C. D., Morrison, D. S. & McMillan, D. C. 2013. Optimization of the systemic inflammation-based Glasgow prognostic score: a Glasgow Inflammation Outcome Study. *Cancer,* 119**,** 2325-32.

Proctor, M. J., McMillan, D. C., Horgan, P. G., Fletcher, C. D., Talwar, D. & Morrison, D. S. 2015. Systemic inflammation predicts all-cause mortality: a glasgow inflammation outcome study. *PLoS One,* 10**,** e0116206.

Proctor, M. J., McMillan, D. C., Morrison, D. S., Fletcher, C. D., Horgan, P. G. & Clarke, S. J. 2012. A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. *Br J Cancer,* 107**,** 695-9.

Proctor, M. J., Morrison, D. S., Talwar, D., Balmer, S. M., Fletcher, C. D., O'reilly, D. S., Foulis, A. K., Horgan, P. G. & McMillan, D. C. 2011a. A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer,* 47**,** 2633-41.

Proctor, M. J., Morrison, D. S., Talwar, D., Balmer, S. M., O'Reilly, D. S., Foulis, A. K., Horgan, P. G. & McMillan, D. C. 2011b. An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study. *Br J Cancer,* 104**,** 726-34.

Proctor, M. J., Talwar, D., Balmar, S. M., O'Reilly, D. S., Foulis, A. K., Horgan, P. G., Morrison, D. S. & McMillan, D. C. 2010. The relationship between the presence and site of cancer, an inflammation-based prognostic score and biochemical parameters. Initial results of the Glasgow Inflammation Outcome Study. *Br J Cancer,* 103**,** 870-6.

Pucher, P. H., Aggarwal, R., Qurashi, M. & Darzi, A. 2014. Meta-analysis of the effect of postoperative in-hospital morbidity on long-term patient survival. *Br J Surg,* 101**,** 1499-508.

Puleo, F. J., Mishra, N. & Hall, J. F. 2013. Use of intra-abdominal drains. *Clin Colon Rectal Surg,* 26**,** 174-7.

Purkayastha, S., Tekkis, P. P., Athanasiou, T., Tilney, H. S., Darzi, A. W. & Heriot, A. G. 2007. Diagnostic precision of magnetic resonance imaging for preoperative prediction of the circumferential margin involvement in patients with rectal cancer. *Colorectal Dis,* 9**,** 402-11.

Rahman, S. H., Evans, J., Toogood, G. J., Lodge, P. A. & Prasad, K. R. 2008. Prognostic utility of postoperative C-reactive protein for posthepatectomy liver failure. *Arch Surg,* 143**,** 247-53; discussion 253.

Ramanathan, M. L., Mackay, G., Platt, J., Horgan, P. G. & McMillan, D. C. 2013. Impact of day 2 C-reactive protein on day 3 and 4 thresholds associated with infective complications following curative surgery for colorectal cancer. *World J Surg,* 37**,** 2705-10.

Ramanathan, M. L., Mackay, G., Platt, J., Horgan, P. G. & McMillan, D. C. 2015. The impact of open versus laparoscopic resection for colon cancer on C-reactive protein concentrations as a predictor of postoperative infective complications. *Ann Surg Oncol,* 22**,** 938-43.

Rao, W., Zhang, X., Zhang, J., Yan, R., Hu, Z. & Wang, Q. 2011. The role of nasogastric tube in decompression after elective colon and rectum surgery: a meta-analysis. *Int J Colorectal Dis,* 26**,** 423-9.

Rawlinson, A., Kang, P., Evans, J. & Khanna, A. 2011. A systematic review of enhanced recovery protocols in colorectal surgery. *Ann R Coll Surg Engl,* 93**,** 583-8.

RCPath. 2014. *Dataset for Colorectal Cancer Histopathology Reports* [Online]. Available: https://www.rcpath.org/resourceLibrary/dataset-for-colorectal-cancer-histopathology-reports--3rd-edition-.html.

Ren, L., Zhu, D., Wei, Y., Pan, X., Liang, L., Xu, J., Zhong, Y., Xue, Z., Jin, L., Zhan, S., Niu, W., Qin, X. & Wu, Z. 2012. Enhanced Recovery After Surgery (ERAS) program attenuates stress and accelerates recovery in patients after radical resection for colorectal cancer: a prospective randomized controlled trial. *World Journal of Surgery,* 36**,** 407-14.

Rennard, S. I., Dale, D. C., Donohue, J. F., Kanniess, F., Magnussen, H., Sutherland, E. R., Watz, H., Lu, S., Stryszak, P., Rosenberg, E. & Staudinger, H. 2015. CXCR2 Antagonist MK-7123. A Phase 2 Proof-of-Concept Trial for Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med,* 191**,** 1001-11.

Retsky, M. W., Demicheli, R., Hrushesky, W. J., Baum, M. & Gukas, I. D. 2008. Dormancy and surgery-driven escape from dormancy help explain some clinical features of breast cancer. *APMIS,* 116**,** 730-41.

Reza, M. M., Blasco, J. A., Andradas, E., Cantero, R. & Mayol, J. 2006. Systematic review of laparoscopic versus open surgery for colorectal cancer. *Br J Surg,* 93**,** 921-8.

Ribeiro, S. C., Ribeiro, R. M., Santos, N. C. & Pinotti, J. A. 2003. A randomized study of total abdominal, vaginal and laparoscopic hysterectomy. *International Journal of Gynecology & Obstetrics,* 83**,** 37-43.

Richards, C. H., Flegg, K. M., Roxburgh, C. S., Going, J. J., Mohammed, Z., Horgan, P. G. & McMillan, D. C. 2012a. The relationships between cellular components of the peritumoural inflammatory response, clinicopathological characteristics and survival in patients with primary operable colorectal cancer. *Br J Cancer,* 106**,** 2010-5.

Richards, C. H., Platt, J. J., Anderson, J. H., McKee, R. F., Horgan, P. G. & McMillan, D. C. 2011. The impact of perioperative risk, tumor pathology and surgical complications on disease recurrence following potentially curative resection of colorectal cancer. *Ann Surg,* 254**,** 83-9.

Richards, C. H., Roxburgh, C. S., Anderson, J. H., McKee, R. F., Foulis, A. K., Horgan, P. G. & McMillan, D. C. 2012b. Prognostic value of tumour necrosis and host inflammatory responses in colorectal cancer. *Br J Surg,* 99**,** 287-94.

Richards, C. H., Roxburgh, C. S., MacMillan, M. T., Isswiasi, S., Robertson, E. G., Guthrie, G. K., Horgan, P. G. & McMillan, D. C. 2012c. The relationships between body composition and the systemic inflammatory response in patients with primary operable colorectal cancer. *PLoS One,* 7**,** e41883.

Richards, C. H., Roxburgh, C. S., Powell, A. G., Foulis, A. K., Horgan, P. G. & McMillan, D. C. 2014. The clinical utility of the local inflammatory response in colorectal cancer. *Eur J Cancer,* 50**,** 309-19.

Rorarius, M. G., Kujansuu, E., Baer, G. A., Suominen, P., Teisala, K., Miettinen, A., Ylitalo, P. & Laippala, P. 2001. Laparoscopically assisted vaginal and abdominal hysterectomy: comparison of postoperative pain, fatigue and systemic response. A case-control study. *Eur J Anaesthesiol,* 18**,** 530-9.

Rosman, A. S. & Korsten, M. A. 2007. Meta-analysis comparing CT colonography, air contrast barium enema, and colonoscopy. *Am J Med,* 120**,** 203-210 e4.

Roth-Isigkeit, A., Brechmann, J., Dibbelt, L., Sievers, H. H., Raasch, W. & Schmucker, P. 1998. Persistent endocrine stress response in patients undergoing cardiac surgery. *J Endocrinol Invest,* 21**,** 12-9.

Rothwell, P. M., Wilson, M., Price, J. F., Belch, J. F., Meade, T. W. & Mehta, Z. 2012. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet,* 379**,** 1591-601.

Roumen, R. M. H., Vanmeurs, P. A., Kuypers, H. H. C., Kraak, W. a. G. & Sauerwein, R. W. 1992. Serum Interleukin-6 and Interleukin-C Reactive Protein Responses in Patients after Laparoscopic or Conventional Cholecystectomy. *European Journal of Surgery,* 158**,** 541-544.

Rowlands, T. E. & Homer-Vanniasinkam, S. 2001. Pro- and anti-inflammatory cytokine release in open versus endovascular repair of abdominal aortic aneurysm. *British Journal of Surgery,* 88**,** 1335-1340.

Roxburgh, C. S., Horgan, P. G. & McMillan, D. C. 2013. The perioperative immune/inflammatory insult in cancer surgery: Time for intervention? *Oncoimmunology,* 2**,** e27324.

Roxburgh, C. S. & McMillan, D. C. 2010. Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. *Future Oncol,* 6**,** 149-63.

Roxburgh, C. S. & McMillan, D. C. 2012. The role of the in situ local inflammatory response in predicting recurrence and survival in patients with primary operable colorectal cancer. *Cancer Treat Rev,* 38**,** 451-66.

Roxburgh, C. S. & McMillan, D. C. 2016. Therapeutics targeting innate immune/inflammatory responses through the interleukin-6/JAK/STAT signal transduction pathway in patients with cancer. *Transl Res,* 167**,** 61-6.

Roxburgh, C. S., McMillan, D. C., Anderson, J. H., Mckee, R. F., Horgan, P. G. & Foulis, A. K. 2010a. Elastica staining for venous invasion results in superior prediction of cancer-specific survival in colorectal cancer. *Ann Surg,* 252**,** 989-97.

Roxburgh, C. S., Salmond, J. M., Horgan, P. G., Oien, K. A. & McMillan, D. C. 2009. Tumour inflammatory infiltrate predicts survival following curative resection for node-negative colorectal cancer. *Eur J Cancer,* 45**,** 2138-45.

Roxburgh, C. S., Wallace, A. M., Guthrie, G. K., Horgan, P. G. & McMillan, D. C. 2010b. Comparison of the prognostic value of tumour- and patient-related factors in patients undergoing potentially curative surgery for colon cancer. *Colorectal Dis,* 12**,** 987-94.

Roy, K. K., Subbaiah, M., Singla, S., Kumar, S., Sharma, J. B. & Mitra, D. K. 2012. Role of serum interleukin-6 in comparing surgical stress after laparoscopic-assisted

vaginal hysterectomy and non-descent vaginal hysterectomy for large uteri. *Arch Gynecol Obstet,* 285**,** 671-6.

Sakuramoto, S., Kikuchi, S., Futawatari, N., Katada, N., Moriya, H., Hirai, K., Yamashita, K. & Watanabe, M. 2009. Laparoscopy-assisted pancreas- and spleen-preserving total gastrectomy for gastric cancer as compared with open total gastrectomy. *Surgical Endoscopy and Other Interventional Techniques,* 23**,** 2416-2423.

Salvans, S., Mayol, X., Alonso, S., Messeguer, R., Pascual, M., Mojal, S., Grande, L. & Pera, M. 2014. Postoperative peritoneal infection enhances migration and invasion capacities of tumor cells in vitro: an insight into the association between anastomotic leak and recurrence after surgery for colorectal cancer. *Ann Surg,* 260**,** 939-44.

Sanchez, M. A., Thomas, C. B. & O'Neal, H. R. 2012. Do aspirin and statins prevent severe sepsis? *Curr Opin Infect Dis,* 25**,** 345-50.

Scarborough, J. E., Mantyh, C. R., Sun, Z., Migaly, J. 2015. Combined mechanical and oral antibiotic bowel preparation reduces incisional surgical site infection and anastomotic leak rates after elective colorectal resection. *Ann Surg*; 262: 331-337.

Schietroma, M., Carlei, F., Cappelli, S., Pescosolido, A., Lygidakis, N. J. & Amicucci, G. 2007. Effects of cholecystectomy (laparoscopic versus open) on PMN-elastase. *Hepatogastroenterology,* 54**,** 342-5.

Schietroma, M., Carlei, F., Cecilia, E. M., Piccione, F., Sista, F., De Vita, F. & Amicucci, G. 2013. A prospective randomized study of systemic inflammation and immune response after laparoscopic nissen fundoplication performed with standard and low-pressure pneumoperitoneum. *Surg Laparosc Endosc Percutan Tech,* 23**,** 189-96.

Schietroma, M., Carlei, F., Franchi, L., Mazzotta, C., Sozio, A., Lygidakis, N. J. & Amicucci, G. 2004a. A comparison of serum interleukin-6 concentrations in patients treated by cholecystectomy via laparotomy or laparoscopy. *Hepatogastroenterology,* 51**,** 1595-9.

Schietroma, M., Carlei, F., Lezoche, E., Agnifili, A., Enang, G. N., Mattucci, S., Minervini, S. & Lygidakis, N. J. 2001. Evaluation of immune response in patients after open or laparoscopic cholecystectomy. *Hepatogastroenterology,* 48**,** 642-6.

Schietroma, M., Carlei, F., Mownah, A., Franchi, L., Mazzotta, C., Sozio, A. & Amicucci, G. 2004b. Changes in the blood coagulation, fibrinolysis, and cytokine profile during laparoscopic and open cholecystectomy. *Surgical Endoscopy and Other Interventional Techniques,* 18**,** 1090-6.

Schlussel, A. T., Gagliano, R. A., Jr., Seto-Donlon, S., Eggerding, F., Donlon, T., Berenberg, J. & Lynch, H. T. 2014. The evolution of colorectal cancer genetics-Part 1: from discovery to practice. *J Gastrointest Oncol,* 5**,** 326-35.

Schmidt, H., Bastholt, L., Geertsen, P., Christensen, I. J., Larsen, S., Gehl, J. & Von Der Maase, H. 2005. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. *Br J Cancer,* 93**,** 273-8.

Schmidt, S. C., Hamann, S., Langrehr, J. M., Hoflich, C., Mittler, J., Jacob, D. & Neuhaus, P. 2007. Preoperative high-dose steroid administration attenuates the surgical stress response following liver resection: results of a prospective randomized study. *Journal of Hepato-Biliary-Pancreatic Surgery,* 14**,** 484-492.

Schrenk, P., Bettelheim, P., Woisetschlager, R., Rieger, R. & Wayand, W. U. 1996. Metabolic responses after laparoscopic or open hernia repair. *Surgical Endoscopy-Ultrasound and Interventional Techniques,* 10**,** 628-632.

Schulze, S., Sommer, P., Bigler, D., Honnens, M., Shenkin, A., Cruickshank, A. M., Bukhave, K. & Kehlet, H. 1992. Effect of combined prednisolone, epidural analgesia, and indomethacin on the systemic response after colonic surgery. *Arch Surg,* 127**,** 325-31.

Schwenk, W., Haase, O., Neudecker, J. & Muller, J. M. 2005. Short term benefits for laparoscopic colorectal resection. *Cochrane Database Syst Rev***,** CD003145.

Schwenk, W., Jacobi, C., Mansmann, U., Bohm, B. & Muller, J. M. 2000. Inflammatory response after laparoscopic and conventional colorectal resections - results of a prospective randomized trial. *Langenbecks Archives of Surgery,* 385**,** 2-9.

Scott, H. R., McMillan, D. C., Forrest, L. M., Brown, D. J., McArdle, C. S. & Milroy, R. 2002. The systemic inflammatory response, weight loss, performance status and survival in patients with inoperable non-small cell lung cancer. *Br J Cancer,* 87**,** 264-7.

Segal, A. W. 2005. How neutrophils kill microbes. *Annu Rev Immunol,* 23**,** 197-223.

Servis, D., Busic, Z., Stipancic, I., Patrlj, L. & Gagro, A. 2008. Serum cytokine changes after gastric resection or gastrectomy for gastric cancer. *Hepatogastroenterology,* 55**,** 1868-72.

Sessler, D. I. 2000. Perioperative heat balance. *Anesthesiology,* 92**,** 578-96.

Shih, L. Y., Wu, J. J. & Yang, D. J. 1987. Erythrocyte sedimentation rate and C-reactive protein values in patients with total hip arthroplasty. *Clin Orthop Relat Res***,** 238-46.

Shinko D., Diakos C. I., Clarke S. J. & Charles K. A. 2017. Cancer-related systemic inflammation: The challenges and therapeutic opportunities for personalised medicine. *Clin Pharmacol Ther, [epub ahead of print]*.

Sietses, C., Wiezer, M. J., Eijsbouts, Q. A., Beelen, R. H., Van Leeuwen, P. A., Von Blomberg, B. M., Meijer, S. & Cuesta, M. A. 1999. A prospective randomized study of the systemic immune response after laparoscopic and conventional Nissen fundoplication. *Surgery,* 126**,** 5-9.

Simmonds, P. C. 2000. Palliative chemotherapy for advanced colorectal cancer: systematic review and meta-analysis. Colorectal Cancer Collaborative Group. *BMJ,* 321**,** 531-5.

Singh, P. P., Zeng, I. S., Srinivasa, S., Lemanu, D. P., Connolly, A. B. & Hill, A. G. 2014. Systematic review and meta-analysis of use of serum C-reactive protein levels to predict anastomotic leak after colorectal surgery. *Br J Surg,* 101**,** 339-46.

Singh, S., Singh, H., Singh, P. P., Murad, M. H. & Limburg, P. J. 2013. Antidiabetic medications and the risk of colorectal cancer in patients with diabetes mellitus: a systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev,* 22**,** 2258-68.

Sista, F., Schietroma, M., Carlei, F., Cecilia, E. M., Piccione, F., De Santis, G., Lancione, L., Iannucci, D., Giuliani, A., Amicucci, G. & Leardi, S. 2013a. The neutrophils response after laparoscopic and open cholecystectomy. *Ann Ital Chir,* 84**,** 153-8.

Sista, F., Schietroma, M., Santis, G. D., Mattei, A., Cecilia, E. M., Piccione, F., Leardi, S., Carlei, F. & Amicucci, G. 2013b. Systemic inflammation and immune response after laparotomy vs laparoscopy in patients with acute cholecystitis, complicated by peritonitis. *World J Gastrointest Surg,* 5**,** 73-82.

Smeets, H. J., Kievit, J., Dulfer, F. T. & Van Kleef, J. W. 1993. Endocrine-metabolic response to abdominal aortic surgery: a randomized trial of general anesthesia versus general plus epidural anesthesia. *World Journal of Surgery,* 17**,** 601-6; discussion 606-7.

Smith, C., Erasmus, P. J. & Myburgh, K. H. 2006. Endocrine and immune effects of dexamethasone in unilateral total knee replacement. *Journal of International Medical Research,* 34**,** 603-11.

Smith, M. D., Mccall, J., Plank, L., Herbison, G. P., Soop, M. & Nygren, J. 2014. Preoperative carbohydrate treatment for enhancing recovery after elective surgery. *Cochrane Database Syst Rev,* 8**,** CD009161.

Solomon, M. J., Young, C. J., Eyers, A. A. & Roberts, R. A. 2002. Randomized clinical trial of laparoscopic versus open abdominal rectopexy for rectal prolapse. *British Journal of Surgery,* 89**,** 35-39.

Spanjersberg, W. R., Reurings, J., Keus, F. & Van Laarhoven, C. J. H. M. 2011. Fast track surgery versus conventional recovery strategies for colorectal surgery. *Cochrane Database of Systematic Reviews*.

SPHO. 2014. *Scottish Public Health Observatory - Colorectal Cancer: Key Points* [Online]. Available: http://www.scotpho.org.uk/health-wellbeing-and-disease/cancer-colorectal/key-points.

Sreeramkumar, V., Adrover, J. M., Ballesteros, I., Cuartero, M. I., Rossaint, J., Bilbao, I., Nacher, M., Pitaval, C., Radovanovic, I., Fukui, Y., Mcever, R. P., Filippi, M. D., Lizasoain, I., Ruiz-Cabello, J., Zarbock, A., Moro, M. A. & Hidalgo, A. 2014. Neutrophils scan for activated platelets to initiate inflammation. *Science,* 346**,** 1234-8.

Sridhar, P., Sistla, S. C., Ali, S. M., Karthikeyan, V. S., Badhe, A. S. & Ananthanarayanan, P. H. 2014. Effect of intravenous lignocaine on perioperative stress response and post-surgical ileus in elective open abdominal surgeries: a double-blind randomized controlled trial. *ANZ J Surg*.

Srinivasa, S., Kahokehr, A. A., Yu, T. C. & Hill, A. G. 2011. Preoperative glucocorticoid use in major abdominal surgery: systematic review and meta-analysis of randomized trials. *Ann Surg,* 254**,** 183-91.

Srinivasa, S., Lemanu, D. P., Singh, P. P., Taylor, M. H. & Hill, A. G. 2013. Systematic review and meta-analysis of oesophageal Doppler-guided fluid management in colorectal surgery. *Br J Surg,* 100**,** 1701-8.

Stage, J. G., Schulze, S., Moller, P., Overgaard, H., Andersen, M., Rebsdorf-Pedersen, V. B. & Nielsen, H. J. 1997. Prospective randomized study of laparoscopic versus open colonic resection for adenocarcinoma. *Br J Surg,* 84**,** 391-6.

STARSURG 2014. Impact of postoperative non-steroidal anti-inflammatory drugs on adverse events after gastrointestinal surgery. *Br J Surg,* 101**,** 1413-23.

Steele, R. J., Mcclements, P. L., Libby, G., Black, R., Morton, C., Birrell, J., Mowat, N. A., Wilson, J. A., Kenicer, M., Carey, F. A. & Fraser, C. G. 2009. Results from the first three rounds of the Scottish demonstration pilot of FOBT screening for colorectal cancer. *Gut,* 58**,** 530-5.

Stein, C. & Kuchler, S. 2013. Targeting inflammation and wound healing by opioids. *Trends Pharmacol Sci,* 34**,** 303-12.

Stephens, M. R., Lewis, W. G., Brewster, A. E., Lord, I., Blackshaw, G. R., Hodzovic, I., Thomas, G. V., Roberts, S. A., Crosby, T. D., Gent, C., Allison, M. C. & Shute, K. 2006. Multidisciplinary team management is associated with improved outcomes after surgery for esophageal cancer. *Dis Esophagus,* 19**,** 164-71.

Sternberg, A., Amar, M., Alfici, R. & Groisman, G. 2002. Conclusions from a study of venous invasion in stage IV colorectal adenocarcinoma. *J Clin Pathol,* 55**,** 17-21.

Stevens, L., Pathak, S., Nunes, Q. M., Pandanaboyana, S., Macutkiewicz, C., Smart, N. & Smith, A. M. 2014. Prognostic significance of pre-operative C-reactive protein and the neutrophil-lymphocyte ratio in resectable pancreatic cancer: a systematic review. *HPB (Oxford)*.

Stewart, C. J., Morris, M., De Boer, B. & Iacopetta, B. 2007. Identification of serosal invasion and extramural venous invasion on review of Dukes' stage B colonic carcinomas and correlation with survival. *Histopathology,* 51**,** 372-8.

Strey, C. W., Marquez-Pinilla, R. M., Markiewski, M. M., Siegmund, B., Oppermann, E., Lambris, J. D. & Bechstein, W. O. 2011. Early post-operative measurement of cytokine plasma levels combined with pre-operative bilirubin levels identify high-risk patients after liver resection. *Int J Mol Med,* 27**,** 447-54.

Su'a, B. U., Pollock, T. T., Lemanu, D. P., Maccormick, A. D., Connolly, A. B. & Hill, A. G. 2015. Chewing gum and postoperative ileus in adults: A systematic literature review and meta-analysis. *Int J Surg,* 14C**,** 49-55.

SurveyMonkey. 2014. *Tips and Tricks to Improve Survey Response Rate* [Online]. Available: https://www.surveymonkey.com/blog/en/blog/2012/03/28/improve-survey-response-rate/ [Accessed 27/05/2014.

Suter, M., Martinet, O. & Spertini, F. 2002. Reduced acute phase response after laparoscopic total extraperitoneal bilateral hernia repair compared to open repair with the Stoppa procedure. *Surgical Endoscopy and Other Interventional Techniques,* 16**,** 1214-9.

Swartbol, P., Norgren, L., Albrechtsson, U., Cwikiel, W., Jahr, J., Jonung, T., Parsson, H., Ribbe, E., Thorne, J., Truedsson, L. & Zdanowski, Z. 1996. Biological responses differ considerably between endovascular and conventional aortic aneurysm surgery. *Eur J Vasc Endovasc Surg,* 12**,** 18-25.

Syk, I., Brunkwall, J., Ivancev, K., Lindblad, B., Montgomery, A., Wellander, E., Wisniewski, J. & Risberg, B. 1998. Postoperative fever, bowel ischaemia and cytokine response to abdominal aortic aneurysm repair - a comparison between endovascular and open surgery. *European Journal of Vascular and Endovascular Surgery,* 15**,** 398-405.

Takahara, T., Uyama, I., Ogiwara, H., Furuta, T. & Iida, S. 1995. Inflammatory responses in open versus laparoscopic herniorrhaphy. *J Laparoendosc Surg,* 5**,** 317-26.

Takahashi, J., Ebara, S., Kamimura, M., Kinoshita, T., Itoh, H., Yuzawa, Y., Sheena, Y. & Takaoka, K. 2001. Early-phase enhanced inflammatory reaction after spinal instrumentation surgery. *Spine,* 26**,** 1698-1704.

Tanaka, T. & Kishimoto, T. 2012. Targeting interleukin-6: all the way to treat autoimmune and inflammatory diseases. *Int J Biol Sci,* 8**,** 1227-36.

Taylor, C., Munro, A. J., Glynne-Jones, R., Griffith, C., Trevatt, P., Richards, M. & Ramirez, A. J. 2010. Multidisciplinary team working in cancer: what is the evidence? *BMJ,* 340**,** c951.

Templeton, A. J., McNamara, M. G., Seruga, B., Vera-Badillo, F. E., Aneja, P., Ocana, A., Leibowitz-Amit, R., Sonpavde, G., Knox, J. J., Tran, B., Tannock, I. F. & Amir, E. 2014. Prognostic Role of Neutrophil-to-Lymphocyte Ratio in Solid Tumors: A Systematic Review and Meta-Analysis. *J Natl Cancer Inst,* 106.

Ten Kate, M., Aalbers, A. G., Sluiter, W., Hofland, L. J., Sonneveld, P., Jeekel, J. & Van Eijck, C. H. 2007. Polymorphonuclear leukocytes increase the adhesion of circulating tumor cells to microvascular endothelium. *Anticancer Res,* 27**,** 17-22.

Thirion, P., Wolmark, N., Haddad, E., Buyse, M. & Piedbois, P. 1999. Survival impact of chemotherapy in patients with colorectal metastases confined to the liver: a re-analysis of 1458 non-operable patients randomised in 22 trials and 4 meta-analyses. Meta-Analysis Group in Cancer. *Ann Oncol,* 10**,** 1317-20.

Thompson, M. R., Perera, R., Senapati, A. & Dodds, S. 2007. Predictive value of common symptom combinations in diagnosing colorectal cancer. *Br J Surg,* 94**,** 1260-5.

Thornton, P. C. & Buggy, D. J. 2011. Local anaesthetic wound infusion for acute postoperative pain: a viable option? *Br J Anaesth,* 107**,** 656-658.

Tjandra, J. J. & Chan, M. K. 2006. Systematic review on the short-term outcome of laparoscopic resection for colon and rectosigmoid cancer. *Colorectal Dis,* 8**,** 375-88.

Toman, N., Buschmann, A. & Muehlberger, T. 2008. Specific C-reactive protein measurements in plastic surgery. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery,* 42**,** 138-144.

Torres, A., Torres, K., Paszkowski, T., Staskiewicz, G. J. & Maciejewski, R. 2007. Cytokine response in the postoperative period after surgical treatment of benign

adnexal masses: comparison between laparoscopy and laparotomy. *Surgical Endoscopy and Other Interventional Techniques,* 21**,** 1841-8.

Tsamis, D., Theodoropoulos, G., Stamopoulos, P., Siakavellas, S., Delistathi, T., Michalopoulos, N. V. & Zografos, G. C. 2012. Systemic inflammatory response after laparoscopic and conventional colectomy for cancer: a matched case-control study. *Surg Endosc,* 26**,** 1436-43.

Tsimogiannis, K. E., Tellis, C. C., Tselepis, A. D., Pappas-Gogos, G. K., Tsimoyiannis, E. C. & Basdanis, G. 2012. Toll-like receptors in the inflammatory response during open and laparoscopic colectomy for colorectal cancer. *Surg Endosc,* 26**,** 330-6.

Turvey, S. E. & Broide, D. H. 2010. Innate immunity. *J Allergy Clin Immunol,* 125**,** S24-32.

Uribe-Querol, E. & Rosales, C. 2015. Neutrophils in Cancer: Two Sides of the Same Coin. *J Immunol Res,* 2015**,** 983698.

Usnlm. 2015. *ClinicalTrials.gov* [Online]. Available: https://clinicaltrials.gov/ct2/show/NCT02370238 2017.

Usui, S., Yoshida, T., Ito, K., Hiranuma, S., Kudo, S. & Iwai, T. 2005. Laparoscopy-assisted total gastrectomy for early gastric cancer - Comparison with conventional open total gastrectomy. *Surgical Laparoscopy Endoscopy & Percutaneous Techniques,* 15**,** 309-314.

Uzunkoy, A., Coskun, A., Akinci, O. F. & Kocyigit, A. 2000. Systemic stress responses after laparoscopic or open hernia repair. *European Journal of Surgery,* 166**,** 467-471.

Vallianou, N. G., Kostantinou, A., Kougias, M. & Kazazis, C. 2014. Statins and cancer. *Anticancer Agents Med Chem,* 14**,** 706-12.

Van't Sant, H. P., Weidema, W. F., Hop, W. C. J., Oostvogel, H. J. M. & Contant, C. M. E. 2010. The Influence of Mechanical Bowel Preparation in Elective Lower Colorectal Surgery. *Ann Surg,* 251**,** 59-63.

Varadhan, K. K., Neal, K. R., Dejong, C. H., Fearon, K. C., Ljungqvist, O. & Lobo, D. N. 2010. The enhanced recovery after surgery (ERAS) pathway for patients undergoing major elective open colorectal surgery: a meta-analysis of randomized controlled trials. *Clin Nutr,* 29**,** 434-40.

Veenhof, A. A., Sietses, C., Von Blomberg, B. M., Van Hoogstraten, I. M., Vd Pas, M. H., Meijerink, W. J., Vd Peet, D. L., Vd Tol, M. P., Bonjer, H. J. & Cuesta, M. A. 2011. The surgical stress response and postoperative immune function after laparoscopic or conventional total mesorectal excision in rectal cancer: a randomized trial. *Int J Colorectal Dis,* 26**,** 53-9.

Veenhof, A. A., Vlug, M. S., Van Der Pas, M. H., Sietses, C., Van Der Peet, D. L., De Lange-De Klerk, E. S., Bonjer, H. J., Bemelman, W. A. & Cuesta, M. A. 2012. Surgical stress response and postoperative immune function after laparoscopy or open surgery with fast track or standard perioperative care: a randomized trial. *Ann Surg,* 255**,** 216-21.

Velasco, E., Thuler, L. C., Martins, C. A., Dias, L. M. & Conalves, V. M. 1996. Risk factors for infectious complications after abdominal surgery for malignant disease. *Am J Infect Control,* 24**,** 1-6.

Velissaris, T., Tang, A. T. M., Murray, M., Mehta, R. L., Wood, P. J., Hett, D. A. & Ohri, S. K. 2004. A prospective randomized study to evaluate stress response during beating-heart and conventional coronary revascularization. *Annals of Thoracic Surgery,* 78**,** 506-512.

Vennix, S., Pelzers, L., Bouvy, N., Beets, G. L., Pierie, J. P., Wiggers, T. & Breukink, S. 2014. Laparoscopic versus open total mesorectal excision for rectal cancer. *Cochrane Database Syst Rev,* 4**,** CD005200.

Ventham, N. T., Hughes, M., O'Neill, S., Johns, N., Brady, R. R. & Wigmore, S. J. 2013. Systematic review and meta-analysis of continuous local anaesthetic wound

infiltration versus epidural analgesia for postoperative pain following abdominal surgery. *British Journal of Surgery,* 100**,** 1280-1289.

Vignali, A., Di Palo, S., Orsenigo, E., Ghirardelli, L., Radaelli, G. & Staudacher, C. 2009. Effect of prednisolone on local and systemic response in laparoscopic vs. open colon surgery: a randomized, double-blind, placebo-controlled trial. *Diseases of the Colon & Rectum,* 52**,** 1080-8.

Vittimberga, F. J., Foley, D. P., Meyers, W. C. & Callery, M. P. 1998. Laparoscopic surgery and the systemic immune response. *Annals of Surgery,* 227**,** 326-334.

Vlug, M. S., Bartels, S. A., Wind, J., Ubbink, D. T., Hollmann, M. W., Bemelman, W. A. & Collaborative, L. S. G. 2012. Which fast track elements predict early recovery after colon cancer surgery? *Colorectal Dis,* 14**,** 1001-8.

Vlug, M. S. & Bemelman, W. A. 2015. Reply to Letter: "The LAFA Study". *Ann Surg,* 261**,** e31-2.

Vlug, M. S., Wind, J., Hollmann, M. W., Ubbink, D. T., Cense, H. A., Engel, A. F., Gerhards, M. F., Van Wagensveld, B. A., Van Der Zaag, E. S., Van Geloven, A. A., Sprangers, M. A., Cuesta, M. A. & Bemelman, W. A. 2011. Laparoscopy in combination with fast track multimodal management is the best perioperative strategy in patients undergoing colonic surgery: a randomized clinical trial (LAFA-study). *Ann Surg,* 254**,** 868-75.

Wakeling, H. G., Mcfall, M. R., Jenkins, C. S., Woods, W. G., Miles, W. F., Barclay, G. R. & Fleming, S. C. 2005. Intraoperative oesophageal Doppler guided fluid management shortens postoperative hospital stay after major bowel surgery. *Br J Anaesth,* 95**,** 634-42.

Walsh, S. R., Cook, E. J., Goulder, F., Justin, T. A. & Keeling, N. J. 2005. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *J Surg Oncol,* 91**,** 181-4.

Walter, V., Jansen, L., Hoffmeister, M. & Brenner, H. 2014. Smoking and survival of colorectal cancer patients: systematic review and meta-analysis. *Ann Oncol,* 25**,** 1517-25.

Walther, A., Johnstone, E., Swanton, C., Midgley, R., Tomlinson, I. & Kerr, D. 2009. Genetic prognostic and predictive markers in colorectal cancer. *Nat Rev Cancer,* 9**,** 489-99.

Wang, G., Jiang, Z. W., Zhao, K., Li, G. L., Liu, F. T., Pan, H. F. & Li, J. S. 2012. Immunologic Response After Laparoscopic Colon Cancer Operation Within an Enhanced Recovery Program. *Journal of Gastrointestinal Surgery,* 16**,** 1379-1388.

Wang, G., Weng, Y., Ishiguro, Y., Sakamoto, H. & Morita, S. 2005. The effect of tramadol on serum cytokine response in patients undergoing pulmonary lobectomy. *Journal of Clinical Anesthesia,* 17**,** 444-50.

Wang, S. C., Chou, J. F., Strong, V. E., Brennan, M. F., Capanu, M. & Coit, D. G. 2015. Pretreatment Neutrophil to Lymphocyte Ratio Independently Predicts Disease-specific Survival in Resectable Gastroesophageal Junction and Gastric Adenocarcinoma. *Ann Surg*.

Warschkow, R., Tarantino, I., Folie, P., Beutner, U., Schmied, B. M., Bisang, P., Schultes, B. & Thurnheer, M. 2012a. C-reactive protein 2 days after laparoscopic gastric bypass surgery reliably indicates leaks and moderately predicts morbidity. *Journal of Gastrointestinal Surgery,* 16**,** 1128-35.

Warschkow, R., Tarantino, I., Torzewski, M., Naf, F., Lange, J. & Steffen, T. 2011. Diagnostic accuracy of C-reactive protein and white blood cell counts in the early detection of inflammatory complications after open resection of colorectal cancer: a retrospective study of 1,187 patients. *International Journal of Colorectal Disease,* 26**,** 1405-13.

Warschkow, R., Tarantino, I., Ukegjini, K., Beutner, U., Muller, S. A., Schmied, B. M. & Steffen, T. 2012b. Diagnostic study and meta-analysis of C-reactive protein as a

predictor of postoperative inflammatory complications after gastroesophageal cancer surgery. *Langenbecks Arch Surg,* 397**,** 727-36.

Weissman, C. 1990. The Metabolic Response to Stress - an Overview and Update. *Anesthesiology,* 73**,** 308-327.

Welsch, T., Muller, S. A., Ulrich, A., Kischlat, A., Hinz, U., Kienle, P., Buchler, M. W., Schmidt, J. & Schmied, B. M. 2007. C-reactive protein as early predictor for infectious postoperative complications in rectal surgery. *International Journal of Colorectal Disease,* 22**,** 1499-507.

Wesolowski, R., Markowitz, J. & Carson, W. E., 3rd 2013. Myeloid derived suppressor cells - a new therapeutic target in the treatment of cancer. *J Immunother Cancer,* 1**,** 10.

White, J., Kelly, M. & Dunsmuir, R. 1998. C-reactive protein level after total hip and total knee replacement. *J Bone Joint Surg Br,* 80**,** 909-11.

WHO-Globocan. 2012. *Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012.* [Online]. Available: http://globocan.iarc.fr/Default.aspx.

Wichmann, M. W., Huttl, T. P., Winter, H., Spelsberg, F., Angele, M. K., Heiss, M. M. & Jaunch, K. W. 2005. Immunological effects of laparoscopic vs open colorectal surgery. *Archives of Surgery,* 140**,** 692-697.

Wiezer, M. J., Meijer, C., Vuylsteke, R., Pullens, R. H., Prins, H. A., Cuesta, M. A., Meijer, S., Hack, C. E. & Van Leeuwen, P. A. 1999. Is major liver surgery associated with an increased systemic inflammatory response? A prospective comparison of hemihepatectomy and other major abdominal surgery. *Liver,* 19**,** 220-7.

Wille-Jorgensen, P. 1991. Prophylaxis of postoperative thromboembolism with combined methods. *Semin Thromb Hemost,* 17 Suppl 3**,** 272-9.

Wilmore, D. W. & Kehlet, H. 2001. Management of patients in fast track surgery. *BMJ,* 322**,** 473-6.

Wilson, K. W., Wilson, M. R. & McGlasson, D. L. 1993. Serum endotoxin, tumor necrosis factor, and interleukin-6 response to total hip arthroplasty. *Mil Med,* 158**,** 712-6.

Wind, J., Polle, S. W., Fung Kon Jin, P. H., Dejong, C. H., Von Meyenfeldt, M. F., Ubbink, D. T., Gouma, D. J. & Bemelman, W. A. 2006. Systematic review of enhanced recovery programmes in colonic surgery. *Br J Surg,* 93**,** 800-9.

Wirtz, D. C., Heller, K. D., Miltner, O., Zilkens, K. W. & Wolff, J. M. 2000. Interleukin-6: a potential inflammatory marker after total joint replacement. *International Orthopaedics,* 24**,** 194-196.

Wong, P. F., Kumar, S., Bohra, A., Whetter, D. & Leaper, D. J. 2007. Randomized clinical trial of perioperative systemic warming in major elective abdominal surgery. *Br J Surg,* 94**,** 421-6.

Wood, J. J., Metcalfe, C., Paes, A., Sylvester, P., Durdey, P., Thomas, M. G. & Blazeby, J. M. 2008. An evaluation of treatment decisions at a colorectal cancer multi-disciplinary team. *Colorectal Dis,* 10**,** 769-72.

Wu, F. P., Sietses, C., Von Blomberg, B. M., Van Leeuwen, P. A., Meijer, S. & Cuesta, M. A. 2003. Systemic and peritoneal inflammatory response after laparoscopic or conventional colon resection in cancer patients: a prospective, randomized trial. *Dis Colon Rectum,* 46**,** 147-55.

Yamauchi, H., Kobayashi, E., Yoshida, T., Kiyozaki, H., Hozumi, Y., Kohiyama, R., Suminaga, Y., Sakurabayashi, I., Fujimura, A. & Miyata, M. 1998. Changes in immune-endocrine response after surgery. *Cytokine,* 10**,** 549-554.

Yang, Z., Wu, Q., Wu, K. & Fan, D. 2010. Meta-analysis: pre-operative infliximab treatment and short-term post-operative complications in patients with ulcerative colitis. *Aliment Pharmacol Ther,* 31**,** 486-92.

Yim, A. P., Wan, S., Lee, T. W. & Arifi, A. A. 2000. VATS lobectomy reduces cytokine responses compared with conventional surgery. *Annals of Thoracic Surgery,* 70**,** 243-7.

Yoshida, S., Ohta, J., Yamasaki, K., Kamei, H., Harada, Y., Yahara, T., Kaibara, A., Ozaki, K., Tajiri, T. & Shirouzu, K. 2000. Effect of surgical stress on endogenous morphine and cytokine levels in the plasma after laparoscopoic or open cholecystectomy. *Surgical Endoscopy and Other Interventional Techniques,* 14**,** 137-40.

Yu, Q., Yu, X. F., Zhang, S. D., Wang, H. H., Wang, H. Y. & Teng, L. S. 2013. Prognostic role of C-reactive protein in gastric cancer: a meta-analysis. *Asian Pac J Cancer Prev,* 14**,** 5735-40.

Yuen, P. M., Mak, T. W., Yim, S. F., Ngan Kee, W. D., Lam, C. W., Rogers, M. S. & Chang, A. M. 1998. Metabolic and inflammatory responses after laparoscopic and abdominal hysterectomy. *Am J Obstet Gynecol,* 179**,** 1-5.

Zengin, K., Taskin, M., Sakoglu, N., Salihoglu, Z., Demiroluk, S. & Uzun, H. 2002. Systemic inflammatory response after laparoscopic and open application of adjustable banding for morbidly obese patients. *Obesity Surgery,* 12**,** 276-9.

Zheng, Z., Zhou, L., Gao, S., Yang, Z., Yao, J. & Zheng, S. 2013. Prognostic role of C-reactive protein in hepatocellular carcinoma: a systematic review and meta-analysis. *Int J Med Sci,* 10**,** 653-64.

Zhu, J. Z., Wang, Y. M., Zhou, Q. Y., Zhu, K. F., Yu, C. H. & Li, Y. M. 2014. Systematic review with meta-analysis: alcohol consumption and the risk of colorectal adenoma. *Aliment Pharmacol Ther,* 40**,** 325-37.

Zieren, J., Jacobi, C. A., Wenger, F. A., Volk, H. D. & Muller, J. M. 2000. Fundoplication: a model for immunologic aspects of laparoscopic and conventional surgery. *J Laparoendosc Adv Surg Tech A,* 10**,** 35-40.

Zijta, F. M., Bipat, S. & Stoker, J. 2010. Magnetic resonance (MR) colonography in the detection of colorectal lesions: a systematic review of prospective studies. *Eur Radiol,* 20**,** 1031-46.

Zmora, O., Madbouly, K., Tulchinsky, H., Hussein, A. & Khaikin, M. 2010. Urinary bladder catheter drainage following pelvic surgery--is it necessary for that long? *Dis Colon Rectum,* 53**,** 321-6.