AN INVESTIGATION INTO THE ROLE OF INTERLEUKIN-6 IN LINKING SYSTEMIC AND LOCAL INFLAMMATORY RESPONSES IN PATIENTS WITH COLORECTAL CANCER

BY

Graeme J.K. Guthrie

BSc (Hons), MBChB, MRCS (Glas)

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MEDICAL DOCTORATE (MD)

TO

THE UNIVERSITY OF GLASGOW

From research conducted in the Academic Department of Surgery and Department of Pathology, Glasgow Royal Infirmary, Faculty of Medical and Veterinary Life Sciences, University of Glasgow.

ABSTRACT

Colorectal cancer is the second most common cause of cancer death in the UK. It is accepted that both tumour and host factors are important determinants of disease progression and survival. While systemic and local inflammatory responses are increasingly recognized to be of particular importance the understanding of the mechanisms linking these important inflammatory processes remains unclear. This thesis examines the prognostic importance of measures of systemic and local inflammation and proposes a hypothesis for a link between tumour necrosis, systemic and local inflammatory responses in patients with colorectal cancer.

Chapter 3 reports the comparison of the prognostic value of longitudinal measurements of systemic inflammation in patients undergoing curative resection of colorectal cancer. In Chapter 3 the results demonstrate that there was no significant overall change in either mGPS or NLR from pre- to post-operatively. This study highlighted the associations between pre- and post-operative mGPS and NLR and T-stage ($p<0.001$), TNM stage ($p<0.005$) and cancer-specific survival. The relationships between pre-operative measurements were examined using multivariate analysis. For pre-operative measurement both mGPS and NLR were associated with cancer-specific survival while when post-operative measures were examined only mGPS was specifically associated with cancer-specific survival (HR 4.81, CI 2.13-10.83, $P<0.001$).

Chapter 4 examines the prognostic value of the Klintrup-Makinen scoring method and the existing limitations with regard to its clinical utility. An automated scoring method using commercially available image analysis software was developed and compared with manual scoring of tumour inflammatory infiltrates. This study demonstrated that both manual K-M scoring ($p<0.001$) and automated K-M scoring ($p<0.05$) had prognostic value in patients who had undergone potentially curative resection of colorectal cancer, and that the novel automated method may provide an objective method of assessment of tumour inflammatory infiltrates using routinely stained haematoxylin and eosin sections of tumour samples.

In chapter 5 a hypothesis was proposed that Interleukin-6 may link tumour necrosis and systemic and local inflammatory responses in patients with colorectal cancer. This chapter examined the basis for this hypothesis, which is presented in figure 5.1. In addition, in chapter 5 the importance of this potential link is examined.

In chapter 6, the hypothesis outlined in chapter 5 was examined in a cohort of patients who had undergone attempted curative resection of colorectal cancer. This study examined the inter-relationships between circulating mediators, in particular IL-6, tumour necrosis and systemic and local inflammatory responses. This results of this study demonstrated that IL-6 was associated with tumour necrosis (<0.001) and mGPS (<0.001) independent of T-stage.

Thus adding weight to the hypothesis that elevated circulating concentrations of IL-6 may play a role in modulating both the systemic and local inflammatory responses in patients with cancer.

Chapter 7 further develops the hypothesis that IL-6 signalling may be important in modulating systemic and local inflammatory responses in patients with colorectal cancer. Further, in chapter 7 the basis for the role of trans-signalling in this signaling pathway was examined. In this study, we reported that neither expression of the soluble IL-6 receptor or soluble gp130 were associated with systemic or local inflammatory responses. As a result the possible reasons for these findings were explored and future work suggested.

A prospective database of patients undergoing attempted curative resection of colorectal cancer in Glasgow Royal Infirmary was used throughout this thesis. This database was created and is maintained regularly by successive research fellows at the Royal Infirmary.

The work presented in this thesis highlights the importance of the host response in the form of systemic and local inflammation in patients with colorectal cancer and proposes a link between these responses and tumour necrosis. In addition, this work adds weight to the body of evidence suggesting that assessment of these host responses may improve stratification to treatment for patients with colorectal cancer. Further, this work proposes a mechanistic link, between tumour necrosis, systemic and local inflammatory responses through Interleukin-6, that merits further investigation.

Table of Contents

List of Tables

List of Figures

ACKNOWLEDGEMENTS

I am sincerely grateful for the assistance provided by the following individuals, each of whom has offered advice, encouragement and guidance throughout the period of research working towards this thesis:

Professor Donald McMillan	Academic Unit of Surgery College of Medical and Veterinary Life Science University of Glasgow Glasgow Royal Infirmary
Professor Paul Horgan	Academic Unit of Surgery University of Glasgow College of Medical and Veterinary Life Science Glasgow Royal Infirmary
Dr. Campbell Roxburgh	Academic Unit of Surgery University of Glasgow College of Medical and Veterinary Life Science Glasgow Royal Infirmary

DECLARATION

The work presented in this thesis was undertaken at the Academic Department of Surgery, Glasgow Royal Infirmary between August 2011 to August 2013. The thesis has been completed whilst working as a Specialty Registrar in General Surgery in the East of Scotland Deanery between 2013 and 2015.

I declare that the work presented in this thesis has been conducted by me, except where indicated below.

- Routine biochemical and haematological measurements used were carried out in the Glasgow Royal Infirmary hospital laboratory by the departments of biochemistry and haematology.

- Blood samples used in cytokine analysis were previously collected and stored by Dr. C. Roxburgh, Clinical Lecturer, Glasgow Royal Infirmary.

- Development of algorithms for image analysis and image analysis were conducted in conjunction with Dr. Clare Orange (University Department of Pathology), and Miss Rebecca Forrest (University of Glasgow Medical Student) using SLIDEPATH imaging software.

PUBLICATIONS

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

1. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. Critical Reviews in Oncology and Haematology. 2013 Oct;88(1):218-30. Epub 2013 Apr 17.

2. Comparison of the prognostic value of longitudinal measurements of systemic inflammation in patients undergoing curative resection of colorectal cancer. Guthrie GJ, Roxburgh CS, Farhan-Alanie OM, Horgan PG, McMillan DC British Journal of Cancer. 2013 Jul 9;109(1):24-8. Epub 2013 Jun 25.

3. Comparison of visual and automated assessment of tumour inflammatory infiltrates in patients with colorectal cancer. Forrest R, Guthrie GJ, Orange C, Horgan PG, McMillan DC, Roxburgh CS. European Journal of Cancer. 2014 Feb;50(3):544-52. Epub 2013 Dec 11.

4. Does Interleukin-6 explain the link between tumour necrosis, local and systemic inflammatory responses and outcome in patients with colorectal cancer? Guthrie GJ, Roxburgh CS, Horgan PG, McMillan DC. Cancer Treatment Reviews. 2013 Feb;39(1):89-96. Epub 2012 Aug 2.

5. Circulating IL-6 concentrations link tumour necrosis and systemic and local inflammatory responses in patients undergoing resection for colorectal cancer. Guthrie GJ, Roxburgh CS, Richards CH, Horgan PG, McMillan DC. British Journal of Cancer. 2013 Jul;109(1):131-7. Epub 2013 Jun 11.

DEDICATION

To My Parents and Paula.

I dedicate this work to you, for your never-ending patience, support and encouragement.

INTRODUCTION

1.1 - EPIDEMIOLOGY OF COLORECTAL CANCER

Colorectal cancer is the third most common cancer in males and females in the UK. Annual incidence of the disease has gradually increased since the mid-1970s, particularly in the male population, with a worldwide annual incidence of approximately 1.24 million. In the UK, annual incidence is approximately 75 per 100,00 males and 56 per 100,000 females. In Scotland, colorectal cancer represents a significant public health problem with a higher incidence than other parts of the UK and most other countries in the Western world, with approximately 3,400 new cases being diagnosed each year [1]. While incidence rates have shown an overall increase since the mid 1970s, more recent data suggests that the incidence is stable in the male population and falling in the female population of Scotland. In 2010, the lifetime risk of developing colorectal cancer was estimated at 1 in 14 for men and 1 in 19 for females.

Colorectal cancer is the second most common cause of cancer death in both men and women in the UK, with 1600 people dying from colorectal cancer in Scotland [1]. Despite improvements in the diagnosis, investigation and treatment of patients with colorectal cancer, outcomes remain poor, with approximately half of those undergoing attempted curative resection dying from the disease, with a 5-year survival of approximately 55% [2].

1.2 - AETIOLOGY OF COLORECTAL CANCER

Despite significant advances in the understanding of the genetic and cellular events involved in colorectal carcinogenesis, the precipitating events causing these remain unclear and are likely to be very complex. Indeed, few precise aetiological agents have been identified for the majority of colorectal cancers. While diet and lifestyle have been strongly implicated in the development of colorectal cancer there remains controversy over a number of specific aetiological agents. The apparent complex nature of colorectal pathogenesis has led many authors to suggest that colorectal cancer is a result of a complex interaction between the host and the environment.

1.2.1 - COLORECTAL CARCINOGENESIS

Cancer is the excessive and uncontrolled proliferation of abnormal cells. The defining characteristic of malignant cells is that they have the ability to infiltrate through the normal structures of parent tissues, in this case the muscularis mucosa, and are able to metastasize to distant sites. Colorectal cancer is epithelial in origin and is thought to be caused by both external and internal factors [3]. The majority of colorectal cancers are adenocarcinoma, subdivisions of which include mucinous and signet cell adenocarcinomas.

Colorectal carcinogenesis begins with transformation of normal mucosa as a result of accumulated genetic and epigenetic alterations, initiated by multiple stressors, resulting in disordered cell replication and abnormal cell proliferation.

While it is thought that colorectal cancers contain a large amount of genetic and molecular alterations that result in cancer progression, key genomic alterations have been identified that are deemed necessary for colorectal carcinogenesis, these include 1) chromosomal instability (CIN), 2) microsatellite instability (MSI), and 3) CpG Island Methylator Phenotype (CIMP+).

- ***Chromosomal Instability (CIN)***

Chromosomal instability refers to alteration in chromosome number, otherwise known as 'aneuploidy'. CIN has been reported to be a key part of the adenoma-carcinoma sequence proposed by Vogelstein et al [4]. This sequence describes a step-wise progression from dysplastic aberrant crypt foci (ACF) to formation of macroscopic adenoma and ultimately colorectal cancer. It is proposed that this 'traditional' pathway from normal mucosa to malignant tumour is associated with the accumulation of genetic alterations including mutation or loss of adenomatous polyposis coli (APC) gene function, mutation of K-ras, and loss of p53, the major tumour suppressor gene [5].

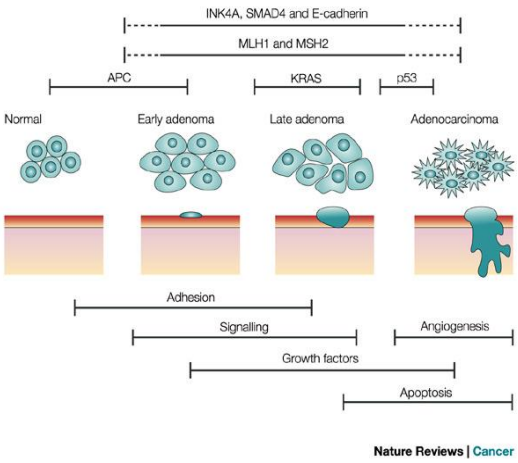


Figure 1.1 – Adenoma Carcinoma Sequence [6]

It is thought that the majority of colorectal cancers may develop this way however, in recent years several studies have suggested more widespread genetic and epigenetic abnormalities and that the 'traditional' pathway may not take into account the complexity of these genetic alterations [7].

- ***Microsatellite Instability***

Another significant contributor to the chromosomal instability observed in colorectal cancer is micro-satellite instability (MSI). Micro-satellites are nucleotide repeat sequences within a DNA sequence. The number of repetitions being variable within the alleles of an individual. MSI refers to a phenotype in which there is failure of the DNA mismatch repair system, with resultant replication of damaged DNA. The contribution of this chromosomal instability is reported to be greatest in Hereditary Non-Polyposis Colon Cancer (HNPCC) where it is observed most commonly [8-10], while that observed in sporadic tumours appears to be a smaller proportion, ranging from 15-20% in the literature [10, 11].

- ***CpG Island Methylator Phenotype***

Gene transcription is regulated by DNA methylation, which occurs where cytosine and guanosine, i.e. CpG, form a dinucleotide pair. CpG methylation occurs frequently throughout healthy DNA [12]. Methylation of CpG islands in promoter sequences occurs in health to silence certain genes however, unregulated methylation of some promoter sequences has been reported to be important in carcinogenesis [13].

It is proposed that hypermethylation of specific genes results in exclusion of these genes to key transcriptional mechanisms with the resultant silencing of these genes, of particular importance is the silencing of tumour suppressor genes in colorectal cancer which may be inactivated by this mechanism [14].

1.2.2 - SPORADIC COLORECTAL CANCER

Sporadic colorectal cancer refers to cancer developing in patients with no genetic predisposing factors. This type of colorectal cancer makes up the majority of cases with 75-80% of cases being classed as sporadic colorectal cancer. More than half of all bowel cancers are linked to major lifestyle and other risk factors. While several factors have been implicated in the aetiology of colorectal cancer no single factor has been confirmed as being causative, and thus an individuals risk of developing colorectal cancer depends on many factors, see Figure 1.2.

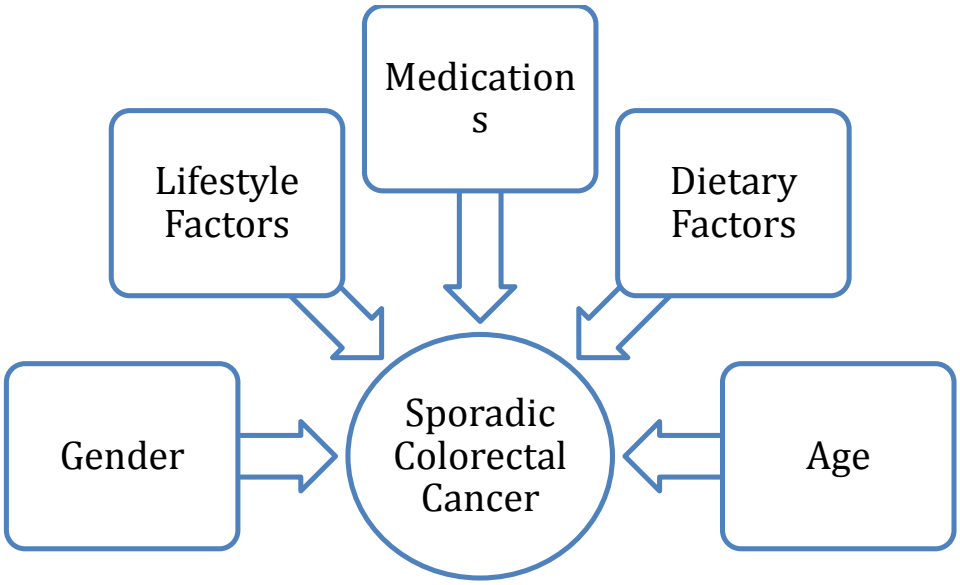


Figure 1.2 – Risk Factors and Sporadic Colorectal Cancer.

Indeed, it has been estimated that avoidable risk factors such as tobacco smoking, alcohol consumption, dietary factors, and bodyweight could account for as much as 34% of all cancers, and approximately 8% of colorectal cancers [15].

1.2.3 - RISK FACTORS IN SPORADIC COLORECTAL CANCER

- ***Age***

There is good evidence that development of colorectal cancer is associated with advancing age, with incidence of colorectal cancer rising significantly after the age of 50 years. Indeed, statistics from UK cancer research between 2009-2011 reported that 95% of colorectal cancers were diagnosed in patients over the age of 50 years, Figure 1.3 [2].

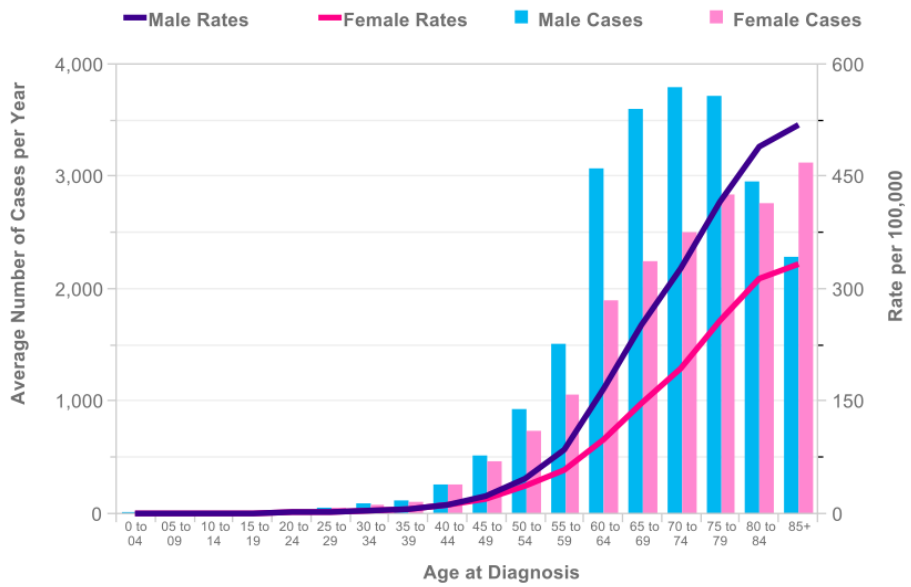


Figure 1.3 - Bowel Cancer - Age-Specific Incidence Rates, UK, 2009-2011.

Source: Cancer Research UK, CancerStats

The association between sporadic colorectal cancer and age is thought to be largely due to the accumulated exposure to environmental risk factors such as those associated with western lifestyle.

- ***Gender***

It is well recognized that the incidence of colorectal cancer is higher in males compared to females. In addition, male patients with colorectal cancer appear to be younger, and tend to have higher incidence of left-sided cancers [16]. Further, deprivation appears to have a greater effect on male patients with higher rates of colorectal cancer observed in male patients from deprived areas while this effect does not appear significant in female patients [17].

- ***Lifestyle Factors***

- Smoking

As for many other cancers, tobacco smoking appears to be one of the most important, avoidable risk factors associated with the development of colorectal cancer. Tobacco smoking is associated with the inhalation of a number of known carcinogenic compounds such as polynuclear aromatic hydrocarbons, heterocyclic amines, nitrosamines, and aromatic amines, and there is good evidence that such carcinogens can reach the colonic mucosa[18]. There is also good epidemiological evidence that smoking increases the likelihood of development of precursor lesions such as adenoma formation [19] and further, the malignant transition of such precursor lesions [18]. In addition, there is evidence that smoking is associated with molecular alterations in key genetic pathways observed in patients with colorectal cancer, including: p53, BRAF, MSI positivity, and CIMP positivity [18]. Also, dose-response variables including daily cigarette consumption, duration of smoking, pack-years and age of initiation have all been reported to be significant [20].

- Alcohol Consumption

A number of epidemiological studies have reported that alcohol intake is associated with increased risk of adenoma formation and transition to colorectal cancer with up to a 2-fold higher risk of colorectal cancer reported in those who drink more than 2 alcoholic drinks per day [21-23]. This has been further supported by molecular studies that have reported the importance of the effect of alcohol on folate and one-carbon metabolism and a potential detrimental effect on immune function through suppression of immune surveillance [22]. The mechanisms by which alcohol exerts its effect may be direct or indirect, however, what is clear is that high intake of alcohol increases risk and therefore recommending reduction of alcohol intake to lower levels may be beneficial.

- Elevated bodyweight, Obesity, and Metabolic Syndrome

Excess bodyweight has consistently been associated with numerous health problems including various cancers. Large cohort studies including the Health Professionals Follow-Up Study (HPFS) have reported a direct association with elevated BMI and increased risk of colorectal cancer [24]. Importantly, a large meta-analysis reported a direct relationship between rising BMI and increasing risk of colorectal cancer [25, 26]. In addition, a large cohort study reported the importance of increasing abdominal obesity and risk of colorectal cancer with risk rising with increased abdominal circumference[26].

These findings are supported by the increasing evidence of an association between the presence of the metabolic syndrome (MS), defined by a cluster of risk factors including abdominal obesity, hyperglycemia, raised blood pressure, elevated triglyceride levels, and low levels of high-density lipoprotein (HDL) levels, and a variety of cancers including colorectal cancer [27]. The mechanisms underlying the link between metabolic syndrome and colorectal cancer may be related to the mitogenic properties of insulin and the proliferative properties of insulin-like growth factor and their affect on colonic mucosal cells [28, 29]. Interestingly, from a mechanistic point of view it has been proposed that obesity reflects a pro-inflammatory state including the presence of elevated circulating cytokine levels which have been implicated in colorectal cancer [30, 31].

- Physical Activity

The role of physical exercise in both primary and secondary prevention for a variety of health problems is well established. With regard to colorectal cancer there is a significant body of evidence that higher levels of exercise are associated with lower incidence of colorectal cancer [32, 33]. Despite the logical extrapolation that an active lifestyle would confer a health advantage, e.g. reduction of obesity, avoidance of metabolic syndrome, several studies suggest that the protective effect is a direct relationship [24, 34] with a dose-response relationship observed across a range of physical activity frequency and intensity [35]. Interestingly, the protective effect of higher levels of exercise may be linked to improved immune system function [36].

- Dietary Factors

Several studies have reported a significant link between dietary patterns and colorectal cancer; in particular the focus has been on the potentially protective affect of dietary fibre and the detrimental effect of red and processed meat. The 'Western diet' comprising large amounts of red and processed meats has been reported to be significant, as has the presence of highly refined carbohydrates [37]. The mechanism underlying the effect of these dietary factors remains unclear.

- Dietary Fibre

The hypothesis that dietary fibre is protective against colorectal cancer relies on its effect on bowel transit. The hypothesis suggests that dietary fibre reduces colonic transit time thereby reducing the time potential carcinogens spend in contact with the colonic mucosa, while diluting/absorbing carcinogens. Despite the plausibility of this mechanism, studies investigating the effect of dietary fibre on colorectal cancer risk have yielded inconsistent results. The majority of studies do report an association between high fibre diets and lower risk of colorectal cancer, with risk reduction of 40-50% reported in some series [38-41]. However, this apparently strong protective effect has been questioned by a series of large prospective cohort studies that have not shown an association between increased dietary fibre and reduction in colorectal cancer risk [42]. Therefore, the evidence regarding dietary fibre intake and colorectal cancer risk remains unclear.

- Red Meat

There is convincing evidence that in populations with Western diets containing high intake of red and processed meats the incidence of colorectal cancer is higher. Many, but not all, epidemiological studies have reported a link between high red meat intake and incidence of both adenoma and colorectal cancer [43-47]. Of significance, a large cohort study of male health professionals, the Health Professionals Follow-up Study (HPFS) provided convincing evidence that men eating large quantities of red or processed meat (>5 times per week) had a 3-fold increase in risk of colon cancer [48]. Despite the strong evidence that high red meat intake is associated with increased risk of polyp and colorectal cancer formation, the mechanism underlying this remains unclear. Several mechanisms have been proposed including: production of carcinogenic hydrocarbons through cooking methods [49, 50]; increased secretion of endogenous insulin (a mitogen), high levels of heme iron [51], heterocyclic amines and N-nitroso compounds [51-53].

- Medications

- Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

In recent years there has been increasing interest in the use of existing non-steroidal anti-inflammatory drugs in the prevention of colorectal cancer [54, 55]. Both epidemiological and randomized trials have provided good evidence that use of aspirin may have a chemo-protective effect and prevent both the formation and recurrence of adenoma and reduce risk of progression of colorectal cancer [56-58].

Further, strong evidence for the protective effect of NSAIDs comes from reports that these drugs were able to cause regression and suppression on lesions seen in the large bowel of patients with familial adenomatous polyposis coli [59, 60]. The most widely accepted mechanism underlying the chemo-preventive effect of NSAIDs in colorectal cancer relates to reduction of prostaglandin synthesis through their inhibitory action on the cyclo-oxygenase pathway (COX) [61]. However, a more complex mechanism may explain the effect more comprehensively, and include modulation of systemic inflammatory responses and direct actions on tumour cells themselves [55, 62]. Despite this strong evidence for the potential role for NSAIDs in the chemoprevention of colorectal cancer there is limited evidence regarding the lowest effective doses required, the duration of treatment required to produce the protective effect, and the populations suitable for targeting with chemoprevention. Further, the side effects of some of these drugs, namely the potential cardiovascular and gastrointestinal side effects, has led to uncertainty with regard to their efficacy in primary prevention and major difficulties in assessing their efficacy in clinical trials.

- Statins

Statins are a class of drug involved in reducing cholesterol synthesis through their inhibitory effect on HMG-CoA Reductase, a key enzyme involved in the rate-limiting step converting HMG-CoA to mevalonate. Downstream products of this pathway have been implicated in carcinogenesis, in particular Ras and Rho proteins have been shown to be important [63].

There are multiple mechanisms implicated in the anti-tumour effects of statins, including induction of apoptosis [64], inhibition of cell proliferation [65], inhibition of angiogenesis [7], and reduction in metastatic capacity [66]. There may also be an immunomodulatory role played by statins, however the exact effect is difficult to quantify due to the myriad of immune cells affected by statin use [67]. The combination of these proposed effects of statin therapy may explain the beneficial effects reported in some clinical trials, however meta-analyses have not found a significant risk reduction in those trials attributable to standard statin therapy [68] and the effect of high dose statin therapy remains unclear.

- Hormonal Treatments

It is consistently reported that colorectal cancer incidence appears to be lower in women compared to men, particularly in the pre-menopausal years. In addition, there is convincing evidence from epidemiological studies that hormone replacement therapy (HRT) may exert a protective effect against colorectal cancer [69, 70]. These reports have been confirmed by randomized clinical trials that showed significant reduction in incidence of colorectal cancer in line with previous reports from observational studies [71]. Potential mechanisms for this effect of hormone therapy include up-regulation of tumour suppressor genes and mismatch repair genes [72, 73]. Despite these encouraging reports, the preparations, dosages and duration of treatment that confer a protective effect remain unclear, and the attendant risk of hormonal treatment in the form of breast cancer and cardiovascular events preclude its use in the setting of primary prevention of colorectal cancer [71].

1.2.4 – NON-SPORADIC COLRECTAL CANCER

While sporadic cancer lacks a clear causal factor and has multiple risk factors that contribute to its development, there are hereditary forms of colorectal cancer and disease processes that are specifically associated with development of colorectal cancer. Hereditary forms of colorectal cancer are reported to make up around 20% of all colorectal cancers. The most well known of these include: Hereditary Non-Polyposis Coli (HNPCC); Familial Adenomatous Polyposis Coli (FAP); and Inflammatory Bowel Disease (IBD).

- ***Hereditary Non-Polyposis Coli (HNPCC)***

Hereditary Non-Polyposis Coli, also known as Lynch syndrome, has clinical, pathological, and molecular features that differ from both sporadic colorectal cancer and other forms of hereditary cancer including FAP. HNPCC tumours tend to affect the right colon, are commonly associated with synchronous and metachronous tumours, are associated with younger patients, and commonly have a better prognosis than sporadic colorectal cancer [74]. HNPCC tumours are often poorly differentiated, have increased signet-cell presence, and have lymphocytic infiltrates similar to that observed in Crohn's disease, including tumour-infiltrating lymphocytes [75]. HNPCC has an autosomal dominant inheritance pattern [76] with high genetic penetrance for colorectal cancer estimated at between 85-90% [77].

Recent work has identified HNPCC specific molecular pathways that differ from that observed in sporadic colorectal cancers. HNPCC is associated with microsatellite instability with mutations observed in specific mismatch repair gene sequences, this includes mutations in 5 DNA mismatch repair gene sequences – hMLH1, hMSH2, hPMS1, hPMS2 [78, 79].

This microsatellite instability however, is not 100% exclusive to HNPCC as it is occasionally observed in sporadic tumours [80], this clearly has implications for the use of genetic testing in HNPCC. Previously a diagnosis of HNPCC colorectal cancer was based purely on clinical criterion and consideration of family history, as set out by the Amsterdam criteria I and II (Table 1.1) [81], and did not take into account the presence of any genetic mutations.

Table 1.1 – The Amsterdam Criteria [81, 82].

AMSTERDAM I	AMSTERDAM II
The following criterion must be met: • At least three relatives with colorectal cancer and, at least one should be a first-degree relative of the other two. • At least two generations of the family should have colorectal cancer. • One of the cases of colorectal cancer should have been diagnosed before the patient was age 50 years. • FAP should have been excluded.	The following criteria must be met: • At least three relatives with an HNPCC-associated cancer (large bowel, endometrium, small bowel, ureter, or renal pelvis, though not including stomach, ovary, brain, bladder, or skin. • One affected person is a first-degree relative of the other two. • At least two successive generations are affected. • One of the cases of colorectal cancer should have been diagnosed before the patient was age 50 years. • FAP should have been excluded. • Tumors have been verified by pathologic examination.

With the subsequent identification of genetic mutations and the ever-expanding list of mutations deemed to be significant in HNPCC, the Bethesda guidelines have developed to aid clinicians with decision-making with regard to which patients merit genetic testing. The main difference between the Amsterdam and the Bethesda guidelines is 1) the purpose for which they were designed, i.e. Amsterdam I & II were designed to identify those patients with colorectal cancer who may have HNPCC tumours, while the Bethesda guidelines aimed to identify those patients with HNPCC who should undergo genetic testing; 2) the Bethesda guidelines also differ in that they take into account pathological criterion, such as micro-satellite status and Crohns-like peri-tumoural reaction. Interestingly, the sensitivity of the Bethesda guidelines appears to outstrip that offered by the Amsterdam criteria (94% versus 72% respectively) [83].

- Familial Adenomatous Polyposis Coli

Familial Adenomatous Polyposis Coli (FAP) is a rare inherited genetic disease predisposing affected patients to colorectal cancer, with an incidence of approximately 1:7000 [84], approximately one third of those with FAP have no family history of the disease. This genetic disease results in polyps developing in the colon and rectum of affected individuals usually in mid-late teens with multiple polyps present by the mid-30s if left untreated. The inheritance pattern is autosomal dominant with mutation of the adenomatous tumour suppressor gene: adenomatous polyposis coli (APC), inherited [85]. The risk of developing colorectal cancer in those with FAP approaches 100% [86]. The diagnosis is made on clinical grounds with endoscopic examination confirming FAP if there are more than 100 colorectal polyps observed at endoscopy.

Genetic testing is then carried out to confirm the presence of the APC gene and family testing and screening of affected individuals carried out. Prophylactic surgery is usually performed to reduce the risk of developing colorectal cancer, however, despite this, the risk of mortality from both gastrointestinal and extra-gastrointestinal malignancies remains [87].

- **Inflammatory Bowel Disease**

It is widely accepted that there is a strong relationship between Ulcerative colitis and Crohn's disease and the development of colorectal cancer [88]. There is good evidence that development of colorectal cancer increases with duration and extent of the disease, with studies reporting a cumulative risk of 2, 8 and 18% after 10, 20, and 30 years respectively for patients with ulcerative colitis [89], and similar cumulative risk in patients with Crohn's disease [88]. Despite the strong association between inflammatory bowel disease (IBD) and colorectal cancer these tumours are reported to have distinct biology and morphology, with increased propensity towards more proximal and synchronous lesions. In addition, these tumours have distinguishing pathological characteristics including increased presence of mucinous and signet cell types with variable patterns of dysplasia [90]. Further, IBD-associated colorectal tumours tend to occur in younger patients and are managed differently with removal of the entire colon being the preferred option due to risk of synchronous tumours [88].

The susceptibility of patients with IBD developing colorectal cancer is likely to be multifactorial and may be explained in both the genetic and pathological changes observed in patients with IBD. Multiple genetic abnormalities have been reported in IBD-associated colorectal cancer, including inactivation of tumour suppressor genes, oncogene mutations, and micro-satellite instability (MSI) [91]. Of interest, are the reported molecular mechanisms implicated in colorectal carcinogenesis in IBD patients related to inflammation. In particular, the theory that oxidant stress results in increased permeability of the intestinal mucosa to bacterial products and that a subsequent abnormal immune response results in the release of a cascade of inflammatory mediators, including interleukin-6 [92, 93].

1.3 CLINICAL FEATURES OF COLORECTAL CANCER

The symptoms of colorectal cancer are dependent on the location and extent of the disease, ranging from occult symptoms to frank 'red-flag' symptoms. While most presentations are due to the effects of the primary tumour, some patients do present with symptoms of secondary deposits.

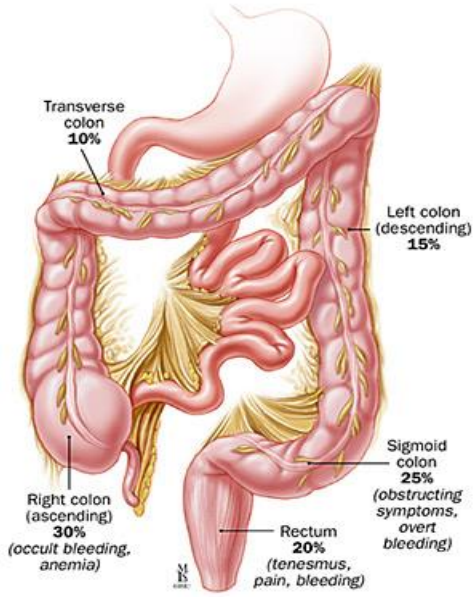


Figure 1.4 - Frequency and Location of Colorectal Cancer

(Johns Hopkins Colon Cancer Centre)

Early colorectal cancer may not cause obvious symptoms and arise insidiously. Common symptoms leading to presentation to primary care include [94, 95]:

- Change in bowel habit – alteration from normal pattern of defaecation to diarrhea, constipation, or change of caliber of the stools.
- Rectal bleeding or presence of blood in stools.
- Cramping abdominal pain and/or bloating.
- Weakness/ Fatigue.
- Unintended Weight loss/ Loss of appetite.

Presentation of appropriate symptoms usually leads to referral to secondary care and appropriate investigation. The more occult symptoms such as weakness and fatigue are often more difficult to detect and many patients present with iron deficiency anaemia having initially presented to their general practitioner with fatigue. Less commonly patients present to secondary care with emergency presentation in the form of bowel perforation or obstruction [96].

1.4 – Diagnosis of Colorectal Cancer

There are clear guidelines with regard to the diagnosis of colorectal cancer [97]. Primary diagnosis of colorectal cancer is achieved by three main methods:

- Endoscopy with biopsy for tissue diagnosis.
- Computed tomography (CT).
- Double contrast barium enema.

Guidance dictates that when colorectal cancer is suspected the whole colon is examined using one or a combination of these modalities [97].

Colonoscopy plays a vital role in the diagnosis of colorectal cancer. It remains the gold standard investigation offering high sensitivity [98]. The major advantage colonoscopy offers over other diagnostic modalities is the ability to directly visualize and biopsy suspicious lesions within the colon and rectum [97]. Disadvantages of this technique include requirement for intravenous sedation, bowel preparation and risk of serious complications including bleeding, perforation and death [99].

Despite the accuracy of endoscopic examination of the colon, other methods including CT colonography and double contrast barium enema do offer highly sensitive, alternative methods of investigation. CT colonography has the benefit of providing diagnostic information from both within and out-with the colon and has been reported to be a safe alternative to colonoscopy, particularly in those not fit for examination using endoscopy [100, 101].

Some reports suggest that CT colonography reaches approximately 93% sensitivity, while double contrast barium enema reaches only approximately 83%. This has led to CT studies largely replacing barium enema in the investigation of colorectal cancer [102],[103].

1.5 – Colorectal Cancer Screening

The principles of cancer screening as defined by the World Health Organisation include [104]:

- The screening programme must address an important health problem.
- The disease should be recognizable at a latent or early stage.
- There must be a good understanding of the natural history of the disease.
- There must be an acceptable form of treatment available.
- The proposed screening test should have high accuracy and be acceptable to the population.
- Facilities must be available to diagnose and treat the disease and the costs must be acceptable.

Colorectal cancer, and the tests and treatments available is a disease that satisfies these conditions. There is now good evidence that colorectal cancer population screening in appropriate age groups reduces both the incidence and mortality associated with colorectal cancer [105, 106].

The screening test used, the faecal occult blood test (FOB), is based on the knowledge that polyps and colorectal cancers may bleed. A positive FOB test result leads to endoscopic examination of the colon and rectum. The UK screening program invites all men and women between age 60-70 years, while the NHS Scotland bowel-screening programme invites all men and women between aged 50-74 to carry out a home faecal-occult blood test every 2 years. Several studies have reported that colorectal cancer screening has reduced cancer-specific mortality in patients with colorectal cancer by approximately 25% in those screened [107].

This is thought to be the result of a significant 'stage-shift', brought about as a direct result of colorectal cancer screening, with more patients being diagnosed at an earlier stage. Importantly, it is widely reported that colorectal cancer screening reduces cancer-specific mortality in patients with colorectal cancer [108, 109]. In addition to population screening, current cancer screening strategies take into account those patient groups that may be at higher risk of developing colorectal cancer as a result of inflammatory bowel disease. As previously described, patients with Ulcerative colitis and Crohn's disease are at increased risk of developing colorectal cancer, and as such screening of these patients is believed to identify tumours at an earlier stage, however reports have not shown that screening of IBD patients reduces mortality [110]. Currently screening colonoscopy is offered to patients with IBD who have had documented disease for 10 years [111].

1.6 – Staging of Colorectal Cancer

Historically, colorectal cancer staging was based on the Dukes classification as described by Cuthbert Dukes in 1932 [112]. This staging system was designed for the classification of rectal cancer and has been modified several times. The Dukes system, most recently modified by Astler and Coller stages colorectal cancer into stage A-D as follows:

Table 1.2 – The Dukes Classification [113].

Dukes Stage	Definition
A	Tumour invades muscularis mucosa and submucosa but does not invade the muscularis propria.
B1	Tumours invade muscularis propria.
B2	Tumours completely penetrate the smooth muscle layer into the serosa.
C	Tumours with any degree of invasion but have regional lymph node involvement.
C1	Tumours invade the muscularis propria with <4 regional lymph nodes involved.
C2	Tumours completely penetrate the smooth muscle layer and serosa with 4 or more regional lymph nodes involved.
D	Tumours with distant metastases present.

The current standard method of staging of colorectal cancer is the tumour, node, metastasis (TNM) system of the American Joint Committee on Cancer (AJCC) and The International Union Against Cancer (UICC) [114], and is widely recognized as the international language of colorectal cancer staging. The TNM system has a comprehensive and consistent set of definitions based on clinical data.

The components of the system include size of the tumour (T), the number of nodes involved (N) and the presence or absence of metastatic disease (M).

Table 1.3 - AJCC and UICC Definitions [114].

	Category	Definition
Primary Tumour (T)	Tx	Primary tumour cannot be assessed
	T0	No evidence of primary tumour
	Tis	Carcinoma in situ (intraepithelial or intramucosal carcinoma)
	T1	Tumour invades the submucosa
	T2	Tumour invades the muscularis propria
	T3	Tumour invades through the muscularis propria into the pericolorectal tissues
	T4a	Tumour penetrates to the surface of the visceral peritoneum
	T4b	Tumour directly invades or is adherent to other organs or structures
Regional Lymph Nodes (N)	Nx	Regional lymph nodes cannot be assessed
	N0	No regional lymph node involvement
	N1	Metastasis in 1-3 lymph nodes
	N1a	Metastasis in 1 regional lymph node
	N1b	Metastasis in 2-3 regional lymph nodes
	N1c	Tumour deposit(s) in the subserosa, mesentery, or non-peritonealized pericolic or perirectal tissues without regional node involvement
	N2	Metastasis in 4 or more regional lymph nodes
	N2a	Metastasis in 4-6 regional lymph nodes
	N2b	Metastasis in 7 or more regional lymph nodes

Distant Metastasis (M)	M0	No distant metastasis
	M1	Distant metastasis
	M1a	Metastasis confined to one organ or site
	M1b	Metastases in more than one organ/site or the peritoneum

The data used to stage patients with colorectal cancer is determined from pathological samples of the primary tumour and nodal tissue and imaging in the form of computed tomography (CT), magnetic resonance imaging (MRI) and in some cases Positron Emission Tomography (PET) and ultrasound. Therefore this method of staging provides a comprehensive, accurate and consistent staging assessment that can be utilized in the multi-disciplinary management of these patients.

1.7 – Surgical Management of Colorectal Cancer

The majority (approximately 80%) of colorectal cancers are resectable by surgery and despite significant advances in the fields of oncology and immunotherapy, surgery remains the definitive treatment of localized colorectal cancer and offers the best attempt at cure. Surgical treatment aims to gain local control of the disease and involves complete en-bloc resection of the primary tumour, its vascular pedicle, and its lymphatic drainage [115].

- Colon Cancer Treatment

The type of surgery for colon cancer is determined by tumour location and stage of disease. Stage I-III tumours can be safely treated by segmental mesocolic resection. For right-sided tumours, right hemicolectomy is appropriate, while left-sided and sigmoid colon cancers are treated by left hemi-colectomy or sigmoid colectomy respectively. There is debate regarding the best modality for stage IV disease but in principle preoperative chemotherapy followed by synchronous or staged colectomy and metastasectomy may be provided [115].

- Rectal Cancer Treatment

Rectal cancer requires careful pre-operative staging to determine appropriate treatment. The use of MRI and the role of neo-adjuvant chemoradiotherapy as an adjunct to attempted curative resection are key to managing patients with rectal cancer. While local excision of rectal tumours is possible for small tumours confined to the mucosa [116], it is not possible to predict by current imaging modalities which of these tumours will have local lymph node involvement and adequacy of resection is determined at pathology.

A proportion of these patients will require further surgery that may include progression to radical surgery if the pathology is not favourable, such as those patients with evidence of tumour close to, or at the resection margin, those with lymphovascular invasion, or a poorly differentiated tumour [97, 117]. The most important surgical principle in the treatment of rectal cancers is use of total mesorectal excision (TME). There is good evidence that adherence to this principle of excision reduces risk of local recurrence by ensuring good circumferential clearance of the tumour [118, 119].

Low anterior resection is the operation of choice for tumours of the upper and middle third of the rectum while tumours of the lower rectum may require more radical surgery in the form of ultra-low anterior resection. Radical abdomino-perineal excision (APR) is reserved for those low rectal tumours thought to be involving the sphincters. The resection technique for extralevator surgery has been refined in recent years with the evolution of the cylindrical APR technique and is thought to confer an oncological advantage with lower circumferential resection margin (CRM) involvement reported [120]. In addition, lower recurrence rates have been reported for those patients with rectal cancer who have received pre-operative radiotherapy [121-123]. Previously post-operative chemoradiotherapy was thought the sole treatment for locally advanced rectal cancers, however in recent years patients with locally advanced rectal tumours have received neo-adjuvant chemoradiotherapy to 'downstage' the tumour and therefore avoid the significant morbidity associated with APR. There is good evidence that such a strategy improved local control in these patients [124].

1.8 – Adjuvant Therapy for Colorectal Cancer

The use of adjuvant chemotherapy and radiotherapy in the treatment of colorectal cancer is determined by multi-disciplinary assessment of individual patients. The aim of adjuvant therapy is to augment the local control achieved by surgery with systemic disease control to prevent the development of metastases and reduce the risk of recurrence.

- Adjuvant Chemotherapy

Adjuvant chemotherapy is used for stage II and III colorectal cancer. While the indications for the use of adjuvant chemotherapy in stage III colorectal cancer are well recognized, its use in patients with stage II disease is less well defined [97]. To date, large-scale trials, including the UK QUASAR [125], and IMPACT study [126] have shown only a small benefit for selected patients with stage II colorectal cancer. It is currently recommended that only those stage II patients with high-risk pathological features fit enough for chemotherapy should receive adjuvant chemotherapy [127],[128]. Several studies have reported a documented risk reduction of death for patients with stage III colorectal cancer [129], with an estimated 5% improvement in 5-year survival for colon cancer and 9% improvement for rectal cancer [130]. Several trials including the National Surgical Adjuvant Breast and Bowel Project (NSABP), and The Netherlands Adjuvant Colorectal Cancer Project (NACCP) found that treatment with 5-Fluorourcail (5-FU) improved 5-year survival and recurrence free survival [131-133].

1.9 – Metastatic Colorectal Cancer

The treatment of metastatic disease is a complex problem and treatment decisions with regard to the best mode of therapy are determined within the context of a colorectal MDT meeting. The most common site for metastatic disease is the liver. The most common methods of diagnosis include CT or MRI [134, 135] and these modalities are key to diagnosing resectability along with adjunctive imaging with FDG-PET scanning [134]. There is good evidence that survival may be improved by synchronous hepatic resection for colorectal liver metastases [136], however only 20-30% of patients with synchronous liver metastases are deemed resectable [137]. Further, recent advancements, including radio-frequency ablation, have led to the consideration of lesions previously thought unresectable to be considered for resection [138].

1.10 – PROGNOSTIC FACTORS IN COLORECTAL CANCER

- 1.10.1 – Tumour Factors

Since the advent of the Dukes' classification, prediction of outcome has been based on high-risk pathological criteria such as the Tumour, Node, Metastasis (TNM) system, and this is widely accepted as the gold-standard.

Table – 1.4 –Colorectal Cancer Survival by Stage.

COLON CANCER	
TNM Stage	5-Year Survival
I	92%
IIa	87%
IIb	63%
IIIa	89%
IIIb	69%
IIIc	53%
IV	11%

RECTAL CANCER	
TNM Stage	5-Year Survival
I	87%
IIa	80%
IIb	49%
IIIa	84%
IIIb	71%
IIIc	58%
IV	12%

Cancer Research UK Statistics [2]

Despite this, it has become increasingly clear that within the Dukes and TNM staging systems there is a wide spectrum of disease with survival being variable, reported to range between 50-85%, particularly in those with Dukes B2 or T3/4 node negative tumours [139],[140],[141]. In an attempt to improve the accuracy of prognostication pathological assessment has been refined. Using a simple, reproducible, cumulative score the Petersen Index combines four pathological factors: peritoneal involvement, venous invasion, spread involving resection margin, and tumour perforation.

- **1.10.1.1 - Peritoneal Involvement**

Peritoneal involvement is defined by presence of tumour cells on the peritoneal surface or within the peritoneal cavity. It has been reported to be a strong independent prognostic factor in both colon and rectal cancer [142, 143]. The recognition of tumour cells on the peritoneal surface is reliant on pathological microscopic assessment and is therefore subject to variability.

- **1.10.1.2 - Venous Invasion**

Venous invasion is a feature of progression of colorectal cancer and is defined as the presence of tumour cells within endothelium lined spaces [144]. Detection of venous invasion requires fastidious pathological assessment, a process that has been hampered in the past by difficulties with staining and sectioning, with wide variability in detection rates from 10-90% [145]. Despite the difficulties with pathological analysis of venous invasion it has been widely reported as a strong prognostic indicator and marker of risk of development of local recurrence and distant metastases [146-148].

- **1.10.1.3 - Involved Resection Margin**

Margin involvement is defined as presence of tumour cells at or within 1mm of the surgical resection margin and is important in staging and prognostication. Most evidence regarding the significance of margin involvement comes from studies involving patients with rectal cancer [149, 150]. Indeed margin involvement is a well recognized, significant adverse prognostic factor [151].

- **1.10.1.4 - Tumour Perforation**

Tumour perforation refers to viscus perforation through the tumour site. It is well recognized that tumour perforation is a significant adverse prognostic factor in patients with colorectal cancer [128].

- **1.10.1.5 - Lymph Node Ratio**

It is an essential part of colorectal cancer surgery that the draining lymphatic tissue is excised along with the tumour. This is based on the recognition that many nodal metastases are found in small lymph nodes (<5mm) [152, 153]. Many factors can affect the lymph node harvest including, age, anatomical variation, and adequacy of surgical resection. Perhaps the most challenging but important factor in lymph node harvest is the ability of the pathologist in identifying and retrieving the lymphatic tissue in resection samples and accurately describing the status of the associated lymph nodes [153].

It is widely accepted that a minimum of 12 lymph nodes must be acquired to provide accurate pathological staging and prognostic information [154-157]. In lymph node positive colorectal cancer, it has been reported that lymph node ratio (LNR), a figure achieved by dividing the number of positive lymph nodes by the number of harvested nodes has been reported to have prognostic significance. It has been reported that LNR provides superior prognostic information compared to N-stage status alone [158-160]. Despite strong evidence that LNR offers superior prognostic information the thresholds used differ between studies thus making its value as a prognostic marker unclear.

- **1.10.1.6 - Angiogenesis**

Angiogenesis is the process whereby a tumour obtains oxygen and nutrients essential to sustain growth and development. Angiogenesis represents one of the hallmarks of cancer [3] and is believed to be a tightly regulated, complex process involving multiple cell-signalling pathways [161]. Angiogenesis in cancer is thought to represent a shift in the delicate balance of pro-angiogenic and anti-angiogenic factors as a result of increased demand for nutrients by rapidly growing tissues. This has been termed the angiogenic switch and is thought key to the malignant process [162] with several subtypes of Vascular Endothelial Growth Factor subtypes (VEGF) reported to be of significance in various tumours. In addition it has been well documented that increased VEGF and its receptors correlate with disease progression and may be useful for predicting prognosis [163-165]. Indeed, several authors have reported that colorectal cancer is an angiogenesis-dependent malignancy and that presence of neovascularization is a poor prognostic sign [166].

- **1.10.1.7 - Tumour Necrosis**

There is good evidence that presence and extent of tumour necrosis is important in determining outcome in many solid organ tumours such as lung [167], urothelial [168], and breast [169] cancers. With reference to colorectal cancer, some studies have reported the presence of tumour necrosis in more than 90% of colorectal cancers [170] thus strongly implicating tumour necrosis in the natural history of colorectal cancer. The importance of tumour necrosis in patients with colorectal cancer was reported in a study by Pollheimer and colleagues who demonstrated that necrosis was significantly associated with tumour-related factors including advanced stage, poor differentiation, venous invasion and larger tumour size [170]. Two recent studies have directly examined the relationship between tumour necrosis and survival in patients with colorectal cancer. Both Pollheimer et al. and Richards et al., have reported that tumour necrosis is a negative prognostic marker in colorectal cancer [170],[171]. In addition, Richards and co-workers reported an association between necrosis and well recognised high-risk pathological variables such as vascular invasion, peritoneal involvement and margin involvement in colorectal cancer [171]. Despite the reported important role of tumour necrosis in colorectal cancer it does not appear to have independent prognostic value [171]. It is hypothesized that tumour necrosis may play a more complex role contributing to the host inflammatory response in patients with colorectal cancer.

1.10.2 Host Factors

- **1.10.2.1 - Cancer Associated Inflammation**

There is now persuasive evidence that cancer survival, especially in early stage disease, is dependent on the tumour-host interaction. In particular, inflammation is now thought to be key in tumourigenesis via DNA damage [172], stimulation of angiogenesis and proliferation, and inhibition of apoptosis [173]. Indeed, the link between inflammatory bowel disease and development of colorectal cancer is well established. For example, many epidemiological studies have reported high frequencies of colorectal cancer among patients with inflammatory bowel disease [174], and animal models of colitis-associated colorectal cancer have been utilized to investigate models of cancer-related inflammation. In addition, it has been reported that IL-6 signalling is key to maintaining mucosal integrity and dysregulation of this key pathway may partly explain a link between inflammatory bowel disease and colorectal cancer [175]. Lastly, in recent years the risk of developing colorectal cancer on a background of inflammatory bowel disease appears to be reducing and this might be explained by improved treatments of colonic mucosal inflammation [176].

Several studies have provided evidence for this crucial role for cancer-associated inflammation, both in resected colorectal tumours and in precursor lesions [177]. Indeed, cancer-associated inflammation has recently been identified as a key determinant of disease progression and survival in colorectal cancer and has been cited as the seventh hallmark of cancer [178-180].

- **1.10.2.2 - The Local Inflammatory Response to Colorectal Cancer**

With reference to the local inflammatory response, more than 100 studies over the last 40 years have reported that inflammatory/immune cells in the immediate tumour microenvironment play an important role in determining colorectal cancer outcome. Recently, Klintrup and co-workers determined, through assessment of the entire immune/inflammatory reaction at both the invasive margin and in the central part of the tumour, that local inflammation was an important prognostic marker predicting both enhanced survival and recurrence-free survival in both colonic and rectal tumours [181]. Recent work confirms that a pronounced tumour inflammatory infiltrate predicts good outcome and it has been proposed this may be used routinely to predict survival [181-183].

- **1.10.2.3 - The Systemic Inflammatory Response to Colorectal Cancer**

The systemic inflammatory response is activated in a number of conditions, however, the pathophysiology is similar regardless of the initiating insult. This response to injury is a complex multi-faceted process involving both humoral and cellular immunity, the complement system and complex cytokine cascades. This non-specific response to injury results in vasodilation, increased vascular permeability, cellular activation and coagulation all aimed at tissue regeneration and repair.

Several pro-inflammatory cytokines play a key role in the activation and propagation of the systemic inflammatory response and are produced by macrophages, monocytes, mast cells, platelets and endothelial cells, with key players including Interleukin-1, and Tumour Necrosis Factor Alpha (TNF-α), which via an action on Nuclear Factor Kappa B (NF-κB), results in the production of further key pro-inflammatory cytokines including Interleukin-6, Interleukin-8, and Interferon-gamma (IFN-**γ**) [184, 185]. It has been reported that IL-6 in particular, is key to the production of C-reactive protein (CRP), one of the acute phase proteins measured clinically in inflammatory conditions [184, 186, 187].

The systemic inflammatory response is normally subject to tight controls by cytokine cascades aimed at dampening the response, in the form of IL-4 and IL-10, which have a direct antagonistic effect on the pro-inflammatory cytokine cascade [185]. While the overall process is normally a balanced response if this balanced response is disrupted and the normal control lost, an exaggerated response results that can have a significant negative impact on body tissues.

With reference to the systemic inflammatory response and cancer, there is now good evidence that it is associated with poor outcome in patients with a variety of common solid organ tumours [188-191]. The systemic inflammatory response, measured as an elevated C-reactive protein (CRP) and hypoalbuminaemia, has consistently been reported as a marker of poor prognosis in primary operable colorectal cancer [192, 193]. The basis of this is not altogether clear, however a marked systemic inflammatory response is associated with important patient-related factors such as nutritional, functional and immunological decline.

In recent years many studies have investigated the most commonly used measures of the systemic inflammatory response and their potential use in stratifying cancer patients. There is good evidence that markers of the acute phase response, particularly CRP and albumin, are both sensitive and reliable markers of systemic inflammation in patients with cancer [193]. It is also well established that the systemic inflammatory response is associated with alterations in circulating white blood cells, specifically the presence of neutrophilia with a relative lymphocytopaenia [186, 194]. Similar to CRP and albumin, haematological tests are carried out routinely for cancer patients in a variety of clinical scenarios, and as such represent an easily measurable objective parameter able to express the severity of the systemic inflammatory response in patients with cancer.

With regard to the acute phase response the last decade has seen the evolution of a prognostic scoring system, the Glasgow Prognostic Score (GPS) based on the combination of these acute phase proteins that provides objective, reliable prognostic information for both operable and inoperable cancers [195]. Further, this scoring system has been validated in a variety of clinical scenarios and is now recognised to have prognostic value, independent of tumour-based factors [195].

The Glasgow Prognostic Score, first reported by Forrest et al. in patients with lung cancer has been reported to compare favourably with other methods of prognostication [196, 197]. The GPS is constructed as set out in below. ≥≤

Table 1.5 – The Glasgow Prognostic Score (GPS) [196].

The Glasgow Prognostic Score	
Variable	Points Allocated
C-Reactive Protein ≤10mg/l and Albumin ≥ 35g/l	0
C-Reactive Protein > 10mg/l	1
Albumin < 35g/l	1
C-Reactive Protein > 10mg/l and Albumin < 35g/l	2

While this scoring system appeared to offer useful independent prognostic value, further evaluation has resulted in modification of the score to take account of the finding that a score of 1 was most often due to an elevated CRP and less commonly due to hypoalbuminaemia, and that hypoalbuminaemia was not a reliable marker of poor prognosis in patients with colorectal cancer [192]. This modification was termed the modified Glasgow Prognostic Score (mGPS), as outlined below.

Table 1.6 – The modified Glasgow Prognostic Score (mGPS) [198].

The modified Glasgow Prognostic Score	
Variable	Points Allocated
C-Reactive Protein ≤ 10mg/l and Albumin ≥ 35g/l	0
C-Reactive Protein > 10mg/l	1
C-Reactive Protein > 10mg/l and Albumin < 35g/l	2

In recent years, and in a similar way to the Glasgow Prognostic Score [195], many research groups have investigated the value of the haematological components of the systemic inflammatory response specifically for use in predicting outcome, and have reported that the individual components of the differential white cell count, specifically the neutrophil and lymphocyte counts, may have clinical utility in predicting survival [199]. The combination of these haematological components of the systemic inflammatory response, as the neutrophil–lymphocyte ratio (NLR), has been reported to have prognostic value in a variety of cancers [193, 200]. Indeed, Walsh et al., investigated the prognostic value of the neutrophil–lymphocyte ratio (NLR), in their centre, because CRP concentrations were not routinely performed as part of pre-treatment assessment [201].

– Studies of the prognostic value of the GPS/mGPS and NLR, in unselected cohorts of patients with cancer.

Recent work has reported the prognostic value of the GPS/mGPS in a variety of solid organ malignancies [198, 202-204]. In addition, as with GPS/mGPS several studies have reported the value of the NLR in unselected cohorts of patients with cancer. Five studies, comprising data on 31,915 patients, have reported the prognostic value of these markers of systemic inflammation in patients with cancer, Table 2.4 [198, 202-206]. In addition, direct comparison studies between well recognized markers of the systemic inflammatory response in cancer, notably CRP, albumin, and platelets and their combinations in the prognostic scores mGPS, NLR, derived NLR and PLR, have consistently reported that GPS/mGPS was a more powerful predictor of survival compared to other markers of the systemic inflammatory response including Neutrophil-Lymphocyte Ratio (NLR), and the Platelet-Lymphocyte Ratio (PLR) and that its predictive value was independent of tumour site [195].

Thus, there is evidence that the NLR has prognostic value in a variety of tumour types. Despite this, there is inconsistency in the thresholds chosen across studies making a direct comparison between these two inflammatory scores difficult. Although the NLR is associated with survival, other markers of the systemic inflammatory response, notably the GPS/mGPS, may be superior predictors of survival. Indeed, a recent large cohort study (Glasgow Inflammation Outcome Study) reported that the mGPS had superior prognostic value over the NLR in differentiating good from poor prognostic groups in a variety of tumour types [203].

More recently, the components of a number of systemic inflammation-based scores (neutrophils, lymphocytes, platelets, CRP and albumin) have been compared in a large unselected cohort of patients with cancer. Of these components, only neutrophils, platelets, CRP and albumin, and not lymphocytes, were shown on multivariate survival analysis to have independent prognostic value [207]. These results may explain the reported superiority of the GPS over the NLR.

Table 1.7 - Studies of the Prognostic Value of the GPS/mGPS and NLR in Patients with Cancer.

Study	Centre	Tumour Site	*n*	HR (p-Value)	Measure (mGPS/NLR)	Comments
Crumley [202]	Glasgow (UK)	Gastro-oesophageal	217	1.7 (<0.001)	mGPS	mGPS predicted survival independent of tumour site/stage/treatment
Proctor [203]	Glasgow (UK)	Various	8759	1.7 (<0.001)	NLR (>5)	mGPS predicted survival superior to NLR, PLR, PI, PNI and elevated NLR (>5) associated with reduced 5-year OS and DFS
Azab[205]	New York (USA)	Breast	316	4.85 (<0.0001)	NLR (>3.33)	Elevated NLR associated with the elderly, large tumours, and more advanced stage
Proctor [206]	Glasgow (UK)	Various	12,118	1.52 (<0.001)	NLR (>4)	NLR and dNLR were associated with reduced OS and DFS independent of age, sex and deprivation. NLR superior to dNLR
Proctor[198]	Glasgow (UK)	Various	9608	1.9 (<0.001)	mGPS	mGPS predicted survival independent of tumour site
Shafique[204]	Glasgow (UK)	Prostate	897	1.8 (<0.05)	mGPS	mGPS predicted survival superior to NLR

- Studies of the prognostic value of the GPS/mGPS and NLR in patients with operable cancer.

With regard to operable cancer, sixty-two studies, comprising data on 20,759 patients in a variety of tumour types have reported the prognostic value of GPS/mGPS and NLR in patients with cancer including colorectal, gastric, oesophageal, pancreatic, liver, urological and gynaecological cancers (Table 2.5) [148, 190, 192, 201, 208-256]. Of note, fourteen studies have reported the prognostic value of GPS/mGPS in patients with operable colorectal cancer. Importantly, these studies reported that GPS/mGPS prognostic value was independent of other well-recognised prognostic markers including tumour stage, pathological features and other measures of systemic inflammation. In comparison, while ten studies report the prognostic value of the NLR and its association with overall and disease-free survival, only four report that NLR had independent prognostic value in patients with colorectal cancer [102, 201, 208-212, 215, 216, 227]. Interestingly, the threshold that defined an elevated NLR differed across these studies, with >5 being the most commonly used threshold (*n* = 16 studies). In patients with operable cancer, NLR was consistently associated with other markers of systemic inflammation, in particular CRP and the modified Glasgow Prognostic Score (mGPS). The relationship between the neutrophil–lymphocyte ratio and pathological features in colorectal cancer was inconsistent with only two studies reporting a significant direct association between the NLR and T-stage while associations with other tumoural factors including tumour size, differentiation and tumour location have been described.

In conclusion, the last decade has seen the accumulation of good evidence supporting associations between the GPS/mGPS, NLR and outcome in patients with operable disease, in particular gastrointestinal cancer. However, while pre-operative NLR is associated with both disease-free and overall-survival in some studies there was a lack of consistent evidence for its value as an independent predictor of survival, particularly in early stage and less aggressive disease. Other markers of the systemic inflammatory response, in particular the mGPS, appear to have stronger prognostic value in these patients.

Table 1.8 - Studies of the prognostic value of the GPS/mGPS and NLR in patients with operable cancer.

Study	Centre	Tumour Site	n	HR (p-value)	Measure GPS/mGPS/NLR	Comments
McMillan [192]	Glasgow (UK)	Colorectal	316	1.7 (<0.001)	mGPS	mGPS predicted survival independent of stage
Leitch [211]	Glasgow (UK)	Colorectal	233	2.1 (<0.001)	mGPS	mGPS predicted survival superior to WCC/lymphocytes
Ishizuka [239]	Tochigi (Japan)	Colorectal	315	1.5 (<0.01)	GPS	GPS predicted survival independent of stage/treatment
Crozier [240]	Glasgow (UK)	Colorectal	188	2.2 (<0.05)	mGPS	mGPS predicted independent of emergency presentation
Roxburgh [148]	Glasgow (UK)	Colorectal	244	2.3 (<0.001)	mGPS	mGPS predicted survival independent of Petersen Index
Moyes [241]	Glasgow (UK)	Colorectal	455	1.8 (<0.01)	mGPS	mGPS predicted post-operative infective complications
Roxburgh [257]	Glasgow (UK)	Colorectal	287	2.7 (<0.001)	mGPS	mGPS predicted survival independent of tumour inflammatory infiltrate
Ishizuka [242]	Tochigi (Japan)	Colorectal Liver	300	2.1 (<0.05)	GPS	GPS predicted survival independent of CLIP score
Ishizuka [243]	Tochigi (Japan)	Colorectal	156	24.5 (<0.05)	GPS	GPS predicted survival in T1/T2 disease
Kobayashi [244]	Tokyo (Japan)	Oesophageal	65	NR (<0.001)	GPS	GPS predicted survival independent of lymph node status
Polterauer [255]	Vienna (Austria)	Cervical	244	NR (<0.05)	GPS	GPS predicted survival independent of FIGO stage
Kobayashi [258]	Tokyo (Japan)	Colorectal Liver	63	3.1 (<0.01)	GPS	GPS predicted survival independent of number of liver metastases
Knight [250]	Manchester (UK)	Pancreas	99	4.3 (<0.05)	GPS	GPS predicted post-operative morbidity

Richards [259]	Glasgow (UK)	Colorectal	320	1.8 (<0.001)	mGPS	mGPS predicted survival independent of POSSUM
Nozoe [245]	Koga (Japan)	Gastric	232	4.1 (<0.001)	mGPS	mGPS predicted survival independent of tumour stage
Moug [260]	Kilmarnock (UK)	Colorectal	206	1.6 (<0.05)	mGPS	mGPS predicted survival independent of LNR
Roxburgh [261]	Glasgow (UK)	Colorectal	302	1.6 (<0.001)	mGPS	mGPS predicted survival independent of comorbidity indices
Vashist [246]	Hamburg (Germany)	Oesophageal	112	3.0 (<0.001)	GPS	GPS predicted peri-operative morbidity and survival
Ishizuka [253]	Tochigi (Japan)	Hepatocellular	300	2.1 (<0.05)	GPS	GPS predicted mortality independent of post-operative morbidity
Dutta [247]	Glasgow (UK)	Oesophageal	112	4.3 (<0.001)	mGPS	mGPS predicted survival independent LNR, NLR and PLR
Jamieson [251]	Glasgow (UK)	Pancreas	135	2.3 (<0.001)	GPS	GPS predicted survival independent margina status/ adjuvant therapy
Ishizuka [254]	Tochigi (Japan)	Hepatocellular	398	2.5 (<0.05)	GPS	GPS predicted survival independent of CLIPS score
Lamb [256]	Glasgow (UK)	Renal	169	5.1 (<0.001)	GPS	GPS predicted survival independent of established scoring systems
La Torre [252]	Rome (Italy)	Pancreas	101	1.8 (<0.01)	mGPS	mGPS predicted survival independent of margin status and LNR
Jamieson [262]	Glasgow (UK)	Pancreas	173	1.8 (<0.01)	mGPS	mGPS predicted survival independent of LNR
Ishizuka [263]	Tochigi (Japan)	Colorectal	271	2.0 (<0.05)	mGPS	mGPS predicted survival in patients with normal CEA
Dutta [248]	Glasgow (UK)	Gastric	120	2.2 (<0.01)	mGPS	mGPS predicted survival independent of LNR, NLR and PLR
Jiang [249]	Tokyo (Japan)	Gastric	1710	1.8 (<0.01)	mGPS	mGPS predicted survival independent of TNM stage

Ding [208]	Guangdong (China)	Colorectal	141	4.88 (0.003)	NLR	Elevated NLR associated with reduced DFS and an independent prognostic factor and not associated with clinicopathological characteristics.
Kwon [264]	Busan (Korea)	Colorectal	200	Non-significant	NLR	Elevated NLR associated with lower OS on univariate analysis only. NLR not associated with clinicopathological factors or stage of disease.
Neal [210]	Leicester (UK)	Colorectal	202	2.05 (0.001)	NLR	NLR associated with postoperative morbidity and OS on univariate analysis.
Walsh [201]	Suffolk (UK)	Colorectal	230	<0.001	NLR	NLR >5 associated with OS and CS survival on univariate analysis.
Leitch [211]	Glasgow (UK)	Colorectal	233	Non-significant	NLR	NLR associated with other measures of systemic inflammation but not prognostic in primary operable disease.
Mallappa [212]	Harrow (UK)	Colorectal	297	1.81 (0.028)	NLR	Elevated NLR independently associated with survival.
Halazun [213]	Leeds (UK)	Colorectal	440	2.26 (<0.001)	NLR	Elevated NLR, age and number of metastases were independent prognostic factors.
Gomez [214]	Leeds (UK)	Colorectal	501	1.3 (0.032)	NLR	Elevated NLR associated with recurrence.
Hung [215]	Tao-Yuan (Taiwan)	Colorectal	1040	1.29 (0.012)	NLR	NLR associated with significantly worse OS (5yrs). NLR associated with advancing age (>65), T4b cancer, elevated CEA and tumour obstruction/perforation. NLR not associated with histological subtype, tumour size,

						tumour grade.
Chiang [216]	Linkou (Taiwan)	Colorectal	3731	1.31 (0.013)	NLR	Elevated NLR was associated with clinicopathological factors associated and with outcome.
Ubukata [217]	Tokyo (Japan)	Gastric	157	5.78 (<0.001)	NLR	NLR independently prognostic and associated with T-stage, tumour size, presence of lymph nodes, and pathological stage.
Aizawa [218]	Kashiwa (Japan)	Gastric	262	2.21 (0.012)	NLR	NLR was an independent prognostic factor. NLR increased in a T-stage dependent manner.
Jung [219]	Gwangju (Korea)	Gastric	293	1.65 (0.019)	NLR	Elevated NLR significantly associated with OS and DFS in later stage gastric cancer. Elevated NLR associated with advanced T-stage, and larger tumour size.
Rashid [220]	Derby (UK)	Oesophageal	294	Non-significant	NLR	NLR not associated with tumour factors or with disease recurrence or survival.
Wang [221]	Guangzhou (China)	Gastric	324	2.32 (0.014)	NLR and GPS	GPS more prognostic than NLR.
Mohri [222]	Tsu (Japan)	Gastric	357	2.78 (<0.0001)	NLR	NLR independently associated with prognosis together with tumour size, advanced T-stage in relatively early stage gastric cancer.
Shimada [223]	Tokyo (Japan)	Gastric	1028	1.85 (0.003)	NLR	NLR was an independent prognostic factor and increased in a t-stage dependent manner.
Garcea [224]	Leicester (UK)	Pancreas	74	0.0057	NLR	NLR significantly associated with recurrence, and was more prognostic than components alone.
Bhatti [225]	Derby (UK)	Pancreas	84	1.78	NLR	NLR reported an independent prognostic factor for

				(0.023)		survival and not associated with tumour characteristics.
Smith [226]	Liverpool (UK)	Pancreas	110	<0.001	NLR	No relationship between NLR and survival.
Wang [221]	Guangzhou (China)	Pancreas	177	2.54 (0.006)	NLR	NLR independently associated with OS.
Gomez [227]	Leeds (UK)	Cholangio-carcinoma	27	1.78 (0.008)	NLR	Elevated NLR associated with poorer DFS, and associated with larger tumours, intrahepatic satellite lesions, microvascular invasion and lymph node involvement.
Kinoshita [228]	Tokyo (Japan)	Liver	150	<0.05	NLR, GPS	Along with mGPS and GPS, NLR was associated with reduced OS. NLR not independently associated with OS.
Motomura [265]	Fukuoka (Japan)	Liver	158	6.24 (0.0002)	NLR	Increasing NLR associated with HCC recurrence. Associated with other markers of systemic inflammation.
Sakai [229]	Aomori (Japan)	Lung	23	Non-significant	NLR	NLR had no significant relationship with recurrence or survival.
Sarraf [230]	London (UK)	Lung	178	1.1 (0.004)	NLR	NLR associated with T-stage and was an independent predictor of outcome.
Tomita [231]	Miyazaki (Japan)	Lung	284	<0.001	NLR	NLR predicted significantly worse 5 yr. survival. NLR only independent risk factor.
Tomita [232]	Miyazaki (Japan)	Lung	301	<0.001	NLR	Combined use of NLR and CRP reported as independent prognostic factor.
Hashimoto [233]	Tokyo (Japan)	Renal	84		NLR	NLR associated with DFS.

				0.026		
Gondo [234]	Tokyo (Japan)	Bladder	189	1.95 (0.0387)	NLR	NLR independent predictor of prognosis.
Ohno [235]	Tokyo (Japan)	Renal	192	2.16 (0.0259)	NLR	Pre-operative NLR associated with CRP. T-stage and elevated NLR were independent predictors of recurrence with decreased survival.
Ohno [236]	Tokyo (Japan)	Renal	250	3.12 (0.0007)	NLR	Elevated NLR associated with DFS and was independent prognostic variable.
Cho [237]	Seoul (Korea)	Ovary	192	8.4 (0.041)	NLR	Elevated NLR, advancing stage, and older age were independent prognostic factors.
Idowu [238]	Liverpool (UK)	Sarcoma	223	5.13 (0.024)	NLR	NLR independently associated with OS and associated with worse DFS.

- Studies of the prognostic value of the GPS/mGPS and NLR in patients with operable cancer who received chemo-radiotherapy.

There are a number of studies (*n=23)* reporting the prognostic value of GPS/mGPS and NLR in patients receiving chemo-radiotherapy for a variety of cancers including gastro-oesophageal [202, 244, 266-268], lung [197, 237, 269-272], hepatocellular [273], pancreatic [274]. Of particular note, there was good evidence from studies in patients with colorectal cancer that both mGPS and NLR have prognostic value in patients with advanced disease receiving chemotherapy [275-280], independent of tumour stage and adjuvant therapy. Similar to patients with operable cancer, the thresholds used to define an elevated NLR are inconsistent while the mGPS thresholds remain constant across all studies. In addition, both pre- and post-treatment NLR have been reported to be of prognostic value in patients with more advanced cancer who receive chemotherapy [268-272, 274-277, 281-283]. Interestingly, at least 3 studies reported that normalisation of the NLR post-treatment was associated with improved survival [270, 284]. Further, a combined scoring system using the NLR and the GPS was reported to be a strong predictor of overall survival [283].

Given the prognostic value of these markers of systemic inflammation and their additional value provided when combined it is suggested by some authors that use of the GPS/mGPS in conjunction with currently used clinical parameters may improve prediction of outcome for those patients undergoing adjuvant therapy, however further work remains to be done.

Table 1.9 - Studies of the prognostic value of the GPS/mGPS and NLR in patients who received chemo-radiotherapy.

Study	Centre	Tumour Site	n	HR (p-value)	Measure GPS/mGPS/NLR	Comments
Forrest [197]	Glasgow (UK)	Lung (NSCLC)	109	1.9 (<0.01)	GPS	GPS predicted survival independent of ECOG-ps/Platinum status
Crumley [202]	Glasgow (UK)	Gastro-oesophageal	65	1.7 (<0.05)	mGPS	mGPS predicted survival independent of ECOG-ps/platinum therapy
Kobayashi [244]	Tokyo (Japan)	Oesophageal	48	5.9 (<0.01)	GPS	GPS predicted toxicity in patients receiving neoadjuvant therapy
Sharma [280]	London/Sydney	Colorectal	52	NR	GPS	GPS predicted toxicity and survival independent of stage/treatment
Ishizuka [278]	Tochigi (Japan)	Colorectal	112	6.0 (<0.01)	GPS	GPS predicted survival in patients receiving adjuvant therapy
Wang [266]	Kaihsiung (Taiwan)	Oesophageal	123	3.4 (<0.001)	GPS	GPS predicted survival in patients receiving radiotherapy
Roxburgh [279]	Glasgow (UK)	Colon	348	3.2 (<0.01)	mGPS	mGPS predicted survival in patients receiving adjuvant therapy
Chua [284]	Sydney (Australia)	Various	68	4.1 (<0.01)	GPS	GPS predicted survival in patients receiving docetaxel
Hwang [267]	Gwangui (South Korea)	Gastric	402	1.8 (<0.01)	GPS	GPS predicted survival independent of performance status
Morimoto [273]	Yokohama (Japan)	Hepatocellular	81	5.5 (<0.001)	GPS	GPS predicted survival in patients receiving sorafenib
Gioulbasanis [285]	Heraklion (Greece)	Lung	96	1.9 (<0.01)	GPS	GPS predicts toxicity and efficacy in platinum-based therapy
Carruthers [275]	Glasgow (UK)	Rectal	115	4.1 (0.002)	NLR	Elevated NLR associated with OS and DFS
Chua [276]	Sydney (Australia)	Appendiceal	174	NR (0.01)	NLR	Low NLR associated with improved survival
Kishi [277]	Texas (USA)	CRC Liver Mets	290	2.22 (0.016)	NLR	Pre- and Post-Treatment NLR associated with survival
Cedres [269]	Barcelona (Spain)	Lung	171	1.5 (0.015)	NLR	NLR associated with T- and N- Stage, OS and DFS

Kao [270]	Concord (Australia)	Lung	173	2.7 (<0.001)	NLR	Low NLR associated with improved survival
Yao [271]	Nanjing (China)	Lung	182	1.81 (0.008)	NLR	Elevated NLR associated with poor OS and DFS
Lee [281]	Goyang (Korea)	Lung	199	1.05 (0.051)	NLR	Pre- and Post- treatment NLR associated with disease progression
Teramukai [272]	Kyoto (Japan)	Lung	388	1.48 (0.013)	NLR	Elevated NLR associated with poorer OS and DFS
Aliustaoglu [268]	Istanbul (Turkey)	Gastric	168	NR (0.001)	NLR	Elevated NLR associated with OS
An [274]	Guandong (China)	Pancreas	95	4.49 (0.013)	NLR	Elevated NLR associated with OS
Keizman [282]	Baltimore (USA)	Renal	133	NR (<0.001)	NLR	NLR associated with OS and DFS
Chua [283]	Sydney (Australia)	Various	68	2 (0.01)	NLR	Combined NLR/GPS score predicted OS

- Studies of the prognostic value of the prognostic value of the GPS/mGPS and NLR in patients with inoperable cancer.

There was good evidence across a range of tumour types that both GPS/mGPS and NLR provide reliable prognostic value in patients with inoperable cancer. With regard to the Glasgow Prognostic Score there was evidence from studies in lung [286-288], breast [191], gastro-oesophageal [289], hepatocellular and pancreatic [290, 291], renal and ovarian cancer [292, 293]. Similarly, several reports described the value of the NLR in predicting outcome in this patient group [294-298]. Of particular note two studies were in advanced colorectal cancer and both reported an association between elevated NLR and poorer survival in advanced colorectal cancer.

In conclusion, in patients with advanced, inoperable disease the GPS/mGPS and NLR reliably predict poorer survival. Despite the recognition that GPS/mGPS and NLR are reliable markers of poor prognosis in advanced disease what remains unclear is how this information can best be used in a clinical setting and further work on the clinical utility of these scores and in particular, the value of mGPS in patients with colorectal cancer is required.

Table 1.10 – Studies of the Prognostic Value of GPS/mGPS and NLR in patients with inoperable cancer.

Study	Centre	Tumour Site	n	HR (p-value)	Measure GPS/mGPS/NLR	Comments
Forrest [286]	Glasgow (UK)	Lung	109	1.7 (<0.001)	GPS	GPS predicted survival independent of ECPG-ps/stage/treatment
Al-Murri [191]	Glasgow (UK)	Breast	96	2.3 (<0.001	GPS	GPS predicted survival independent of stage/treatment
Crumley [289]	Glasgow (UK)	Gastro-oesophageal	258	1.5 (<0.001)	GPS	GPS predicted survival independent of stage/treatment
Glen [290]	Glasgow (UK)	Pancreas	187	1.7 (<0.001)	GPS	GPS predicted survival independent of stage
Read [299]	Sydney (Australia)	Colorectal	84	2.3 (<0.05)	GPS	GPS predicted survival independent of stage/treatment
Ramsey [293]	Glasgow (UK)	Renal	119	2.4 (<0.001)	GPS	GPS predicted survival independent of scoring systems
Sharma [292]	Sydney (Australia)	Ovarian	154	1.7 (<0.01)	GPS	GPS predicted survival independent of stage/treatment
Pinato [287]	London (UK)	Lung	171	2.6 (<0.001)	mGPS	mGPS predicted survival independent of NLR and EPS
Leung [288]	Glasgow (UK)	Lung	261	1.7 (<0.001)	mGPS	mGPS predicted survival independent of ECOG-ps/stage/treatment
Pinato [291]	London (UK)	Hepatocellular	578	2.7 (<0.01)	GPS	GPS predicted survival in training and validation datasets
Partridge [300]	Edinburgh (UK)	5-sites	102	2.7 (<0.01)	mGPS	mGPS predicted survival independent of tumour site in palliative care
Kaneko [294]	Tokyo (Japan)	Colorectal	50	4.39 (0.0013)	NLR	NLR independently associated with hypoalbuminaemia, OS, DFS
Chua [283]	Sydney (Australia)	Colorectal	349	1.6 (0.01)	NLR	NLR independent predictor of OS
McNally [295]	Ohio (USA)	Hepatocellular	103	NR (0.021)	NLR	NLR independent predictor of OS
Huang [296]	Guangzhou (China)	Hepatocellular	145	NR (0.041)	NLR	NLR independently associated with poor survival
Jeong [297]	Seoul (Korea)	Gastric	104	NR (0.037)	NLR/mGPS	Elevated and mGPS independently prognostic.
Wang [298]	Dalian (China)	Variety	497	1.35 (0.014)	NLR	Elevated NLR associated with survival.

- Studies of the prognostic value of the NLR in patients with operable cancer who received neoadjuvant therapy

While there are reports regarding the prognostic value of the NLR in patients undergoing neoadjuvant treatment prior to resection, there are no reports of the prognostic value of the mGPS in this group of patients. Six studies, comprising data on 1044 patients have reported the prognostic value of the NLR in patients who received neo-adjuvant therapy and subsequently underwent cancer resection [301-306]. Among these studies it was consistently reported that elevated NLR was associated with increased recurrence and death in patients with hepatocellular cancer [301, 302, 306]. Although the thresholds for an elevated NLR differed in one of these studies, it was consistently reported that elevated NLR was an independent predictor of both disease-free and overall survival in patients with hepatocellular cancer. Interestingly, two of these studies reported a significant relationship between NLR and microvascular invasion in HCC. In addition, studies of patients with oesophageal cancer receiving neoadjuvant treatment have reported the prognostic value of the NLR [303-305]. In patients receiving neoadjuvant chemotherapy followed by resection it was reported that elevated NLR was associated with survival. Interestingly, while the study by Sato and colleagues reports an independent association between the NLR and pathological response to treatment, the study by Sharaiha and colleagues reports no association between the NLR and response to treatment. However, both these studies reported an association between elevated NLR and poor prognosis with Sharaiha and colleagues reporting elevated NLR as an independent prognostic factor regardless of tumour type [305].

In conclusion, in patients receiving neoadjuvant chemotherapy followed by surgery it is consistently reported that NLR predicts recurrence and overall survival. Interestingly, there was inconsistency with regard to the ability of the NLR to predict pathological response to treatment in this group of patients.

Table 1.11 - Studies of the prognostic value of the NLR in patients with operable cancer who received neoadjuvant therapy.

Study	Centre	Tumour Site	*n*	HR (p-value)	Threshold	Comments
Wang [306]	Guangdong (China)	HCC	101	2.65 (<0.001)	NLR	Elevated NLR independently associated with poor DFS.
Halazun [301]	New York (USA)	Liver	150	19.99 (0.005)	NLR	Elevated NLR associated with increased risk of recurrence and death.
Bertuzzo [302]	Bologna (Italy)	Liver	219	19.14 (<0.001)	NLR	Elevated NLR associated with lower OS. Elevated NLR and MVI negatively affected DFS. Elevated NLR and MVI were independent prognostic factors.
Sato [303]	Shizuoka (Japan)	Oesophageal	83	2.83 (0.043)	NLR	NLR associated with pathological response to neoadjuvant chemotherapy.
Miyata [304]	Osaka (Japan)	Oesophageal	152	- Non-significant	NLR	Elevated NLR associated with survival but not independently prognostic.
Sharaiha [305]	New York (USA)	Oesophageal	339	2.26 (<0.001)	NLR	Elevated NLR associated with worse DFS and OS independent of tumour type.

- Relationships between clinicopathological factors and GPS/mGPS and NLR in patients with cancer.

A plethora of clinicopathological factors are reported to be important in patients with cancer. Therefore, studies examining the prognostic value of markers of the systemic inflammatory response have reported on the associations between several pathological variables and GPS/mGPS and NLR. Several tumour factors have been associated with GPS/mGPS including T-stage, tumour necrosis and angiogenesis [171, 195, 307]. In addition, several studies report a strong association between cancer cachexia and an elevated systemic inflammatory response with at least seven studies reporting an association between nutritional status, cancer cachexia and GPS/mGPS [307-313]. Interestingly, elevated GPS/mGPS has been reported to be associated with both haematological and biochemical changes in patients with cancer [314], including stage dependent alterations in cytokine concentrations [315, 316].

Several studies have reported factors associated with an elevated NLR. In patients with breast cancer elevated NLR has been associated with advancing age, larger tumours, and stage of disease [205]. Further, increasing tumour stage was also associated with elevated NLR in patients with operable cancers, namely colorectal, gastro-oesophageal, hepatocellular, lung [201, 208, 217, 228, 230]. In addition, factors that represent more aggressive tumour behaviour, such as increased tumour size, microvascular and lymphatic invasion, lymph node involvement, number of metastatic lesions and elevated CEA concentrations, were associated with elevated NLR [213, 215-217].

Conversely, a number of studies failed to report a relationship between NLR and tumour characteristics [209]. Only one study in patients receiving chemotherapy (either neoadjuvant or combined chemotherapy/radiotherapy) has reported that NLR was associated with T-stage and nodal status but not with number of metastatic lesions, performance status, type of chemotherapy or use of glucocorticoid medication [269]. Further, only a single study in inoperable cancer examined factors associated with NLR and reported that NLR > 3 was associated with increased tumour stage, tumour type, increasing age, and female gender, but not lymph node metastasis or high CEA or alkaline phosphatase concentrations [298].

- **1.10.2.4 - Summary**

The hypothesis that markers of the systemic inflammatory response, both in the form of the GPS/mGPS and NLR, reliably predict survival in patients with malignancy is one that has garnered considerable interest in the last decade. Many groups have investigated the prognostic value of the NLR in a variety of tumours and at differing stages of disease. While these observations may be of clinical importance, it is important to note that with regard to NLR that there is considerable heterogeneity in the thresholds used to determine an elevated NLR across studies and a variety of thresholds have been reported both in operable disease, in those receiving chemotherapy, and in inoperable disease. The heterogeneity of the thresholds used makes a conclusion regarding the clinical utility of the NLR somewhat difficult and further work utilising the most common threshold of >5 should be considered in an attempt to refine whether this simple measure of the systemic inflammatory response is reliable as a prognostic marker in the clinical setting. Despite this, and the heterogeneous groups of cancer patients within which these relationships have been examined, a consistent finding is that NLR may reflect a more advanced stage of disease with potentially more aggressive tumour behavior, while GPS/mGPS may offer more robust, superior prognostic value in early stage disease, for example, in colorectal cancer only four of eleven studies reported NLR as independently prognostic. In addition, it appears that the NLR is more consistently independently prognostic in patients with upper gastrointestinal malignancy, a group of solid organ malignancies that tend to present at a later stage with more advanced features.

The clinical utility of such a finding has great importance, as the ability to determine more accurately which patients should receive treatments for advanced disease, such as chemotherapy is important for both quality of life and survival as reported in recent study by Temel et al. [317]. However, the literature regarding post-treatment measurement of the NLR appears limited and inconsistent and therefore further longitudinal studies of the prognostic value of the NLR are warranted.

Given the observation, that NLR is more consistently prognostic in more advanced states such as those patients requiring chemotherapy or who have inoperable disease compared to the GPS/mGPS. This may be particularly relevant given the expected stage shift occurring with colorectal cancer to that of earlier stage disease as a result of colorectal cancer screening. Further, it is therefore reasonable to propose that NLR could be used as a biomarker in patients who require adjunctive treatment or who do not appear clinically to be suitable for surgical intervention and would therefore be useful in the improved stratification of patients with cancer.

Interestingly, recent work has proposed that the combination of measures of the systemic inflammatory response may be a powerful predictor of outcome in cancer. At least one study has proposed the use of a combined score using the NLR and markers of the acute phase response and reported that it has improved value in patients with cancer. Further, a recent large prospective cohort study has reported the use of a combined biomarker score that has prognostic value in patients with cancer [317].

Therefore, further studies are required to investigate the prognostic value of such a combined score using established routinely measured prognostic markers of the systemic inflammatory response, namely C-reactive protein, albumin, neutrophil and lymphocyte counts.

2.0 – SUMMARY AND AIMS

2.1 Summary

Colorectal cancer is the third most common cancer in males and females in the UK and, despite advances in treatment it remains the second most common cancer death in both men and women. It is anticipated that the introduction and development of colorectal cancer screening will result in a significant stage-shift in the pattern of presentation of the disease to increasing numbers of patients with node-negative colorectal cancer. This has significant implications in the clinical arena, as stratification of patients to the ever-increasing armoury of treatments will become increasingly challenging. Therefore, the development of reliable prognostic indices is key to the evolution of high quality patient care.

While pathological criteria including the Dukes' classification and more recently the AJCC TNM system have been used to provide prognostic information there is an increasing need for improved prognostication. There is now good evidence that cancer progression and survival is determined by a range of factors. In particular, it has become increasingly clear that systemic and local inflammatory responses are key determinants of progression and outcome in patients with colorectal cancer. There is now a significant body of evidence reporting the importance of the systemic inflammatory response measured as both alterations in the acute phase proteins including CRP and albumin, and alterations in haematological markers of systemic inflammation including circulating neutrophils, lymphocytes and platelets and their combination in a variety of scoring systems.

The most widely documented prognostic scores that have been developed and refined taking into account the importance of the systemic inflammatory response are the modified Glasgow Prognostic Score and the Neutrophil-Lymphocyte Ratio.

While the evidence of the prognostic value of these scoring systems has increased significantly in recent years, their routine use in clinical practice remains elusive. The clinical utility of such systemic inflammation-based prognostic scores would be enhanced if they were shown to have prognostic value over time and after therapeutic intervention. This would provide the basis of interventions directed at systemic inflammation, maintaining low levels of systemic inflammation and active surveillance, following resection of colorectal cancer. No study has directly compared the longitudinal measurement of the mGPS and NLR in patients with colorectal cancer.

There is good evidence that the local inflammatory response plays a key role in determining progression and survival. While the Klintrup-Makinen criteria has been reported to have prognostic value in patients with colorectal cancer and has been validated in numerous cohorts of patients with colorectal cancer it is not used in routine clinical practice and conventional staging methods remain the accepted paradigm. The reasons that this semi-quantitative method of examining the local inflammatory response are not routinely used in clinical practice include: 1) The complexity and lack of reproducibility of scoring the inflammatory cell infiltrate caused by differences in immunohistochemical staining methods between different units;

2) The different cell types present and importantly, 3) The subjectivity of assessing the tumour inflammatory cell infiltrate. Therefore a reliable and accurate measure of the tumour inflammatory cell infiltrate may be useful in the refinement of staging the host inflammatory response in clinical pathological practice.

The development of image analysis software that is capable of point-scoring inflammatory cells could provide a reliable and accurate measure of the tumour inflammatory cell infiltrate that may be useful in the refinement of staging the host inflammatory response in clinical pathological practice. This modality could offer a method of standardizing the assessment of the local inflammatory cell infiltrate in patients with colorectal cancer.

Further, the mechanisms underlying the interaction between systemic and local inflammatory processes are likely to be very complex and are not well understood. It has become increasingly recognized that these process may interact and impact on the host's response to cancer by way of an altered immune response. The modulation of this host immune response to cancer is key to progression and outcome and as such a link between these key inflammatory processes may provide both useful prognostic information to allow better stratification of patients to treatments, and provide novel therapeutic targets. Recent work has implicated tumour necrosis as a link between the systemic and local inflammatory responses, however its lack of independent prognostic value has led some authors to hypothesise that these two processes may be linked at a molecular level.

Key to this hypothesis is the observation that that the 'dirty' cell death observed in tumour necrosis may stimulate inflammatory pathways including the pro-inflammatory cytokines that are known to be crucial in the induction and maintenance of systemic inflammatory responses and have been implicated in modulating immune cell responses.

A plausible mediator in this pathway is the IL-6 signalling pathway. To date, no studies have examined the hypothesis that Interleukin-6 may provide a link between tumour necrosis and the systemic and local inflammatory responses in patients with colorectal cancer and merits further examination. In addition downstream signaling pathways have also been strongly implicated in a mechanistic explanation for the induction, maintenance and interaction between these inflammatory processes. No studies have assessed these downstream signaling pathways including the IL-6 trans-signalling pathway in the context of associations with tumour necrosis and systemic and local inflammatory responses in patients with colorectal cancer.

2.2 – Aims

To examine the areas of uncertainty detailed above, in patients undergoing potentially curative resection of colorectal cancer, the following studies were carried out:

1. To investigate the prognostic value of longitudinal measurements of systemic inflammation in patients undergoing curative resection for colorectal cancer.

2. To develop an automated scoring method to enable consistent and reproducible assessment of tumour inflammatory infiltrates in colorectal cancer.

3. To evaluate the evidence for the role of Interleukin-6 in linking tumour necrosis and systemic and local inflammatory responses in patients with colorectal cancer.

4. To examine whether circulating mediators, in particular IL-6, may be a link between tumour necrosis and local and systemic inflammatory responses in patients undergoing curative resection for colorectal cancer.

5. To examine the role the soluble IL-6 receptor/gp130 trans-signalling pathway may play in linking tumour necrosis, local and systemic inflammatory responses in patients with colorectal cancer.

3.0 – COMPARISON OF THE PROGNOSTIC VALUE OF LONGITUDINAL MEASUREMENTS OF SYSTEMIC INFLAMMATION IN PATIENTS UNDERGOING CURATIVE RESECTION OF COLORECTAL CANCER.

- 3.1 – Introduction

As described in Chapter 2 many studies have shown that the host response in the form of systemic inflammation is a key factor in determining outcomes in colorectal cancer, and as such the measurement of various circulating markers of systemic inflammation are useful in predicting survival. Indeed, it has been shown that a simple objective scoring system, that is, the modified Glasgow Prognostic Score, which measures systemic inflammation using CRP and albumin, is effective at predicting overall and cancer-specific survival in a variety of solid organ malignancies including colorectal cancer [195]. Similarly, it has become clear that the systemic inflammatory response results in changes in circulating white blood cells, and that components of the full blood count, in particular the neutrophil-lymphocyte ratio (NLR), also are useful in predicting overall and cancer-specific survival in a variety of solid organ malignancies, including colorectal cancer as described in chapter one.

Recently, these systemic inflammation-based prognostic scores, mGPS and NLR were compared in a large cross-sectional cohort of unselected patients with cancer[203]; however, to our knowledge, there has been no direct comparison of the longitudinal measurement of the mGPS and NLR in patients with colorectal cancer.

The clinical utility of such systemic inflammation-based prognostic scores would be enhanced if they were shown to have prognostic value over time and after therapeutic intervention. This would provide the basis of interventions directed at systemic inflammation, maintaining low levels of systemic inflammation and active surveillance, following resection of colorectal cancer.

Therefore the aim of the present study was to compare the prognostic value of longitudinal measurements of systemic inflammation in patients undergoing curative resection of colorectal cancer.

- **3.2 – Patients and Methods**

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and pre-operative abdominal computed tomography, were considered to have undergone potentially curative resection between 2006 and 2010 in a single surgical unit at Glasgow Royal Infirmary were included in the study. The patients were identified from a prospectively maintained database and included both elective and emergency resections. Exclusion criteria were: clinical evidence of active infection, presence of a chronic inflammatory condition, and death within 30 days of surgery. Tumours were staged using the conventional tumour, node, metastasis (TNM) staging system, 7th Edition, 2010 [318].

Longitudinal measurements of a differential white cell count, neutrophil and lymphocyte counts, CRP and albumin were recorded before surgery and between 3- and 6- months (median 3.5 months) following surgery.

The mGPS was constructed using CRP and albumin using previously described thresholds[195]. A score of 2 was allocated to those patients with both elevated CRP (>10mg/l^{-1}) and hypoalbuminaemia (<35g^{-1}), those with only an elevated CRP (>10mg/l^{-1}) were allocated a score of 1, whereas those with normal CRP ($\leq$10mg/l^{-1}) were allocated a score of 0. The NLR was constructed using previously documented thresholds. Briefly, NLR was determined by dividing the absolute neutrophil count by the absolute lymphocyte count: the NLR was then dichotomized using the most commonly used threshold: <5:1 = low = 0, >5:1 = high = 1.

Date and cause of death was crosschecked with the data held by the National Cancer Registry and the Registrar General (Scotland), and 1st December 2011 served as the censor date. Cancer-specific and overall survival was calculated from the date of post-operative blood sample until the date of death.

The prognostic value of the pre-operative measure of the systemic inflammatory response was assessed using survival calculated from the date of the pre-operative blood test until date of death. While the prognostic value of the post-operative measure of the systemic inflammatory response was assessed using survival calculated from the date of the post-operative blood test until the date of death.

Univariate survival analysis was carried out using the Kaplan-Meier method with the log-rank test and associations between categorical variables examined using the χ^2 test for linear trend. The Wilcoxon Rank test was used to test the related samples pre-operative mGPS and post-operative mGPS, and pre-operative NLR and post-operative NLR. Cox proportional hazard regression was used to calculate hazard ratio (HR) and 95% confidence intervals (95% CI). A p-value of <0.05 was considered to be significant. Analysis was performed using SPSS software version 19 (SPSS Inc., Chicago, IL, USA).

The study was approved by the Research and Ethics Committee, Glasgow Royal Infirmary.

- **3.3 - Results**

Three hundred and twenty-six patients undergoing potentially curative resection for colorectal cancer were identified from a prospectively maintained database and studied. Two patients had metatstatic disease, however they underwent attempted surgery with curative intent and therefore were included with the analysis. Full biochemical and haematological data both pre- and post-operatively were available for 206 patients. The patient characteristics of those patients with longitudinal measurements were not significantly different to those without (data not shown).

The baseline characteristics of patients for whom full pre- and post-operative biochemical and haematological data were available are shown in Table 3.1. One hundred and twenty-nine (63%) patients underwent surgery for colonic tumours, while 77 (37%) had surgery for rectal tumours. The majority of patients were more than 65 years old (64%) were male (58%), had TNM stage I/II disease, and were carried out electively. Fifty-eight (28%) patients received adjuvant therapy following resection of the primary tumour. Twenty-five patients received neoadjuvant therapy. While pre- ($p<0.005$) and post- operative ($p<0.001$) NLR were associated with administration of neoadjuvant therapy, there was no association between pre- or post-operative mGPS.

The majority of patients (91%) included in the present study were elective resections. Emergency presentation was significantly associated with a higher pre-operative mGPS ($p<0.001$), while pre-operative NLR was not ($p=0.116$). However, in the present, relatively small study, mode of presentation itself was not significantly associated with cancer-specific survival and was not significantly associated with either post-operative mGPS or NLR.

With regard to more traditional predictors of poor outcome neither pre- or post-operative mGPS, nor pre- or post-operative NLR were associated with TNM stage while only pre-operative NLR ($p<0.05$) and post-operative mGPS ($p<0.05$) were associated with margin status.

In the 206 patients with longitudinal measurements, 74 (36%) patients had an elevated mGPS, pre-operatively. Following resection, 66 patients (32%) had an elevated mGPS. With regard to the NLR 46 (22%) had an elevated NLR pre-operatively while 36 (18%) had an elevated NLR post-operatively.

Analysis of the relationship between the pre- and post-operative samples using the Wilcoxon Rank Test, showed no significant difference between both the pre- and post-operative mGPS, and the pre- and post-operative NLR (Table 3.2, both $p > 0.10$).

Of the 132 patients with an mGPS of 0 pre-operatively, 98 (74%) had an mGPS of 0 on follow-up, 9 (7%) had an mGPS of 1 on follow-up, while 25 (19%) had an mGPS of 2 on follow-up. Of the 33 patients with an mGPS of 1 pre-operatively, 20 (61%) had an mGPS of 0 on follow-up, 3 (9%) patients had an mGPS of 1 on follow-up, while 10 (30%) had an mGPS of 2 on follow-up. Of the 41 patients with an mGPS of 2 pre-operatively, 22 (54%) had an mGPS of 0 on follow-up, 6 (15%) had an mGPS of 1 on follow-up, while 13 (32%) had an mGPS of 2 on follow-up. Of the 161 patients with an NLR of 0 pre-operatively, 139 (86%) had an NLR of 0 on follow-up, while 22 (14%) patients had an NLR of 1 on follow-up. Of the 45 patients with an NLR of 1 pre-operatively, 31 (69%) patients had an NLR of 0 on follow-up, while 14 (31%) had an NLR of 1 on follow-up.

Similarly, there was no significant difference between both the pre- and post-operative mGPS and the pre- and post-operative NLR in patients with node negative disease (both $p>0.10$).

The relationship between clinico-pathological characteristics and survival in patients undergoing potentially curative resection for colorectal cancer are shown in Table 3.2. In the present study the minimum follow-up period was 12 months and the median follow-up period of those who remained alive was 36 months (range 12-71) months. On follow-up, 41 patients died, of which 29 patients died of colorectal cancer and 12 from other causes. No patients were lost to follow-up.

On univariate survival analysis in 206 patients, T-stage ($p < 0.001$), TNM stage ($p = 0.005$), pre-operative mGPS ($p < 0.05$, Figure 3.1a) and NLR ($p < 0.05$, Figure 3.1b) were significantly associated with cancer-specific survival. Similarly, on univariate survival analysis post-operative mGPS ($p < 0.001$, Figure 3.2a) and NLR ($p < 0.005$, Figure 3.2b) were both significantly associated with cancer-specific survival.

On multivariate survival analysis, comparing pre-operative mGPS and NLR, both pre-operative mGPS (HR 1.97, C.I. 1.16-3.34, $p <0.05$) and NLR (HR 3.07, C.I. 1.23-7.63, $p <0.05$) were independently associated with reduced cancer-specific survival (mGPS, and NLR). When the same multivariate comparison was carried out on post-operative measurements, only the post-operative mGPS was independently associated with cancer-specific survival (HR 4.81, C.I. 2.13-10.83, $p < 0.001$).

On univariate survival analysis in 117 node negative patients, only pre-operative mGPS ($p < 0.05$) was associated with cancer-specific survival. On univariate analysis using date of post-operative sample to date of death only post-operative mGPS ($p < 0.001$) was significantly associated with cancer-specific survival.

- **3.4 – Discussion**

In the present study, we report, for the first time, a longitudinal comparison of systemic inflammation-based prognostic scores in patients with colorectal cancer. The results are consistent with previous studies that have reported the prognostic value of the pre-operative assessment of mGPS and the NLR. Further, the post-operative assessments (3-6 months) also had prognostic value in patients with colorectal cancer. Therefore, it is clear that the persistent elevation of the systemic inflammatory response, before and following resection of colorectal cancer, is associated with poor survival. Taken together, there is clearly a role for the routine monitoring of the systemic inflammatory response in patients undergoing potentially curative resection of colorectal cancer.

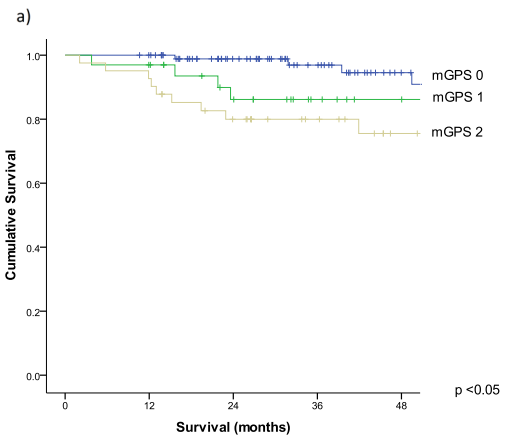
In the present study, it was of interest that the systemic inflammatory response, whether measured using the mGPS or NLR, was not subject to significant overall change from the pre- to post- operative period. The majority of patients, before surgery, had no evidence of a systemic inflammatory response. In these patients, the majority remained systemically non-inflamed following surgery. Of the patients who had evidence of a systemic inflammatory response before surgery, a small proportion became systemically non-inflamed following resection of the primary tumour. The consequence of these changes may be that the post-operative measurement of the systemic inflammatory response better predicts outcome. Indeed, these results are consistent with previous studies that reported the prognostic value of CRP alone, both pre- and post-operatively. [188, 190]. When the post-operative mGPS and NLR were compared, it was clear that the mGPS had superior prognostic value. Further studies are warranted.

Recently, it has been reported that the NLR was associated with a higher mortality in patients with coronary artery disease [319]. Given that this is a major cause of non-cancer death, it is possible that some of the patients in the present study died of coronary artery disease. However, the small numbers of non-cancer deaths in the present study precluded meaningful statistical analysis.

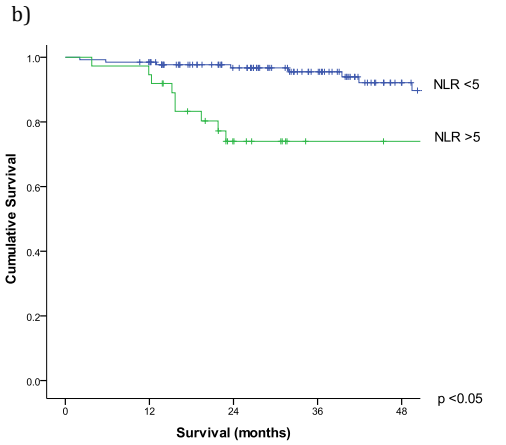
In the present study, we assessed the prognostic value of longitudinal measurements of systemic inflammation in patients undergoing curative resection of colorectal cancer. Although there is good evidence that presence of the systemic inflammatory response, evidenced by mGPS or NLR, is associated with poorer outcomes in patients with cancer [195], the mechanism underlying persistent activation of the systemic inflammatory response in patients who have undergone resection of the primary tumour remains unclear. It may be secondary to chronic dysregulation of immune and inflammatory responses owing to activation by micrometastatic disease or non-malignant disease invoking tissue injury/necrosis. Plausible mediators of these immune and inflammatory responses are the pro-inflammatory cytokines that have been reported to be present at elevated concentrations in the circulation of patients with colorectal cancer [316]. In particular, the pleiotropic pro-inflammatory cytokine interleukin-6, upregulated by tissue injury/necrosis and inflammatory cells such as macrophages, have the ability to activate and maintain the systemic inflammatory response through trans-signalling pathways involving the soluble IL-6 receptor [195].

The reasons for those patients with persistently activated systemic inflammatory responses having worse prognosis is becoming increasingly clear. In addition to the recognized detrimental effect on nutritional and functional status[195] , there is increasing evidence that an elevated systemic inflammatory response is dysregulated, in particular with upregulation of innate immune responses and downregulation of adaptive immune responses promoting tumour dissemination and progression [193].

In summary, the results of the present study support the longitudinal assessment of the systemic inflammatory response, in particular using the mGPS, in patients undergoing potentially curative resection for colorectal cancer. Further work using serial measurements of markers of systemic inflammation following resection may add further weight to the hypothesis that such markers of systemic inflammation may be useful in predicting survival.

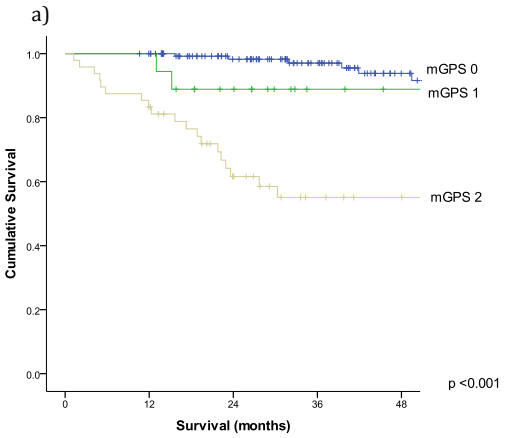


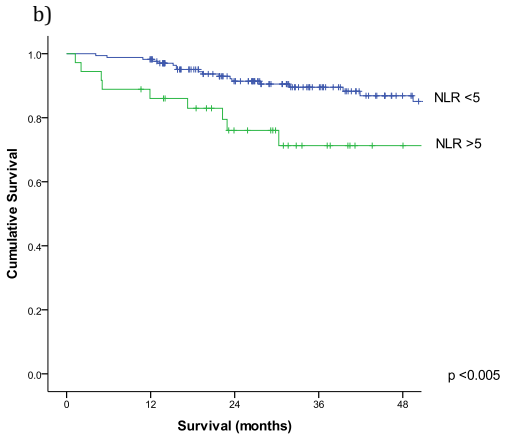
No. at risk					
	0	12	24	36	48
mGPS 0	132	125	86	54	32
mGPS 1	33	32	23	15	11
mGPS2	41	38	29	21	13



No. at risk					
	0	12	24	36	48
NLR <5	161	151	111	75	43
NLR >5	45	44	27	15	13

Figure 3.1 – The relationship between pre-operative assessment of systemic inflammation as evidenced by mGPS (a) and NLR (b) and cancer specific survival in patients undergoing potentially curative resection of colorectal cancer.





No. at risk					
	0	12	24	36	48
mGPS 0	140	120	90	59	39
mGPS 1	18	15	10	3	2
mGPS2	48	32	20	12	9

No. at risk					
	0	12	24	36	48
NLR <5	170	141	102	65	46
NLR >5	36	26	18	9	4

Figure 3.2 – The relationship between post-operative assessment of systemic inflammation as evidence by mGPS (a) and NLR (b) and cancer specific survival in patients undergoing potentially curative resection of colorectal cancer.

Table 3.1 - Baseline clinicopathological characteristics of patients undergoing potentially curative resection for colorectal cancer.

	No. of Patients (n = 206)		
Age <65/65-74/>75	74 (36%)/79 (38%)/53 (26%)		
Sex Male/Female	120 (58%)/86 (42%)		
Presentation Elective/Emergency	187 (91%)/ 19 (9%)		
Site Colon/Rectum	129 (63%) 77 (37%)		
T-stage	**Colon**	**Rectum**	
T1	8 (4%)	6 (3%)	
T2	12 (6%)	9 (4%)	
T3	63(31%)	49 (24%)	
T4	46(22%)	12 (6%)	
TNM stage I II III IV	32 (16%) 85 (41%) 87 (42%) 2 (1%)		
Adjuvant Chemotherapy No/Yes	148(72%)/58(28%)		
Measurement of Systemic Inflammatory Response (TNMI-IV)	Pre-operative (n = 206)	Post-operative (n = 206)	p-Value*
mGPS 0 1 2	 132 (64%) 33 (16%) 41 (20%)	 140 (68%) 18 (9%) 48 (23%)	 p = 0.926
NLR 0 (<5) 1 (>5)	 161 (78%) 45 (22%)	 170 (82%) 36 (18%)	 p = 0.216
Measurement of Systemic Inflammatory Response (TNM I/II)	Pre-operative (n = 117)	Post-operative (n = 117)	
mGPS 0 1 2	 74 (63%) 17 (15%) 26 (22%)	 82 (70%) 10 (9%) 25 (21%)	 p = 0.427
NLR 0 (<5) 1 (>5)	 90 (77%) 27 (23%)	 93 (79%) 24 (21%)	 p = 0.577

*Wilcoxon Rank Test

Table 3.2 – The relationship between clinicopathological characteristics and survival in patients undergoing potentially curative resection for colorectal cancer.

	All patients (n=206)			**Node negative (n = 117)**		
	Cancer-Specific Survival			Cancer-Specific Survival		
	Univariate Analysis			Univariate Analysis		
	HR	95% CI	p-Value	HR	95% CI	p-Value
Age (<65/ 65-74/ >75)	0.79	0.49-1.27	0.330	0.10	0.24-1.14	0.101
Gender (Male/ Female)	1.24	0.58-2.63	0.570	4.23	0.92-19.41	0.064
Site (Colon/ Rectum)	1.03	0.49-2.19	0.930	1.55	0.49-4.80	0.450
T-stage (T1/ T2/ T3/ T4)	2.75	0.49-5.07	0.001	1.69	0.77-3.70	0.190
TNM Stage I/ II/ III/ IV	2.31	1.28-4.17	0.005	4.13	0.53-32.08	0.180
Pre-operative mGPS (0/ 1/ 2)	1.94	1.17-3.21	0.010	2.57	1.10-5.96	0.028
Pre-operative NLR (<5/ >5)	3.28	1.36-7.93	0.008	2.08	0.62-2.09	0.233
Post-operative mGPS (0/ 1/ 2)	3.31	2.15-5.09	<0.001	4.81	2.13-10.83	<0.001
Post-operative NLR (<5/ >5)	3.07	1.42-6.62	0.004	3.10	0.98-9.84	0.054

4.0 – COMPARISON OF VISUAL AND AUTOMATED ASSESSMENT OF TUMOUR INFLAMMATORY INFILTRATES IN PATIENTS WITH COLORECTAL CANCER.

- 4.1 - Introduction

As described in Chapter one it has become increasingly clear that local inflammatory responses are a key determinant of progression and survival in patients with colorectal cancer [183]. There is consistent evidence that the presence of a high-grade local inflammatory cell infiltrate both within the tumour and in the immediate microenvironment predicts survival independent of tumour stage in colorectal cancer [181-183]. Many studies have reported that increasing density of inflammatory cells in and around the tumour is associated with improved outcome in patients with colorectal cancer and this is thought to represent the host anti-tumour response [183]. Further, there is good evidence that the immune classification of tumours has independent and superior prognostic value when compared to traditional staging methods [182].

Despite the strong evidence supporting the prognostic value of inflammatory cell infiltrates, and the existence of well-described methods for the semi-quantitative assessment of inflammatory cell infiltration, [181, 182], the extent of the local inflammatory cell infiltrate is not routinely considered in clinical practice and conventional staging systems such as TNM stage remain the mainstay in clinical practice.

Reasons for this include the complexity and lack of reproducibility of scoring the inflammatory cell infiltrate caused by differences in immunohistochemical staining methods between different units, the different cell types present and importantly, the subjectivity of assessing the tumour inflammatory cell infiltrate. Therefore a reliable and accurate measure of the tumour inflammatory cell infiltrate may be useful in the refinement of staging the host inflammatory response in clinical pathological practice.

There is now image analysis software capable of point-scoring cells in routinely processed H&E tumour sections. Recent studies have reported that computer-aided analysis has significant advantages over manual scoring methods including: objectivity, accuracy and reproducibility[320-323]. Therefore, this modality may offer a method of standardizing the assessment of the local inflammatory cell infiltrate in patients with colorectal cancer.

The aim of the present study was to compare visual and automated assessment of tumour inflammatory cell infiltration in patients with colorectal cancer.

- **4.2 – Patients and Methods**

Patients with colorectal cancer who, on the basis of preoperative staging and laparotomy findings, were considered to have undergone an elective, potentially curative resection of colorectal cancer between 1997 and 2006 in a single surgical unit at Glasgow Royal Infirmary were included in the study. Tumours were staged using the conventional tumour, node, metastasis (TNM) staging system, 7th Edition, [318]. Patients with conditions known to elicit an acute or chronic systemic inflammatory response were excluded. These were namely (i) pre-operative chemoradiotherapy, (ii) clinical evidence of active pre-operative infection, or (iii) chronic active inflammatory diseases such as rheumatoid arthritis.

The study was approved by the Research Ethics Committee, Glasgow Royal Infirmary, Glasgow.

Visual Assessment of Tumour Inflammatory Cell Infiltration Assessment of the tumour inflammatory cell infiltrate was performed on original haematoxylin and eosin-stained full-sections of the tumour, considered to be representative of the specimen. The local inflammatory response was evaluated previously in this cohort (GJKG and CSDR) using the method described by Klintrup and Makinen [181].

Briefly, K-M criteria is a four-point scale, a score of 0 indicates no increase in inflammatory cells at the invasive margin, a score of 1 indicates presence of a mild/patchy increase in inflammatory cell reaction at the invasive margin but no destruction of invading cancer cell islets, a score of 2 indicates observation of a band-like inflammatory reaction at the invasive margin, and a score of 3 indicates observation of a florid inflammatory reaction with cup-like inflammatory infiltrate at the invasive margin. The manual scores were then dichotomised to 'high' and 'low' grade inflammation in line with the previously published literature [181].

- **Slide Scamming and Automated Assessment**

The routine Haematoxylin & Eosin stained tumour sections used for the visual assessment were scanned using a Hamamatsu NanoZoomer (Welwyn Garden City,Hertfordshire, UK). Visualization and image analysis assessment was carried out using Slidepath Digital Image Hub, version 4.0.1, (Slidepath, Leica Biosystems, Milton Keynes, UK). Visual assessment of inflammatory infiltrates at the deepest point of the invasive margin was performed on a high definition monitor. The *'Measure stained cells algorithm'*, Tissue Image Analysis, version 2.0 (Slidepath, Leica Biosystems, Milton Keynes, UK), was then used to assess the sections for immune cell infiltrates at the point felt to represent the deepest point of tumour invasion. The algorithm quantifies nuclear staining to derive a numeric score for each selected sample area. The default algorithm preferences were modified to count only inflammatory cells at the invasive margin and exclude other cell types including stromal fibroblasts and tumour cells.

This distinction between different cell types was based on different staining intensities, cell size and size of nuclei. The optimized algorithm parameters are shown in Appendix 1. Visual validation of the algorithm was performed to ensure that only inflammatory cells were counted and other cell types were excluded from the analysis. The analysis scale was performed at 20x magnification for all scoring.

In order for the software system to be guided to analyse the inflammatory infiltrate at the invasive margin it was necessary to annotate the H&E sections. Two different methods of annotation were used and their accuracy assessed. Three slides were annotated and scored for each specimen and a total of 160 tumour specimens were scored.

- **Annotation Methods**

Freehand annotations

- Using the sealed freehand annotation tool provided by Slidepath software. The area to be analyzed was manually selected by drawing around the inflammatory infiltrate at the invasive margin. All freehand annotations were drawn at 20x magnification.

- One freehand annotation was created for each slide.

- The optimized algorithm was selected and the algorithm customized to analyze only the annotated region at the invasive margin.

- Upon completion of automatic cell counting, manual export of the data in .csv format allowed for further analysis.

- Cell counts were expressed as total number of positive nuclei (Âµm) and number of positive stained cells/mm^2. The total number of positive nuclei was obtained from the Slidepath data output and the number of cells/ mm^2 was calculated by dividing the mean total number of cells by the mean total tissue area analyzed (mm^2).

- Three slides were scored for each tumour specimen and the mean score of the slides was taken as the final score for that specimen.

Rectangular Box annotations

- The area from the invasive margin to be analyzed was identified visually and the magnification was then set to 20x at the selected area.

- The 'Tissue IA optimizer' icon was selected and the optimized 'Measure stained cells algorithm' was chosen to analyze the area of the invasive margin visible on-screen. This generated rectangular boxes to indicate the position of the area analyzed. Results were recorded and stored for each rectangular area.

- Cell counts were expressed as the total number of positive nuclei (Âµm) and number of positive stained cells/mm2. The total number of positive nuclei was obtained from the Slidepath data output and the number of cells/ mm2 was calculated by dividing the mean total number of cells by the mean total tissue area analyzed (mm2).

- To ensure reproducibility of measurements, three rectangles were assessed along the invasive margin of each tumour using this method. The mean score of the three boxes was taken as the final score for that slide.

- Three slides were scored for each tumour specimen and the mean score of the slides was taken as the final score for that specimen.

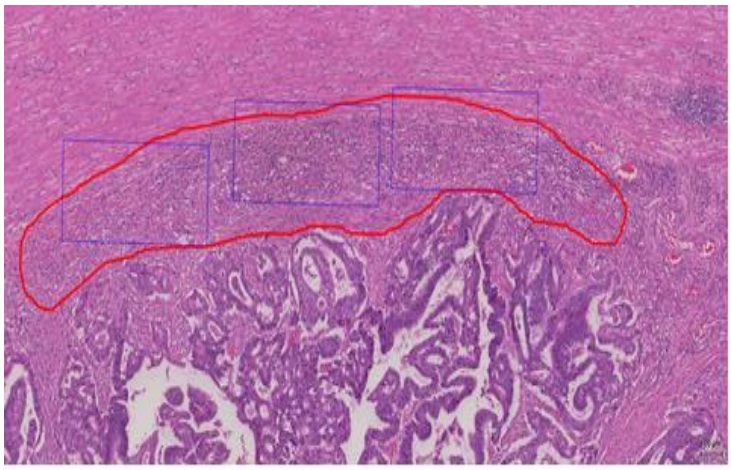


Figure 4.0– Image illustrating annotation methods

Statistical Analysis Associations between visual K-M scores and automated inflammatory cell counts were examined using boxplots. An analysis of variance between the automated cell counts associated with each K-M score were performed using the Kruskal-Wallis test. The inflammatory cell counts were categorised into groups using quartiles. All other variables were grouped according to standard or previously published thresholds. Associations between automated scoring and tumour variables were examined using Chi-square tests for trend. Kaplan-Meier survival curves with log-rank tests were used to perform univariate survival analyses. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS® version 19.0 (IBM SPSS, Chicago, Illinois, USA).

- **4.3 - Results**

One hundred and fifty-four patients who underwent potentially curative resection of colorectal cancer were included in the study. Summary characteristics of patients included in the study are shown in Tables 4.1 and 4.2. The majority of patients were 65 years or older (70%) with similar numbers of men (52%) and women (48%). The majority of resections were carried out electively (87%) and were for colon cancer (78%). Pathological reports classified the tumours as T-stage 1 (1%), 2 (3%), 3 (72%), 4 (24%). On routine pathological analysis, the minority of patients had evidence of 'high-risk' pathological features including poor tumour differentiation (10%), extramural vascular invasion (31%), peritoneal involvement (22%), margin involvement (6%), and therefore the majority were classed as 'low' Petersen Index (88%). On visual assessment using Klintrup's criteria, the majority of patients (69%) were given a score of 0 or 1 (low-grade inflammation) and the remainder (31%) were given a score of 2 or 3 (high-grade inflammation).

The relationships between K-M scoring and the automated inflammatory cell counts were examined using boxplots. The automated inflammatory cell counts assessed using the freehand annotation method were significantly associated with both K-M score ($p<0.001$) and K-M grade ($p<0.001$). The automated inflammatory cell counts assessed using the rectangular box method were also significantly associated with both K-M score ($p<0.001$) and K-M grade ($p<0.001$). The median numbers of inflammatory cells associated with each K-M score and each K-M grade are shown in Table 4.3.

An analysis of variance was performed to assess for significant differences in the median number of inflammatory cells associated with each K-M score. The freehand annotation method demonstrated significant differences in the number of inflammatory cells between all K-M scores, with the exception of scores 2 and 3. The rectangular box method demonstrated significant differences in the number of inflammatory cells between all K-M scores, with the exception of the number of cells per mm^2 between scores 2 and 3, (Table 4.3). Whilst both methods demonstrated significant variation across K-M categories, there was greater discrimination using the rectangular box method in comparison to the freehand annotation method (Chi-square 86.2 vs 52.6). Therefore, the total number of inflammatory cells at the invasive margin assessed using the rectangular boxes method was selected for further survival analysis.

The inflammatory cell counts were then divided into categories to group tumours with similar inflammatory cell densities. Given that there were significant differences in the number of inflammatory cells associated with each K-M score, using the rectangular boxes method, the automated cell counts were grouped into four separate categories using quartiles. To test how strongly the automated Klintrup quartiles were associated with the visually scored K-M scores the intra-class correlation coefficient (ICC) was calculated and there was good agreement with an ICC of 0.82.

Similar to the visual K-M scoring system, the automated K-M classification of the inflammatory cell counts, using quartiles, was significantly associated with venous invasion ($p<0.05$), and mGPS ($p\leq0.05$), Table 4.4.

An assessment of the prognostic value of the automated classification of inflammatory cell counts was then performed. The median follow-up was 107 months (range 44–178). During this period, 43 patients died from colorectal cancer and 38 patients died from other causes. The relationship between baseline clinicopathological characteristics and cancer-specific survival are shown in table 4.4.

On univariate analysis, age ($p<0.05$), T-stage ($p<0.005$), N-stage ($p<0.005$), TNM stage ($p<0.005$), venous invasion ($p<0.05$), peritoneal involvement ($p<0.05$), margin involvement ($p<0.005$), mGPS ($p<0.005$), and visual K-M category ($p<0.005$) and visual K-M grade ($p<0.005$) were significantly associated with cancer specific survival. Further, on univariate survival analysis, both automated K-M category ($p<0.05$) and automated K-M grade ($p<0.005$) were associated with cancer-specific survival.

Kaplan-Meier survival analysis using the manually acquired scores demonstrated that patients whose tumours had high-grade inflammatory/immune cell infiltrate had improved cancer-specific survival compared with those with low-grade inflammatory cell infiltrate (Figures 4.1a and 4.1b) as previously reported by Klintrup and colleagues. Similarly, on Kaplan-Meier survival analysis, both automated Klintrup Category ($p<0.05$) and automated Klintrup Grade ($p<0.05$) demonstrated that those patients with high inflammatory cell counts had improved cancer-specific survival when compared with those patients with lower inflammatory cell counts, (Figures 4.2a and 4.2b).

- **4.4 – Discussion**

The results of the present study demonstrate that using commercially available image analysis software, an automated objective assessment of the peritumoural inflammatory cell infiltrate at the invasive margin was obtained and that such an assessment had prognostic value in patients with colorectal cancer.

In order for assessment of the local inflammatory response to be adopted in routine clinical pathological practice, the method used must be practical, reproducible and accurate on routinely stained H&E tumour sections. In the present study, whilst both automated methods, rectangular boxes and freehand annotations, demonstrated significant variation across K-M categories, there was greater discrimination using the rectangular box method. The rectangular box method allowed more precise and selective sampling of inflammatory infiltrates along the tumour invasive margin.

The freehand method required sampling from a larger, continuous area of the invasive margin, therefore these samples were likely to include more background 'noise' making it difficult to accurately point score the inflammatory cells. In addition, the freehand annotations were not of a standardized shape or size. In the present small study, the rectangular box automated scoring method had a tendency to over-score the local inflammatory cell infiltrate compared to the visual K-M scoring system (4/47 graded as K-M high grade were scored by automated method as 'low inflammatory cell count' and 15/93 graded as K-M low grade were scored by the automated method as 'high inflammatory cell count').

These discrepancies are likely to be related to the algorithm, as selection of valid algorithm parameters is of utmost importance. The algorithm used in the present study distinguished inflammatory cells from other cell types found at the invasive margin, including stromal fibroblasts and tumour cells. This distinction was based on different staining intensities, cell size, and size of nuclei, amongst other parameters, and the algorithm was optimized to work adequately across all tumour specimens.

In the present study, the good agreement observed between the visual K-M scoring method and the automated inflammatory cell counts support the accuracy of the image analysis scoring method for the assessment of the local inflammatory response. In addition, and providing further validation of accuracy, the automated inflammatory cell counts were associated with a number of pathological characteristics including T stage, TNM stage, venous invasion, peritoneal and margin involvement. A key advantage of the automated method is that it provides reliable numeric quantification of the inflammatory cell density and thus provides an objective measure that may also provide an improved degree of accuracy and sensitivity over the more subjective visual method. Indeed, several authors have proposed that automated assessment of inflammatory infiltrates offers advantages over visual assessment including, consistency, exactness, objectivity and time-efficiency [322-324].

Previous studies examining the role of computer-aided assessment of tumour inflammatory infiltrates have focused on the use automated cell counting of imunohistochemically stained tumour sections [322-325].

However, very few immunohistochemical stains are used in routine clinical practice, therefore the use of such stains to aid automated cell counting adds a layer of complexity to automated assessment that may preclude its use in routine clinical pathological practice in most institutions. While the H&E protocol used in our institution has been consistent during the study period, we recognize that other centres may have slightly different staining protocols that may result in different staining intensities that may affect the reproducibility of this method if the same algorithm was to be applied to other cohorts. While we acknowledge this limitation, we propose that minor adjustment of the image analysis algorithm may be able to allow for this.

An automated scoring method will not replace the need for a human observer to determine that the images analyzed are representative of the lesion, do not have significant background staining, and ensure areas of necrosis are avoided but will provide a degree of consistency in observation not possible with purely visual assessment. In addition, the computer-based scoring method used in this study takes advantage of existing software available and is not a 'custom' system, and is therefore easy to use and relatively inexpensive and could easily be acquired by pathology departments for routine use.

Importantly, in terms of potential clinical utility, the automated assessment of the local inflammatory infiltrate using the K-M criteria is simple, practical and quick to perform. In addition, further software developments including refinements to image annotation may enhance clinical utility of the methodology further.

Such developments, for example the placeable grid, may facilitate an even more practical and time effective assessment of the local inflammatory response that can be used widely in the prognostic assessment of patients with colorectal cancer.

Given the importance of the host local immune response in colorectal cancer progression, there have been calls to incorporate an assessment of this into routine clinical practice. Indeed, assessment of the local inflammatory cell response has the potential to predict outcome and identify patients at risk of recurrence and for whom adjuvant treatment should be considered [326].

In summary, the results of the present study demonstrate that automated assessment appears to effectively recapitulate the clinical value of visual assessment of the local inflammatory cell infiltrate at the invasive margin of colorectal tumours. In addition, the present study demonstrates it is possible to obtain an objective assessment of tumour inflammatory infiltrates using routinely stained H&E sections and that an automated, computer-based scoring method is therefore a workable and cost-effective approach to the clinical assessment of local immune cell infiltrates in patients with colorectal cancer. This study was an exploratory pilot study to assess the potential of an automated method of scoring inflammatory infiltrates. Given the apparent value of this method, a larger cohort study could be pursued to validate the described technique and assessment of its value within a multivariate may form the basis of further work in this area. In addition, a study examining the value of this automated system in a cohort of patients enrolled in a clinical trial may add value to the preliminary work described here.

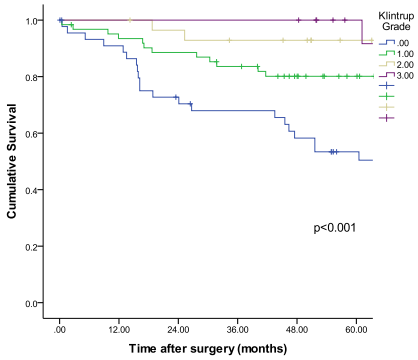


Figure 4.1a – Kaplan-Meier survival curve demonstrating the relationship between Manual Klintrup-Makinen grade and cancer-specific survival.

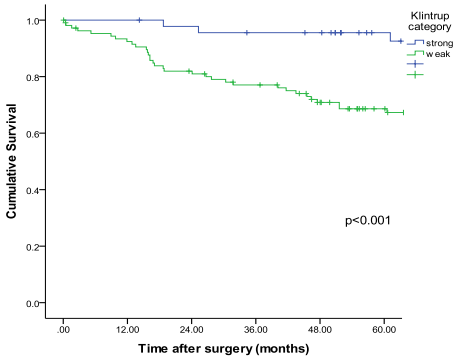


Figure 4.1b – Kaplan-Meier survival curve demonstrating the relationship between Manual Klintrup-Makinen category (weak/strong) and cancer-specific survival.

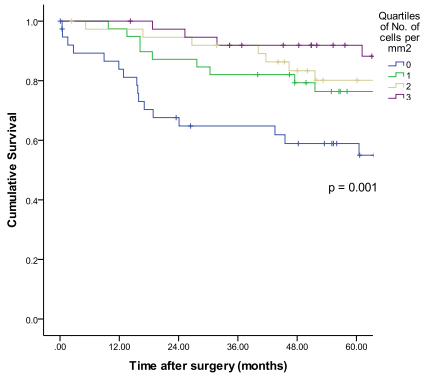


Figure 4.2a – Kaplan-Meier survival curve demonstrating the relationship between Automated Klintrup-Makinen grade and cancer-specific survival.

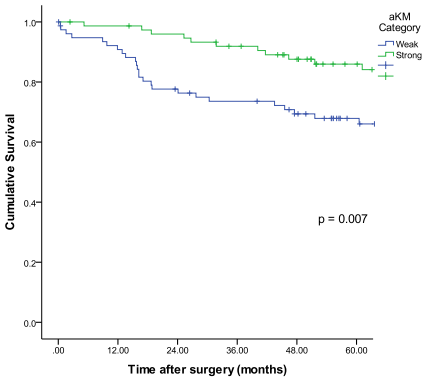


Figure 4.2b – Kaplan-Meier survival curve demonstrating the relationship between Automated Klintrup-Makinen category (weak/strong) and cancer-specific survival.

Table 4.1 - The clinicopathological characteristics of patients with primary operable colorectal cancer.

Clinicopathological characteristics	**Patients (*n = 154*)**
Age (<65/65-74/ >75 years)	46 (30%)/58 (38%)/ 50 (32%)
Sex (Male/Female)	84 (55%)/ 70 (46%)
Presentation (Elective/Emergency)	135 (87%)/ 16 (10%)
Tumour Characteristics	
Tumour Site (Colon/Rectum)	125 (81%)/ 29(19%)
T-stage ($T_1/T_2/T_3/T_4$)	1 (1%)/ 4 (3%)/ 107 (69%)/ 42 (27%)
N-stage ($N_0/N_1/N_2$)	129 (84%)/ 16 (10%)/ 9 (6%)
TNM Stage	4 (3%)/ 125 (81%)/ 25 (16%)
Differentitation (Poor/Moderate-Well)	13 (9%)/ 141 (91%)
Venous Invasion (Absent/Present)	103 (67%)/ 51 (33%)
Peritoneal Involvement (Absent/Present)	116 (75%)/ 38 (25%)
Margin Involvement (Absent/Present)	146 (95%)/ 8 (5%)
Tumour Perforation (Absent/Present)	148 (96%)/ 6 (4%)
Systemic Responses	
Modified Glasgow Prognostic Score (0/1/2)	84 (54%)/ 41 (27%)/ 29 (19%)
Neutrophil_Lymphocyte Ratio (<5/>5)	93 (60%)/ 31 (20%)
Local Inflammatory Cell Infiltrate	
Visual Klintrup-Makinen Grade (0/1/2/3)	46 (30%)/ 62 (40%)/ 29 (19%)/ 17 (11%)
Visual Klintrup-Makinen Category (Weak/Strong)	108 (70)/ 46 (30%)

Table 4.2 – Baseline clinicopathological characteristics and relationships with cancer-specific survival (n=154).

	Cancer-Specific Survival		
	Univariate Analysis		
	HR	**95% CI**	**p-Value**
Patient Factors			
Age (<65/ 65-74/ >75)	1.85	(1.22-2.80)	0.004
Sex (Male/Female)	0.862	(0.47-1.60)	0.634
Presentation (Elective/Emergency	1.87	(0.78-4.48)	0.161
Tumour Factors			
Tumour Site (Colon/Rectum)	1.40	(0.69-2.86)	0.354
T-stage ($T_1/T_2/T_3/T_4$)	2.76	(1.52-5.02)	0.001
N-stage ($N_0/N_1/N_2$)	1.89	(1.23-2.89)	0.004
TNM-Stage (I/II/III)	2.75	(1.43-5.28)	0.002
Differentiation (Poor/Moderate-Well)	1.32	(0.47-3.71)	0.599
Venous Invasion (Absent/ Present)	2.54	(1.37-4.73)	0.003
Peritoneal Involvement (Absent/ Present)	2.51	(1.33-4.73)	0.004
Margin Involvement (Absent/Present)	3.70	(1.45-9.47)	0.006
Tumour Perforation (Absent/ Present)	0.69	(0.09-5.04)	0.717
Systemic Inflammatory Response			
mGPS (0/1/2)	1.80	(1.23-2.64)	0.002
NLR (<5/>5)	2.03	(0.97-4.28)	0.061
Local Inflammatory Cell Infiltrate			
Manual Klintrup-Makinen Grade (0/1/2/3)	0.39	(0.26-0.60)	<0.001
Automated Klintrup-Makinen Grade (Quartiles - 0/1/2/3)	0.59	(0.43-0.80)	0.001

Table 4.3 - Median number of inflammatory cells associated with K-M scoring.

	Total Inflammatory Cells (Median and Range)	Total Inflammatory Cells (P-Value)	Inflammatory cells per mm2 (Median and Range)	Inflammatory cells per mm² (P-Value)
Freehand Annotation Method				
Manual K-M Score				
0	670 (13-6524)		0.75 (0-3.65)	
1	1814 (17-7906)	<0.001[a]	2.02 (0-4.75)	<0.001[e]
2	3115 (1222-6806)	<0.001[b]	2.86 (1.49-4.47)	<0.001[f]
3	3107 (671-6483)	0.425[c]	3.06 (0.89-5.36)	0.690[g]
Manual K-M Grade				
Weak	1489 (13-7906)		1.64 (0.3-4.75)	
Strong	3107 (671-6806)	<0.001[d]	2.9 (0.89-5.36)	<0.001[h]
Rectangular Box Method				
Manual K-M Score				
0	91 (0-583)		0.45 (0-3.38)	
1	346 (2-1043)	<0.001[a]	1.81 (0.02-4.97)	<0.001[e]
2	682 (369-1068)	<0.001[b]	3.61 (1.46-5.6)	<0.001[f]
3	852 (145-1227)	<0.05[c]	3.95 (0.76-5.9)	<0.347[g]
Manual K-M Grade				
Weak	273 (0-1043)		1.27 (0-4.97)	
Strong	742 (145-1227)	<0.001[d]	3.7 (0.76-5.96)	<0.001[d]

Freehand Annotation Method – analysis of variance using Kruskal-Wallis test between:

a groups 0 and 1 (total inflammatory cells)

b groups 1 and 2 (total inflammatory cells)

c groups 2 and 3 (total inflammatory cells)

e groups 0 and 1 (inflammatory cells per mm²)

f groups 1 and 2 (inflammatory cells per mm²)

g groups 2 and 3 (inflammatory cells per mm²)

d Analysis of variance between counts in weak and strong category (total inflammatory cells, and inflammatory cells permm²)

Rectangular Box Method - analysis of variance using Kruskal-Wallis test between:

a groups 0 and 1 (total inflammatory cells)

b groups 1 and 2 (total inflammatory cells)

c groups 2 and 3 (total inflammatory cells)

e groups 0 and 1 (inflammatory cells per mm²)

f groups 1 and 2 (inflammatory cells per mm²)

g groups 2 and 3 (inflammatory cells per mm²)

d Analysis of variance between counts in weak and strong category (- total inflammatory cells, and - inflammatory cells permm²)

Table 4.4 – Relationships between automated Klintrup-Makinen (aKM) score using rectangular box method and clinicopathological variables.

	Automated Klintrup-Makinen Score (aKM)				
	Quartiles				
	0 (n = 39)	**1 (n = 39)**	**2 (n = 38)**	**3 (n = 38)**	**p-Value**
Patient Factors					
Age (<65/ 65-74/ ≥75 years)	6/16/17	14/17/8	10/14/14	16/11/11	0.085
Sex (male/ female)	24/15	17/22	20/18	23/15	0.875
Presentation (Elective/Emergency)	31/8	34/5	37/1	36/2	0.007
Tumour characteristics					
Tumour Site (Colon/ Rectum)	36/3	30/9	29/9	30/8	0.148
T-stage (T_1/ T_2/ T_3/ T_4)	0/1/21/17	0/0/29/10	0/1/31/6	1/2/26/9	0.010
N-stage (N_0/ N_1/ N_2)	35/1/3	31/6/2	32/5/1	31/4/3	0.646
TNM Stage	1/34/4	0/31/8	1/31/6	2/29/7	0.757
Differentiation (Poor/Moderate-Well)	5/34	2/37	2/36	4/34	0.731
Venous Invasion (Absent/Present)	18/21	28/11	28/10	29/9	0.006
Peritoneal Involvement (Absent/Present)	24/15	30/9	32/6	30/8	0.055
Margin Involvement (Absent/Present)	36/3	36/3	37/1	37/1	0.207
Tumour Perforation (Absent/Present)	37/2	36/3	38/0	37/1	0.278
Systemic Responses					
Modified Glasgow Prognostic Score (0/1/2)	14/13/12	25/9/5	23/8/7	22/11/5	0.050
Neutrophil-Lymphocyte Ratio (<5:1/>5:1)	26/10	23/9	22/6	22/6	0.463

5.0 – The link between local and systemic inflammatory responses

- **5.1 – Cancer-associated inflammation and circulating cytokines in colorectal cancer.**

As described, there is a clear association between tumour necrosis and the local and systemic inflammatory responses, but the lack of independent prognostic value suggests that necrosis although an important feature, reflects the production of a mediator of local and systemic inflammatory responses. One possibility is that necrosis may stimulate mediators that down-regulate the local inflammatory response and up-regulate the systemic inflammatory response. Therefore, the delineation of such a mediator of the relationship between tumour necrosis and these inflammatory responses may provide some unique insight into the natural history of colorectal cancer and provide potential therapeutic targets.

The molecular links between tumour necrosis and the inflammatory responses are likely to be very complex. However, it is recognized that tumour necrosis is likely to be the result of a tumour out-growing its blood supply, becoming relatively hypoxic and inducing the up-regulation of cellular stress genes in the tumour and the inflammatory cell infiltrate. Indeed, it has been postulated that the combination of inflammation and necrosis provides an environment in which the epigenetic regulation of genes, cell death, cell proliferation and mutagenesis occurs [178].

At sites of chronic inflammation, cells are continuously dying as a consequence of hypoxic stress, an event in turn promoting growth and proliferation of the local epithelium. The apoptotic to necrotic conversion that is associated with unscheduled cell death and the subsequent release of necrotic mediators is recognized not to be a 'clean' death, but instead stimulates inflammatory pathways [178]. These inflammatory pathways are now recognized to be important for angiogenesis, stromagenesis and the promotion of epithelial proliferation, all of which are required for tumour growth [180].

An important hypoxic stress pathway is regulated by hypoxia-inducible-factor-1-alpha (HIF-1α) [327] that is, in turn, a potent stimulator of Interleukin-6 (IL-6) production from the tumour and inflammatory infiltrate cells [328]. It is of interest, therefore, that the IL-6/JAK/STAT pathway has emerged as a key player in cancer-associated inflammation [329-332].

IL-6 is a multi-functional pro-inflammatory cytokine that has crucial roles in tumour progression through growth-promotion, anti-apoptotic activity, and modulation of immune function and thus is a strong candidate for mediating both local and systemic cancer-associated inflammatory responses. The persistently elevated concentrations of IL-6 may then be maintained by failure of negative feedback systems or through the establishment of uncontrolled production of IL-6 and subsequent activation of the JAK/STAT/HIF-1α system.

The increased local and systemic IL-6, have been proposed to play a key role in inflammatory processes that are known to be key to cancer development and progression, including immune-regulation and angiogenesis [3, 333-335] and may also result in an impaired local inflammatory response and an elevated systemic inflammatory response (see Figure 5.1).

Further, experimental models of cancer have added weight to the involvement of IL-6 in inflammation associated forms of cancer, e.g. an in-vivo model of hepatocellular carcinoma proposed IL-6 as a key mediator in cancer-associated inflammation and interestingly, the mechanism involved tissue necrosis [336].

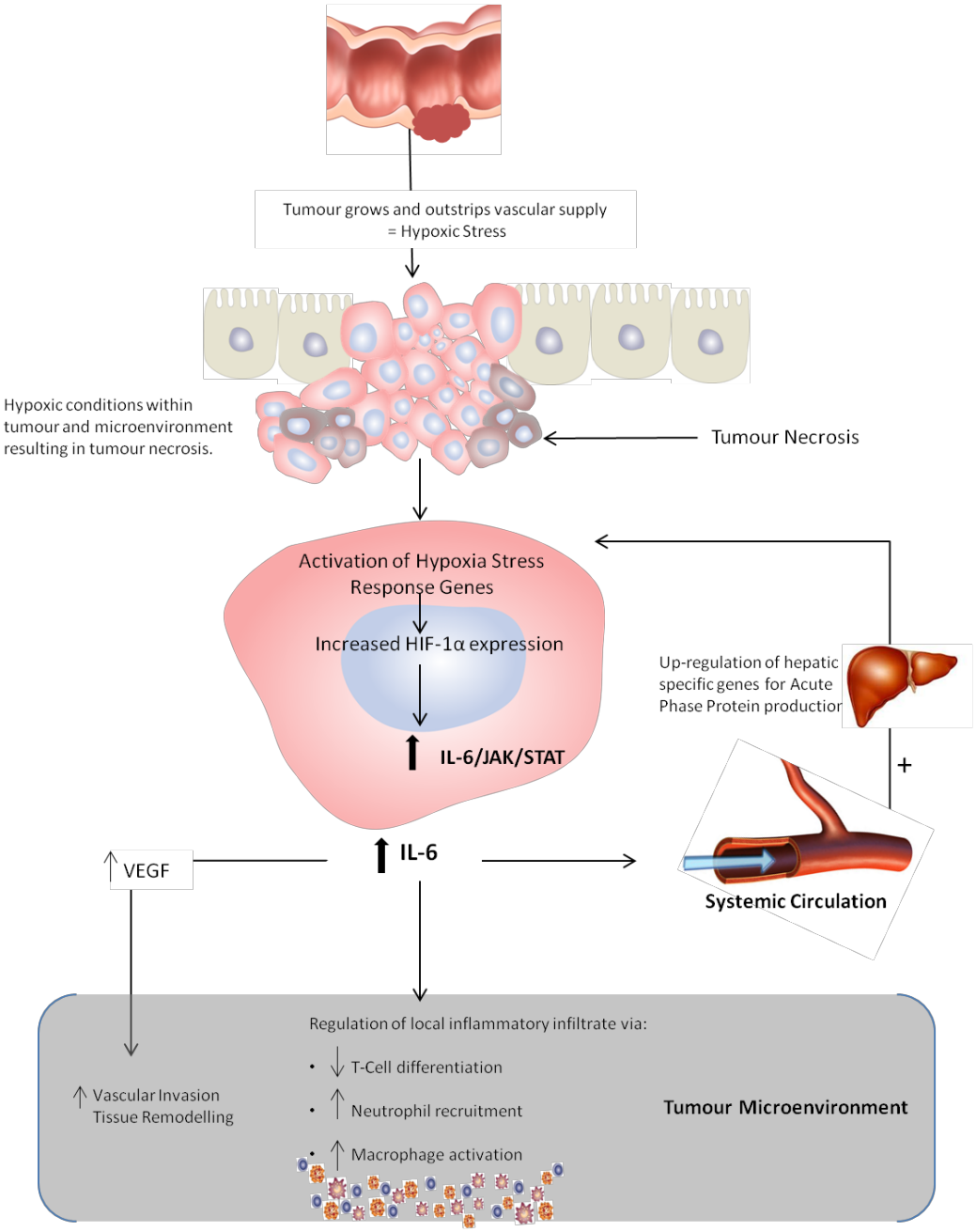


Figure 5.1 – Proposed pathway linking tumour necrosis and the local and systemic inflammatory processes in colorectal cancer.

- **5.2 – Stage-dependent alterations in circulating cytokines**

In addition to in-vivo work suggesting the role of IL-6 in cancer-associated inflammation, recent work has suggested that colorectal cancer is associated with extensive alterations in the serum cytokine environment [316]. In particular, it has been reported that IL-6, similar to tumour necrosis, is associated with advancing T-stage and, interestingly, a strong association between the systemic inflammatory response, as evidenced by the modified Glasgow Prognostic Score (mGPS) [315]. Further, it is proposed that the association between the systemic inflammatory response and serum cytokine alterations provides insight into the inflammatory cells associated with up-regulation of the systemic inflammatory response, in particular the postulated role of macrophages in the tumour microenvironment [316]. These findings add weight to the hypothesis that tumour necrosis has an important role in linking local and systemic inflammatory response in patients with colorectal cancer [171, 337].

- **5.3 – IL-6 and the local inflammatory response in patients with colorectal cancer.**

It is now well-recognised that the local inflammatory response is associated with colorectal cancer survival [183]. There is good evidence that a strong local inflammatory reaction in the form of high numbers of tumour infiltrating lymphocytes (TILs) is a sign of good prognosis [181, 182]. Increasingly, it has been recognized that a delicate balance exists within the tumour microenvironment with regard to local inflammatory responses [338, 339]. Indeed, early studies reported the importance particularly of Th1-polarised cytotoxic T-cells and T memory cells [182]. Importantly, there are studies that report local lymphatic activation, immune deviation, and presence of T-regulatory cells, a cell type associated with disease progression and poor prognosis [340].

In addition, it has been proposed that the genotype of colorectal cancer may influence the local inflammatory infiltrate. Indeed, it has been reported that tumours with high frequency microsatellite instability (MSI-H) are characterised by more abundant intra-tumoural and peri-tumoural lymphocyte infiltrates [341-343]. A possible explanation is that MSI-H tumours are more antigenic than their more genotypically stable counterparts and may passively activate effector T-cells [342-344]. However, despite the association between MSI-H and increased T-lymphocyte infiltrate there appears to be potential for alternative mechanisms modulating the local inflammatory response, potentially via a cytokine mediated pathway that links systemic and local inflammation.

Evidence for a critical role for IL-6 in the modulation of immune cells comes from studies of acute inflammation that have shown IL-6 and the soluble IL-6 receptor play a significant part in the modulation of recruitment of inflammatory cells [345-348]. Also, recent work has reported that IL-6 plays an important role in differentiation of myeloid-derived suppressor cells that may be important with regard to intra-tumoural inflammatory processes [349-351].

Specifically, it has been reported that IL-6 is critical in modulation of T-cell responses in colorectal cancer, particularly affecting Th17 and T-regulatory cells, and also in the trafficking and recruitment of immune cells that produce pro-inflammatory cytokines [352]. Therefore, it may be that IL-6 provides a key regulatory signal in the T-cell adaptive immune response with effects focused on the modulation of T-cell differentiation. Indeed, IL-6 is cited as one of the critical determinants of differentiation of T cells [346, 353, 354]. As a consequence, it is plausible that IL-6 has unique properties that may influence the constituents of the tumour microenvironment, specifically the down-regulation of T-lymphocytic function. However, the exact mechanisms involved remain unclear.

Due to its relatively low molecular weight (21–28 kD), IL-6 is able to rapidly diffuse through cells and tissues and makes up part of a complex milieu of cytokines present in the tumour microenvironment. This property makes this cytokine a good candidate for modulation of inflammatory responses in the tumour microenvironment in colorectal cancer.

Since its discovery in 1986 there have been significant advances in understanding the plethora of roles played by IL-6, including key roles in inflammation, modulation of immune function, and more recently cancer [355]. Interestingly, with regard to cancer, IL-6 has been recognised to play a crucial role in growth-promotion and inhibition of apoptosis, and has been linked to the pathogenesis of various cancers [335]. It has also been known for some time that several human tumour cell lines produce IL-6 [356-358]. In vitro and in vivo studies have suggested a critical role for IL-6 in tumour cell proliferation [359, 360].

Recent studies have provided good evidence for the presence of IL-6 in the tumour microenvironment of various solid organ malignancies. IL-6 has been shown to be expressed at increased concentrations in resected specimens of renal cancer, with strong expression reported in both the epithelium and stroma of the tumour compartment [361]. Interestingly, this group noted that surrounding normal peritumoural tissue showed less expression of IL-6 [361]. Further evidence comes from a recent study by Bellone and colleagues who, for the first time, reported expression of a number of pro-inflammatory cytokines, including IL-6, in the tissue microenvironment of pancreatic cancer[362]. Interestingly, an immunosuppressive state has been reported in the microenvironment of pancreatic tumours and IL-6 modulation of the immune response may explain a reduction in immune surveillance.

With regard to colorectal cancer there is now good evidence that colorectal cancer tissue express IL-6 at increased concentrations. An early study by Kinoshita and co-workers reported that tumoural expression of IL-6 was significantly increased compared to normal tissue [363]. More recently, other groups reported over-expression of IL-6 in colorectal cancer tissues [364, 365]. Chung and colleagues reported a significant association between tumour over-expression of IL-6 and poorer survival [365]. These studies provide further evidence that IL-6 is linked to progression of colorectal cancer. Indeed, they reported that elevated tissue expression of IL-6 was associated with high-risk pathological factors including T-stage, nodal status and vascular invasion [365]. In addition to the over-expression of IL-6 in tissue specimens, recent work has reported tissue expression of Signal Transducer and Activator of Transcription 3 (STAT-3) in colitis-associated colorectal cancer [175]. It is reported the JAK/STAT pathway, specifically STAT-3, is critical in the regulation of inflammation. IL-6 is a key component of this pathway and notably it has been proposed that this cytokine has the potential to activate or inhibit T-cell functions and modulate inflammatory responses [366].

Therefore aberrant IL-6/JAK/STAT signaling in colorectal tumours may be crucial in the development and progression of colorectal cancer. Interestingly, the study by Li et al. also reported that IL-6 is localized at sites of macrophage infiltration thus suggesting an interaction between this cytokine and immune cells in the tumour microenvironment[175].

Thus it seems plausible that IL-6 has unique properties that may influence the constituents of the tumour microenvironment specifically by down-regulating T-lymphocytic function, thus enabling the tumour cells to escape immune surveillance and survive and progress. However the exact mechanisms involved remain unclear and require further investigation.

- **5.4 – IL-6 and the systemic inflammatory response in patients with colorectal cancer.**

The mechanisms for the up-regulation of systemic IL-6 production in patients with colorectal cancer also remain unclear. Indeed, a key question with regard to the involvement of IL-6 in the systemic inflammatory response is whether the tumour microenvironment is the sole source of this pro-inflammatory cytokine in colorectal cancer. In addition, it is proposed that polymorphisms of the IL-6 gene may be involved.

In order to assess the biological plausibility of a link between IL-6 and the systemic and local inflammatory responses we must consider the potential associations between genetic predisposition for an exaggerated IL-6 response in patients developing cancer. Mendelian randomization studies provide an approach to examine this in humans, however, these studies require large numbers of patients. Indeed, this approach has been reported in the investigation of targeting the IL-6 receptor for prevention of coronary heart disease[367]. With reference to polymorphisms of the IL-6 gene, these have been associated with tumourigenesis in a variety of solid organ malignancies, including colorectal cancer, and also with circulating levels of C-reactive protein[368]. Interestingly, a study by Slattery and colleagues reported that there was a significant interaction between Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) use and two of the four recognized IL-6 polymorphisms, and that patients with the C allele who used aspirin/NSAID had lower risk of colorectal cancer[369], further implicating IL-6 and inflammatory-related pathways in colorectal cancer biology.

However, further investigation of the polymorphisms that increase circulating IL-6 concentrations and their relationship with outcome in patients with colorectal cancer is required.

Nevertheless, it is now widely accepted that IL-6 is one of the main pro-inflammatory signals generated by the body to elicit systemic inflammatory responses [186]. Experimental evidence in the early 1990s confirmed a significant role for IL-6 in systemic inflammation. Murine studies clearly demonstrated that genetic deletion of IL-6 led to abolishment of acute phase responses modulated by the liver, and interestingly, impaired T-cell immunity[370]. Thus providing strong evidence that this pleiotropic cytokine is critical to the systemic inflammatory response. Of particular interest is the role IL-6 plays in the up-regulation of hepatic specific genes involved in the production of C-reactive protein (CRP). It is known that this potent pro-inflammatory cytokine is crucial to the up-regulation of the acute phase response by inducing the synthesis and secretion of the acute phase proteins by hepatocytes [186]. In addition, the other components of IL-6 signalling, specifically those involved in trans-signalling: the soluble IL-6 receptor (sIL-6R) and gp130, are reported to be important in the regulation of the acute phase response [352, 371, 372].

There is consistent evidence that circulating IL-6 is important with regard to survival in a number of solid organ malignancies. Several investigators have shown an association between elevated circulating IL-6 and poorer survival in breast cancer.

Ravishanka and colleagues demonstrated that elevated circulating concentrations of IL-6 was directly associated with tumour invasion, lymph node status, and TNM stage and poorer survival [373]. Additionally, a similar relationship between circulating IL-6 and survival in prostate cancer has been reported [374, 375]. Other work has reported an association between more advanced disease, tumour size and presence of metastases [376]. It is of interest that Ramsey et al. reported that IL-6 concentrations did not normalize following resection of renal tumours, and neither did C-reactive protein [377]. In addition, CRP concentrations have been shown to remain elevated following resection of pancreatic and colorectal cancers [188, 378]. Thus it is plausible that IL-6 concentrations may be determined by tissues other than that of the tumour.

With reference to colorectal cancer, since the initial report [379], there has been consistent evidence that circulating IL-6 concentrations are elevated compared with normal controls [363, 380-386]. These studies have also reported associations between IL-6 and more traditional clinicopathological parameters. Specifically, elevated concentrations of IL-6 have been reported to be associated with tumour size and poorer survival [381, 383]. It is of interest therefore that Galizia and co-workers reported that elevated pre-operative circulating IL-6 concentrations were significantly associated with T-stage and predicted curative and non-curative surgery rates [381]. Interestingly, this group noted a drop in circulating IL-6 following surgical resection, however the IL-6 concentrations did not normalize [381].

Similarly, Chung and co-workers, reported elevated circulating IL-6 concentrations in colorectal cancer patients compared to normal controls, and that elevated circulating IL-6 was significantly associated with T-stage[383]. Further, Esfandi et al. assessed IL-6 concentrations at different tumour stages and reported that circulating IL-6 increases in a T-stage related manner [385]. Indeed, this finding is corroborated by other studies[383, 385, 387]. Therefore it can be concluded that circulating IL-6 concentrations are associated with tumour size but it is not clear whether the tumour is the main source of IL-6 in patients with colorectal cancer.

To date, with reference to M-stage, circulating IL-6 concentrations have been reported to be significantly higher in patients with both colorectal liver and lung metastasis[363, 383, 388]. The majority of studies have investigated IL-6 with regard to presence of liver metastasis, indeed three studies have shown there to be a significant association between elevated circulating IL-6 concentrations and the presence of liver metastasis [363, 383].

Several studies have reported elevated circulating IL-6 concentrations to be directly associated with elevated levels of C-reactive protein in various cancers [376, 377, 389, 390]. Most recently, Ravishankaran and colleagues reported a positive correlation between elevated IL-6 concentrations and CRP ($r = 0.579$) in breast cancer patients [373]. Also, studies in prostate cancer have reported a correlation between elevated circulating IL-6 and other markers of systemic inflammation, namely erythrocyte sedimentation rates (ESR), hypoalbuminaemia, thrombocytosis, leukocytosis, and anaemia [391-393].

Also there is consistent finding of a correlation between IL-6 and CRP in renal malignancy [376, 394-396]. Interestingly, Blay et al. report both IL-6 and CRP concentrations fell following administration of anti-IL-6 antibody and increased following withdrawal of this treatment [397].

Martin and colleagues reported elevated IL-6 concentrations in lung cancer patients and noted that IL-6 appeared to enhance the acute phase response in this group of patients[398], while McKeown et al. reported IL-6 to be the only pro-inflammatory cytokine independently associated with CRP ($r = 0.616$) in patients with lung cancer[389].

With reference to patients with colorectal cancer, it has been reported that increased circulating concentrations of IL-6 are associated with increased CRP [383, 399]. It has been proposed that the association between IL-6 and C-reactive protein in colorectal cancer may reflect uncontrolled up-regulation of IL-6 signalling pathway[356, 399]. Despite these findings, no studies have addressed the potential relationship between tumour necrosis and circulating concentrations of IL-6 and the effect on the systemic and local inflammatory responses.

From the above therefore, it is conceivable that elevated circulating IL-6 may originate from the tumour, the tumour microenvironment, or both. In this regard further work is required to assess the role played by tumour necrosis and the immune cells in the tissue microenvironment in the upregulation of IL-6 signalling. Indeed, there is evidence that macrophages may drive IL-6 signalling in the tumour microenvironment[400].

While these studies have been informative regarding the role of circulating IL-6 in advanced disease, the relationship between circulating IL-6 and prognosis in patients with early stage disease has not been extensively investigated in colorectal cancer and requires further investigation.

- **5.5 – Summary**

There is now a significant body of literature implicating IL-6 as a key mediator in the natural history of a variety of common solid tumours, in particular colorectal cancer. With reference to colorectal cancer both tumour tissue and circulating IL-6 concentrations have been reported to be elevated.

Furthermore, elevated tumour concentrations have been associated with increased tumour proliferation, differentiation and vascular invasion and circulating concentrations have been associated with systemic effects such as weight loss, fatigue and the production of acute phase proteins. Taken together these results support the concept that the pleiotropic cytokine, IL-6, may be involved in modulation of both the systemic and local inflammatory responses in a variety of tumours including colorectal cancer. However, it is not clear whether the elevated tumour and circulating concentrations of interleukin-6 are due to the presence of necrosis or some other constitutive lesion. Furthermore, the role of IL-6 and its downstream signaling pathways, such as the JAK/STAT pathways need further investigation in colorectal tumours and inflammatory infiltrate. Such information will provide new insight into the natural history of colorectal cancer and may help to provide a means of identifying those patients with early stage disease who may be at risk of disease progression.

Additionally, if IL-6 were proven to be important in this regard, it may become an important therapeutic target in patients with colorectal cancer. Indeed, in recent years multiple cell line and animal studies have reported consistent evidence that anti-IL-6 therapies represent a promising treatment strategy in various cancers[401, 402]. With regard to cancer-associated inflammation it is becoming more apparent that targeted therapies against IL-6 will not only target malignant tumour cells but also target the crucial interaction between the tumor and the host in the tumour microenvironment.

Of particular interest, is the finding that inhibition of IL-6 signalling specifically suppressed the phosphorylation and nuclear translocation of STAT-3, which has been strongly implicated in the tumour-host interaction [403]. Over the last decade several monoclonal antibodies and conjugated toxins have been developed for inhibition of IL-6 signalling in humans.

More recently, some of these compounds have entered phase I/II trials, with notable results including the finding that reduction in circulating IL-6 concentrations was associated with better response to therapy [404-406]. Despite these encouraging results the clinical efficacy of these treatments are yet to be fully examined. It is also of interest that while clinical cancer studies have investigated the effect of monoclonal antibodies to directly inhibit IL-6, it is also known that drugs currently in routine clinical practice, particularly non-immunologic therapies including corticosteroids and non-steroidal drugs, are capable of inhibiting IL-6 signalling. This observation requires further examination.

6.0 – CIRCULATING IL-6 CONCENTRATIONS LINK TUMOUR NECROSIS AND SYSTEMIC AND LOCAL INFLAMMATORY RESPONSES IN PATIENTS UNDERGOING RESECTION OF COLORECTAL CANCER.

- 6.1 - Introduction

Despite the strong evidence linking the local and systemic inflammatory responses to colorectal cancer survival and their apparent independent prognostic value, the mechanisms by which these two related inflammatory processes are activated, maintained, and interact are not clear. Recent work has highlighted tumour necrosis as a prognostic factor in colorectal cancer and its close associations with the local and systemic inflammatory responses [171, 407]. However, what remains unclear is the mechanism linking tumour necrosis and the local and systemic inflammatory responses.

One hypothesis is that tumour necrosis may link these two key inflammatory processes via immunological mediators, in particular interleukin-6, present in the tumour microenvironment and in the circulation [402].

The molecular links between tumour necrosis and these key inflammatory processes are likely to be complex. The 'dirty', hypoxia-driven, unscheduled cell death occurring in tumour necrosis makes it likely that this process may stimulate a variety of inflammatory mediators that can influence both the systemic and local inflammatory responses. Other potential candidates for such mediators include circulating IL-10 and vascular endothelial growth factor (VEGF) [408, 409].

Therefore, the aim of the present study was to examine whether circulating mediators, in particular IL-6, may be a link between tumour necrosis and local and systemic inflammatory responses in patients undergoing curative resection for colorectal cancer.

- **6.2 – Patients and Methods**

Patients with colorectal cancer who, on the basis of preoperative staging and laparotomy findings, were considered to have under- gone an elective, potentially curative resection of colorectal cancer between April 2004 and July 2009 in a single surgical unit at Glasgow Royal Infirmary were included in the study. Tumours were staged using the conventional tumour, node, metastasis (TNM) staging system, 7th edition, 2010 [318]. Patients with conditions known to elicit an acute or chronic systemic inflammatory response were excluded. These were namely (1) preoperative chemoradiotherapy, (2) clinical evidence of active preoperative infection, or (3) chronic active inflammatory diseases such as rheumatoid arthritis.

Blood samples were collected before surgery for routine laboratory analysis of full blood count, white cell and lymphocyte counts, albumin, and C-reactive protein. The mGPS and NLR ratio were constructed using the parameters described in Chapter 3 (page 93), while the NLR ratio was constructed using previously documented thresholds.

Blood samples were centrifuged and the serum stored at -80^{0}C before analysis of IL-6, IL-10, and VEGF. Circulating concentrations of IL-6, IL-10, and VEGF were measured using commercially available human colorimetric enzyme-linked immunosorbent assays (Quantikine ELISA, R&D Systems, Europe Ltd, Abingdon, UK). The minimum detectable concentrations were 2pgml-1 for IL-6, 4pgml-1 for IL-10, and 9pgml-1 for VEGF.

Assessment of the local inflammatory cell infiltrate and tumour necrosis was performed on original haematoxylin and eosin stained full sections of the tumour, felt to represent the maximum depth of tumour invasion. The local inflammatory response was evaluated previously in this cohort using the method described by Klintrup and Makinen as in Chapter 4 [181]. The scores were then dichotomised to 'high' and 'low' grade inflammation in line with the previously published literature [181].

Tumour necrosis was assessed using the method described by Pollheimer et al[407]. Briefly, at X40 magnification, the full sections were examined for evidence of tumour necrosis. Tumour necrosis was graded as 'absent' (none), 'focal' (<10% of tumour surface area), 'moderate' (10–30% tumour surface area), or 'extensive' (>30% of tumour surface area) in each section before an assessment of overall extent of necrosis was made. To test the reliability of the evaluation of necrosis, sections of 30 patients (average of 3 slides per patient) were examined independently by two observers (GJKG and CSDR) blinded to clinical outcome and clinicopathological variables. The intraclass correlation coefficient (ICC) for the assessment of local inflammatory cell infiltrate was 0.81 and for tumour necrosis was 0.70.

To evaluate metabolic upset, the body composition parameters, body mass index (BMI), total body fat, subcutaneous body fat, visceral fat, and skeletal muscle mass, as previously described [313], were used to assess the relationship between systemic and local inflammation, tumour necrosis and circulating immunological factors, and cancer cachexia. Body composition data was only available for approximately one third of patients in this cohort.

Data are presented as median and range. Grouping of variables was carried out using standard thresholds for laboratory parameters. As there are no widely accepted thresholds for IL-6, IL-10, and VEGF, the values were grouped as tertiles [410]. The concentrations of IL-6, IL-10, and VEGF below the threshold of sensitivity of the respective assays were expressed as equal to this threshold. The relationships between the groups of patients was carried out using Mantel–Haenszel (w2) test for trend and the Kruskal–Wallis test and Spearman's rank correlation as appropriate. The relationship between IL-6 and cancer-specific survival was examined using the Kaplan–Meier method. Analysis was performed using SPSS software version 19 (SPSS Inc., Chicago, IL, USA).

The study was approved by the Research Ethics Committee, Glasgow Royal Infirmary, Glasgow.

- **6.3 - Results**

The majority of patients were >65 years old (66%), were male (51%), had TNM stage I/II disease (62%), and the majority had C-reactive protein (68%) and albumin (72%) concentrations in the normal range and a normal mGPS (68%). Of the 33 patients with hypoalbuminaemia, 16 (48%) had an elevated C-reactive protein. The majority of patients also had total white cell counts (71%), neutrophil counts (89%), lymphocyte counts (82%), and platelet counts (87%) in the normal range and a normal NLR (80%).

Serum IL-6 concentrations were divided into tertiles. The tertiles had approximately equal numbers of patients: tertile 1 (39 patients), tertile 2 (39 patients), and tertile 3 (40 patients). Tertile 1 (median concentration 2pgml^{-1}, range 0.8–3.38) the lowest, tertile 2 moderately elevated concentration (median concentration 4.85pgml^{-1}, range 3.43–6.01), and tertile 3 (median concentration (9.97 pg ml^{-1}, range 6.1–252.46) the highest.

On assessment of tumour necrosis, 48% had no evidence of tumour necrosis, 29% had focal areas of tumour necrosis, 11% had moderate tumour necrosis, whereas 12% had extensive tumour necrosis. On assessment of the local tumour inflammatory cell response using the K-M criteria, the majority of patients were considered to have a high-grade inflammatory cell infiltrate (55%).

On Spearman's rank correlation of individual values, there were significant associations between circulating concentrations of IL-6 and IL-10 (r = 0.56, P<0.001), C-reactive protein (r = 0.45, *P*<0.005), albumin (r = -0.65, *P*<0.001), and the skeletal muscle index (r = -0.38, *P* = 0.056). Circulating concentrations of IL-10 were significantly associated with albumin (r = 0.40, *P*<0.05). Circulating concentrations of VEGF were significantly associated with C-reactive protein (r = 0.32, *P*<0.05).

The relationship between circulating IL-6 tertiles, tumour characteristics, and systemic responses in all patients with colorectal cancer are shown in Table 6.1. When all patients were considered, circulating IL-6 was associated with gender (P<0.05), increased IL-10 (*P*<0.001), VEGF (*P*<0.001), tumour site (*P*<0.05), increased T stage (*P*<0.01), tumour necrosis (*P*<0.001), increased mGPS (*P*<0.001), increased white cell (*P*<0.05) and platelet (*P*<0.05) counts, and low skeletal muscle index (*P*<0.01).

When all patients were considered together, tumour necrosis was associated with increased T stage (*P*<0.005), decreased local inflammatory cell infiltrate (*P*<0.05), increased IL-6 (*P*<0.001), increased IL-10 (*P*<0.005), increased VEGF (*P*<0.001), mGPS (*P*<0.001), anaemia (*P*<0.05), elevated white cell (*P*<0.005), neutrophil count (*P*<0.05), and platelet counts (*P*<0.001), and skeletal muscle index (*P*<0.001).

In the present study, the median follow-up for the survivors was 53 (range 25–91) months. During this period, 29 patients died from colorectal cancer and 11 from other causes. Neither IL-6 and IL-10 nor VEGF were significantly associated with cancer-specific survival.

In order to account for the effect of T stage, the relationship between tumour characteristics, circulating mediators, and systemic responses in patients with stage T3 colorectal cancer (n = 73) was examined (Table 6.2). When T3 colon tumours were considered alone, the relationships between IL-6 and IL-10 ($P<0.001$), VEGF ($P<0.001$), mGPS ($P<0.001$), platelet count ($P<0.05$), and tumour necrosis ($P<0.001$) remained significant.

- **6.4 - Discussion**

The results of the present study show that tumour necrosis, independent of tumour size, was significantly associated with elevated concentrations of IL-6, IL-10, VEGF, mGPS, NLR, platelet count, and the skeletal muscle index.

Furthermore, IL-6 concentrations were significantly associated with IL-10, VEGF, platelet counts, mGPS, and the skeletal muscle index. In addition, IL-10 and VEGF were significantly associated with each other and both were significantly associated with systemic inflammation, as evidenced by the mGPS. Taken together, the results of the present study indicate that IL-6 is indeed a plausible mediator of the relationship between tumour necrosis and systemic inflammatory responses in patients with operable colorectal cancer.

In the present study, circulating IL-6 concentrations were not significantly associated with the extent of the general local inflammatory cell infiltrate as evidenced by K-M criteria or cancer-specific survival. This does not preclude IL-6 concentrations being associated with specific inflammatory cell types. Also, the relatively small number of cancer deaths limits the conclusions that can be made about the prognostic value of IL-6. Furthermore, although most inflammatory cell types increase with a high K-M grade, macrophage counts have recently been reported to be similar in both low- and high-grade K-M grades [337]. It was therefore of interest that circulating concentrations of IL-6 were directly associated with IL-10 as tumour IL-10 levels can be sustained by the production of IL-6 by tumour-infiltrating macrophages [408, 411].

Although the exact mechanism by which IL-10 exerts its effect in the tumour microenvironment is unclear, it is accepted that this cytokine has multiple stimulatory and inhibitory effects on innate and adaptive immune responses respectively. Indeed, it has been proposed that IL-10 is a key anti-inflammatory cytokine involved in the switch from Th1 to Th2 immune responses [412]. Therefore, it may be the balance between IL-6 and IL-10 in the tumour microenvironment that may be important in determining the nature of local and systemic inflammatory responses in colorectal cancer.

Recent work has highlighted a direct relationship between the systemic inflammatory responses (mGPS) and the loss of skeletal muscle in patients with colorectal cancer [313]. In the present study, although there was a relatively small number of observations, there was also an association between elevated concentrations of circulating IL-6 and a low skeletal muscle mass. These results are consistent with work in experimental models of cancer cachexia that have implicated proinflammatory cytokines and their downstream signalling cascades in cancer-associated muscle wasting [413-415]. Prominent among these is the IL-6/JAK/STAT3 cascade [415] and therefore confirms the plausibility of the present hypothesis in patients with colorectal cancer.

The mechanism whereby tumour necrosis results in an increase in circulating IL-6 concentrations is not clear. One plausible hypothesis is that hypoxic tumour cells activate hypoxia-inducible factors that result in the production of IL-6 and VEGF and that these spill into the circulation raising circulating concentrations (see Figure 6.1).

However, it is of interest that IL-6 concentrations do not appear to normalise following potentially curative resection of tumours, similar to CRP. Thus, it is also plausible that IL-6 concentrations may be determined by tissues other than that of the tumour. Indeed, it has been reported that tumour-associated macrophages (TAMs) may play an important role, as they are known to produce significant amounts of IL-6 and are reported to be a major source of IL-6 in both the serum and tumour microenvironment of patients with colorectal cancer [416, 417]. Indeed, numerous studies have reported the presence of macrophages in the tumour microenvironment [183]. Despite this, the prognostic value of tumour-infiltrating macrophages remains unclear. At least nine studies have previously reported a relationship between TAMs and survival. Of these, five studies reported a strong association between the density of macrophages at the invasive margin and survival, whereas the remainder reported no significant association with survival [183].

Although some studies have reported that high macrophage infiltration is associated with improved survival [418-423], recent work that has identified the two main subpopulations of macrophages present in the tumour microenvironment suggests a more complex relationship between macrophage infiltration and survival [424, 425]. Indeed, it has been reported that the distribution of the M1 and M2 subpopulations of macrophages in the tumour microenvironment may influence survival and that these subpopulations may localise to different areas of the tumour depending on the prevailing conditions, including tissue hypoxia [426-428].

Indeed, it has long been recognised that TAMs localise to hypoxic regions of the tumour microenvironment [429]. Therefore, it is plausible that the relative density of macrophages (perhaps M2) are important in such cytokine alterations and the elaboration of the systemic inflammatory response in patients with colorectal cancer and therefore this requires further investigation.

Interestingly, recent work by Kantola et al. has highlighted the potential relationship between the systemic inflammatory response, as evidenced by mGPS, and alterations in a variety of serum cytokine concentrations and this, along with the results of the present study, may provide new insight into the inflammatory cells associated with the upregulation of the systemic inflammatory response in patients with colorectal cancer [315, 316].

Indeed, with the exception of macrophages, few inflammatory cells can produce such a spectrum of cytokines and growth factors, and this is consistent with recent reports that macrophages are abundant in tumour microenvironments even in the absence of other inflammatory cells [337, 430]. Therefore, the above findings are also consistent with the hypothesis that tumour necrosis (increasing with T stage) and IL-6 play an important role in linking local and systemic inflammatory responses in patients with colorectal cancer [171].

It has also previously been suggested that IL-6 may stimulate systemic inflammatory responses through the trans-signalling pathway involving the soluble IL-6 receptor and it is plausible that intra-tumoural and circulating macrophages may contribute to this trans-signalling pathway [401, 431], and therefore play a key role in the evolution of a chronic systemic inflammatory response. Indeed, Chua et al (2011) proposed a similar scheme that proinflammatory cytokines may modulate both the local tumour microenvironment and a chronic systemic inflammatory response that affects normal organs, including liver and muscle [283]. Irrespective, the relationship between this 'IL-6 trans-signalling' pathway and both local and systemic inflammatory responses in patients with colorectal cancer also merits further evaluation.

In summary, the present study therefore provides, for the first time, supportive evidence for the hypothesis that tumour necrosis, independent of T stage, elevates circulating IL-6 concentrations, thereby modulating both local and systemic inflammatory responses including angiogenesis that, in turn, may promote tumour progression and metastases. Further evaluation of the relationships between cells that produce IL-6 (e.g. macrophages) in the tumour microenvironment and in the circulation is of considerable interest. A limitation of the present study is the lack of data on body composition on all patients in this cohort, however, these results are encouraging and further work to firmly establish this link between elevated circulating levels of IL-6 and low skeletal muscle index.

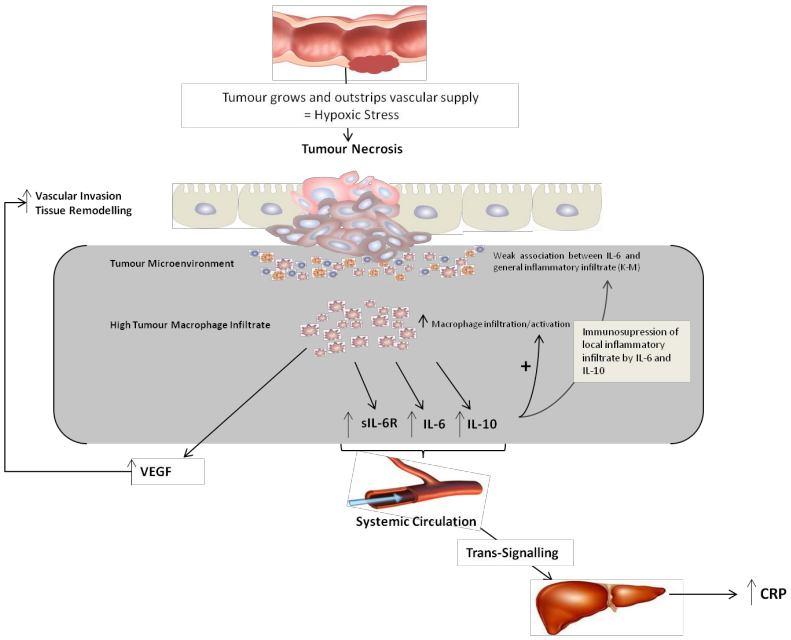


Figure 6.1 Proposed pathway linking tumour necrosis, IL-6, and systemic and local inflammatory responses in patients with colorectal cancer.

Table 6.1 - The relationships between circulating interleukin-6, tumour characteristics and systemic responses in patients with colorectal cancer.

	Circulating Interleukin-6 (pg/ ml)			
Patient Characteristics	**< 3.4pg/ ml (n=39)**	**3.4-6.0pg/ ml (n=39)**	**>6.0pg/ml (n= 40)**	**p-Value**
Age (<65/ 65-74/ ≥75 years)	18/13/8	12/16/11	10/18/12	0.080
Sex (male/ female)	25/14	20/19	15/25	0.019
Circulating mediators				
Interleukin-10 (tertiles)	19/12/7	11/12/7	5/9/20	<0.001
VEGF (tertiles pg/ ml)	25/0/0	10/29/0	0/5/35	<0.001
Tumour characteristics				
Presentation (Elective/ Emergency)	36/1	37/0	32/1	0.948
Tumour Site (Colon/ Rectum)	17/21	21/17	24/10	0.028
T-stage (T_1/ T_2/ T_3/ T_4)	4/7/23	2/5/25/7	1/2/25/12	0.007
N-stage (N_0/ N_1/ N_2)	29/4/6	23/12/4	21/14/5	0.237
Tumour necrosis (Low/High)	24/13	24/12	6/34	<0.001
Vascular invasion (Yes/ No)	14/25	19/20	14/26	0.927
Inflammatory cell infiltrate (K-M – Low-grade/High-grade)	19/20	18/19	13/24	0.239
Systemic responses				
Systemic inflammatory response (mGPS low/high)	34/5	31/8	15/25	<0.001
Neutrophil Lymphocyte Ratio (NLR) (Low<5/High>5)	34/5	30/9	30/10	0.182
Anaemia >13g/dl (men), >11.5g/dl (women) <13g/dl men, <11.5g/dl (women)	12/27	13/26	11/29	0.751
White Cell count (<8.5/ 8.5-11.5/ >11.5 x10^9/l)	30/6/3	32/6/1	22/11/7	0.030
Neutrophil count (<7.5/ >7.5x10^9/l)	35/4	38/1	32/8	0.164
Lymphocyte count (≥1/ <1 10^9/l)	6/33/0	9/28/2	4/36/0	0.545
Monocyte Count (<1/>1x10^9/l)	38/1	34/5	34/6	0.069
Platelets (≤400/ >400 x10^6/L)	37/2	35/4	31/9	0.021
BMI (kg/ m^2) (Normal weight, Overweight, Obese)	1/6/2	6/2/5	8/7/8	0.818
Total Fat Index (cm^2/m^2) (Low/Medium/High)	0/3/6	6/4/3	8/6/9	0.161
Subcutaneous fat index (cm^2/m^2) Low/Medium/High)	0/4/5	6/3/4	11/2/10	0.163
Visceral Fat Index (cm^2/m^2) (Low/Medium/High)	1/5/3	6/6/1	8/7/8	0.795
Skeletal Muscle Index (cm^2/m^2) (Low/Medium/High)	0/1/8	2/5/6	7/10/6	0.002

Abbreviations: BMI=body mass index; IL-6=interleukin-6; IL-10=interleukin-10; K-M=Klintrup and Makinen criteria; mGPS=modified Glasgow Prognostic Score; NLR=neutrophil– lymphocyte ratio; VEGF = vascular endothelial growth factor.

Table 6.2 - The interrelationships between different pathological and clinical parameters in patients with T3 stage disease undergoing potentially curative resection for colorectal cancer (n = 73).

	Tumour Characteristics		Circulating Mediators			Systemic Responses			
	Tumour Necrosis	**K-M (Low/High Grade)**	**IL-6**	**IL-10**	**VEGF**	**Platelet Count**	**mGPS**	**NLR**	**Skeletal Muscle Index**
Site	0.111	0.787	0.023	0.644	0.008	0.173	0.042	0.868	0.427
Tumour Necrosis		0.140	<0.001	0.008	<0.001	0.004	<0.001	0.044	0.009
K-M (Low/High Grade)			0.768	0.837	0.428	0.719	0.320	0.886	0.496
IL-6				<0.001	<0.001	0.038	<0.001	0.301	0.133
IL-10					0.004	0.222	0.017	0.904	0.297
VEGF						0.017	<0.001	0.161	0.195
Platelet Count							0.001	0.619	0.537
mGPS								0.339	0.600

7.0 – THE ROLE OF THE sIL-6/GP130 TRANS-SIGNALLING SYSTEM IN LINKING TUMOUR NECROSIS, SYSTEMIC AND LOCAL INFLAMMATION IN PATIENTS WITH COLORECTAL CANCER.

- 7.1 – Introduction

Recent work has proposed that local and systemic inflammatory processes in patients with colorectal cancer may be linked through tumour necrosis [171]. Studies in colorectal cancer have strongly implicated tumour necrosis in the natural history of colorectal cancer with some reporting presence of tumour necrosis in more than 90% of colorectal cancers [407] and that presence of tumour necrosis has prognostic value in patients with colorectal cancer [171, 407]. Studies have shown that tumour necrosis was directly associated with an increased systemic inflammatory response and a decreased local inflammatory response and while tumour necrosis did not have independent prognostic value it is plausible that tumour necrosis may play a causal role in linking these two key inflammatory processes [171].

While the molecular mechanism by which tumour necrosis links these inflammatory processes is likely to be very complex, a plausible hypothesis is that tumour hypoxia, that results in the disorganised, 'dirty' process of unscheduled cell death, releases necrotic mediators that modulate inflammatory pathways that, in turn, promote tumour growth [178]. A plausible mechanism involves the hypoxic stress pathway involving Hypoxia Inducing Factor-1-alpha (HIF-1α) that in turn is reported to stimulate production of Interleukin-6 from tumour and inflammatory cells [327, 328].

There is good evidence that circulating IL-6 is important in patients with colorectal cancer [363, 380-383] and it has been proposed that increased local and systemic IL-6 may play a key role in regulating key processes in cancer development including immune-regulation, angiogenesis, and modulation of systemic inflammatory responses [186, 335, 339], thus making IL-6 a strong candidate for mediating both local and systemic inflammatory responses. The classical IL-6 signalling pathway is well known to mediate IL-6 signalling by binding to a membrane bound receptor (mIL-6R), however this pathway is restricted to cells expressing both mIL-6R and its effector unit gp130. While there are studies reporting increased IL-6 expression in tissues of patients with colorectal cancer [365, 432], the evidence for increased expression of the membrane-bound receptor is less robust [365]. A potential alternative explanation for IL-6 signalling includes the trans-signalling pathway. This pathway, involving the soluble IL-6 receptor (sIL-6R) and its signal transducer gp130, have been implicated in IL-6 signalling in a variety of cancers [386, 389, 433]. Further, this trans-signalling pathway is well recognised to be a key part of the STAT3 signalling pathway that has recently been strongly implicated in tumour growth [433]. In addition, sIL-6R and sgp130 are well-recognised to be important in IL-6 mediated regulation of the systemic inflammatory response, in particular the systemic inflammatory response [352, 371, 372].

Therefore the IL-6 trans-signalling pathway is a plausible pathway in the IL-6 signalling cascade in patients with colorectal cancer. Despite this, there has been limited investigation of the role of the IL-6 trans-signalling pathway in patients with colorectal cancer.

The aim of the present study was to examine whether elevated concentrations of the soluble receptors, sIL-6R and gp130, were present in patients with colorectal cancer and examine whether they form part of the link between tumour necrosis, and local and systemic inflammatory responses in patients undergoing curative resection for colorectal cancer.

- **7.2 – Patients and Methods**

Patients with colorectal cancer who, on the basis of preoperative staging and laparotomy findings, were considered to have undergone an elective, potentially curative resection of colorectal cancer between July 2010 and June 2011 were included in the study. Tumours were staged using the conventional tumour, node, metastasis (TNM) staging system, 7th Edition [318]. Patients with conditions known to elicit an acute or chronic systemic inflammatory response were excluded. These were namely (i) pre-operative chemoradiotherapy, (ii) clinical evidence of active pre-operative infection, or (iii) chronic active inflammatory diseases such as rheumatoid arthritis.

Blood samples were collected prior to surgery for routine laboratory analysis of full blood count, white cell and lymphocyte counts, albumin and C-reactive protein. A further blood sample was taken at the time and stored. Blood samples were centrifuged and the serum stored at -80^{o}C prior to analysis of soluble interleukin-6 receptor (sIL-6R) and sgp130.

Circulating concentrations of sIL-6R and soluble gp130 were measured using commercially available Human soluble IL-6R and Human soluble gp130 Quantikine enzyme-linked immunosorbent assays (R&D Systems, Europe Ltd, Abingdon, UK). Using this assay the values corresponded to the total amount of sIL-6R and sgp130 present in the samples, i.e. the amount of free receptors plus the amount of the receptors bound. All samples were thawed only once and assayed in duplicate.

The modified Glasgow Prognostic Score (mGPS) was constructed based on routine pre-operative blood tests[195]. Briefly, patients with both an elevated C-reactive protein (>10mgl^{-1}) and low albumin (<35gl^{-1}) were allocated a score of 2; patients in whom only C-reactive protein was elevated (>10mgl^{-1}) were allocated a score of 1 and those with a normal C-reactive protein were allocated a score of 0. The neutrophil-lymphocyte (NLR) ratio was constructed using previously documented thresholds. Briefly, NLR was determined by dividing the absolute neutrophil count by the absolute lymphocyte count, the NLR data was then dichotomised, and given a score of 0 (<5:1), and 1 (>5:1).

Assessment of the local inflammatory cell infiltrate and tumour necrosis was performed on original haematoxylin and eosin-stained full-sections of the tumour, felt to represent the maximum depth of tumour invasion. The local inflammatory response was evaluated previously in this cohort using the method described by Klintrup and Makinen [181]. Briefly, K-M criteria is a four-point scale, a score of 0 indicates no increase in inflammatory cells at the invasive margin, a score of 1 indicates presence of a mild/patchy increase in inflammatory cell reaction at the invasive margin but no destruction of invading cancer cell islets, a score of 2 indicates observation of a band-like inflammatory reaction at the invasive margin, and a score of 3 indicates observation of a florid inflammatory reaction with cup-like inflammatory infiltrate at the invasive margin. The scores were then dichotomised to 'high' and 'low' grade inflammation in line with the previously published literature [181].

Tumour necrosis was assessed using the method described by Pollheimer and colleagues [407]. Briefly, at x40 magnification the full sections were examined for evidence of tumour necrosis. Tumour necrosis was graded as 'absent' (none), 'focal' (less than 10 per cent of tumour surface area), 'moderate' (10-30 per cent of tumour surface area), or 'extensive' (more than 30 per cent of tumour surface area) in each section before an assessment of overall extent of necrosis was made.

To test the reliability of the evaluation of necrosis, sections of 30 patients (average of 3 slides per patient) were examined independently by two observers (GJKG and CSDR) blinded to clinical outcome and clinicopathological variables. The intra-class correlation coefficient (ICC) for the assessment of local inflammatory cell infiltrate was 0.81 and for tumour necrosis was 0.70.

The study was approved by the Research Ethics Committee, Glasgow Royal Infirmary, Glasgow.

- **7.3 - Results**

Seventy-four patients who underwent potentially curative resection for colorectal cancer between June 2004 and June 2009 were included in the study. The majority of patients were 65 years or older (66%), male (57%%), TNM stage I/II (64%%) and the majority had C-reactive protein (76%) and albumin (55%) concentrations in the normal range and a normal mGPS (76%). Of the 33 patients with hypoalbuminaemia, 14 (19%) had an elevated C-reactive protein. The majority of patients also had total white cell counts (80%), neutrophil counts (92%), lymphocyte counts (92%), and platelet counts (92%) in the normal range. On assessment of the local tumour inflammatory cell response using the Klintrup-Makinen criteria, the majority of patients were considered to have a high-grade inflammatory cell infiltrate (52%). On assessment of tumour necrosis, 30% had no evidence of tumour necrosis, 42% had focal areas of tumour necrosis, 20% had moderate tumour necrosis, while 8% had extensive tumour necrosis.

As no established thresholds exist for circulating concentrations of soluble IL6 receptor and soluble gp130 were grouped as tertiles. The relationship between circulating sIL-6 receptor and soluble gp130 concentrations in patients with colorectal cancer is shown in Tables 7.2 and 7.3. Increased concentration of circulating sIL6 receptor was associated with anaemia only ($p<0.05$).

Tumour necrosis was associated with vascular invasion ($p<0.05$), local inflammatory infiltrate (<0.05), T-stage, margin positivity ($p<0.05$), peritoneal involvement ($p<0.05$).

With regard to the systemic inflammatory response tumour necrosis was associated with increased C-reactive protein ($p<0.001$), hypoalbuminaemia ($p<0.05$), increased mGPS ($p<0.001$), anaemia ($p<0.05$) and increased platelets ($p<0.05$). In contrast, sIL6 receptor was not associated with patient or tumour characteristics or systemic responses with the exception of an association with anaemia ($p=0.020$). Similarly, soluble gp130 was not associated with patient, tumour characteristics or systemic responses.

- **7.4 – Discussion**

In chapter six we highlighted that serum IL-6 concentrations were elevated in patients with colorectal cancer and proposed that IL-6 may be an important link between tumour necrosis and systemic and local inflammatory responses. Given these findings we proposed that IL-6 trans-signalling may be a plausible pathway for transduction of IL-6 signalling.

In the present study, neither soluble IL6 receptor nor soluble gp130 is associated with local or systemic inflammatory responses in patients with colorectal cancer. In addition, in the present study this study reports no significant association between these two soluble factors of this trans-signalling pathway. In this study, our findings suggest that neither sIL-6R nor sgp130 are associated with any tumour characteristics or measured elements of the systemic inflammatory response. This is similar to recent findings by Yeh et al. who reported no association between pathological variables, including T-stage in patients with colorectal cancer [433].

A possible limitation of this study relates to the thresholds used for detecting elevated concentrations of sIL-6R and sgp130. These thresholds are slightly higher than reported in studies of patients with breast cancer and myeloma. Despite this, our thresholds used are somewhat lower than those used by Yeh et al.

While is it widely accepted that IL-6 is one of the main pro-inflammatory signals involved in modulation of systemic inflammatory responses the source and downstream signalling pathway involved remains unclear. The findings of this study are not supportive of a role for trans-signalling in our cohort of patients with colorectal cancer, however it cannot exclude this pathway in playing a role in modulation of systemic and local inflammatory responses in patients with cancer. These findings may reflect the fact that the effect of trans-signalling is low or that current ELISA methods are not able to detect the various forms of these soluble components of trans-signalling. This merits further evaluation.

Table 7.1 – Baseline characteristics of patients undergoing potentially curative resection for colorectal cancer.

	No. of Patients (n=74)
Patient Characteristics	
Age (<65/ 65-74/>75)	25/32/17
Sex (Male/Female)	42/32
Tumour Characteristics	
Tumour Site (Colon/ Rectum)	44/30
T-stage (T_1/ T_2/ T_3/ T_4)	9/11/40/14
N-stage (N_0/ N_1/ N_2)	48/18/8
TNM Stage (I/II/III)	17/30/27
Tumour necrosis (Absent/Focal/Moderate/Extensive)	22/31/15/6
Vascular invasion (Yes/ No)	32/42
Margin Involvement (Yes/No)	8/66
Tumour Perforation (Yes/No)	8/66
Peritoneal Involvement (Yes/No)	11/63
Tumour Inflammatory Cell Infiltrate	
Inflammatory Cell Infiltrate (K-M grade 0/1/2/3)	5/31/32/6
Inflammatory Cell Infiltrate (K-M – Low-grade/High-grade)	38/36
Trans-Signalling Receptors	
sIL-6 Receptor (pg/ml) Median and Range	398.50 (214-898)
sgp130 (ng/ml) Median and Range	301 (190-493)
Systemic Responses	
C-Reactive Protein (<10/>10mg)	56/18
Albumin (>35g/L/<35g/L)	41/33
Systemic inflammatory response (mGPS 0/1/2)	56/5/13
Neutrophil Lymphocyte Ratio (NLR) (Low<5/High>5)	63/11
Platelet Lymphocyte Ratio (PLR)(<150:1/150-300:1/>300:1)	31/29/11
Anaemia (Yes/No) >13g/dl (men), >11.5g/dl (women), <13g/dl men, <11.5g/dl (women)	41/33

Table 7.2 – The relationships between circulating soluble IL6 receptor, tumour characteristics, and systemic responses in patients with colorectal cancer.

	Circulating sIL6-R (ng/ml)			
	(n=27) <352 ng/ml	(n=24) 348-484 ng/ml	(n=23) >484 ng/ml	*P*-value
Patient Characteristics				
Age	11/9/7	6/12/6	8/11/4	0.935
Sex (male/female)	14/13	17/7	11/12	0.836
Tumour Characteristics				
Tumour Site (colon/rectum)	15/12	15/9	14/9	0.529
T-stage (T1,T2,T3,T4)	5/5/10/7	1/5/12/6	3/1/18/1	0.744
N-stage N0,N1,N2	17/8/2	17/4/3	14/6/3	0.704
Tumour Necrosis	6/11/7/3	9/6/6/3	7/14/2/0	0.068
Vascular Invasion	8/19	11/13	13/10	0.056
Inflammatory cell infiltrate (K-M low grade/high grade)	13/12	11/14	12/12	0.884
Trans-signalling receptors				
Circulating sgp130 (ng/ml)	8/8/11	9/10/5	8/7/8	0.597
Systemic Responses				
Systemic Inflammatory Response (mGPS 0/1/2)	19/2/6	17/1/6	20/2/1	0.129
Neutrophil Lymphocyte Ratio (NLR) (Low<5/High>5)	22/5	20/4	21/2	0.341
Anaemia <13g/dl men <11.5g/dl (women)	12/15	11/13	18/5	0.020
White Cell count (<8.5/ 8.5-11.5/ >11.5 $x10^9$/l)	21/4/2	18/3/3	20/3/0	0.339
Neutrophil count (<7.5/ >7.5$x10^9$/l)	24/3	22/2	22/1	0.388
Lymphocyte count ($\underline{\geq}$1/ <1 10^9/l)	26/1	21/3	21/2	0.495
Monocyte Count (<1/>1$x10^9$/l)	26/1	22/2	23/0	0.550
Platelets ($\underline{\leq}$400/ >400 $x10^6$/L)	24/3	22/2	22/1	0.388

Table 7.3 - The relationships between circulating soluble sgp130 concentrations, tumour characteristics and systemic responses in patients with colorectal cancer.

	Circulating sgp130 (ng/ ml)			
	(n=25) <302ng/ml	(n=25) 302-353ng/ml	(n=24) >353ng/ml	**p-Value**
Patient Characteristics				
Age (<65/ 65-74/ ≥75 years)	9/13/3	6/11/8	10/8/6	0.719
Sex (male/ female)	7/18	12/13	13/11	0.065
Tumour Characteristics				
Presentation (Elective/ Emergency)	23/2	25/0	24/0	0.084
Tumour Site (Colon/ Rectum)	15/10	14/9	13/11	0.683
T-stage (T_1/ T_2/ T_3/ T_4)	3/1/16/5	5/5/11/4	1/5/13/5	0.973
N-stage (N_0/ N_1/ N_2)	14/8/3	18/4/3	16/6/2	0.461
Tumour necrosis (Absent/Focal/Moderate/Extensive)	7/9/7/2	8/12/3/2	7/10/5/2	0.762
Vascular invasion (Yes/ No)	12/13	15/10	15/9	0.307
Inflammatory Cell Infiltrate (K-M – Low-grade/High-grade)	13/12	11/14	12/12	0.884
Trans-signalling receptors				
Circulating sIL6r (pg/ml)	8/9/8	8/10/7	11/5/8	0.597
Systemic Responses				
Systemic Inflammatory Response (mGPS 0/1/2)	19/1/5	20/2/3	17/2/5	0.794
Neutrophil Lymphocyte Ratio (NLR) (Low<5/High>5)	22/3	20/5	21/3	0.953
Anaemia >13g/dl (men), >11.5g/dl (women) <13g/dl men, <11.5g/dl (women)	13/12	15/10	13/11	0.874
White Cell count (<8.5/ 8.5-11.5/ >11.5 x10^9/l)	22/1/2	17/5/3	20/4/0	0.857
Neutrophil count (<7.5/ >7.5x10^9/l)	23/2	23/2	22/2	0.966
Lymphocyte count (≥1/ <1 10^9/l)	24/1	22/3	22/2	0.574
Monocyte Count (<1/>1x10^9/l)	24/1	23/2	24/0	0.490
Platelets (≤400/ >400 x10^6/L)	23/2	22/1	20/3	0.547

8.0 - Conclusions

It is clear that colorectal cancer remains a significant healthcare burden, and despite advances in the understanding of this disease a large number of patients are still dying prematurely. The advent of colorectal cancer screening in recent years is set to impact the natural history of the disease with the predicted 'stage-shift' resulting in the number of node negative cancers being detected and treated increasing. This 'stage-shift' has several effects not least the increasing need for more accurate, objective and reliable stratification of patients with colorectal cancer to allow allocation to treatment to deliver high quality patient care.

Of particular relevance is the increasing acknowledgement globally that both host and tumour-related factors, in particular the importance of inflammatory responses have a significant impact on disease progression and outcome [148, 179, 195]. Indeed the number of articles has increased dramatically with search terms including cancer and inflammation returning more than double the number of articles now, compared to a decade ago [434]. With regard to local inflammatory responses there is a clear association between improved survival and high-grade inflammatory responses at the tumour margin [181, 183]. Indeed, more than 100 studies have described not only the importance of the general inflammatory response but also the type, density and location of immune cells in the peri-tumoural environment. Further, there is increasing interest in the clinical utility of assessments of the inflammatory infiltrate in the tumour microenvironment.

In addition, there is a large body of evidence that systemic inflammatory responses, measured as the well-described modified Glasgow Prognostic Score (mGPS) and the Neutrophil-Lymphocyte Ratio (NLR) have strong prognostic value in patients with colorectal cancer [195].

Despite the wide recognition of the importance of the key players including tumour necrosis, systemic and local inflammatory responses in patients with colorectal cancer, several questions remain unanswered. Firstly, while markers of systemic and local inflammation are well recognised to have significant prognostic value, the application of these scores in a clinical setting could be enhanced if it could be shown these scores had prognostic value over time following therapeutic intervention. At the outset of this period of research this significant question remained unanswered. To this end, Chapter 3 aimed to address the paucity of literature in this area. The work described in Chapter 3 is not only consistent with previous literature confirming the prognostic value of preoperative assessment of mGPS and NLR but reports that longitudinal measurements of systemic inflammation also have prognostic value in patients undergoing curative resection for colorectal cancer. Furthermore, this work confirms previous observations that mGPS may offer superior prognostic value in patients with colorectal cancer compared to NLR, and for the first time suggests it also may be superior in the setting of active surveillance. This not only suggests that routine prolonged monitoring of the mGPS and NLR may be beneficial for prognostic purposes but also may provide a basis for interventions directed at systemic inflammation being used to maintain low levels of inflammation following resection of colorectal cancer.

Since the work carried out described in this thesis, further work has led to the adoption of platelets in a novel score termed the neutrophil-platelet score (NPS). Further, systemic inflammation-based scores are being increasingly adopted to help stratify patients receiving chemotherapy, e.g. in clinical trials such as the FOCUS-4 trial. Indeed the FOCUS-4 trial, currently recruiting, uses platelets $<400x10^9/L$ as an inclusion criteria. In addition to the use of neutrophils and platelets in stratifying patients to adjuvant therapy, there is also recognition that lymphocyte and monocyte numbers may also have prognostic value and further work in this area has recently been reported. Work by Clarke and colleagues has recently reported that the lymphocyte-monocyte ratio (LMR) has independent prognostic value in patients undergoing curative resection for colorectal cancer. Therefore further work using these scores in the context of clinical trials will be increasingly important."

Secondly, while numerous authors have reported the importance of the local inflammatory response in and around colorectal tumours, the use of these scores in the clinical setting is not routine and does not currently form part of the routine clinical assessment in patients with colorectal cancer. Chapter 4 aimed to address both the reasons for its lack of use and examine solutions to this problem. Review of the literature confirmed there is good evidence for the importance of the general local inflammatory cell infiltrate [181, 183], and it became apparent that the routine use of scores of local inflammatory infiltrates in the clinical setting is limited by a number of factors including, complexity and subjectivity of the scoring system, and lack of reproducibility caused by differing staining techniques in different units.

The study described in Chapter 4 aimed to address this problem through the development of an automated, objective assessment of local inflammatory infiltrates using commercially available image analysis software, and comparing this with well recognised methods of assessment of the local inflammatory response in patients with colorectal cancer. The results of this study reported, for the first time, that an accurate, objective assessment was not only possible, but also had prognostic value comparable to the manual method. While it is acknowledged that human input is still required in this method, this method of assessment of local inflammatory responses appears to be simple, practical and quick. It is also proposed that with further advances in imaging technology, refinement of this method may enhance its clinical utility. The reproducibility and reliability of this method remains to be tested, and validation this automated assessment of tumour inflammatory cell infiltrates merits further examination. Moreover, in the context of the interaction between the tumour, stromal cells and inflammatory cells this approach is of increasing interest. Indeed, recent work has led to development of a tumour microenvironment score that is reported to offer prognostic value suitable for clinical utility [435].

Despite the acknowledgement that tumour necrosis, systemic and local inflammatory responses have prognostic value in patients with colorectal cancer, the understanding of the possible links between these processes remains unclear.

After review of the literature it was apparent that tumour necrosis may link systemic and local inflammatory responses [171], however it is observed that the lack of independent prognostic value of tumour necrosis may suggest that this 'dirty' process of disordered cell death may result in production of a mediator, proposed to be Interleukin-6, that could induce and maintain the interaction between systemic and local inflammatory responses in patients with colorectal cancer. In Chapter 5, while it is acknowledged that molecular links between these processes are likely to be complex, key players are identified including hypoxic stress pathways involving HIF-1α and the IL-6/JAK/STAT pathway. As a result, a diagram (Figure 5.1) reflecting this hypothesis was formulated.

Given previous observations that tumour necrosis is an important prognostic markers in patients with colorectal cancer [402] and our hypothesis that IL-6 may be one of the important mediators of this link, Chapter 6 aimed to directly address this hypothesis.

This study, for the first time, described that tumour necrosis, independent of tumour size, was significantly associated with IL-6 and other circulating pro-inflammatory mediators. In addition, we reported that increasing concentrations of circulating IL-6 were significantly associated with mGPS and this study is consistent with a contemporaneous study by Kantola and co-workers [316].

This study represents the first description of IL-6 as a mediator in the link between tumour necrosis and cancer-associated inflammatory responses, in particular the systemic inflammatory response. This also raises the possibility that IL-6 may be produced by tumour cells themselves, as previously described. Despite this, a limitation of the present work may be the lack of examination of this proposed relationship, however both experience from preliminary work carried out during the course of this thesis and prior work have shown that assessment of IL6 concentrations at the tissue level is persistently hampered by deep background staining in tissue samples. Further work could therefore be aimed at refining the techniques of IL-6 detection in tissue samples to further examine tumour expression of IL-6.

This is of increasing interest as there are now several compounds aimed at targeting not only IL-6 but also its downstream signaling components. Of particular note, there is now increasing interest in targeting inflammatory responses in patients with cancer, in particular recent work targeting the JAK/STAT mechanism as a means of modulating inflammatory responses has garnered interest and several clinical trials are ongoing [436].

This study raised the question with regard to the possible downstream signaling pathways that may be involved in linking these important inflammatory responses and tumour necrosis. The mechanism linking IL-6 with systemic inflammatory responses is also likely to be a complex relationship.

Previous work by Scheller and Rose-John and colleagues had reported a possible mechanism involving the IL-6 trans-signalling pathway involving the soluble-IL-6 receptor and soluble gp130 in patients with cancer [401, 431]. Given this existing evidence, the relationship between the IL-6 trans-signalling pathway required further examination.

Chapter 7 therefore describes our study examining the proposed relationship between the sIL-6 receptor, gp130 and systemic and local inflammatory responses. This small study failed to demonstrate a link between these inflammatory processes and the trans-signalling pathway and while this study did not demonstrate a direct link, it cannot exclude a more minor role for this pathway in modulating immune responses. This may be because the pathway by which IL-6 exerts its immunomodulatory influence is more complex or perhaps occurs at the cellular level. Further work examining the IL-6 receptor in tumour samples may merit further investigation. Also, interventions targeting the IL-6/JAK/STAT pathway may shed more light on the importance of IL-6 trans-signalling.

In summary, this thesis highlights the importance of systemic and local inflammatory responses in patients with colorectal cancer, and has added weight to the body of evidence suggesting the value of markers of systemic inflammation, in the form of the mGPS, and that have clinical utility. With the advent of a number of drugs targeting the IL-6/JAK/STAT pathway the basis of such prognostic value will become increasingly clear.

Both from the work presented in this thesis and from the literature reviewed in the course of this work it is becoming increasingly recognised that cancer associated inflammation and immune responses are linked in a complex manner. The work presented here highlights the potential role of interleukin-6 in patients with colorectal cancer and its interaction with inflammatory processes, in particular, the systemic and local inflammatory processes. Given this potentially important role it seems plausible that therapies targeting interleukin-6 and its downstream pathways to downregulate inflammatory pathways may be relevant in the treatment of these patients. Several non-selective agents are known to affect these pathways including corticosteroids, non-steroidal anti-inflammatory drugs, statins and cytotoxic anti-inflammatory drugs have all been proposed to be of potential therapeutic value. In addition, selective blockade of interleukin-6 using drugs such as siltuximab and tocilizumab and are currently under investigation. The work described here, in particular the association between circulating levels of IL-6 and surrogate markers of systemic inflammatory responses adds weight to the hypothesis that IL-6 may play a pivotal role and may provide further insight into the complex interaction between the tumour and the host in patients with colorectal cancer.

Further work could focus on the use of selective inhibitors of not only IL-6 but also its downstream pathways including the JAK/STAT system. Indeed, two licensed selective JAK 1/2 inhibitors have been examined and further work is merited."

9.0 - References

1. Scotland, I., *Cancer Statistics: Colorectal Cancer.* 2013: p. http://www.isdscotland.org/Health-Topics/Cancer/Cancer-Statistics/Colorectal/ - colorectal.
2. UK, C.R., *Bowel Cancer Incidence Rates.* CRUK Website, 2011: p. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer/incidence - heading-One.
3. Hanahan D, Weinberg RA. *The Hallmarks of Cancer.* Cell, 2000. **100** (100): p. 57-70.
4. Fearon ER, Vogelstein B, *A genetic model for colorectal tumorigenesis. .* Cell 1990. **61**: p. 759-767.
5. Grady, *Genomic instability and colon cancer. .* Cancer Metastasis Rev 2004. **23**: p. 11-27.
6. Kerr D, *Clinical development of gene therapy for colorectal cancer.* Nature Reviews Cancer. **3**: p. 615-22.
7. Staton CA, Chetwood AS, Cameron IC, *The angiogenic switch occurs at the adenoma stage of the adenoma carcinoma sequence in colorectal cancer.* Gut, 2007. **56**: p. 1426–32.
8. Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR, *Clues to the pathogenesis of familial colorectal cancer.* Science, 1993. **260**(5109): p. 812-16.
9. Thibodeau SN, French AJ, Cunningham JM, Tester D, Burgart LJ, Roche PC, McDonnell SK, Schaid DJ, Vockley CW, Michels VV, Farr Jr. GH, O'Connell MJ, *Microsatellite Instability in Colorectal Cancer: Different Mutator Phenotypes and the Principal Involvement of hMLH1.* Cancer Res 1998. **58**: p. 1713.
10. Soreide K, Janssen EA, Soiland H, Korner H, Baak JP. , *Microsatellite instability in colorectal cancer. .* Br J Surg, 2006. **93**: p. 395-406.
11. Hoeijmakers J, *Genome maintenance mechanisms for preventing cancer. .* Nature Reviews Cancer, 2001. **411**: p.:366-374.
12. Illingworth RS, Bird RS, *CpG islands - 'A Rough Guide'.* FEBS Letters, 2009. **583**: p. 1713-1720.
13. Bariol C, Suter C, Cheong K, Ku SL, Meagher A, Hawkins N, Ward R., *The relationship between hypomethylation and CpG island methylation in colorectal neoplasia. .* Am J Pathol 2003. **162**: p. 1361-1371.
14. Jones PA, Baylin SB, *The fundamental role of epigenetic events in cancer. .* Nat Rev Genet 2002. **3**: p. 415-428.
15. Parkin DM, Boyd L, Walker LC, *The fraction of cancer attributable to lifestyle and environmental factors in the UK in 2010.* British Journal of Cancer, 2011. **105**: p. S77-81.
16. McArdle CS, Hole DJ, *Outcome following surgery for colorectal cancer.* British Medical Bulletin, 2002. **64**((1)): p. 119-125.
17. Oliphant R, Brewster DH, Morrison DS, *The changing association between socioeconomic circumstances and the incidence of colorectal cancer: a*

population-based study. British Journal of Cancer, 2011. **104**((11)): p. 1791-1796.
18. Chen K, XiaG, Zhang C, Sun Y., *Correlation between smoking history and molecular pathways in sporadic colorectal cancer: a meta-analysis.* International Journal of Clinical and Experimental Medicine, 2015. **8** (3): p. 3241-3257.
19. Botteri E, Iodice S, Bagnardi V, Maisonneuve P, Lowenfels AB., *Cigarette smoking and adenomatous polyps: a meta-analysis.* Gastroenterology, 2008. **134**: p. 388-395.
20. Liang PS, ChenTY, Giovannucci E., *Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis.* International Journal of Cancer, 2009. **124**: p. 2406-2415.
21. Cho E, Smith-Warner SA, Ritz J, van den Brandt PA, Colditz GA, Folsom AR, Freudenheim JL, Giovannucci E, Goldbohm RA, Graham S, Holmberg L, Kim DH, Malila N, Miller AB, Pietinen P, Rohan TE, Sellers TA, Speizer FE, Willett WC, Wolk A, Hunter DJ, *Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies.* Annals Internal Medicine 2004. **140**: p. 603–613.
22. Kune GA, Vitetta L., *Alcohol consumption and the etiology of colorectal cancer: a review of the scientific evidence from 1957 to 1991.* Nutrition and Cancer, 1992. **18**((2)): p. 97-111.
23. Ferrari P, Jenab M., Norat T, Moskal A, Slimani N, Olsen A, Tjonneland A, Overvad K, Jensen MK, Boutron-Ruault MC, Clavel-Chapelon F, Morois S, Rohrmann S, Linseisen J, Boeing H, Bergmann M, Kontopoulou D, Trichopoulou A, Kassapa C, Masala G, Krogh V, Vineis P, Panico S, Tumino R, van Gils CH, Peeters P, Bueno-de-Mesquita HB, Ocke MC, Skeie G, Lund E, Agudo A, Ardanaz E, Lopez DC, Sanchez MJ, Quiros JR, Amiano P, Berglund G, Manjer J, Palmqvist R, Van Guelpen B, Allen N, Key T, Bingham S, Mazuir M, Boffetta P, Kaaks R, Riboli E. , *Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European prospective investigation into cancer and nutrition (EPIC).* International Journal of Cancer, 2007. **121**: p. 2065–2072.
24. Giovannucci E, Ascherio A., Rimm EB, Colditz GA, Stampfer MJ, Willett WC, *Physical activity, obesity, and risk for colon cancer and adenoma in men.* Annals of Internal Medicine, 1995. **122**: p. 327–334.
25. Ning Y, Wang L, Giovannucci EL, *A quantitative analysis of body mass index and colorectal cancer: findings from 56 observational studies.* Obesity Review, 2009.
26. Moghaddam AA, Woodward M, Huxley R, *Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events.* Cancer Epidemiology Biomarkers Preview, 2007. **16**(12): p. 2533-47.
27. Cremers MI., *Metabolic syndrome, synchronous gastric and colorectal neoplasms: an ominous triad.* Journal of Gastroenterology and Hepatology, 2012. **27**: p. 1411–1412.
28. La Vecchia C, Negri E, Decarli A, Franceschi S, *Diabetes mellitus and colorectal cancer risk.* Cancer Epidemiology Biomarkers Preview, 1997. **6**(12): p. 1007-10.

29. McCarl M, Harnack L, Limburg PJ, Anderson KE, Folsom AR, *Incidence of colorectal cancer in relation to glycemic index and load in a cohort of women.* Cancer Epidemiology Biomarkers Preview, 2006. **15**(5): p. 892-6.
30. Shoelson SE, Herrero L, Naaz A., *Obesity, inflammation, and insulin resistance.* Gastroenterology, 2007. **132**: p. 2169–2180.
31. Kim S, Keko T, Martin C, Galanko J, Woosley JT, Schroeder JC, Satia JA, Halabi S, Sandler RS, *Circulating levels of inflammatory cytokines and risk of colorectal adenomas.* Cancer Research, 2008. **68**: p. 323–328.
32. Colditz G, Cannuscio C, Frazier A., *Physical activity and reduced risk of colon cancer: implications for prevention. Cancer Causes Control.* Cancer Causes Control, 1997. **8**: p. 649–667.
33. Colbert LH, Hartman TJ, Malila N, Limburg PJ, Pietinen P, Virtamo J, Taylor PR, Albanes D, *Physical activity in relation to cancer of the colon and rectum in a cohort of male smokers.* Cancer Epidemiology Biomarkers Preview, 2001. **10**: p. 265–268.
34. Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma K-N, Berry TD, *Energy balance and colon cancer--beyond physical activity.* Cancer Research, 1997. **57**: p. 75–80.
35. Wolin KY, Yan Y, Colditz GA, Lee IM, *Physical activity and colon cancer prevention: a meta-analysis.* British Journal of Cancer, 2009. **100**: p. 611–616.
36. Samad AK, Taylor RS, Marshall T, Chapman MA, *A meta-analysis of the association of physical activity with reduced risk of colorectal cancer.* Colorectal Disease, 2005. **7**(3): p. 204-13.
37. Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN. , *Eating patterns and risk of colon cancer. .* American Journal of Epidemiology, 1998. **148**: p. 4–16.
38. Trock B, Lanza E, Greenwald P. 82, *Dietary fiber, vegetables, and colon cancer: critical review and meta-analyses of the epidemiologic evidence.* Journal of National Cancer Institute, 1990. **82**: p. 650-661.
39. Howe GR, Benito E, Castelleto R, Cornee J, Esteve J, Gallagher RP, Iscovich JM, Deng-ao J, Kaaks R, Kune GA, et al. , *Dietary intake of fiber and decreased risk of cancers of the colon and rectum: evidence from the combined analysis of 13 case-control studies.* Journal of the Natlonal Cancer Institute 1992. **84**: p. 1887–1896.
40. Levi F, Pasche C, Lucchini F, La Vecchia C., *Dietary fibre and the risk of colorectal cancer.* European Journal of Cancer, 2001. **37**: p. 2091–2096.
41. Bingham SA, Day NE, Luben R, Ferrari P, Slimani N, Norat T, Clavel-Chapelon F, Kesse E, Nieters A, Boeing H, Tjonneland A, Overvad K, Martinez C, Dorronsoro M, Gonzalez CA, Key TJ, Trichopoulou A, Naska A, Vineis P, Tumino R, Krogh V, Bueno-de-Mesquita HB, Peeters PH, Berglund G, Hallmans G, Lund E, Skeie G, Kaaks R, Riboli E. Lancet, *Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study.* The Lancet, 2003. **361**: p. 1496–1501.
42. Fuchs CS, Giovanucci EL, Colditz GA, Hunter DJ, Stampfer MJ, Rosner B, Speizer FE, Willett WC, *Dietary fiber and the risk of colorectal cancer and adenoma in women.* New England Journal of Medicine, 1999. **340**: p. 169–176.

43. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE, *Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women.* New England Journal of Medicine, 1990. **323**: p. 1664–1672.
44. Goldbohm RA, van den Brandt PA, van 't Veer P, Brants HA, Dorant E, Sturmans F, Hermus RJ., *A prospective cohort study on the relation between meat consumption and the risk of colon cancer. Cancer Res.* **54** p. 718–723.
45. Neugut AI, Garbowski GC, Lee WC, Murray T, Nieves JW, Forde KA, Treat MR, Waye JD, Fenoglio-Preiser C, *Dietary risk factors for the incidence and recurrence of colorectal adenomatous polyps. A case-control study.* Annals of Internal Medicine, 1993. **118**: p. 91–95.
46. Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R., *A prospective study of red and processed meat intake in relation to cancer risk.* PLoS Med, 2007. **4**.
47. Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M, Overvad K, Olsen A, Tjonneland A, Clavel F, Boutron-Ruault MC, Kesse E, Boeing H, Bergmann MM, Nieters A, Linseisen J, Trichopoulou A, Trichopoulos D, Tountas Y, Berrino F, Palli D, Panico S, Tumino R, Vineis P, Bueno-de-Mesquita HB, Peeters PH, Engeset D, Lund E, Skeie G, Ardanaz E, Gonzalez C, Navarro C, Quiros JR, Sanchez MJ, Berglund G, Mattisson I, Hallmans G, Palmqvist R, Day NE, Khaw KT, Key TJ, San Joaquin M, Hemon B, Saracci R, Kaaks R, Riboli E., *Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition.* Journal of the Nationall Cancer Institute, 2005. **97**: p. 906–916.
48. Giovannucci E, Willett WC, *Dietary factors and risk of colon cancer.* Annals of Medicine, 1994. **26**: p. 443–452.
49. Gerhardsson de Verdier M, Hagman U, Peters RK, Steineck G, Overvik E, *Meat, cooking methods and colorectal cancer: a case-referent study in Stockholm.* International Journal of Cancer, 1991. **49**: p. 520–525.
50. Martinez ME, Jacobs ET, Ashbeck EL, Sinha R, Lance P, Alberts DS, Thompson PA, *Meat intake, preparation methods, mutagens and colorectal adenoma recurrence.* Carcinogenesis, 2007. **28**: p. 2019–2027.
51. Cross AJ, Pollock JR, Bingham SA, *Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat.* Cancer Research, 2003. **15**(63): p. 2358-60.
52. Sugimura, *Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. .* Mutation Research, 1985. **150**: p. 33–41.
53. Wakabayashi K, Nagao M, Esumi H, Sugimura T, *Food-derived mutagens and carcinogens.* Cancer Research, 1992. **52** p. 2092s–2098s.
54. Grau MV, Sandler RS, McKeown-Eyssen G, Bresalier RS, Haile RW, Barry EL, Ahnen DJ, Gui J, Summers RW, Baron JA., *Nonsteroidal Anti-inflammatory Drug Use After 3 Years of Aspirin Use and Colorectal Adenoma Risk: Observational Follow-up of a Randomized Study.* Journal of the National Cancer Institute, 2009. **101**(4): p. 267-76.
55. Chan AT, Arber N, Burn J, Chia WK, Elwood P, Hull MA, Logan RF, Rothwell PM, Schrör K, Baron JA, *Aspirin in the Chemoprevention of Colorectal Neoplasia: An Overview.* Cancer Prevention Research, 2011. **5**: p. 164-78.

56. Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeown-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Saibil F, van Stolk RU., *A randomized trial of aspirin to prevent colorectal adenomas.* New England Journal of Medicine, 2003. **348**(10): p. 891-9.
57. Baron JA, *Epidemiology of Non-Steroidal Anti-Inflammatory Durgs and Cancer.* Progress in Experimental Tumour Research, 2003. **37**: p. 1-24.
58. Flossmann E, Rothwell PM, *Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies.* Lancet, 2007. **9573**: p. 1603-1613.
59. Giardiello FM, Hamilton SR, Krush AJ, et al., *Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis.* New Engl J Med, 1993. **328**(18): p. 1313-1316.
60. Labayle D, Fischer D, Vielh P, et al. , *Sulindac causes regression of rectal polyps in familial adenomatous polyposis. .* Gastroenterology, 1991. **101**(3): p. 635-639.
61. Wu AW, Gu J, Ji JF, Li ZF, Xu GW., *Role of COX-2 in carcinogenesis of colorectal cancer and its relationship with tumor biological characteristics and patients' prognosis.* World Journal of Gastroenterology, 2003. **9**(9): p. 1990-4.
62. Park JH, McMillan DC, Horgan PG, Roxburgh CS, *The impact of anti-inflammatory agents on the outcome of patients with colorectal cancer.* Cancer Treatment Reviews, 2014. **40**(1): p. 68-77.
63. Wong WW, Dimitroulakos J, Minden MD, et al., *HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumor-specific apoptosis. .* Leukemia 2002. **16**: p. 508–19.
64. Agarwal B, Halmos B, Feoktistov AS, et al., *Mechanism of lovastatin-induced apoptosis in intestinal epithelial cells. .* Carcinogenesis, 2002. **23**: p. 521–8.
65. Hong MY, Seeram NP, Zhang Y, et al., *Anticancer effects of Chinese red yeast rice versus monacolin K alone on colon cancer cells.* J Nutr Biochem, 2008. **19**: p. 448–58.
66. Wang HB, Liu XP, Liang J, et al. , *Expression of RhoA and RhoC in colorectal carcinoma and its relations with clinicopathological parameters. .* Clin Chem Lab Med 2009. **47**: p. 811–17.
67. Bardou M, Barkun A, Martel M, *Effect of statin therapy on colorectal cancer.* Gut, 2010. **59**: p. 1572-85.
68. Bonovas S, Filioussi K, Christodoulos S,Sitaras F, Sitaris M, *Statins and the Risk of Colorectal Cancer: A Meta-Analysis of 18 Studies Involving More Than 1.5 Million Patients.* Journal of Clinical Oncology, 2007. **25**(23): p. 3462-8.
69. Delellis Henderson K, Duan L, Sullivan-Halley J, Ma H, Clarke CA, Neuhausen SL, Templeman C, Bernstein L, *Menopausal hormone therapy use and risk of invasive colon cancer: the California Teachers Study.* Am J Epidemiol. , 2010. **171**(4): p. 415-25.

70. Nelson HD, Humphrey L, Nygren P, Teutsch SM, Allan JD, *Postmenopausal hormone replacement therapy: scientific review.* JAMA, 2002. **288**(7): p. 872-81.
71. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators, *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.* JAMA, 2002. **288**(3): p. 321-33.
72. Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B, *Suppression of human colorectal carcinoma cell growth by wild-type p53.* Science, 1990. **249**(4971): p. 912-5.
73. Slattery ML, Curtin K, Anderson K, Ma KN, Ballard L, Edwards S, Schaffer D, Potter J, Leppert M, Samowitz WS, *Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors.* J Natl Cancer Inst., 2000. **92**(22): p. 1831-6.
74. Lynch HT, Smyrk TC, Cavalieri J, Lynch JF., *Identification of an HNPCC family.* Am J Gastroenterol, 1994. **89**(4): p. 605-9.
75. Liu X, Guo J, Zhao Z, Landau M, Heald B, Pai R, Lin J., *Distinct clinicohistologic features of inflammatory bowel disease-associated colorectal adenocarcinoma: in comparison with sporadic microsatellite-stable and Lynch syndrome-related colorectal adenocarcinoma.* Am J Surg Pathol, 2012. **36**(8): p. 1228-33.
76. Lynch HT, Shaw MW, Magnuson CW, Larsen AL, Krush AJ., *Hereditary factors in cancer. Study of two large midwestern kindreds.* Arch Intern Med. , 1966. **117**(2): p. 206-12.
77. Lynch HT, de laChapelle A, *Genetic susceptibility to non-polyposis colorectal cancer.* J Med Genet, 1999. **36**(11): p. 801-18.
78. de la Chapelle A, Peltomaki P, *Genetics of hereditary colon cancer. .* Annu Rev Genet 1995. **29**: p. 329–348. .
79. Peltomäki P, de la Chapelle A, *Mutations predisposing to hereditary nonpolyposis colorectal cancer.* Advances in Cancer Research, 1997: p. 93–119.
80. Möslein G, Tester DJ, Lindor NM, et al. , *Microsatellite instability and mutation analysis of hMSH2 and hMLH1 in patients with sporadic, familial and hereditary colorectal cancer. .* Hum Mol Genet 1996. **5**: p. 1245–1252. .
81. Vasen, Mecklin, Khan, Lynch, *The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). .* Dis. Colon Rectum 1991. **34**: p. 424-425.
82. Vasen, H., Watson, P., Mecklin, J.-P., & Lynch, H. (1999). , *New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC. .* Gastroenterology. **116**(6): p. 1453–1456.
83. Syngal S, Fox EA, Eng C, Kolodner, Garber JE, *Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1.* J. Med. Genet. , 2000. **37**: p. 641-645.
84. Bulow S, Faurschou Nielsen T et al., *The incidence rate of familial adenomatous polyposis. Results from the Danish Polyposis Register. .* Int J Colorectal Dis., 1996. **11**: p. 88-91.

85. Groden J, Thilveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M, et al., *Identification and characterization of the familial adenomatous polyposis coli gene.* Cell, 1991. **66**(3): p. 589-600.
86. Debinski HS, Love S, Spigelman AD, Phillips RK., *Colorectal polyp counts and cancer risk in familial adenomatous polyposis.* Gastroenterology, 1996. **110**(4): p. 1028-30.
87. Galle TS, Juel K, Bülow S., *Causes of death in familial adenomatous plyposis.* Scand J Gastroenterol., 1999. **34**(8): p. 808-12.
88. Gillen CD, Wwalmsley RS, Prior P, Andrews HA, Allan RN, *Ulcerative colitis and Crohn's disease: a comparisonofthecolorectalcancer riskin extensive colitis.* Gut, 1994. **34**: p. 1590-1592.
89. Ekbom A, Helmick C, Zack M, Adami HO., *Ulcerative colitis and colorectal cancer. A population-based study. .* N Engl J Med 1990. **323**: p. 1228–1233.
90. Azer, *Overview of molecular pathways in inflammatory bowel disease associated with colorectal cancer development.* European Journal of Gastroenterology and Hepatology, 2013. **25**: p. 271-281.
91. Itzkowitz, *Molecular biology of dysplasia and cancer in inflammatory bowel disease.* Gastroenterol Clin North Am 2006. **35**: p. 553–571.
92. Banan A, Choudhary S, Zhang Y, Fields JZ, Keshavarzian A. , *Oxidant-induced intestinal barrier disruption and its prevention by growth factors in a human colonic cell line: role of the microtubule cytoskeleton.* Free Radic Biol Med 2000. **28**: p. 727–738.
93. Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, Nusrat A. , *Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. .* J Immunol, 2003. **171**: p. 6164–6172.
94. Hamilton W, Round A., Sharp D, Peters TJ, *Clinical features of colorectal cancer before diagnosis: a population-based case–control study.* British Journal of Cancer, 2005. **93**: p. 399-405.
95. Thompson MR, Perera R, Senapati A, Dodds S., *Predictive value of common symptom combinations in diagnosing colorectal cancer.* Br J Surg, 2007. **94**(10): p. 1260-5.
96. Bass G, Fleming C, Conneely J, Martin Z, Mealy K., *Emergency first presentation of colorectal cancer predicts significantly poorer outcomes: a review of 356 consecutive Irish patients.* Dis Colon Rectum, 2009. **52**(4): p. 678-84.
97. (SIGN), S.I.G.N., *Diagnosis and Management of Colorectal Cancer.* SIGn Guideline, 2011(126).
98. Rex D, Rahmani E, Haseman JH, Lemmel GT, Kaster S, Buckley JS, *Relative sensitivity of colonoscopy and barium enema for detection of colorectal cancer in clinical practice. .* Gastroenterology, 1997. **112**(1): p. 17-23.
99. Waye J, Kahn O, Auerbach ME. , *Complications of colonoscopy and flexible sigmoidoscopy.* Gastrointest Endosc Clin N Am, 1996. **6**(2): p. 343-77.
100. Regge D, Laudi C, Galatola G, Della Monica P, Bonelli L, Angelelli G, et al., *Diagnostic accuracy of computed tomographic colonography for the detection of advanced neoplasia in individuals at increased risk of colorectal cancer. .* JAMA, 2009. **301**(23): p. 2453-61.

101. Koo BC, Ng C, J UK-I, Prevost AT, Freeman AH., *Minimal preparation CT for the diagnosis of suspected colorectal cancer in the frail and elderly patient. .* Clin Radiol 2006. **61**(2): p. 127-39.
102. Halligan S, Altman DG, Taylor SA, Mallett S, Deeks JJ, Bartram CI, Atkin W., *CT colonography in the detection of colorectal polyps and cancer: systematic review, meta-analysis, and proposed minimum data set for study level reporting.* Radiology, 2005. **237**(3): p. 893-904.
103. Rosman AS, Korsten MA, *Meta-analysis comparing CT colonography, air contrast barium enema, and colonoscopy. .* Am J Med 2007. **120**(3): p. 203-10.
104. World Health Organization, *Principles and Practice of Screening for Disease.* Public Health Papers, 1968. **Report No.34**.
105. Hardcastle JD, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, James PD, Mangham CM., *Randomised controlled trial of faecal-occult-blood screening for colorectal cancer.* Lancet, 1996. **30**(348): p. 1472-7.
106. Scholefield JH, Moss S, Sufi F, Mangham CM, Hardcastle JD., *Effect of faecal occult blood screening on mortality from colorectal cancer: results from a randomised controlled trial.* Gut, 2002. **50**(6): p. 840-4.
107. Hewitson P, Glasziou P, Watson E, Towler B, Irwig L, *Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update.* Am J Gastroenterol, 2008. **103**(6): p. 1541-9.
108. Mansouri D, McMillan DC, Crearie C, Morrison DS, Crighton EM, Horgan PG, *Temporal trends in mode, site and stage of presentation with the introduction of colorectal cancer screening: a decade of experience from the West of Scotland.* Br J Cancer, 2015. **113**(3): p. 556-61.
109. Roxburgh CS, McTaggart F, Balsitis M, Diament RH., *Impact of the bowel-screening programme on the diagnosis of colorectal cancer in Ayrshire and Arran.* Colorectal Dis, 2013. **15**(1): p. 34-41.
110. Collins PD, Mpofu C, Watson AJ, Rhodes JM. , *Strategies for detecting colon cancer and/or dysplasia in patients with inflammatory bowel disease. .* Cochrane Database of Systematic Reviews, 2006(2).
111. Cairns S, Scholefield JH, Steele RJ, Dunlop MG, Thomas HJ, Evans GD, Eaden JA, Rutter MD, Atkin WP, Saunders BP, Lucassen A, Jenkins P, Fairclough PD, Woodhouse CR; British Society of Gastroenterology; Association of Coloproctology for Great Britain and Ireland. , *Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002).* Gut, 2010. **59**(5): p. 666-89.
112. Dukes, *The classification of cancer of the rectum.* The Journal of Pathology and Bacteriology, 1932. **35**(3): p. 323-332.
113. Astler VB, Coller FA, *The prognostic significance of direct extension of carcinoma of the colon and rectum.* Ann Surg, 1954. **139**(6): p. 846-52.
114. Edge SB, Ccompton C, *The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM.* Ann Surg Oncol, 2010. **17**((6)): p. 1471-4.
115. Scottish Intercollegiate Guideline Network, *SIGN 126 - Diagnosis and Management of Colorectal Cancer.* 2015.

116. Langer C, Liersch T, Süss M, Siemer A, Markus P, Ghadimi BM, Füzesi L, Becker H., *Surgical cure for early rectal carcinoma and large adenoma: transanal endoscopic microsurgery (using ultrasound or electrosurgery) compared to conventional local and radical resection.* Int J Colorectal Dis., 2003. **18**(3): p. 222-9.
117. Coutsoftides T, Sivak MV, Jr, Benjamin SP, Jagelman D, *Colonoscopy and the management of polyps containing invasive carcinoma.* Annals of Surgery, 1978. **188**(5): p. 638-641.
118. Heald R, Moran BJ, Ryall RD, Sexton R, MacFarlane JK, *Rectal cancer: the Basingstoke experience of total mesorectal excision, 1978-1997. .* Arch Surg 1998. **133**(8): p. 894-9.
119. Wiig JN, Carisen E, Soreide O., *Mesorectal excision for rectal cancer: a view from Europe.* Semin Surg Oncol 1998. **15**(2): p. 78-86.
120. Holm T, Ljung A, Häggmark T, Jurell G, Lagergren J., *Extended abdominoperineal resection with gluteus maximus flap reconstruction of the pelvic floor for rectal cancer.* Br J Surg, 2007. **94**(2): p. 232-8.
121. Wong RK, Tandan V, De Silva S, Figueredo A., *Pre-operative radiotherapy and curative surgery for the management of localized rectal carcinoma. .* Cochrane Database of Systematic Reviews 2007. **2**.
122. Sebag-Montefiore DSR, Steele RJ, Monson J, Grieve R, Khanna S, et al. , *Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial.* Lancet, 2009. **373**(9666): p. 811-20.
123. van Gijn W, Marijnen CAM, Nagtegaal ID, Kranenbarg EM-K, Putter H, Wiggers T, et al. , *Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12- year follow-up of the multicentre, randomised controlled TME trial. .* Lancet Oncol, 2011. **12**(6): p. 575-82.
124. Sauer R, Becker H., *Preoperative versus Postoperative Chemoradiotherapy for Rectal Cancer.* New England Journal of Medicine, 2004. **351**: p. 1731-40.
125. Gray R, Barnwell J, Hills R, McConkey C, Williams N, Kerr D. , *QUASAR: A randomized study of adjuvant chemotherapy (CT) vs observation including 3238 colorectal cancer patients. .* Proc Am Soc Clin Onco 2004. **23**.
126. IMPACT, *Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer.* Journal of Clinical Oncology, 1999. **17**: p. 1356-63.
127. Morris EJ, Maughan NJ, Forman D, Quirke P., *Who to treat with adjuvant therapy in Dukes B/stage II colorectal cancer? The need for high quality pathology. .* Gut, 2007. **56**(10): p. 1419-25.
128. Petersen VC, Baxter KJ, Love SB, ShepherdNA., *Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer.* Gut, 2002. **51**(1): p. 65-9.
129. Buyse M, Zeleniuch Jacquotte A, Chalmers TC. , *Adjuvant therapy of colorectal cancer. Why we still don't know. .* JAMA 1988. **259**: p. 3571–8.
130. Dube S, Heyden F, Jenicek M. , *Adjuvant chemotherapy in colorectal carcinoma; results of a meta-analysis. .* Dis Colon Rectum 1997. **40**: p. 35-41.

131. Francini G, Petrioli R, Lorenzini L, et al., *Folinic acid and 5-fluorouracil as adjuvant chemotherapy in colon cancer.* Gastroenterology, 1994. **106**: p. 899-906.
132. Wolmark N, Fisher B, Rockette H, et al. , *Postoperative adjuvant chemotherapy or BCG for colon cancer: results from NSABP protocol C-01. .* J Natl Cancer Inst 1988. **80**: p. 30–6.
133. Taal BG, van Tinteren H, Zoetmulder FA., *Adjuvant 5FU plus levamisole in colonic or rectal cancer: improved survival in stage II and III. .* Br J Cancer, 2001. **85**: p. 1437-43.
134. Morris KT, Song TJ, Fong Y, *Recent advancements in diagnosis and treatment of metastatic colorectal cancer to the liver.* Surg Oncol, 2006. **15**(3): p. 129-34.
135. Schima W, Kulinna C, Langenberger H, Ba-Ssalamah A, *Liver metastases of colorectal cancer: US, CT or MR?* Cancer Imaging, 2005. **23**(5): p. S149-56.
136. Scheele J, Stang R, Altendorf-Hofmann A, Paul M, *Resection of colorectal liver metastases.* World J Surg., 1995. **19**(1): p. 59-71.
137. Stangl R, Altendorf-Hofman A, Charnley RM, Scheele J, *Factors influencing the natural history of colorectal liver metastases.* Lancet, 1994. **343**(8910): p. 1405-10.
138. Leung EY, Roxburgh CS, Leen E, Horgan PG, *Combined resection and radiofrequency ablation for bilobar colorectal cancer liver metastases.* Hepatogastroenterology, 2010. **57**(97): p. 41-6.
139. Nauta, R, Stablein DM, Holyoke ED., *Survival of Patients With Stage B2 Colon Carcinoma: The Gastrointestinal Tumor Study Group Experience.* Arch Surg, 1989. **124**(2): p. 180-182.
140. O'Connell JB, Maggard MA, Ko CY., *Colon Cancer Survival Rates With the New American Joint Committee on Cancer Sixth Edition Staging.* Journal of the National Cancer Institute, 2004. **96**(19): p. 1420-1425.
141. McArdle CS, Hole DJ, *Outcome following surgery for colorectal cancer.* British Medical Bulletin, 2002. **64**(1): p. 119-125.
142. Shepherd NA, Baxter KJ, Love SB., *Influence of local peritoneal involvement on pelvic recurrence and prognosis in rectal cancer.* J Clin Pathol, 1995. **48**(9): p. 849-55.
143. Shepherd NA, Baxter KJ, Love SB, *The prognostic importance of peritoneal involvement in colonic cancer: a prospective evaluation.* Gastroenterology, 1997. **112**(4): p. 1096-102.
144. Sternberg A, Amar M., Alfici R, Groisman G, *Conclusions from a study of venous invasion in stage IV colorectal adenocarcinoma.* J Clin Pathol, 2002. **55**: p. 17-21.
145. Vass DG, Ainsworth R, Anderson JH, Murray D, Foulis AK, *The value of an elastic tissue stain in detecting venous invasion in colorectal cancer.* J Clin Pathol, 2004. **57**: p. 769-772.
146. Horn A , Dahl O, Morild I., *Venous and neural invasion as predictors of recurrence in rectal adenocarcinoma. .* Dis Colon Rectum, 1991. **34**: p. 798-804.
147. Ouchi K , Sugawara T, Ono H, et al., *Histologic features and clinical significance of venous invasion in colorectal carcinoma with hepatic metastasis.* Cancer, 1996. **78**: p. 2313-17.

148. Roxburgh CSD, Wallace AM, Guthrie GJK, Horgan PG, McMillan DC, *Comparison of the prognostic value of tumour-based and patient-related factors in patients undergoing potentially curative surgery for colon cancer.* Colorectal Dis, 2009. **12**(10): p. 987-994.
149. Quirke P, Durdey P, Dixon MF, Williams NS, *Local recurrence of rectal adenocarcinoma due to inadequate surgical resection. Histopathological study of lateral tumour spread and surgical excision.* Lancet, 1986. **2**(8514): p. 996-9.
150. Adam IJ, Mohamdee M, Martin IG, Scott N, Finan PJ, Johnston D, Dixon MF, Quirke P, *Role of circumferential margin involvement in the local recurrence of rectal cancer.* Lancet, 1994. **344**(8924): p. 707-11.
151. Birbeck KF, Macklin CP, Tiffin NJ, Parsons W, Dixon MF, Mapstone NP, Abbott CR, Scott N, Finan PJ, Johnston D, Quirke P., *Rates of Circumferential Resection Margin Involvement Vary Between Surgeons and Predict Outcomes in Rectal Cancer Surgery.* Ann Surg, 2002. **235**(4): p. 449-457.
152. Herrera-Ornelas L, Justiniano J, Castillo N, et al., *Metastases in small lymph nodes from colon cancer.* Arch Surg, 1987. **122**: p. 1253-1256.
153. Brown HG, Luckasevic TM, Medich DS, et al., *Efficacy of manual dissection of lymph nodes in colon cancer resections.* Mod Pathol, 2004. **17**: p. 402-406.
154. Tepper JE, O'Connell MJ, Niedzwiecki D, et al., *Impact of number of nodes retrieved on outcome in patients with rectal cancer.* J Clin Oncol, 2001. **19**: p. 157-163.
155. Le Voyer TE, Sigurdson ER, Hanlon AI, et al., *Colon cancer survival is associated with increasing number of lymph nodes analyzed: a sec- ondary survey of Intergroup Trial Int-0089. .* J Clin Oncol, 2003. **21**: p. 2912-2919.
156. Swanson RS, Compton CC, Stewart AK, Bland KI., *The prognosis of T3N0 colon cancer is dependent upon the number of lymph nodes examined.* Ann Surg Oncol, 2003. **10**: p. 65-71.
157. Joseph NE, Sigurdson ER, Hanlon AL, et al. , *Accuracy of determining nodal negativity in colorectal cancer on the basis of the number of nodes retrieved on resection.* Ann Surg Oncol, 2003. **10**: p. 213-218.
158. De Ridder M, Vinh-Hung V, Van Nieuwenhove Y, Hoorens A, Sermeus A, Storme G., *Prognostic value of the lymph node ratio in node positive colon cancer.* Gut, 2006. **55**(11): p. 1681.
159. Rosenberg R, Friederichs J, Schuster T, Gertler R, Maak M, Becker K, Grebner A, Ulm K, Höfler H, Nekarda H, Siewert JR., *Prognosis of patients with colorectal cancer is associated with lymph node ratio: a single-center analysis of 3,026 patients over a 25-year time period.* Ann Surg, 2008. **248**(6): p. 968-78.
160. Ceelen W, Van Nieuwenhove Y, Pattyn P., *Prognostic value of the lymph node ratio in stage III colorectal cancer: a systematic review.* Ann Surg Oncol, 2010. **17**(11): p. 2847-55.
161. Folkman, *Fundamental concepts of the angiogenic process.* Curr Mol Med, 2003. **3**(7): p. 643-651.
162. Carmeliet, *Angiogenesis in life, disease and medicine.* Nature, 2005. **15**(438 (7070)): p. 932-6.

163. Takahashi Y, Tucker SL, Kitadai Y, Koura AN, Bucana CD, Cleary KR, Ellis LM, *Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer.* Arch Surg, 1997. **132**(5): p. 541-6.
164. Lee JC, Chow NH, Wang ST, Huang SM., *Prognostic of vascular endothelial growth factor expression in cancer.* European Journal of Cancer, 2000. **36**(6): p. 748-753.
165. De Vita F, Orditura M, Lieto E, Infusino S, Morgillo F, Martinelli E, Castellano P, Romano C, Ciardiello F, Catalano G, Pignatelli C, Galizia G, *Elevated perioperative serum vascular endothelial growth factor levels in patients with colon carcinoma.* Cancer, 2004. **100**(2): p. 270-8.
166. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, MorereJ-F, Benamouzig R, Breau J-L, Perret G-Y, *Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature.* Br J Cancer, 2006. **94**(12): p. 1823-1832.
167. Swinson, D.E., et al., *Tumour necrosis is an independent prognostic marker in non-small cell lung cancer: correlation with biological variables.* Lung Cancer, 2002. **37**(3): p. 235-40.
168. Langner, C., et al., *Tumor Necrosis as Prognostic Indicator in Transitional Cell Carcinoma of the Upper Urinary Tract.* The Journal of Urology, 2006. **176**(3): p. 910-914.
169. Leek, R.D., et al., *Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast.* Br J Cancer, 1999. **79**(5-6): p. 991-5.
170. Pollheimer, M.J., et al., *Tumor necrosis is a new promising prognostic factor in colorectal cancer.* Hum Pathol, 2010. **41**(12): p. 1749-57.
171. Richards, C.H., et al., *Prognostic value of tumour necrosis and host inflammatory responses in colorectal cancer.* Br J Surg, 2012. **99**(2): p. 287-94.
172. Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ, *Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric-oxide dependent mechanism.* Cancer Res, 2000. **60**(1): p. 184-90.
173. Jackson JR, Seed MP, Kircher CH, Willoughby DA, Winkler JD., *The codependence of angiogenesis and chronic inflammation.* FASEB, 1997. **11**(6): p. 457-65.
174. Danese S, Montovani A., *Inflammatory bowel disease and intestinal cancer: a paradigm of the Yin–Yang interplay between inflammation and cancer.* Oncogene, 2010. **29**(23): p. 3313-23.
175. Li Y, de HaarC, Chen M, Deuring J, Gerrits MM, Smits R, Xia B, Kuipers EJ, van der Woude CJ., *Disease-related expression of the IL6/STAT3/SOCS3 signalling pathway in ulcerative colitis and ulcerative colitis-related carcinogenesis.* Gut, 2010. **59**(2): p. 227-35.
176. Jess T, Simonsen J, Jørgensen KT, Pedersen BV, Nielsen NM, Frisch M., *Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years.* Gastroenterology, 2012. **143**(2): p. 375-81.
177. Higaki S, Akazawa A, Nakamura H, Yanai H, Yoshida T, Okita K, *Metaplastic polyp of the colon develops in response to inflammation.* J Gastroenterol Hepatol., 1999. **14**(7): p. 709-14.

178. Vakkila J, Lotze MT, *inflammation and necrosis promote tumour growth.* Nat Rev Immunol., 2004. **4**(8): p. 641-8.
179. Hanahan D, Weinberg RA, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.
180. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A., *Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability.* Carcinogenesis, 2009. **30**(7): p. 1073-81.
181. Klintrup K, Makinen JM, Kauppila S, Väre PO, Melkko J, Tuominen H, Tuppurainen K, Mäkelä J, Karttunen TJ, Mäkinen MJ., *Inflammation and prognosis in colorectal cancer.* Eur J Cancer., 2005. **41**(17): p. 2645-54.
182. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F., *Type, density, and location of immune cells within human colorectal tumors predict clinical outcome.* Science, 2006. **313**(5795): p. 1960-4.
183. Roxburgh CS, McMillan DC, *The role of the in situ local inflammatory response in predicting recurrence and survival in patients with primary operable colorectal cancer. Cancer Treat Rev 2011;38(5):451–66.* Cancer Treat Rev, 2011. **38**(5): p. 451-66.
184. Heinrich PC, Castell JV, Andus T., *Interleukin-6 and the acute phase response.* Biochem J., 1990. **265**(3): p. 621-36.
185. Bone RC., *Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation.* Crit Care Med., 1996. **24**(1): p. 163-72.
186. Gabay C, Kushner I, *Acute-phase proteins and other systemic responses to inflammation.* N Engl J Med, 1999. **340**(6): p. 448-54.
187. Castell JV, Gomez-Lechon MJ, David M, Fabra R, Trullenque R, Heinrich PC., *Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6.* Hepatology., 1990. **12**(5): p. 1179-86.
188. McMillan DC, Canna K, McArdle CS. , *Systemic inflammatory response predicts survival following curative resection of colorectal cancer.* Br J Surg, 2003. **90**(2): p. 215-9.
189. Crumley AB, McMillan DC, McKernan M, Going JJ, Shearer CJ, Stuart RC., *An elevated C-reactive protein concentration, prior to surgery, predicts poor cancer-specific survival in patients undergoing resection for gastro-oesophageal cancer.* Br J Cancer., 2006. **94**(11): p. 1568-71.
190. Jamieson NB, Glen P, McMillan DC, McKay CJ, Foulis AK, Carter R, Imrie CW., *Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma head of pancreas.* Br J Cancer., 2005. **92**(1): p. 21-3.
191. Al Murri AM, Bartlett JM, Canney PA, Doughty JC, Wilson C, McMillan DC., *Evaluation of an inflammation-based prognostic score (GPS) in patients with metastatic breast cancer.* Br J Cancer. 2006 Jan 30;94(2):227-30., 2006. **94**(2).
192. McMillan DC, Crozier JE, Canna K, Angerson WJ, McArdle CS., *Evaluation of an inflammation-based prognostic score (GPS) in patients undergoing resection for colon and rectal cancer.* Int J Colorectal Dis., 2007. **22**(8): p. 881-6.

193. Roxburgh CS, McMillan DC, *Role of systemic inflammatory response in predicting survival in patients with primary operable cancer.* Future Oncol., 2010. **6**(1): p. 149-63.
194. Zahorec R., *Ratio of neutrophil to lymphocyte counts – rapid and sim- ple parameter of systemic inflammation and stress in critically ill.* Bratislavske Lekarske Listy., 2001. **102**(1): p. 5-14.
195. McMillan DC, *The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer.* Cancer Treat Rev., 2013. **39**(5): p. 534-40.
196. Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ., *Evaluation of cumulative prognostic scores based on the systemic inflammatory response in patients with inoperable non-small-cell lung cancer.* Br J Cancer., 2003. **89**(6): p. 1028-30.
197. Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ., *Comparison of an inflammation-based prognostic score (GPS) with performance status (ECOG) in patients receiving platinum-based chemotherapy for inoperable non-small-cell lung cancer.* Br J Cancer., 2004. **90**(9): p. 1704-6.
198. Proctor MJ, McMillan DC, Talwar D, Balmer SM, O'Reilly DS, Foulis AK, Horgan PG, McMillan DC., *An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study.* Br J Cancer., 2011. **104**(4): p. 726-34.
199. Schmidt H, Suciu S, Punt CJA, Gore M, Kruit W, Patel P., *Pretreatment levels of peripheral neutrophils and leukocytes as independent predictors of overall survival in patients with ameri- can joint committee on cancer stage iv melanoma: results of the EORTC 18951 biochemotherapy trial.* Journal Clin Onc., 2007. **25**(12): p. 1562-9.
200. Clarke SJ, Chua W, Moore M, Kao S, Phan V, Tan C, Charles K, McMillan DC., *Use of inflammatory markers to guide cancer treatment.* Clin Pharmacol Ther., 2011. **90**(3): p. 475-8.
201. Walsh SR, Cook E, Goulder F, Justin TA, Keeling NJ., *Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer.* J Surg Oncol., 2005. **91**(3): p. 181-4.
202. Crumley AB, Stuart RC, McKernan M, Going JJ, Shearer CJ, McMillan DC., *Comparison of pre-treatment clinical prognostic factors in patients with gastro-oesophageal cancer and proposal of a new staging system.* J Gastrointest Surg., 2010. **14**(5): p. 781-7.
203. Proctor MJ, McMillan DC, Talwar D, Balmer SM, Fletcher CD, O'reilly DS, Foulis AK, Horgan PG, McMillan DC, *A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study.* Eur J Cancer., 2011. **47**: p. 2633-2641.
204. Shafique K, Proctor MJ, McMillan DC, Qureshi K, Leung H, Morrison DS., *Systemic inflammation and survival of patients with prostate cancer: evidence from the Glasgow Inflammation Outcome Study.* Prostate Cancer Prostatic Dis., 2012. **15**(2): p. 195-201.
205. Azab B, Bhatt V, Phookan J, Murukutla S, Kohn N, Terjanian T, Widmann WD., *Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients.* Ann Surg Oncol., 2012. **19**(1): p. 217-24.

206. Proctor MJ, McMillan DC, Morrison DS, Fletcher CD, Horgan PG, Clarke SJ., *A derived neutrophil to lymphocyte ratio predicts sur- vival in patients with cancer.* British Journal of Cancer 2012. **107**(4): p. 695-9.
207. Proctor MJ, Horgan PG, Talwar D, Fletcher CD, Morrison DS, McMillan DC., *Optimization of the systemic inflammation-based Glasgow prognostic score: a Glasgow Inflammation Outcome Study.* Cancer., 2013. **119**(12): p. 2325-32.
208. Ding PR, An X, Zhang RX, Fang YJ, Li LR, Chen G, Wu XJ, Lu ZH, Lin JZ, Kong LH, Wan DS, Pan ZZ., *Elevated preoperative neutrophil to lymphocyte ratio predicts risk of recurrence following curative resection for stage IIA colon cancer.* Int J Colorectal Dis., 2010. **25**(12): p. 1427-33.
209. Kwon HC, Kim SH, Oh SY, Lee S, Lee JH, Choi HJ, Park KJ, Roh MS, Kim SG, Kim HJ, Lee JH., *Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer.* Biomarkers., 2012. **17**(3): p. 216-22.
210. Neal CP, Mann CD, Garcea G, Briggs CD, Dennison AR, Berry DP., *Preoperative systemic inflammation and infectious complications after resection of colorectal liver metastases.* Arch Surg., 2011. **146**(4): p. 471-8.
211. Leitch EF, Chakrabarti M, Crozier JE, McKee RF, Anderson JH, Horgan PG, McMillan DC., *Comparison of the prognostic value of selected markers of the systemic inflammatory response in patients with colorectal cancer.* Br J Cancer., 2007. **97**(9): p. 1266-70.
212. Mallappa S, Sinha A, Gupta S, Chadwick SJ., *Preoperative neutrophil to lymphocyte ratio >5 is a prognostic factor for recurrent colorectal cancer.* Colorectal Dis., 2013. **15**(3): p. 323-8.
213. Halazun KJ, Aldoori A, Malik HZ, Al-Mukhtar A, Prasad KR, Toogood GJ, Lodge JP., *Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases.* Eur J Surg Oncol. **34**(1): p. 55-60.
214. Gomez D, Morriss-Stiff G, Wyatt J, Toogood GJ, Lodge JP, Prasad KR., *Surgical technique and systemic inflammation influ- ences long-term disease-free survival following hepatic resection for colorectal metastasis.* Journals of Surgical Oncology, 2008. **98**(5): p. 371-6.
215. Hung HY, Chen JS, Yeh CY, Changchien CR, Tang R, Hsieh PS, Tasi WS, You JF, You YT, Fan CW, Wang JY, Chiang JM., *Effect of preoperative neutrophil-lymphocyte ratio on the surgical outcomes of stage II colon cancer patients who do not receive adjuvant chemotherapy.* Int J Colorectal Dis., 2011. **26**(8): p. 1059-65.
216. Chiang SF, Hung HY, Tang R, Changchien CR, Chen JS, You YT, Chiang JM, Lin JR., *Can neutrophil-to-lymphocyte ratio predict the survival of colorectal cancer patients who have received curative surgery electively?* Int J Colorectal Dis., 2012. **27**(10): p. 1347-57.
217. Ubukata H, Motohashi G, Tabuchi T, Nagata H, Konishi S. , *Evaluations of interferon-gamma/interleukin-4 ratio and neutrophil/lymphocyte ratio as prognostic indicators in gastric cancer patients.* Journal of Surgical Oncology., 2010. **102**(7): p. 742-7.
218. Aizawa M, Gotohda N, Takahashi S, Konishi M, Kinoshita T. , *Predictive value of baseline neutrophil/lymphocyte ratio for T4 disease in wall-penetrating gastric cancer.* World J Surgery, 2011. **35**(12): p. 2717-22.

219. Jung MR, Park YK, Jeong O, Seon JW, Ryu SY, Kim DY, Kim YJ., *Elevated preoperative neutrophil to lymphocyte ratio predicts poor survival following resection in late stage gastric cancer.* J Surg Oncol., 2011. **104**(5): p. 504-10.
220. Rashid F, Waraich N, Bhatti I, Saha S, Khan RN, Ahmed J, Leeder PC, Larvin M, Iftikhar SY., *A pre-operative elevated neutrophil: lymphocyte ratio does not predict survival from oesophageal cancer resection.* World J Surg Oncol. 2010 Jan 6;8:1. p. 1477.
221. Wang DS, Ren C, Qiu MZ, Luo HY, Wang ZQ, Zhang DS, Wang FH, Li YH, Xu RH., *Comparison of the prognostic value of various preoperative inflammation-based factors in patients with stage III gastric cancer.* Tumour Biol., 2012. **33**(3): p. 749-56.
222. Mohri Y, Tanaka K, Ohi M, Yokoe T, Miki C, Kusunoki M., *Prognostic significance of host- and tumor-related factors in patients with gastric cancer. .* World J Surgery, 2010. **34**(2): p. 285-90.
223. Shimada H, Takiguchi N, Kainuma O, Soda H, Ikeda A, Cho A, Miyazaki A, Gunji H, Yamamoto H, Nagata M., *High preoperative neutrophil-lymphocyte ratio predicts poor survival in patients with gastric cancer.* Gastric Cancer., 2010. **13**(3): p. 170-6.
224. Garcea G, Ladwa N, Neal C, Metcalfe M, Dennison A, Berry D. , *Preoperative neutrophil-to-lymphocyte ratio (NLR) is associated with reduced disease-free survival following curative resection of pancreatic adenocarcinoma. .* World Journal of Surgery, 2011. **35**(4): p. 868-72.
225. Bhatti I, Peacock O, Lloyd G, Larvin M, Hall RI., *Preopera-tive hematologic markers as independent predictors of prognosis in resected pancreatic ductal adenocarcinoma: neutrophil–lymphocyte versus platelet–lymphocyte ratio.* American Journal of Surgery, 2010. **200**(2): p. 197-203.
226. Smith RA, Bosonnet L, Raraty M, Sutton R, Neoptolemos JP, Campbell F, Ghaneh P., *Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma.* Am J Surg., 2009. **197**(4): p. 466-72.
227. Gomez D, Morris-Stiff G, Toogood GJ, Lodge JP, Prasad KR., *Impact of systemic inflammation on outcome following resection for intrahepatic cholangiocarcinoma. .* Journal of Surgical Oncology, 2008. **97**(6): p. 513-8.
228. Kinoshita A, Onoda H, Imai N, Iwaku A, Oishi M, Fushiya N, Koike K, Nishino H, Tajiri H., *Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma.* Br J Cancer., 2012. **107**(6): p. 988-93.
229. Sakai T, Tsushima T, Kimura D, Hatanaka R, Yamada Y, Fukuda I. , *A clinical study of the prognostic factors for postoperative early recur- rence in patients who underwent complete resection for pulmonary adenocarcinoma. .* Annals of Thoracic and Cardiovascular Surgery, 2011. **17**(6): p. 539-43.
230. Sarraf KM, Belcher E, Raevsky E, Nicholson AG, Goldstraw P, Lim E. , *Neutrophil/lymphocyte ratio and its association with survival after complete resection in non-small cell lung cancer. .* Journal of Thoracic and Cardiovascular Surgery, 2009. **137**(2): p. 425-8.

231. Tomita M, Shimizu T, Ayabe T, Yonei A, Onitsuka T. , *Preoperative neutrophil to lymphocyte ratio as a prognostic predictor after cura- tive resection for non-small cell lung cancer. .* Anticancer Research, 2011. **31**(9): p. 2995-8.
232. Tomita M, Shimizu T, Ayabe T, Onitsuka T. , *Elevated preoperative inflammatory markers based on neutrophil-to-lymphocyte ratio and C-reactive protein predict poor survival in resected non-small cell lung cancer.* Anticancer Research, 2012. **32**(8): p. 3535-8.
233. Hashimoto T, Ohno Y, Nakashima J, Gondo T, Ohori M, Tachibana M. , *Clinical significance of preoperative peripheral blood neutrophil count in patients with non-metastatic upper urinary tract carcinoma. .* World Journal of Urology, 2013. **31**(4): p. 953-8.
234. Gondo T, Nakashima J, Ohno Y, Choichiro O, Horiguchi Y, Namiki K, Yoshioka K, Ohori M, Hatano T, Tachibana M., *Prognostic value of neutrophil-to-lymphocyte ratio and establishment of novel preoperative risk stratification model in bladder cancer patients treated with radical cystectomy.* Urology., 2012. **79**(5): p. 1085-91.
235. Ohno Y, Nakashima J, Ohori M, Hatano T, Tachibana M., *Pretreatment neutrophil-to-lymphocyte ratio as an independent predictor of recurrence in patients with nonmetastatic renal cell carcinoma.* J Urol., 2010. **184**(3): p. 873-8.
236. Ohno Y, Nakashima J, Ohori M, Gondo T, Hatano T, Tachibana M. , *Follow-up of neutrophil-to-lymphocyte ratio and recurrence of clear cell renal cell carcinoma. .* The Journal of Urology., 2012. **187**(2): p. 411-7.
237. Cho H, Hur HW, Kim SW, Kim SH, Kim JH, Kim YT, Lee K., *Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment.* Cancer Immunol Immunother., 2008. **58**(1): p. 15-23.
238. Idowu OK, Ding Q, Taktak AF, Chandrasekar CR, Yin Q., *Clinical implication of pretreatment neutrophil to lympho- cyte ratio in soft tissue sarcoma.* Biomarkers., 2012. **17**(6): p. 539-44.
239. Ishizuka M, Nagata H, Takagi K, Horie T, Kubota K., *Inflammation-based prognostic score is a novel predictor of postoperative outcome in patients with colorectal cancer.* Ann Surg, 2007. **246**(1047-51.).
240. Crozier JE, Leitch EF, McKee RF, Anderson JH, Horgan PG, McMillan DC., *Relationship between emergency presentation, systemic inflammatory response, and cancer-specific survival in patients undergoing potentially curative surgery for colon cancer.* Am J Surg., 2009. **197**: p. 544-9.
241. Moyes LH, Leitch EF, McKee RF, Anderson JH, Horgan PG, McMillan DC., *Preoperative systemic inflammation predicts postoperative infectious complications in patients undergoing curative resection for colorectal cancer. .* Br J Cancer, 2009. **100**: p. 1236-9.
242. Ishizuka M, Kita J, Shimoda M, Rokkaku K, Kato M, Sawada T, Kubota K., *Systemic inflammatory response predicts postoperative outcome in patients with liver metastases from colorectal cancer.* J Surg Oncol., 2009. **100**(1): p. 38-42.
243. Ishizuka M, Nagata H, Takagi K, Kubota K. , *Systemic inflammatory response associated with distant metastasis of T1 or T2 colorectal cancer.* Dig Dis Sci., 2010. **55**: p. 3181-7.

244. Kobayashi T, Teruya M, Kishiki T, Kaneko S, Endo D, Takenaka Y, Miki K, Kobayashi K, Morita K., *Inflammation-based prognostic score and number of lymph node metastases are independent prognostic factors in esophageal squamous cell carcinoma.* Dig Surg., 2010. **27**(3): p. 232-7.
245. Nozoe T, Iguchi T, Egashira A, Adachi E, Matsukuma A, Ezaki T., *Significance of modified Glasgow prognostic score as a useful indicator for prognosis of patients with gastric carcinoma.* Am J Surg., 2011. **201**(2): p. 186-91.
246. Vashist YK, Loos J, Dedow J, Tachezy M, Uzunoglu G, Kutup A, Yekebas EF, Izbicki JR., *Glasgow Prognostic Score is a predictor of perioperative and long-term outcome in patients with only surgically treated esophageal cancer.* Ann Surg Oncol., 2011. **18**(4): p. 1130-8.
247. Dutta S, Crumley AB, Fullarton GM, Horgan PG, McMillan DC., *Comparison of the prognostic value of tumour- and patient-related factors in patients undergoing potentially curative resection of oesophageal cancer.* World J Surg., 2011. **35**(8): p. 1861-6.
248. Dutta S, Crumley AB, Fullarton GM, Horgan PG, McMillan DC., *Comparison of the prognostic value of tumour and patient related factors in patients undergoing potentially curative resection of gastric cancer.* Am J Surg., 2012. **204**(3): p. 294-9.
249. Jiang X, Hiki N, Nunobe S, Kumagai K, Kubota T, Aikou S, Sano T, Yamaguchi T., *Prognostic importance of the inflammation-based Glasgow prognostic score in patients with gastric cancer.* Br J Cancer., 2012. **107**(2): p. 275-9.
250. Knight BC, Kausar A, Manu M, Ammori BA, Sherlock DJ, O'Reilly DA., *Evaluation of surgical outcome scores according to ISGPS definitions in patients undergoing pancreatic resection.* Dig Surg., 2010. **27**(5): p. 367-74.
251. Jamieson NB, Denley SM, Logue J, MacKenzie DJ, Foulis AK, Dickson EJ, Imrie CW, Carter R, McKay CJ, McMillan DC., *A prospective comparison of the prognostic value of tumor- and patient-related factors in patients undergoing potentially curative surgery for pancreatic ductal adenocarcinoma.* Ann Surg Oncol., 2011. **18**(8): p. 2318-28.
252. La Torre M, Nigri G, Cavallini M, Mercantini P, Ziparo V, Ramacciato G., *The glasgow prognostic score as a predictor of survival in patients with potentially resectable pancreatic adenocarcinoma.* Ann Surg Oncol., 2012. **19**(9): p. 2917-23.
253. Ishizuka M, Kubota K, Kita J, Shimoda M, Kato M, Sawada T., *Usefulness of a modified inflammation-based prognostic system for predicting postoperative mortality of patients undergoing surgery for primary hepatocellular carcinoma. .* J Surg Oncol., 2011. **103**: p. 801-6.
254. Ishizuka M, Kubota K, Kita J, Shimoda M, Kato M, Sawada T., *Impact of an inflammation-based prognostic system on patients undergoing surgery for hepatocellular carcinoma: a retrospective study of 398 Japanese patients.* Am J Surg., 2012. **203**(1): p. 101-6.
255. Polterauer S, Grimm C, Seebacher V, Rahhal J, Tempfer C, Reinthaller A, Hefler L., *The inflammation-based Glasgow Prognostic Score predicts survival in patients with cervical cancer.* Int J Gynecol Cancer., 2010. **20**(6): p. 1052-7.

256. Lamb GW, Aitchison M, Ramsey S, Housley SL, McMillan DC., *Clinical utility of the Glasgow Prognostic Score in patients undergoing curative nephrectomy for renal clear cell cancer: basis of new prognostic scoring systems.* Br J Cancer., 2012. **106**(2): p. 279-83.
257. Roxburgh CS, Salmond JM, Horgan PG, Oien KA, McMillan DC., *Comparison of the prognostic value of inflammation-based pathologic and biochemical criteria in patients undergoing potentially curative resection for colorectal cancer.* Annals of Surgery, 2009. **249**(5): p. 788-93.
258. Kobayashi T, Teruya M, Kishiki T, Endo D, Takenaka Y, Miki K, Kobayashi K, Morita K., *Elevated C-reactive protein and hypoalbuminemia measured before resection of colorectal liver metastases predict postoperative survival.* Dig Surg, 2010. **27**(4): p. 285-90.
259. Richards CH, Leitch EF, Horgan PG, Anderson JH, McKee RF, McMillan DC., *The relationship between patient physiology, the systemic inflammatory response and survival in patients undergoing curative resection of colorectal cancer.* Br J Cancer, 2010. **103**(9): p. 1356-61.
260. Moug SJ, McColl G, Lloyd SM, Wilson G, Saldanha JD, Diament RH., *Comparison of positive lymph node ratio with an inflammation-based prognostic score in colorectal cancer.* Br J Cancer, 2011. **98**(2): p. 282-6.
261. Roxburgh CS, Platt JJ, Leitch EF, Kinsella J, Horgan PG, McMillan DC, *Relationship between preoperative comorbidity, systemic inflammatory response, and survival in patients undergoing curative resection for colorectal cancer. .* Ann Surg Oncol, 2011. **18**: p. 997–1005.
262. Jamieson NB, Oien KA, Foulis AK, Dickson EJ, Imrie CW, et al. *The relationship between tumor inflammatory cell infiltrate and outcome in patients with pancreatic ductal adenocarcinoma.* Ann Surg Oncol, 2012. **19**(11): p. 3581-90.
263. Ishizuka M, Nagata H, Takagi K, Iwasaki Y, Kubota K, *Inflammation-based prognostic system predicts postoperative survival of colorectal cancer patients with a normal preoperative serum level of carcinoembryonic antigen.* Ann Surg Oncol 2012 [Epub ahead of print] PubMed PMID: 22576063., 2012. **19**(11): p. 3422-31.
264. Kwon, H.C., et al., *Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer.* Biomarkers, 2012. **17**(3): p. 216-22.
265. Motomura T, Shirabe K, Mano Y, Muto J, Toshima T, Umemoto Y, Fukuhara T, Uchiyama H, Ikegami T, Yoshizumi T, Soejima Y, Maehara Y., *Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment.* J Hepatol., 2013. **58**(1): p. 58-64.
266. Wang CY, Hsieh M, Chiu YC, Li SH, Huang HW, Fang FM, Huang YJ., *Higher serum C-reactive protein concentration and hypoalbuminemia are poor prognostic indicators in patients with esophageal cancer undergoing radiotherapy.* Radiother Oncol., 2009. **92**(2): p. 270-5.
267. Hwang JE, Kim HN, Kim DE, Choi HJ, Jung SH, Shim HJ, Bae WK, Hwang EC, Cho SH, Chung IJ., *Prognostic significance of a systemic inflammatory response in patients receiving first-line palliative chemotherapy for recurred or metastatic gastric cancer.* BMC Cancer., 2011. **11**: p. 489.

268. Aliustaoglu M, Bilici A, Ustaalioglu BB, Konya V, Gucun M, Seker M, Gumus M., *The effect of peripheral blood values on prognosis of patients with locally advanced gastric cancer before treatment.* Med Oncol., 2010. **27**(4): p. 1060-5.
269. Cedrés S, Torrejon D, Martínez A, Martinez P, Navarro A, Zamora E, Mulet-Margalef N, Felip E., *Neutrophil to lymphocyte ratio (NLR) as an indicator of poor prognosis in stage IV non-small cell lung cancer.* Clin Transl Oncol., 2012. **14**(11): p. 864-9.
270. Kao SC, Paviakis N, Harvie R, Vardy JL, Boyer MJ, van Zandwijk N, Clarke SJ., *High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy.* Clin Cancer Res., 2010. **16**(23): p. 5805-13.
271. Yao Y, Yuan D, Liu H, Gu X, Song Y., *Pretreatment neutrophil to lymphocyte ratio is associated with response to therapy and prognosis of advanced non-small cell lung cancer patients treated with first-line platinum-based chemotherapy.* Cancer Immunol Immunother., 2013. **62**(3): p. 471-9.
272. Teramukai S, Kitano T, Kishida Y, Kawahara M, Kubota K, Komuta K, Minato K, Mio T, Fujita Y, Yonei T, Nakano K, Tsuboi M, Shibata K, Furuse K, Fukushima M., *Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: an analysis of Japan Multinational Trial Organisation LC00-03.* Eur J Cancer., 2009. **45**(11): p. 1950-8.
273. Morimoto M, Numata K, Moriya S, Kondo M, Nozaki A, Morioka Y, Maeda S, Tanaka K., *Inflammation-based prognostic score for hepatocellular carcinoma patients on sorafenib treatment.* Anticancer Res., 2012. **32**(2): p. 619-23.
274. An X, Ding P, Li YH, Wang FH, Shi YX, Wang ZQ, He YJ, Xu RH, Jiang WQ., *Elevated neutrophil to lymphocyte ratio predicts survival in advanced pancreatic cancer.* Biomarkers., 2010. **15**(6): p. 516-22.
275. Carruthers R, Tho LM, Brown J, Kakumanu S, McCartney E, McDonald AC., *Systemic inflammatory response is a predictor of outcome in patients undergoing preoperative chemoradiation for locally advanced rectal cancer.* Colorectal Dis, 2012. **14**(10): p. e701-7.
276. Chua TC, Chong CH, Liauw W, Zhao J, Morris DL. , *Inflammatory markers in blood and serum tumor markers predict survival in patients with epithelial appendiceal neoplasms undergoing surgical cytoreduction and intraperitoneal chemotherapy.* Ann Surg, 2012. **256**(2): p. 342-9.
277. Kishi Y, Kopetz S, Chun YS, Palavecino M, Abdalla EK, Vauthey JN. , *Blood neutrophil-to-lymphocyte ratio predicts survival in patients with colorectal liver metastases treated with systemic chemotherapy.* Annals of Surgical Oncology, 2009. **16**(3): p. 614-22.
278. Ishizuka M, Nagata H, Takagi K, Kubota K., *Influence of inflammation-based prognostic score on mortality of patients undergoing chemotherapy for far advanced or recurrent unresectable colorectal cancer.* Ann Surg., 2009. **250**(2): p. 268-72.
279. Roxburgh C, McDonald A, Salmond J, Oien K, Anderson J, McKee R, Horgan P, McMillan D., *Adjuvant chemotherapy for resected colon cancer: comparison of the prognostic value of tumour and patient related factors.* Int J Colorectal Dis., 2011. **26**(4): p. 483-92.

280. Sharma R, Zucknick M, London R, Kacevska M, Liddle C, Clarke SJ., *Systemic inflammatory response predicts prognosis in patients with advanced-stage colorectal cancer.* Clin Colorectal Cancer., 2008. **7**(5): p. 331-7.
281. Lee Y, Kim SH, Han JY, Kim HT, Yun T, Lee JS. , *Early neutrophil-to-lymphocyte ratio reduction as a surrogate marker of prognosis in never smokers with advanced lung adenocarci- noma receiving gefitinib or standard chemotherapy as first-line therapy.* Journal of Cancer Research and Clinical Oncology, 2012. **138**(12): p. 2009-16.
282. Keizman D, Ish-Shalom M, Huang P, Eisenberger MA, Pili R, Hammers H, Carducci MA., *The association of pre-treatment neutrophil to lymphocyte ratio with response rate, progression free survival and overall survival of patients treated with sunitinib for metastatic renal cell carcinoma.* Eur J Cancer., 2012. **48**(2): p. 202-8.
283. Chua W, Charles KA, Baracos VE, Clarke SJ., *Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer.* Br J Cancer., 2011. **104**(8): p. 1288-95.
284. Chua W, Clarke SJ, Charles KA, *Systemic inflammation and prediction of chemotherapy outcomes in patients receiving docetaxel for advanced cancer.* Support Care Cancer, 2012. **20**(8): p. 1869-74.
285. Gioulbasanis I, Pallis A, Vlachostergios PJ, Xyrafas A, Giannousi Z, Perdikouri IE, Makridou M, Kakalou D, Georgoulias V, *The Glasgow Prognostic Score (GPS) predicts toxicity and efficacy in platinum-based treated patients with metastatic lung cancer.* Lung Cancer, 2012. **77**(2): p. 383-8.
286. Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dagg K, Scott HR., *A prospective longitudinal study of performance status, an inflammation-based score (GPS) and survival in patients with inoperable non-small-cell lung cancer.* Br J Cancer., 2005. **92**(10): p. 1834-6.
287. Pinato DJ, Mauri FA, Ramakrishnan R, Wahab L, Lloyd T, Sharma R., *Inflammation-based prognostic indices in malignant pleural mesothelioma.* J Thorac Oncol., 2012. **7**(3): p. 587-94.
288. Leung EY, Scott HR, McMillan DC., *Clinical utility of the pretreatment glasgow prognostic score in patients with advanced inoperable non-small cell lung cancer.* J Thorac Oncol., 2012. **7**(4): p. 655-62.
289. Crumley AB, McMillan DC, McKernan M, McDonald AC, Stuart RC., *Evaluation of an inflammation-based prognostic score in patients with inoperable gastro-oesophageal cancer.* Br J Cancer., 2006. **94**(5): p. 637-41.
290. Glen P, J.N., McMillan DC, Carter R, Imrie CW, McKay CJ., *Evaluation of an inflammation-based prognostic score in patients with inoperable pancreatic cancer.* Pancreatology., 2006. **6**(5): p. 450-3.
291. Pinato DJ, Stebbing J, Ishizuka M, Khan SA, Wasan HS, North BV, Kubota K, Sharma R., *A novel and validated prognostic index in hepatocellular carcinoma: the inflammation based index (IBI).* J Hepatol., 2012. **57**(5): p. 1013-20.
292. Sharma R, Hook J, Kumar M, Gabra H., *Evaluation of an inflammation-based prognostic score in patients with advanced ovarian cancer.* Eur J Cancer., 2008. **44**(2): p. 251-6.

293. Ramsey S, Lamb G, Aitchison M, Graham J, McMillan DC., *Evaluation of an inflammation-based prognostic score in patients with metastatic renal cancer.* Cancer., 2007. **109**(2): p. 205-12.
294. Kaneko M, Nozawa H, Sasaki K, Hongo K, Hiyoshi M, Tada N, Murono K, Nirei T, Kawai K, Sunami E, Tsuno NH, Kitayama J., *Elevated neutrophil to lymphocyte ratio predicts poor prognosis in advanced colorectal cancer patients receiving oxaliplatin-based chemotherapy.* Oncology., 2012. **82**(5): p. 261-8.
295. McNally ME, Martinez A, Khabiri H, Guy G, Michaels AJ, Hanje J, Kirkpatrick R, Bloomston M, Schmidt CR., *Inflammatory markers are associated with outcome in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization.* Ann Surg Oncol., 2013. **20**(3): p. 923-8.
296. Huang ZL, Luo J, Chen MS, Li JQ, Shi M., *Blood neutrophil-to-lymphocyte ratio predicts survival in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization.* J Vasc Interv Radiol., 2011. **22**(5): p. 702-9.
297. Jeong JH, Lim SM, Yun JY, Rhee GW, Lim JY, Cho JY, Kim YR., *Comparison of two inflammation-based prognostic scores in patients with unresectable advanced gastric cancer.* Oncology., 2012. **83**(5): p. 292-9.
298. Wang S, Zhang Z, Fang F, Gao X, Sun W, Liu H. , *The neutrophil/lymphocyte ratio is an independent prognostic indicator in patients with bone metastasis. .* Oncology Letters., 2011. **2**(4): p. 735-40.
299. Read JA, Choy S, Beale PJ, Clarke SJ, *Evaluation of nutritional and inflammatory status of advanced colorectal cancer patients and its correlation with survival.* Nutr Cancer, 2006. **55**: p. 78-85.
300. Partridge M, Fallon M, Bray C, McMillan D, Brown D, Laird B, *Prognostication in advanced cancer: a study examining an inflammation-based score.* J Pain Symptom Manage, 2012. **44**(2): p. 161-7.
301. Halazun KJ, Hardy M, Rana AA, Woodland DC 4th, Luyten EJ, Mahadev S, Witkowski P, Siegel AB, Brown RS Jr, Emond JC., *Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma.* Ann Surg., 2009. **250**(1): p. 141-51.
302. Bertuzzo VR, Cescon M, Ravaioli M, Grazi GL, Ercolani G, Del Gaudio M, Cucchetti A, D'Errico-Grigioni A, Golfieri R, Pinna AD., *Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers.* Transplantation., 2011. **91**(11): p. 1279-85.
303. Sato H, Ttsubosa Y, Kawano T., *Correlation between the pretherapeutic neutrophil to lymphocyte ratio and the pathologic response to neoadjuvant chemotherapy in patients with advanced esophageal cancer.* World Journal of Surgery, 2012. **36**(3): p. 617-22.
304. Miyata H, Yamasaki M, Kurokawa Y, Takiguchi S, Nakajima K, Fujiwara Y, Mori M, Doki Y., *Prognostic value of an inflammation-based score in patients undergoing pre-operative chemotherapy followed by surgery for esophageal cancer.* Exp Ther Med., 2011. **2**(5): p. 879-885.

305. Sharaiha RZ, Halazun KJ, Mirza F, Port JL, Lee PC, Neugut AI, Altorki NK, Abrams JA., *Elevated preoperative neutrophil:lymphocyte ratio as a predictor of postoperative disease recurrence in esophageal cancer.* Ann Surg Oncol., 2011. **18**(12): p. 3362-9.
306. Wang GY, Yang Y, Li H, Zhang J, Jiang N, Li MR, Zhu HB, Zhang Q, Chen GH., *A scoring model based on neutrophil to lymphocyte ratio predicts recurrence of HBV-associated hepatocellular carcinoma after liver transplantation.* PLoS One., 2011. **6**(9): p. e25295.
307. Krzystek-Korpacka M, Matusiewicz M, Diakowska D, Grabowski K, Blachut K, Kustrzeba-Wojcicka I, Terlecki G, Gamian A., *Acute-phase response proteins are related to cachexia and accelerated angiogenesis in gastroesophageal cancers.* Clin Chem Lab Med., 2008. **46**(3): p. 359-64.
308. Kerem M, Ferahkose Z, Yilmaz UT, Pasaoglu H, Ofluoglu E, Bedirli A, Salman B, Sahin TT, Akin M., *Adipokines and ghrelin in gastric cancer cachexia.* World J Gastroenterol., 2008. **14**(23): p. 3633-41.
309. Meek CL, Wallace AM, Forrest LM, McMillan DC., *The relationship between the insulin-like growth factor-1 axis, weight loss, an inflammation-based score and survival in patients with inoperable non-small cell lung cancer.* Clin Nutr., 2010. **29**(2): p. 206-9.
310. Skipworth RJ, Deans D, Tan BH, Sangster K, Paterson-Brown S, Brown DA, Hunter M, Breit SN, Ross JA, Fearon KC., *Plasma MIC-1 correlates with systemic inflammation but is not an independent determinant of nutritional status or survival in oesophago-gastric cancer.* Br J Cancer., 2010. **102**(4): p. 665-72.
311. Diakowska D, Krzystek-Korpacka M, Markocka-Maczka K, Diakowski W, Matusiewicz M, Grabowski K., *Circulating leptin and inflammatory response in esophageal cancer, esophageal cancer-related cachexia-anorexia syndrome (CAS) and non-malignant CAS of the alimentary tract.* Cytokine., 2010. **51**(2): p. 132-7.
312. Giannousi Z, Gioulbasanis I, Pallis AG, Xyrafas A, Dalliani D, Kalbakis K, Papadopoulos V, Mavroudis D, Georgoulias V, Papandreou CN., *Nutritional status, acute phase response and depression in metastatic lung cancer patients: correlations and association prognosis.* Support Care Cancer., 2012. **20**(8): p. 1823-9.
313. Richards CH, Roxburgh CS, Macmillan MT, Isswiasi S, Robertson EG, Guthrie GK, Horgan PG, Mcmillan DC, *The relationships between body composition and the systemic inflammatory response in patients with primary operable colorectal cancer.* PLoS One, 2012. **7**(8): p. e41883.
314. Brown DJ, Milroy R, Preston T, McMillan DC., *The relationship between an inflammation-based prognostic score (Glasgow Prognostic Score) and changes in serum biochemical variables in patients with advanced lung and gastrointestinal cancer.* J Clin Pathol., 2007. **60**(6): p. 705-8.
315. Kantola T, Klintrup K, Väyrynen JP, Vornanen J, Bloigu R, Karhu T, Herzig K-H, Näpänkangas J, Mäkelä J, Karttunen TJ, Tuomisto A, Mäkinen MJ, *Reply: Comment on 'Stage-dependent alterations of the serum cytokine pattern in colorectal carcinoma'.* British Journal of Cancer, 2013. **108**: p. 1917-1918.

316. Kantola T, Klintrup K, Väyrynen JP, Vornanen J, Bloigu R, Karhu T, Herzig KH, Näpänkangas J, Mäkelä J, Karttunen TJ, Tuomisto A, Mäkinen MJ., *Stage-dependent alterations of the serum cytokine pattern in colorectal carcinoma.* Br J Cancer., 2012. **107**(10): p. 1729-36.
317. Temel JS, Greer JA, Muzikansky A, Gallagher ER, Admane S, Jackson VA, Dahlin CM, Blinderman CD, Jacobsen J, Pirl WF, Billings JA, Lynch TJ., *Early palliative care for patients with metastatic non-small-cell lung cancer.* N Engl J Med., 2010. **363**(8): p. 733-42.
318. American Joint Committee on Cancer (AJCC), *Colon and Rectum Cancer Staging.* AJCC Cancer Staging Manual., 2010. **7th Edition**.
319. Park JJ, Jang H.-J, Oh I-Y, Yoon C-H, Suh J-W, Cho Y-S, Youn T-J, Cho G-Y, Chae I-H, Choi D-J, *Prognostic value of neutrophil to lymphocyte ratio in patients presenting with st-elevation myocardial infarction undergoing primary percutaneous coronary intervention. .* Am J Cardiol., 2013. **111**: p. 636-642.
320. Taylor CR, Levenson RM, *Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II.* Histopathology., 2006. **49**(4): p. 411-24.
321. Gokhale S, Rosen D, Sneige N, Diaz LK, Resetkova E, Sahin A, Liu J, Albarracin CT., *Assessment of two automated imaging systems in evaluating estrogen receptor status in breast carcinoma.* Appl Immunohistochem Mol Morphol., 2007. **15**(4): p. 451-5.
322. Allard MA, Bachet J, Beauchet A, Julie C, Malafosse R, Penna C, Nordlinger B, Emile JF., *Linear quantification of lymphoid infiltration of the tumor margin: a reproducible method, developed with colorectal cancer tissues, for assessing a highly variable prognostic factor.* Diagn Pathol., 2012. **7**(156).
323. Väyrynen JP, Vornanen JO, Sajanti S, Böhm JP, Tuomisto A, Mäkinen MJ., *An improved image analysis method for cell counting lends credibility to the prognostic significance of T cells in colorectal cancer.* Virchows Arch., 2012. **460**(5): p. 455-65.
324. Rizzardi AE, Johnson AT, Vogel RI, Pambuccian SE, Henriksen J, Skubitz AP, Metzger GJ, Schmechel SC., *Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring.* Diagn Pathol., 2012. **7**(42).
325. Halama N, Zoernig I, Spille A, Westphal K, Schirmacher P, Jaeger D, Grabe N., *Estimation of immune cell densities in immune cell conglomerates: an approach for high-throughput quantification.* PLoS One., 2009. **4**(11): p. e7847.
326. Galon J, Pages F, Marincola FM, Thurin M, Trinchieri G, Fox BA, Gajewski TF, Ascierto PA., *The immune score as a new possible approach for the classification of cancer.* J Transl Med., 2012. **10**(1).
327. Poon E, Harris A, Ashcroft M., *Targeting the hypoxia-inducible factor (HIF) pathway in cancer.* Expert Rev Mol Med., 2009. **11**(e26).

328. Lang SA, Moser C, Gaumann A, Klein D, Glockzin G, Popp FC, Dahlke MH, Piso P, Schlitt HJ, Geissler EK, Stoeltzing O., *Targeting heat shock protein 90 in pancreatic cancer impairs insulin-like growth factor-I receptor signaling, disrupts an interleukin-6/signal-transducer and activator of transcription 3/hypoxia-inducible factor-1alpha autocrine loop, and reduces orthotopic tumor growth.* Clin Cancer Res., 2007. **13**(21): p. 6459-68.
329. Bromberg J, *Stat proteins and oncogenesis.* J Clin Invest., 2002. **109**(9): p. 1139-42.
330. Mantovani A, Allavena P, Sica A, Balkwill F., *Cancer-related inflammation.* Nature, 2008. **454**(7203): p. 436-44.
331. Grivennikov SI, Karin M, *Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer.* Cytokine Growth Factor Rev. 2010 Feb;21(1):11-9.
332. Li N, Grivennikov SL, Karin M., *The unholy trinity: inflammation, cytokines, and STAT3 shape the cancer microenvironment.* Cancer Cell., 2011. **19**(4): p. 429-31.
333. Taga T, Kishimoto T, *Gp130 and the interleukin-6 family of cytokines.* Annu Rev Immunol., 1997. **15**: p. 797-819.
334. Lauta VM, *A review of the cytokine network in multiple myeloma: diagnostic, prognostic, and therapeutic implications.* Cancer, 2003. **97**(10): p. 2440-52.
335. Hong DS, Angelo L, Kurzrock R., *Interleukin-6 and its receptor in cancer: implications for translational therapeutics.* Cancer., 2007. **110**(9): p. 1911-28.
336. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M., *Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production.* Science. 2007 Jul 6;317(5834):121-4., 2007. **317**(5834): p. 121-4.
337. Richards CH, Flegg KM, Roxburgh CS, Going JJ, Mohammed Z, Horgan PG, McMillan DC., *The relationships between cellular components of the peritumoural inflammatory response, clinicopathological characteristics and survival in patients with primary operable colorectal cancer.* Br J Cancer. 2012. **106**(12): p. 2010-5.
338. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A., *Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes.* Trends Immunol., 2002. **23**(11): p. 549-55.
339. Ben-Baruch A, *Inflammation-associated immune suppression in cancer: the roles played by cytokines, chemokines and additional mediators.* Semin Cancer Biol. 2006. **16**(1): p. 38-52.
340. Pagès F, Galon J, Fridman WH., *The essential role of the in situ immune reaction in human colorectal cancer.* J Leukoc Biol., 2008. **84**(4): p. 981-7.
341. Dolcetti R, Viel A, Doglioni C, Russo A, Guidoboni M, Capozzi E, Vecchiato N, Macrì E, Fornasarig M, Boiocchi M., *High prevalence of activated intraepithelial cytotoxic T lymphocytes and increased neoplastic cell apoptosis in colorectal carcinomas with microsatellite instability.* Am J Pathol., 1999. **154**(6): p. 1805-13.

342. Phillips SM, Banerjea A, Feakins R, Li SR, Bustin SA, Dorudi S., *Tumour-infiltrating lymphocytes in colorectal cancer with microsatellite instability are activated and cytotoxic.* Br J Surg., 2004. **91**(4): p. 469-75.
343. Kumar S, Chang EY, Frankhouse J, Dorsey PB, Lee RG, Johnson N., *Combination of microsatellite instability and lymphocytic infiltrate as a prognostic indicator for adjuvant therapy in colon cancer.* Arch Surg., 2009. **144**(9): p. 835-40.
344. Saurer L, Mueller C, *T cell-mediated immunoregulation in the gastrointestinal tract.* Allergy., 2009. **64**(4): p. 505-19.
345. Hurst SM, Wilkinson TS, McLoughlin RM, Jones S, Horiuchi S, Yamamoto N, Rose-John S, Fuller GM, Topley N, Jones SA., *Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation.* Immunity., 2001. **14**(6): p. 705-14.
346. Naugler WE, Karin M, *The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer.* Trends Mol Med., 2008. **14**(3): p. 109-19.
347. Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, Faggioni R, Luini W, van Hinsbergh V, Sozzani S, Bussolino F, Poli V, Ciliberto G, Mantovani A., *Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment.* Immunity., 1997. **6**(3): p. 315-25.
348. Fielding CA, McLoughlin RM, McLeod L, Colmont CS, Najdovska M, Grail D, Ernst M, Jones SA, Topley N, Jenkins BJ., *IL-6 regulates neutrophil trafficking during acute inflammation via STAT3.* J Immunol., 2008. **181**(3): p. 2189-95.
349. Marigo I, Boslo E, Solito S, Mesa C, Fernandez A, Dolcetti L, Ugel S, Sonda N, Bicciato S, Falisi E, Calabrese F, Basso G, Zanovello P, Cozzi E, Mandruzzato S, Bronte V., *Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor.* Immunity., 2010. **32**(6): p. 790-802.
350. Peranzoni E, Zilio S, Marigo I, Dolcetti L, Zanovello P, Mandruzzato S, Bronte V., *Myeloid-derived suppressor cell heterogeneity and subset definition.* Curr Opin Immunol., 2010. **22**(2): p. 238-44.
351. Gabrilovich DI, Nagaraj S, *Myeloid-derived suppressor cells as regulators of the immune system.* Nat Rev Immunol., 2009. **9**(3): p. 162-74.
352. Jones SA, Richards PJ, Scheller J, Rose-John S., *IL-6 transsignaling: the in vivo consequences.* J Interferon Cytokine Res., 2005. **25**(5): p. 241-53.
353. Pasare C, Medhitov R, *Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells.* Science, 2003. **299**(5609): p. 1033-6.
354. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK., *Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells.* Nature, 2006. **441**(7090): p. 235-8.
355. Lin WW, Karin M, *A cytokine-mediated link between innate immunity, inflammation, and cancer.* J Clin Invest., 2007. **117**(5): p. 1175-83.

356. Miki S, Iwano M, Miki Y, Yamamoto M, Tang B, Yokokawa K, Sonoda T, Hirano T, Kishimoto T., *Interleukin-6 (IL-6) functions as an in vitro autocrine growth factor in renal cell carcinomas.* FEBS Lett., 1989. **250**(2): p. 607-10.
357. Siegall CB, Schwab G, Nordan RP, FitzGerald DJ, Pastan I., *Expression of the interleukin 6 receptor and interleukin 6 in prostate carcinoma cells.* Cancer Res., 1990. **50**(24): p. 7786-8.
358. Oka M, Iizuka N, Yamamoto K, Gondo T, Abe T, Hazama S, Akitomi Y, Koishihara Y, Ohsugi Y, Ooba Y, Ishihara T, Suzuki T., *The influence of interleukin-6 on the growth of human esophageal cancer cell lines.* J Interferon Cytokine Res., 1996. **16**(12): p. 1001-6.
359. Schneider MR, Hoeflich A, Fischer JR, Wolf E, Sordat B, Lahm H., *Interleukin-6 stimulates clonogenic growth of primary and metastatic human colon carcinoma cells.* Cancer Lett., 2000. **151**(1): p. 31-8.
360. Lahm H, Petral-Malec D, Yilmaz-Ceyhan A, Fischer JR, Lorenzoni M, Givel JC, Odartchenko N., *Growth stimulation of a human colorectal carcinoma cell line by interleukin-1 and -6 and antagonistic effects of transforming growth factor beta 1.* Eur J Cancer., 1992. **28A**(11): p. 1894-9.
361. Cardillo MR, Ippoliti F, *Interleukin-6, interleukin-10 and heat shock protein-90 expression in renal epithelial neoplasias and surrounding normal-appearing renal parenchyma. .* Int J Immunopathol Pharmacol., 2007. **20**(1): p. 37-46.
362. Bellone G, Smirne C, Mauri FA, Tonel E, Carbone A, Buffolino A, Dughera L, Robecchi A, Pirisi M, Emanuelli G., *Cytokine expression profile in human pancreatic carcinoma cells and in surgical specimens: implications for survival.* Cancer Immunol Immunother., 2006. **55**(6): p. 684-98.
363. Kinoshita T, Ito H, Miki C., *Serum interleukin-6 level reflects the tumor proliferative activity in patients with colorectal carcinoma.* Cancer, 1999. **85**(12): p. 2526-31.
364. Maihöfner C, Charalambous MP, Bhambra U, Lightfoot T, Geisslinger G, Gooderham NJ; Colorectal Cancer Group., *Expression of cyclooxygenase-2 parallels expression of interleukin-1beta, interleukin-6 and NF-kappaB in human colorectal cancer.* Carcinogenesis., 2003. **24**(4): p. 665-71.
365. Chung YC, Chaen YL, Hsu CP., *Clinical significance of tissue expression of interleukin-6 in colorectal carcinoma.* Anticancer Res., 2006. **26**(5b): p. 3905-11.
366. Sansone P, Bromberg J, *Targeting the interleukin-6/Jak/stat pathway in human malignancies.* J Clin Oncol., 2012. **30**(9): p. 1005-14.
367. The Interleukin-6 Receptor Mendelian Randomisation Analysis Consortium, *The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis.* Lancet, 2012. **379**(9822): p. 1214-24.
368. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL., *Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women.* J Clin Endocrinol Metab., 2003. **88**(1): p. 255-9.

369. Slattery ML, Wolff RK, Herrick JS, Caan BJ, Potter JD., *IL6 genotypes and colon and rectal cancer.* Cancer Causes Control., 2007. **18**(10): p. 1095-105.
370. Heinrich PC, Castell JV, Andus T., *Interleukin-6 and the acute phase response.* Biochem J, 1990. **265**(3): p. 621-36.
371. Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM., *The soluble interleukin 6 receptor: mechanisms of production and implications in disease.* FASEB J., 2001. **15**(1): p. 43-58.
372. Jones SA, Rose-John.S., *The role of soluble receptors in cytokine biology: the agonistic properties of the sIL-6R/IL-6 complex.* Biochim Biophys Acta, 2002. **1592**(3): p. 251-63.
373. Ravishankaran P, Karunanithi R, *Clinical significance of preoperative serum interleukin-6 and C-reactive protein level in breast cancer patients. .* World J Surg Oncol., 2011. **9**(1): p. 18.
374. Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, Murai M., *Serum interleukin 6 as a prognostic factor in patients with prostate cancer.* Clin Cancer Res., 2000. **6**(7): p. 2702-6.
375. Wise GJ, Marella V, Talluri G, Shirazian D., *Cytokine variations in patients with hormone treated prostate cancer.* J Urol. 2000. **164**(3 Pt 1): p. 722-5.
376. Yoshida N, Ikemoto S, Narita K, Sugimura K, Wada S, Yasumoto R, Kishimoto T, Nakatani T., *Interleukin-6, tumour necrosis factor alpha and interleukin-1beta in patients with renal cell carcinoma.* Br J Cancer. 2002 May 6;86(9):1396-400. 2002. **86**(9): p. 1396-400.
377. Ramsey S, Lamb G, Aitchison M, McMillan DC., *The longitudinal relationship between circulating concentrations of C-reactive protein, interleukin-6 and interleukin-10 in patients undergoing resection for renal cancer.* Br J Cancer., 2006. **95**(8): p. 1076-80.
378. Shiu YC, Lin JK, Huang CJ, Jiang JK, Wang LW, Huang HC, Yang SH., *Is C-reactive protein a prognostic factor of colorectal cancer?* Dis Colon Rectum., 2008. **51**(4): p. 443-9.
379. Fearon KC, McMillan DC, Preston T, Winstanley FP, Cruickshank AM, Shenkin A., *Elevated circulating interleukin-6 is associated with an acute-phase response but reduced fixed hepatic protein synthesis in patients with cancer.* Ann Surg., 1991. **213**(1): p. 26-31.
380. Belluco C, Nitti D, Frantz M, Toppan P, Basso D, Plebani M, Lise M, Jessup JM., *Interleukin-6 blood level is associated with circulating carcinoembryonic antigen and prognosis in patients with colorectal cancer.* Ann Surg Oncol., 2000. **7**(2): p. 133-8.
381. Galizia G, Orditura M, Romano C, Lieto E, Castellano P, Pelosio L, Imperatore V, Catalano G, Pignatelli C, De Vita F., *Prognostic significance of circulating IL-10 and IL-6 serum levels in colon cancer patients undergoing surgery.* Clin Immunol., 2002. **102**(2): p. 169-78.
382. Nakagoe T, Tsuji T, Sawai T, Tanaka K, Hidaka S, Shibasaki S, Nanashima A, Yamaguchi H, Yasutake T, Sugawara K, Inokuchi N, Kamihira S., *The relationship between circulating interleukin-6 and carcinoembryonic antigen in patients with colorectal cancer.* Anticancer Res., 2003. **23**(4): p. 3561-4.
383. Chung YC, Chang YF., *Serum interleukin-6 levels reflect the disease status of colorectal cancer.* J Surg Oncol 2003. **83**(4): p. 222-6.

384. Nikiteas NI, Tzanakis N, Gazouli M, Rallis G, Daniilidis K, Theodoropoulos G, Kostakis A, Peros G, *Serum IL-6, TNFalpha and CRP levels in Greek colorectal cancer patients: prognostic implications.* World J Gastroenterol., 2005. **11**(11): p. 1639-43.
385. Esfandi F, Mohammadzadeh Ghobadloo S, Basati G., *Interleukin-6 level in patients with colorectal cancer.* Cancer Lett., 2006. **244**(1): p. 76-8.
386. Yeh KY, Li YY, Hsieh LL, Lu CH, Chou WC, Liaw CC, Tang RP, Liao SK., *Analysis of the effect of serum interleukin-6 (IL-6) and soluble IL-6 receptor levels on survival of patients with colorectal cancer.* Jpn J Clin Oncol., 2010. **40**(6): p. 580-7.
387. Kaminska J, Nowacki MP, Kowalska M, Rysinska A, Chwalinski M, Fuksiewicz M, Michalski W, Chechlinska M., *Clinical significance of serum cytokine measurements in untreated colorectal cancer patients: soluble tumor necrosis factor receptor type I–an independent prognostic factor.* Tumour Biol., 2005. **26**(4): p. 186-94.
388. Ueda T, Shimada E, Urakawa T, *Serum levels of cytokines in patients with colorectal cancer: possible involvement of interleukin-6 and interleukin-8 in hematogenous metastasis.* J Gastroenterol., 1994. **29**(4): p. 423-9.
389. McKeown DJ, Brown DJ, Kelly A, Wallace AM, McMillan DC., *The relationship between circulating concentrations of C-reactive protein, inflammatory cytokines and cytokine receptors in patients with non-small-cell lung cancer.* Br J Cancer., 2004. **91**(12): p. 1993-5.
390. McArdle PA, McMillan DC, Sattar N, Wallace AM, Underwood MA., *The relationship between interleukin-6 and C-reactive protein in patients with benign and malignant prostate disease.* Br J Cancer., 2004. **91**(10): p. 1755-7.
391. Johansson JE, Andersson SO, Holmberg L, Bergström R., *Prognostic factors in progression-free survival and corrected survival in patients with advanced prostatic cancer: results from a randomized study comprising 150 patients treated with orchiectomy or estrogens.* J Urol., 1991. **146**(5): p. 1327-32.
392. Johansson JE, Sigurdsson T, Holmberg L, Bergström R., *Erythrocyte sedimentation rate as a tumor marker in human prostatic cancer. An analysis of prognostic factors in 300 population-based consecutive cases.* Cancer., 1992. **70**(6): p. 1556-63.
393. Twillie DA, Elsenberger M, Carducci MA, Hseih WS, Kim WY, Simons JW., *Interleukin-6: a candidate mediator of human prostate cancer morbidity.* Urology., 1995. **45**(3): p. 542-9.
394. Blay JY, Negrier S., Combaret V, Attali S, Goillot E, Merrouche Y, Mercatello A, Ravault A, Tourani JM, Moskovtchenko JF, et al., *Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma.* Cancer Res., 1992. **52**(12): p. 3317-22.
395. Takenawa J, Kaneko Y, Fukumoto M, Fukatsu A, Hirano T, Fukuyama H, Nakayama H, Fujita J, Yoshida O., *Enhanced expression of interleukin-6 in primary human renal cell carcinomas.* J Natl Cancer Inst., 1991. **83**(22): p. 1668-72.

396. Ljungberg B, Grankvist K, Rasmuson T., *Serum interleukin-6 in relation to acute-phase reactants and survival in patients with renal cell carcinoma.* Eur J Cancer., 1997. **33**(11): p. 1794-8.
397. Blay JY, Rossi JF, Wijdenes J, Menetrier-Caux C, Schemann S, Négrier S, Philip T, Favrot M., *Role of interleukin-6 in the paraneoplastic inflammatory syndrome associated with renal-cell carcinoma.* Int J Cancer., 1997. **72**(3): p. 424-30.
398. Martín F, Santolaria F, Batista N, Milena A, González-Reimers E, Brito MJ, Oramas J., *Cytokine levels (IL-6 and IFN-gamma), acute phase response and nutritional status as prognostic factors in lung cancer.* Cytokine., 1999. **11**(1): p. 80-6.
399. Miki C, Konishi N, Ojima E, Hatada T, Inoue Y, Kusunoki M., *C-reactive protein as a prognostic variable that reflects uncontrolled up-regulation of the IL-1-IL-6 network system in colorectal carcinoma.* Dig Dis Sci., 2004. **49**(6): p. 970-6.
400. Li YY, Hsieh LL, Tang RP, Liao SK, Yeh KY., *Interleukin-6 (IL-6) released by macrophages induces IL-6 secretion in the human colon cancer HT-29 cell line.* Hum Immunol., 2009. **70**(3): p. 151-8.
401. Rose-John S, Waetzig GH, Scheller J, Grötzinger J, Seegert D., *The IL-6/sIL-6R complex as a novel target for therapeutic approaches.* Expert Opin Ther Targets., 2007. **11**(5): p. 613-24.
402. Trikha M, Corringham R, Klein B, Rossi JF., *Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence.* Clin Cancer Res., 2003. **9**(13): p. 4653-65.
403. Guo Y, Xu F, Lu T, Duan Z, Zhang Z., *Interleukin-6 signaling pathway in targeted therapy for cancer.* Cancer Treat Rev., 2012. **38**(7): p. 904-10.
404. Fizazi K, Lipton A, Mariette X, Body JJ, Rahim Y, Gralow JR, Gao G, Wu L, Sohn W, Jun S., *Randomized phase II trial of denosumab in patients with bone metastases from prostate cancer, breast cancer, or other neoplasms after intravenous bisphosphonates.* J Clin Oncol., 2009. **27**(10): p. 1564-71.
405. Rossi JF, Negrier S, James ND, Kocak I, Hawkins R, Davis H, Prabhakar U, Qin X, Mulders P, Berns B., *A phase I/II study of siltuximab (CNTO 328), an anti-interleukin-6 monoclonal antibody, in metastatic renal cell cancer.* Br J Cancer., 2010. **103**(8): p. 1154-62.
406. Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, Thompson R, Schioppa T, Nemeth J, Vermeulen J, Singh N, Avril N, Cummings J, Rexhepaj E, Jirström K, Gallagher WM, Brennan DJ, McNeish IA, Balkwill FR., *Interleukin-6 as a therapeutic target in human ovarian cancer.* Clin Cancer Res. **17**(18): p. 6083-96.
407. Pollheimer MJ, Kornprat P, Lindtner RA, Harbaum L, Schlemmer A, Rehak P, Langner C., *Tumor necrosis is a new promising prognostic factor in colorectal cancer.* Hum Pathol., 2010. **41**(12): p. 1749-57.
408. Mocellin S, Marincola F, Young HA, *Interleukin-10 and the immune response against cancer: a counterpoint.* J Leukoc Biol., 2005. **78**: p. 1043-1051.
409. Hsu CP, Chung Y, *Influence of interleukin-6 on the invasiveness of human colorectal carcinoma.* Anticancer Res., 2006. **26**(6B): p. 4607-4614.

410. Lee JK, Bettencourt R, Brenner D, Le TA, Barrett-Connor E, Loomba R., *Association between serum interleukin-6 concentrations and mortality in older adults: the Rancho Bernardo study.* PLoS One., 2012. **7**(e34128).
411. Herbeuval J-P, Lelievre E, Lambert C, Dy M, Genin C (2004) J Immunol 172: 4630–4636., *Recruitment of STAT3 for production of IL-10 by colon carcinoma cells induced by macrophage-derived IL-6.* J Immunol., 2004. **172**(7): p. 4630-6.
412. De Vita F, Orditura M, Galizia G, Romano C, Lieto E, Iodice P, Tuccillo C, Catalano G., *Serum interleukin-10 is an independent prognostic factor in advanced solid tumors.* Oncol Rep., 2000. **7**(2): p. 357-61.
413. Strassmann G, Fong M, Kenney JS, Jacob CO., *Evidence for the involvement of interleukin 6 in experimental cancer cachexia.* J Clin Invest., 1992. **89**(5): p. 1681-4.
414. Argilés JM, Busquets S, López-Soriano FJ., *The pivotal role of cytokines in muscle wasting during cancer.* Int J Biochem Cell Biol., 2005. **37**(10): p. 2036-46.
415. Bonetto A, Aydogdu T, Jin X, Zhang Z, Zhan R, Puzis L, Koniaris LG, Zimmers TA., *JAK/STAT3 pathway inhibition blocks skeletal muscle wasting downstream of IL-6 and in experimental cancer cachexia.* Am J Physiol Endocrinol Metab., 2012. **303**(3).
416. Lieubeau B, Heymann MF, Henry F, Barbieux I, Meflah K, Grégoire M., *Immunomodulatory effects of tumor-associated fibroblasts in colorectal-tumor development.* Int J Cancer., 1999. **81**(4): p. 629-36.
417. Piancatelli D, Romano P, Sebastiani P, Adorno D, Casciani CU., *Local expression of cytokines in human colorectal carcinoma: evidence of specific interleukin-6 gene expression.* J Immunother., 1999. **22**(1): p. 25-32.
418. Lackner C, Jukic Z, Tsybrovskyy O, Jatzko G, Wette V, Hoefler G, Klimpfinger M, Denk H, Zatloukal K., *Prognostic relevance of tumour-associated macrophages and von Willebrand factor-positive microvessels in colorectal cancer.* Virchows Arch., 2004. **445**(2): p. 160-7.
419. Forssell J, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R., *High macrophage infiltration along the tumor front correlates with improved survival in colon cancer.* Clin Cancer Res., 2007. **13**(5): p. 1472-9.
420. Zhou Q, P.R., Wu XJ, Xia Q, Hou JH, Ding Y, Zhou QM, Zhang X, Pang ZZ, Wan DS, Zeng YX, Zhang XS., *The density of macrophages in the invasive front is inversely correlated to liver metastasis in colon cancer.* J Transl Med.2010. **8**(13).
421. Algars A, Irjala H, Vaittinen S, Huhtinen H, Sundström J, Salmi M, Ristamäki R, Jalkanen S., *Type and location of tumor-infiltrating macrophages and lymphatic vessels predict survival of colorectal cancer patients.* Int J Cancer., 2012. **131**(4): p. 864-73.
422. Edin S, Wikberg M, Dahlin AM, Rutegard J, Oberg A, Oldenborg PA, Palmqvist R, *he distribution of macrophages with a M1 or M2 phenotype in relation to prognosis and the molecular characteristics of colorectal cancer.* PLoS One, 2012. **7**(e7045.).

423. Kinouchi M, Miura K, Mizoi T, Ishida K, Fujibuchi W, Sasaki H, Ohnuma S, Saito K, Katayose Y, Naitoh T, Motoi F, Shiiba K, Egawa S, Shibata C, Unno M., *Infiltration of CD40-positive tumor-associated macrophages indicates a favorable prognosis in colorectal cancer patients.* Hepatogastroenterology., 2013. **60**(121): p. 83-8.
424. Sica A, Schioppa T, Mantovani A, Allavena P., *Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy.* Eur J Cancer., 2006. **42**(6): p. 717-27.
425. Solinas G, Germano G, Mantovani A, Allavena P., *Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation.* J Leukoc Biol., 2009. **86**(5): p. 1065-73.
426. Movahedi K, Laoui D, Gysemans C, Baeten M, Stangé G, Van den Bossche J, Mack M, Pipeleers D, In't Veld P, De Baetselier P, Van Ginderachter JA., *Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes.* Cancer Res., 2010. **70**(14): p. 5728-39.
427. Laoui D, Van Overmeire E, Movahedi K, Van den Bossche J, Schouppe E, Mommer C, Nikolaou A, Morias Y, De Baetselier P, Van Ginderachter JA., *Mononuclear phagocyte heterogeneity in cancer: different subsets and activation states reaching out at the tumor site.* Immunobiology., 2011. **216**(11): p. 1192-202.
428. Richards DM, Hettinger J, Feuerer M., *Monocytes and macrophages in cancer: development and functions.* Cancer Microenviron., 2013. **6**(2): p. 179-91.
429. Harris, *Hypoxia-a key regulatory factor in tumour growth.* Nat Rev Cancer., 2002. **2**(1): p. 38-47.
430. Mohammed ZM, Going JJ, Edwards J, Elsberger B, Doughty JC, McMillan DC., *The relationship between components of tumour inflammatory cell infiltrate and clinicopathological factors and survival in patients with primary operable invasive ductal breast cancer.* Br J Cancer., 2012. **107**(5): p. 864-73.
431. Scheller J, Ohnesorge N, Rose-John S., *Interleukin-6 trans-signalling in chronic inflammation and cancer.* Scand J Immunol., 2006. **63**(5): p. 321-9.
432. Komoda H, Tanaka Y, Honda M, Matsuo Y, Hazama K, Takao T., *Interleukin-6 levels in colorectal cancer tissues.* World J Surg., 1998. **22**(8): p. 895-8.
433. Kun-Yun Yeh, Ying-Ying L, Ling-Ling Hsieh, Chang-Hsien Lu, Wen-Chi Chou, Chuang-Chi Liaw, Rei-Ping Tang, Shuen-Kuei Liao., *Analysis of the Effect of Serum Interleukin-6 (IL-6) and Soluble IL-6 Receptor Levels on Survival of Patients with Colorectal Cancer.* Japanese Journal of Clinical Oncology, 2010. **40**(6): p. 580-587.
434. Roxburgh CS, McMillan DC, *Cancer and systemic inflammation: treat the tumour and treat the host.* Br J Cancer., 2014. **110**(6): p. 1409-12.
435. Park JH, McMillan DC, Powell AG, Richards CH, Horgan PG, Edwards J, Roxburgh CS., *Evaluation of a tumor microenvironment-based prognostic score in primary operable colorectal cancer.* Clin Cancer Res., 2015. **21**(4): p. 882-8.

436. Hurwitz HI, Uppal Nikhil, Wagner SA, Bendell JC, Beck JT, Wade SM 3rd, Nemunaitis JJ, Stella PJ, Pipas JM, Wainberg ZA, Manges R, Garrett WM, Hunter DS, Clark J, Leopold L, Sandor V, Levy RS., *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., 2015. **33**(34): p. 4039-47.

Appendix 1

'Measure stained cells algorithm' **- optimized algorithm parameters**	
Measurement Units 0=Âµm, 1=mm, 2=pixels	0
Tissue Threshold Segment Tissue from Background by Intensity	220
Nuclei Heterogeneity 0=Nuclei are similar, >=1, Nuclei increasingly diverse (darkest to lightest)	2
Strength Of Nuclear Counterstaining 0=Strong Nuclear Counterstaining, 2=Weak Nuclear Counterstaining	0
Nuclear Window Radius Size Values in units	37
Nuclear Area Low/High Threshold Eliminate nuclei with area outside this range (specified in units squared)	0-2235
Nuclei Per Window Low/High Threshold Eliminate nuclei with density outside this range	0-101
% Of Nuclear Area Per Window Low/High Threshold Eliminate nuclei with nuclear area density outside this range (specified in units squared)	0-100
Cell Area Low/High Threshold Eliminate cells with area outside this range (specified in units squared)	0-2005
Maximum Cell Radius Values in units	63
Nuclear Staining Intensity Cutoff Above this value pixels are identified as negative	163
% Of Stained Area In A Nucleus Cutoff Eliminate nuclei with a % below this value	51
Strong/Moderate/Weak Nuclear Staining Intensity Cutoff Identify nuclei having strong/moderate/weak staining intensity	0-248
Cytoplasmic Staining Intensity Cutoff Above this value pixels are identified as negative	220
% Of Cytoplasmic Stained Area In A Cell Cutoff Eliminate areas with a % below this value	75
Strong/Moderate/Weak Cytoplasmic Staining Intensity Cutoff Identify areas having strong/moderate/weak staining intensity	54-133
Membrane Staining Intensity Cutoff Above this value pixels are identified as negative	220
% Of Membrane Stained Area In A Cell Cutoff Eliminate areas with a % below this value	75
Strong/Moderate/Weak Membrane Staining Intensity Cutoff Identify areas having strong/median/weak staining intensity	160
Nuclear Staining Filter 0 = Include All Cells, 1 = Include only Positive Cells, 2 = Include only Negative Cells	1
Cytoplasmic Staining Filter 0 = Include All Cells, 1 = Include only Positive Cells, 2 = Include only Negative Cells	0
Membrane Staining Filter 0 = Include All Cells, 1 = Include only Positive Cells, 2 = Include only Negative Cells	0
Default Calibration	1
Nuclear Counterstain	Deconvolution - H&E
Nuclear Marker	Deconvolution

	– H&E