AN INVESTIGATION OF THE DETERMINANTS OF THE LOCAL AND SYSTEMIC INFLAMMATORY RESPONSES IN PATIENTS WITH COLORECTAL CANCER.

BY

Colin H. Richards

BSc MBChB MRCS

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ABSTRACT

Colorectal cancer is the second most common cause of cancer death in the Western world but the factors that determine disease progression remain poorly understood. At the outset of this thesis it was recognised that tumour growth and metastases were determined by complex interactions between tumour and host. It was evident that a systemic inflammatory response was associated with poor prognosis in colorectal cancer while a strong local immune cell response conferred a favourable outcome.

This thesis investigated this topic by examining the factors responsible for activating, maintaining and regulating these inflammatory responses and drew the following conclusions:

Chapter 3 concluded that abnormal patient physiology, in particular the presence of anaemia and cardiac disease, was strongly associated with a systemic inflammatory response in patients with colorectal cancer. Targeting specific physiological parameters may therefore be a novel way to improve a patients' inflammatory status. Chapter 5 used CT image analysis to confirm a strong relationship between systemic inflammation and reduced skeletal muscle mass in patients with colorectal cancer. This offered insight into the underlying basis of cancer-related weight loss and suggested attenuation of the host inflammatory response may be a therapeutic target in cancer cachexia. Chapter 6 built on these results with a detailed examination of the relative importance of pre-, intra- and post-operative factors in patients undergoing surgery for colorectal cancer. Rather than being the cause of disease recurrence, surgical complications appeared to be a consequence of pre-existing physiological disturbance and systemic inflammation, supporting a concept whereby pre-operative status is

of paramount importance to long-term cancer outcomes. Chapter 7 investigated possible links between the local and systemic inflammatory responses. The pathological feature of tumour necrosis was confirmed as both an independent prognostic indicator in colorectal cancer and the first documented link between local and systemic inflammation. A model was proposed whereby failure of local anti-tumour control leads to rapid growth, tissue hypoxia and cellular necrosis, triggering the host to initiate a systemic inflammatory response. The local inflammatory response in colorectal cancer was then considered. Chapter 8 confirmed that, while a strong local response was primarily the result of lymphocyte infiltration, the examination of individual cell types did not add prognostic value compared to an overall grade of peritumoural inflammation. Chapter 9 built on this knowledge to examine the clinical utility of the local inflammatory response in colorectal cancer. It was clear that the density of cellular infiltrate was more important than the type or location of individual immune cells. After comparing a number of methodologies, an overall grade of peritumoural inflammation, using the Klintrup-Makinen (K-M) criteria, was established as the preferred technique for assessing the local inflammatory response in colorectal cancer.

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DECLARATION

The work presented in this thesis was undertaken during a period of research between 2009 and 2011 in the University Departments of Surgery and Pathology at Glasgow Royal Infirmary. The work has been completed whilst working as a Specialty Registrar in General Surgery in the North of Scotland deanery between 2011 and 2013. The censor date for the literature search undertaken at the outset of this thesis was 1st September 2009. The word 'current' used throughout the thesis therefore refers to this date.

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

- All data collection, analysis, execution and manuscript preparation for work relating to this thesis.
- The maintenance of the colorectal cancer database at Glasgow Royal Infirmary (2009 – 2011)
- The retrieval of medical case records and the calculation of the POSSUM score in 250/320 patients (CHAPTER 3)
- The calculation of all mortality prediction scoring systems (CHAPTER 4)
- The development of a technique to analyse body composition using ImageJ software and the measurement of all fat parameters (CHAPTER 5)
- All data relating to pre-operative risk factors and complications (CHAPTER 6)
- The retrieval of pathological sections and scoring of tumour necrosis (CHAPTER 7)
- The development of a technique to analyse the cellular components of the peritumoural inflammatory response (CHAPTER 8)
- The immunohistochemical staining of CD8 and FOXP3 (CHAPTER 9)

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- 70/320 POSSUM scores were calculated by Fiona Leitch (CHAPTER 3)
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PUBLICATIONS

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

1. The clinical utility of the local inflammatory response in colorectal cancer
   Richards CH, Roxburgh CSD, Powel AG, Foulis AK, Horgan PG, McMillan DC
   Eur J Cancer. 2013 Oct 5. S0959-8049. Epub 2013 Sept 29

2. The relationships between cellular components of the peritumoural inflammatory response, clinicopathological characteristics and survival in patients with primary operable colorectal cancer.
   Richards CH, Flegg KM, Roxburgh CS, Going JJ, Mohammed Z, Horgan PG, McMillan DC.
   Br J Cancer. 2012 Jun 5;106(12):2010-5. Epub 2012 May 17.

3. The relationships between body composition and the systemic inflammatory response in patients with primary operable colorectal cancer.
   Richards CH, Roxburgh CS, MacMillan MT, Isswiasi S, Robertson EG, Guthrie GK, Horgan PG, McMillan DC.
   PLoS One. 2012;7(8):e41883. Epub 2012 Aug 3.

4. The prognostic value of tumour necrosis and host inflammatory responses in colorectal cancer.
   Richards CH, Roxburgh CSD, Anderson JH, McKee RF, Horgan PG, McMillan DC.
   Br J Surg. 2012 Feb;99(2):287-94.

5. The prognostic value of histological tumour necrosis in solid organ malignant disease: a systematic review.
   Richards CH, Mohammed Z, Qayyum T, Horgan PG, McMillan DC.
   Future Oncol. 2011 Oct;7(10):1223-35.

6. The impact of perioperative risk, tumor pathology and surgical complications on disease recurrence following potentially curative resection of colorectal cancer.
Richards CH, Platt JJ, Anderson JH, McKee RF, Horgan PG, McMillan DC.
Ann Surg. 2011 Jul;254(1):83-9.

7. The Revised ACPGBI Model is a Simple and Accurate Predictor of Operative Mortality After Potentially Curative Resection of Colorectal Cancer.
Richards CH, Leitch EF, Anderson JH, McKee RF, McMillan DC, Horgan PG.
Ann Surg Oncol. 2011 Dec;18(13)3680-5.

8. The relationship between patient physiology, the systemic inflammatory response and survival in patients undergoing curative resection of colorectal cancer.
Richards CH, Leitch EF, Horgan PG, Anderson JH, McKee RF, McMillan DC.
Br J Cancer. 2010 Oct 26;103(9):1356-61.

9. A systematic review of POSSUM and its related models as predictors of post-operative mortality and morbidity in patients undergoing surgery for colorectal cancer.
Richards CH, Leitch FE, Horgan PG, McMillan DC.
J Gastrointest Surg. 2010 Oct;14(10):1511-20.

PRESENTATIONS

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

1. The clinical utility of the local inflammatory response in colorectal cancer.
   Digestive Diseases Week, Florida, USA, 2013 (poster of distinction)

2. The prognostic significance and clinicopathological associations of inflammatory cell infiltration at the invasive margin of colorectal tumours.
   Association of Surgeons of Great Britain and Ireland, Liverpool, 2012 (Moynihan prize session)

3. The relationships between body composition and the systemic inflammatory response in patients with primary operable colorectal cancer.
   Association of Surgeons of Great Britain and Ireland, Liverpool, 2012 (oral)

4. The prognostic influence and interrelationships of $CD8^+$ T-cell infiltration in malignant colorectal tumors.
   American Society of Clinical Oncology, Chicago, 2011 (poster)

5. Predicting post-operative mortality in colorectal cancer surgery: A systematic review of the accuracy of POSSUM, P-POSSUM and CR-POSSUM
   Digestive Diseases Week, New Orleans, USA, 2010 (oral)

6. The relationships between patient physiology, the systemic inflammatory response and survival in patients undergoing curative resection of colorectal cancer.
   Digestive Diseases Week, New Orleans, USA, 2010 (oral)

7. Tumour necrosis represents a novel link between the systemic and local inflammatory response to colorectal cancer.
   Association of Surgeons of Great Britain and Ireland, Bournemouth, 2011 (poster)

8. The revised ACPGBI model is an accurate predictor of operative mortality following colorectal cancer surgery with curative intent.
   Association of Coloproctology of Great Britain and Ireland, Birmingham, 2011 (poster)

9. The impact of postoperative complications on short and long term survival following colorectal cancer surgery.
   West of Scotland Surgical Association, Glasgow 2011 (oral)

DEDICATION

To my wife Helen, who has provided me with never ending help, support and encouragement during this period of research.

1.0 INTRODUCTION

1.1 EPIDEMIOLOGY OF COLORECTAL CANCER

1.1.1 Disease burden worldwide

Colorectal cancer is the third most common cancer in the world with a prevalence of over 3 million people in 2006 (Kamangar, Dores et al. 2006). Worldwide, the annual incidence is estimated at over 1.2 million with the highest rates seen in Australasia, Western Europe and North America. The African nations have the lowest incidence although countries with a rapid 'westernisation' of diet and lifestyle, such as Japan, have seen a substantial increase in the number of new cases of colorectal cancer. Worldwide, the disease accounts for more than 600,000 deaths each year, making it the fourth commonest cause of cancer death (Parkin, Pisani et al. 1999).

1.1.2 Disease burden in the United Kingdom

In the United Kingdom (UK), colorectal cancer is the third most common cancer in men and the second most common cancer in women. The incidence rates in the UK are estimated to be the 14th (males) and 12th (females) highest in the European Union. Each year over 40,000 new cases of colorectal cancer are diagnosed in the UK and the disease accounts for over 16,000 deaths (CRUK). A north-south divide in the incidence of colorectal cancer currently exists, especially for men, with higher rates seen in Scotland and the North of England compared to London and the South East. Overall, the incidence of the disease has increased since the 1970's. For men, age-standardised incidence rates increased by 27% between 1975-1977 and 2007-2009 but for women the rise has been much smaller at around 8% (CRUK).

Despite the increased number of new cases diagnosed each year, mortality from colorectal cancer has fallen across all age groups since the 1970's. Mortality rates decreased steadily from the early 1970's to the early 1990's and have decreased more rapidly since then. The largest improvements have been seen in younger patients with mortality rates dropping by over 50% in 45-59 year olds between 1971 and 2008.

1.2. COLORECTAL CARCINOGENESIS

Colorectal cancer is a heterogenous disease which can arise from different pathological precursors. There are at least three major molecular pathways through which colorectal mucosa can undergo malignant transformation; (1) the chromosomal instability (CIN) pathway, (2) the microsatellite instability (MSI) pathway and (3) the CpG island methylator phenotype (CIMP+) pathway. Each of these pathways are characterised by distinct molecular signatures and involve different mechanisms of carcinogenesis (Worthley, Whitehall et al. 2007).

1.2.1 Chromosomal instability

In the late 1980's Vogelstein and colleagues proposed a model for colorectal carcinogenesis whereby a series of genetic alterations leads to the transformation of benign colorectal adenoma to adenocarcinoma (the adenoma-carcinoma sequence) (Vogelstein, Fearon et al. 1988). This transition from normal epithelium to malignant tumour is associated with a number of specific molecular events including alterations in chromosome number (aneuploidy), activation of oncogenes and mutation of p53. Among the earliest events in this pathway are deletions of the adenomatous polyposis coli (APC) gene. This initial defect occurs in over 60% of colorectal neoplasms although the order and timing of subsequent molecular events is inconsistent. Mutations in oncogenes such as K-ras result in further growth and progression of the adenoma with the final transition to adenocarcinoma mediated through the inactivation or mutation of p53. It is now accepted that this original model may be too simplistic and recent evidence suggests carcinogenesis to be an extremely complex process involving cumulative mutations in a growing number of oncogenes and tumour suppressor genes (Staton, Chetwood et al. 2007). It is hypothesised that the character of

individual mutations may influence the type of pathological change and rate of tumour growth seen in sporadic colorectal cancers. Figure 1.1 summarises the order of the adenoma to carcinoma sequence along with the key genetic mutations.

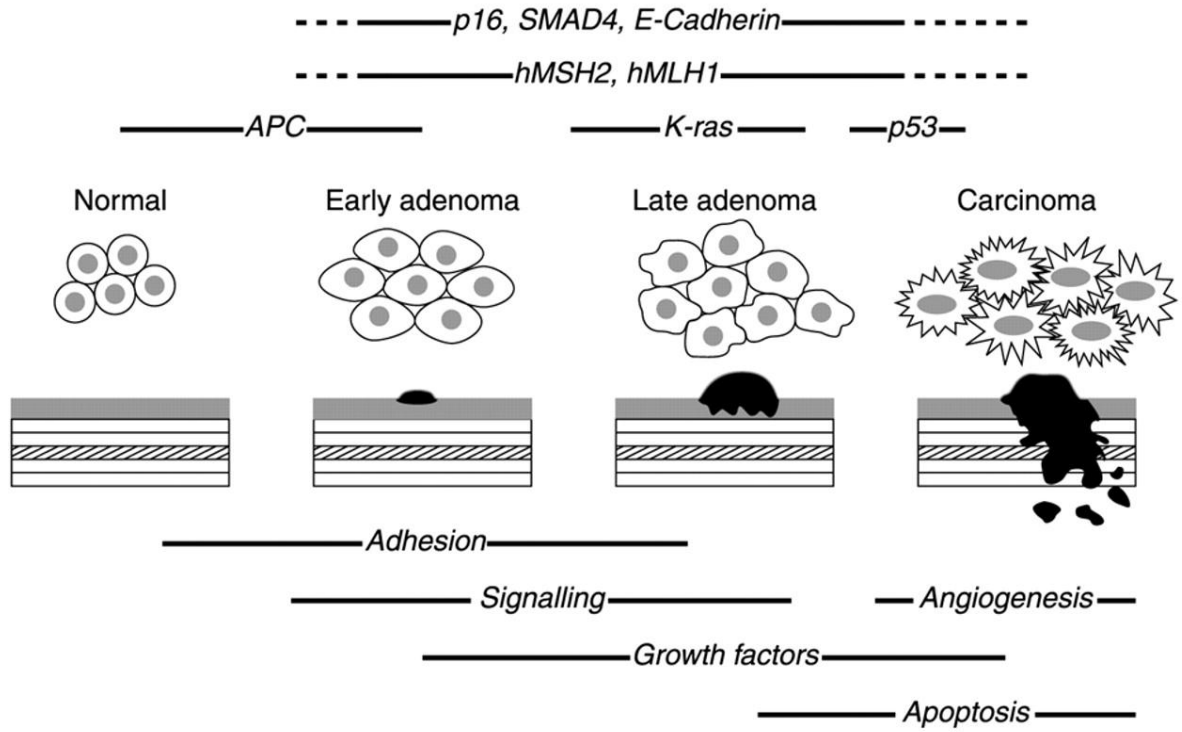


Figure 1.1. The adenoma-carcinoma sequence in sporadic colorectal cancer. The order in which key genes may be affected are shown above the stage during which they are thought to occur. Functional pathways affected are at the bottom of the diagram. Adapted from Fearnhead et al.

1.2.2 Microsatellite instability

Microsatellites are repetitive sequences of DNA distributed throughout the human genome. Microsatellite instability (MSI) is a form of genomic instability associated with defective DNA mismatch repairs (MMR) and results from failings within the MMR system to repair errors that occur during DNA replication. This results in the accumulations of base pair mismatches and alterations in the length of the microsatellite sequences, ultimately leading to protein truncations. These genetic defects were first discovered in the 1990's and are broadly linked to the pathogenesis of cancer. MSI is observed in the majority of Hereditary Non-polyposis Colon Cancer (HNPCC) tumours (discussed below) but is also found in a smaller proportion of sporadic colorectal cancers (Aaltonen, Peltomaki et al. 1993; Ionov, Peinado et al. 1993; Thibodeau, French et al. 1998).

Since its discovery, numerous studies began to describe the presence of MSI in different tumour types. However, initial variability in study methodology saw different panels of molecular markers used to define the phenomenon until an international consensus on the definition of MSI was finally reached in 1998 (Boland, Thibodeau et al. 1998). To grade microsatellite instability, five standard markers known collectively known as the Bethesda panel are now assessed (D2S123, D5S346, D17S250, BAT-25 and BAT-26). Tumours are described as having high frequency microsatellite instability (MSI-H) if two or more loci are unstable and low frequency microsatellite instability (MSI-L) if one is unstable. Overall, it is estimated that MSI is present in approximately 15% of colorectal tumours. Tumours without evidence of MMR defects can be referred to as microsatellite stable (MSS) tumours (Poynter, Siegmund et al. 2008).

Colorectal cancers associated with MSI tend to show a stable karyotype without the chromosomal instability seen in sporadic tumours. More than 30 different genetic mutations have been identified in MMR deficient tumours with the proteins they encode involved in a variety of cellular functions; DNA repair (MRE11A), growth factor receptors (IGF receptor II) and pro-apoptotic factors (BAX). Other notable differences in MSI tumours are a lower prevalence of KRAS mutations and more mutations in the phophatidylinositol 3-kinase (PI3K) pathways, a known driver of tumourigenesis (Vivanco and Sawyers 2002).

From a clinical perspective MSI tumours tend to be right-sided and are often diagnosed at an earlier stage. In addition, the tumours are often poorly differentiated, have a strong infiltration of inflammatory cells and tend to have less tumour necrosis (Greenson, Bonner et al. 2003). The clinical implications of determining the MSI status of colorectal cancer are described in more detail below.

1.2.3 Hypermethylation

In addition to chromosomal and microsatellite instability, a third carcinogenic pathway, known as hypermethylation, has recently been described. The precursor lesions for the development of carcinomas via this route are not adenomas but serrated polyps and include hyperplastic aberrant crypt foci, serrated adenomas and hyperplastic polyps. These tumours are thought to develop along a pathway where hypermethylation rather than genetic mutation is responsible for the inactivation of tumour suppressor gene function (Esteller, Fraga et al. 2002; Ferracin, Gafa et al. 2008). A subset of colorectal tumours have been shown to exhibit such hypermethylation at specific gene reporters and are referred to as CpG island methylator phenotype (CIMP+) (Issa 2004). Akin to cancers resulting from microsatellite instability, CIMP+ tumours also display certain clinicopathological features, including proximal tumour

location, poor differentiation and a high frequency of BRAF mutations (van Rijnsoever, Grieu et al. 2002; Weisenberger, Siegmund et al. 2006). Furthermore, CIMP+ colorectal cancers often lack the alteration of p53 or APC seen in tumours resulting from chromosomal instability. Work is ongoing in an attempt to standardise the classification of CIMP+ colorectal cancer.

1.3 AETIOLOGY OF COLORCETAL CANCER

The aetiology of colorectal cancer is still poorly understood. The majority of colorectal tumours (>90%) are termed 'sporadic' and are thought to result from complex interactions between host and environmental factors. In a small number of cases, the pathogenesis of colorectal cancer can be attributed to specific aetiological factors such as inflammatory bowel disease or inherited genetic mutations (Figure 1.2) (Ponz de Leon, Benatti et al. 2004). The aetiology of sporadic and non-sporadic colorectal cancer is described below.

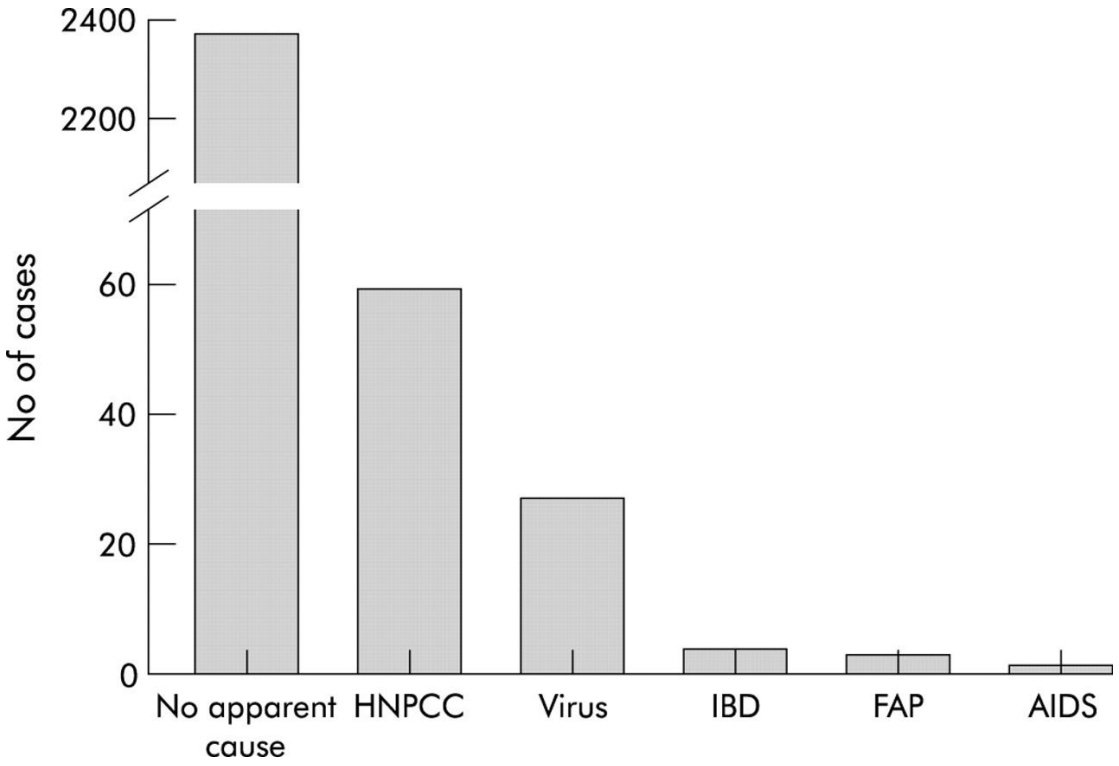


Figure 1.2. Frequency of the main known causes of colorectal cancer. Cancers with 'no apparent cause' are often referred to as sporadic. Adapted from Ponz de Leon 2004.
HNPCC: hereditary non-polyposis colorectal cancer, IBD: inflammatory bowel disease, FAP: familial adenomatous polyposis, AIDS: acquired immunodeficiency syndrome

1.3.1 SPORADIC COLORECTAL CANCER

A large number of factors have been implicated in the development of sporadic colorectal cancer but few have been confirmed as causative (Table 1.1). Epidemiological studies have consistently reported associations between colorectal cancer and certain ill-defined risk factors, such as diet and Western lifestyle. Over the years the role of these host and environmental factors has been extensively investigated but no clear conclusions have yet been drawn. Many studies have relied on epidemiological data and there are often conflicting reports. The factors described below are those that have been commonly associated with an increased risk of colorectal cancer.

1.3.1.1 Age

Age is the single biggest risk factor for sporadic colorectal cancer. The incidence of the disease increases with age and over 85% of cases are diagnosed in patients aged 60 years or older (CRUK). The reasons for this association with age are likely to be a result of increased exposure to the environmental risk factors described below, in particular an increased time for chromosomal mutations to develop. There is now evidence that age-related degradation of telomeres, molecular 'caps' which act to protect chomosomes' structural integrity during cell division, may be one mechanism through which cancer risk increases with time (Hoeijmakers 2007). However, shortening of telomeres does not occur at a uniform rate, leading some to suggest that biological age is more important to the development of colorectal cancer than chronological age (Mayor 2009). It is of interest that the relationship between CRC and age appears to be restricted to sporadic cancers. The incidence of HNPCC tumours, for example, occurs during the 5^{th} decade of life before reducing again (Umar, Risinger et al. 2004).

Table 1.1. Environmental and host factors associated with the development of sporadic colorectal cancer.

Environmental factors	
Western lifestyle	- diet
	- smoking
	- alcohol
	- sedentary lifestyle
Diet	- red meat
	- fibre
	- carotenoids, vitamins and anti-oxidants
Drugs	- Aspirin (reduced risk)
	- NSAID's (reduced risk)
	- Statins (reduced risk)
	- HRT (reduced risk)
Host factors	
Host physiology	- age
	- comorbidity
	- cardiovascular disease
	- obesity and body habitus
Inflammatory response	- systemic
	- local

NSAID: non-steroidal anti-inflammatory drugs
HRT: hormone replacement therapy

1.3.1.2 Western Lifestyle

The highest rates of colorectal cancer are found in Western countries and up to 15-fold differences in age-standardised incidence rates are observed between different geographical locations across the world (Muir and Parkin 1985). Studies on migrant populations have demonstrated that the incidence rates of the host country are adopted within a generation (Haenszel and Kurihara 1968; Potter, Slattery et al. 1993). This has led to a widely held belief that a Western lifestyle is responsible for the development of CRC in many cases. Many

studies have attempted to focus on specific components of a Western lifestyle although multiple interactions between individual factors mean an integrated picture is still lacking (Slattery, Edwards et al. 1999).

1.3.1.3 Dietary fibre

The role of diet as a risk factor for colorectal cancer has been extensively investigated over the years. As early as the 1970's a link with dietary fibre was suggested after incidence rates of CRC were noted to be significantly lower in populations with a high fibre intake (Burkitt 1971). The suggested mechanism was one by which high dietary fibre would mean ingested food moved more rapidly through the gastrointestinal tract; giving less time for carcinogens to be in contact with the mucosa thereby reducing the likelihood of carcinogenesis. This hypothesis has since been tested by several large prospective studies with somewhat conflicting results. A study by Fuchs and colleagues examining the diets of almost 90,000 women over a 16 year period found no association between dietary fibre intake and the risk of colorectal cancer (Fuchs, Giovannucci et al. 1999). However, these results were challenged by the European Prospective Investigation into Cancer and Nutrition (EPIC) study, an investigation of over 500,000 individuals, which reported that doubling the intake of dietary fibre could reduce the risk of colorectal cancer by 40% (Bingham, Day et al. 2003). Finally, a pooled analysis of over 13 prospective studies (>700,000 men and women) concluded that, after accounting for other dietary risk factors, high fibre intake was not associated with a reduced risk of colorectal cancer (Park, Hunter et al. 2005). Currently, it is therefore unclear whether dietary fibre is an independent risk factor for the development of colorectal cancer.

1.3.1.4 Red meat consumption

Over the past 30 years epidemiological studies have consistently observed that countries with a high intake of red meat and animal fat have a higher incidence of colorectal cancer (Armstrong and Doll 1975; Graham and Mettlin 1979). These early studies generated the hypothesis that meat consumption was associated with the development of gastrointestinal malignancy in general and colorectal cancer in particular. However, despite over 50 studies investigating this hypothesis, the relationships between red meat consumption and colorectal cancer are equivocal. In the majority of studies the impact of meat consumption is relatively weak (RR<1.5) and there is no clear dose-response relationship (Sandhu, White et al. 2001). Despite this, the World Cancer Research Fund (WCRF) in conjunction with the American Institute for Cancer Research (AICR) released a consensus statement in 2007 describing red meat as a convincing cause of colorectal cancer and suggested individuals should limit their intake to 500g per week (AICR 2007). In contrast to the wealth of epidemiological evidence, studies evaluating the mechanisms by which red meat may be linked to tumour development have been sparse. Some studies have suggested that cooking meat at high temperature may release carcinogenic hydrocarbons while others have postulated that haem iron (Cross, Pollock et al. 2003) or the N-nitroso compounds found in processed meats are to blame (Santarelli, Pierre et al. 2008). Overall, current evidence does not support a clear association between red meat intake and the development of colorectal cancer.

1.3.1.5 Exercise and sedentary lifestyle

The role of exercise in reducing the risk of colorectal cancer is now well established (AICR 2007). The majority of studies have reported individuals with high levels of daily activity to have a significantly lower risk than those with sedentary lifestyles (White, Jacobs et al. 1996; Samad, Taylor et al. 2005). In 2009, a meta-analysis concluded that regular exercise reduced

the risk of colon cancer by almost 25% in both men and women (Wolin, Lee et al. 2007). The optimum type, intensity and duration of exercise remains unclear but it is apparent that any regular exercise bestows significant benefits in terms of cancer reduction. Furthermore, this effect appears to be independent of potentially confounding variables such as cardiovascular health, diet and obesity (Colditz, Cannuscio et al. 1997). Suggested mechanisms to explain the effect of exercise include lowering levels of prostaglandins, decreasing gut transit time and improving immune function (Samad, Taylor et al. 2005).

1.3.1.6 Coronary artery disease

Coronary artery disease has been shown to have similar risk factors as colorectal cancer (Neugut, Jacobson et al. 1995). It has been demonstrated that obesity, sedentary lifestyle (Giovannucci, Ascherio et al. 1995), diabetes (Larsson, Giovannucci et al. 2005), a high fat diet (Stemmermann, Nomura et al. 1984) and smoking (Le Marchand, Wilkens et al. 1997) are associated with both disease processes, leading some to propose that cardiovascular disease itself may be implicated in the aetiology of CRC. This is further supported by autopsy studies which have reported that atherosclerosis and colorectal adenomatous polyps tend to occur in the same individuals (Correa, Strong et al. 1982; Stemmermann, Heilbrun et al. 1986). Kune and co-workers, however, found no association between coronary artery disease and the presence of colorectal cancer in a case matched study of over 1400 individuals (Kune, Kune et al. 1988).

1.3.1.7 Obesity and insulin resistance

Obesity is now well established as a risk factor for colorectal cancer. In 2007, a meta-analysis from Moghaddam and colleagues estimated that individuals with a Body Mass Index (BMI) $\geq 30 kg/m^2$ had a 20% greater risk of developing CRC compared to normal weight controls

(Moghaddam, Woodward et al. 2007). There appeared to be a dose-response relationship with central obesity in particular, and every 2cm increment in waist circumference increased the risk of CRC by 4%. This association with central obesity, a surrogate marker for levels of metabolically-active visceral fat, may give insight into the mechanisms through which excess weight can increase cancer risk. There are now a growing number of reports that suggest insulin resistance plays a key role in this association (McKeown-Eyssen 1994; Giovannucci 1995). Not only have studies shown close relationships between glucose levels, diabetes and malignancy (Larsson, Giovannucci et al. 2005), experimental work has also demonstrated that insulin and insulin-like growth factor (IGF-1) can stimulate the proliferation of both normal colonic mucosal cells and carcinoma cell lines (La Vecchia, Negri et al. 1997; Limburg, Anderson et al. 2005).

1.3.1.8 Smoking

Cigarette smoking is associated with an increased risk of a number of malignancies including lung, stomach, kidney, bladder and pancreas. There is now consistent evidence that cigarette smoking also causes colorectal cancer. Almost all studies that have investigated the impact of smoking on precursor lesions have concluded that cigarette smoking increases the likelihood of colorectal adenoma formation albeit only after decades of exposure (Giovannucci and Martinez 1996). Dose-response relationships with CRC have also been reported when studies have assessed smoking duration, cigarette pack years and smoking intensity (Wu, Paganini-Hill et al. 1987; Le Marchand, Wilkens et al. 1997; Slattery, Potter et al. 1997). The relative importance of intensity and duration of tobacco use are unresolved but it is clear that long term heavy smokers are at highest risk. Indeed, some reports estimate that up to 20% of colorectal cancers in the United States are directly attributable to cigarette smoking (Heineman, Zahm et al. 1994).

1.3.1.9 Alcohol

Alcohol has been implicated in the aetiology of colorectal cancer. The EPIC trail investigated the impact of alcohol consumption on a cohort of almost half a million subjects over a 6 year period and concluded that both lifetime and baseline alcohol intake increased the risk of colon and rectal cancer (Ferrari, Jenab et al. 2007). Furthermore, a pooled analysis of fourteen separate studies suggested that a high alcohol intake, defined as more than 100g/week, was associated with a 19% increase in the risk of colon cancer in men and women (Moskal, Norat et al. 2007). The mechanisms through which alcohol leads to tumour development have yet to be elucidated but may include a direct carcinogenic effect of acetaldehyde, the primary metabolite of alcohol and a compound known to alter DNA.

1.3.1.10 Hormone replacement therapy

In 1999 a large meta-analysis reported that the risk of colorectal cancer was significantly lower in postmenopausal women who had taken Hormone Replacement Therapy (HRT) compared to those who had never received such treatment (Grodstein, Newcomb et al. 1999). In addition to this epidemiological evidence, there are several biological reasons why endogenous hormones may be protective. Oestrogens decrease the production of bile acids which have been implicated in initiating and promoting malignant change of colonic epithelium (McMichael and Potter 1980). The presence of oestrogen also decreases serum levels of insulin-like grown factor-1 (IGF-1), an important mitogen required for cellular proliferation and subsequent malignant transformation (Campagnoli, Biglia et al. 1993).

1.3.1.11 Non-steroidal anti-inflammatory drugs

There is good evidence that patients taking non-steroidal anti-inflammatory drugs (NSAIDs) reduce their risk of developing colorectal cancer. In 2003, a randomised controlled trial of

over 1000 patients concluded that daily aspirin reduced the risk of colorectal adenoma formation in patients with a history of polyps (Baron, Cole et al. 2003). These findings are supported by epidemiological data which suggests that NSAIDs not only reduce the incidence of adenomas (Arber 2000) but also reduce the risk of progression to adenocarcinoma (Peleg, Maibach et al. 1994). Despite this evidence, the cardiovascular and gastrointestinal side effects of these drugs have meant that trials investigating them as colorectal cancer chemoprevention have failed to recommend their routine use (Baron, Sandler et al. 2006; Bertagnolli, Eagle et al. 2006). The precise mechanisms which explain the anti-carcinogenic effect of NSAIDs have yet to be clarified although it is recognised that this class of drugs reduces the synthesis of prostaglandins through inhibition of cyclooxygenase (COX) enzymes. One hypothesis is that they exert their beneficial effect by direct inhibition of COX-2, the COX isoform implicated in carcinogenesis (Wu, Gu et al. 2003). An alternative explanation is that NSAIDs work by modulating the local and systemic inflammatory responses, recognised to be associated with the development and progression of colorectal cancer (see below) (McMillan, Canna et al. 2003; Erlinger, Platz et al. 2004).

1.3.1.12 Statins

Statins are a class of drug which inhibit HMG-CoA reductase, an enzyme important in the synthesis of cholesterol, and were originally designed as lipid-lowering agents. However, HMG-CoA is over-expressed in colorectal cancer cell lines (Hentosh, Yuh et al. 2001) and statins were shown to induce apoptosis of tumour cells in vitro (Rao, Newmark et al. 2002). With this hypothesis in mind, Poynter and colleagues analysed the drug histories of almost 4000 patients and reported that statin use was associated with a significant reduction in the relative risk of developing colorectal cancer (Poynter, Gruber et al. 2005). A subsequent meta-analysis of 18 studies, involving over 1.5 million patients, concluded that statins may be

associated with a reduction in the risk of colorectal cancer although the relationship was not as strong as first reported (Bonovas, Filioussi et al. 2007). More recently, studies have suggested that the impact of statins on tumour development may be mediated by their anti-inflammatory properties. The inhibition of HMG-CoA prevents the synthesis of mevalonic acid a downstream pre-requisite for a number of molecular processes including inflammation, cellular proliferation and angiogenesis (Zhu, Daghini et al. 2008). There is also evidence that statins may act to prevent distant metastases through the sensitization of colorectal tumours to chemotherapeutic agents (Siddiqui, Nazario et al. 2009).

1.3.1.13 Systemic inflammatory response

A number of studies have suggested that the risk of CRC is higher in individuals with evidence of a pre-existing systemic inflammatory response. (Tsilidis, Branchini et al. 2008). In a prospective study of over 22,000 patients, Erlinger and co-workers discovered that plasma C-reactive protein (CRP) levels were consistently elevated among people who subsequently developed colon cancer (Erlinger, Platz et al. 2004). The authors suggested that inflammation may therefore play a role in carcinogenesis but pointed out that systemic inflammation was also associated with other potential risk factors such as obesity, smoking and coronary artery disease. Such associations have been confirmed by other studies (Ross 1999; Visser, Bouter et al. 1999; Freeman, Norrie et al. 2002) and raise the possibility that systemic inflammation may simply represent a surrogate marker for a variety of different CRC risk factors (Figure 1.3). However, regardless of whether a systemic inflammatory response represents a final common pathway or is considered an independent risk factor, it is clear that inflammation and carcinogenesis are intimately related. In experimental models, pro-inflammatory cytokines have been shown to damage DNA, promote cellular proliferation

and inhibit apoptosis (Jaiswal, LaRusso et al. 2000; Meira, Bugni et al. 2008; Davies, Powell et al. 2009). It remains to be established whether inflammation is a cause or a consequence of cancer development, but their intimate relationship has led to inflammation recently being proposed as an inherent hallmark of cancer (Colotta, Allavena et al. 2009)

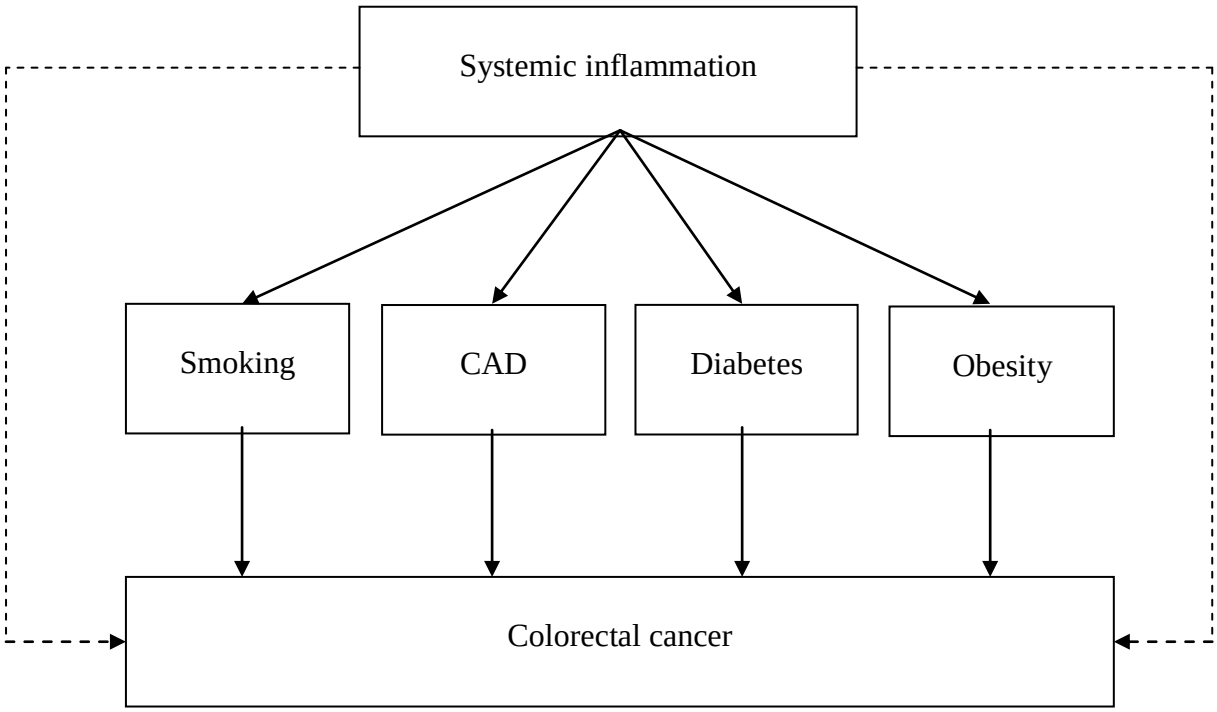


Figure 1.3. Systemic inflammation as a risk factor for colorectal cancer. The solid arrows represent an association through established colorectal cancer risk factors while the dashed arrows represent a possible independent association.

1.3.2 NON-SPORADIC COLORECTAL CANCER

In a small number of cases the aetiological factors implicated in the development of colorectal cancer are more clearly defined. This category, termed 'non-sporadic' colorectal cancer includes hereditary forms of the disease as well as specific disease processes associated with the development of colorectal malignancy.

1.3.2.1 Inflammatory bowel disease

Patients with inflammatory bowel disease, namely Crohn's disease (CD) or ulcerative colitis (UC), have an increased risk of developing colorectal cancer. A meta-analysis of over 60,000 patients concluded that the risk of CRC in patients with CD was 2.59, although this risk increased in patients with severe or long-standing colitis (Canavan, Abrams et al. 2006). The risk of CRC in patients with UC is related to the duration of symptoms and is estimated at 2% after 10 years, 8% after 20 years and 18% after 30 years (Eaden, Abrams et al. 2001). The predisposition to cancer in patients with inflammatory bowel disease does not appear to have a specific genetic basis but instead is assumed to be the result of chronic inflammation (Triantafillidis, Nasioulas et al. 2009). The hypothesis of inflammation as the precursor of tumour development is supported by epidemiological data including evidence that cancer risk increases with both the severity (Gupta, Harpaz et al. 2007) and duration of colitis (Lakatos and Lakatos 2008). It should be emphasised that the same carcinogenic pathways, namely chromosomal instability and microsatellite instability, lead to the development of both sporadic and colitis-associated colorectal cancer. The complex relationships between inflammation and cancer development are described in more detail below but it is thought that the inflammatory process can interact directly with genes involved in the carcinogenic pathways, including p53 and DNA MMR genes (Itzkowitz and Yio 2004).

1.3.2.2 Hereditary Non-Polyposis Colon Cancer

Hereditary Non-Polyposis Colon Cancer (HNPCC) is an autosomal dominant disease which predisposes carriers to the development of several different tumour types but primarily to those of the colorectum (Umar, Risinger et al. 2004). It is the most common cause of so-called 'familial CRC' and was recognised as distinct from Familial Adenomatous Polyposis (FAP) in the 1960's by Henry Lynch; for this reason the disease is sometimes referred to as Lynch syndrome (Lynch, Shaw et al. 1966). HNPCC has an incidence of approximately 1:1000 in the general population and bestows an estimated 80% lifetime risk of colorectal cancer on carriers (Lynch and de la Chapelle 1999). In addition, such individuals have an increased risk of endometrial, stomach, ovarian, biliary and urothelial tumours. Patients with HNPCC differ from those with sporadic CRC in a number of ways; they are often diagnosed at an earlier age, the tumours are often right-sided, they have an increased risk of synchronous or metachronous tumours and their disease often carries a better prognosis (Vasen, Mecklin et al. 1991).

The key to understanding these differences is the recognition that HNPCC cancers arise through a different molecular pathway than sporadic tumours. As opposed to the chromosomal mutations seen in sporadic CRC, HNPCC cancers are the result of microsatellite instabilities and are therefore associated with certain molecular and pathological characteristics (see 'microsatellite instability' below). At a molecular level, HNPCC is caused by germline mutations in any of 5 DNA MMR genes – MSH2, MLH1, MSH6, PMS2 or PMS1 (Kolodner, Tytell et al. 1999). The resultant microsatellite instability (MSI) is often used as a surrogate marker for HNPCC although it should be emphasised that the disease can occasionally occur without mismatch-repair mutations; conversely a

proportion of sporadic colorectal cancers will have MMR deficiency, preventing MSI status being used as the sole diagnostic criteria for HNPCC.

The diagnosis of HNPCC is therefore based on a set of international guidelines, known collectively as the 'Amsterdam' and 'Bethesda' guidelines (Table1.2). In essence, the condition is usually diagnosed with a detailed family history followed by genetic testing of potentially susceptible individuals. The Amsterdam criteria were originally developed in the early 1990's to determine whether a family should be classified as having HNPCC and were subsequently revised in 1998 (Amsterdam II criteria) (Vasen, Watson et al. 1999). The Bethesda guidelines were developed with the purpose of deciding whether to genetically test individuals with cancer in their family who do not satisfy the Amsterdam criteria; these guidelines were also subsequently revised in 2003 (Umar, Boland et al. 2004).

1.3.2.3 Familial Adenomatous Polyposis

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disease characterized by the development of hundreds of adenomas in the colon and rectum during the second decade of life. The disease is rare, with an estimated incidence of 1:8000 and accounts for <1% of all cases of colorectal cancer (Fearnhead, Wilding et al. 2002). The condition results from a mutation in the adenomatous polyposis (APC) gene, a tumour suppressor gene located on chromosome 5, leading to chromosomal instability and the development of CRC along the CIN pathway (Groden, Thliveris et al. 1991). Almost all patients with FAP will go on to develop CRC if they are not diagnosed and treated at an early age. Disease registries now mean it is unusual for people with FAP mutations to remain undiagnosed and most undergo regular surveillance and, ultimately, prophylactic colectomy. Despite this, the association with extracolonic malignancies including pancreatic mucinous adenocarcinoma,

hepatoblastoma and desmoid tumours means that a significant number of patients with FAP still die from malignant disease (Belchetz, Berk et al. 1996).

Table 1.2. International criteria used in establishing a diagnosis of HNPCC.

Amsterdam II criteria (1998)
Three or more relatives with an HNPCC associated cancer plus all of the following: - one affected individual should be a first degree relative of the other two - two or more successive generations should be affected - cancer in one or more affected relative should be diagnosed before the age of 50 years - FAP should be excluded in cases of colorectal cancer - tumours should be verified by pathological examination
Revised Bethesda guidelines (2003)
Just one of the following criteria need to be met: - diagnosed with colorectal cancer before the age of 50 - synchronous or metachronous HNPCC associated cancer, regardless of age - colorectal cancer with MSI-H morphology, diagnosed before the age of 60 - colorectal cancer with one or more first degree relative with an HNPCC associated tumour, diagnosed before the age of 50 - colorectal cancer with two or more relatives with an HNPCC associated tumour, regardless of age

Hereditary non-polyposis colorectal cancer
FAP: Familial adenomatous polyposis
MSI-H: high levels of microsatellite instability

1.3.2.4 Hamartomatous Polyposis Syndromes

A number of different syndromes have been described whereby patients have a propensity to develop multiple hamartomatous polyps in the gastrointestinal tract. The majority of these syndromes are inherited in an autosomal dominant fashion and include Juvenile Polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), hereditary mixed polyposis syndrome

(HMPS) and the PTEN hamartoma tumour syndromes (Calva and Howe 2008). Although the clinical features of these syndromes are variable, all give patients an increased risk of developing colorectal cancer. It is estimated that JPS carries a 38% risk of colorectal cancer (Howe, Mitros et al. 1998) while a diagnosis of PJS bestows an 84 fold relative risk of developing colon cancer when compared to the general population (Giardiello, Welsh et al. 1987). The progression of hamartomatous polyps to cancer is poorly understood but probably represents a different mechanism to that observed in the adenoma to carcinoma sequence described above. The histological changes are thought mainly to affect the lamina propria, a process described as 'landscaping' which leads to the development of epithelial cancers. In addition to an increased risk of colorectal cancer, patients with multiple hamartomas' are prone to malignancies of the stomach, pancreas and small bowel (Kinzler and Vogelstein 1998).

1.3.3 Summary – Aetiology of colorectal cancer

The development of colorectal cancer occurs following complex interactions between host and environmental factors. In a small number of cases, specific genetic mutations mean the disease is inevitable but in the majority of people the development of colorectal cancer is difficult to predict. Environmental factors such as a Western diet rich in red meat and low in fibre may predispose an individual but tumour development does not always occur. Individual susceptibility appears largely to depend on host factors such as age, an absence of comorbidity or pre-existing systemic inflammation. It is of particular interest that inflammation has been associated with many other individual risk factors for colorectal cancer and raises the possibility that a final common pathway is responsible for both tumour development and the generation of a systemic inflammatory response.

1.4 MANAGEMENT PRINCIPLES IN COLORECTAL CANCER

1.4.1 Presentation

The presentation of colorectal cancer is dependent on the site of tumour and extent of disease. Many patients with early cancers have no symptoms and a diagnosis is only made via population screening. Common symptoms associated with colorectal cancer include abdominal pain, rectal bleeding, altered bowel habit and involuntary weight loss (Thompson, Perera et al. 2007). The likelihood of individual symptoms varies by tumour location. Proximal cancers for example, rarely cause gross rectal bleeding because the blood tends to mix with the stool and degrade during colonic transit. This occult blood loss means such patients often present with iron deficiency anaemia (Harewood and Ahlquist 2000). In contrast, distal rectal tumours may present with fresh rectal bleeding, pelvic pain or tenesmus (Cappell 2005). In a few cases, patients without recent symptoms present as an emergency with intestinal obstruction, fistulation or perforation (Bass, Fleming et al. 2009).

1.4.2 Diagnosis

The diagnostic work up for colorectal cancer depends on the mode of presentation. If a patient presents as an emergency with symptoms and signs of peritonitis, a diagnosis of colorectal cancer may only be made incidentally during operative intervention. However, in the elective setting, a histological diagnosis should be made and the disease fully staged before treatment is commenced (ACPGBI). The investigations used in the diagnosis of colorectal cancer are detailed below.

1.4.3 Diagnostic modalities

Colonoscopy is the gold standard investigation of the colon and rectum allowing direct visualisation of the mucosal surface and offering the capacity to obtain tissue for histological

diagnosis. Colonoscopy is highly sensitive at detecting both malignant tumours and benign adenomas (Rex 1995). Colonoscopy has a number of disadvantages including the impact on healthcare resources, its invasive nature and a small but significant risk of serious complications (Scholefield 2000). Flexible sigmoidoscopy, examining only the distal colon, is an alternative to colonoscopy and is effective in diagnosing the majority of colorectal tumours (Thompson, Flashman et al. 2008). To examine the right colon, however, this test must be augmented with additional investigations, such as barium enema.

Double contrast barium enema is a radiological technique offering the capacity to image the entire colon. The presence of alternative colonic pathology such as diverticular disease, however, can make interpretation difficult and often necessitates the need for direct endoscopic visualisation. A review of over 2000 consecutive cases estimated that barium enema had a sensitivity of 83% for the detection of colorectal cancer compared to 95% for colonoscopy. In addition, barium enema was less adept in the identification of early stage tumours (Rex, Rahmani et al. 1997).

CT colonography is a minimally invasive technique increasingly used as an alternative to colonoscopy for examining the lower gastrointestinal tract. Individual protocols vary but the technique involves the administration of bowel preparation followed by high resolution CT scanning. The sensitivity of CT colonography for the detection of colorectal cancer was estimated by one systematic review as 93%, a figure comparable to conventional colonoscopy (Halligan, Altman et al. 2005). This modality has the added benefit of offering simultaneous staging information (Chung, Huh et al. 2005).

1.4.4 Staging of colorectal cancer

The staging of colorectal cancer quantifies the extent of disease and provides a framework for selecting the appropriate treatment. A number of staging systems exist but across the world the most common is the Tumour, Node Metastases (TNM) system produced by the American Joint Committee on Cancer (AJCC). Using this system, the stage of colorectal cancer has three components, the primary tumour (T), the regional lymph nodes (N) and the presence of metastatic disease (M), which are combined to form stage groupings. Current practice in the UK dictates that colorectal cancers are staged according to the 5th edition of the AJCC/TNM classification (Fleming ID 1997). An alternative staging system for colorectal cancer also exists in the UK, commonly referred to as Dukes staging. The original system, proposed by Cuthbert Dukes in the 1930's (Dukes 1937) for the classification of rectal cancer, has been modified on several occasions and now encompasses both colon and rectal cancer and includes a "D" stage for the presence of distant metastases (Table 1.3).

Final staging of colorectal cancer relies on the pathological assessment of the resected tumour and can only be completed after surgery. Pre-treatment staging, used to select the most appropriate management strategy relies on a combination of physical examination, visualisation of the colon and radiological imaging of the chest, abdomen and pelvis. In patients with newly diagnosed colorectal cancer, abdominal, pelvic and chest CT is used to define the extent of local tumour extension and establish the presence or absence of regional lymphatic spread and distant metastases. It is preferable to obtain these scans prior to, rather than after operation, as the results may influence surgical planning. If a patient requires an emergency operation for complications associated with a recently diagnosed colorectal cancer, staging may be completed in the post-operative period.

In patients with colon cancer, additional staging modalities such as contrast-enhanced magnetic resonance imaging (MRI) and Positron Emission Tomography (PET) are not routinely used but may be employed if there is diagnostic uncertainty regarding the presence of metastatic disease.

In patients with rectal cancer, current Royal College of Radiology guidelines state that pre-operative staging should include pelvic MRI to assess the circumferential resection margin and exclude disease outwith the mesorectum. Endoanal and endorectal ultrasound may also be employed to assess the depth of invasion through the bowel wall and involvement of mesorectal lymph nodes. Accurate staging of the rectum is important for decision-making regarding the provision of neo-adjuvant treatment in rectal cancer (Brown 2005).

Table 1.3. Comparison of colorectal cancer staging systems currently used in the UK.

	Staging systems			
TNM Stage	**T**	**N**	**M**	**Dukes**
Stage 0	Tis	N0	M0	-
Stage I	T1	N0	M0	A
Stage II	T2	N0	M0	A
Stage IIA	T3	N0	M0	B
Stage IIB	T4	N0	M0	B
Stage IIIA	T 1-2	N1	M0	C
Stage IIIB	T 3-4	N1	M0	C
Stage IIIC	Any T	N2	M0	C
Stage IV	Any T	Any N	M1	D

TNM: AJCC Tumour, Node and Metastases staging system
Dukes: Modified Dukes classification of colorectal cancer

1.4.5 Management principles in colon cancer

Approximately 80% of colon cancers are localised to the bowel wall and can be surgically resected. These operations are undertaken with curative intent and involve complete removal of the tumour, the vascular pedicle and the lymphatic drainage of the affected colonic segment. In most cases intestinal continuity can be restored with a primary anastomosis but in the presence of unfavourable circumstances a diverting stoma may be employed. Laparoscopic-assisted colectomy is an acceptable alternative to open surgery and follows the same oncological principles. There is some evidence that recovery may be quicker with laparoscopic surgery while morbidity, mortality and oncological outcomes appear comparable (Delaney, Chang et al. 2008).

1.4.6 Polyp cancers

Benign polyps or those with carcinoma in situ can be managed with endoscopy resection (polypectomy). If invasive cancer is discovered in a polyp then management decisions are centred on the choice between endoscopic polypectomy versus formal surgical resection of the affected colonic segment. Endoscopic resection is an acceptable strategy for early stage polyp cancers with no adverse pathological features. However, in the presence of unfavourable characteristics including poorly differentiated histology, lymphovascular invasion, tumour cells at the stalk margin or cancer in a sessile polyp, radical surgical resection is indicated (Coutsoftides, Sivak et al. 1978; Kikuchi, Takano et al. 1995).

1.4.7 Management principles in rectal cancer

Surgical resection is also the cornerstone of potentially curative treatment for rectal cancer. The choice of operation is dependent on the size, stage and position of the tumour. Small

tumours that are confined to the mucosa may be effectively managed by local excision using techniques such as transanal endoscopic microsurgery (TEMS) (Langer, Liersch et al. 2003; Lee, Lee et al. 2003) while larger tumours require more radical resection. Invasive rectal cancers are ideally removed using a sphincter-sparing procedure provided the distal margin of resection is histologically clear of tumour. Tumours of the upper and middle third of rectum are usually managed with a low anterior resection while those in the distal rectum may be amenable to ultra low anterior resection with colonic pouch. For low-lying rectal tumours with confirmed sphincter invasion or in whom a clear distal resection margin cannot be guaranteed, the operation of choice is an abdomino-perineal resection (APR). Recently, a more radical approach to low rectal tumours using an extralevator or 'cylindrical' APR has been reported to be oncologically superior with lower rates of positive circumferential resection margins (CRM) (Holm, Ljung et al. 2007). In order to achieve the greatest likelihood of complete tumour clearance in rectal cancer surgery, it is important that any potentially curative operation for invasive disease includes total mesorectal excision (TME) (Heald, Husband et al. 1982; Law and Chu 2004).

1.4.8 Neo-adjuvant treatment in rectal cancer

Management of cancer in the low rectum presents clinicians with the dual challenge of preserving anal sphincter function while removing local tumour. In many cases an APR offers the greatest chance of curative surgery but leaves patients with a permanent colostomy. For patients with large or low-lying tumours initially precluding sphincter-sparing surgery, neo-adjuvant treatment may enhance the prospects of resection with curative intent but its provision is unlikely to avoid the need for APR. The indications for neo-adjuvant chemoradiotherapy include T3/4 tumours, positive mesorectal nodes on preoperative imaging and tumours threatening or involving the mesorectal fascia (Sauer, Becker et al. 2004; Rodel,

Martus et al. 2005). The optimal regime for neo-adjuvant chemoradiotherapy in rectal cancer has yet to be established and is the subject of ongoing clinical trials.

1.4.9 Adjuvant chemotherapy

In patients with colon cancer who have undergone potentially curative surgery, disease recurrence is presumed to be the result of clinically occult metastases that are present at the time of resection. The goal of adjuvant chemotherapy is to eliminate these tumour cells and thereby increase the likelihood of cure. A 5 year survival advantage after adjuvant chemotherapy has been clearly demonstrated in stage III (node-positive) colon cancer but its benefit in node-negative disease has yet to be confirmed (Wolmark, Fisher et al. 1988; Moertel, Fleming et al. 1995; Wolmark, Wieand et al. 2000). Current recommendations are that only patients with Stage II colon cancer who have high risk pathological features should be considered for adjuvant chemotherapy (ACPGBI ; Benson, Schrag et al. 2004). The status of the inflammatory response is not currently a criteria for the consideration of adjuvant chemotherapy in patients with colorectal cancer.

Adjuvant chemotherapy is usually started within 6 to 8 weeks or when patients have recovered from surgery. Although there is no agreement as to the optimum timing of chemotherapy, there is consensus that excessive treatment delays can have a negative impact on survival (Dahl, Fluge et al. 2009).

The choice of chemotherapy regime for patients who have undergone potentially curative colorectal cancer resection is not well established but effectiveness, drug toxicity and patient fitness are taken into account. Combination therapy where oxaliplatin, cetuximab or bevacizumab are given along with 5-Fluorouracil (5-FU) are reported to offer superior disease free survival compared to 5-FU alone (Andre, Boni et al. 2004; Gill, Loprinzi et al.

2004). Alternative oral agents such as capecitabine can be considered for patients unable to tolerate intravenous regimes (Twelves, Wong et al. 2005).

There is uncertainty as to whether adjuvant chemotherapy offers a survival advantage to patients with rectal cancer who have previously undergone preoperative treatment. One school of thought suggests that those with a good tumour response do not require further treatment postoperatively (Fietkau, Barten et al. 2006) while others argue that this demonstrates a tumour cell sensitivity to chemotherapy that should be utilised (Bosset, Collette et al. 2006).

1.4.10 Metastatic disease

Approximately 20% of patients with colorectal cancer have evidence of metastatic disease at the time of diagnosis. In selected patients with limited metastases, surgical resection still provides a potentially curative option. Long term survival can be achieved with aggressive surgical treatment of both the primary tumour and secondary deposits. Such a strategy is usually combined with chemotherapy although the optimal timing of surgery in relation to oncological treatment has yet to be determined. In patients who present with colorectal cancer and synchronous resectable hepatic metastases, the management options include simultaneous colonic and liver resection (Tanaka, Shimada et al. 2004; de Santibanes, Fernandez et al. 2010) or a staged approach (Jamison, Donohue et al. 1997; Choti, Sitzmann et al. 2002).

1.4.11 Colorectal cancer screening programme

Colorectal cancer has a number of features that make it an attractive candidate for population screening. These include the fact that most cases develop slowly over a number of years and the disease is more effectively treated when diagnosed at an earlier stage. In addition, there is

a safe screening test which is acceptable to the majority of the population. Most importantly, trials in North America and Europe reported that the incidence and mortality of colorectal cancer was reduced in screened populations (Selby, Friedman et al. 1992; Hardcastle, Chamberlain et al. 1996; Scholefield, Moss et al. 2002). In 2006 the NHS Bowel Cancer Screening Programme commenced by inviting all men and women aged 60 to 69 (50 – 74 in Scotland) to submit a faecal occult blood (FOB) test every 2 years. Local screening centres have been established to provide endoscopy for people who have an abnormal test result. In the UK, the expectation of the colorectal cancer screening programme is that more tumours will be detected at an earlier stage and the incidence of advanced disease will reduce (Kaye and Shulman 1992). This so-called 'stage shift' means the ability of clinicians to risk stratify and treat patients with node-negative disease will become increasingly important (see below).

1.5 PROGNOSTIC FACTORS IN COLORECTAL CANCER

The prognosis of colorectal cancer is often summarised according to tumour stage at diagnosis. Five year survival rates in the United Kingdom vary from over 90% for patients with tumours confined to the mucosa to less than 10% for those with metastatic disease (CRUK). However, it is now recognised that the prognosis of patients with colorectal cancer is not governed exclusively by pathological stage. Rather, disease progression appears to be determined by complex interactions between both tumour and host characteristics. Tumour factors include specific pathological features, molecular markers or genetic mutations. Host factors include age, physiological function, local immune cell response and the presence of a systemic inflammatory response.

1.5.1 TUMOUR FACTORS AND COLORECTAL CANCER PROGNOSIS

A large number of tumour characteristics have been described as having prognostic value in colorectal cancer. These range from gross pathological features such as evidence of lymph node involvement right through to the presence or absence of specific molecular markers or genetic mutations. The following summarises those tumour factors reported to influence disease progression and survival in colorectal cancer.

1.5.1.1 Pathological stage

The pathological stage of the tumour is widely regarded as the single biggest determinant of outcome in colorectal cancer. The staging systems most commonly employed in the UK are the Dukes and TNM classifications.

Dukes system

As described above, Dukes' original classification of rectal cancer has undergone a series of modifications in an attempt to improve prognostic stratification and encompass both colon and rectal tumours. This system stratifies tumours according to the depth of invasion into the bowel wall and the presence or absence of lymph node or distant metastases. Dukes A describes a cancer confined to the colorectal mucosa or submucosa, Dukes B1 extends to the muscularis propria while Dukes B2 penetrates the muscularis propria. By definition, tumours termed Dukes C have evidence of lymph node involvement with C1 tumours confined to the bowel wall and C2 tumours penetrating through the bowel wall. More recently, an additional stage, termed Dukes D, was added to describe the presence of distant metastases. The Dukes system is adept at giving a gross description of the extent of the primary tumour and is still used by some clinicians in the UK as a prognostic indicator. The 5 year survival rates of patients with colorectal cancer, stratified by Dukes stage are summarised in Table 1.4.

TNM system

Although the Dukes system is still used by some, confusion over modifications and terminology (Mainprize, Mortensen et al. 2002) mean that the system has been superseded by the Tumour Node Metastases (TNM) system. This system was developed and is maintained by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) and is based on the extent of the primary tumour (T), the extent of regional lymph node involvement (N) and the presence or absence of distant metastases (M). The rationale behind this standardised system is to indicate prognosis, plan and assess the response to treatment and compare data objectively between centres. The TNM system is regularly updated and is currently on its 7th edition. Despite this, in the UK the Royal College of Pathologists (RCPath) still currently recommends the 5th edition as the optimal staging

system for colorectal cancer (Table 1.5). The reasons behind this decision include disputes over evidence underlying recent amendments as well as the difficulties in standardising long term clinical trials using a continuingly evolving system (Quirke, Williams et al. 2007). For example, a given stage may have quite different prognosis depending on which staging edition is used, an effect that has been termed 'stage migration'.

Although both the Dukes and TNM system provide useful prognostic information they have a number of potential problems. To allocate an accurate stage, both systems are reliant on the quality of surgical excision and the subsequent pathological assessment of the specimen (Johnson, Porter et al. 2006). For example, an adequate assessment of nodal status requires the surgeon to harvest a minimum number of lymph nodes and the pathologist to accurately identify and stage each individual node. Low lymph node counts can under-stage a tumour and have been suggested as an independent risk factor for disease recurrence (Chang, Rodriguez-Bigas et al. 2007). Lymph node number has therefore become a surrogate marker for quality of surgical resection and pathological assessment and current guidelines recommend that a minimum of 12 nodes are required to allow accurate staging (RCPath).

The other primary problem with the Dukes and TNM systems is that survival rates vary considerably within and across each prognostic category. Indeed, it is now accepted that patients with node-negative disease (Stage II) who have certain high risk pathological features such as venous invasion have a worse survival than some patients with lymph node metastases (Stage III) (Petersen, Baxter et al. 2002; Morris, Maughan et al. 2007).

In summary, the pathological stage of the tumour provides important prognostic information in colorectal cancer and is the standard against which all other prognostic factors are measured. The TNM system is employed throughout the world but despite continuous

modifications, cannot yet accurately identify all patients who will ultimately succumb to their disease.

Table 1.4. Five year survival rates of patients with colorectal cancer, stratified by TNM and Dukes stage at diagnosis. Adapted from the CRUK cancer statistics website.

TNM stage	Dukes stage	% of all cases	5 year survival
Stage I	A	8.7%	93.2%
Stage IIA	B	24.2%	77.0%
Stage IIB	B		
Stage IIIA	C	23.6%	47.7%
Stage IIIB	C		
Stage IIIC	C		
Stage IV	D	9.2%	6.6%
Unknown	Unknown	34.3%	N/A*

*Not applicable.

Table 1.5. AJCC/TNM (5th edition) system for the classification of colorectal cancer.

pT	**Primary tumour**
pTx	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour invades submucosa
pT2	Tumour invades muscularis propria
pT3	Tumour invades through muscularis propria
pT4	Tumour directly invades other organs (pT4a) or visceral peritoneum (pT4b)
pN	**Regional lymph nodes**
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastases
pN1	Metastases in 1-3 regional lymph nodes
pN2	Metastases in 4 or more regional lymph nodes
pM	**Distant metastases**
pMX	Distant metastases cannot be assessed
pM0	No distant metastases
pM1	Distant metastases

1.5.1.2 High risk pathological characteristics

In addition to conventional pathological stage, as described by the Dukes or TNM system, a number of other pathological characteristics have been reported to affect prognosis. These high risk tumour features, which can occur alone or in combination, are described below.

Tumour grade

Tumour grade describes how well the tumour is differentiated and is reported subjectively by the pathologist examining the specimen. Colorectal tumours are generally categorized as low grade (well or moderately differentiated) or high grade (poorly differentiated). A number of

studies have suggested that tumour grade is a prognostic factor in colorectal cancer. For example, in a study of over 100,000 patients O'Connell and colleagues reported reduced survival in patients with high grade tumours compared to low grade tumours in stage II – IV colon cancer (O'Connell, Maggard et al. 2004). Similar results have been reported in rectal cancer with poorly differentiated tumours displaying an increased risk of local recurrence and reduced 5 year survival (McDermott, Hughes et al. 1984). However, there remains concern that the histological grading of tumours in this way is subject to significant inter-observer variability (Thomas, Dixon et al. 1983). In conclusion, there is evidence that tumour grade influences survival in colorectal cancer although its effect appears to be small and is not likely to apply to stage I disease.

Venous invasion

The microscopic diagnosis of venous invasion is made when tumour cells are identified within a endothelium lines space surrounded by a rim of smooth muscle and/or containing red blood cells (Sternberg, Amar et al. 2002). Venous invasion is an established predictor of poor prognosis in colorectal cancer and its presence is associated with an increased incidence of disease recurrence and reduced survival (Krasna, Flancbaum et al. 1988; Minsky, Mies et al. 1988; Ouchi, Sugawara et al. 1996; Stewart, Morris et al. 2007). The reported incidence of venous invasion within colorectal tumours varies widely between studies from as low as 10% to as high as 89% (Dirschmid, Lang et al. 1996; Sternberg, Amar et al. 2002). Venous emboli are more prevalent in advanced disease and can occur in any part of the tumour so may be missed during routine pathological sectioning. However, the most decisive factor influencing the variance in reporting rates between different laboratories is likely to be the pathological technique employed. While some centres rely on haematoxylin and eosin (H&E) staining alone (Horn, Dahl et al. 1990), others employ sensitive immunohistochemistry

techniques to stain elastin fibres (elastase), greatly aiding the identification of venous emboli (Minsky, Mies et al. 1988; Dirschmid, Lang et al. 1996). In conclusion, there is strong evidence that venous invasion is an important prognostic factor in colorectal cancer but its clinical application is hampered by variations in reporting rates and techniques of assessment.

Perineural invasion

Perineural invasion (PNI) is a pathological process whereby tumour cells invade nervous tissues and spread along nerve sheaths (Batsakis 1985). It is recognised to represent an aggressive tumour phenotype in other solid organ cancers (Soo, Carter et al. 1986; de la Taille, Katz et al. 1999) and its presence in colorectal tumours is reported to be a poor prognostic sign. Several reports now indicate that PNI is a high risk feature in colorectal cancer and is associated with local recurrence and reduced survival (Krasna, Flancbaum et al. 1988; Ross, Rusnak et al. 1999; Liebig, Ayala et al. 2009). The evidence appears to be particularly strong for rectal cancer, perhaps reflecting the dense network of autonomic nerves in the pelvis. Despite this evidence, the RCPath do not currently recommend the assessment of PNI as core data in the reporting of colorectal tumour pathology. It is likely therefore that the incidence of PNI is under-estimated. Indeed, a retrospective examination of 269 colorectal cancer cases by Leibig and co-workers identified evidence of PNI in 22% of tumours compared to an incidence of just 0.5% on the original reports (Liebig, Ayala et al. 2009). In conclusion, PNI should be considered a high risk pathological feature in colorectal cancer but its prognostic utility is limited until its presence is routinely reported.

Peritoneal involvement

Serosal or peritoneal involvement is said to be present if tumour cells are visible either on the peritoneal surface or free in the peritoneal cavity. It is regarded as a poor prognostic sign in

both colon and rectal cancer and is associated with disease recurrence and metastatic spread (Shepherd, Baxter et al. 1997; Stewart, Morris et al. 2007). The identification of peritoneal involvement is reliant on accurate pathological assessment and there is some evidence that rates may be under-reported in many centres (Stewart, Morris et al. 2007).

Tumour perforation

Tumour perforation is defined as a visible defect through the tumour such that the bowel lumen is in communication with the external surface of the resected specimen. It is widely recognised as a high risk pathological characteristic and has been associated with increased risk of disease recurrence and reduced survival, independent of tumour stage, in patient with colorectal cancer (Petersen, Baxter et al. 2002; Benson, Schrag et al. 2004).

Margin involvement

Tumour cells present at or within 1mm of the surgical margin indicate inadequate tumour excision and are an exceedingly poor prognostic indicator. Studies have consistently demonstrated that involvement of the circumferential resection margin (CRM) is one of the strongest predictors of disease recurrence after rectal cancer resection (Adam, Mohamdee et al. 1994; Birbeck, Macklin et al. 2002). It is intuitive that residual tumour in situ results in a poor outcome and many clinicians therefore consider margin involvement as a marker of surgical quality rather than a true histological characteristic.

1.5.1.3 Petersen Index

Petersen and co-workers set out to identify objective and easily determined pathological features that could help identify which patients with Dukes B colon cancer may benefit from chemotherapy. After a meticulous pathological review of 268 consecutive cases the authors concluded that four factors – venous invasion, peritoneal involvement, tumour perforation

and margin involvement – were independent prognostic markers on multivariate analysis (Petersen, Baxter et al. 2002). Combining these factors into a cumulative scoring system stratified patients effectively into low risk (score 0 – 2) or high risk (score 3 – 5) categories. The prognostic value of the Petersen Index (PI) was subsequently confirmed in a large validation cohort of patients with Dukes B disease (Morris, Maughan et al. 2007).

1.5.1.4 Lymph node ratio

The lymph node ratio (LNR), calculated by dividing the number of lymph nodes with confirmed metastatic disease by the total number of lymph nodes sampled, has received attention as a possible prognostic indicator in colorectal cancer. A study of over 26,000 patients by De Ridder and colleagues suggested that the LNR could be used effectively to stratify patients with node positive colon cancer into two distinct prognostic groups (De Ridder, Vinh-Hung et al. 2006). These results are supported by other work which suggests that the LNR provides superior prognostic information compared to N stage alone (Le Voyer, Sigurdson et al. 2003; Berger, Sigurdson et al. 2005). The rationale behind the prognostic utility of the LNR is that patients with inadequate lymph node resection are at risk of being under-staged and receiving less adjuvant treatment. Using the ratio of metastatic to examined nodes reduces the likelihood of disease misclassification and under-treatment. Despite evidence that the LNR may provide additional prognostic information to TNM stage, there is little agreement as to which thresholds to use. The study by De Ridder outlined above used a LNR cut-off of 0.4 to split patients into two groups while other studies have used different thresholds (Peschaud, Benoist et al. 2008) or have stratified patients into three prognostic groups (Rosenberg, Friederichs et al. 2008). In conclusion, the LNR clearly has no role to play in predicting disease outcomes in patients with node negative disease. It may represent

an additional prognostic marker for patients with positive lymph nodes although agreement has yet to be reached regarding the optimum thresholds.

1.5.1.5 Microsatellite instability

A large number of studies have now investigated the prognostic implications of determining the miscrosatellite instability (MSI) status of colorectal tumours. As described previously, MSI tumours tend to be located in the right colon and are often poorly differentiated with higher numbers of inflammatory cells present in the tumour microenvironment (Greenson, Bonner et al. 2003). In general, MSI tumours have a favourable prognosis, respond well to 5-FU chemotherapy (Ribic, Sargent et al. 2003) and have a lower metastatic potential than sporadic MSS cancers. In 2005, a meta-analysis of 32 studies by Popat and colleagues confirmed that patients with MSI tumours had a survival advantage over those with MSS tumours, particularly for those with node-negative disease (Popat, Hubner et al. 2005). Despite these findings, MSI testing has yet to be incorporated into routine clinical practice.

1.5.1.6 Molecular markers

A large number of molecular markers have been proposed as prognostic indicators in colorectal cancer. The majority of these molecules are confined to experimental studies and only a small number, such as carcinoembryonic antigen and K-ras, are ever used in clinical practice. The following provides a summary of the prognostic value of the most common molecular markers used in colorectal cancer.

Carcinoembryonic antigen

Carcinoembryonic antigen (CEA) was first described in 1965 by Gold and Freedman as a molecule found only in foetal colon and colonic adenocarcinoma (Gold and Freedman 1965) although it has subsequently been discovered in very low concentrations in other tissues

(Boucher, Cournoyer et al. 1989). CEA is a glycoprotein with considerable heterogeneity whose primary function is thought to involve binding to bacteria within the gut, either as a means of facilitating bacterial colonization or to prevent infection (Duffy 2001). The discovery that CEA could be detected in the serum of patients with colorectal cancer but not in healthy controls prompted its promotion as a marker for colorectal cancer (Thomson, Krupey et al. 1969) and it is still one of the most widely used tumour markers in the world.

CEA has been proposed for roles in both the diagnosis and prognosis of colorectal cancer but recently its clinical utility has been questioned. The low positive predictive value of CEA in unselected patient populations mean it is unsuitable for use as a screening tool (Fletcher 1986) and an unacceptable sensitivity and specificity profile mean its use in the diagnosis of colorectal cancer is similarly limited (Begent 1984). In terms of the prognostic value of CEA in patients with known colorectal cancer, the evidence has been conflicting. A number of studies have shown that patients with high preoperative concentration of CEA have a worse outcome than those with low levels (Grem 1997). However, the value of the molecule over and above conventional pathological staging has yet to be proven and studies have failed to agree as to whether CEA is useful in stratifying patients with node-negative disease (Moertel, O'Fallon et al. 1986; Harrison, Guillem et al. 1997). Currently, CEA is most often measured postoperatively as a means of detecting disease recurrence or monitoring response to treatment (Graham, Wang et al. 1998). In conclusion, the available evidence suggests that CEA has no role in the diagnosis or preoperative prognostic stratification of patients with colorectal cancer. It may have a role in the surveillance of patients postoperatively but no large randomised trial has yet addressed the effect of CEA testing on quality of life, cost of care or overall survival (Duffy 2001).

Carbohydrate antigen 19-9

Carbohydrate (CA) 19-9, an adhesion molecule detectable in serum, is raised in a variety of gastrointestinal malignancies including pancreatic and colorectal cancer (Koprowski, Herlyn et al. 1981). A small number of studies have suggested that pre-operative levels of Ca19-9 provide stage independent prognostic information in patients with colorectal cancer (Filella, Molina et al. 1992; Reiter, Stieber et al. 2000). However, conflicting evidence suggests that serum level do not accurately predict disease recurrence (Morita, Nomura et al. 2004) and the routine use of Ca 19-9 is not currently recommended for the diagnosis or prognostic stratification of colorectal cancer (Locker, Hamilton et al. 2006).

Proliferation indices

Cellular proliferation can be measured using a number of techniques from complex reverse transcriptase polymerase chain reaction (PCR) assays to simple immunohistochemical staining for Ki-67. Data relating to the prognostic value of Ki-67 in colorectal cancer has been inconsistent and often underpowered (Chen, Henk et al. 1997; Garrity, Burgart et al. 2004; Zlobec, Baker et al. 2008). For example, Allegra and colleagues initially examined Ki-67 in 703 patients with colorectal cancer and observed a positive prognostic effect using an arbitrary cutoff of 40% (Allegra, Paik et al. 2003). However, the same team could not replicate these results on a different cohort derived from five different clinical trials (Allegra, Parr et al. 2002). Cellular proliferation as measured by Ki-67 cannot yet be considered a useful prognostic marker in colorectal cancer. Standardisation of methodology and identification of optimum thresholds may increase the clinical utility of proliferation indices.

Angiogenesis

New blood vessels are essential to the growth of any solid organ tumour (Folkman, Cole et al. 1966). In colorectal cancer the process of angiogenesis is thought to increase the metastatic potential of tumours and as such its occurrence was proposed to have potential prognostic utility. Indeed, an early study by Frank et al reported that increased microvessel density (MVD), a surrogate marker of neovascularisation, was associated with decreased survival in patients with Dukes B colon cancer (Frank, Saclarides et al. 1995). Subsequent studies have examined angiogenesis by measuring growth factors associated with the process, such as vascular endothelial growth factor (VEGF) (Bhatavdekar, Patel et al. 2001). A subsequent meta-analysis (45 studies examining MVD; 27 studies examining VEGF) concluded that angiogenesis was associated with reduced recurrence free survival in patients with colorectal cancer (Des Guetz, Uzzan et al. 2006). However, the authors commented that the methodology varied considerably between studies. In conclusion, there is evidence that angiogenesis is a poor prognostic indicator in colorectal tumours but its assessment requires standardisation if it is to be useful in clinical practice.

1.5.1.7 Oncogenes and tumour suppressor genes

Mutations in p53, a tumour suppressor gene located on chromosome 17p, are common in colorectal tumours with a prevalence estimated at 40 – 50% (Scott, Sagar et al. 1991). The prognostic value of detecting such p53 mutations has yet to be determined and much of the evidence to date is conflicting. Several studies have suggested that tumours with p53 mutations carry a poorer prognosis (Yamaguchi, Kurosaka et al. 1992; Hamelin, Laurent-

Puig et al. 1994; Houbiers, van der Burg et al. 1995). However, other authors report no correlation between p53 expression and survival (Scott, Sagar et al. 1991; Bell, Scott et al. 1993; Kressner, Lindmark et al. 1996). A recent review of the literature identified 35 studies which reported p53 mutations to be associated with poor outcome and 24 studies in which no correlation with survival was observed (Mutch 2007). These aberrant results may be due to variability of the detection and retrieval systems used to quantify p53 (Wynford-Thomas 1992).

K-ras is one of the most commonly mutated oncogenes in colorectal cancer and is associated with cellular proliferation and early tumourogenesis (Forrester, Almoguera et al. 1987). To date, no consistent results have been observed regarding the prognostic significance of K-ras mutations. Some studies have proposed that K-ras mutations are associated with reduced overall survival in patients with colorectal cancer (Bazan, Migliavacca et al. 2002; Conlin, Smith et al. 2005) while others have found no such correlation (Pricolo, Finkelstein et al. 1996; Andersen, Lovig et al. 1997).

In conclusion, the prognostic value of genetic mutations in colorectal cancer has yet to be confirmed. Much of the evidence is conflicting and results are blighted by study heterogeneity and methodological variability. Unless a consensus regarding techniques and thresholds can be reached genetic profiling cannot be considered a useful prognostic for colorectal cancer.

1.5.1.8 Tumour necrosis

Tumour necrosis is a common histological feature of many solid organ tumour types and has been proposed as a marker of poor prognosis in renal (Frank, Blute et al. 2002), breast (Fisher, Palekar et al. 1978) and lung cancer (Swinson, Jones et al. 2002). The presence of

necrosis has associated with unfavourable host characteristics including increasing age, elevated white cell count and anaemia (Edwards, Swinson et al. 2003; Sengupta, Lohse et al. 2005) and one hypothesis is that necrosis may impact survival by influencing the host inflammatory response. Summary data describing the prognostic value of tumour necrosis in solid organ tumours is given in Table 1.6.

Five studies, comprising data on a total of 1,051 patients, have reported the prognostic value of tumour necrosis in colorectal malignancy (Table 1.7). All studies used a similar semi-quantitative assessment of necrosis with the most common method a four group extent-based classification. The largest study to date was conducted by Pollheimer and colleagues who reported necrosis to be an independent predictor of cancer specific survival in a cohort of 381 patients with TNM Stage I – IV disease (Pollheimer, Kornprat et al. 2010). These results supported an earlier study by Gao and coworkers who reported necrosis to reduce overall survival, independent of pathological stage, in 300 patients with colorectal cancer (Gao, Arbman et al. 2005). Two of the studies related specifically to node negative disease. An early study by Svennevig et al (Svennevig, Lunde et al. 1984) reported no relationship between necrosis and survival in 100 patients with Dukes B cancer while a subsequent study reported tumour necrosis and perineural invasion as the only independent predictors of survival in 117 patients with Dukes B disease (Mulcahy, Toner et al. 1997). The relationship between tumour necrosis and disease recurrence was examined by Knutsen and colleagues in a study of 153 patients with rectal cancer. The authors reported a higher rate of recurrence in patients with extensive necrosis but also observed an association with preoperative radiotherapy which may partly explain why necrosis did not have independent prognostic value (Knutsen, Adell et al. 2006).

In terms of pathological associations, the majority of studies reported a relationship between tumour necrosis and aggressive characteristics including large size, high grade, poor differentiation and venous invasion (Mulcahy, Toner et al. 1997; Gao, Arbman et al. 2005; Pollheimer, Kornprat et al. 2010).

With regard to relationships with the host inflammatory response, reports have been conflicting. One study linked tumour necrosis with the local inflammatory response as assessed by cyclooxygenase-2 (COX-2) expression (Knutsen, Adell et al. 2006) while another study reported that tumours with a strong inflammatory cell infiltrate had less necrosis (Gao, Arbman et al. 2005). Meanwhile, other studies have reported no relationships between necrosis and either a general inflammatory reaction or lymphocytic infiltration (Svennevig, Lunde et al. 1984; Pollheimer, Kornprat et al. 2010).

In conclusion, there is evidence that tumour necrosis is associated with outcome in a range of solid organ tumour types. A limited number of studies suggest an association between tumour necrosis and poor prognosis in patients with colorectal cancer. More research is needed to establish whether necrosis exerts a prognostic effect independently of other high risk pathological features. To date, evidence regarding the interaction between tumour necrosis and the host inflammatory response has been contradictory.

Table 1.6. Summary characteristics of studies reporting the prognostic value of tumour necrosis in solid organ tumour types.

Tumour type	No. of studies	Total no. of patients	Relationship with outcome	Associations
Renal	23	15,852	Predicted poor prognosis (n=19) No relationship with outcome (n=4)	Increasing tumour grade/stage Poor performance status, High WCC/ESR Ki-67 expression
Breast	13	9,277	Predicted poor prognosis (n=9) No relationship with outcome (n=4)	Increasing tumour size/grade Increasing age High microvessel density, High macrophage count, Angiogenesis
Lung	7	1218	Predicted poor prognosis (n=6) No relationship with outcome (n=1)	Increasing tumour stage High platelet count, Anaemia P53 expression, High VEGF expression
Sarcoma	4	1208	Predicted poor prognosis (n=4)	Not related to tumour size/grade
Thyroid	2	241	Predicted poor prognosis (n=2)	Not reported
Pancreas	1	348	Predicted poor prognosis (n=1)	Increasing tumour size/grade Venous invasion Hypoxic foci
Hepatocellular	1	33	No relationship with outcome (n=1)	Not reported

WCC: white cell count
ESR: erthythrocyte sedimentation rate
VEGF: vascular endothelial growth factor

Table 1.7. Studies reporting the prognostic value of tumour necrosis in colorectal cancer.

Year	Author	Sample Size	Population Studied	Necrosis Method	Relationship with Outcome	Associations
1984	Svennevig	100	Dukes B colorectal cancer	Semi-quantitative (weak/moderate/extensive)	Not significant	Not related to inflammatory cell infiltrate
1997	Mulcahy	117	Dukes B colorectal cancer	Semi-quantitative (minimum/extensive)	Reduced overall survival (multivariate analysis)	Increasing tumour size
2005	Gao	300	Dukes A – D colorectal cancer	Semi-quantitative (absent/<10%/10-30%/>30%)	Reduced overall survival (multivariate analysis)	Increasing tumour stage, Poor differentiation
2006	Knutsen	153	Dukes A – D rectal cancer	Semi-quantitative (<5%/>5%)	Higher rate of recurrence (univariate analysis)	Preoperative radiotherapy, Higher COX-2 expression
2010	Pollheimer	381	TNM Stage I – IV colorectal cancer	Semi-quantitative (absent/<10%/10-30%/>30%)	Reduced cancer specific survival (multivariate analysis)	Increasing tumour size/grade/stage, Venous invasion, Not related to lymphocytic infiltrate

TNM: AJCC Tumour, Node, Metastases staging system
COX: Cyclooxygenase

1.5.1.9 Summary – Tumour factors and colorectal cancer prognosis

The prognosis of colorectal cancer is based primarily on pathological stage as described by the Dukes or TNM staging systems. A number of additional pathological characteristics, including tumour grade, venous invasion, peritoneal involvement, tumour perforation and margin involvement, have the capacity to stratify patients with node negative disease and may be useful in the allocation of adjuvant treatment. Despite the obvious benefits of identifying these high risk characteristics, their assessment is variable and depends almost exclusively on accurate pathological processing and reporting.

A host of other molecular and genetic markers have been proposed to have predictive value in colorectal cancer but so far none have been incorporated into routine clinical practice. The reasons for this include a failure to provide prognostic information independent of stage, a lack of standardized methodology, disagreement regarding optimum cutoffs and conflicting results between centres. This is exemplified by that fact that none of the markers described above is recommended for routine use by the American Society of Clinical Oncologists (ASCO), the American Joint Committee on Cancer (AJCC) or the Royal College of Pathologists (RCPath) in the United Kingdom.

1.5.2 HOST FACTORS AND COLORECTAL CANCER PROGNOSIS

It is now recognised that disease progression in colorectal cancer is influenced by complex interactions between both tumour- and host-related factors. The tumour characteristics described above, while providing a degree of prognostic information, cannot fully explain the survival differences observed in patients with cancers of the same pathological stage. It is increasingly apparent that host factors, defined as patient characteristics that promote or inhibit tumour growth, are equally important determinants of outcome. These patient factors include inherent characteristics such as chronological age as well as potentially modifiable traits such as physiological function and the host inflammatory response. The latter has received particular attention in relation to cancer outcomes and may represent the intrinsic ability of a person to generate an anti-tumour response.

1.5.2.1 Inflammation and cancer

Links between inflammation and cancer were described as far back as the 19th century and epidemiological evidence now confirms that inflammatory diseases increase the risk of developing many different types of cancer (Balkwill and Mantovani 2001). It is also well recognised that anti-inflammatory drugs reduce the risk of developing certain cancers (Thun, Namboodiri et al. 1993; Baron, Cole et al. 2003) and targeting inflammatory mediators decreases the incidence and spread of a number of different malignant tumours (Burton and Libutti 2009). These links between inflammation and cancer are further strengthened by the fact that immune cells and inflammatory mediators are often observed in tumour tissue and the cellular processes usually associated with chronic inflammation are also active in the tumour microenvironment (Mantovani, Allavena et al. 2008). Inflammation is now recognised as a key component of the biological capabilities that are acquired during the

development of human tumours (Colotta, Allavena et al. 2009). These capabilities, described as the 'hallmarks' of cancer, enable tumour cells to survive, proliferate and disseminate. The relationships between inflammation and the Hallmarks of Cancer are represented in Figure 1.3.

1.5.2.2 The host immune response

The human immune system comprises a number of interdependent organs, cells and processes that collectively protect the body from foreign pathogens. Broadly categorised into innate (non-specific) and adaptive (acquired) immunity, the system functions to recognise and destroy antigens associated with bacterial, viral or fungal infections. The immune system can also recognise cancer-specific antigens, allowing the identification and destruction of tumour cells in a process known as immunosurveillance (see below). Paradoxically, some cellular processes associated with inflammation can promote tumour progression (Vakkila and Lotze 2004) and it is therefore the balance of pro- and anti-tumour factors that many believe to be of primary importance in determining cancer outcomes (Zlobec and Lugli 2009).

Innate immunity

The innate immune system, comprising phagocytic cells (neutrophils and macrophages), degranulating cells (basophils, eosinophils and mast cells) and natural killer (NK) cells as well as humoral (complement) components, provides a crucial first line of defence against common microorganisms. Bacteria that successfully penetrate the epithelial surfaces of the body are met by macrophages, bound by cell surface receptors and engulfed in a process known as phagocytosis. This is followed by the release of biologically active molecules, known as chemokines and cytokines, which generate an inflammatory response. Although

most infectious agents and/or tissue damage initially induce this non-specific response, the innate system may subsequently activate an adaptive immune response (Janeway and Medzhitov 2002; Medzhitov 2007).

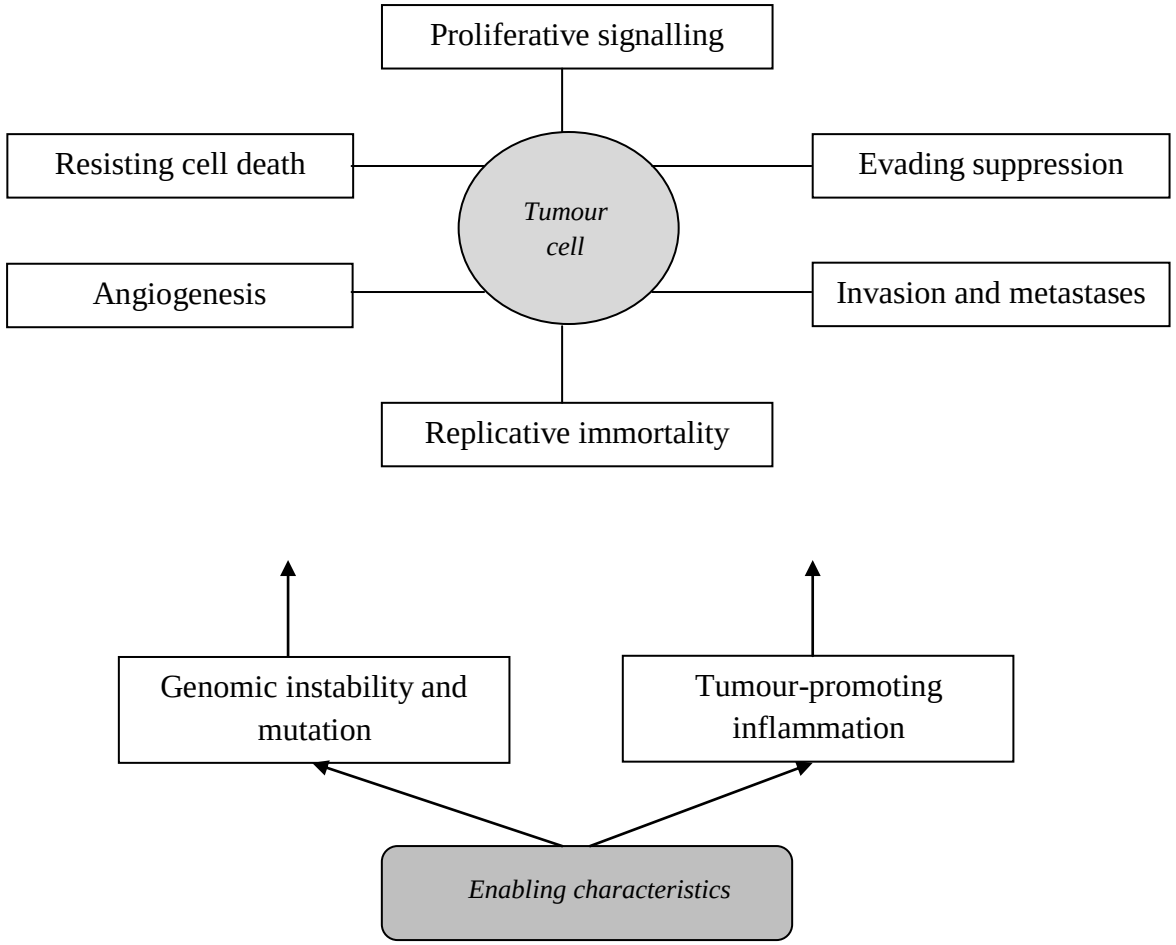


Figure 1.4. The relationships between inflammation and the Hallmarks of Cancer. Adapted from Hanahan et al.

Adaptive immunity

The adaptive immune system is composed primarily of lymphocytes and serves to aid in the recognition of 'non-self', eliminate specific pathogens and produce an immunological memory of previously encountered antigens. Activation of adaptive immunity is usually triggered by the presentation of antigens by specialised cells associated with the innate immune system known as antigen-presentation cells (APC). Adaptive immunity can be divided into humoral and cell-mediated immunity although many of the processes and cell types are inter-dependent. B cells are the major cell types in humoral immunity and produce antibodies, known as immunoglobulins, which recognise and bind to specific antigens, making them easy targets for phagocytes and triggering the complement cascade (Janeway 2001). T lymphocytes, identified by the presence of specific T-cell receptors (TCR), are responsible for coordinating cell-mediated immunity and can be categorised into a number of subsets; helper T cells ($CD4^+$), cytotoxic T cells ($CD8^+$), memory T cells ($CD45R0^+$) and regulatory T cells ($FOXP3^+$). Each subset plays a specific role in the identification and destruction of antigens. CD8+ T cells are the effector cells of adaptive immunity, inducing cell death through the release of cytotoxins such as peforin, granzyme B and granulysin (Janeway 2001).

1.5.2.3 Cancer immunoediting

As described above, cancer immunosurveillance is the process whereby tumour-specific antigens provoke an effective immunological reaction in the host thereby preventing the development of otherwise inevitable malignancy (Burnet 1957). The concept is not new but advances in genetic understanding have now validated the hypothesis and expanded it to include contributions from both the innate and adaptive immune systems (Dunn, Old et al. 2004). However, there is growing recognition that the relationship between cancer and the

immune response is yet more complex still and may involve the promotion as well as prevention of tumourogenesis (Shankaran, Ikeda et al. 2001; Schreiber and Podack 2009). A broader concept has therefore been developed, termed 'cancer immunoediting', which describes a dynamic process composed of three phases: elimination, equilibrium and escape. Elimination represents the original idea of cancer immunosurveillance, equilibrium is the period of latency after incomplete tumour destruction and escape refers to the final growth and dissemination of cancer cells (Dunn, Old et al. 2004). The immune response in cancer is thus now recognised as a complex relationship between pro- and anti-tumour factors with the potential to impact outcome in either a positive or negative manner. The host response can be broadly categorised into the systemic inflammatory response (describing a prolonged and inappropriate activation of the acute phase response) and the local inflammatory response (describing the infiltration of immune cells in the tumour microenvironment). These responses and their relationships with the prognosis of patients with colorectal cancer are described below.

1.5.2.4 The tumour microenvironment

The tumour microenvironment can be defined as the tissue medium in which tumour cells grow and develop. It is a complex and unique environment comprised of the invasive margin, proliferating tumour cells, tumour stroma, blood vessels, tissue cells and inflammatory cells (Figure 1.4). The tumour microenvironment represents a dynamic interface between tumour and host and it is postulated that the molecular events which occur here dictate whether a tumour progresses or is successfully eliminated by the host (Whiteside 2008). As described above, the host does not allow tumour development to progress unchecked but instead attempts to mount an effective immune response. This local inflammatory response can be considered an attempt to destroy tumour cells but it is often attenuated. The methods through

which tumour cells evade host immunity are referred to as ‘escape mechanisms’ and are thought to include direct interference with immune cell signalling capabilities, an accumulation of T regulatory cells which can suppress T cell function and downregulation of HLA expression on tumour cells resulting in inadequate recognition by the hosts immune cells (Ferrone and Whiteside 2007).

The changes that occur in the tumour microenvironment over time are similar to those seen in chronic inflammation and, once established, become dominated by pro-tumour processes. One of the earliest events is tissue hypoxia resulting in relative cellular ischaemia (Denko, Fontana et al. 2003). This favours the influx of macrophages which become activated and propagate hypoxia through the generation of reactive oxygen species (ROS). Mediated through NF-*k*B, a protein complex that controls the transcription of DNA, a number of signalling events then take place in both cancer cells and surrounding inflammatory cells (Karin and Greten 2005; Lluis, Buricchi et al. 2007). Pro-inflammatory cytokines, including TNF-α, are produced which alter the microenvironment to benefit the tumour. This cascade of cytokines influences a variety of key events including angiogenesis, cellular proliferation and matrix re-modelling, ultimately resulting in tumour growth and progression (Balkwill and Coussens 2004). It is evident that infiltrating immune cells are one of the most important components of the tumour microenvironment. Indeed, the nature, function, density and localization of immune cells within the tumour microenvironment have all been reported to influence tumour progression and clinical outcome in human colorectal cancer (Galon, Costes et al. 2006). The function and prognostic value of individual immune cell types are considered below.

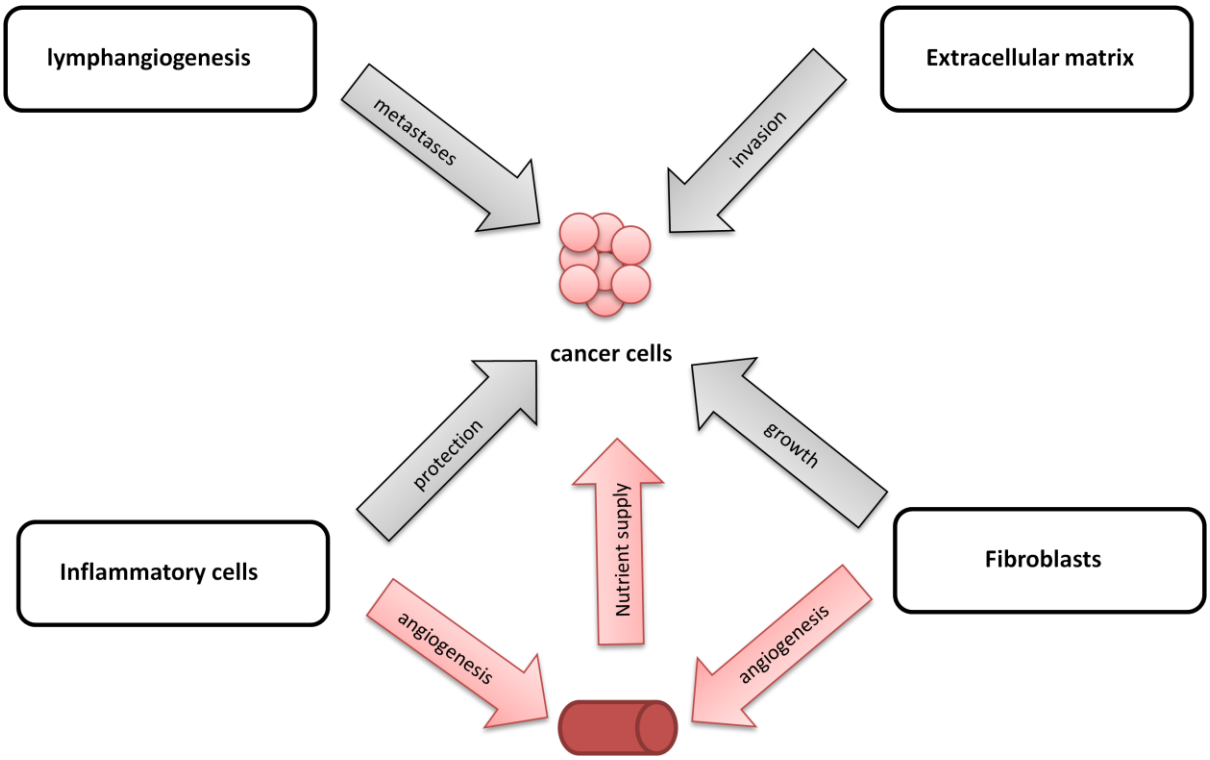


Figure 1.5. How components of the tumour microenvironment influence tumour growth and metastases. Adapted from Leyva-Illades et al.

1.5.2.5 The local inflammatory response

A strong inflammatory response at a local level has been consistently associated with improved clinical outcomes in patients with colorectal cancer. As far back as the 1970's it was observed that tumours with a high concentration of inflammatory cells often carried a favourable prognosis (House and Watt 1979) and a strong local response was hypothesised to represent effective anti-tumour immunity. This idea has now been supported by a wealth of studies which have examined the prognostic implications of inflammatory cell infiltration in the tumour microenvironment (Table 1.8). While it is generally accepted that a local inflammatory response is beneficial to patients with colorectal cancer the relative importance of type, density and location of individual immune cells has yet to be established. Similarly, the factors responsible for inhibiting or promoting an in-situ immune response are unclear and there is no agreement as to how the local inflammatory response should be defined.

Measuring the local inflammatory response

Over the past 40 years, a large number of studies, often using different methodologies, have examined the prognostic implications of the local inflammatory response in colorectal cancer. These studies have explored inflammatory cells in different areas of the tumour microenvironment, including the invasive margin (peritumoural), tumour stroma and cancer cell nests. The latter components may be combined into an area termed 'intratumoural' (Figure 1.5). Different methods for defining the local inflammatory response along with their prognostic value in colorectal cancer are summarised below.

Table 1.8. Summary of studies reporting the associations of the local inflammatory response with survival in patients with colorectal cancer. Adapted from Roxburgh et al .

Measure of the local inflammatory response	Total number of studies	% of studies reporting an association with survival
Generalised inflammatory cell infiltrate	39	92%
T lymphocytes		
CD3+ expression	12	83%
CD4+ expression	5	20%
CD8+ expression	25	80%
CD45R0+ expression	8	100%
FOXP3+ expression	7	43%
B lymphocytes	1	0%
Natural Killer (NK) cells	4	75%
Tumour associated macrophages (TAM's)	13	69%
Neutrophils	4	75%
Mast cells	7	86%
Dendritic cells	6	67%
Eosinophils	6	83%

Generalised inflammatory cell infiltrate

In the 1980's, Jass and colleagues suggested that local infiltration of immune cells had independent prognostic value in patients with rectal cancer. Using a semi-quantitative assessment of lymphocytic infiltrate, they reported five year survival rates of 92% for those with a pronounced lymphocyte response compared to 36% for those with a weak response (Jass 1986; Jass, Love et al. 1987). This was followed in the 1990's with a series of studies describing the association of lymphoid aggregates around the tumour, termed the Crohn's-Like reaction (CLR), with improved outcomes in patients with colorectal cancer (Graham and Appelman 1990; Harrison, Dean et al. 1995; Adams and Morris 1997). Since this time, further methods for the assessment of a generalised inflammatory cell reaction have been undertaken by several groups including Nagtegaal and co-workers (Nagtegaal, Marijnen et al. 2001) and Ogino and co-workers (Ogino, Nosho et al. 2009) who both reported associations with survival. A particularly simple technique for assessing local inflammation has recently been proposed by Klintrup and Makinen. Using a semi-quantitative assessment of peritumoural inflammatory infiltrate on haematoxylin and eosin (H&E) stained sections, the authors reported high-grade inflammation at the invasive margin to be an important prognostic indicator in patients with node negative colorectal cancer (Klintrup, Makinen et al. 2005). These findings were subsequently validated in an external cohort of patients with node-negative disease (Roxburgh, Salmond et al. 2009). Overall, there is consistent evidence that a generalised increase in inflammatory cell infiltrate is associated with improved prognosis in patients with colorectal cancer.

Tumour infiltrating lymphocytes

Cells associated with the adaptive immune system have been extensively studied in colorectal cancer. The majority of studies have reported that high numbers of tumour infiltrating

lymphocytes (TILs) are associated with a favourable prognosis but there is debate about which T cell subtypes are most important. The prognostic value of sub-populations of T lymphocytes in colorectal cancer is discussed below.

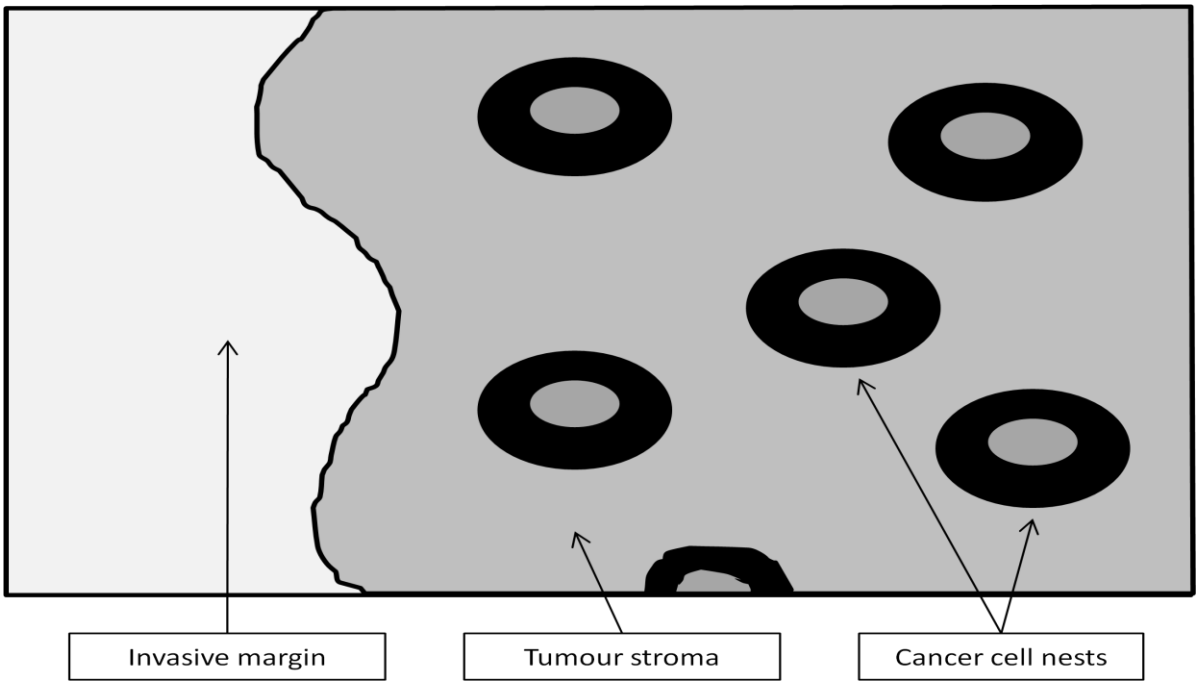


Figure 1.6. Schematic representation of different components within the tumour microenvironment.

CD3+ cells

TILs are predominantly T cells characterized by the presence of the cluster of differentiation 3 (CD3) surface protein. CD3+ antibody is thus used as a marker of global T cell infiltration and has been studied by a number of groups in relation to colorectal cancer outcome. Nagtegaal and colleagues were one of the first to report that CD3+ infiltration was associated with early tumour stage in patients with rectal cancer. Of particular interest was their finding that tumours with low levels of TILs were more likely to have evidence of distant metastases (Nagtegaal, Marijnen et al. 2001). After similar results were reported by other authors (Guidoboni, Gafa et al. 2001; Baeten, Castermans et al. 2006), attention was focused on which areas of the tumour were most important. Galon and co-workers reported that CD3+ infiltration at both the invasive margin and central tumour was associated with reduced recurrence and longer survival in 415 patients with colorectal cancer (Galon, Costes et al. 2006). Furthermore, the authors suggested that an 'immune score' based on the relative densities of CD3+ and CD45RO+ (memory T cells) was a more accurate predictor of survival than tumour stage. One potential confounding factor in the assessment of TIL's as prognostic markers is their association with microsatellite status. It is recognised that lymphocyte infiltration is marked in tumours with high frequency microsatellite instability (MSI-H) and this may explain the better clinical outcomes seen in these patients (Kumar, Chang et al. 2009). However, a number of studies have now reported that while lymphocyte infiltration is higher in MSI-H tumours, the survival relationships of CD3+ are independent of microsatellite status (Guidoboni, Gafa et al. 2001; Laghi, Bianchi et al. 2009; Sinicrope, Rego et al. 2009).

CD8+ cells

The effector cells of the cell mediated immune response, CD8+ cells are often referred to as cytotoxic T lymphocytes (CTLs). Antigens are presented to CD8+ cells in a complex with human leukocyte antigen (HLA) class 1 proteins. Upon encounter with this antigen/HLA complex, CD8+ cells expand and differentiate into CTLs with the capacity to directly destroy tumour cells. This destructive capacity is mediated by the release of perforins which disrupt the cell membrane allowing enzymatic proteases (such as granzyme B) to enter and induce apoptosis of the target cell (Loose and Van de Wiele 2009). The prognostic value of CD8+ cells in colorectal cancer has been studied over a number of years. One of the first to report the value of this T cell subtype was Naito et al who demonstrated that a dense CD8+ infiltration was associated with improved survival in 139 patients with colorectal cancer (Naito, Saito et al. 1998). Looking specifically at the location of lymphocytes in the tumour microenvironment, the group concluded that cytoxic T cells in the cancer cell nests were most closely associated with outcome. Galon and co-workers subsequently investigated the capacity of the adaptive immune response to control tumour behaviour. They demonstrated that an increased intratumoural expression of CD8+ was significantly associated with the absence of early metastatic events and with a decreased rate of disease recurrence in 415 patients with colorectal cancer (Galon, Costes et al. 2006). These findings have been corroborated by a host of other studies and it is now evident that CD8 infiltration in both the invasive margin and central tumour is a marker of good prognosis in colorectal cancer (Guidoboni, Gafa et al. 2001; Nagtegaal, Marijnen et al. 2001; Menon, Janssen-van Rhijn et al. 2004; Baker, Zlobec et al. 2007). Akin to CD3+ infiltration, there has been debate as to whether CD8+ cells are simply a surrogate marker for MSI status. However, there is reliable

evidence that the beneficial effect of CD8+ infiltration is observed in both MSS and MSI colorectal tumours with (Prall, Duhrkop et al. 2004).

Memory T cells

Memory T cells are a subset of T lymphocytes that have previously encountered and responded to an antigen. At a second encounter with the same antigen, these cells can reproduce quickly to mount a faster and stronger immune cell reaction. Memory CD8+ cells are sensitive to such re-stimulation and can become cytotoxic again in a short space of time. Similarly, memory CD4+ cells have comparable characteristics to CD4+ cells but require additional re-stimulation before acting on target cells. The expression of cell surface molecules changes with the loss of L seletin and an alteration of the CD45 isoform from CD45RA+ to CD45RO+. These cells can then be described as 'activated' memory T cells and have the capacity to become armed effector cells on re-exposure to antigen. There is now some evidence that high densities of CD45RO+ cells are beneficial to the host anti-tumour immune response. Indeed, a study conducted by Pages and colleagues reported that high numbers of activated memory T cells in colorectal tumours were associated with fewer indicators of metastatic potential, including less venous and lymphatic invasion (Pages, Berger et al. 2005). Galon and co-workers followed this up with a detailed investigation of the prognostic values of different immune cells in a cohort of over 400 patients with colorectal cancer, concluding that CD45RO+ and CD3+ cells at the invasive margin and central tumour were strong and stage-independent indicators of good prognosis (Galon, Costes et al. 2006).

T regulatory cells

T regulatory cells (Tregs) are a heterogenous group of T lymphocytes which play a unique role in the modulation and control of cell mediated immunity. Originally identified as a subset of CD4+ CD25+ cells, they were thought to act primarily as immunosuppressants. In experimental models, Tregs were shown to reduce the activity of cytotoxic T cells (Chen, Pittet et al. 2005) and their presence was associated with adverse outcomes in breast and ovarian cancer (Curiel, Coukos et al. 2004; Bates, Fox et al. 2006). These findings appeared to support a hypothesis whereby Tregs acted to dampen down any effective host immune response but their role in cancer immunotherapy has recently been re-examined (Zou 2006; Curiel 2007). The transcription forkhead box P3 (FOXP3+) has been identified as a more sensitive marker of Tregs (Sinicrope, Rego et al. 2009) and there is now some evidence that their presence in colorectal tumours may be beneficial. Despite a small-scale study reporting that FOXP3+ expression was not related to survival (Loddenkemper, Schernus et al. 2006), Salama et al recently reported that a high density of intratumoural FOXP3+ cells was associated with improved survival in 967 patients with colorectal cancer (Salama, Phillips et al. 2009). These seemingly contradictory reports regarding the prognostic value of Tregs in different cancer types may be explained by a number of parameters. First, the precise role of Tregs may differ according to tumour stage. Second, because it is supposed that the deleterious effect of Tregs is mediated by their inhibition of effector T cells, it may be that studies which have reported Treg numbers without knowledge of CD8+ density have drawn inaccurate conclusions. Finally, it is recognised that tumour cells and T cells, with or without suppressive functions, may transiently express FOXP3+ (Badoual, Hans et al. 2009). In summary, Tregs may play a role in determining outcome in colorectal cancer but their prognostic value is not well established.

B Lymphocytes

In contrast to the wealth of studies which have examined T cells, few have investigated the prognostic effect of B lymphocytes in human colorectal cancer. A small study by Baeten and co-workers reported that patients with high intratumoural $CD20^+$ counts showed a trend towards improved survival although this did not reach statistical significance (Baeten, Castermans et al. 2006). One reason for this may be that although the CD20 protein is found on the majority of B cells it is not expressed on plasma cells, the effector cell responsible for antibody production.

Natural Killer cells

Natural Killer (NK) cells are part of the innate immune system and, unlike T lymphocytes, have the capacity to eliminate tumour cells that do not express the HLA complex. NK cells can interact with macrophages to incite phagocytosis but also have direct cytotoxic capabilities, particularly in the cytokine-rich tumour environment (Loose and Van de Wiele 2009). Several studies have reported that a strong infiltration of intratumoural NK cells is associated with improved survival and reduced recurrence in patients with colorectal cancer (Nagtegaal, Marijnen et al. 2001; Menon, Janssen-van Rhijn et al. 2004; Atreya and Neurath 2008).

Macrophages

Macrophages are a prevalent inflammatory cell and play an indispensible role in both innate and cell mediated immunity. Macrophages can be activated by a variety of stimuli to differentiate into two functionally different phenotypes. Classically activated macrophages (M1) express a series of pro-inflammatory mediators such as IL-2, IL-23 and TNF-α. In contrast, alternatively activated macrophages (M2) express a wide array of anti-inflammatory

molecules (Mantovani, Sozzani et al. 2002). The prognostic role of tumour-associated macrophages (TAMs) in colorectal cancer has thus far been controversial, perhaps reflecting the different roles of these distinct phenotypes. While TAMs have been associated with proliferation, angiogenesis and tumour growth in a variety of cancers (Siveen and Kuttan 2009; Solinas, Germano et al. 2009), several studies have reported that high numbers of TAMs are associated with improved prognosis in colorectal cancer (Oberg, Samii et al. 2002; Forssell, Oberg et al. 2007). One hypothesis is that M1 TAMs are predominant in colorectal tissue, promoting an anti-tumour immune response (Dumont, Berton et al. 2008)[Dumont 2008]. However, reports have not been consistent and several authors have reported no correlation between TAM infiltration and survival (Baeten, Castermans et al. 2006; Nagorsen, Voigt et al. 2007). The role of TAMs in tumour progression thus appears complex and variable depending on the phenotype involved.

Neutrophils

Neutrophils and polymorphonuclear cells (PMC) are intimately associated with the innate immune system and are some of the first inflammatory cells to respond to tissue damage. Their prognostic value in colorectal cancer has been poorly studied, although there is limited evidence that high numbers of neutrophils are associated with increased survival (Klintrup, Makinen et al. 2005; Baeten, Castermans et al. 2006) and reduced recurrence (Nagtegaal, Marijnen et al. 2001). These studies have observed that while neutrophils are present at the invasive margin, they appear to provide less rigorous prognostic information than other inflammatory cell types.

Mast cells

Mast cells play a key role in the inflammatory response and, when activated, degranulate to release inflammatory mediators including histamine, prostaglandins and leukotrienes. In 1999, a study of nearly 600 patients with colorectal cancer reported that high mast cell numbers were associated with improved survival, independent of tumour stage (Nielsen, Hansen et al. 1999). Subsequent studies have reported that mast cell infiltration in either the central tumour or invasive margin is a marker of good prognosis (Nagtegaal, Marijnen et al. 2001; Acikalin, Oner et al. 2005; Tan, Fan et al. 2005).

Eosinophils

A small number of studies have examined the relationships between eosinophil infiltration and colorectal cancer survival. These studies have used quantitative and semi-quantitative techniques to examine eosinophils in both the tumour centre and the invasive margin. Despite study heterogeneity, there is consistent evidence that a strong infiltration of eosinophils is beneficial (Fisher, Paik et al. 1989; Nielsen, Hansen et al. 1999; Klintrup, Makinen et al. 2005) with the largest study to date suggesting the survival relationships are independent of tumour stage (Nagtegaal, Marijnen et al. 2001).

1.5.2.6 The systemic inflammatory response

Inflammation is the natural reaction to tissue injury caused by mechanical, chemical or microbial stimuli. The systemic inflammatory response is usually a rapid and non-specific response involving a number of key events: vasodilation, increased vascular permeability, cellular activation and coagulation. The complement, kinin and coagulation cascades are triggered, phagocytes activated and, in some cases, an adaptive immune cell response is mounted. The normal physiological response to inflammation is one of stress and consists of alterations in cardiovascular function (increased heart rate and blood pressure) and neuroendocrine control (release of catecholamines, cortisol, antidiuretic hormone, growth hormone, insulin and glucagon). Cytokines, the principle molecules responsible for the initiation and maintenance of inflammation, are released and include interleukins (IL-1 and IL-6), tumour necrosis factor (TNF) and interferons (Heinrich, Castell et al. 1990). The cellular effectors of the inflammatory response, polymorphonucleocytes (PMNs), macrophages and endothelial cells, then come into play and their activation results in the synthesis and secretion of secondary inflammatory mediators such as prostaglandins, leukotrienes, proteases and free radicals. Finally, activation of the coagulation cascade and local thrombosis develops as the injured tissues attempt to wall off damaged areas and prevent blood loss (Davies and Hagen 1997).

Inflammation is thus a normal and usually beneficial physiological response to injury. Problems for the host can arise however if the normal tight controls of the inflammatory response are lost. Loss of these controls results in an exaggerated inflammatory response, clinically identified as systemic inflammatory response syndrome (SIRS) (Figure 1.6). SIRS is often seen as the result of an amplified response to infection (sepsis) but can be triggered by a host of alternative stimuli such as drugs, trauma or malignancy. With a failure of normal

homeostasis there is a flood of inflammatory mediators and the predominant effects of cytokines start to become destructive rather than protective. The uncontrolled vasodilation, fluid shifts, thrombosis and anaerobic metabolism ultimately result in end-organ damage (Bone, Grodzin et al. 1997).

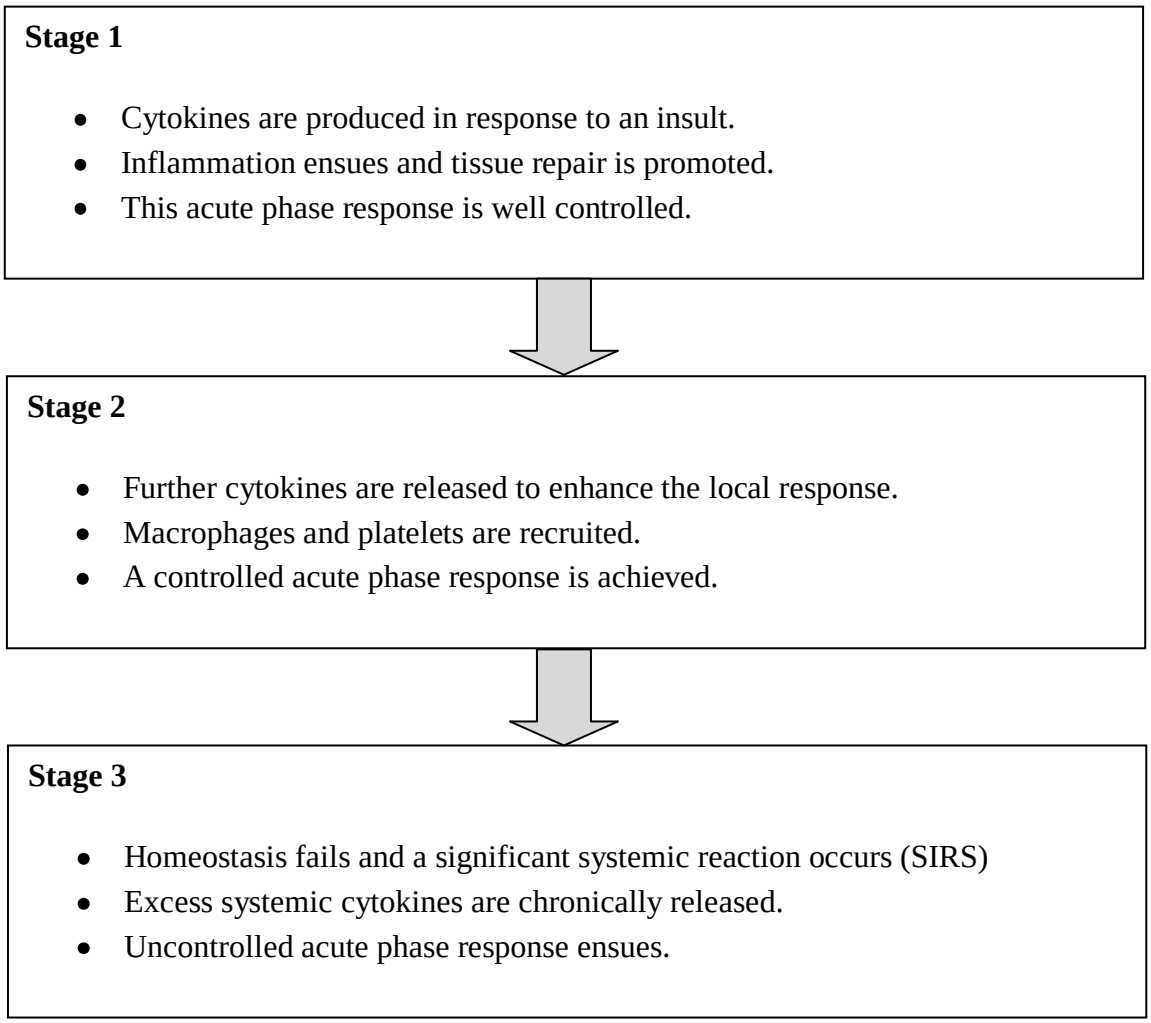


Figure 1.7. Steps involved in the development of a systemic inflammatory response syndrome (SIRS).

Systemic inflammation in the context of malignancy

In recent years it has become clear that the response of the body to cancer is not a unique process but shares many parallels with inflammation and the physiological responses to tissue damage described above (Mantovani, Allavena et al. 2008). The presence of a systemic inflammatory response is a common finding in patients with cancer and malignant tumours have even been described as 'wounds that do not heal' (Dvorak 1986). The reasons that cancers induce an inflammatory response are likely to be complex although it is recognised that some tumours directly produce and secrete growth factors and proinflammatory cytokines (Burke, Relf et al. 1996). Tumour-associated leukocytes and platelets represent alternative inflammatory stimuli while other cytokines and chemokines are inducible by hypoxia, a major physiological difference between tumours and normal tissue (Koong, Denko et al. 2000).

The inflammatory cytokines associated with malignancy may influence the growth, mutation, proliferation and survival of both tumour and surrounding stromal cells. These far reaching effects are mediated through a number of mechanisms including DNA damage, the inactivation of p53, autocrine growth factor functions, angiogenesis and metastatic invasion (Germano, Allavena et al. 2008). Indeed, chemokines may induce cellular proliferation, migration and adhesion (Tricot 2000) and direct evidence for their role in the secondary localisation of cancer has been obtained in mouse models (Wang, Chertov et al. 1998).

Regardless of the initial catalyst, the presence of a systemic inflammatory response in patients with cancer is almost universally considered an indicator of poor prognosis. There is evidence that systemic inflammation is associated with the cachexia and functional decline of patients with advanced disease (McMillan, Preston et al. 1994) and measures of the systemic

inflammatory response have been reported as prognostic markers in a variety of tumour types including lung (Forrest, McMillan et al. 2004), breast (Al Murri, Bartlett et al. 2006) and pancreatic cancer (Glen, Jamieson et al. 2006). The prognostic value of the systemic inflammatory response in colorectal cancer is discussed in below.

Measuring the systemic inflammatory response

The systemic inflammatory response can be measured using a variety of biochemical or haematological markers. One option for detecting the presence of inflammation is to measure the serum concentrations of acute phase proteins; a class of proteins synthesised in the liver whose concentrations change in the presence of inflammation. Positive acute phase proteins including C-reactive protein (CRP), complement and ferritin increase during an inflammatory response while negative acute phase proteins such as albumin and transferrin decrease (Gruys, Toussaint et al. 2005). CRP in particular is now recognised as a sensitive biomarker of inflammation and demonstrates marked and measurable changes in serum concentration (Hogarth, Gallimore et al. 1997). Measuring the numbers of inflammatory cells present in the bloodstream represents an alternative technique for quantifying the presence of an inflammatory response in patients. Total white cell count (WCC), neutrophils, lymphocytes and platelets can all be detected using standard laboratory techniques. In an effort to standardise the measurement of the systemic inflammatory response in patients with cancer, a number of simple 'inflammatory scores' have been described whose values have been shown to correlate directly with clinical outcomes. The modified Glasgow Prognostic Score (mGPS) combines circulating CRP and albumin concentrations (McMillan, Crozier et al. 2007) while the neutrophil lymphocyte ratio (NLR) measures the relative values of neutrophil and lymphocyte counts (Walsh, Cook et al. 2005). Alternative inflammatory scores include total while cell count (Shankar, Wang et al. 2006) and the platelet to lymphocyte ratio (PLR)

(Smith, Ghaneh et al. 2008). The thresholds and allocated values used in these scoring systems are shown in Table 1.9.

Table 1.9. Scoring systems used to describe and measure the systemic inflammatory response.

Scoring system	Score
White cell count (WCC)	
WCC <8.5 (10^9/l)	0
WCC 8.5 - 11.0 (10^9/l)	1
WCC >11 (10^9/l)	2
Neutrophil:Lymphocyte Ratio (NLR)	
NLR <5:1	0
NLR ≥5:1	1
Platelet:Lymphocyte Ratio (PLR)	
PLR < 150:1	0
PLR 150-300:1	1
PLR >300:1	2
The modified Glasgow Prognostic Score (mGPS)	
C-reactive protein ≤10 mg/l and albumin ≥35 g/l	0
C-reactive protein ≤10 mg/l and albumin <35 g/l	0
C-reactive protein >10 mg/l	1
C-reactive protein >10 mg/l and albumin <35 g/l	2

Prognostic value of the systemic inflammatory response in colorectal cancer

It is now clear that inflammation plays a critical role in the pathogenesis, control and eventual metastasis of cancers. Although particular aspects of the host immune response may protect against disease progression (see below), there is evidence that an ongoing systemic inflammatory response is consistently associated with poor outcomes in patients with cancer. These relationships were first described in the early 1980's when concentrations of acute phase proteins were observed to be elevated in patients with a variety of malignancies, especially in those with evidence of metastatic spread (Weinstein, Skinner et al. 1984). Following these initial observations, the prognostic value of the systemic inflammatory response has been reported in a wide variety of tumour types (McMillan, Elahi et al. 2001; Jamieson, Glen et al. 2005; Hara, Matsuzaki et al. 2007). These relationships have been particularly well described in gastrointestinal tumours and there is now evidence that a systemic inflammatory response is associated with impaired response to chemotherapy, early disease recurrence and reduced long term survival in colon and rectal cancer (Goransson, Jonsson et al. 1996; Longo, Virgo et al. 2000; Canna, McMillan et al. 2004; Miki, Konishi et al. 2004; Ishizuka, Nagata et al. 2007; Sharma, Zucknick et al. 2008). Inflammation-based prognostic scores, in particular the mGPS, thus hold the promise of identifying patients at increased risk of disease progression as well as providing well-defined therapeutic targets for future clinical trials.

1.5.2.7 Summary – Host factors and colorectal cancer prognosis

There is consistent evidence that host factors are important determinants of prognosis in patients with colorectal cancer. In particular, the host inflammatory response appears to play a critical role in the development, control and progression of colorectal cancer. Although the relationships between host and tumour are complex, encompassing an array of pro- and anti-tumour responses, certain consistencies in observations are now emerging. It is apparent that a prolonged and inappropriate activation of the systemic inflammatory response is associated with poor outcomes in many solid organ tumour types, including colorectal cancer. Equally apparent are the observations that a strong and coordinated inflammatory response at a local level is beneficial to survival. The cell mediated immune response in particular appears to play a prominent role in protecting against tumour growth and dissemination in colorectal cancer. Overall, emergent evidence suggests that the host inflammatory response in colorectal cancer is an equally, if not more, important determinant of outcome than pathological tumour stage.

2.0 SUMMARY AND AIMS

2.1 Summary

Colorectal cancer is the second most common cause of cancer death in the Western world. Advances in molecular biology have increased our understanding of the genetic pathways involved in colorectal carcinogenesis but the factors which determine disease progression are still unclear. Pathological stage remains the mainstay of colorectal cancer prognosis and decisions regarding the provision of adjuvant chemotherapy are still based on the presence or absence of tumour characteristics first described over 80 years ago. The need for improved prognostic stratification is evidenced by the disparate outcomes observed between tumours of the same AJCC/TNM stage and by the fact that, even in patients undergoing surgery with curative intent, only half will survive to five years.

It is now recognised that tumour growth, recurrence and metastases in colorectal cancer are determined by complex interactions between tumour- and host-related characteristics. A broad concept, deemed 'immunoediting', now exists to describe the process whereby the host immune system may act to either suppress or facilitate tumour progression. With this model in mind, there is consistent evidence that activation of the systemic inflammatory response is associated with poor prognosis in colorectal cancer while an effective local immune cell response confers a favourable outcome. Despite this knowledge, the factors responsible for regulating the systemic and local inflammatory responses in patients with colorectal cancer have yet to be determined.

The underlying basis of the systemic inflammatory response in patients with colorectal cancer is unclear. Previous reports have linked systemic inflammation with increasing burden of comorbidity in patients with benign disease (Sin and Man 2003) but it is uncertain whether

these associations are dependent on particular aspects of host physiology. In addition, there is evidence that inflammation and cancer cachexia are closely related (Argiles, Busquets et al. 2005) and it may be that an activation of the systemic inflammatory response is indicative of, or indeed responsible for, the changes in body composition seen in cancer patients. Finally, septic complications in the postoperative period, associated with early disease recurrence in colorectal cancer (McArdle, McMillan et al. 2005), are recognised to induce a profound inflammatory response (Moyes, Leitch et al. 2009). No study to date has investigated the inter-relationships between systemic inflammation, postoperative complications and disease recurrence in a single cohort of patients with colorectal cancer.

Links between the systemic inflammatory response and the local infiltration of immune cells in patients with colorectal cancer are similarly unclear. Although previous work has suggested that there is no direct relationship, certain aspects of tumour pathology, such as T stage, have been associated with both systemic and local inflammation. Tumour necrosis, a pathological characteristic extensively studied in breast and renal cancer, has recently been linked to certain aspects of inflammation but has been poorly studied in colorectal cancer. No study to date has examined the relationships between tumour necrosis and measures of the systemic and local inflammatory responses in patients with colorectal cancer.

It is now recognised that a strong infiltration of immune cells in and around colorectal tumours is a favourable prognostic sign. Previous work has demonstrated that a strong peritumoural inflammatory response is a stage-independent prognostic factor but the prevalence of individual immune cells and the relative importance of cellular subtypes within this reaction are unknown.

In patients with colorectal cancer the presence of high numbers of tumour infiltrating lymphocytes (TILs) is generally perceived as beneficial but here again there remains a number of outstanding questions. Akin to the systemic response, the factors responsible for initiating and/or maintaining a local inflammatory response are unknown. Many previous studies have investigated lymphocyte subtypes in isolation or have failed to describe their localization within the tumour microenvironment. Fewer still have analysed the relationships between individual immune cells and patient-related characteristics. Finally, although a large number of studies have examined the prognostic impact of immune cells in colorectal tumours, there is considerable disparity in the methodologies and definitions used. No study has yet compared the prognostic utility of different methods of assessing the local inflammatory response in patients with colorectal cancer.

2.2 Aims

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

1. To examine the relationships between patient physiology, the systemic inflammatory response and survival.

2. To compare the performance of surgical scoring systems in high risk patient subgroups, including those with systemic inflammation.

3. To examine the relationships between parameters of body composition and the systemic inflammatory response.

4. To investigate the impact of preoperative risk factors, tumour pathology and postoperative complications on disease recurrence and survival in patients wtih colorectal cancer.

5. To investigate the prognostic value of tumour necrosis in colorectal cancer and to examine its relationships with the systemic and local inflammatory responses.

6. To examine the relationships between an overall measure of peritumoural inflammation, the prevalence of individual immune cells and survival in patients with colorectal cancer.

7. To investigate the clinical utility of the local inflammatory response in primary operable colorectal cancer.

3.0 THE RELATIONSHIPS BETWEEN PATIENT PHYSIOLOGY, THE SYSTEMIC INFLAMMATORY RESPONSE AND SURVIVAL IN PATIENTS WITH PRIMARY OPERABLE COLORECTAL CANCER.

3.1 Introduction

Following potentially curative surgery for colorectal cancer, the prognostic stratification and provision of adjuvant therapy is usually guided by tumour pathology (Figueredo, Coombes et al. 2008). It is increasingly recognised, however, that pathological stage is not the sole determinant of outcome and host-related factors, in particular the systemic inflammatory response, also appear to be important. There is now a considerable body of evidence that markers of systemic inflammation such as the modified Glasgow Prognostic Score can predict survival in patients with primary operable colorectal cancer (Roxburgh and McMillan 2010). This effect seems to be independent of TNM stage or other high-risk pathological features (Ishizuka, Nagata et al. 2007; Koike, Miki et al. 2008).

The basis of the relationship between systemic inflammation and cancer survival is not clear. It has yet to be established which host characteristics, if any, an elevated inflammatory response may represent. It is of interest that a systemic inflammatory response has been reported to predict cardiac events (Lloyd-Jones and Levy 2003) and is associated with patient-related factors such as obesity (Ridker, Buring et al. 2003), diabetes (Dehghan, Kardys et al. 2007) and smoking (Frohlich, Sund et al. 2003). One hypothesis, therefore, is that systemic inflammation may reflect altered patient physiology. Indeed, several studies have reported that abnormal physiology scores are associated with reduced long term survival in patients with colorectal cancer (Brosens, Oomen et al. 2006; Jenkins, O'Neill et al. 2007).

The aim of the present study was to examine the relationship between patient physiology, the systemic inflammatory response and survival in patients undergoing potentially curative resection of colorectal cancer.

3.2 Materials and Methods

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and pre-operative staging CT scan, were considered to have undergone potentially curative resection for colorectal cancer (Stage I – III) between January 1997 and December 2006 in a single surgical unit at Glasgow Royal Infirmary were included in the study. This cohort was identified from a prospectively maintained database and included both elective (> 24 hours from admission) and emergency (< 24 hours from admission) operations. The identification of patients for the study was made primarily through the multi-disciplinary team (MDT) gastrointestinal cancer meetings and it is possible that some emergency cases were not included. Patients with conditions known to acutely or chronically evoke a systemic inflammatory response were excluded. These included (i) pre-operative chemo-radiotherapy, (ii) clinical evidence of infection and (iii) chronic active inflammatory disease such as active rheumatoid arthritis. Patients who died within 30 days of surgery were excluded from the survival analysis. The tumours were staged according to conventional AJCC/TNM classification (5^{th} Edition) (Fleming ID 1997).

Prospectively collected data included patient demographics, pathological characteristics of the tumour, haemotology and biochemistry results. The medical notes were then retrieved and data extracted on patient physiological status. The case notes included surgical pre-operative assessment including details of known comorbidity, smoking status, anaesthetic assessment of cardiovascular function and ECG interpretation, nursing notes and drug prescription charts. Patient physiology was assessed by scoring patients according to the Physiological and Operative Severity Score for the enumeration of Mortality and Morbidity (POSSUM) criteria (Copeland, Jones et al. 1991). The original POSSUM model was chosen as a basis for assessing physiological function for two reasons; (1) the same physiological

variables are also used in the P-POSSUM model which has proved accurate in predicting post-operative mortality following colorectal cancer resection (Poon, Chan et al. 2005; Slim, Panis et al. 2006; Ugolini, Rosati et al. 2009) and (2) the original POSSUM model is the only model developed to predict both morbidity and mortality after surgical resection. Age was excluded from the physiological component of POSSUM and analysed as an independent variable, in line with previous work (Tekkis, Prytherch et al. 2004). The remaining eleven physiological parameters (cardiac disease, respiratory disease, ECG changes, pulse, blood pressure, haemoglobin, white cell count, sodium, potassium, urea and Glasgow Coma Scale) were used to construct a POSSUM physiology score (Table 3.1). Patients were then assigned to one of four groups (score 11 – 14, 15 – 20, 21 – 30, > 30) as previously described (Tekkis, Prytherch et al. 2004).

Deprivation was defined using the Carstairs Deprivation Index (Morris and Carstairs 1991). This is composed of four indicators of deprivation (car ownership, overcrowded housing, Registrar General social class and male unemployment) and has been validated for use within central Scotland (Hole and McArdle 2002). Deprivation scores were based on the postcode of the patients' residence at the time of surgery.

The development and rationale behind the Glasgow Prognostic Score has been described previously (McMillan 2008). Briefly, patients with both an elevated C-reactive protein (>10mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of '2'. Patients in whom neither of these abnormalities was present were allocated a score of '0'. In line with the recent modification of the Glasgow Prognostic Score, patients with an elevated C-reactive protein alone were assigned a score of '1' while those with hypoalbuminaemia alone were

assigned a score of '0'. All measurements of C-reactive protein and albumin were taken within a 24 hour period prior to surgery.

Patients received regular follow-up (3 months, 6 months and then annually to five years) with CT scanning each year and regular colonoscopic surveillance until 5 years post surgery. Information on date and cause of death was cross-checked with that received by the cancer registration system and the Registrar General (Scotland). Overall survival analysis evaluated deaths from any cause in the follow up period. Cancer specific survival evaluated deaths only as a direct result of colorectal cancer. Cancer specific survival was measured from the date of surgery to the date of death from colorectal cancer, with patients who died of other causes censored in the analysis. The study was approved by the West of Scotland Research Ethics Committee (WoSREC), Glasgow.

Statistics

Grouping of all variables was carried out using standard or previously published thresholds. Deaths up to September 2009 were included in the survival analysis. Univariate survival analysis was carried out with Kaplan-Meier curves and long rank testing. Multivariate survival analysis, using the Cox model and a stepwise backward procedure, was carried out for all variables showing a significant association on univariate analysis. Hazard ratios (HR) and 95% confidence intervals (C.I.) were calculated. A p-value of less than 0.05 was considered statistically significant. Inter-relationships between variables were assessed using contingency table analysis with the chi-square test for trend as appropriate. Analysis was performed using SPSS® version 19.0 (IBM SPSS, Illinois, USA).

3.3 Results

Baseline clinico-pathological characteristics for the 320 included patients are shown in Table 3.2. All elective operations were carried out by one of four colorectal surgeons while emergency operations were carried out by on-call general surgeons. All operations were open and details of operative technique were at the discretion of the operating surgeon. The majority of patients were aged 65 years or older (65%), lived in deprived areas (65%) and were current or previous smokers (58%). There was a significant association between smoking history (current or ex) and increasing deprivation (p=0.04). The majority of patients underwent elective operations (96%), had colonic tumours (62%), had well to moderately differentiated tumours (89%) and had node negative disease (60%). The distribution of patients by systemic inflammatory response (mGPS) and POSSUM physiology score is summarised in Table 3.2.

The minimum follow up was 32 months; the median follow up of the survivors was 74 months. During this period 83 patients died of colorectal cancer and there were 53 non-cancer related deaths. The relationships between clinico-pathological characteristics and survival are shown in Table 3.2. On univariate analysis, age (p=0.001), smoking history (p=0.037), presentation (p<0.001), TNM stage (p<0.001), mGPS (p<0.001) and POSSUM physiology score (p<0.001) were significantly associated with cancer specific survival. Age (p<0.001), smoking history (p=0.004), presentation (p=0.001), TNM stage (p=0.004), mGPS (p<0.001) and POSSUM physiology score (p<0.001) were significantly associated with overall survival. The Kaplan Meier survival curves demonstrating the relationships between POSSUM physiology score and both cancer specific (p<0.001; log-rank test) and overall survival (p<0.001; log-rank test) are shown in Figures 3.1 and 3.2 respectively.

Multivariate survival analysis was then carried out. Using cox regression analysis, age (HR 1.46, p<0.01), emergency presentation (HR 2.08, p=0.08), TNM stage (HR 2.39, p<0.001), mGPS (HR 1.78, p<0.001), and POSSUM physiology score (HR 1.38, p=0.02) were independently associated with cancer specific survival. Age (HR 1.64, p<0.001), smoking history (HR 1.52, p=0.02), TNM stage (HR 1.64, p<0.001), mGPS (HR 1.60, p<0.001), and POSSUM physiology score (HR 1.27, p=0.03) were independently associated with overall survival (Table 3.3).

In the group of patients with Stage III disease, we noted a significant association between POSSUM physiology score and the likelihood of adjuvant therapy being administered (X^2 = 9.94, df = 3, p = 0.019). Of the 129 patients with Stage III disease, 46 patients (36%) received adjuvant therapy and 83 patients (64%) did not. In patients with physiology score 11 – 14, 21 patients (51%) received adjuvant therapy; physiology score 15 – 20, 19 patients (35%) received adjuvant therapy; physiology score 21 – 30, 6 patients (21%) received adjuvant therapy; physiology score > 30, no patient received adjuvant therapy. However, there was no significant association between the systemic inflammatory response and the likelihood of adjuvant therapy being administered in patients with Stage III.

The relationships between POSSUM physiology score and clinico-pathological characteristics are shown in Table 3.4. There was a significant relationship between POSSUM physiology score and mGPS (p=0.006). A higher POSSUM physiology score was also significantly associated with increasing age (p<0.001), tumours of colonic origin (p<0.001) and advanced TNM stage (p<0.05). POSSUM physiology score was significantly related to all its component variables except potassium level (p=0.11) and Glasgow Coma Scale, the latter of which was uniformly normal. The individual physiological variables that

contributed most to elevated POSSUM physiology score were low haemoglobin level (202/320), abnormal systolic blood pressure (192/320) and impaired cardiac function (166/320). Those that contributed least were sodium level (25/320), potassium level (21/320) and GCS (0/320). (Table 3.4).

The relationships between POSSUM physiology score and mGPS were then examined in more detail by calculating the mean score for each of the physiological variables. This demonstrated significant associations between mGPS and the individual physiological variables of abnormal pulse rate ($p=0.008$), raised white cell count ($p=0.05$), low sodium ($p<0.001$), raised potassium ($p=0.01$) and low haemoglobin (<0.001) (Figure 3.3).

3.4 Discussion

The results of the present study show that pre-operative measures of impaired patient physiology, such as elevated POSSUM physiology scores, are significantly associated with poorer cancer specific and overall survival in patients undergoing potentially curative resection of colorectal cancer. However, when considered with age, TNM stage, smoking status and the systemic inflammatory response (mGPS), the POSSUM physiology score was reduced in statistical significance. Although the POSSUM physiology score was strongly associated with mGPS, multivariate survival analysis demonstrates that both were independent predictors, suggesting that poor patient physiology alone cannot fully explain the relationship between systemic inflammation and reduced survival.

The results of the present study are consistent with previous work. Jenkins and co-workers (2007) reported that, using the same thresholds, there was a significant association between an elevated POSSUM physiology score and poorer cancer specific survival in 432 patients with colorectal cancer (Jenkins, O'Neill et al. 2007). Brosens and colleagues (2006) also reported that, in 542 colorectal cancer patients, there was an association between POSSUM physiology score and overall survival using 'low' and 'high' risk groups based on the median physiology score (Brosens, Oomen et al. 2006).

Given that the POSSUM score was developed to predict post-operative mortality and morbidity, the basis of this relationship with long term survival is not clear. One explanation is that poor patient physiology is associated with an increased likelihood of post-operative complications such as an anastomotic leak; recognised to be associated with early recurrence and cancer death, independent of tumour stage (McArdle, McMillan et al. 2005; Jung, Yu et al. 2008; Marra, Steffen et al. 2009). Another possible explanation, examined in the present

study, is that a pre-operative systemic inflammatory response reflects, in part, abnormal patient physiology. It is of interest therefore that Moyes and coworkers recently reported that, in 455 patients undergoing colorectal cancer surgery, the pre-operative mGPS was independently associated with an increased risk of developing post-operative infectious complications (Moyes, Leitch et al. 2009). It remains to be determined whether infectious complications are the basis of the relationships between patient physiology, systemic inflammation and cancer specific survival. Of interest, we noted that patients with deranged physiology were significantly less likely to receive adjuvant therapy for Stage III tumours. However, when patients with node-negative disease were considered separately, patient physiology was again found to predict cancer specific survival, suggesting the survival benefit of good physiology is independent of the influence of chemotherapy.

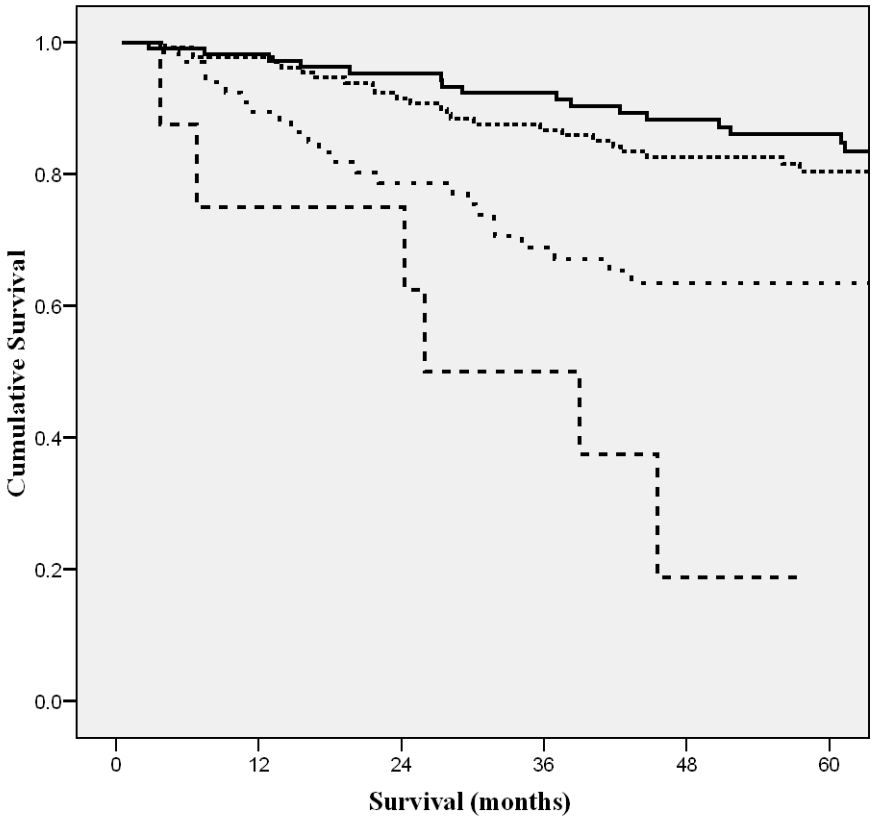
In the present study the individual physiological components associated with the mGPS were an elevated pulse rate, low haemoglobin and high WCC, as well as the biochemical abnormalities of low sodium and raised potassium. It may be that poor cardiac function in these patients, combined with anaemia, leads to relative tissue hypoxia. Indeed, it is recognised that tissue hypoxia is a potent stimulator of local and systemic inflammation (Wouters 2005; Zinkernagel, Johnson et al. 2007). If this were to be the case, it might be expected that systemic inflammation would be closely associated with pathological markers of tissue hypoxia such as tumour necrosis. Further work is needed to define such relationships.

The results from the present study have a number of implications. The POSSUM scoring systems have already proven accurate in predicting post-operative mortality (Senagore, Warmuth et al. 2004; Ferjani, Griffin et al. 2007) and morbidity (Menon and Farouk 2002;

Valenti, Hernandez-Lizoain et al. 2009) after colorectal cancer surgery. Clearly, a single scoring system that would allow assessment of post-operative outcomes and predict long-term cancer survival would be advantageous. Both the POSSUM score and mGPS have the potential to offer such an assessment. However, the POSSUM physiology score has eleven component variables, some of which may not be routinely recorded. In contrast, the mGPS has only two components, is easier to construct and may therefore be less subject to interpretative error. It remains to be determined which system will be most useful in predicting both short term and long outcome in patients undergoing surgery for colorectal cancer.

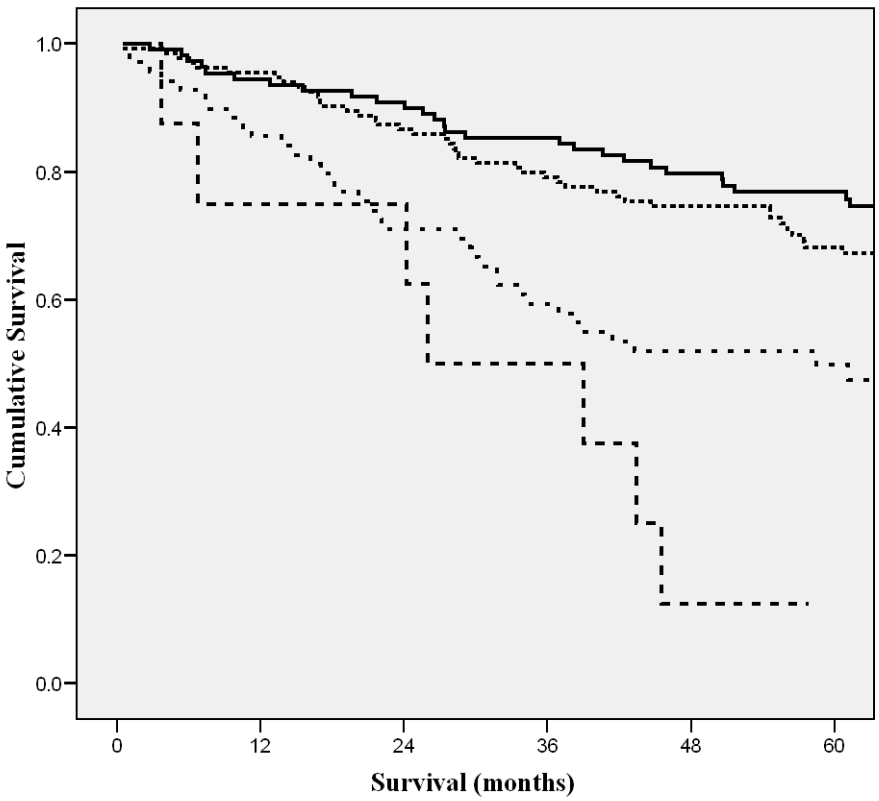
These results also suggest that the pre-operative targeting of patient physiological and inflammatory status may represent a novel approach to improving outcomes in patients with cancer. There is already some evidence that 'host-related' targets may be of considerable importance. For example, the use of statins has recently been reported to improve survival from colorectal cancer, possibly by improvement in cardiovascular status (Siddiqui, Nazario et al. 2009). The attenuation of the systemic inflammatory response and the improvement of oxygen delivery to the tissues represent other possibilities.

In summary, patient physiology and the systemic inflammatory response are strongly associated. However, POSSUM physiology score and mGPS were independent predictors of cancer specific and overall survival in patients with primary operable colorectal cancer.



Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5
Group 1	109	102	97	91	78
Group 2	134	126	113	103	85
Group 3	69	57	47	39	29
Group 4	8	6	6	3	1

Figure 3.1. The relationship between POSSUM physiology score and cancer-specific survival in patients undergoing potentially curative resection for colorectal cancer. Groups 1 – 4 are shown top to bottom ($p<0.001$; log-rank test).



Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5
Group 1	109	103	99	92	78
Group 2	134	128	116	104	88
Group 3	69	59	48	40	29
Group 4	8	6	6	4	1

Figure 3.2. The relationship between POSSUM physiology score overall survival in patients undergoing potentially curative resection for colorectal cancer. Groups 1 – 4 are shown top to bottom ($p<0.001$; log-rank test).

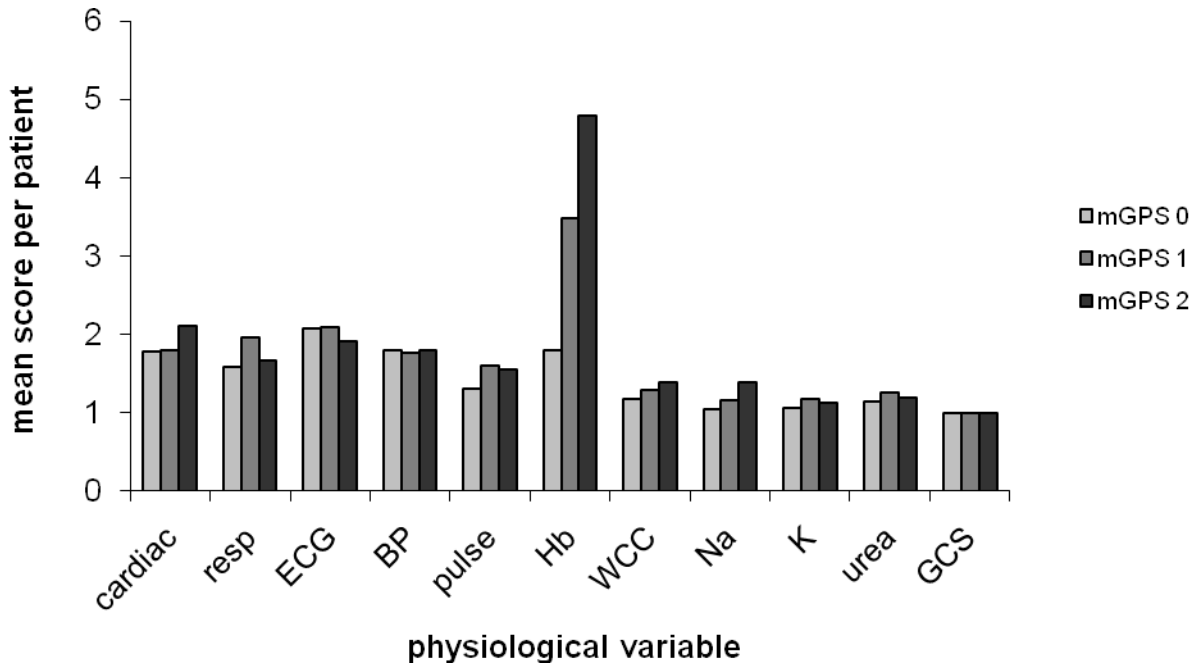


Figure 3.3. Graphic representation demonstrating the relationship between mGPS and individual POSSUM physiological variables.

Table 3.1. Physiological variables used in the construction of the POSSUM physiology score. (Age is excluded from the original physiology score and is analysed in the study as an independent variable).

POSSUM physiology score	**1**	**2**	**4**	**8**
			Oedema	JVP
Cardiac	normal	Cardiac drugs		
			Warfarin	Cardiomegaly
		SOB exertion	SOB stairs	SOB rest
Respiratory	normal			
		Mild COPD	Mod COPD	Fibrosis
E.C.G.	normal	-	AF (60-90)	Other abnormality
Systolic B.P.		131-170	≥ 171	
	110-130			≤ 89
(mmHg)		100-109	90-99	
Pulse		81-100		≥ 120
	50-80		101-120	
(beats/min)		40-49		≤ 39
Haemoglobin		11.5-12.9	10-11.4	≤ 9.9
	13-16			
(g/dL)		16.1-17	17.1-18	≥ 18.1
White cell count		10.1-20	≥ 20.1	
	4-10			-
($x10^{12}$/L)		3.1-3.9	≤ 3	
Sodium				
	≥ 136	131-135	126-130	≤ 125
(mmol/L)				
Potassium		3.2-3.4	2.9-3.1	≤ 2.8
	3.5-5			
(mmol/L)		5.1-5.3	5.4-5.9	≥ 6
Urea				
	≤ 7.5	7.6-10	10.1-15	≥ 15.1
(mmol/L)				
G.C.S.	15	12-14	9-11	≤ 8

Table 3.2. The relationship between clinico-pathological variables and survival in patients undergoing potentially curative resection for colorectal cancer; univariate survival analysis.

Variable	**320 (%)**	**Cancer-specific survival**		**Overall survival**	
		Hazard ratio	**p-value**	**Hazard ratio**	**p-value**
		(95% C.I.)		**(95% C.I.)**	
Age					
≤64	111 (35)				
65-74	102 (32)				
≥75	107 (33)	1.66 (1.26, 2.18)	<0.001	1.80 (1.45, 2.23)	<0.001
Sex					
Male	170 (53)				
Female	150 (47)	1.30 (0.84, 2.01)	0.25	1.18 (0.84, 1.66)	0.34
Deprivation					
1-2	12 (4)				
3-5	99 (31)				
6-7	209 (65)	1.11 (0.74, 1.65)	0.46	0.98 (0.72, 1.32)	0.77
Smoking					
Never	135 (42)				
Current/previous	185 (58)	1.62 (1.02, 2.55)	0.04	1.67 (1.17, 2.39)	0.004
Presentation					
Elective	307 (96)				
Emergency	13 (4)	3.93 (1.80, 8.56)	<0.001	3.00 (1.53, 5.94)	0.001
Tumour site					
Colon	197 (62)				
Rectum	123 (38)	0.84 (0.53, 1.32)	0.45	1.09 (0.77, 1.54)	0.62
Differentiation					
Well/moderate	286 (89)				
Poor	34 (11)	1.26 (0.63, 2.52)	0.51	1.58 (0.96, 2.59)	0.07
TNM stage					
Stage I	38 (12)				
Stage II	153 (48)				
Stage III	129 (40)	2.21 (1.51, 3.21)	<0.001	1.60 (1.21, 2.10)	0.004
Adjuvant therapy					
No	254 (79)				
Yes	66 (21)	1.00 (0.59, 1.69)	0.99	0.90 (0.59, 1.37)	0.61
mGPS					
Low (0)	194 (61)				
Intermediate (1)	90 (28)				
High (2)	36 (11)	1.71 (1.29, 2.27)	<0.001	1.60 (1.28, 2.01)	<0.001
POSSUM physiology score					
Group 1 (11-14)	109 (34)				
Group 2 (15-20)	134 (42)				
Group 3 (21-30)	69 (21)				
Group 4 (>30)	8 (3)	1.73 (1.33, 2.25)	<0.001	1.59 (1.29, 1.96)	<0.001

Table 3.3. The relationship between clinico-pathological variables and survival in patients undergoing potentially curative resection for colorectal cancer; multivariate survival analysis.

Variable	Cancer-specific survival		Overall survival	
	Hazard ratio (95% C.I.)	p-value	Hazard ratio (95% C.I.)	p-value
Age	1.46 (1.10, 1.94)	<0.01	1.64 (1.32, 2.05)	<0.001
Smoking	1.46 (0.92, 2.32)	0.10	1.52 (1.06, 2.18)	0.02
Presentation	2.08 (0.91, 4.76)	0.08	1.70 (0.84, 3.45)	0.14
TNM stage	2.39 (1.59, 3.59)	<0.001	1.64 (1.22, 2.20)	<0.001
mGPS	1.78 (1.32, 2.41)	<0.001	1.60 (1.26, 2.02)	<0.001
POSSUM physiology score	1.38 (1.05, 1.82)	0.02	1.27 (1.02, 1.58)	0.03

Table 3.4. The relationships between POSSUM physiology score and clinico-pathological characteristics in patients undergoing potentially curative resection for colorectal cancer.

Variable	Group 1 11 - 14 (n=109)	Group 2 15 - 20 (n=134)	Group 3 21 - 30 (n=69)	Group 4 > 30 (n=8)	p-value
POSSUM variables					
Cardiac (1/2/4/8)	82/27/0/0	66/60/8/0	6/40/20/3	0/1/5/2	<0.001
Resp. (1/2/4/8)	86/23/0/0	80/39/13/2	32/15/19/3	2/2/3/1	<0.001
E.C.G. (1/4/8)	109/0/0	125/3/6	26/12/31	2/1/5	<0.001
S.B.P. (1/2/4/8)	59/50/0/0	43/75/15/1	26/35/7/1	0/4/4/0	<0.001
Pulse (1/2/4/8)	83/26/0/0	80/46/8/0	37/27/5/0	4/3/1/0	0.01
Hb. (1/2/4/8)	71/37/1/0	33/36/43/22	14/14/13/28	0/3/2/3	<0.001
W.C.C. (1/2/4)	104/4/1	103/28/3	49/19/1	1/6/1	<0.001
Sodium (1/2/4/8)	107/2/0/0	121/10/3/0	62/5/2/0	5/2/1/0	0.008
Potassium (1/2/4/8)	106/3/0/0	123/7/4/0	64/4/1/0	6/1/1/0	0.11
Urea (1/2/4/8)	107/2/0/0	114/16/4/0	62/6/1/0	2/1/4/1	<0.001
G.C.S. (1/2/4/8)	109/0/0/0	134/0/0/0	69/0/0/0	8/0/0/0	N/A
Age					
≤64/65-74/≥75	57/31/21	41/41/52	13/27/29	0/3/5	<0.001
Sex					
Male/female	64/45	64/70	38/31	4/4	0.38
Smoking					
Never/current or ex	54/55	55/79	25/44	1/7	0.09
Presentation					
Elective/emergency	107/2	127/7	66/3	7/1	0.34
Tumour site					
Colon/rectum	51/58	85/49	54/15	7/1	<0.001
Deprivation					
1-2/3-5/6-7	5/42/62	5/39/93	2/19/48	0/2/6	0.47
Differentiation					
Well or mod/poor	101/8	122/12	57/12	6/2	0.08
TNM stage					
I/II/III	22/46/41	13/67/54	3/37/29	0/3/5	0.03
mGPS					
0/1/2	80/22/7	77/44/13	34/21/14	3/3/2	0.006

4.0 THE ACCURACY OF SCORING SYSTEMS IN THE PREDICTION OF OPERATIVE MORTALITY FOLLOWING RESECTION OF COLORECTAL CANCER.

4.1 Introduction

The majority of patients with diagnosis of colorectal cancer undergo surgical resection and hospitals increasingly require robust and accurate systems for recording operative outcomes (Russell, Bruce et al. 2003). Operative mortality is often used as an indirect measure of quality of care but population heterogeneity means comparing crude mortality rates can be misleading. The use of standardised risk-adjustment models allows fair comparison of outcome by correcting for the confounding effects of case-mix (Sagar, Hartley et al. 1994; Jones and de Cossart 1999).

Several different predictive models have been proposed for patients undergoing surgical resection of colorectal cancer. One of the earliest, the Physiologic and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM) model (Copeland, Jones et al. 1991), was developed for use within general operative practice. The original model, however, consistently over-predicted death in low risk patients, leading to the Portsmouth modification (P-POSSUM) (Whiteley, Prytherch et al. 1996; Prytherch, Whiteley et al. 1998). Recently, a specialty-specific model known as colorectal POSSUM (CR-POSSUM) has been developed for use within benign and malignant colorectal surgery (Tekkis, Prytherch et al. 2004).

Conflicting reports regarding the ability of P-POSSUM and CR-POSSUM to predict colorectal cancer outcome (Senagore, Warmuth et al. 2004; Slim, Panis et al. 2006; Leung, Ferjani et al. 2009), coupled with the complexity of their construction, has limited the use of either model in routine clinical practice. In an effort to combat these shortcomings, a

simplified risk-adjustment model was developed by the Association of Coloproctology of Great Britain and Ireland (ACPGBI) (Tekkis, Poloniecki et al. 2003). Dedicated to estimating operative mortality following colorectal cancer surgery, the model was designed for use in both clinical audit and preoperative counselling and required only five widely recorded variables; age, American Society of Anaesthesiology (ASA) grade, cancer stage, operative urgency and resection status. In 2010, the online version of the ACPGBI model was revised to include age, ASA grade, cancer stage, operative urgency and operation type (minor/intermediate/major/complex) (http://www.riskprediction.ork.uk). To our knowledge, the revised ACPGBI model has yet to be externally validated in a cohort of patients undergoing colorectal cancer resection at a single institution.

The aim of the present study, therefore, was to compare the performance of the revised ACPGBI model, the original ACPGBI model, P-POSSUM and CR-POSSUM in the prediction of operative mortality following potentially curative resection of colorectal cancer. Specifically, we wanted to assess model performance in high risk subgroups, including emergency cases and patients with high ASA grades or evidence of an elevated systemic inflammatory response.

4.2 Materials and Methods

Patients with colorectal cancer who were considered to have undergone potentially curative resection of colorectal cancer between January 1st 1997 and December 31st 2007 at Glasgow Royal Infirmary were identified from the same prospective database described in Chapter 3.

Preoperative patient characteristics were recorded including age, sex, smoking status, mode of presentation, ASA grade and systemic inflammatory response as measured by the modified Glasgow Prognostic Score (mGPS). Operative details included tumour site (colon or rectum) and operation type. The tumours were staged according to conventional AJCC/TNM classification (5th Edition) (Fleming ID 1997).

The risk-adjustment models were then constructed. Physiological, operative and pathological variables were recorded according to the P-POSSUM, CR-POSSUM, ACPGBI (original) and ACPGBI (revised) criteria (Table 4.1). Variables required for the construction of the ACPGBI models were collected prospectively as part of the core data set within the colorectal cancer database. Additional data required for the construction of the P-POSSUM and CR-POSSUM models was recorded retrospectively from health records. Missing data, limited to 'evidence of peritoneal soiling' in 20 cases (5%) and 'blood loss' in 14 cases (3%), were allocated normal values and included in the analysis, in line with published recommendations (Senagore, Warmuth et al. 2004). The predicted risk of mortality using each model was generated using an online calculator according to the equations shown in Table 4.1. The study was approved by the West of Scotland Research Ethics Committee (WoSREC), Glasgow.

Statistics

Observed operative mortality was defined as death within 30 days of operation. Predicted mortality rates for each model are represented as the mean value with 95% confidence intervals (CI). Observed and expected mortality rates were compared to generate observed to expected (O:E) ratios. Model calibration (the ability of the model to assign the correct probabilities of outcome to individual patients) was assessed using the Hosmer-Lemeshow goodness-of-fit test. Model discrimination (the ability of the model to assign higher probabilities of outcome to patients who died than to those who did not) was assessed by measuring the area under the Receiver Operator Characteristic curve (AUC). Statistical analysis was performed using SPSS® version 19.0 (IBM SPSS, Illinois, USA).

4.3 Results

A total of 423 patients who underwent potentially curative resection of colorectal cancer were included. Baseline clinical, pathological and operative characteristics of the patients are shown in Table 4.2. The majority of patients were younger than 75 years (65%) with a similar number of males and females. Preoperative assessment recorded 43% of patients as having never smoked and 54% of patients as ASA grade I or II. In terms of systemic inflammatory response 58% were mGPS 0, 27% were mGPS 1 and 15% were mGPS 2. The majority of operations (93%) were undertaken on an elective basis and all resections were performed via open surgery. The site of the primary tumour was the colon in 62% and the rectum in 38%. Ninety percent of operations involved an anastomosis with the remainder either a Hartmann`s procedure (3%) or an abdominoperineal resection of rectum (APR) (7%). Pathological reports classified the tumours as Stage 1 (12%), Stage II (44%) or Stage III (44%) (Table 4.2).

In the postoperative period a total of seventeen patients died (30 day mortality = 4.0%). The causes of death were cardiovascular complications in 8 patients, respiratory complications in 5 patients, intra-abdominal sepsis in 3 patients and cerebrovascular accident (CVA) in 1 patient. The relationships between clinico-pathological variables and operative mortality are shown in Table 4.2. The patient subgroups identified as at significantly increased risk of operative mortality were elderly patients ($p<0.001$) and those with high ASA grade ($p=0.008$). There was a trend between both emergency presentation ($p=0.073$) and increased mGPS ($p=0.072$) and increased rates of operative mortality although this did not reach statistical significance.

The summary analyses of model performance in the prediction of operative mortality are shown in Table 4.3. In terms of numbers of deaths, the rate expected by the revised ACPGBI model was closest to the observed rate although there was no statistically significant difference in the rates predicted by the revised ACPGBI model and P-POSSUM. In terms of mortality ratios, the calculated O:E ratio was closest to '1' using the revised ACPGBI model. In terms of discrimination, CR-POSSUM attained the largest AUC (0.84) but there was no significant difference between any of the models with overlapping confidence intervals in all cases. In terms of calibration, the revised ACPGBI model attained the largest p-value (0.20) but all four models fitted the data adequately ($p \geq 0.05$, demonstrating no significant lack of fit) (Table 4.3).

The analyses of model performance across high and low risk patient subgroups are shown in Table 4.4. Considering the highest risk subgroups; in patients 75 years or older, the revised ACPGBI model predicted mortality most closely; in emergency presentation, the revised ACPGBI model and P-POSSUM predicted mortality equally well; in patients with high ASA grades, CR-POSSUM predicted mortality most closely and in patients with systemic inflammation the revised ACPGBI performed the most accurately. Considering the lowest risk subgroups; in patients 64 years or younger, in those presenting electively and in those with ASA grade I, the revised ACPGBI model again predicted mortality most closely (Table 4.4).

4.4 Discussion

The present study reports the revised ACPGBI risk-adjustment model to be a simple and accurate predictor of operative mortality in patients undergoing potentially curative resection of colorectal cancer. Furthermore, the recent revision appears to have improved the performance of the model.

The ability of risk-adjustment models to accurately predict outcome is often judged by their performance across low or high risk patient subgroups. The present study confirmed operative mortality rates to be highest in elderly patients, those with high ASA grades and in those presenting as an emergency. In addition, a novel finding of the present study was that patients with evidence of a preoperative systemic inflammatory response appear to be at increased risk of death in the postoperative period. Although no single model performed consistently well across all these high risk subgroups, the revised ACPGBI model performed well in elderly patients, emergency cases and in those with systemic inflammation. Previously, it has been reported that the original ACPGBI model performed poorly in emergency colorectal cancer surgery (Metcalfe, Norwood et al. 2005; Ferjani, Griffin et al. 2007); an observation confirmed in the present study. The reasons behind an improved performance of the revised model in the emergency setting are not entirely clear. One explanation may be that all patients included in the present study underwent primary resection. The revised ACPGBI model, which substitutes 'resection status' for 'operation type', may therefore have offered additional stratification to the emergency cases.

The accurate prediction of mortality in low risk groups has also been a documented weakness of previous risk-adjustment models. Indeed, the phenomenon of over-prediction of events in low risk groups was the original stimulus for the development of P-POSSUM (Whiteley,

Prytherch et al. 1996). Although all four models examined in the present study over-predicted death in the youngest patients and in those undergoing elective surgery, the revised ACPGBI model performed best in both instances. The mortality rates following elective colorectal cancer surgery are often extremely low; possibly the result of sub-specialisation (Zorcolo, Covotta et al. 2003), high volume centres (Hillner, Smith et al. 2000) or improvements in perioperative management (de Leon, Pezzi et al. 2009). For a model to perform well in low risk patients, the lowest attainable score must reflect this level of risk and it appears the revised ACPGBI model achieves this.

The present study suggests the revised ACPGBI model has more predictive value in colorectal cancer surgery than the specialty-specific model, CR-POSSUM. A possible explanation for the poor performance of CR-POSSUM in the present cohort may be gained by examining the dataset on which the model was developed. The CR-POSSUM model was based on the examination of surgical outcome following approximately 7000 colorectal operations which included a large proportion of minor and benign cases (Tekkis, Prytherch et al. 2004). This population heterogeneity, albeit within a colorectal specialty, may compromise the models' accuracy when applied to major colorectal cancer resection. The revised ACPGBI model, in contrast, was developed using data from over 7000 patients exclusively undergoing resection of colorectal cancer which may explain its enhanced performance in the present cohort.

This study refers only to patients undergoing open colorectal cancer resection and these results may not be applicable to patients undergoing laparoscopic resection. It may be surmised, however, that as none of the variables in the ACPGBI model are attributable to operative technique, the scores generated for individual patients would be unaltered. If this

were so, the performance of the model would only be changed if laparoscopic resection impacted on operative mortality rates; current evidence suggests this not to be the case (Reza, Blasco et al. 2006). Future studies should aim to assess the performance of the model in patients undergoing laparoscopic resection of colorectal cancer.

The accuracy of all predictive models, however, must be interpreted with caution. Although the reporting of O:E ratios is a simple method for comparing model performance, it must be remembered that disparate observed and expected rates may be a reflection of surgical under- or over-performance rather than model inaccuracy. The present study attempted to counter this by supplying additional data on the discrimination and calibration of each model. Although such statistics can help quantify the ability of a model to assign the correct probabilities of outcome to each patient, it should be remembered that these models were designed to predict population rather than individual mortality risk.

The development of an accurate scoring system for the prediction of operative mortality following colorectal cancer surgery is a major challenge. An ideal system would be simple to construct, rely on readily available objective data and perform consistently across low and high risk patient subgroups. The revised ACPGBI model fulfils many of these criteria and may have several important clinical applications. First, consistent performance across low risk elective patients means the model could be an important audit tool for comparing individual surgeon or hospital performance. An accurate estimation of mortality for high risk patients would not only facilitate the consent process but could play an active role in surgical decision-making. Currently, clinicians often rely on subjective judgment in such cases, while patients often misinterpret the level of risk (Ravitch 1989). The addition of a predictive

model would add an important objective element to this process, benefiting both patient and clinician.

In summary, the present study reports the revised ACPGBI model to be the most simple and accurate predictor of operative mortality following potentially curative resection of colorectal cancer.

Table 4.1. The variables used in the construction of the P-POSSUM, CR-POSSUM and ACPGBI models including the equations used to calculate the risk (R) of mortality.

Model	Physiological variables	Operative variables
P-POSSUM	Age (years)	Operation category
	Cardiac failure	Number. of procedures
	Respiratory status	Total blood loss (ml)
	E.C.G.	Peritoneal soiling
	Systolic B.P. (mmHg)	Cancer stage
	Pulse (beats/min)	Operative urgency
	Haemoglobin (g/dL)	
	White cell count ($x10^{12}$/L)	
	Sodium (mmol/L)	
	Potassium (mmol/L)	
	Urea (mmol/L)	
	Glasgow Coma Score	
Mortality equation: Log [R/(1-R)] = -9.065 + (0.16 x physiological score) + (0.15 x operative score)		
CR-POSSUM	Age (years)	Operative severity
	Cardiac failure	Peritoneal soiling
	Systolic B.P. (mmHg)	Total blood loss (ml)
	Pulse (beats/min)	Operative urgency
	Urea (mmol/L)	Cancer stage
	Haemoglobin (g/dL)	
Mortality equation: Log [R/(1-R)] = -9.167 + (0.33 x physiological score) + (0.30 x operative score)		
ACPGBI (original)	Age (years)	Cancer stage
	ASA grade	Operative urgency
		Cancer resection status
Mortality equation: Log [R/(1-R)] = (-4.859 + total score)		
ACPGBI (revised)	Age (years)	Cancer stage
	ASA grade	Operative urgency
		Operative procedure
Mortality equation: Log [R/(1-R)] = (-4.859 + total score)		

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Table 4.2. The relationships between clinico-pathological characteristics and 30-day mortality in patients undergoing colorectal cancer surgery with curative intent.

Variable	Patient group	423 (%)	30 day mortality n (%)	p-value
Age (years)	≤ 64	140 (33)	0 (0)	
	65 – 74	135 (32)	4 (3)	
	≥ 75	148 (35)	13 (9)	<0.001
Sex	Male	230 (54)	12 (5)	
	Female	193 (46)	5 (3)	0.171
Smoking Status	Non-smoker	183 (43)	5 (3)	
	Ex-smoker	150 (36)	8 (5)	
	Current smoker	90 (21)	4 (4)	0.381
Presentation	Elective	395 (93)	14 (4)	
	Emergency	28 (7)	3 (10)	0.073
ASA Grade	ASA I	52 (12)	0 (0)	
	ASA II	178 (42)	5 (3)	
	ASA III	168 (40)	9 (5)	
	ASA IV	25 (6)	3 (12)	0.008
Tumour Site	Colon	264 (62)	11 (4)	
	Rectum	159 (38)	6 (4)	0.842
Operation	Right hemicolectomy	130 (31)	3 (2)	
	Sigmoid colectomy	79 (16)	3 (4)	
	Subtotal colectomy	13 (3)	1 (8)	
	Colonic unspecified	44 (10)	2 (7)	
	Hartmann’s procedure	12 (3)	1 (8)	
	Anterior resection	126 (30)	3 (2)	
	APR	28 (7)	3 (11)	0.416
TNM stage	Stage I	51 (12)	5 (10)	
	Stage II	186 (44)	5 (3)	
	Stage III	186 (44)	7 (4)	0.211
mGPS	0	246 (58)	8 (3)	
	1	114 (27)	3 (3)	
	2	63 (15)	6 (10)	0.072

Table 4.3. Comparison of P-POSSUM, CR-POSSUM and ACPGBI models in the prediction of 30-day operative mortality following colorectal cancer surgery with curative intent.

Model	Observed Mortality[a]	Performance Indicators			
		Exp. Mortality[b]	O:E ratio[c]	Discrimation[d]	Calibration[e]
P-POSSUM	4.0	4.6 (3.79-5.34)	0.87	0.79 (0.71-0.88)	10.63, p = 0.06
CR-POSSUM	4.0	6.3 (5.65-6.94)	0.63	0.84 (0.79-0.90)	15.84, p = 0.05
ACPGBI (original)	4.0	6.9 (6.31-7.55)	0.58	0.76 (0.68-0.84)	14.23, p = 0.08
ACPGBI (revised)	4.0	3.8 (3.43-4.27)	1.05	0.73 (0.63-0.82)	11.02, p = 0.20

[a] Observed 30 day mortality rate
[b] Expected 30 day mortality rate (95% confidence intervals)
[c] O:E ratio represents the observed to expected mortality ratio.
[d] Discrimination is measured by the area under the Receiver-Operator Characteristic curve (95% confidence intervals): higher values represent better model discrimination.
[e] Calibration is measured by the Hosmer-Lemeshow statistic: smaller values and larger p-values represent better model calibration.

Table 4.4. Comparison of the performance of P-POSSUM, CR-POSSUM and ACPGBI models in high risk patient subgroups.

Patient subgroup	Observed Mortality (%)	P-POSSUM		CR-POSSUM		ACPGBI (original)		ACPGBI (revised)	
		Exp[a]	O:E ratio[b]	Exp[a]	O:E ratio[b]	Exp[a]	O:E ratio[b]	Exp[a]	O:E ratio[b]
Age									
≤ 64	0	2.0	N/A	2.5	N/A	2.4	N/A	1.5	N/A
65 – 74	3.0	5.3	0.57	5.0	0.60	6.9	0.43	3.3	0.91
≥ 75 years	8.8	6.4	1.38	11.1	0.79	11.3	0.78	6.6	1.33
Mode of Presentation									
Elective	3.5	4.1	0.85	5.9	0.59	6.2	0.56	3.3	1.06
Emergency	10.7	11.8	0.91	12.2	0.88	17.1	0.63	11.8	0.91
ASA Grade									
ASA 1	0	1.2	N/A	2.6	N/A	1.1	N/A	0.8	N/A
ASA 2	2.8	2.2	1.27	5.3	0.53	3.7	0.68	2.0	1.15
ASA 3	5.4	5.9	0.92	7.7	0.70	9.8	0.55	5.1	1.06
ASA 4	12.0	19.6	0.61	11.6	1.03	23.0	0.52	14.5	0.83
mGPS									
0	3.3	3.2	1.03	5.5	0.60	5.8	0.57	2.9	1.14
1	2.6	5.1	0.51	6.8	0.38	7.4	0.35	4.0	0.65
2	9.5	9.0	1.05	8.5	1.12	12.1	0.78	8.0	1.19
All patients	4.0	4.6	0.87	6.3	0.63	6.9	0.58	3.8	1.05

[a]Exp represent the expected mortality (mean value).
[b]O:E ratio represents the observed to expected mortality ratio.

5.0 THE RELATIONSHIPS BETWEEN BODY COMPOSITION AND THE SYSTEMIC INFLAMMATORY RESPONSE IN PATIENTS WITH PRIMARY OPERATIVE COLORECTAL CANCER.

5.1 Introduction

Approximately 1 in 3 people in the United Kingdom will develop cancer during their lifetime (Bosanquet and Sikora 2004). Of these, almost half will experience a progressive involuntary weight loss with their disease, termed cancer cachexia. The degree of weight loss varies by tumour type but gastrointestinal tumours have a particularly high prevalence (Dewys, Begg et al. 1980). Indeed, it is estimated that up to half of patients with colorectal cancer have experienced weight loss by the time of presentation (Khalid, Spiro et al. 2007).

Cachexia has long been recognised as a marker of poor prognosis in cancer patients; associated with an increased risk of surgical complications (Peng, van Vledder et al. 2011) , resistance to chemotherapy (Ross, Ashley et al. 2004; Prado, Baracos et al. 2007), reduced quality of life (Dewys, Begg et al. 1980) and decreased survival (Andreyev, Norman et al. 1998; O'Gorman, McMillan et al. 2000; van Vledder, Levolger et al. 2012). The clear link between weight loss, reduced performance status, impaired response to treatment and poor prognosis in such patients may be due to the preferential loss of skeletal muscle. It has been suggested that, although the loss of adipose tissue accounts for the majority of the weight loss, it is the loss of muscle which impacts upon morbidity and mortality (Kotler 2000; Morley, Thomas et al. 2006; Fearon, Strasser et al. 2011). This has led some to describe the phenomenon of cancer-related weight loss as ‘sarcopenia’; a term originally employed to describe the gradual loss of skeletal muscle seen with ageing. The aetiological factors responsible for these changes in body composition are unclear but previous observations indicate there may be an association with inflammation. Indeed, there is now evidence that the systemic inflammatory response, already recognized as a marker of poor prognosis in

patients with gastrointestinal cancer (Proctor, Morrison et al. 2011), is associated with the cardinal features of cachexia (Argiles, Busquets et al. 2005; McMillan 2009). Previous work has demonstrated an association between systemic inflammation and a loss of lean tissue as measured using a total body potassium scanner (McMillan, Scott et al. 1998) although such equipment is not routinely available, is unlikely to be useful in clinical practice and has been superseded by the advent of cross-sectional imaging.

The aim of the present study, therefore, was to examine the relationships between CT measured parameters of body composition and the systemic inflammatory response in patients with primary operable colorectal cancer.

5.2 Materials and Methods

Patients with colorectal cancer who were considered to have undergone potentially curative resection for colorectal cancer between January 1st 2003 and December 31st 2010 at Glasgow Royal Infirmary were identified from the same prospective database described in Chapter 3. Of these, only patients with recorded height data and CT images taken preoperatively for diagnostic or staging purposes and stored in an electronic format suitable for image analysis were included in the study.

Patient height and weight was recorded from preoperative assessment health records and included only if documented within 30 days of CT scan. Patients were classified by body mass index (BMI) as underweight (BMI<18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9) or obese (BMI>30) according to World Health Organisation (WHO) criteria. The tumours were staged according to conventional AJCC/TNM classification (5th Edition) (Fleming ID 1997).

Preoperative systemic inflammatory response in the present study was assessed using three different measures (Chapter 1, Table 1.9). These were (1) serum white cell count (WCC) (Maltoni, Caraceni et al. 2005), (2) neutrophil to lymphocyte ratio (NLR) (Walsh, Cook et al. 2005) and (3) the modified Glasgow Prognostic Score (mGPS) (McMillan 2008).

The image analysis of CT scans was undertaken using medical imaging software. To test the reliability of different software packages, one commercially available program (Slice-O-Matic, version 4.3, Tomovision) and one governmental free-ware program (NIH ImageJ, version 1.44, http://rsbweb.nih.gov.ij/), were compared. Two trained investigators (CSDR and MTM) analysed a random sample of 50 cases using each of the software packages with the following results. (1) CSDR versus MTM using Slice-O-Matic software, mean difference

of 4.51 cm^2, limits of agreement -1.67 cm^2 to 10.69 cm^2, interclass correlation coefficient (ICC) = 0.977, (2) CSDR versus MTM using ImageJ software, mean difference of 1.52 cm^2, limits of agreement -8.81 cm^2 to 11.85 cm^2, ICC = 0.987, (3) Slice-O-Matic versus ImageJ software, mean difference of 7.50 cm^2, limits of agreement -13.63 cm^2 to 28.64 cm^2, ICC = 0.953. After establishing that both software packages provided reliable measurements, ImageJ was used for the entire cohort. Figure 5.1 provides an example of CT image analysis using NIH ImageJ software.

Total fat, subcutaneous fat, visceral fat and skeletal muscle cross-sectional areas (cm^2) were measured at the level of L3 using standard Hounsfield unit ranges (adipose tissue: -190 to -30; skeletal muscle: -29 to +150) (Mitsiopoulos, Baumgartner et al. 1998). Each parameter was then normalized for patient stature, as is conventional for BMI, and designated as total fat index (cm^2/m^2), subcutaneous fat index (cm^2/m^2), visceral fat index (cm^2/m^2), skeletal muscle index (cm^2/m^2). To further test inter-observer agreement, each parameter was again measured independently by two investigators in a random sample of 50 cases (total fat index, ICC = 0.982; subcutaneous fat index, ICC = 0.992; visceral fat index, ICC = 0.955; skeletal muscle index, ICC = 0.987). The study was approved by the West of Scotland Research Ethics Committee (WoSREC), Glasgow.

Statistics

Body composition parameters are presented as mean values with standard deviation (SD) and are categorised into sex-specific tertiles (low/medium/high). Grouping of other variables was carried out using standard or previously published thresholds. Relationships between continuous and categorical variables were examined using X^2 linear-by-linear analysis, non-parametric tests and Pearson correlation coefficients (r) as appropriate. *P* values of less than

0.05 were considered statistically significant. Statistical analysis was performed using SPSS® version 19.0 (IBM SPSS, Illinois, USA).

5.3 Results

A total of 548 patients underwent potentially curative resection of colorectal cancer during the study period. Of these, 374 patients were excluded (314 patients did not have an electronic version of their CT scans available for image analysis and 60 patients did not have any height data recorded) and 174 patients were included. Figure 5.2 summarises the study selection process. Baseline clinico-pathological characteristics of the included cohort are shown in Table 5.1. Approximately one third of patients were 75 years or older with a similar number of males and females. The majority of patients had no evidence of a systemic inflammatory response prior to surgery. According to WHO BMI classification, 3% of patients were underweight, 36% normal weight, 33% overweight and 28% obese. The operations were carried out for colon cancer in 66% of cases and rectal cancer in 34%. Pathology reports classified 16% of the tumours as stage I, 44% as stage II and 40% as stage III (Table 5.1).

The body composition parameters of the patients are shown in Table 5.2. There were no sex differences in BMI. Females had significantly more total fat (150.3cm^2/m^2 versus 124.1 cm^2/m^2, $p<0.001$) and subcutaneous fat (104.4cm^2/m^2 versus 73.7cm^2/m^2, $p<0.001$) while males had significantly more skeletal muscle (46.2cm^2/m^2 versus 36.9cm^2/m^2, $p<0.001$). These differences justified the use sex-specific tertiles in the study i.e. data relating to body composition is thus corrected for sex (Table 5.2).

The relationships between parameters of body composition and measures of the systemic inflammatory response in patients with primary operable cancer are shown in Table 5.3. There were no relationships between any parameter of body composition and serum WCC or

NLR. However, there was a significant relationship between an elevated mGPS and a low skeletal muscle index (p=0.001) (Table 5.3).

To further examine this relationship, absolute values of C-reactive protein and albumin were correlated with each parameter of body composition. With regard to C-reactive protein, there were no relationships with total fat index, subcutaneous fat index or visceral fat index but there was a significant negative correlation with skeletal muscle index (r=-0.21, p=0.005). With regard to albumin, there were no relationships with total fat index or subcutaneous fat index but there were significant positive correlations with visceral fat index (r=0.18, p=0.02) and skeletal muscle index (r=0.31, p<0.001). Scatterplots demonstrating these correlations are shown in Figure 5.3.

The relationships between skeletal muscle index and clinicopathological characteristics of the patients are shown in Table 5.4. There were significant associations between a low skeletal muscle index and increasing age (p<0.001) and presence of anaemia (p=0.029). There were no associations between skeletal muscle index and any of the tumour-related variables (Table 5.4).

The relationships between BMI classification and skeletal muscle index are illustrated in Figure 5.4. At least some patients from all the BMI categories fell within the lowest tertile of skeletal muscle index. In females, this meant a total of 24 patients (30%) with a normal, overweight or obese BMI were within the lowest tertile of skeletal muscle index. In males, 31 patients (33%) with a normal, overweight or obese BMI were within the lowest tertile of skeletal muscle index (Figure 5.4).

5.4 Discussion

The results of the present study demonstrate a strong association between low skeletal muscle mass and the presence of a systemic inflammatory response, as measured by mGPS, in patients with primary operable colorectal cancer. Furthermore, there were no direct relationships between skeletal muscle mass and any tumour-related variables, including tumour stage or nodal status. Taken together, these results would suggest that the loss of lean tissue in cancer cachexia may be driven by the host systemic inflammatory response.

The negative impact of systemic inflammation on cancer outcome has been reported previously; associated with an increased risk of septic complications (Moyes, Leitch et al. 2009), functional decline and decreased survival (Richards, Platt et al. 2011). The present study confirms that, using a different methodological approach, systemic inflammation plays a role in the development of muscle wasting in patients with colorectal cancer. This is supported by experimental models whereby pro-inflammatory cytokines, including interleukin-1 (IL-1), IL-6 and tumour necrosis factor-ά (TNF), have been reported as mediators of both anorexia and skeletal muscle proteolysis (Argiles, Busquets et al. 2005), the key components of weight loss in patients with cancer. Furthermore, the present study points to such inflammatory mediators having an effect on the liver, key to the elaboration of the systemic inflammatory response (Gabay and Kushner 1999). In addition to the hepatic production of acute phase proteins and their influence on skeletal muscle metabolism, there is also an increase in liver enzyme activity associated with an elevated mGPS (Brown, Milroy et al. 2007; Roxburgh, Wallace et al. 2010). Overall, these results highlight the potential importance of a liver-derived systemic inflammatory response in the progressive nutritional and functional decline of patients with colorectal cancer. It should be emphasised that these findings may also be applicable to benign disease. Indeed, similar observations regarding the

depletion of skeletal muscle being associated with activation of the systemic inflammatory response have been made in non-cancer cohorts, including patients with renal failure and chronic obstructive airways disease (Kotler 2000; Morley, Thomas et al. 2006).

Several previous studies investigating the clinical impact of cancer cachexia have focused specifically on the loss of lean tissue (Pichard, Kyle et al. 2004; Peng, van Vledder et al. 2011). However, in cancer patients, muscle wasting can occur with or without the loss of adipose tissue while in non-cancer patients there is evidence that obesity and visceral adipose tissue in particular are associated with a low grade inflammatory state (Saijo, Kiyota et al. 2004; Trayhurn and Wood 2004). In order to examine these relationships in detail we included measures of both adipose tissue and skeletal muscle and can now report that a systemic inflammatory response in patients with colorectal cancer is associated with a reduction in skeletal muscle as opposed to an increase in visceral adiposity.

It is clear from the present study that a simple measure of BMI is insufficient to detect the changes in body composition associated with malignant disease. This is particularly true in populations with an increasing prevalence of obesity; it is of interest that only 3% of patients in the present study were classified as underweight according to WHO classification. Even the application of a cutoff value of <20, as suggested by Fearon and co-workers (Fearon, Strasser et al. 2011) as a more sensitive indicator of cachexia, increased this figure to only 5%. It is evident that traditional descriptors of body composition, such as BMI, do not have the capacity to adequately identify patients with reduced levels skeletal muscle (Thibault, Genton et al. 2012). The present study, therefore, supports the use of cross-sectional imaging to assess the body composition of patients with malignant disease (Thibault and Pichard 2012). By comparing two widely-available software packages, we have demonstrated that

such analysis of CT scans is an objective and reproducible method of quantifying body composition.

In the present study we chose to use sex-specific tertiles rather than specific cutoff values to define levels of adiposity and sarcopenia. The most common current definition of sarcopenia is an appendicular skeletal muscle index more than two SDs below that of healthy adults (5.45 kg/m2 for females and 7.26 kg/m2 for males) (Baumgartner, Koehler et al. 1998). These values relate to dual-energy x-ray (DEXA) scanning and may not be readily applied to cross-sectional imaging. Prado and co-workers, using CT image analysis, defined a skeletal muscle index of 52.4cm^2/m^2 in men and 38.5cm^2/m^2 in women as associated with mortality (Prado, Lieffers et al. 2008). However, the population on which these cutoff values were developed was highly selective, consisting of 250 patients with an obese BMI (≥30) and a heterogeneous selection of respiratory tract and gastrointestinal cancers. Application of these cutoff values to the present cohort would have resulted in over 70% of patients being classified as 'sarcopenic'; a figure which highlights the need for additional reference values for cross-sectional imaging modalities. Indeed, an international consensus group on the diagnostic criteria for cancer cachexia concluded that definitive cutoffs for the diagnosis of sarcopenia still need to be determined from large contemporary datasets (Fearon, Strasser et al. 2011).

This study has a number of limitations. Height and weight data were primarily based on patient-reported values, although these have proven reliable in previous studies (Stunkard and Albaum 1981; Perry, Byers et al. 1995). Electronic records of CT images were difficult to access prior to 2006 and only routinely available after 2008, meaning long term outcomes could not be assessed. In addition, although cancer-related weight loss is a continuous

process, this study only assessed body composition at a single point in time. The changes in adipose tissue and skeletal muscle mass which occur over time and the relationships with cancer survival are of considerable interest and will be the subject of future work.

The present study adds important objective evidence to what is often empirically accepted; that patients with cancer preferentially lose lean tissue during the cachectic process. In addition, these results highlight a direct relationship between low levels of skeletal muscle and the presence of a systemic inflammatory response in patients with primary operable colorectal cancer.

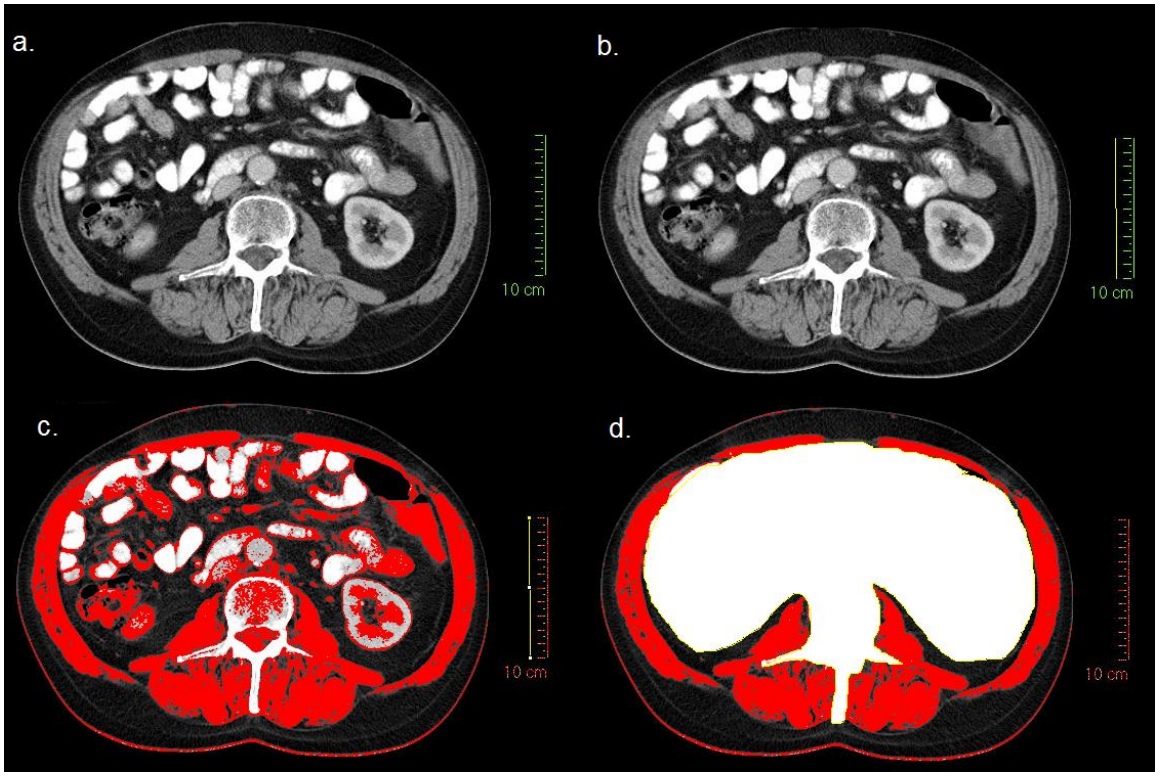


Figure 5.1. An example of CT image analysis using NIH ImageJ software. (a) the original CT image in JPEG format, (b) the scale is set using a known distance (10cm) from the original CT image, (c) skeletal muscle thresholds (-29 to +150 HU) are applied, (d) the abdominal contents and L3 vertebrae are cropped and the skeletal muscle cross sectional area calculated in cm^2.

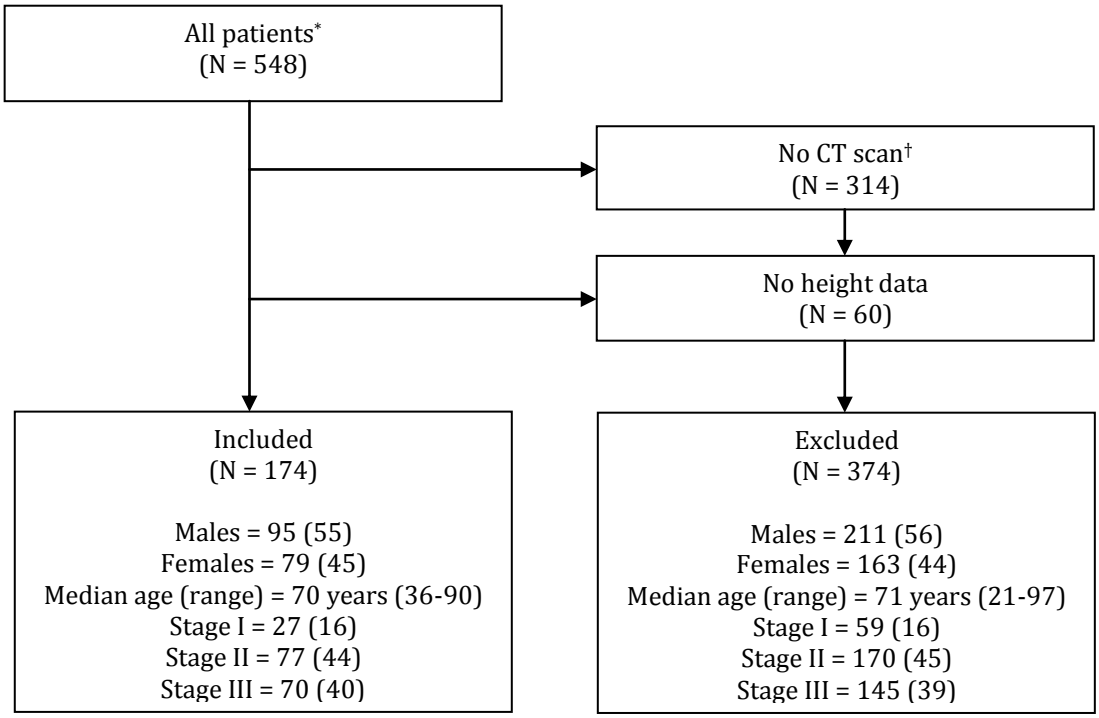
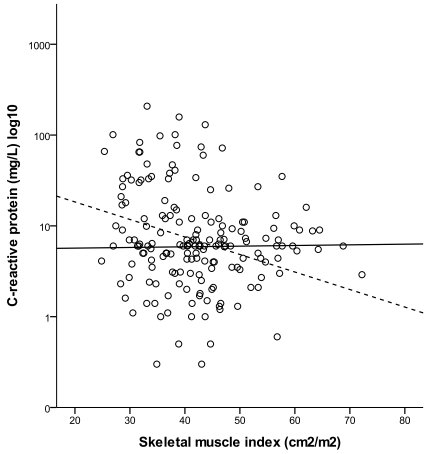
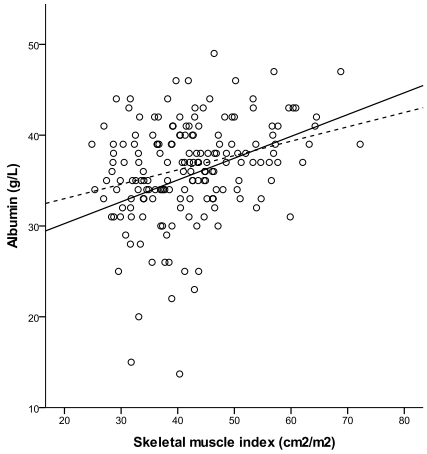


Figure 5.2. Flow chart representing the study selection process.
* All patients undergoing potentially curative resection of colorectal cancer January 1st 2003 and December 31st 2010.
† No CT scan stored in an electronic format suitable for image analysis.

r = -0.21



r = 0.31



r = -0.46

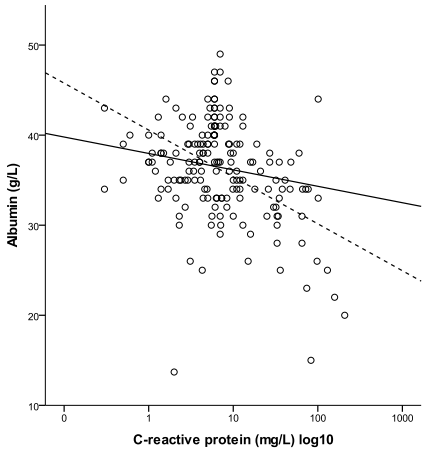


Figure 5.3. Scatterplots of the associations between C-reactive protein, albumin and skeletal muscle index. Fit lines are shown for male (—) and female (-----) patients.
r = Pearsons correlation coefficient for all patients.

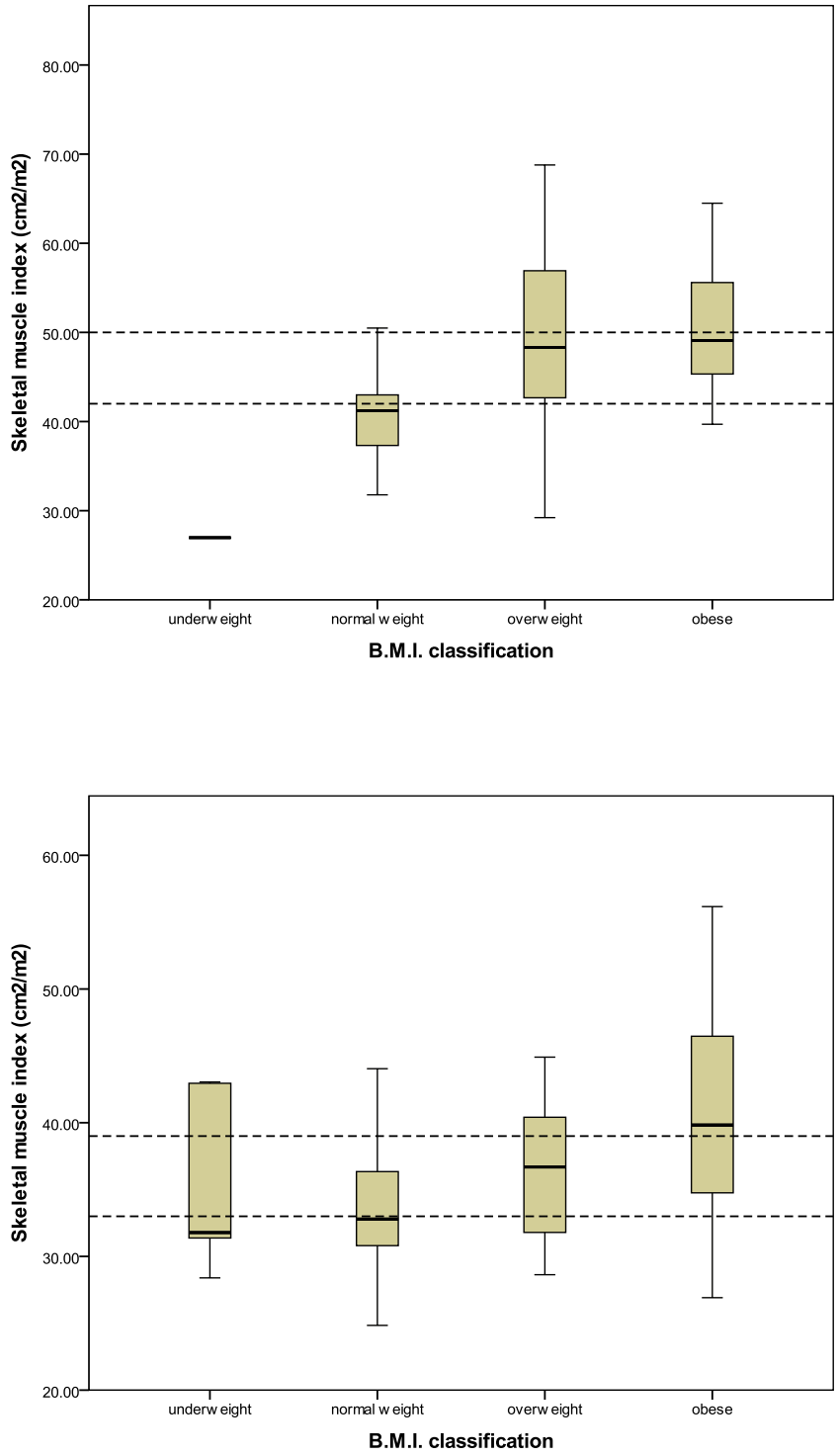


Figure 5.4. The relationship between B.M.I. classification and skeletal muscle index in male (top panel) and female (bottom panel) patients with primary operable colorectal cancer. Dashed lines represent cutoff values of the sex-specific tertiles.

Table 5.1. Clinicopathological characteristics of patients with primary operable colorectal cancer.

Variable		N = 174 (%)
Clinical variables		
Age	≤ 64	51 (29)
	65 – 74	63 (36)
	≥ 75	60 (35)
Sex	Female	79 (45)
	Male	95 (55)
ASA grade*	1 / 2	77 (44)
	3 / 4	68 (39)
Presentation	Elective	165 (95)
	Emergency	9 (5)
Anaemia*	None	93 (53)
	Mild	50 (29)
	Severe	30 (17)
Smoking status*	Never	74 (43)
	Ex	64 (37)
	Current	33 (19)
Inflammatory variables		
White cell count ($x10^9$/L)*	< 8.5	112 (64)
	8.5 – 11	34 (20)
	> 11	15 (9)
Neutrophil:lymphocyte ratio*	< 5	118 (68)
	> 5	34 (20)
mGPS	0	123 (71)
	1	20 (12)
	2	31 (18)
Pathological variables		
Tumour site	Colon	115 (66)
	Rectum	59 (34)
T stage	T 1/2	33 (19)
	T 3	94 (54)
	T 4	47 (27)
N Stage	N 0	105 (60)
	N 1	48 (28)
	N 2	21 (12)
TNM stage	Stage I	27 (16)
	Stage II	77 (44)
	Stage III	70 (40)
Venous invasion	Absent	77 (44)
	Present	97 (56)
Differentiation	Well/mod	163 (94)
	Poor	11 (6)
Lymph nodes retrieved	> 12	130 (75)
	< 12	44 (25)

* Missing values: ASA (n=29), anaemia (n=1), smoking (n=3), white cell count (n=13), neutrophil:lymphocyte ratio (n=22)

Table 5.2. Body composition parameters of patients with primary operable colorectal cancer.

Parameter	Male		Female		p^*
	value	N (%)	value	N (%)	
Body mass index (kg/m^2)					
Mean (SD)	27.7 (6.8)		26.9 (6.2)		0.59
Range	18.5 – 64.5		14.5 – 47.6		
Underweight	< 18.5	1 (1)	< 18.5	5 (6)	
Normal weight	18.5 – 24.9	33 (35)	18.5 – 24.9	30 (38)	
Overweight	25.0 – 29.9	37 (39)	25.0 – 29.9	20 (25)	
Obese	> 30	24 (25)	> 30	24 (30)	
Total fat index (cm^2/m^2)					
Mean (SD)	124.1 (52.2)		150.3 (58.6)		<0.001
Range	38.1 – 309.7		29.5 – 318.2		
Sex-specific tertile "Low"	38.0 – 101.0	32 (34)	29.5 – 130.5	27 (34)	
Sex-specific tertile "Medium"	101.0 – 134.5	32 (34)	130.5 – 177.5	27 (34)	
Sex-specific tertile "High"	134.5 – 310.0	31 (32)	177.5 – 318.5	25 (32)	
Subcutaneous fat index (cm^2/m^2)					
Mean (SD)	73.7 (37.5)		104.4 (44.6)		<0.001
Range	24.4 – 231.4		14.9 – 207.9		
Sex-specific tertile "Low"	24.0 – 58.5	32 (34)	14.5 – 85.5	27 (34)	
Sex-specific tertile "Medium"	58.5 – 73.5	32 (34)	85.5 – 129.5	27 (34)	
Sex-specific tertile "High"	73.5 – 231.5	31 (32)	129.5 – 208.0	25 (32)	
Visceral fat index (cm^2/m^2)					
Mean (SD)	50.4 (21.8)		45.9 (22.9)		0.13
Range	10.8 – 134.9		5.9 – 114.4		
Sex-specific tertile "Low"	10.5 – 40.5	32 (34)	5.5 – 37.5	27 (34)	
Sex-specific tertile "Medium"	40.5 – 55.5	32 (34)	37.5 – 50.5	27 (34)	
Sex-specific tertile "High"	55.5 – 135.0	31 (32)	50.5 – 114.5	25 (32)	
Skeletal muscle index (cm^2/m^2)					
Mean (SD)	46.2 (8.6)		36.9 (7.8)		<0.001
Range	26.9 – 68.8		24.8 – 72.2		
Sex-specific tertile "Low"	26.5 – 42.0	32 (34)	24.5 – 32.5	27 (34)	
Sex-specific tertile "Medium"	42.0 – 49.5	32 (34)	32.5 – 39.0	27 (34)	
Sex-specific tertile "High"	49.5 – 69.0	31 (33)	39.0 – 72.5	25 (32)	

* Mann-Whitney U test.

Table 5.3. The relationships between parameters of body composition and measures of the systemic inflammatory response in patients with primary operable colorectal cancer.

Inflammatory response	Body mass index (kg/m^2)		Total fat index (cm^2/m^2)		Subcutaneous fat index (cm^2/m^2)		Visceral fat index (cm^2/m^2)		Skeletal muscle index (cm^2/m^2)	
	under/norm/over/obese	p^*	low/med/high	p^*	low/med/high	p^*	low/med/high	p^*	low/med/high	p^*
WCC										
< 8.5	4/43/39/26		39/39/34		41/35/36		38/44/30		39/37/36	
8.5 – 11	0/10/7/17		5/14/15		7/11/16		9/8/17		8/14/12	
> 11	0/5/6/4	0.08	6/4/5	0.34	3/8/4	0.18	4/6/5	0.15	7/6/2	0.51
NLR										
< 5	3/38/42/35		34/44/40		36/41/41		38/38/42		40/38/40	
> 5	0/15/7/12	0.94	14/10/10	0.28	13/11/10	0.41	11/15/8	0.44	9/17/8	0.85
mGPS										
0	3/41/46/33		41/46/36		39/45/39		39/47/37		35/41/47	
1	1/5/5/9		4/4/12		6/4/10		5/5/10		7/7/6	
2	2/17/6/6	0.09	14/9/8	0.76	14/9/8	0.40	15/7/9	0.50	17/11/3	0.001

* X^2 linear-by-linear analysis
WCC = white cell count
NLR = neutrophil;lymphocyte ratio
mGPS = modified Glasgow Prognostic Score

Table 5.4. The relationship between skeletal muscle index and clinico-pathological characteristics in patients with primary operable colorectal cancer.

Variable		Skeletal muscle index (cm^2/m^2) Low (n = 59)	Medium (n = 59)	High (n = 56)	p *
Age	≤ 64	8 (16)	14 (27)	29 (57)	
	65 – 74	22 (35)	24 (38)	17 (27)	
	≥ 75	29 (48)	21 (35)	10 (17)	<0.001
ASA grade	1 / 2	26 (34)	26 (34)	25 (32)	
	3 / 4	22 (32)	23 (34)	23 (34)	0.84
Presentation	Elective	53 (32)	58 (35)	54 (33)	
	Emergency	6 (67)	1 (11)	2 (22)	0.11
Anaemia	None	25 (27)	30 (32)	38 (41)	
	Mild	22 (44)	17 (34)	11 (22)	
	Severe	11 (37)	12 (40)	7 (23)	0.029
Smoking status	Never	22 (30)	30 (40)	22 (30)	
	Ex	26 (41)	17 (27)	21 (33)	
	Current	9 (27)	11 (33)	13 (39)	0.64
Tumour site	Colon	36 (31)	40 (35)	39 (34)	
	Rectum	23 (39)	19 (32)	17 (29)	0.33
T stage	T 1/2	8 (24)	9 (27)	16 (49)	
	T 3	35 (37)	32 (34)	27 (29)	
	T 4	16 (34)	18 (38)	13 (28)	0.08
N stage	N 0	35 (33)	34 (32)	36 (34)	
	N 1	18 (38)	17 (35)	13 (27)	
	N 2	6 (29)	8 (38)	7 (33)	0.85
TNM stage	Stage I	6 (22)	7 (26)	14 (52)	
	Stage II	29 (38)	26 (34)	22 (29)	
	Stage III	24 (34)	26 (37)	20 (29)	0.14
Venous invasion	Absent	24 (31)	28 (36)	25 (33)	
	Present	35 (36)	31 (32)	31 (32)	0.66
Differentiation	Well/mod	55 (34)	54 (33)	54 (33)	
	Poor	4 (36)	5 (46)	2 (18)	0.49
Lymph nodes retrieved	> 12	42 (32)	47 (36)	41 (32)	
	< 12	17 (39)	12 (27)	15 (34)	0.79

* X^2 linear-by-linear analysis.

6.0 THE IMPACT OF PREOPERATIVE RISK FACTORS, TUMOUR PATHOLOGY AND POSTOPERATIVE COMPLICATIONS ON DISEASE RECURRENCE FOLLOWING POTENTIALLY CURATIVE RESECTION OF COLORECTAL CANCER.

6.1 Introduction

As described in Chapter 1, the identification of patients with colorectal cancer who are at risk of developing disease recurrence following potentially curative surgery is currently reliant on tumour stage or the presence of 'high risk' pathological criteria such as vascular invasion, perineural invasion and resection margin status.

Recently, there has been considerable interest in the impact of postoperative complications on colorectal cancer recurrence. McArdle and coworkers initially reported that, in a prospective cohort of 2,235 patients undergoing potentially curative resection, the presence of anastomotic leak was associated with poorer disease specific survival, independent of tumour stage (McArdle, McMillan et al. 2005). This observation has been repeated by a number of studies reporting an association between surgical complications and increased risk of colorectal cancer recurrence (Bell, Walker et al. 2003; Walker, Bell et al. 2004).

Although the impact of complications on postoperative mortality is intuitive, the mechanism by which they contribute to reduced long-term survival has not been defined. One hypothesis is that pro-inflammatory cytokines released during major infective complications stimulate residual tumour growth and result in higher rates of disease recurrence (Balkwill and Mantovani 2001). An alternative hypothesis is that postoperative complications are simply a surrogate marker for underlying preoperative patient-related risk factors, and it is these that are the true determinants of outcome. Indeed, there is now evidence that an elevated preoperative systemic inflammatory response, a predictor of survival following colorectal

cancer resection (Chapter 1), is also associated with the development of postoperative infective complications (Moyes, Leitch et al. 2009). Furthermore, altered preoperative patient physiology, itself a risk factor for surgical complications, appears to also predict long term survival following colorectal cancer surgery (Chapter 3).

Despite the above evidence, few studies have assessed the relative importance of preoperative, pathological and postoperative variables in a single cohort. The aim of the present study was, therefore, to examine the impact of preoperative risk factors, tumour pathology and postoperative complications on disease recurrence following potentially curative resection of colorectal cancer.

6.2 Materials and Methods

Patients with histologically proven colorectal cancer who were considered to have undergone potentially curative resection for colorectal cancer between January 1st 1997 and December 31st 2007 were identified from the same prospective database described in Chapter 3. The tumours were staged according to conventional AJCC/TNM classification (5th Edition) (Fleming ID 1997).

Preoperative patient physiology was assessed according to the POSSUM criteria described in Chapter 3. Preoperative systemic inflammatory response was assessed using the modified Glasgow Prognostic Score (mGPS) (Chapter 3).

Postoperative mortality and morbidity were recorded as in-hospital rates. All postoperative complications were recorded and categorised as infective or non-infective. Infective complications were classified as surgical site infections (SSI) or remote site infections (RSI) while non-infective complications were classified by system (cardiovascular, respiratory) as suggested by the Centers for Disease Control and Prevention (CDC) (Mangram, Horan et al. 1999).

Patients who survived to discharge were reviewed at one month postoperatively, at 6 month intervals for 2 years and then annually until five years post surgery. The follow up regime included annual computed tomography of the chest, abdomen and pelvis and colonoscopic surveillance every 3 years. Disease recurrence was defined as local (colon, pelvis or peritoneum) or systemic (hepatic, pulmonary or multi-organ) on the basis of clinical, endoscopic or radiological findings at the time of diagnosis.

All survival analysis was carried out after excluding postoperative deaths. Information on date and cause of death was cross-checked with that received by the cancer registration system and the Registrar General (Scotland). Death records were complete until 31st July 2010, which served as the censor date. Disease-free survival was measured from the date of surgery until the date of documented disease recurrence or death from colorectal cancer; overall survival from the date of surgery to the date of death from any cause. The study was approved by the West of Scotland Research Ethics Committee (WoSREC), Glasgow.

Statistics

Grouping of variables was carried out using standard or previously published thresholds. Comparison of categorical variables was performed using binary logistic regression; variables significant on univariate analysis were entered into a multivariate model. All survival analyses were performed using Cox proportional hazards regression; variables significant on univariate analysis were entered into a multivariate model, using a backward conditional method. *P* values of less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS® version 19.0 (IBM SPSS, Illinois, USA).

6.3 Results

A total of 423 patients who underwent potentially curative surgery for colorectal cancer were included. Baseline preoperative, pathological and postoperative characteristics of the patients are shown in Table 6.1. The majority of patients were older than 65 years (67%) with a similar number of males and females. More than half the patients (59%) lived in areas with the highest deprivation scores and were either ex or current smokers (57%). The majority of operations were elective (93%) and all resections were performed via open surgery. The operations were carried out for both colonic (62%) and rectal (38%) tumours and the overall rate of neo-adjuvant therapy was 5% (13% of rectal tumours). The majority of patients had no evidence of preoperative systemic inflammation (mGPS) (58%) and had physiology scores between 11 and 20 (75%). Pathological reports classified 12% of tumours as Stage I, 44% as Stage II and 44% as Stage III. The rates of vascular (intramural and extramural) and perineural invasion were 43% and 11% respectively. The tumour extended to the surgical resection margins in 13% of cases (this included cases where tumour cells were present on the peritoneal surface of the resected specimen). In the postoperative period, 17 patients died (in-hospital mortality rate = 4%) and a 142 patients developed at least one complication (in-hospital morbidity rate = 34%). A total of 20% of patients received adjuvant therapy and 35% of patients developed disease recurrence in the follow up period (Table 6.1).

The details and classification of postoperative complications are shown in Table 6.2. The majority of complications were infective (74%) with surgical site infections (SSI) the most prevalent subtype (43%). Remote site infections (RSI) were predominantly pneumonia (20%) and urinary tract infection (6%). Approximately one third (32%) of complications were non-infective; the majority of which related to the cardiovascular (18%) and respiratory systems (5%) (Table 6.2).

The relationships between preoperative variables and the development of postoperative complications are shown in Table 6.3. On univariate analysis, age ($p<0.05$), smoking status ($p<0.01$), emergency presentation ($p<0.01$), systemic inflammation (mGPS) ($p<0.01$) and POSSUM physiology score ($p<0.001$) were significantly associated with postoperative complications. On multivariate analysis, smoking status (OR 1.38, 95% CI 1.06 – 1.82, $p=0.019$) and POSSUM physiology score (OR 1.66, 95% CI 1.17 – 2.27, $p<0.001$) remained independently associated with the development of postoperative complications (Table 6.3). When infective complications were considered alone; univariate analysis reported an association with deprivation ($p<0.05$), smoking status ($p<0.01$), emergency presentation ($p<0.01$), systemic inflammation (mGPS) ($p<0.01$) and POSSUM physiology score ($p<0.05$). On multivariate analysis, smoking status (OR 1.70, 95% CI 1.27 – 2.29, $p<0.001$) and emergency presentation (OR 3.27, 95% CI 1.45 – 7.36, $p=0.004$) remained independently associated with the development of infective complications. When non-infective complications were considered alone; univariate analysis reported an association with age ($p<0.001$), smoking status ($p<0.05$) and POSSUM physiology score. On multivariate analysis, age (OR 2.42, 95% CI 1.51 – 3.90, $p<0.001$) and POSSUM physiology score (OR 1.55, 95% CI 1.03 – 2.32, $p=0.036$) remained independently associated with the development of non-infective complications.

The median follow up for survivors was 80 months (range 37 – 158). During this period 142 patients developed disease recurrence, 124 patients died from colorectal cancer and 72 patients died from other causes. The relationships between preoperative, pathological and postoperative variables and disease-free survival are shown in Table 6.4a. On univariate analysis, age ($p<0.05$), deprivation ($p<0.01$), smoking ($p<0.05$), presentation ($p<0.001$), systemic inflammation (mGPS) ($p<0.001$), POSSUM physiology score ($p<0.001$), TNM

stage (p<0.001), vascular invasion (p<0.001), perineural invasion (p<0.001) and margin involvement (p<0.001) were significantly associated with disease-free survival. On multivariate analysis, smoking status (HR 1.25, 95% CI 1.01 – 1.55, p=0.043), systemic inflammation (mGPS) (HR 1.31, 95% CI 1.04 – 1.65, p=0.021), POSSUM physiology score (HR 1.31, 95% CI 1.06 – 1.63, p=0.012), TNM stage (HR 1.87, 95% CI 1.37 – 2.55, p<0.001) and margin involvement (HR 4.72, 95% CI 3.20 – 6.96, p<0.001) were independently associated with disease-free survival (Table 6.4a).

The relationships between preoperative, pathological and postoperative variables and overall survival are shown in Table 6.4b. On univariate analysis, age (p<0.001), deprivation (p<0.01), smoking (p<0.01), presentation (p<0.01), systemic inflammation (mGPS) (p<0.001), POSSUM physiology score (p<0.001), TNM stage (p<0.001), vascular invasion (p<0.001), perineural invasion (p<0.001), margin involvement (p<0.001) and any postoperative complication (p<0.05) were significantly associated with overall survival. On multivariate analysis, age (HR 1.48, 95% CI 1.23 – 1.79, p<0.001), smoking status (HR 1.33, 95% CI 1.10 – 1.60, p=0.003), systemic inflammation (mGPS) (HR 1.28, 95% CI 1.05 – 1.57, p=0.015), POSSUM physiology score (HR 1.24, 95% CI 1.03 – 1.49, p=0.025), TNM stage (HR 1.52, 95% CI 1.17 – 1.96, p=0.002) and margin involvement (HR 3.62, 95% CI 2.50 – 5.25, p<0.001) were independently associated with overall survival (Table 6.4b).

The site of recurrence was classified as local in 36 patients (25%), including metastases to the colon (n=17), pelvis (n=9) or peritoneum (n=10). Recurrence was classified as systemic in 101 patients (71%), including metastases to the liver (n=55), lung (n=13) or multiple organs (n=33). In the remaining 5 patients (4%), disease recurrence was diagnosed clinically with no imaging to confirm the site.

The relationships between site of disease recurrence and the variables associated with reduced disease-free survival are shown in Table 6.5. On univariate analysis, smoking status ($p<0.05$) and vascular invasion ($p<0.05$) were significantly associated with systemic, rather than local, disease recurrence. No other variables were associated with recurrence to a particular site.

6.4 Discussion

The results of the present study show that smoking status, patient physiology and systemic inflammation are associated with early disease recurrence, independent of tumour stage, following potentially curative resection of colorectal cancer. Furthermore, these same preoperative variables are associated with the development of postoperative complications. Taken together, these results suggest that preoperative patient-related factors are important determinants of both short and long term outcome following colorectal cancer resection.

In Chapter 3, patient physiology was shown to be an independent predictor of survival following potentially curative resection of colorectal cancer. These results now demonstrate that altered physiology is a risk factor for both postoperative complications and early disease recurrence. In addition, despite considerable evidence that the systemic inflammatory response is associated with poor outcome from a range of solid organ tumours (Chapter 1), the present study confirms, for the first time, an association between systemic inflammation and early recurrence following colorectal cancer resection.

With reference to smoking, our results are in line with previous reports. There is now consistent evidence that cigarette smoking increases the risk of postoperative complications following a range of surgical procedures, including colorectal cancer resection (Sorensen, Jorgensen et al. 1999; Nickelsen, Jorgensen et al. 2005). There is also evidence that smoking shortens survival following curative resection of colorectal cancer (Munro, Bentley et al. 2006; Carsin, Sharp et al. 2008). The present study suggests the mechanism behind this association may be an increased risk of early systemic metastases.

Previous studies have reported that postoperative complications are associated with poorer long term survival after major surgery (Khuri, Henderson et al. 2005). In the present study,

the development of a postoperative complication was not independently associated with reduced disease-free or overall survival after colorectal cancer resection. Several possibilities may be considered to explain these apparent differences. The study by Khuri and co-workers, one of the largest to date, reported only overall survival while the present study investigated disease specific outcome. Indeed, the present results did initially suggest an association between complications and overall survival; this only became non-significant after correction for preoperative risk factors. Importantly, the number of patients in the current study precluded meaningful sub-analysis of individual complications and it remains a possibility that only major infective complications, such as anastomotic leak, have an adverse influence on long term outcomes.

Results from the present study have implications for our understanding of how morbidity following cancer surgery may influence survival. Previous hypotheses relating postoperative complications to poorer survival were based on the paradigm that infective complications initiated an inflammatory cascade, including the release of pro-inflammatory cytokines and vascular growth factors, which promoted tumour growth and dissemination (Abramovitch, Marikovsky et al. 1999). In the event of an anastomotic leak, it has been suggested that malignant cells may 'spill' into the pelvis with subsequent implantation and local recurrence (O'Dwyer and Martin 1989). The present results suggest that, rather than being the cause of disease recurrence, surgical complications are a consequence of poor patient physiology coupled with a pro-inflammatory state; the true determinants of long term outcome.

The mechanisms underlying the relationship between smoking, patient physiology, the systemic inflammatory response and disease recurrence are likely to be complex. There is now considerable evidence that a strong inflammatory cell infiltrate within and around the

tumour has a protective effect on disease progression in colorectal cancer (Chapter 1). One hypothesis, therefore, is that physiological dysfunction or a systemic inflammatory response impairs the ability of the host to mount an effective local immune response. Further work is needed to clarify the relationships between physiological function, the systemic inflammatory response and immune response within the tumour microenvironment.

The reported impact of preoperative factors on disease recurrence may also have implications for staging and pre-surgical optimization of patients about to undergo potentially curative resection of colorectal cancer. With reference to staging, there is now evidence that the combination of positron emission tomography and computed tomography (PET/CT) can increase the detection rate of colorectal metastases prior to surgery (Orlacchio, Schillaci et al. 2009). It may be that patients with a pronounced inflammatory response represent those who already have tumour dissemination, undetectable with current imaging modalities. Patients with an elevated mGPS prior to surgery thus represent a cohort who may benefit from such additional staging modalities.

With regard to improving preoperative physiological function, more emphasis should be placed on 'multimodal' approach; utilizing medication review, exercise programmes, smoking cessation and a period of intensive inpatient cardiovascular optimization for those at highest risk (Khoo, Vickery et al. 2007).

From the present and previous studies there is now consistent evidence that preoperative risk factors predict short and long term outcome after colorectal cancer surgery. Despite this, few studies have been undertaken to test whether manipulation of such factors is associated with improved outcome. There is some evidence that smoking cessation (Kenfield, Stampfer et al. 2008), exercise programmes (Meyerhardt, Giovannucci et al. 2009) or the routine

administration of anti-inflammatory medication (Chan, Ogino et al. 2009) has a positive impact on colorectal cancer survival but further prospective studies, specifically targeting the risk factors highlighted in the current study, are warranted.

In summary, the present study reports that preoperative risk factors, including smoking status, patient physiology and systemic inflammation, are associated with the both the development of postoperative complications and early disease recurrence following potentially curative resection of colorectal cancer.

Table 6.1. Preoperative, pathological and postoperative characteristics of 423 patients undergoing potentially curative resection of colorectal cancer.

Variable	Patient group	423 (%)
Preoperative variables		
Age	≤ 64	140 (33)
	65 – 74	135 (32)
	≥ 75	148 (35)
Sex	Male	230 (54)
	Female	193 (46)
Deprivation score	1 - 2	20 (5)
	3 - 5	150 (36)
	6 - 7	241 (59)
Smoking status	Non smoker	183 (43)
	Ex smoker	150 (36)
	Current smoker	90 (21)
Presentation	Elective	395 (93)
	Emergency	28 (7)
Tumour site	Colon	254 (62)
	Rectum	159 (38)
Neo-adjuvant therapy	Yes	21 (5)
	No	402 (95)
Systemic inflammation	mGPS = 0	246 (58)
	mGPS = 1	114 (27)
	mGPS = 2	63 (15)
POSSUM physiology score	11 – 14	143 (33)
	15 – 20	177 (42)
	21 – 30	92 (22)
	> 30	11 (3)
Pathological criteria		
TNM stage	Stage I	51 (12)
	Stage II	186 (44)
	Stage III	186 (44)
Vascular invasion	Yes	181 (43)
	No	242 (57)
Perineural invasion	Yes	48 (11)
	No	371 (88)
Margin involvement	Yes	54 (13)
	No	366 (87)
Postoperative outcome		
In-hospital mortality	Yes	17 (4)

	No	406 (96)
In-hospital morbidity	Yes	142 (34)
	No	281 (66)
Adjuvant therapy	Yes	86 (20)
	No	337 (80)
Disease recurrence	Yes	142 (35)
	No	264 (65)
Status at censor date	Alive	210 (52)
	Colorectal cancer death	124 (30)
	Non-cancer death	72 (18)

Table 6.2. Classification of postoperative complications recorded in 423 patients following potentially curative resection of colorectal cancer.

Classification of complication	**142 (%)**
Infective	**105 (74)***
Surgical site infection	61 (43)
- Anastomotic leak	18 (13)
- Intra-abdominal abcess	33 (23)
- Wound	31 (22)
Remote site infection	46 (32)
- Pneumonia	29 (20)
- UTI	8 (6)
- GI tract	7 (5)
- Other	5 (4)
Non-infective	**45 (32)***
Cardiovascular	25 (18)
- Atrial fibrillation	11 (8)
- Acute Coronary Syndrome	10 (7)
- Other	4 (3)
Respiratory	7 (5)
- Pulmonary Embolus	3 (2)
- Pulmonary Oedema	3 (2)
- Pleural Effusion	1 (1)
Miscellaneous	13 (9)

* Values do not equal 100% as several patients had more than one complication

Table 6.3. The relationships between preoperative variables and the development of postoperative complications.

Variable	Patient Group	Patients with complication n (%)	Univariate		Multivariate	
			OR (95% C.I.)	*p*	OR (95% C.I.)	*p*
Age	< 65	38 (27)				
	65 – 74	44 (33)				
	≥ 75	60 (40)	1.36 (1.06, 1.74)	0.016		0.127
Sex	Male	84 (36)				
	Female	58 (30)	1.34 (0.89, 2.01)	0.161		
Deprivation score	1 – 2	5 (25)				
	3 – 5	45 (30)				
	6 - 7	86 (36)	1.29 (0.90, 1.85)	0.162		
Smoking status	Non	50 (27)				
	Ex	52 (35)				
	Current	40 (44)	1.45 (1.12, 1.89)	0.005	1.38 (1.06, 1.82)	0.019
Presentation	Elective	126 (32)				
	Emergency	16 (57)	2.85 (1.31, 6.20)	0.008		0.097
Tumour site	Colon	88 (33)				
	Rectum	54 (35)	1.13 (0.75, 1.70)	0.581		
Neo-adjuvant Rx	Yes	6 (29)				
	No	99 (25)	1.23 (0.50, 3.04)	0.653		
Systemic inflammation	mGPS = 0	70 (28)				
	mGPS = 1	44 (39)				
	mGPS = 2	28 (44)	1.45 (1.11, 1.90)	0.007		0.192
POSSUM physiology score	11 – 14	33 (23)				
	15 – 20	61 (35)				
	21 – 30	41 (45)				
	> 30	7 (64)	1.68 (1.30, 2.16)	<0.001	1.66 (1.17, 2.27)	<0.001

Table 6.4a. The relationships between preoperative, pathological and postoperative variables and disease-free survival; cox regression analysis (In-hospital mortality has been excluded).

Variable	**Univariate HR (95% C.I.)**	***p***	**Multivariate HR (95% C.I.)**	***p***
Preoperative variables				
Age	1.25 (1.03, 1.52)	0.028		0.660
Sex	1.30 (0.94, 1.80)	0.116		
Deprivation	1.56 (1.15, 2.13)	0.004		0.183
Smoking status	1.25 (1.02, 1.53)	0.035	1.25 (1.01, 1.55)	0.043
Presentation	2.60 (1.52, 4.43)	<0.001		0.422
Tumour site	0.77 (0.55, 1.08)	0.134		
Neo-adjuvant therapy	1.39 (0.73, 2.65)	0.313		
mGPS	1.42 (1.15, 1.74)	<0.001	1.31 (1.04, 1.65)	0.021
POSSUM physiology score	1.46 (1.20, 1.79)	<0.001	1.31 (1.06, 1.63)	0.012
Pathological criteria				
TNM stage	2.16 (1.63, 2.85)	<0.001	1.87 (1.37, 2.55)	<0.001
Vascular invasion	2.49 (1.79, 3.46)	<0.001		0.117
Perineural invasion	2.52 (1.67, 3.79)	<0.001		0.525
Margin involvement	5.56 (3.85, 8.03)	<0.001	4.72 (3.20, 6.96)	<0.001
Postoperative variables				
Any complication	1.25 (0.89, 1.77)	0.197		
Infective complication	1.06 (0.72, 1.56)	0.762		
Non-infective complication	1.28 (0.75, 2.18)	0.371		
Adjuvant therapy	1.14 (0.77, 1.67)	0.517		

Table 6.4b. The relationships between preoperative, pathological and postoperative variables and overall survival; cox regression analysis (In-hospital mortality has been excluded).

Variable	**Univariate**		**Multivariate**	
	HR (95% C.I.)	***p***	**HR (95% C.I.)**	***p***
Preoperative variables				
Age	1.55 (1.30, 1.85)	<0.001	1.48 (1.23, 1.79)	<0.001
Sex	1.22 (0.92, 1.61)	0.171		
Deprivation	1.44 (1.10, 1.87)	0.007		0.242
Smoking status	1.30 (1.09, 1.55)	0.004	1.33 (1.10, 1.60)	0.003
Presentation	2.34 (1.42, 3.86)	0.001		0.370
Tumour site	1.02 (0.77, 1.36)	0.883		
Neo-adjuvant therapy	0.96 (0.51, 1.82)	0.902		
mGPS	1.39 (1.16, 1.67)	<0.001	1.28 (1.05, 1.57)	0.015
POSSUM physiology score	1.47 (1.23, 1.75)	<0.001	1.24 (1.03, 1.49)	0.025
Pathological criteria				
TNM stage	1.73 (1.38, 2.18)	<0.001	1.52 (1.17, 1.96)	0.002
Vascular invasion	1.99 (1.50, 2.64)	<0.001		0.337
Perineural invasion	2.13 (1.44, 3.14)	<0.001		0.295
Margin involvement	4.62 (3.27, 6.53)	<0.001	3.62 (2.50, 5.25)	<0.001
Postoperative variables				
Any complication	1.36 (1.01, 1.82)	0.044		0.788
Infective complication	1.26 (0.91, 1.74)	0.163		
Non-infective complication	1.18 (0.73, 1.92)	0.499		
Adjuvant therapy	0.86 (0.61, 1.23)	0.420		

Table 6.5. The relationships between variables significantly associated with reduced disease-free survival and site of disease recurrence; linear-by-linear association.

Variable	Patient group	Local recurrence 36 (%)	Systemic recurrence 101 (%)	*p*
Age	< 65	11 (24)	34 (76)	
	65 – 74	10 (23)	34 (77)	
	≥ 75	15 (31)	33 (69)	0.451
Deprivation	1 – 2	0 (0)	3 (100)	
	3 – 5	14 (33)	28 (67)	
	6 - 7	21 (24)	67 (76)	0.609
Smoking	Non	19 (37)	33 (63)	
	Ex	11 (22)	40 (78)	
	Current	6 (18)	28 (82)	0.041
Presentation	Elective	30 (25)	92 (75)	
	Emergency	6 (40)	9 (60)	0.202
Systemic inflammation	mGPS = 0	22 (30)	51 (70)	
	mGPS = 1	9 (23)	30 (77)	
	mGPS = 2	5 (20)	20 (80)	0.270
POSSUM physiology score	11 – 14	12 (32)	26 (68)	
	15 – 20	9 (16)	48 (84)	
	21 – 30	14 (40)	21 (60)	
	> 30	1 (14)	6 (86)	0.802
TNM stage	Stage I	1 (14)	6 (86)	
	Stage II	13 (29)	32 (71)	
	Stage III	22 (26)	63 (74)	0.869
Vascular invasion	No	20 (35)	37 (65)	
	Yes	16 (20)	64 (80)	0.048
Perineural invasion	No	29 (27)	79 (73)	
	Yes	7 (25)	21 (75)	0.844
Margin involvement	No	26 (26)	72 (74)	
	Yes	10 (26)	29 (74)	0.915

7.0 THE RELATIONSHIPS BETWEEN TUMOUR NECROSIS AND HOST INFLAMMATORY RESPONSES IN PRIMARY OPERABLE COLORECTAL CANCER.

7.1 Introduction

It is now recognized that colorectal cancer outcome is dependent on interactions between tumour and host related factors. The host inflammatory response plays a key role in disease progression and has the capacity to either promote or inhibit tumour growth. There is now good evidence that an elevated systemic inflammatory response is detrimental to survival while a pronounced inflammatory cell infiltrate at a local level has been consistently associated with improved clinical outcome (Chapter 1). The evidence to date suggests that these host responses are acting independently and as yet there has been no documented link between local and systemic inflammation.

Tumour necrosis has recently been proposed as a prognostic marker in colorectal cancer (Pollheimer, Kornprat et al. 2010). This follows a number of similar studies reporting necrosis as a marker of poor prognosis in renal, breast and lung carcinoma (Fisher, Palekar et al. 1978; Frank, Blute et al. 2002; Swinson, Jones et al. 2002). The mechanisms underpinning the relationship between necrosis and cancer survival, however, are unclear. One plausible hypothesis is that tumour necrosis may impact on colorectal cancer survival by influencing the host inflammatory responses. Indeed, there is evidence from other cancer types that tumour necrosis is associated with markers of systemic inflammation, such as serum white cell count and erythrocyte sedimentation rate (ESR) (Sengupta, Lohse et al. 2005), as well as alterations in the local recruitment of inflammatory cells (Carlomagno, Perrone et al. 1995). The aim of the present study was to investigate the prognostic value of tumour necrosis in colorectal cancer and to examine its relationships with the host systemic and local inflammatory responses.

7.2 Materials and Methods

Patients with colorectal cancer who were considered to have undergone potentially curative resection of colorectal cancer between January 1997 and December 2007 were identified from the same prospective database described in Chapter 3.

The tumours were staged according to conventional AJCC/TNM classification (5^{th} Edition) (Fleming ID 1997). Additional high risk pathological features were recorded from reports issued at the time. The Petersen Index (PI) was constructed from scores allocated to four selected pathological variables present in the tumour specimen. Intra or extramural vascular invasion, peritoneal involvement and margin involvement were allocated a score of 1 and tumour perforation was allocated a score of 2. The cumulative total is calculated and the PI considered low risk (score 0 – 1) or high risk (score 2 – 5) (Petersen, Baxter et al. 2002).

Preoperative systemic inflammatory response was assessed using the modified Glasgow Prognostic Score (mGPS) as described in Chapter 1. Routine laboratory measurements of haemoglobin (Hb), white cell count (WCC), albumin and C-reactive protein (CRP) were recorded prior to surgery. Using local reference ranges, anaemia was defined as a Hb concentration <13.0g/dl in males and <11.5g/dl in females. Severe anaemia was defined as a Hb measurement <11.0g/dl in males and <10.0g/dl in females (Hamilton, Lancashire et al. 2008).

Assessment of both the local inflammatory cell infiltrate and tumour necrosis was undertaken on the same original haematoxylin and eosin (H&E) sections. The sections were selected from areas of the central tumour felt to represent the maximum depth of tumour invasion. A median of 3 sections (range 2 – 5) were examined per patient. The local inflammatory cell infiltrate had been previously assessed in the cohort according to the Klintrup-Makinen (K-

M) criteria (Roxburgh, Salmond et al. 2009). Briefly, the invasive margin of each tumour was scored according to a 4 point scale. A score of 0 indicated there was no increase in inflammatory cells; score 1 denoted a mild or patchy increase; score 2 denoted a prominent inflammatory reaction and score 3 denoted a florid 'cup-like' inflammatory infiltrate. The inflammatory cell infiltrate was subsequently classified as low grade (score 0 - 1) or high grade (score 2 - 3). The inter-observer intra-class correlation coefficient (ICC) for the assessment of the inflammatory cell infiltrate was 0.81.

Assessment of tumour necrosis was undertaken according to the methodology of Pollheimer and coworkers (Pollheimer, Kornprat et al. 2010). The sections were examined at low magnification (x40) for evidence of tumour necrosis. The extent of necrosis was semiquantitatively assessed and, using published thresholds, graded as 'absent' (none), 'focal' (< 10% of tumour area), 'moderate' (10 - 30% of tumour area) or 'extensive' (> 30% of tumour area) in each section before an assessment was made of the overall extent of necrosis. To test consistency of scoring, 50 cases were examined independently by two observers (CHR and CSDR) blinded to clinical outcome. The ICC for the assessment of tumour necrosis was 0.86. CHR then scored all cases and these data were used in the analysis. Figure 7.1 provides examples of the four necrosis categories.

Information on date and cause of death was cross-checked with that received by the cancer registration system and the Registrar General (Scotland). Death records were complete until 1st December 2010, which served as the censor date. Cancer specific survival was measured from the date of surgery until the date of death from colorectal cancer. The study was approved by the West of Scotland Research Ethics Committee (WoSREC), Glasgow.

Statistics

Grouping of variables was carried out using standard or previously published thresholds. Associations between categorical variables were examined using chi square tests for linear trend. Survival analyses were performed using Cox proportional hazards regression to calculate hazard ratios (HR). All variables significant on univariate analysis were entered into a multivariate model using a backwards conditional method. *P* values of less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS® version 19.0 (IBM SPSS, Illinois, USA).

7.3 Results

A total of 343 patients who underwent potentially curative resection of colorectal cancer were included. Summary characteristics of the cohort are shown in Table 7.1. The majority of patients were 65 years or older (65%) with a similar number of males and females. The majority of operations were carried out electively (94%) and all were performed by open surgery. The resections were carried out for both colonic (69%) and rectal (31%) cancer and pathological reports classified the tumours as Stage I (7%), Stage II (49%) or Stage III (44%). The Petersen Index classified 81% of the tumours as low risk and 19% as high risk. There was no evidence of a systemic inflammatory response in the majority of patients (56%) while the local inflammatory cell infiltrate was classified as low grade in 65% of cases and high grade in 35% (Table 7.1).

Tumour necrosis was graded as 'absent' in 32 cases (9%), 'focal' in 166 cases (48%) , 'moderate' in 101 cases (29%) and 'extensive' in 44 cases (13%). The relationships between tumour necrosis and clinico-pathological variables in all patients are shown in Table 7.1. There were significant relationships between tumour necrosis and both systemic inflammatory response ($p<0.001$) and local inflammatory cell infiltrate ($p=0.004$). To examine whether these associations were independent of tumour stage, the analyses were repeated in patients with node negative disease (Table 7.2).

The median follow up for the survivors was 96 months (range 45 – 167 months). During this period, 103 patients died from colorectal cancer and 78 patients died from other causes. The Kaplan-Meier survival curve, demonstrating a significant association between tumour necrosis and reduced cancer specific survival, is shown in Figure 7.2.

The multivariable survival analyses for all patients are shown in Table 7.3. When all variables were considered (Model 1), age (HR 1.29, p=0.050), systemic inflammatory response (HR 1.74, p=0.001), low grade local inflammatory cell infiltrate (HR 2.65, p=0.001), TNM stage (HR 1.55, p=0.041) and high risk Petersen Index (HR 3.50, p<0.001) were independently associated with reduced cancer specific survival. When the systemic and local inflammatory responses were removed from the model (Model 2), age (HR 1.28, p=0.062), TNM stage (HR 1.62, p=0.020), high risk Petersen Index (HR 3.43, p<0.001) and tumour necrosis (HR 1.35, p=0.027) were independently associated with cancer specific survival (Table 7.3).

These analyses were then repeated in patients with node negative disease (Table 7.4). When all variables were considered (Model 1), age (HR 1.78, p=0.005), systemic inflammatory response (HR 1.71, p=0.019), low grade local inflammatory cell infiltrate (HR 3.33, p=0.002) and high risk Petersen Index (HR 4.67, p<0.001) were independently associated with reduced cancer specific survival. When the systemic and local inflammatory responses were removed from the model (Model 2), age (HR 1.84, p=0.004) and high risk Petersen Index (HR 4.07, p<0.001) were independently associated with cancer specific survival (Table 7.4).

7.4 Discussion

The present study confirms tumour necrosis as a marker of poor prognosis, independent of pathological stage, in patients with primary operable colorectal cancer. Necrosis is directly associated with both an elevation of the systemic inflammatory response and an attenuation of the local inflammatory cell infiltrate. This suggests that the impact of tumour necrosis on colorectal cancer survival may be explained by close relationships with the host inflammatory responses.

Previously, tumour necrosis has shown prognostic value in a variety of solid organ tumours including renal (Frank, Blute et al. 2002), breast (Fisher, Palekar et al. 1978), lung (Swinson, Jones et al. 2002) and colorectal malignancy (Gao, Arbman et al. 2005; Pollheimer, Kornprat et al. 2010). It is clear from these studies that necrosis is not an isolated pathological feature but is strongly related to aggressive tumour characteristics including size, grade and pathological stage. The present study now confirms that tumour necrosis is associated specific pathological characteristics pertinent to colorectal cancer progression, namely vascular invasion, peritoneal involvement, margin involvement and tumour perforation. The relationship with these pathological variables, however, cannot adequately explain the influence of tumour necrosis on colorectal cancer outcome. Indeed, the present results confirm necrosis as a marker of poor prognosis, independent of tumour stage. We therefore hypothesized that the mechanisms underpinning the relationships between necrosis and survival may instead involve interactions with patient related variables, in particular those relating to the inflammatory response.

The impact of inflammation on cancer outcome has received significant attention in recent years. Studies now indicate that a preoperative systemic inflammatory response is one of the

most important factors in determining both short and long term outcomes in patients undergoing surgery for colorectal cancer. In contrast, a pronounced inflammatory response at a local level has been consistently associated with improved clinical outcome in patients with colorectal cancer (Chapter 1). For example, there is evidence that a pronounced inflammatory cell infiltrate is associated with an absence of vascular and lymphatic emboli; the earliest signs of tumour invasion and dissemination (Pages, Berger et al. 2005). Despite these established findings, the initial stimulus for the development of an inflammatory response in patients with colorectal cancer has not been clear. The present results suggest that the presence of tumour necrosis, itself associated with a weak local inflammatory reaction, may represent a trigger for the host to initiate a systemic inflammatory response.

The relationships between tumour necrosis and the inflammatory response might be further explained by how malignant cells respond to hypoxic stress. Indeed, it has been postulated that the combination of inflammation and necrosis provide an environment in which the epigenetic regulation of genes, cell death, cell proliferation and mutagenesis occurs (Vakkila and Lotze 2004). 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. Cells that die due to hypoxic stress have limited ways of initiating the apoptotic cascade because important pathways are blocked by endogenous inhibitors. 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 further stimulates inflammatory pathways. 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 (Colotta, Allavena et al. 2009).

With regard to other patient variables, the present study reports an association between tumour necrosis and the presence of preoperative anaemia, supporting similar observations in both renal cell carcinoma (Sengupta, Lohse et al. 2005) and malignant mesothelioma (Edwards, Swinson et al. 2003). Necrosis is generally attributed to rapid tumour growth resulting in vascular insufficiency and tissue hypoxia. It is evident, however, that necrosis can occur even in small tumours, suggesting that impaired oxygen delivery to the tissues may be a contributory factor in the development of necrosis. Alternatively, the reduced haemoglobin concentrations observed in such patients may simply be a consequence of cancer-associated inflammation (Spivak 2005).

To further our understanding of the relationships between tumour necrosis and inflammation, future studies should include a detailed examination of the associations between tumour necrosis and cell signalling pathways, genetic changes and markers of tumour cell growth. In addition, a detailed investigation of the individual immune cell types most strongly associated with necrosis is needed.

The present study has a number of limitations. First, despite much of the data, including the blood tests used in the calculation of the systemic inflammatory response, being collected prospectively, the analysis of tumour necrosis was undertaken in a retrospective fashion. In addition, the grading of both the inflammatory cell infiltrate and tumour necrosis was undertaken using a semiquantitative technique, introducing the possibility of sampling bias. To minimize this risk, two independent observers examined between two and five sections per case before assigning each patient an overall score. Indeed, the high level of inter-observer agreement in the grading of both the inflammatory cell infiltrate and tumour necrosis suggests these techniques to be simple and reproducible. Finally, the distribution of

inflammatory cells and necrotic areas is variable and analysing only a small number of pathological sections may not be representative of the whole tumour.

In summary, the present study confirms tumour necrosis as a stage independent prognostic marker in colorectal cancer. Furthermore, these results indicate that the impact of tumour necrosis on colorectal cancer survival may be explained by close relationships with the host inflammatory responses.

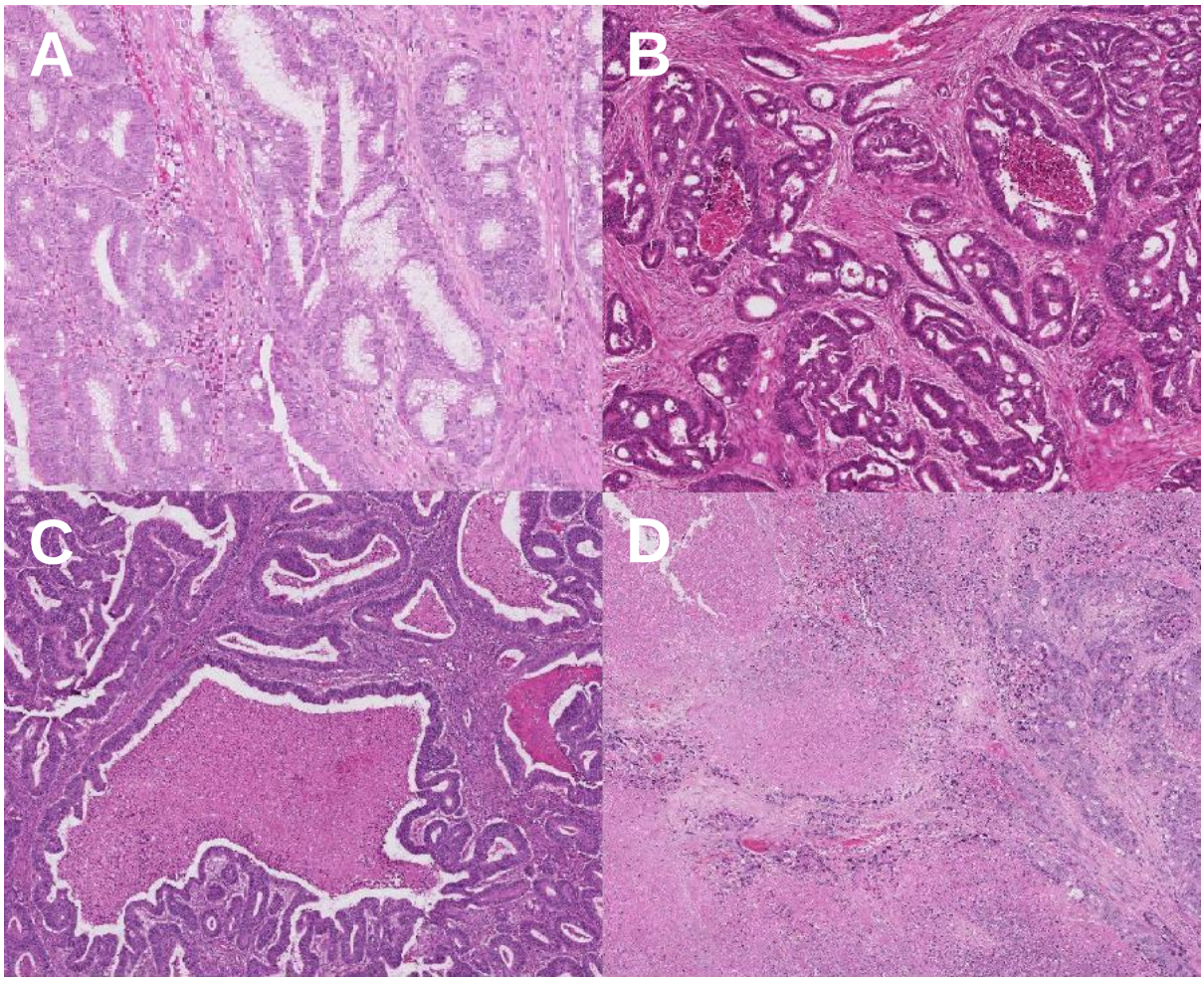
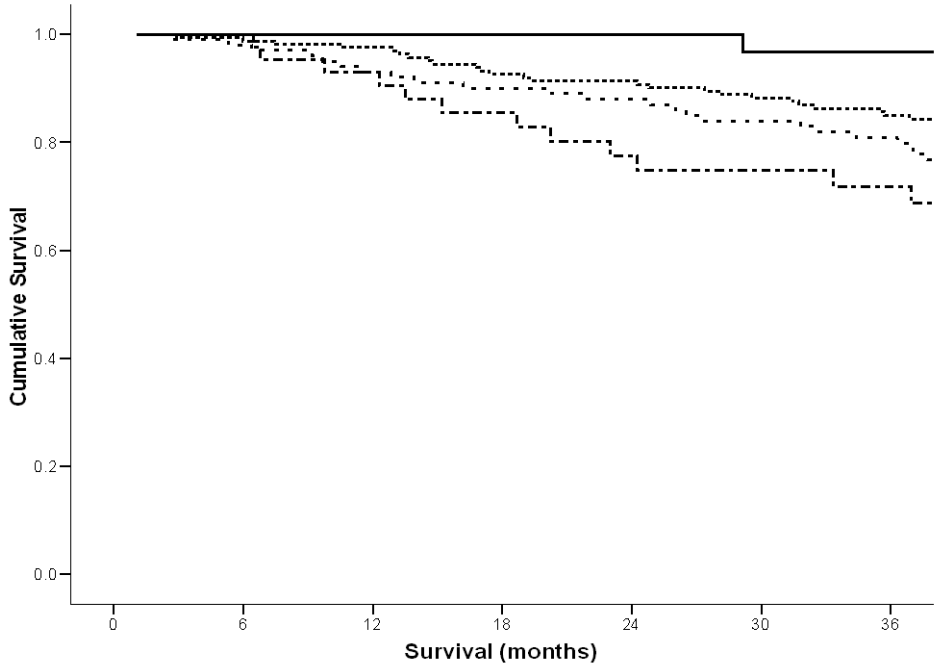


Figure 7.1. Examples of the four grades of histological tumour necrosis in H&E sections; Panel A = 'absent' (none) necrosis, Panel B = 'focal' (< 10%) necrosis, Panel C = 'moderate' (10 - 30%) necrosis, Panel D = 'extensive' (> 30%) necrosis.



Number at risk	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Absent	32	31	31	30	30
Focal	166	163	158	148	144
Moderate	101	99	93	90	87
Extensive	44	42	37	33	28

Figure 7.2. The relationship between tumour necrosis and cancer specific survival in all patients (n=343); necrosis groups ‘absent’, ‘focal’, ‘moderate’ and ‘extensive’ are shown top to bottom (Kaplan-Meier; $p<0.001$, log-rank test).

Table 7.1. The relationships between tumour necrosis and clinico-pathological variables in all patients (n = 343).

Variable		Tumour necrosis				
		Absent (n=32)	**Focal (n=166)**	**Moderate (n=101)**	**Extensive (n=44)**	***p*** [†]
Patient related variables						
Age	≤ 64	11 (9)	62 (52)	34 (29)	12 (10)	
	65 – 74	15 (14)	49 (44)	29 (26)	18 (16)	
	≥ 75	6 (5)	55 (49)	38 (34)	14 (12)	0.214
Sex	Female	13 (8)	75 (48)	45 (29)	23 (15)	
	Male	19 (10)	91 (49)	56 (30)	21 (11)	0.390
Presentation	Elective	29 (9)	158 (49)	95 (30)	39 (89)	
	Emergency	3 (14)	8 (36)	6 (27)	5 (23)	0.438
Tumour site	Colon	24 (10)	109 (46)	71 (30)	33 (14)	
	Rectum	8 (8)	57 (54)	30 (28)	11 (10)	0.526
Anaemia[*]	None	15 (10)	82 (57)	35 (24)	13 (9)	
	Mild	6 (8)	28 (39)	27 (38)	11 (15)	
	Severe	8 (11)	29 (40)	23 (32)	13 (18)	0.022
White cell count [**]	< 8.5 ($x10^9$/L)	22 (12)	90 (50)	54 (30)	15 (8)	
	8.5-11 ($x10^9$/L)	4 (6)	33 (49)	19 (28)	12 (18)	
	> 11($x10^9$/L)	5 (9)	20 (37)	17 (32)	12 (22)	0.006
Systemic inflammatory response	mGPS 0	22 (11)	106 (55)	51 (26)	15 (8)	
	mGPS 1	7 (6)	48 (43)	35 (31)	22 (20)	
	mGPS 2	3 (8)	12 (32)	15 (41)	7 (19)	<0.001
Local inflammatory cell infiltrate (K-M)	Low grade	15 (7)	108 (48)	62 (28)	38 (17)	
	High grade	17 (14)	58 (48)	39 (33)	6 (5)	0.004
Tumour related variables						
T stage	T1	2 (25)	5 (63)	0 (0)	1 (13)	
	T2	7 (25)	18 (64)	2 (7)	1 (4)	
	T3	18 (9)	99 (50)	63 (32)	20 (10)	
	T4	5 (5)	44 (41)	36 (34)	22 (21)	<0.001
N stage	N0	23 (12)	94 (49)	50 (26)	26 (14)	
	N1	7 (6)	54 (48)	37 (33)	15 (13)	
	N2	2 (5)	18 (49)	14 (38)	3 (8)	0.322
TNM stage	Stage I	6 (23)	17 (65)	2 (8)	1 (4)	
	Stage II	17 (10)	77 (46)	48 (29)	25 (15)	
	Stage III	9 (6)	72 (48)	51 (34)	18 (12)	0.015
Petersen Index	Low risk	30 (11)	140 (50)	78 (28)	31 (11)	
	High risk	2 (3)	26 (41)	23 (36)	13 (20)	0.003

[†] chi square linear by linear association
[*] available in 290/343 patients, [**] available in 303/343 patients

Table 7.2. The relationships between tumour necrosis and clinico-pathological variables in patients with node negative disease (n = 193).

Variable		Tumour necrosis				
		Absent (n=23)	**Focal (n=94)**	**Moderate (n=50)**	**Extensive (n=26)**	***p*†**
Patient related variables						
Anaemia*	None	11 (15)	42 (58)	15 (21)	5 (7)	
	Mild	6 (13)	20 (44)	13 (28)	7 (15)	
	Severe	6 (14)	16 (36)	13 (30)	9 (21)	0.020
White cell count **	< 8.5 ($x10^9$/L)	15 (14)	53 (51)	29 (28)	7 (7)	
	8.5-11 ($x10^9$/L)	3 (8)	19 (53)	8 (22)	6 (17)	
	> 11($x10^9$/L)	5 (16)	10 (31)	8 (25)	9 (28)	0.022
Systemic inflammatory response	mGPS 0	15 (14)	62 (56)	23 (21)	10 (9)	
	mGPS 1	6 (10)	23 (38)	18 (30)	13 (22)	
	mGPS 2	2 (9)	9 (39)	9 (39)	3 (13)	0.014
Local inflammatory cell infiltrate (K-M)	Low grade	11 (10)	55 (49)	24 (21)	22 (20)	
	High grade	12 (15)	39 (48)	26 (26)	4 (14)	0.061
Tumour related variables						
T stage	T1	2 (40)	3 (60)	0 (0)	0 (0)	
	T2	4 (19)	14 (67)	2 (10)	1 (5)	
	T3	12 (10)	60 (48)	38 (31)	14 (11)	
	T4	5 (12)	17 (40)	10 (23)	11 (26)	0.001
Petersen Index	Low risk	21 (12)	86 (50)	46 (27)	20 (12)	
	High risk	2 (10)	8 (40)	4 (20)	6 (30)	0.114

† chi square linear by linear association
* available in 163/193 patients, ** available in 172/193 patients

Table 7.3. The relationships between clinico-pathological variables and cancer specific survival in all patients (n = 343).

Variable	Multivariable anlaysis	
	HR (95% C.I.)	***p***
Model 1 (all variables)		
Age (≤64/65-74/≥75)	1.29 (1.00, 1.66)	0.050
Systemic inflammatory response (mGPS 0/1/2)	1.74 (1.27, 2.39)	0.001
Local inflammatory cell infiltrate (K-M low grade/high grade)	2.65 (1.52, 4.63)	0.001
TNM stage (I/II/III)	1.55 (1.02, 2.35)	0.041
Petersen Index (low risk/high risk)	3.50 (2.21, 5.55)	<0.001
Model 2 (without inflammatory variables)		
Age (≤64/65-74/≥75)	1.28 (0.99, 1.66)	0.062
TNM stage (I/II/III)	1.62 (1.08, 2.43)	0.020
Petersen Index (low risk/high risk)	3.43 (2.16, 5.45)	<0.001
Tumour necrosis (absent/focal/moderate/extensive)	1.35 (1.04, 1.77)	0.027

Table 7.4. The relationships between clinico-pathological variables and cancer specific survival in patients with node negative disease (n = 193).

Variable	Multivariable analysis	
	HR (95% C.I.)	***p***
Model 1 (all variables)		
Age (≤64/65-74/≥75)	1.78 (1.19, 2.65)	0.005
Systemic inflammatory response (mGPS 0/1/2)	1.71 (1.09, 2.66)	0.019
Local inflammatory cell infiltrate (K-M low grade/high grade)	3.33 (1.55, 7.13)	0.002
Petersen Index (low risk/high risk)	4.67 (2.23, 9.77)	<0.001
Model 2 (without inflammatory variables)		
Age (≤64/65-74/≥75)	1.84 (1.22, 2.77)	0.004
Petersen Index (low risk/high risk)	4.07 (1.94, 8.57)	<0.001

8.0 THE RELATIONSHIP BETWEEN CELLULAR COMPONENTS OF THE PERITUMOURAL INFLAMMATORY RESPONSE, CLINICOPATHOLOGICAL CHARACTERISTICS AND SURVIVAL IN PRIMARY OPERABLE COLORECTAL CANCER.

8.1 Introduction

Local infiltration of inflammatory cells in the tumour microenvironment is associated with improved survival in patients with colorectal cancer (Chapter 1). However, despite extensive investigation over a 40 year period, a reliable measure of the local inflammatory cell infiltrate has yet to be incorporated into clinical practice. In order to establish routine clinical utility there is therefore a need to standardize the assessment of the local inflammatory cell response in colorectal tumours.

A logical starting point would be to compare the prognostic value and clinicopathological associations of individual immune cells with a more generalised assessment of local inflammation. Indeed, a global assessment of peritumoural inflammatory cell infiltrate, using routinely stained sections, has been proposed by Klintrup and Makinen (K-M grade) (Klintrup, Makinen et al. 2005) and independently validated (Roxburgh, Salmond et al. 2009).

The aim of the present study, therefore, was to examine the relationships between individual inflammatory cells, overall K-M grade and survival in patients with primary operable colorectal cancer.

8.2 Materials and Methods

Patients with colorectal cancer who were considered to have undergone potentially curative resection of colorectal cancer between January 1997 and December 2006 were identified from the same prospective database described in Chapter 3.

The tumours were staged according to conventional AJCC/TNM classification (5^{th} Edition) (Fleming ID 1997). Additional pathological data were taken from reports issued at the time of resection. With regard to venous invasion, cases in the present study which pre-dated the introduction of routine elastica staining at Glasgow Royal Infirmary in 2003 were stained and reported retrospectively. Tumour necrosis was assessed as described in Chapter 7.

The systemic inflammatory response was assessed using the modified Glasgow Prognostic Score, as described in Chapter 1. The peritumoural inflammatory cell infiltrate was assessed according to the K-M criteria, as described in Chapter 7.

The following method was then used to identify individual inflammatory cells. The original sections used for the K-M grading were retrieved from pathology archives and a single representative slide chosen for more detailed analysis. This section was converted to electronic format using a high resolution digital scanner (Slidepath Digital Image Hub v3) before five distinct areas (560μm x 250μm) were selected at intervals along the invasive margin. Gridlines (42μm x 42μm) were digitally superimposed and individual cells counted in ten random boxes within each of these areas. This resulted in a total of 50 boxes (approximately 0.09mm^2) being analysed per patient. For the purposes of deciding if a cell which straddled a gridline was within a box or not, two perpendicular lines were considered 'inclusion' lines and only cells touching these lines were included. Cellular identification put each cell into one of six categories: lymphocyte, plasma cell, neutrophil, macrophage

(including mast cells), eosinophil or 'other' which included neoplastic, stromal, endothelial, necrosed or unidentifiable cells. The cells were counted using image analysis software (ImageJ available at http://rsbweb.nih.gov/ij/). A total of 20 cases were scored independently by two observers to confirm consistency of scoring. The inter-observer intraclass correlation coefficients for each cell type were: lymphocytes 0.92, plasma cells 0.80, neutrophils 0.65, macrophages 0.40, eosinophils 0.92. Figure 8.1 shows an example of how five distinct areas were chosen from along the length of the invasive margin.

Patients were followed up for five years after surgery. Information on date and cause of death was cross-checked with that received by the cancer registration system and the Registrar General (Scotland). Death records were complete until 1st December 2010, which served as the censor date. Cancer-specific survival was measured from the date of surgery until the date of death from colorectal cancer. The study was approved by the West of Scotland Research Ethics Committee (WoSREC), Glasgow.

Statistical analysis

The inflammatory cell types were divided into two equal groups termed 'low' and 'high' based on the median cell count. Grouping of other variables was carried out using standard or previously published thresholds. Associations between categorical and continuous variables were examined using chi-squared tests for linear trend and non-parametric tests respectively. Survival analyses were performed using the Kaplan-Meier method and Cox proportional hazards regression. Variables significant on univariate analysis were entered into a multivariate model using a backwards conditional method. $P<0.050$ was considered statistically significant. Statistical analyses were performed using SPSS® version 19.0 (IBM SPSS, Illinois, USA).

8.3 Results

A total of 130 patients who underwent potentially curative resection of colorectal cancer were included. The majority of patients were 65 years or older (68%) with a similar number of males (52%) and females (48%). Most operations were elective (94%) and were carried out for both colon (68%) and rectal cancer (32%). The preoperative systemic inflammatory response was graded as mGPS 0 in 68 patients (52%), mGPS 1 in 47 (36%) and mGPS 2 in 15 (12%). Original pathological reports classified 8% of the tumours as Stage I, 49% as Stage II and 43% as Stage III. Using elastin staining, there was evidence of intra- or extramural venous invasion in 43 of the tumours (33%). In the postoperative period, a total of 38 patients (29%) received adjuvant chemotherapy.

Application of the K-M criteria graded the peritumoural inflammatory cell response as 'low grade' in 63 patients (48%) and 'high grade' in 67 patients (52%). The distribution of individual inflammatory cell types in the invasive margin are summarised in Table 8.1. The cells identified were primarily macrophages, lymphocytes and neutrophils with relatively few plasma cells or eosinophils (Table 8.1).

The relationships between overall K-M grade and individual inflammatory cell types are shown in Figure 8.2. There were significant relationships between high K-M grade and increased numbers of lymphocytes ($p<0.001$), plasma cells (<0.001), neutrophils ($p<0.01$) and eosinophils ($p<0.01$). There was no relationship between K-M grade and macrophage count (Figure 8.2).

The median follow-up for the survivors was 105 months (range 55 - 163). During this period, 37 patients died from colorectal cancer and 34 patients died from other causes. The survival analyses for K-M grade and individual inflammatory cell types are shown in Table 8.2. On

univariate analysis, K-M grade ($p<0.01$), lymphocyte infiltration ($p<0.05$) and plasma cell infiltration ($p<0.01$) were significantly associated with cancer-specific survival. The Kaplan-Meier survival curves demonstrating these relationships are shown in Figure 8.3.

K-M grade, lymphocyte infiltration and plasma cell infiltration were then entered into a multivariate survival model with standard clinical and pathological variables (Table 8.3). This demonstrated that systemic inflammatory response (mGPS) (HR 2.27, $p<0.01$), TNM stage (HR 1.97, $p<0.05$), venous invasion (HR 2.03, $p<0.05$), tumour necrosis (HR 1.54, $p<0.05$) and K-M grade (HR 2.38, $p<0.05$) were independently associated with cancer-specific survival (Table 3). When patients with node-negative disease were considered alone, only systemic inflammatory response (mGPS) (HR 2.46, $p<0.05$) and K-M grade (HR 3.67, $p<0.05$) were independently associated with cancer-specific survival (data not shown).

The relationships between K-M grade, individual inflammatory cell types and patient-related variables are shown in Table 8.4. No relationships were observed between either K-M grade or lymphocyte infiltration and any of the patient-related variables studied, including markers of the systemic inflammatory response. There was a significant association between plasma cell infiltration and serum neutrophil count ($p<0.05$) (Table 8.4).

The relationships between K-M grade, individual inflammatory cell types and tumour-related variables are shown in Table 8.5. There were significant relationships between K-M grade and T stage ($p<0.01$), N stage ($p<0.05$), TNM stage ($p<0.05$), venous invasion ($P<0.05$), tumour necrosis ($p<0.05$) and margin characteristics ($p<0.001$). For individual cell types, there were significant relationships between lymphocyte infiltration, venous invasion ($p<0.05$) and margin characteristics ($p<0.01$). Similarly, there were significant relationships

between plasma cell infiltration and N stage ($p<0.05$), TNM stage ($p<0.05$), venous invasion ($p<0.01$), tumour necrosis ($p<0.05$) and margin characteristics ($p<0.05$) (Table 8.5).

8.4 Discussion

Results from the present study demonstrate that a strong infiltration of inflammatory cells in the invasive margin of colorectal tumours confers a distinct survival advantage for patients with primary operable colorectal cancer. Furthermore, although a strong overall inflammatory cell infiltrate is a superior predictor of prognosis than the analysis of individual cell types, lymphocytes and plasma cells appear particularly important and are associated with a number of favourable pathological characteristics. Taken together, these results indicate a prominent role for a coordinated adaptive immune response in the prevention of tumour progression in colorectal cancer.

A large number of previous studies, published over 40 year period, have examined the prognostic value of inflammatory cell infiltration in colorectal cancer (Roxburgh and McMillan 2011). Despite this volume of work, there is still no standardised method for the assessment of the local inflammatory response in colorectal tumours. This lack of consensus may be partly explained by the fact that many previous studies have concentrated on single cell types (Naito, Saito et al. 1998; Forssell, Oberg et al. 2007), have relied on tissue microarrays (TMA's) (Galon, Costes et al. 2006; Salama, Phillips et al. 2009) or have employed immunohistochemical techniques (Menon, Janssen-van Rhijn et al. 2004; Sandel, Dadabayev et al. 2005). Importantly, few previous studies have directly compared different methods for assessing the local inflammatory response on full sections.

If such assessments are to move from experimental research into clinical practice, the technique employed must be simple, reproducible and easy to incorporate into existing pathological staging systems. The present study suggests that a simplified overall assessment

of peritumoural inflammation, using the K-M grade, fulfils these criteria and is a superior predictor of prognosis than an assessment of individual inflammatory cells.

In addition to comparing the prognostic value of the above methods, the present study also included a detailed examination of the cellular composition of the invasive margin of colorectal tumours. When all patients were considered, macrophages were the most prevalent cell type, followed closely by lymphocytes and neutrophils. When the cellular composition was re-examined in patients with a high grade peritumoural inflammatory response, the relative proportion of lymphocytes increased while the proportions of neutrophils and macrophages fell. These findings suggests that such patients are mounting a coordinated inflammatory response mediated primarily though lymphocytes.

The mechanisms by which a strong local adaptive immune response improves prognosis in patients with colorectal cancer are not clear. The present study found no association between an infiltration of inflammatory cells and any of the patient-related variables examined. In particular, there were no direct relationships between local inflammation and serum white cell count or systemic inflammatory response. These findings, therefore, suggest a model whereby the initial stimulus for the development of a local inflammatory cell response is evoked by events within the tumour and its microenvironment (Whiteside 2008). In the case of a non-specific immune cell reaction, this may include local tissue damage caused directly by tumour invasion with subsequent hypoxia, cellular necrosis and the release of pro-inflammatory cytokines (Chapter 7). Alternatively, a beneficial adaptive immune cell response may be triggered by altered antigenicity of the tumour cells themselves (Goedegebuure and Eberlein 1995). Indeed, the presence of lymphocytes in the present study was associated with a number of favourable tumour characteristics including an expanding

rather than infiltrative growth pattern, a feature previously reported as an independent prognostic factor in colorectal cancer (Cianchi, Messerini et al. 1997). An association between intra-tumoural lymphocytes and lower levels of venous invasion has been reported previously (Pages, Berger et al. 2005) and the present results suggest this relationship also exists with lymphocytes in the invasive margin. That this finding has not been reported previously may be explained by the use of elastica staining in the present study to aid the detection of venous emboli; a technique resulting in a higher prevalence of venous invasion than seen in many previous studies (Roxburgh and Foulis 2011).

In contrast to cells associated with the adaptive immune response, an abundance of neutrophils or macrophages at the tumour border did not influence survival in the present cohort. Evidence regarding the prognostic value of these cell types, intimately associated with the innate immune response, has been conflicting. Although a number of studies have suggested that a strong infiltration of neutrophils (Baeten, Castermans et al. 2006) and macrophages (Forssell, Oberg et al. 2007) is beneficial to patients with colorectal cancer, others have reported no relationship with survival (Nagtegaal, Marijnen et al. 2001; Nagorsen, Voigt et al. 2007). Indeed, in certain situations tumours may exploit these innate inflammatory cells to promote tumour proliferation and invasion (Pollard 2004). Rather than reflecting a protective host response, the presence of these cell types in the tumour microenvironment then favours tumour growth and dissemination (Liotta and Kohn 2001; Whiteside 2008). However, using H&E stained slides it is difficult to identify and assess the degree of macrophage infiltration. Further work using immunohistochemistry may be required to examine the prognostic value of tumour associated macrophages (TAM's) although there should be careful consideration of the markers to be used since some, such as

CD68, may be expressed by other non myeloid tissues in cancer specimens (Gottfried, Kunz-Schughart et al. 2008).

The present study has a number of limitations. The identification and classification of individual cell types on H&E stained sections is a time consuming process, limiting patient numbers and restricting potential clinical application. However, this level of detail was required to compare the two methods and we can now confirm that a laborious examination of individual cells offers no additional prognostic information compared to a simplified global assessment of inflammation. The present study focused only on the invasive margin and did not assess inflammatory cells within the tumour itself. The primary reason for this approach was that the tumour border is felt to represent a critical interface between pro- and anti-tumour factors (Zlobec and Lugli 2009). Furthermore, inflammatory cells within the tumour itself are difficult to identify on H&E-stained sections and there is currently no global assessment of intra-tumoural inflammation against which to make a comparison. Nevertheless, an examination of the prognostic value of intra-tumoural inflammatory cells, using immunohistochemical techniques, is of considerable interest and will be the subject of future work.

In summary, the present study confirms that a simple assessment of peritumoural inflammation, using the K-M grade, has independent prognostic value in patients with primary operable colorectal cancer. Examination of individual cell types does not improve prediction of outcome but does suggest a prominent role for lymphocytes in the prevention of tumour progression in these patients. Taken together these findings give additional support to the prognostic significance of the local inflammatory response in colorectal cancer and to the

idea that a simple overall assessment of peritumoral inflammation could be applied in clinical practice.

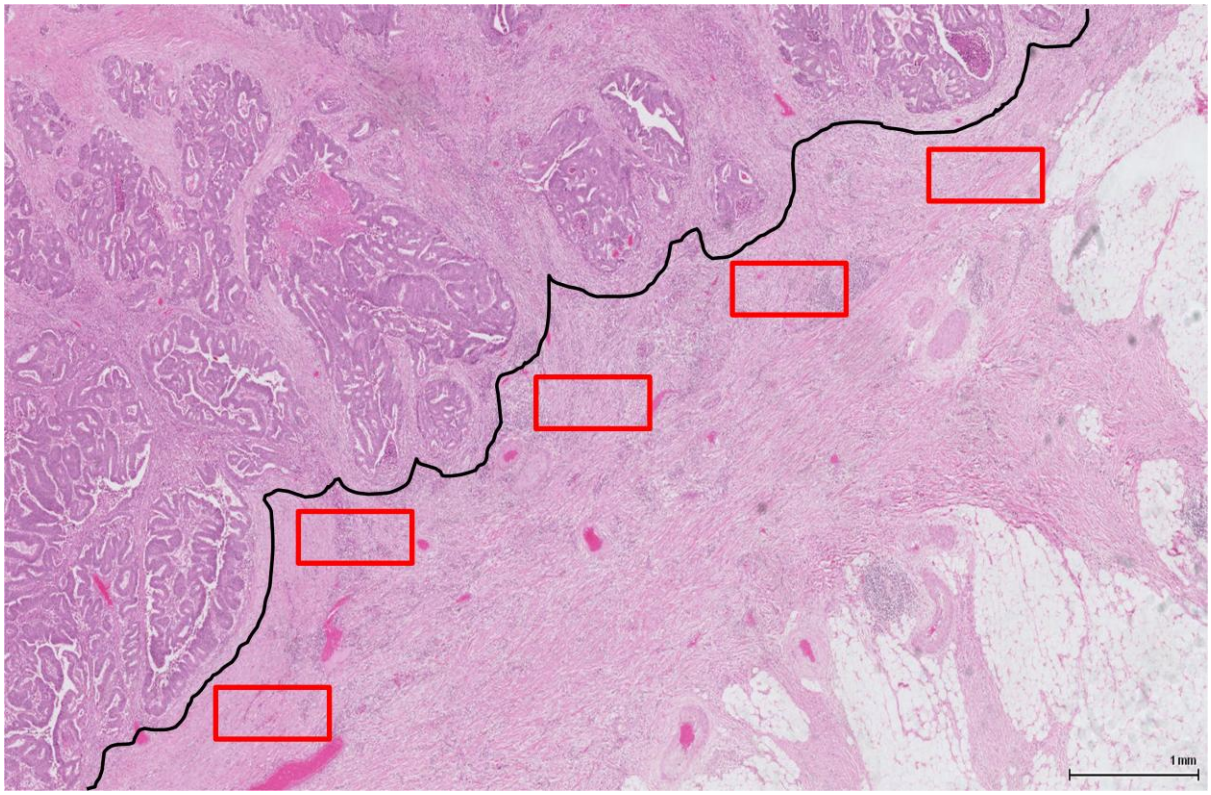


Figure 8.1. Example of how 5 distinct areas (560μm x 250μm) were chosen from the invasive margin (black line) of each H&E stained section. The inflammatory cells in each of these areas were then categorised and counted.

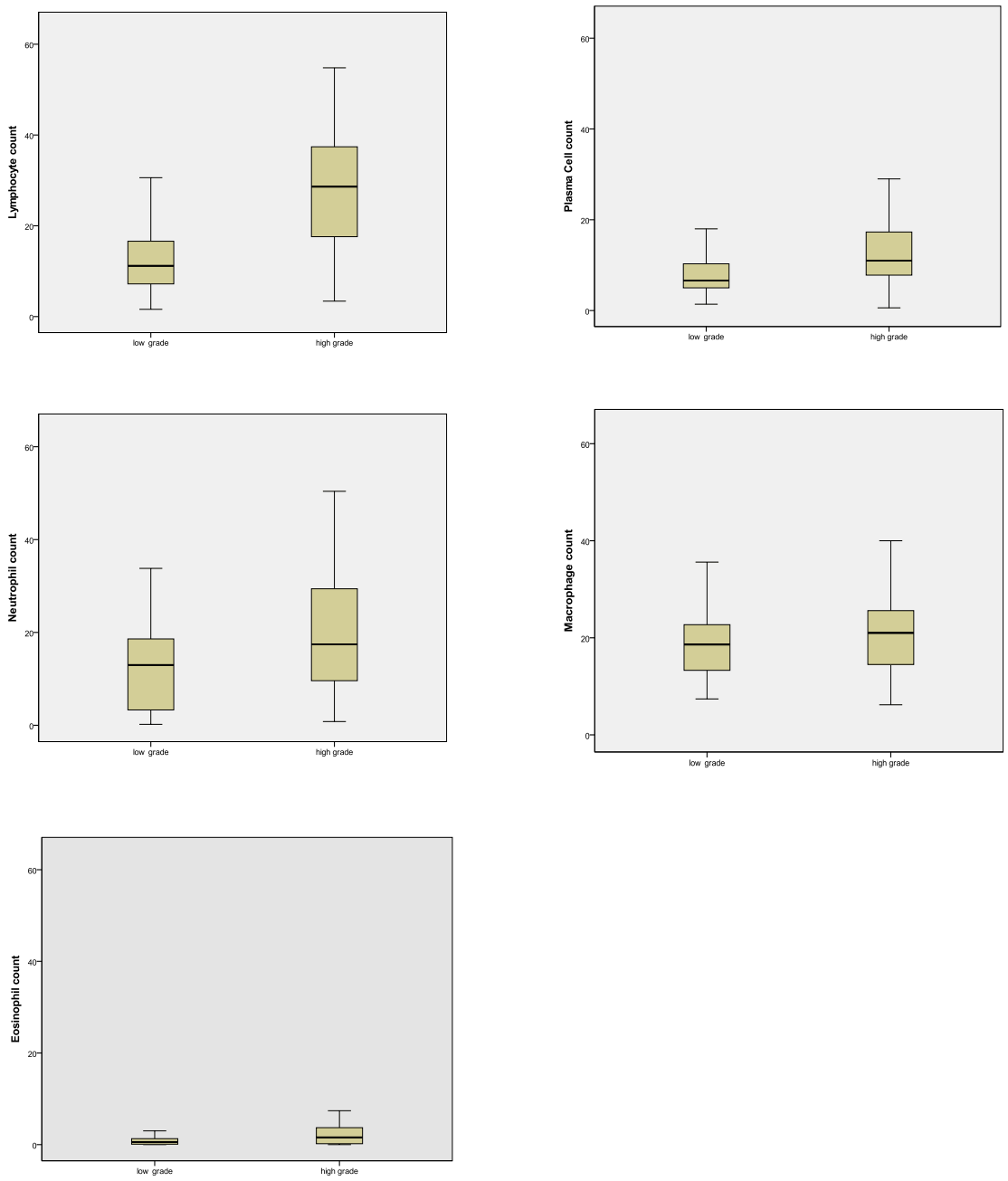
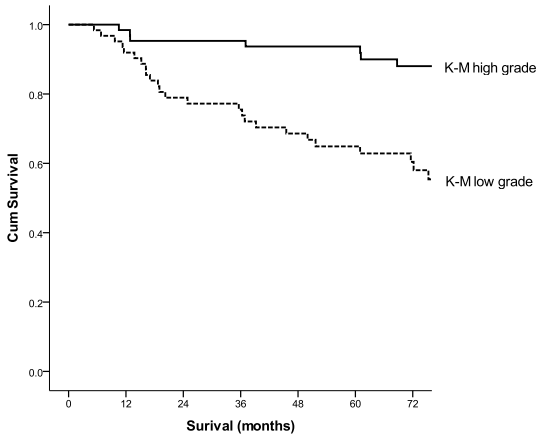
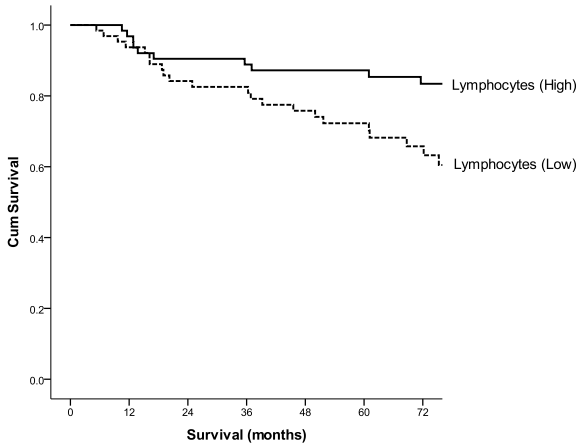


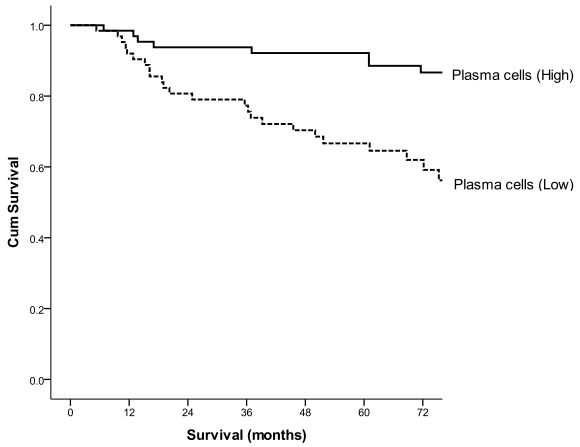
Figure 8.2. Boxplot representation of the relationships between individual inflammatory cell types and K-M grade; lymphocytes ($p<0.001$, Mann-Whitney U test), plasma cells ($p<0.001$), neutrophils ($p=0.002$), macrophages ($p=0.21$), eosinophils ($p=0.001$).



Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5
K-M high grade	67	63	62	58	53
K-M low grade	63	56	47	43	36



Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5
Lymphocytes (high)	65	61	56	53	48
Lymphocytes (Low)	65	59	51	49	41



Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5
Plasma cells (high)	65	63	59	57	53
Plasma cells (low)	65	57	48	44	37

Figure 8.3. Kaplan-Meier survival curves demonstrating the relationships between K-M grade (p=0.001, log-rank test), lymphocyte infiltration (p=0.041, log-rank test), plasma cell infiltration (p=0.001, log-rank test) and cancer-specific survival.

Table 8.1. Distribution of individual inflammatory cell types in the invasive margin of colorectal tumours.

Cell type	N	Cell count*		% of all cells	
		Median	(range)	Mean	(95% C.I.)
Macrophages	130	19	(6 - 49)	22	(21 - 23)
Lymphocytes	130	17	(2 - 86)	21	(20 - 23)
Neutrophils	130	15	(1 - 78)	18	(16 - 20)
Plasma Cells	130	9	(1 - 41)	11	(10 - 12)
Eosinophils	130	1	(0 - 14)	2	(1 - 2)
Others†	130	19	(5 - 55)	22	(20 - 24)

* Cell count/ 0.018mm^2
† Including neoplastic cells, stromal cells and endothelial cells.

Table 8.2. The relationships between K-M grade, individual inflammatory cell types and cancer-specific survival (univariate survival analysis).

Variable		Univariate HR (95% C.I.)	*p*
Peritumoural inflammation	K-M low grade	3.13 (1.53, 6.38)	0.002
	K-M high grade	1.00	
Lymphocytes	Low	1.98 (1.02, 3.86)	0.045
	High	1.00	
Plasma cells	Low	2.99 (1.49, 5.99)	0.002
	High	1.00	
Neutrophils	Low	1.45 (0.75, 2.81)	0.27
	High	1.00	
Macrophages	Low	1.38 (0.71, 2.68)	0.34
	High	1.00	
Eosinophils	Low	1.72 (0.89, 3.35)	0.11
	High	1.00	

Table 8.3. The relationships between clinico-pathological characteristics and cancer-specific survival (multivariate survival analysis).

Variable	Univariate HR (95% C.I.)	*p*	Multivariate HR (95% C.I.)	*p*
Sex (female/male)	1.15 (0.60, 2.19)	0.68		
Age (≤64/65-74/≥75)	1.30 (0.87, 1.96)	0.20		
Presentation (elective/emergency)	1.79 (0.55, 5.87)	0.34		
Smoking (never/ex/current)	1.19 (0.73, 1.95)	0.49		
Anaemia (none/mild/severe)	0.97 (0.60, 1.57)	0.91		
Systemic inflammatory response (mGPS 0/1/2)	2.40 (1.55, 3.73)	<0.001	2.27 (1.36, 3.80)	0.002
Tumour site (colon/rectum)	0.72 (0.34, 1.53)	0.39		
TNM stage (I/II/III)	2.25 (1.24, 4.05)	0.007	1.97 (1.01, 3.82)	0.046
Differentiation (well or mod/poor)	2.47 (1.12, 5.43)	0.024		0.12
Venous invasion (no/yes)	2.38 (1.24, 4.54)	0.009	2.03 (1.02, 4.06)	0.044
Tumour necrosis (absent/focal/moderate/extensive)	2.02 (1.36, 2.99)	<0.001	1.54 (1.02, 2.33)	0.038
Character or margin (expanding/infiltrating)	2.25 (1.16, 4.34)	0.016		0.29
Peritumoural inflammation (K-M high grade/low grade)	3.13 (1.53, 6.38)	0.002	2.38 (1.08, 5.22)	0.031
Lymphocytes (high/low)	1.98 (1.02, 3.86)	0.045		0.36
Plasma cells (high/low)	2.99 (1.49, 5.99)	0.002		0.54

Table 8.4. The relationships between K-M grade, lymphocyte infiltration, plasma cell infiltration and patient-related variables.

Variable	K-M grade (low/high)	p	Lymphocytes (low/high)	p	Plasma cells (low/high)	p
Sex		0.71		1.00		0.48
Male	34/34		34/34		32/36	
Female	29/33		31/31		33/29	
Age		0.92		0.91		0.59
≤ 64	19/22		17/24		19/22	
65 - 74	25/22		30/17		23/24	
> 75	19/23		18/24		23/19	
Presentation		0.17		1.00		0.47
Elective	61/61		61/61		60/62	
Emergency	2/6		4/4		5/3	
Smoking status		0.28		0.13		0.37
Never	21/30		21/30		24/27	
Ex	18/15		20/13		14/19	
Current	11/10		12/9		13/8	
Anaemia		0.56		0.41		0.41
Mild	25/31		27/29		30/26	
Moderate	8/15		14/9		8/15	
Severe	15/13		10/18		13/15	
Serum leukocytes*						
White cell count	9.3/8.9	0.63	9.3/8.8	0.50	9.7/8.4	0.11
Neutrophils	6.8/5.9	0.12	6.8/5.8	0.10	6.9/5.7	0.037
Lymphocytes	1.5/1.6	0.21	1.5/1.6	0.64	1.6/1.5	0.24
Systemic inflammatory response		0.50		0.70		0.16
mGPS = 0	30/38		35/33		30/38	
mGPS = 1	26/21		23/24		26/21	
mGPS = 2	7/8		7/8		9/6	

*$x10^9$/l

Table 8.5. The relationships between K-M grade, lymphocyte infiltration, plasma cell infiltration and tumour-related variables.

Variable	K-M grade (low/high)	p	Lymphocytes (low/high)	p	Plasma cells (low/high)	p
Tumour site		0.67		0.09		0.85
Colon	42/47		40/49		44/45	
Rectum	21/20		25/16		21/20	
T stage		0.001		0.41		0.06
T1/2	1/14		6/9		4/11	
T3/4	62/53		59/56		61/54	
N stage		0.039		0.72		0.03
N0	30/44		38/36		31/43	
N1/2	33/23		27/29		34/22	
TNM stage		0.006		0.78		0.024
I	1/9		5/5		3/7	
II	29/35		33/31		28/36	
III	33/23		27/29		34/22	
Differentiation		0.72		0.48		0.83
Well/moderate	53/57		54/56		55/55	
Poor	10/9		11/8		9/10	
Venous invasion		0.008		0.016		0.005
No	35/52		37/50		36/51	
Yes	28/15		28/15		29/14	
Tumour necrosis		0.018		0.11		0.010
Absent	3/7		6/4		2/8	
Focal	26/33		26/33		27/32	
Moderate	20/22		19/23		23/19	
Extensive	13/4		14/3		12/5	
Character or margin		<0.001		0.009		0.016
Expanding	25/53		32/46		32/46	
Infiltrating	37/14		33/18		32/19	

9.0 THE CLINICAL UTILITY OF THE LOCAL INFLAMMATORY RESPONSE IN PRIMARY OPERABLE COLORECTAL CANCER.

9.1 Introduction

It is now recognized that the host immune response is an important determinant of outcome in human cancers (Hanahan and Weinberg 2011). A number of studies have demonstrated that infiltration of inflammatory cells in colorectal tumours is associated with improved survival, regardless of pathological stage (Roxburgh and McMillan 2011). It is generally assumed that the presence of these cells is a manifestation of an effective immune response although it is unclear whether this reflects distinct tumour biology or particular host characteristics.

Despite the potential to improve risk stratification for patients with colorectal cancer, a reliable measure of the local inflammatory response has yet to be incorporated into clinical practice. The reasons for this are likely to include the multitude of individual cell types or 'immune scores' that have been proposed as prognostic as well as the inherent complexities of immunohistochemistry (Galon, Pages et al. 2012). In particular, there is a need to clarify whether lymphocyte subtyping adds additional prognostic information beyond the evaluation of inflammatory cells on routinely stained sections (Huh, Lee et al. 2012).

The aims of the present study, therefore, were to evaluate the type, density and location of tumour infiltrating lymphocytes (TIL's) in patients with primary operable colorectal cancer and to examine relationships with both tumour and host characteristics. Furthermore, we sought to compare the prognostic value of individual T-cell subtypes, an immunohistochemistry-based score and a simple histopathological assessment of inflammatory cell infiltrate.

9.2 Materials and Methods

Patients with colorectal cancer who were considered to have undergone potentially curative resection of colorectal cancer between January 1997 and December 2006 were identified from the same prospective database described in Chapter 3.

Prospectively collected data included patient demographics, pathological characteristics and laboratory measurements; haemoglobin (Hb), white cell count (WCC), albumin, C-reactive protein (CRP), urea and electrolytes. Medical records were reviewed retrospectively to record deprivation index, American Society of Anesthesiologists (ASA) grade, smoking status and POSSUM physiology scores, as described in Chapter 3. Preoperative systemic inflammatory response was assessed using three validated measures; (1) serum white cell count (WCC) (Maltoni, Caraceni et al. 2005), (2) neutrophil to lymphocyte ratio (NLR) (Walsh, Cook et al. 2005) and (3) the modified Glasgow Prognostic Score (mGPS) (McMillan 2008).

Histopathology and immunohistochemistry

Tumours were staged according to the 5th edition of the AJCC/TNM staging system (Fleming ID 1997). Additional pathological features, including tumour differentiation and venous invasion, were taken from contemporary reports. Tumour necrosis was graded semiquantitatively as 'absent' (none), 'focal' (< 10% of tumour area), 'moderate' (10–30%) or 'extensive' (>30%), as described in Chapter 7.

Archived paraffin embedded blocks of the central tumour were then retrieved to perform immunohistochemistry. One block, representative of the point of deepest tumour invasion, was chosen per case. Consecutive blank 4μm sections were cut and mounted on silanized slides before being dewaxed in xylene and rehydrated using graded alcohol washes. Heat-

induced antigen retrieval was performed by microwaving under pressure using a citrate or Tris/EDTA buffer before endogenous peroxidase activity was blocked (5% normal goat serum in TRIS buffered saline (TBS)) and the following primary antibodies applied; $CD3^+$ (Vector Labs, code VP-RM01, 1/100 dilution), $CD8^+$ (DakoCytomation, code M7103, 1/100 dilution), $CD45R0^+$ (DakoCytomation, code M0742, 1/150 dilution) and $FOXP3^+$ (Abcam, code 20034, 1/200 dilution). Sections were washed with TBS, incubated with Dako Envision, washed again and had 3'3' diaminobenzidine (DAB) applied. Finally, sections were washed with water, counterstained with haemotoxylin, dehydrated and mounted (Appendix 1 – 4 describe the full immunohistochemistry protocols)

Evaluation of T-cell density was carried out by investigators blinded to clinicopathologic information. Density was graded semi-quantitatively as absent, weak, moderate or strong in three separate tumour compartments; (1) invasive margin (IM), (2) tumour stroma (ST) and (3) cancer cell nests (CCN). Figure 9.1 shows examples of different patterns of T-cell infiltration in the tumour microenvironment. To confirm consistency of grading, 100 cases were scored independently by two investigators.

Immune Scores

In addition to assessing individual T-cell subtypes, two previously proposed 'immune scores' for the assessment of the local inflammatory response in colorectal cancer were applied; (1) The Galon Immune Score, a composite immunohistochemistry-based score which grades $CD45R0^+$ and $CD8^+$ infiltration in the invasive margin and central tumour (Mlecnik, Tosolini et al. 2011) and (2) the Klintrup-Makinen (K-M) grade, a global assessment of inflammatory cell infiltration at the invasive margin using haematoxylin and eosin (H&E) stained sections (Klintrup, Makinen et al. 2005).

Statistical analysis

All variables were grouped according to standard or previously published thresholds. Associations were examined using Chi-square tests for linear trend and Mann Whitney tests. Death records were complete until 1st December 2011, which served as the censor date. Univariate survival analyses were performed using Kaplan–Meier survival curves with log-rank tests. Multivariate analyses were performed using Cox proportional hazards regression with a backwards conditional method. $p<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS® version 19.0 (IBM SPSS, Chicago, Illinois, USA).

9.3 Results

A total of 365 patients who underwent potentially curative resection of colorectal cancer in the study period were included. Clinical data was unavailable in a number of cases; deprivation index (2%), ASA grade (22%), smoking status (17%), POSSUM physiology score (27%), WCC (14%), NLR (23%) and anaemia (16%). Immunohistochemistry and/or pathological data was missing in 12% (staining errors, broken slides and inability to clearly identify the invasive margin).

Table 9.1 summarizes the clinical and pathological characteristics of the cohort. The majority of patients were 65 years or older (67%) with a similar number of males (55%) and females (45%). Approximately two thirds of operations were for colon cancer and one third for rectal cancer. Conventional staging confirmed that 208/365 patients (57%) had undergone surgery for node-negative disease (Table 9.1).

The type, density and location of T-cells in the tumour microenvironment are shown in Table 9.2. The distribution of T-cells in each tumour compartment varied between cellular subtype; there was a strong infiltration of $CD3^+$, $CD45R0^+$ and $CD8^+$ cells at the invasive margin (IM) in approximately 10-15% of cases and within the cancer cell nests (CCN) in approximately 10% of cases. The distribution of T-cells within the tumour stroma varied considerably. Of note, the densities of $FOXP3^+$ infiltration were lower than other T-cell subtypes in all tumour compartments and were rarely found within the cancer cell nests (Table 9.2). The inter-observer agreements for each T-cell subtype are also shown in Table 9.2. There was excellent agreement (ICC>0.80) in the grading of all T-cell subtypes except $FOXP3^+$ cells. It should be noted that the ICC (0.81) of the K-M grade has been reported previously in this

cohort (Roxburgh, Salmond et al. 2009) while the Galon Immune Score is a combination of T-cell subtypes with individual inter-observer agreements (Table 9.2).

The inter-relationships between T-cell subtypes in the tumour compartments are shown in Table 9.3. Despite individual variations in patterns of infiltration, there were highly significant positive relationships between infiltration of all T-cell subtypes in all tumour compartments (all $p<0.001$, chi-square tests for linear trend). Similarly, the K-M grade and Galon Immune Score were significantly related to all T-cell subtypes in all tumour compartments (all $p<0.001$,chi-square tests for linear trend) (Table 9.3).

The median follow-up for the survivors in the study was 115 months (range 59 - 179). During this period, 137 patients died from colorectal cancer and 71 patients died from inter-current disease. Table 9.4 shows the relationships between T-cell infiltration and cancer-specific survival. On univariate analysis there were significant relationships between infiltration of all T-cell subtypes and cancer-specific survival (all $p<0.01$). To examine which subtype had the strongest prognostic value in each tumour compartment the invasive margin, tumour stroma and cancer cell nests were then considered separately. On multivariate analysis, $CD3^+$ (HR 0.49, 95% CI 0.38-0.63, $p<0.001$) was the strongest predictor of survival at the invasive margin, CD 3^+ (HR 0.58, 95% CI 0.46-0.75, $p<0.001$) the strongest in the tumour stroma and $CD8^+$ (HR 0.68, 95% CI 0.50-0.90, $p=0.008$) the strongest within the cancer cell nests (Table 9.4).

Having established the prognostic value of $CD3^+$ IM and $CD8^+$ CCN in particular, their relationships with host characteristics and tumour biology were examined. There were no significant relationships between $CD3^+$ IM or $CD8^+$ CCN infiltration and patient demographics, markers of physiological health or any assessment of the systemic

inflammatory response (Table 9.5a). In terms of tumour biology, significant associations were observed between $CD3^+$ IM and T stage ($p<0.001$), N stage ($p=0.026$), TNM stage ($p=0.008$), venous invasion ($p=0.038$) and growth pattern ($p=0.001$). Similarly, $CD8^+$ CCN was significantly associated with T stage ($p=0.014$), N stage (<0.001), TNM stage ($p=0.001$), venous invasion ($p=0.002$) and growth pattern ($p=0.003$) (Table 9.5b).

Comparison was then made between the prognostic value of individual T-cell subtypes ($CD3^+$ IM, $CD3^+$ ST and $CD8^+$ CCN), a composite immune score (Galon Immune Score) and a histopathological assessment of inflammatory cell infiltrate (K-M grade) (Table 9.6). Four models were constructed to compare prognostic value in the following patient subsets; (1) stage I-III colorectal cancer, (2) stage I-II colorectal cancer, (3) colon cancer and (4) rectal cancer. On univariate analysis, significant survival relationships for $CD3^+$ IM (all $p<0.001$), $CD3^+$ ST (all $p<0.001$), $CD8^+$ CCN (all $p<0.01$), K-M grade (all $p<0.01$) and Galon Immune Score (all $p<0.01$) were observed across all patient subsets. On multivariate analysis, $CD3^+$IM (HR 0.72, 95% CI 0.52-0.99, $p=0.045$) and $CD8^+$CCN (HR 0.58, 95% CI 0.44-0.77, $p<0.001$) were independently associated with cancer specific survival in stage I-III colorectal cancer; $CD3^+$ST (HR 0.61, 95% CI 0.39-0.93, $p=0.020$) and $CD8^+$CCN (HR 0.56, 95% CI 0.36-0.86, $p=0.009$) were independently associated with cancer specific survival in node-negative colorectal cancer; $CD3^+$ IM (HR 0.61, 95% CI 0.39-0.96, $p=0.031$) and $CD8^+$ CCN (HR 0.55, 95% CI 0.39-0.79, $p=0.001$) were independently associated with cancer specific survival in colon cancer; $CD3^+$ ST (HR 0.57, 95% CI 0.35-0.94, $p=0.027$) and $CD8^+$ CCN (HR 0.45, 95% CI 0.26-0.78, $p=0.005$) were independently associated with cancer specific survival in rectal cancer (Table 9.6).

Kaplan-Meier curves demonstrating cancer specific survival according to the different methods of assessing the local inflammatory response in colorectal cancer are demonstrated in Figure 9.2; $CD3^+$ IM ($p<0.001$, log-rank test), $CD8^+$ CCN ($p<0.001$, log-rank test), K-M grade ($p<0.001$, log-rank test) and Galon Immune Score ($p<0.001$, log-rank test).

9.4 Discussion

The results of the present study confirm and extend our previous study of the prognostic value of inflammatory cells at the invasive margin of colorectal tumours, using H&E analysis (Chapter 8). In particular, by using immunohistochemistry in a larger cohort we have identified that increased T-cell infiltrate, whether in the invasive margin, tumour stroma or cancer cell nests is consistently associated with improved cancer specific survival independent of nodal status or tumour site. Taken together with previous work (Nielsen, Hansen et al. 1999; Roxburgh and McMillan 2011), these results suggest that the type and location of inflammatory cells are subordinate to the density of infiltration. Indeed, a dense infiltrate of T-cells, indicative of a coordinated adaptive immune response, appears to be one of the most important factors in predicting outcome in patients undergoing potentially curative resection for colorectal cancer. With a view to developing a standardised assessment of tumour inflammatory cell infiltrate which can be used in clinical practice, the observation that density is paramount provides a solid basis on which to base future methodological approaches.

A large number of previous studies have examined the prognostic value of inflammatory cell infiltration in colorectal cancer (Roxburgh and McMillan 2011). However, despite accumulating evidence that effector/cytotoxic ($CD3^+/CD8^+$)(Naito, Saito et al. 1998; Galon, Costes et al. 2006), memory ($CD45R0^+$) (Pages, Berger et al. 2005) and regulatory ($FOXP3^+$) (Salama, Phillips et al. 2009) T-cells are important components of an anti-tumour response, there is no agreement as to which individual cell type(s) are most important. This is likely to be due to many studies only reporting the prognostic value of certain cell subtypes or selected cell groups in a variety of locations; often failing to differentiate between different tumour compartments (Naito, Saito et al. 1998; Nagtegaal, Marijnen et al. 2001; Diederichsen,

Hjelmborg et al. 2003). In addition, many studies have relied on tissue microarrays (TMA's) (Pages, Berger et al. 2005; Galon, Costes et al. 2006; Salama, Phillips et al. 2009) that, given the heterogenous patterns of inflammatory cell infiltration observed in the present study, may not be representative of full sections. It is clear that for individual studies to be compared objectively results must include a precise description of the type, density and location of individual inflammatory cells; we sought to achieve this by examining a panel of T-cell markers and describing infiltration separately within the invasive margin, tumour stroma and cancer cell nests. Interestingly, our results suggest that strong inter-relationships exist between all T-cell subtypes in all tumour compartments, perhaps explaining why each subtype has been reported as having individual prognostic value.

The mechanisms by which a strong local immune response improves prognosis in patients with colorectal cancer are not clear. The present study found no association between the strength of the local inflammatory response and surrogate markers of global health such as age, deprivation score or physiological function. Similarly, there were no relationships between T-cell infiltration and serum leukocyte count or levels of circulating cytokines. These findings therefore support a model whereby a beneficial local inflammatory response is not solely reliant on a patients' inherent immunity but may rather be evoked by events within the tumour and its microenvironment (Nagtegaal, Marijnen et al. 2001). If host characteristics cannot explain the presence or absence of a local immune response, our examination of relationships with pathological features offers some insight into the mechanisms by which survival is improved. It has been shown that the microscopic characteristics of the invasive margin have prognostic significance in colorectal cancer(Jass, Love et al. 1987; Hase, Shatney et al. 1993; Kanazawa, Mitomi et al. 2008) and in the present study we observed that low levels of $CD3^+$ IM were associated with a more aggressive

infiltrative growth pattern. A strong tumour inflammatory cell infiltrate in the mesenchyme may therefore protect against direct tumour growth and extension; a hypothesis supported by the strong association with T stage.

In contrast, a strong infiltration of intra-tumoural T-cells was more closely associated with lymph node status, an indicator of metastatic spread rather than direct tumour growth. This raises the possibility that the mechanisms by which TIL's improve outcome may vary depending on their location within the microenvironment. Indeed, the present study found that a strong infiltration of CD8+ cells within the cancer cell nests was associated with a number of favourable pathological characteristics, including significantly lower levels of venous invasion. This supports the work of Pages and colleagues (Pages, Berger et al. 2005) and suggests that intra-tumoural lymphocytes may confer a survival advantage through the prevention of vascular emboli, the earliest sign of metastatic invasion. Further work is required to confirm such associations and investigate their biological relevance.

Considering the importance of the host immune response in the control of tumour progression, it is now essential to incorporate a measure of this in the classification and prognostic stratification of colorectal cancer. Not only does infiltration of inflammatory cells predict outcome in node negative disease, thereby having the potential to identify patients who may benefit from adjuvant chemotherapy, it has the advantage of representing a possible target for novel therapies. A major barrier to inclusion in therapeutic trials or clinical practice, however, is the fact that no standardised methodology exists. Indeed, Galon and colleagues (Galon, Pages et al. 2012) have identified the 'harmonization' of methods to assess the local inflammatory response as essential in improving clinical decision-making for patients with colorectal cancer. To our knowledge, this is the first study to directly compare

the prognostic value of individual T-cell subtypes, a composite immune score and a histopathological assessment on the same cohort of patients. We have deliberately tried to avoid concluding that one method is better than another; instead recognizing that, in addition to predictive ability, factors such as simplicity, variability and ease of incorporation into existing staging systems must be taken into account when developing a standardized method of grading the local inflammatory response.

The present study has a number of limitations. Data relating to several clinical variables was collected retrospectively and was incomplete in a number of cases. Despite this, the study included a detailed examination of relationships with patient-related characteristics, including markers of physiological health and the systemic inflammatory response. We also chose to grade T-cell infiltrate semi-quantitatively rather that utilize automated cell counting software, introducing the possibility of observer variability. However, inter-observer agreement was generally good and the technique permits a broader examination of full sections, allowing tumour compartments to be identified accurately and necrotic areas to be avoided. Given the heterogeneity of T-cell density within single sections the authors believe this technique may be more representative than automated TMA analysis. Finally, data on molecular features such as microsatellite instability (MSI) and genetic mutations were not available in the present study. Although an association between lymphocyte infiltration and MSI has been shown in colorectal tumours (Jenkins, Hayashi et al. 2007), a well-powered study by Ogino and coworkers recently demonstrated that the survival benefit of T-cell infiltration was independent of any molecular or genetic features including MSI status and *KRAS* mutations (Ogino, Nosho et al. 2009).

In summary, the present study has shown that increased T-cell infiltrate in either the invasive margin, tumour stroma or cancer cell nests is consistently associated with improved survival, independent of nodal status or tumour site, in patients with primary operable colorectal cancer. These results provide a solid foundation on which to develop a standardised method for the routine assessment of tumour inflammatory cell infiltrate.

Table 9.1. Clinico-pathological characteristics of the 365 patients with primary operable colorectal cancer.

Variable		**365 (%)**
Age	≤ 64	119 (33)
	65 – 74	124 (34)
	≥ 75	122 (33)
Sex	Male	201 (55)
	Female	164 (45)
Presentation	Elective	339 (93)
	Emergency	26 (7)
Deprivation score	1 - 2	17 (5)
	3 - 5	148 (41)
	6 - 7	193 (54)
POSSUM physiology score	11 – 14	47 (18)
	15 – 20	113 (43)
	21 – 30	93 (35)
	> 30	13 (5)
ASA grade	1 – 2	175 (61)
	3 – 4	110 (39)
Smoking status	Non smoker	125 (41)
	Ex smoker	108 (36)
	Current smoker	71 (23)
Anaemia	None	157 (51)
	Mild	74 (24)
	Severe	75 (25)
White cell count	< 8.5 ($x10^9$/L)	192 (61)
	8.5-11 ($x10^9$/L)	73 (23)
	> 11($x10^9$/L)	50 (16)
NLR	< 5:1	216 (77)
	≥ 5:1	64 (23)
mGPS	0	212 (58)
	1	103 (28)
	2	50 (14)
Tumour site	Colon	236 (65)
	Rectum	129 (35)
T stage	T 1	10 (3)
	T 2	26 (7)
	T 3	219 (60)
	T 4	110 (30)
N Stage	N 0	208 (57)
	N 1	114 (31)
	N 2	43 (12)
TNM stage	Stage I	26 (7)
	Stage II	182 (50)
	Stage III	157 (43)
Venous invasion	No	243 (67)
	Yes	122 (33)
Differentiation	Well / Moderate	320 (88)
	Poor	45 (12)
Growth pattern	Expanding	189 (54)
	Infiltrative	163 (46)
Tumour necrosis	Absent	29 (9)
	Focal	162 (49)
	Moderate	95 (29)
	Extensive	41 (13)

Table 9.2. Type, location and density of inflammatory cell infiltration in the microenvironment of colorectal tumours with inter-observer variability testing.

Cell type	Location	Density				ICC	*p*[*]
		Absent N (%)	Weak N (%)	Moderate N (%)	Strong N (%)		
$CD3^+$	Margin	39 (12)	148 (47)	95 (30)	35 (11)	0.828	<0.001
	Stroma	23 (7)	137 (42)	116 (35)	53 (16)	0.879	<0.001
	CC nests	82 (25)	134 (41)	67 (20)	46 (14)	0.865	<0.001
$CD45R0^+$	Margin	36 (11)	141 (44)	94 (30)	48 (15)	0.883	<0.001
	Stroma	8 (2)	142 (43)	116 (35)	64 (19)	0.898	<0.001
	CC nests	85 (26)	145 (44)	69 (21)	31 (9)	0.872	<0.001
$CD8^+$	Margin	61 (20)	134 (43)	90 (29)	27 (9)	0.833	<0.001
	Stroma	85 (26)	160 (49)	61 (19)	21 (6)	0.867	<0.001
	CC nests	107 (33)	123 (38)	60 (18)	37 (11)	0.873	<0.001
$FOXP3^+$	Margin	63 (20)	122 (39)	126 (41)	0 (0)	0.823	<0.001
	Stroma	71 (22)	122 (38)	130 (40)	0 (0)	0.727	<0.005
	CC nests	166 (51)	157 (49)	0 (0)	0 (0)	0.422	0.134
K-M grade[†]	Margin	72 (21)	160 (46)	86 (25)	28 (8)	0.81	<0.001
Galon Immune Score[††]	All	91 (25)	106 (29)	53 (15)	58 (16)	N/A	N/A

[†] Includes all inflammatory cell types
[††]Composite score of $CD45R0^+$ and $CD8^+$ infiltration in the invasive margin and central tumour and graded as (0)-Hi, (1-2)-Hi, (3)-Hi and (4)-Hi
ICC=Inter-observer intraclass correlation coefficient
[*] *p* value of the F-test corresponding to the ICC

Table 9.3. Contingency table analysis demonstrating the inter-relationships between T-cell subtypes, K-M grade and the Galon Immune Score in colorectal tumours.

		CD3+			CD45R0+			CD8+			FOXP3+		
		Margin	**Stroma**	**CC nests**	**Margin**	**Stroma**	**CC nests**	**Margin**	**Stroma**	**CC nests**	**Margin**	**Stroma**	**CC nests**
CD3+	**Margin**												
	Stroma	<0.001											
	CC nests	<0.001	<0.001										
CD45R0+	**Margin**	<0.001	<0.001	<0.001									
	Stroma	<0.001	<0.001	<0.001	<0.001								
	CC nests	<0.001	<0.001	<0.001	<0.001	<0.001							
CD8+	**Margin**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001						
	Stroma	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
	CC nests	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				
FOXP3+	**Margin**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001			
	Stroma	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
	CC nests	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	
K-M grade		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Galon Immune Score		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

p values represent chi square tests for linear trend with all relationships positive unless stated

Table 9.4. The relationships between T-cell infiltration and cancer specific survival in patients with primary operable colorectal cancer (Model 1; TIL's in the invasive margin, Model 2; TIL's in the tumour stroma, Model 3; TIL's in the cancer cell nests).

			Univariate			Multivariate		
Location	**Type**	**Density**	**HR**	**95% CI**	***p****	**HR**	**95% CI**	***p****
Invasive margin								
Margin	$CD3^+$	Absent/weak/mod/strong	0.51	(0.40, 0.64)	<0.001	0.49	(0.38, 0.63)	<0.001
Margin	$CD45R0^+$	Absent/weak/mod/strong	0.62	(0.50, 0.77)	<0.001			0.45
Margin	$CD8^+$	Absent/weak/mod/strong	0.53	(0.42, 0.66)	<0.001			0.61
Margin	$FOXP3^+$	Absent/weak/mod/strong	0.66	(0.51, 0.84)	0.001			0.18
Tumour stroma								
Stroma	$CD3^+$	Absent/weak/mod/strong	0.54	(0.43, 0.67)	<0.001	0.58	(0.46, 0.75)	<0.001
Stroma	$CD45R0^+$	Absent/weak/mod/strong	0.64	(0.51, 0.82)	<0.001			0.94
Stroma	$CD8^+$	Absent/weak/mod/strong	0.70	(0.55, 0.88)	0.002			0.79
Stroma	$FOXP3^+$	Absent/weak/mod	0.67	(0.53, 0.84)	0.001			0.06
Cancer cell nests								
CC nests	$CD3^+$	Absent/weak/mod/strong	0.54	(0.44, 0.67)	<0.001	0.73	(0.55, 0.97)	0.030
CC nests	$CD45R0^+$	Absent/weak/mod/strong	0.64	(0.51, 0.79)	<0.001			0.61
CC nests	$CD8^+$	Absent/weak/mod/strong	0.53	(0.42, 0.66)	<0.001	0.68	(0.50, 0.90)	0.008
CC nests	$FOXP3^+$	Absent/weak	0.52	(0.36, 0.75)	0.001			0.08

* Cox proportional hazards regression

Table 9.5a. The relationships between $CD3^+$ IM, $CD8^+$ CCN and host characteristics in patients with primary operable colorectal cancer.

	$CD3^+$ IM					**$CD8^+$ CCN**				
	Absent	**Weak**	**Moderate**	**Strong**	***p****	**Absent**	**Weak**	**Moderate**	**Strong**	***p****
Age (≤64/65-74/≥75)	12/10/17 (31/26/44)	44/48/56 (30/32/38)	32/36/27 (34/38/28)	8/14/13 (23/40/37)	0.58	38/30/39 (36/28/36)	34/46/43 (28/37/35)	23/20/17 (38/33/28)	9/13/15 (24/35/41)	0.77
Sex (male/female)	20/19 (51/49)	85/63 (57/43)	51/44 (54/46)	17/18 (49/51)	0.61	59/48 (55/45)	65/58 (53/47)	32/28 (53/47)	22/15 (60/40)	0.79
Presentation (elective/emergency	33/6 (85/15)	139/9 (94/6)	91/4 (96/4)	33/2 (94/6)	0.10	97/10 (91/9)	119/4 (97/3)	53/7 (88/12)	35/2 (95/5)	0.84
Deprivation score (1-2/3-5/6-7)	2/14/23 (5/36/59)	7/55/82 (5/38/57)	5/46/42 (5/50/45)	1/15/19 (3/43/54)	0.35	7/49/49 (7/47/47)	4/48/49 (3/40/57)	1/25/33 (2/42/56)	3/12/21 (8/33/58)	0.20
POSSUM physiology score (11-14/15-20/21-30/>30)	5/16/9/2 (16/50/28/6)	16/42/39/5 (16/41/38/5)	10/31/28/4 (14/43/38/5)	6/9/8/1 (25/38/33/4)	0.90	15/35/22/6 (19/45/28/8)	14/42/33/2 (15/46/36/2)	8/17/15/3 (19/40/35/7)	3/10/13/1 (11/37/48/4)	0.29
ASA grade (1-2/3-4)	16/16 (50/50)	63/50 (56/44)	52/24 (68/32)	18/9 (67/33)	0.05	48/32 (60/40)	57/40 (59/41)	32/18 (64/36)	21/9 (70/30)	0.32
Smoking status (non/ex/current)	13/11/8 (41/34/25)	44/45/33 (36/37/27)	42/26/14 (51/32/17)	13/11/5 (45/38/17)	0.10	41/22/23 (48/26/27)	38/37/24 (38/37/24)	23/24/7 (43/44/13)	14/12/8 (41/35/24)	0.82
Anaemia (none/mild/severe)	19/6/10 (54/17/29)	56/31/29 (48/27/25)	43/16/23 (52/20/28)	14/11/5 (47/37/17)	0.83	44/23/19 (51/27/22)	55/21/28 (53/20/27)	29/11/12 (56/21/23)	10/13/9 (31/41/28)	0.31
White cell count (<8.5/8.5-11/>11)	21/9/8 (55/24/21)	75/27/20 (62/22/16)	47/22/13 (57/27/16)	22/5/3 (73/17/10)	0.23	55/18/16 (62/20/18)	65/28/16 (60/26/15)	31/13/9 (59/25/17)	19/9/5 (58/27/15)	0.87
NLR (<5:1/≥5:1)	24/8 (75/25)	83/26 (76/24)	59/19 (76/24)	22/8 (73/27)	0.86	59/17 (78/22)	78/25 (76/24)	40/8 (83/17)	21/12 (64/36)	0.38
mGPS (0/1/2)	18/14/7 (46/36/18)	82/46/20 (55/31/14)	61/23/11 (64/24/12)	20/10/5 (57/29/14)	0.19	54/35/18 (51/33/17)	70/37/16 (57/30/13)	40/13/7 (67/22/12)	18/13/6 (49/35/16)	0.45

* Chi square test for linear trend

Table 9.5b. The relationships between $CD3^+$ IM, $CD8^+$ CCN and tumour biology in patients with primary operable colorectal cancer.

	$CD3^+$ IM				**p***	**$CD8^+$ CCN**				**p***
	Absent	**Weak**	**Moderate**	**Strong**		**Absent**	**Weak**	**Moderate**	**Strong**	
Tumour site (colon/rectum)	24/15 (62/39)	93/55 (63/37)	62/33 (64/34)	27/8 (77/23)	0.16	64/43 (60/40)	81/42 (66/34)	37/23 (62/38)	30/7 (81/19)	0.07
T stage (1/2/3/4)	1/1/22/15 (3/3/56/39)	1/8/94/45 (1/5/64/30)	3/9/59/24 (3/10/62/25)	3/5/21/6 (9/14/60/17)	0.001	0/6/64/37 (0/6/60/34)	3/10/76/34 (2/8/62/28)	4/5/36/15 (7/8/60/25)	2/4/21/10 (5/11/57/27)	0.014
N Stage (0/1/2)	16/18/5 (41/46/13)	81/50/17 (55/34/12)	57/28/10 (60/30/10)	24/9/2 (69/26/6)	0.026	47/39/21 (44/36/20)	75/38/10 (61/31/8)	37/18/5 (62/30/8)	27/10/0 (73/27/0)	<0.001
TNM stage (I/II/III)	2/14/23 (5/36/59)	7/74/67 (5/50/45)	8/49/38 (8/52/40)	4/20/11 (11/57/31)	0.008	5/42/60 (5/39/56)	9/66/48 (7/54/39)	5/32/23 (8/53/38)	5/22/10 (13/60/27)	0.001
Differentiation (well or mod/poor)	33/6 (85/15)	129/19 (87/13)	87/8 (92/8)	28/7 (80/20)	0.98	91/16 (85/15)	113/10 (92/8)	54/6 (90/10)	28/9 (76/24)	0.43
Venous invasion (no/yes)	25/14 (64/36)	94/54 (64/37)	67/28 (71/29)	29/6 (83/17)	0.038	64/43 (60/40)	81/42 (66/34)	46/14 (77/23)	31/6 (84/16)	0.002
Growth pattern (expanding/infiltrative)	10/28 (26/74)	73/66 (53/47)	59/35 (63/37)	22/13 (63/37)	0.001	39/64 (38/62)	70/50 (58/42)	39/18 (68/32)	20/16 (56/44)	0.003
Tumour necrosis (absent/focal/mod/ext)	1/23/9/3 (3/64/25/8)	14/54/42/24 (10/40/31/18)	7/51/23/7 (8/58/26/8)	2/24/8/1 (6/69/23/3)	0.07	6/53/30/11 (6/53/30/11)	9/59/30/17 (8/51/26/15)	8/26/13/6 (15/49/25/11)	2/16/14/2 (6/47/41/6)	0.65

* Chi square test for linear trend

Table 9.6. Comparison of the prognostic value of different methods of assessing the local inflammatory response in patients with primary operable colorectal cancer (Model 1; stage I-III colorectal cancer, Model 2; stage I-II colorectal cancer, Model 3; colon cancer, Model 4; rectal cancer).

		Univariate			Multivariate	
Immune Score	**HR**	**95% CI**	***p****	**HR**	**95% CI**	***p****
Colorectal cancer (stage I-III)						
$CD3^+$ IM	0.51	(0.40, 0.64)	<0.001	0.72	(0.52, 0.99)	0.045
$CD3^+$ ST	0.54	(0.43, 0.67)	<0.001			0.07
$CD8^+$ CCN	0.53	(0.42, 0.66)	<0.001	0.58	(0.44, 0.77)	<0.001
K-M grade	0.54	(0.43, 0.68)	<0.001			0.20
Galon Immune Score	0.72	(0.63, 0.82)	<0.001			0.18
Colorectal cancer (stage I-II)						
$CD3^+$ IM	0.52	(0.35, 0.77)	0.001			0.35
$CD3^+$ ST	0.44	(0.30, 0.63)	<0.001	0.61	(0.39, 0.93)	0.020
$CD8^+$ CCN	0.49	(0.38, 0.70)	<0.001	0.56	(0.36, 0.86)	0.009
K-M grade	0.56	(0.39, 0.79)	0.001			0.41
Galon Immune Score	0.74	(0.60, 0.90)	0.003			0.45
Colon cancer						
$CD3^+$ IM	0.56	(0.41, 0.74)	<0.001	0.61	(0.39, 0.96)	0.031
$CD3^+$ ST	0.57	(0.43, 0.76)	<0.001			0.07
$CD8^+$ CCN	0.53	(0.41, 0.70)	<0.001	0.55	(0.39, 0.79)	0.001
K-M grade	0.58	(0.42, 0.74)	<0.001			0.15
Galon Immune Score	0.75	(0.64, 0.88)	<0.001			0.08
Rectal cancer						
$CD3^+$ IM	0.43	(0.28, 0.64)	<0.001			0.22
$CD3^+$ ST	0.45	(0.30, 0.67)	<0.001	0.57	(0.35, 0.94)	0.027
$CD8^+$ CCN	0.51	(0.35, 0.76)	0.001	0.45	(0.26, 0.78)	0.005
K-M grade	0.52	(0.35, 0.76)	0.001			0.49
Galon Immune Score	0.68	(0.54, 0.85)	0.001			0.40

*Cox proportional hazards regression

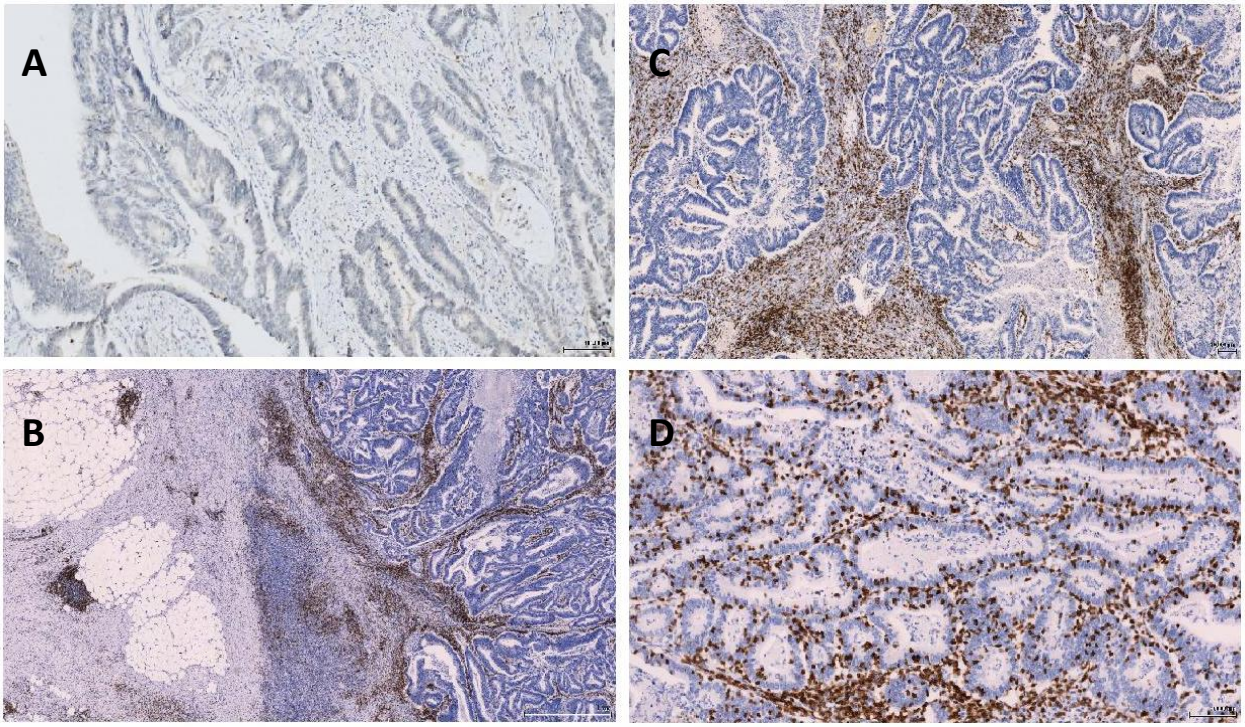
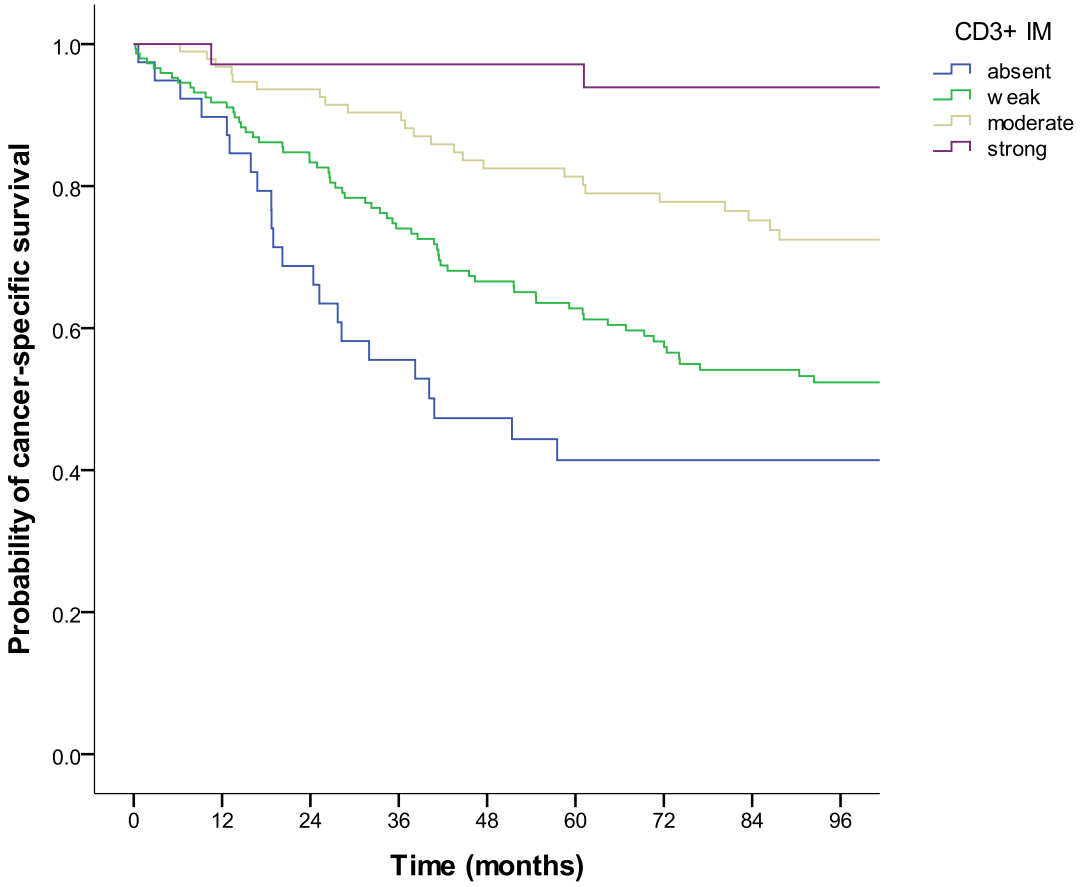
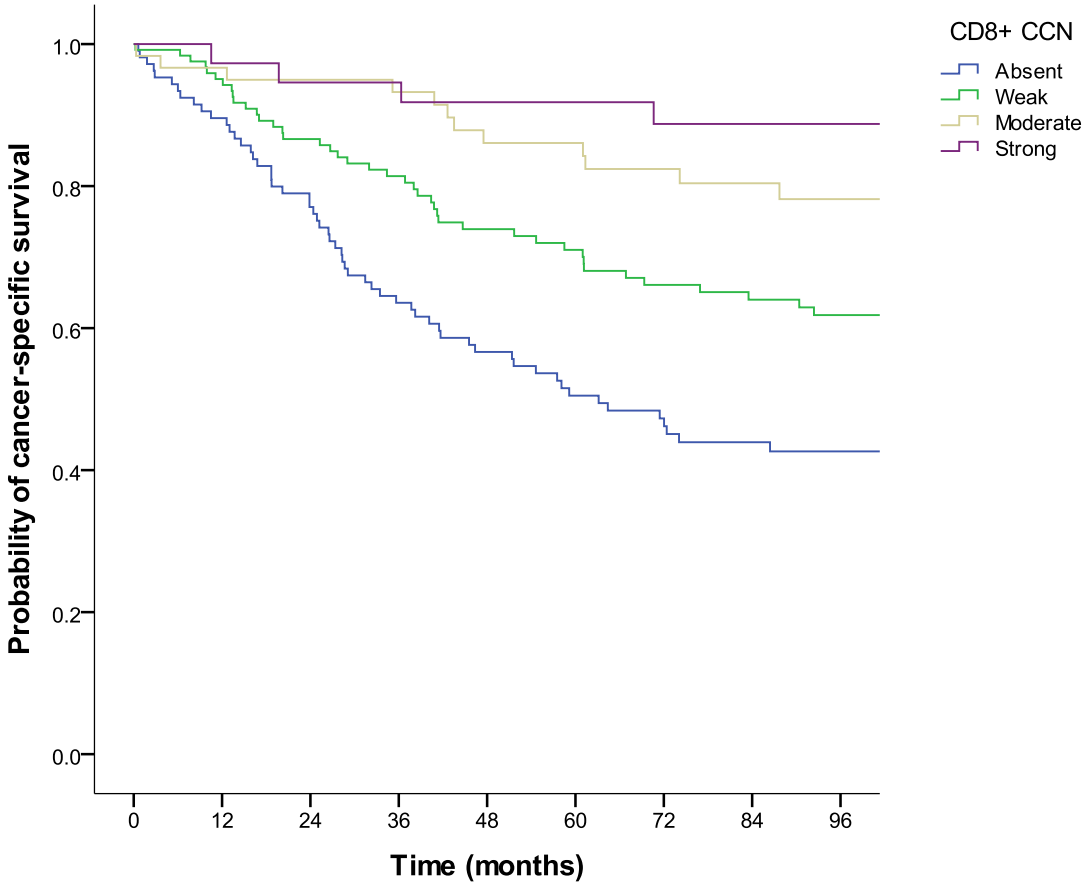


Figure 9.1. Examples of stained sections demonstrating different patterns of T-cell infiltration in the microenvironment of colorectal tumours. Absence of T-cell infiltration (Panel A); Strong infiltration of $CD3^+$ cells at the invasive margin (Panel B); Strong infiltration of $CD3^+$ cells in the tumour stroma with relative 'sparing' of the cancer cell nests (Panel C); and strong infiltration of $CD8^+$ cells in the cancer cell nests (Panel D).



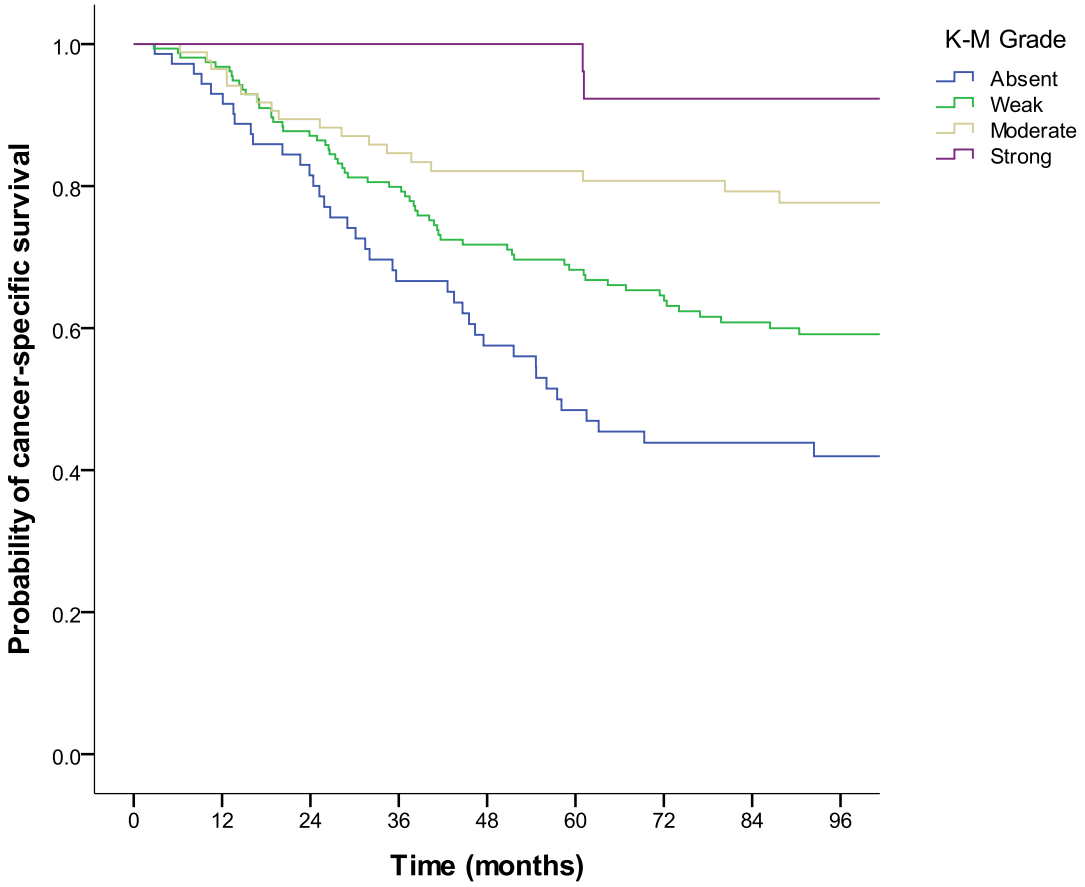
Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
Absent	39	34	25	20	16	14	13	6
Weak	148	130	115	100	85	80	71	60
Moderate	95	90	86	82	72	69	62	55
Strong	35	34	34	33	31	29	27	24

Figure 9.2. Kaplan-Meier survival curves demonstrating the relationship between cancer-specific survival in patients with colorectal cancer according to the application of different measures of the local inflammatory response. Shown on this panel; $CD3^{+}$ IM ($p<0.001$, log-rank test)



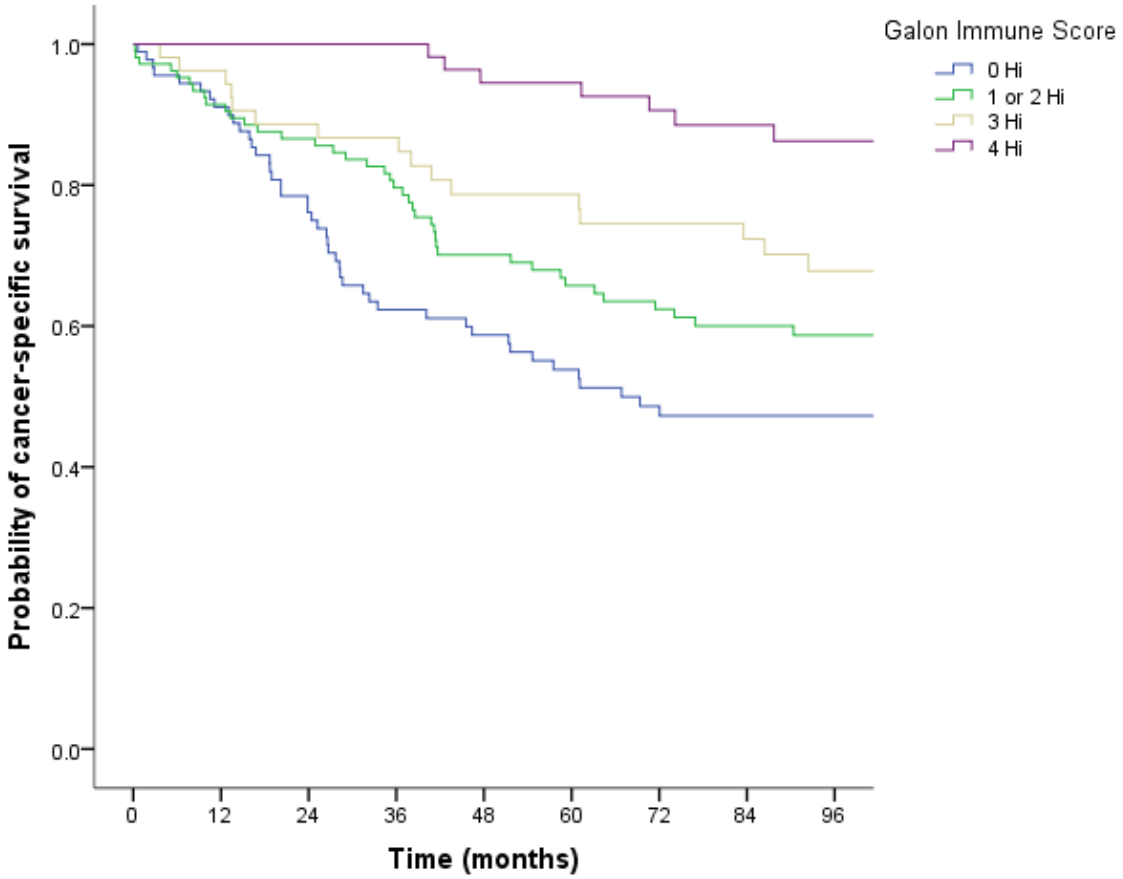
Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
Absent	107	92	78	65	54	47	41	34
Weak	123	112	99	87	65	72	65	57
Moderate	60	57	55	53	48	46	41	37
Strong	37	36	34	34	32	30	29	27

Figure 9.2 (cont). Kaplan-Meier survival curves demonstrating the relationship between cancer-specific survival in patients with colorectal cancer according to the application of different measures of the local inflammatory response. Shown on this panel; $CD8^+$ CCN ($p<0.001$, log-rank test)



Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
Absent	72	65	54	43	37	32	27	23
Weak	160	149	132	118	100	94	85	71
Moderate	86	80	75	68	62	59	55	50
Strong	28	28	28	27	27	26	23	22

Figure 9.2 (cont). Kaplan-Meier survival curves demonstrating the relationship between cancer-specific survival in patients with colorectal cancer according to the application of different measures of the local inflammatory response. Shown on this panel; K-M grade ($p<0.001$, log-rank test)



Number at risk	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
0 Hi	91	79	64	52	46	42	35	31
1 or 2 Hi	106	94	88	76	63	59	52	45
3 Hi	53	50	46	43	39	38	35	32
4 Hi	58	57	56	55	50	48	45	39

Figure 9.2 (cont). Kaplan-Meier survival curves demonstrating the relationship between cancer-specific survival in patients with colorectal cancer according to the application of different measures of the local inflammatory response. Shown on this panel; Galon Immune Score ($p<0.001$, log-rank test)

10.0 CONCLUSIONS

At the beginning of this period of research it was clear that a significant proportion of patients with colorectal cancer, despite undergoing potentially curative surgery, were nevertheless dying prematurely from their disease. It was recognized that disease progression in these patients was dependent not only on pathological stage but on complex interactions between tumour- and host-related factors. A substantial body of evidence already existed confirming an elevated systemic inflammatory response, as measured by the mGPS, as a reliable indicator of poor prognosis in these patients. Similarly, data assembled over a 40 year period concluded that a strong infiltration of inflammatory cells in the tumour microenvironment was associated with favourable outcomes in patients with colorectal cancer (Chapter 1.0).

Despite this knowledge, at the outset of this thesis, several key questions remained unanswered. First, the underlying basis of the systemic inflammatory response in patients with colorectal cancer was unclear. Similarly, the factors associated with an effective local immune cell reaction remained undetermined. Many studies in this field had concentrated almost exclusively on tumour biology and few had investigated the potential role of host factors. It was also unclear whether the local and systemic inflammatory responses were in some way linked and a detailed investigation of these relationships was lacking. Finally, although the benefits of an effective anti-tumour response in these patients were not in doubt, no study had ever compared the prognostic value of different methods of assessing the local inflammatory response in a single cohort.

This thesis started with an attempt to gain insight into the patient factors associated with systemic inflammation in patients with colorectal cancer (Chapter 3.0). The results demonstrated, for the first time, that abnormal patient physiology, in particular the presence

of anaemia and cardiac disease, was strongly associated with a systemic inflammatory response. These relationships raised the possibility that systemic inflammation in these patients was a result of relative tissue hypoxia, perhaps initiated by rapid tumour growth and aggravated by impaired oxygen delivery; a hypothesis that was to be investigated subsequently. When the long term outcomes of patients with impaired physiology were considered, it was apparent that they were dying prematurely from their disease. However, both physiology scores and mGPS were independently associated with cancer specific survival, suggesting that poor physiology alone could not fully explain the relationship between inflammation and cancer outcomes. Nonetheless, the results of this study did suggest that targeting patient physiology in the pre-operative period may be a novel way to improve outcomes in patients with colorectal cancer. In relation to future work, it would be of considerable interest to investigate whether improving these physiological parameters, for example through a period of intensive cardiovascular optimization, would lead to better outcomes via an attenuation of the systemic inflammatory response.

Chapter 5.0 examined the influence of systemic inflammation on the body composition of patients with malignant disease. The results demonstrated a strong association between low skeletal muscle mass and the presence of a systemic inflammatory response in patients with primary operable colorectal cancer. Many of these patients had a BMI in the normal range, promoting the view that the analysis of cross-sectional imaging is a more accurate way to quantify body composition in patients with cancer. It was of particular interest that advanced tumour stage was not directly related to significantly lower skeletal muscle mass; a result which suggests the loss of lean tissue in cancer cachexia is driven not by tumour biology but rather through the host systemic inflammatory response. The negative clinical impact of cancer-related weight loss has been well documented and these results not only offer insight

into the underlying basis of cancer cachexia, they identify the attenuation of the systemic inflammatory response as a potential therapeutic target. Future work in this area should focus on whether early changes in body composition, detectable from staging CT scans, can predict long term outcomes.

Chapter 6.0 examined the question of whether surgical complications were truly responsible for reduced survival in patients with colorectal cancer or whether these associations could be explained by pre-existing patient-related factors. The results demonstrated that smoking, impaired physiology and systemic inflammation were associated not only with the development of septic complications but also with reduced long term survival. Rather than being the cause of disease recurrence, surgical complications appeared to be a consequence of poor physiology or a pro-inflammatory state; the true determinants of long term outcome. These results support a concept whereby a patients' pre-operative status is of paramount importance. Attention should be directed towards identifying these high risk patients early and intervening where possible. Such interventions should include smoking cessation in all patients with colorectal cancer and, as far as possible, the correction of physiological parameters in the pre-operative period. Targeting the systemic inflammatory response through the administration of aspirin, statins or non-steroidal anti-inflammatory drugs offers another potential therapeutic strategy.

The relationships between the systemic inflammatory response and local immune cell infiltrates were explored in Chapter 7.0. We chose to examine these relationships with specific reference to tumour necrosis because this histological feature, recently reported as prognostic in colorectal cancer, has also been associated with serum markers of inflammation in other tumour types. The study validated a semi-quantative analysis of tumour necrosis as

an independent prognostic marker in patients with primary operable colorectal cancer. Furthermore, necrosis was directly associated with both an elevation of the systemic inflammatory response and an attenuation of the local immune cell infiltrate. This represents the first documented link between local and systemic inflammation in patients with colorectal cancer. One hypothesis is that failure of local anti-tumour control leads to rapid tumour growth, tissue hypoxia and cellular necrosis. The presence of necrosis may then act as a trigger for the host to initiate a systemic inflammatory response. Indeed, it was evident that a strong linear relationship existed between the degree of tumour necrosis and circulating levels of serum CRP. To further our understanding of the relationships between necrosis and inflammation, future work should be directed towards a detailed examination of the associations between tumour necrosis and cell signalling pathways, genetic mutations, including microsatellite status and molecular markers of tumour cell proliferation.

Previous work had established that a global assessment of peritumoural inflammation, using the K-M grade, was an independent prognostic marker in patients with primary operable colorectal cancer. As a logical starting point for developing a clinically relevant method of assessing the local inflammatory response, Chapter 8.0 sought to investigate which cellular components of the peritumoural infiltrate were most relevant to prognosis. The study demonstrated that individual immune cells could be reliably identified and categorized on H&E stained sections and suggested that a strong K-M reaction was primarily the result of lymphocyte infiltration. Although examination of individual cell types did not improve prediction of outcome compared to overall K-M grade, those tumours with a strong lymphocytic infiltrate were noted to have distinctly favourable pathological characteristics. This indicated a prominent role for the adaptive immune response in the prevention of tumour progression in colorectal cancer and allowed a subsequent analysis to focus on specifically on

lymphocytes. Advances in the digital image analysis of pathological sections also mean that there is scope to develop an automated assessment of K-M grade on either full sections or tissue microarrays; a development with the potential to further standardise research practice.

Chapter 9.0 sought to build on this knowledge by examining the clinical utility of the local inflammatory response in colorectal cancer. This was the first study to directly compare different methodologies for assessing the local inflammatory cell infiltrate and confirmed that all three measures were able to predict outcome in primary operable colorectal cancer. Indeed, strong inter-relationships existed between individual inflammatory cells, leading to the conclusion that the density of cellular infiltrate is the critical component of the local inflammatory response. Future work in this area should continue on two fronts. First, efforts must be made to achieve consensus on the measurement of the local inflammatory response in patients with colorectal cancer. The fact that the K-M grade offers a simple and reproducible way to grade inflammatory cell density on routinely stained sections promotes its use in all future research. Additional consideration must also be given to the underlying factors responsible for producing an effective immune cell response in the tumour microenvironment. One possibility is that $CD8^+$ lymphocytes are responding to specific antigens and a detailed examination of tumour cell HLA expression is warranted.

In summary, it is apparent that cancer-associated alterations in patient immune and inflammatory responses are complex (Figure 10). Disease progression in these patients is not a tumour-cell autonomous process but is rather the result of a multitude of molecular interactions between tumour and host. In patients with primary operable colorectal cancer both the local and systemic inflammatory responses are important predictors of outcome. The aim of this thesis was to investigate the factors responsible for activating and maintaining

these responses and, with this in mind, several conclusions may now be drawn. It seems likely that key events within the tumour microenvironment initially dictate whether a colorectal tumour develops or is successfully eliminated by the host. The work presented above clearly demonstrates that the local infiltration of immune cells is effective in preventing tumour growth and metastases. A strong local response, coordinated primarily through lymphocytes, is associated with favourable pathological characteristics, including lower levels of venous invasion, and translates into a lower incidence of disease recurrence and improved patient survival. In the alternative scenario, should malignant cells successfully evade host immunity, the microenvironment becomes dominated instead by molecular processes which promote tumour growth. In this situation, rather than being protective, the inflammatory response becomes destructive, facilitating angiogenesis and stimulating cellular proliferation. The resultant rapid growth results in tissue hypoxia and areas of necrosis develop within the central tumour. Results from this thesis suggest that the presence of tumour necrosis is one mechanism through which the host is stimulated to mount a systemic inflammatory response. However, it may be that this is only one of many ways through which an uncontrolled acute phase response is generated. Recently, there has been increased interest in the role of the stroma in influencing the growth and spread of malignant tumours. In particular, the presence of increased numbers of myofibroblasts has been associated with shorter disease-free survival in patients with colorectal cancer, leading to the hypothesis that the stroma itself is a key determinant of growth, invasion and metastases. The relationships between the tumour stroma and the inflammatory cells that surround and infiltrate have yet to be investigated but represent an intriguing avenue of research.

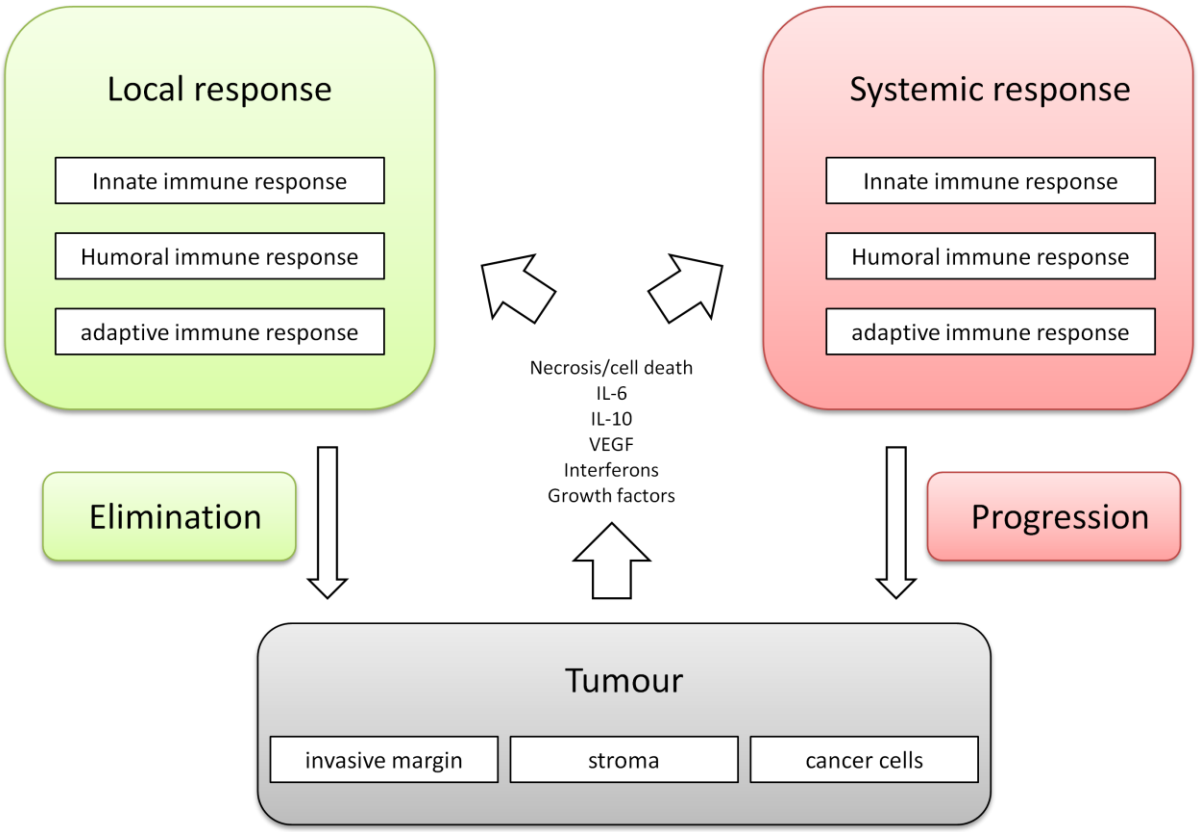


Figure 10. Cancer immunity and inflammation; a schematic representation of the complex interactions between the tumour and host.

Regardless of the initial stimulus, this thesis supports the idea that systemic inflammation in the context of malignancy represents an ultimate failure of homeostasis. This is evidenced by its association with a diverse range of patient-related factors, including deranged physiology, cardiovascular comorbidity, anaemia and a loss of skeletal muscle mass. The presence of a systemic inflammatory response in patients with colorectal cancer is thus a useful and universal indicator of poor prognosis; associated with early disease recurrence, distant metastases and reduced survival.

This thesis suggests that inflammation affects nearly every facet of tumour development. Ultimately, an increased understanding of the factors which govern the local and systemic inflammatory responses is fundamental to improving outcomes in patients with colorectal cancer.

11.0 REFERENCES

Aaltonen, L. A., P. Peltomaki, et al. (1993). "Clues to the pathogenesis of familial colorectal cancer." Science **260**(5109): 812-816.

Abramovitch, R., M. Marikovsky, et al. (1999). "Stimulation of tumour growth by wound-derived growth factors." Br J Cancer **79**(9-10): 1392-1398.

Acikalin, M. F., U. Oner, et al. (2005). "Tumour angiogenesis and mast cell density in the prognostic assessment of colorectal carcinomas." Dig Liver Dis **37**(3): 162-169.

ACPGBI "Guidelines for the management of bowel cancer. London 2007. Available from http://www.acpgbi.org.uk/assets/documents/COLOguides.pdf."

Adam, I. J., M. O. Mohamdee, et al. (1994). "Role of circumferential margin involvement in the local recurrence of rectal cancer." Lancet **344**(8924): 707-711.

Adams, W. J. and D. L. Morris (1997). "Pilot study--cimetidine enhances lymphocyte infiltration of human colorectal carcinoma: results of a small randomized control trial." Cancer **80**(1): 15-21.

AICR (2007). "Food, Nutrition, Physical Activity and the prevention of Cancer: A Global Perspective." AICR.

Al Murri, A. M., J. M. Bartlett, et al. (2006). "Evaluation of an inflammation-based prognostic score (GPS) in patients with metastatic breast cancer." Br J Cancer **94**(2): 227-230.

Allegra, C. J., S. Paik, et al. (2003). "Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with Dukes' B and C colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project collaborative study." J Clin Oncol **21**(2): 241-250.

Allegra, C. J., A. L. Parr, et al. (2002). "Investigation of the prognostic and predictive value of thymidylate synthase, p53, and Ki-67 in patients with locally advanced colon cancer." J Clin Oncol **20**(7): 1735-1743.

Andersen, S. N., T. Lovig, et al. (1997). "K-ras mutations and prognosis in large-bowel carcinomas." Scand J Gastroenterol **32**(1): 62-69.

Andre, T., C. Boni, et al. (2004). "Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer." N Engl J Med **350**(23): 2343-2351.

Andreyev, H. J., A. R. Norman, et al. (1998). "Why do patients with weight loss have a worse outcome when undergoing chemotherapy for gastrointestinal malignancies?" Eur J Cancer **34**(4): 503-509.

Arber, N. (2000). "Do NSAIDs prevent colorectal cancer?" Can J Gastroenterol **14**(4): 299-307.

Argiles, J. M., S. Busquets, et al. (2005). "The pivotal role of cytokines in muscle wasting during cancer." Int J Biochem Cell Biol **37**(10): 2036-2046.

Armstrong, B. and R. Doll (1975). "Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices." Int J Cancer **15**(4): 617-631.

Atreya, I. and M. F. Neurath (2008). "Immune cells in colorectal cancer: prognostic relevance and therapeutic strategies." Expert Rev Anticancer Ther **8**(4): 561-572.

Badoual, C., S. Hans, et al. (2009). "Revisiting the prognostic value of regulatory T cells in patients with cancer." J Clin Oncol **27**(19): e5-6; author reply e7.

Baeten, C. I., K. Castermans, et al. (2006). "Proliferating endothelial cells and leukocyte infiltration as prognostic markers in colorectal cancer." Clin Gastroenterol Hepatol **4**(11): 1351-1357.

Baker, K., I. Zlobec, et al. (2007). "Differential significance of tumour infiltrating lymphocytes in sporadic mismatch repair deficient versus proficient colorectal cancers: a potential role for dysregulation of the transforming growth factor-beta pathway." Eur J Cancer **43**(3): 624-631.

Balkwill, F. and L. M. Coussens (2004). "Cancer: an inflammatory link." Nature **431**(7007): 405-406.

Balkwill, F. and A. Mantovani (2001). "Inflammation and cancer: back to Virchow?" Lancet **357**(9255): 539-545.

Baron, J. A., B. F. Cole, et al. (2003). "A randomized trial of aspirin to prevent colorectal adenomas." N Engl J Med **348**(10): 891-899.

Baron, J. A., R. S. Sandler, et al. (2006). "A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas." Gastroenterology **131**(6): 1674-1682.

Bass, G., C. Fleming, et al. (2009). "Emergency first presentation of colorectal cancer predicts significantly poorer outcomes: a review of 356 consecutive Irish patients." Dis Colon Rectum **52**(4): 678-684.

Bates, G. J., S. B. Fox, et al. (2006). "Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse." J Clin Oncol **24**(34): 5373-5380.

Batsakis, J. G. (1985). "Nerves and neurotropic carcinomas." Ann Otol Rhinol Laryngol **94**(4 Pt 1): 426-427.

Baumgartner, R. N., K. M. Koehler, et al. (1998). "Epidemiology of sarcopenia among the elderly in New Mexico." Am J Epidemiol **147**(8): 755-763.

Bazan, V., M. Migliavacca, et al. (2002). "Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype." Ann Oncol **13**(9): 1438-1446.

Begent, R. H. (1984). "The value of carcinoembryonic antigen measurement in clinical practice." Ann Clin Biochem **21 (Pt 4)**: 231-238.

Belchetz, L. A., T. Berk, et al. (1996). "Changing causes of mortality in patients with familial adenomatous polyposis." Dis Colon Rectum **39**(4): 384-387.

Bell, S. M., N. Scott, et al. (1993). "Prognostic value of p53 overexpression and c-Ki-ras gene mutations in colorectal cancer." Gastroenterology **104**(1): 57-64.

Bell, S. W., K. G. Walker, et al. (2003). "Anastomotic leakage after curative anterior resection results in a higher prevalence of local recurrence." Br J Surg **90**(10): 1261-1266.

Benson, A. B., 3rd, D. Schrag, et al. (2004). "American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer." J Clin Oncol **22**(16): 3408-3419.

Berger, A. C., E. R. Sigurdson, et al. (2005). "Colon cancer survival is associated with decreasing ratio of metastatic to examined lymph nodes." J Clin Oncol **23**(34): 8706-8712.

Bertagnolli, M. M., C. J. Eagle, et al. (2006). "Celecoxib for the prevention of sporadic colorectal adenomas." N Engl J Med **355**(9): 873-884.

Bhatavdekar, J. M., D. D. Patel, et al. (2001). "Molecular markers are predictors of recurrence and survival in patients with Dukes B and Dukes C colorectal adenocarcinoma." Dis Colon Rectum **44**(4): 523-533.

Bingham, S. A., N. E. Day, et al. (2003). "Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study." Lancet **361**(9368): 1496-1501.

Birbeck, K. F., C. P. Macklin, et al. (2002). "Rates of circumferential resection margin involvement vary between surgeons and predict outcomes in rectal cancer surgery." Ann Surg **235**(4): 449-457.

Boland, C. R., S. N. Thibodeau, et al. (1998). "A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer." Cancer Res **58**(22): 5248-5257.

Bone, R. C., C. J. Grodzin, et al. (1997). "Sepsis: a new hypothesis for pathogenesis of the disease process." Chest **112**(1): 235-243.

Bonovas, S., K. Filioussi, et al. (2007). "Statins and the risk of colorectal cancer: a meta-analysis of 18 studies involving more than 1.5 million patients." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **25**(23): 3462-3468.

Bosanquet, N. and K. Sikora (2004). "The economics of cancer care in the UK." Lancet Oncol **5**(9): 568-574.

Bosset, J. F., L. Collette, et al. (2006). "Chemotherapy with preoperative radiotherapy in rectal cancer." N Engl J Med **355**(11): 1114-1123.

Boucher, D., D. Cournoyer, et al. (1989). "Studies on the control of gene expression of the carcinoembryonic antigen family in human tissue." Cancer Res **49**(4): 847-852.

Brosens, R. P., J. L. Oomen, et al. (2006). "POSSUM predicts decreased overall survival in curative resection for colorectal cancer." Dis Colon Rectum **49**(6): 825-832.

Brown, D. J., R. Milroy, et al. (2007). "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 **60**(6): 705-708.

Brown, G. (2005). "Thin section MRI in multidisciplinary pre-operative decision making for patients with rectal cancer." The British journal of radiology **78 Spec No 2**: S117-127.

Burke, F., M. Relf, et al. (1996). "A cytokine profile of normal and malignant ovary." Cytokine **8**(7): 578-585.

Burkitt, D. P. (1971). "Epidemiology of cancer of the colon and rectum." Cancer **28**(1): 3-13.

Burnet, M. (1957). "Cancer; a biological approach. I. The processes of control." Br Med J **1**(5022): 779-786.

Burton, E. R. and S. K. Libutti (2009). "Targeting TNF-alpha for cancer therapy." J Biol **8**(9): 85.

Calva, D. and J. R. Howe (2008). "Hamartomatous polyposis syndromes." Surg Clin North Am **88**(4): 779-817, vii.

Campagnoli, C., N. Biglia, et al. (1993). "Differential effects of oral conjugated estrogens and transdermal estradiol on insulin-like growth factor 1, growth hormone and sex hormone binding globulin serum levels." Gynecol Endocrinol **7**(4): 251-258.

Canavan, C., K. R. Abrams, et al. (2006). "Meta-analysis: colorectal and small bowel cancer risk in patients with Crohn's disease." Aliment Pharmacol Ther **23**(8): 1097-1104.

Canna, K., D. C. McMillan, et al. (2004). "Evaluation of a cumulative prognostic score based on the systemic inflammatory response in patients undergoing potentially curative surgery for colorectal cancer." Br J Cancer **90**(9): 1707-1709.

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

Carlomagno, C., F. Perrone, et al. (1995). "Prognostic significance of necrosis, elastosis, fibrosis and inflammatory cell reaction in operable breast cancer." Oncology **52**(4): 272-277.

Carsin, A. E., L. Sharp, et al. (2008). "Inequity in colorectal cancer treatment and outcomes: a population-based study." Br J Cancer **99**(2): 266-274.

Chan, A. T., S. Ogino, et al. (2009). "Aspirin use and survival after diagnosis of colorectal cancer." JAMA **302**(6): 649-658.

Chang, G. J., M. A. Rodriguez-Bigas, et al. (2007). "Lymph node evaluation and survival after curative resection of colon cancer: systematic review." J Natl Cancer Inst **99**(6): 433-441.

Chen, M. L., M. J. Pittet, et al. (2005). "Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo." Proc Natl Acad Sci U S A **102**(2): 419-424.

Chen, Y. T., M. J. Henk, et al. (1997). "Prognostic significance of tumor markers in colorectal cancer patients: DNA index, S-phase fraction, p53 expression, and Ki-67 index." J Gastrointest Surg **1**(3): 266-272; discussion 273.

Choti, M. A., J. V. Sitzmann, et al. (2002). "Trends in long-term survival following liver resection for hepatic colorectal metastases." Ann Surg **235**(6): 759-766.

Chung, D. J., K. C. Huh, et al. (2005). "CT colonography using 16-MDCT in the evaluation of colorectal cancer." AJR. American journal of roentgenology **184**(1): 98-103.

Cianchi, F., L. Messerini, et al. (1997). "Character of the invasive margin in colorectal cancer: does it improve prognostic information of Dukes staging?" Dis Colon Rectum **40**(10): 1170-1175; discussion 1175-1176.

Colditz, G. A., C. C. Cannuscio, et al. (1997). "Physical activity and reduced risk of colon cancer: implications for prevention." Cancer Causes Control **8**(4): 649-667.

Colotta, F., P. Allavena, et al. (2009). "Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability." Carcinogenesis **30**(7): 1073-1081.

Conlin, A., G. Smith, et al. (2005). "The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma." Gut **54**(9): 1283-1286.

Copeland, G. P., D. Jones, et al. (1991). "POSSUM: a scoring system for surgical audit." Br J Surg **78**(3): 355-360.

Correa, P., J. P. Strong, et al. (1982). "Atherosclerosis and polyps of the colon. Quantification of precursors of coronary heart disease and colon cancer." J Chronic Dis **35**(5): 313-320.

Coutsoftides, T., M. V. Sivak, Jr., et al. (1978). "Colonoscopy and the management of polyps containing invasive carcinoma." Ann Surg **188**(5): 638-641.

Cross, A. J., J. R. Pollock, et al. (2003). "Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat." Cancer Res **63**(10): 2358-2360.

CRUK "Bowel cancer incidence 2009. Available at: http://www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/incidence."

CRUK " Bowel cancer mortality 2010. Available from: http://www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/mortality/."

Curiel, T. J. (2007). "Tregs and rethinking cancer immunotherapy." J Clin Invest **117**(5): 1167-1174.

Curiel, T. J., G. Coukos, et al. (2004). "Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival." Nat Med **10**(9): 942-949.

Dahl, O., O. Fluge, et al. (2009). "Final results of a randomised phase III study on adjuvant chemotherapy with 5 FU and levamisol in colon and rectum cancer stage II and III by the Norwegian Gastrointestinal Cancer Group." Acta Oncol **48**(3): 368-376.

Davies, M. G. and P. O. Hagen (1997). "Systemic inflammatory response syndrome." Br J Surg **84**(7): 920-935.

Davies, P. S., A. E. Powell, et al. (2009). "Inflammation and proliferation act together to mediate intestinal cell fusion." PLoS One **4**(8): e6530.

de la Taille, A., A. Katz, et al. (1999). "Perineural invasion on prostate needle biopsy: an independent predictor of final pathologic stage." Urology **54**(6): 1039-1043.

de Leon, M. P., A. Pezzi, et al. (2009). "Survival, surgical management and perioperative mortality of colorectal cancer in the 21-year experience of a specialised registry." Int J Colorectal Dis **24**(7): 777-788.

De Ridder, M., V. Vinh-Hung, et al. (2006). "Prognostic value of the lymph node ratio in node positive colon cancer." Gut **55**(11): 1681.

de Santibanes, E., D. Fernandez, et al. (2010). "Short-term and long-term outcomes after simultaneous resection of colorectal malignancies and synchronous liver metastases." World J Surg **34**(9): 2133-2140.

Dehghan, A., I. Kardys, et al. (2007). "Genetic variation, C-reactive protein levels, and incidence of diabetes." Diabetes **56**(3): 872-878.

Delaney, C. P., E. Chang, et al. (2008). "Clinical outcomes and resource utilization associated with laparoscopic and open colectomy using a large national database." Ann Surg **247**(5): 819-824.

Denko, N. C., L. A. Fontana, et al. (2003). "Investigating hypoxic tumor physiology through gene expression patterns." Oncogene **22**(37): 5907-5914.

Des Guetz, G., B. Uzzan, et al. (2006). "Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature." Br J Cancer **94**(12): 1823-1832.

Dewys, W. D., C. Begg, et al. (1980). "Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group." Am J Med **69**(4): 491-497.

Diederichsen, A. C., J. B. Hjelmborg, et al. (2003). "Prognostic value of the CD4+/CD8+ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells." Cancer Immunol Immunother **52**(7): 423-428.

Dirschmid, K., A. Lang, et al. (1996). "Incidence of extramural venous invasion in colorectal carcinoma: findings with a new technique." Hum Pathol **27**(11): 1227-1230.

Duffy, M. J. (2001). "Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful?" Clin Chem **47**(4): 624-630.

Dukes, C. (1937). "Histological Grading of Rectal Cancer: (Section of Pathology)." Proc R Soc Med **30**(4): 371-376.

Dumont, P., A. Berton, et al. (2008). "Expression of galectin-3 in the tumor immune response in colon cancer." Lab Invest **88**(8): 896-906.

Dunn, G. P., L. J. Old, et al. (2004). "The immunobiology of cancer immunosurveillance and immunoediting." Immunity **21**(2): 137-148.

Dvorak, H. F. (1986). "Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing." N Engl J Med **315**(26): 1650-1659.

Eaden, J. A., K. R. Abrams, et al. (2001). "The risk of colorectal cancer in ulcerative colitis: a meta-analysis." Gut **48**(4): 526-535.

Edwards, J. G., D. E. Swinson, et al. (2003). "Tumor necrosis correlates with angiogenesis and is a predictor of poor prognosis in malignant mesothelioma." Chest **124**(5): 1916-1923.

Erlinger, T. P., E. A. Platz, et al. (2004). "C-reactive protein and the risk of incident colorectal cancer." JAMA **291**(5): 585-590.

Esteller, M., M. F. Fraga, et al. (2002). "Cancer epigenetics and methylation." Science **297**(5588): 1807-1808; discussion 1807-1808.

Fearnhead, N. S., J. L. Wilding, et al. (2002). "Genetics of colorectal cancer: hereditary aspects and overview of colorectal tumorigenesis." British medical bulletin **64**: 27-43.

Fearon, K., F. Strasser, et al. (2011). "Definition and classification of cancer cachexia: an international consensus." Lancet Oncol **12**(5): 489-495.

Ferjani, A. M., D. Griffin, et al. (2007). "A newly devised scoring system for prediction of mortality in patients with colorectal cancer: a prospective study." Lancet Oncol **8**(4): 317-322.

Ferracin, M., R. Gafa, et al. (2008). "The methylator phenotype in microsatellite stable colorectal cancers is characterized by a distinct gene expression profile." J Pathol **214**(5): 594-602.

Ferrari, P., M. Jenab, et al. (2007). "Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European prospective investigation into cancer and nutrition (EPIC)." Int J Cancer **121**(9): 2065-2072.

Ferrone, S. and T. L. Whiteside (2007). "Tumor microenvironment and immune escape." Surgical oncology clinics of North America **16**(4): 755-774, viii.

Fietkau, R., M. Barten, et al. (2006). "Postoperative chemotherapy may not be necessary for patients with ypN0-category after neoadjuvant chemoradiotherapy of rectal cancer." Dis Colon Rectum **49**(9): 1284-1292.

Figueredo, A., M. E. Coombes, et al. (2008). "Adjuvant therapy for completely resected stage II colon cancer." Cochrane Database Syst Rev(3): CD005390.

Filella, X., R. Molina, et al. (1992). "Prognostic value of CA 19.9 levels in colorectal cancer." Ann Surg **216**(1): 55-59.

Fisher, E. R., S. M. Paik, et al. (1989). "Prognostic significance of eosinophils and mast cells in rectal cancer: findings from the National Surgical Adjuvant Breast and Bowel Project (protocol R-01)." Hum Pathol **20**(2): 159-163.

Fisher, E. R., A. S. Palekar, et al. (1978). "Pathological findings from the national surgical adjuvant breast project (Protocol No. 4). IV. Significance of tumor necrosis." Hum Pathol **9**(5): 523-530.

Fleming ID, C. J., Henson DE, Hutter RVP, Kennedy BJ, Murphy GP, O'Sullivan B, Sobin LH, Yarbro JW (eds.). (1997). AJCC 5th Edition Cancer Staging Manual. Philadelphia, Lippincott-Raven.

Fletcher, R. H. (1986). "Carcinoembryonic antigen." Ann Intern Med **104**(1): 66-73.

Folkman, J., P. Cole, et al. (1966). "Tumor behavior in isolated perfused organs: in vitro growth and metastases of biopsy material in rabbit thyroid and canine intestinal segment." Ann Surg **164**(3): 491-502.

Forrest, L. M., D. C. McMillan, et al. (2004). "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 **90**(9): 1704-1706.

Forrester, K., C. Almoguera, et al. (1987). "Detection of high incidence of K-ras oncogenes during human colon tumorigenesis." Nature **327**(6120): 298-303.

Forssell, J., A. Oberg, et al. (2007). "High macrophage infiltration along the tumor front correlates with improved survival in colon cancer." Clin Cancer Res **13**(5): 1472-1479.

Frank, I., M. L. Blute, et al. (2002). "An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy based on tumor stage, size, grade and necrosis: the SSIGN score." J Urol **168**(6): 2395-2400.

Frank, R. E., T. J. Saclarides, et al. (1995). "Tumor angiogenesis as a predictor of recurrence and survival in patients with node-negative colon cancer." Ann Surg **222**(6): 695-699.

Freeman, D. J., J. Norrie, et al. (2002). "C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study." Diabetes **51**(5): 1596-1600.

Frohlich, M., M. Sund, et al. (2003). "Independent association of various smoking characteristics with markers of systemic inflammation in men. Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95)." Eur Heart J **24**(14): 1365-1372.

Fuchs, C. S., E. L. Giovannucci, et al. (1999). "Dietary fiber and the risk of colorectal cancer and adenoma in women." N Engl J Med **340**(3): 169-176.

Gabay, C. and I. Kushner (1999). "Acute-phase proteins and other systemic responses to inflammation." N Engl J Med **340**(6): 448-454.

Galon, J., A. Costes, et al. (2006). "Type, density, and location of immune cells within human colorectal tumors predict clinical outcome." Science **313**(5795): 1960-1964.

Galon, J., F. Pages, et al. (2012). "The immune score as a new possible approach for the classification of cancer." J Transl Med **10**: 1.

Gao, J. F., G. Arbman, et al. (2005). "Relationships of tumor inflammatory infiltration and necrosis with microsatellite instability in colorectal cancers." World J Gastroenterol **11**(14): 2179-2183.

Garrity, M. M., L. J. Burgart, et al. (2004). "Prognostic value of proliferation, apoptosis, defective DNA mismatch repair, and p53 overexpression in patients with resected Dukes' B2 or C colon cancer: a North Central Cancer Treatment Group Study." J Clin Oncol **22**(9): 1572-1582.

Germano, G., P. Allavena, et al. (2008). "Cytokines as a key component of cancer-related inflammation." Cytokine **43**(3): 374-379.

Giardiello, F. M., S. B. Welsh, et al. (1987). "Increased risk of cancer in the Peutz-Jeghers syndrome." N Engl J Med **316**(24): 1511-1514.

Gill, S., C. L. Loprinzi, et al. (2004). "Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much?" J Clin Oncol **22**(10): 1797-1806.

Giovannucci, E. (1995). "Insulin and colon cancer." Cancer Causes Control **6**(2): 164-179.

Giovannucci, E., A. Ascherio, et al. (1995). "Physical activity, obesity, and risk for colon cancer and adenoma in men." Ann Intern Med **122**(5): 327-334.

Giovannucci, E. and M. E. Martinez (1996). "Tobacco, colorectal cancer, and adenomas: a review of the evidence." J Natl Cancer Inst **88**(23): 1717-1730.

Glen, P., N. B. Jamieson, et al. (2006). "Evaluation of an inflammation-based prognostic score in patients with inoperable pancreatic cancer." Pancreatology **6**(5): 450-453.

Goedegebuure, P. S. and T. J. Eberlein (1995). "The role of CD4+ tumor-infiltrating lymphocytes in human solid tumors." Immunol Res **14**(2): 119-131.

Gold, P. and S. O. Freedman (1965). "Specific carcinoembryonic antigens of the human digestive system." J Exp Med **122**(3): 467-481.

Goransson, J., S. Jonsson, et al. (1996). "Pre-operative plasma levels of C-reactive protein, albumin and various plasma protease inhibitors for the pre-operative assessment of operability and recurrence in cancer surgery." Eur J Surg Oncol **22**(6): 607-617.

Gottfried, E., L. A. Kunz-Schughart, et al. (2008). "Expression of CD68 in non-myeloid cell types." Scand J Immunol **67**(5): 453-463.

Graham, D. M. and H. D. Appelman (1990). "Crohn's-like lymphoid reaction and colorectal carcinoma: a potential histologic prognosticator." Mod Pathol **3**(3): 332-335.

Graham, R. A., S. Wang, et al. (1998). "Postsurgical surveillance of colon cancer: preliminary cost analysis of physician examination, carcinoembryonic antigen testing, chest x-ray, and colonoscopy." Ann Surg **228**(1): 59-63.

Graham, S. and C. Mettlin (1979). "Diet and colon cancer." Am J Epidemiol **109**(1): 1-20.

Greenson, J. K., J. D. Bonner, et al. (2003). "Phenotype of microsatellite unstable colorectal carcinomas: Well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability." Am J Surg Pathol **27**(5): 563-570.

Grem, J. (1997). "The prognostic importance of tumor markers in adenocarcinomas of the gastrointestinal tract." Curr Opin Oncol **9**(4): 380-387.

Groden, J., A. Thliveris, et al. (1991). "Identification and characterization of the familial adenomatous polyposis coli gene." Cell **66**(3): 589-600.

Grodstein, F., P. A. Newcomb, et al. (1999). "Postmenopausal hormone therapy and the risk of colorectal cancer: a review and meta-analysis." Am J Med **106**(5): 574-582.

Gruys, E., M. J. Toussaint, et al. (2005). "Acute phase reaction and acute phase proteins." J Zhejiang Univ Sci B **6**(11): 1045-1056.

Guidoboni, M., R. Gafa, et al. (2001). "Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis." Am J Pathol **159**(1): 297-304.

Gupta, R. B., N. Harpaz, et al. (2007). "Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study." Gastroenterology **133**(4): 1099-1105; quiz 1340-1091.

Haenszel, W. and M. Kurihara (1968). "Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States." J Natl Cancer Inst **40**(1): 43-68.

Halligan, S., D. G. Altman, et al. (2005). "CT colonography in the detection of colorectal polyps and cancer: systematic review, meta-analysis, and proposed minimum data set for study level reporting." Radiology **237**(3): 893-904.

Hamelin, R., P. Laurent-Puig, et al. (1994). "Association of p53 mutations with short survival in colorectal cancer." Gastroenterology **106**(1): 42-48.

Hamilton, W., R. Lancashire, et al. (2008). "The importance of anaemia in diagnosing colorectal cancer: a case-control study using electronic primary care records." British journal of cancer **98**(2): 323-327.

Hanahan, D. and R. A. Weinberg (2011). "Hallmarks of cancer: the next generation." Cell **144**(5): 646-674.

Hara, M., Y. Matsuzaki, et al. (2007). "Preoperative serum C-reactive protein level in non-small cell lung cancer." Anticancer Res **27**(4C): 3001-3004.

Hardcastle, J. D., J. O. Chamberlain, et al. (1996). "Randomised controlled trial of faecal-occult-blood screening for colorectal cancer." Lancet **348**(9040): 1472-1477.

Harewood, G. C. and D. A. Ahlquist (2000). "Fecal occult blood testing for iron deficiency: a reappraisal." Dig Dis **18**(2): 75-82.

Harrison, J. C., P. J. Dean, et al. (1995). "Impact of the Crohn's-like lymphoid reaction on staging of right-sided colon cancer: results of multivariate analysis." Hum Pathol **26**(1): 31-38.

Harrison, L. E., J. G. Guillem, et al. (1997). "Preoperative carcinoembryonic antigen predicts outcomes in node-negative colon cancer patients: a multivariate analysis of 572 patients." J Am Coll Surg **185**(1): 55-59.

Hase, K., C. Shatney, et al. (1993). "Prognostic value of tumor "budding" in patients with colorectal cancer." Dis Colon Rectum **36**(7): 627-635.

Heald, R. J., E. M. Husband, et al. (1982). "The mesorectum in rectal cancer surgery--the clue to pelvic recurrence?" Br J Surg **69**(10): 613-616.

Heineman, E. F., S. H. Zahm, et al. (1994). "Increased risk of colorectal cancer among smokers: results of a 26-year follow-up of US veterans and a review." Int J Cancer **59**(6): 728-738.

Heinrich, P. C., J. V. Castell, et al. (1990). "Interleukin-6 and the acute phase response." Biochem J **265**(3): 621-636.

Hentosh, P., S. H. Yuh, et al. (2001). "Sterol-independent regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in tumor cells." Molecular carcinogenesis **32**(3): 154-166.

Hillner, B. E., T. J. Smith, et al. (2000). "Hospital and physician volume or specialization and outcomes in cancer treatment: importance in quality of cancer care." J Clin Oncol **18**(11): 2327-2340.

Hoeijmakers, J. H. (2007). "Genome maintenance mechanisms are critical for preventing cancer as well as other aging-associated diseases." Mechanisms of ageing and development **128**(7-8): 460-462.

Hogarth, M. B., R. Gallimore, et al. (1997). "Acute phase proteins, C-reactive protein and serum amyloid A protein, as prognostic markers in the elderly inpatient." Age Ageing **26**(2): 153-158.

Hole, D. J. and C. S. McArdle (2002). "Impact of socioeconomic deprivation on outcome after surgery for colorectal cancer." Br J Surg **89**(5): 586-590.

Holm, T., A. Ljung, et al. (2007). "Extended abdominoperineal resection with gluteus maximus flap reconstruction of the pelvic floor for rectal cancer." Br J Surg **94**(2): 232-238.

Horn, A., O. Dahl, et al. (1990). "The role of venous and neural invasion on survival in rectal adenocarcinoma." Dis Colon Rectum **33**(7): 598-601.

Houbiers, J. G., S. H. van der Burg, et al. (1995). "Antibodies against p53 are associated with poor prognosis of colorectal cancer." Br J Cancer **72**(3): 637-641.

House, A. K. and A. G. Watt (1979). "Survival and the immune response in patients with carcinoma of the colorectum." Gut **20**(10): 868-874.

Howe, J. R., F. A. Mitros, et al. (1998). "The risk of gastrointestinal carcinoma in familial juvenile polyposis." Ann Surg Oncol **5**(8): 751-756.

Huh, J. W., J. H. Lee, et al. (2012). "Prognostic significance of tumor-infiltrating lymphocytes for patients with colorectal cancer." Arch Surg **147**(4): 366-372.

Ionov, Y., M. A. Peinado, et al. (1993). "Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis." Nature **363**(6429): 558-561.

Ishizuka, M., H. Nagata, et al. (2007). "Inflammation-based prognostic score is a novel predictor of postoperative outcome in patients with colorectal cancer." Ann Surg **246**(6): 1047-1051.

Issa, J. P. (2004). "CpG island methylator phenotype in cancer." Nat Rev Cancer **4**(12): 988-993.

Itzkowitz, S. H. and X. Yio (2004). "Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation." Am J Physiol Gastrointest Liver Physiol **287**(1): G7-17.

Jaiswal, M., N. F. LaRusso, et al. (2000). "Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism." Cancer Res **60**(1): 184-190.

Jamieson, N. B., P. Glen, et al. (2005). "Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma head of pancreas." Br J Cancer **92**(1): 21-23.

Jamison, R. L., J. H. Donohue, et al. (1997). "Hepatic resection for metastatic colorectal cancer results in cure for some patients." Arch Surg **132**(5): 505-510; discussion 511.

Janeway, C. A., Jr. (2001). "How the immune system protects the host from infection." Microbes Infect **3**(13): 1167-1171.

Janeway, C. A., Jr. and R. Medzhitov (2002). "Innate immune recognition." Annu Rev Immunol **20**: 197-216.

Jass, J. R. (1986). "Lymphocytic infiltration and survival in rectal cancer." J Clin Pathol **39**(6): 585-589.

Jass, J. R., S. B. Love, et al. (1987). "A new prognostic classification of rectal cancer." Lancet **1**(8545): 1303-1306.

Jenkins, J. T., G. O'Neill, et al. (2007). "The relationship between patient physiology and cancer-specific survival following curative resection of colorectal cancer." Br J Cancer **96**(2): 213-217.

Jenkins, M. A., S. Hayashi, et al. (2007). "Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study." Gastroenterology **133**(1): 48-56.

Johnson, P. M., G. A. Porter, et al. (2006). "Increasing negative lymph node count is independently associated with improved long-term survival in stage IIIB and IIIC colon cancer." J Clin Oncol **24**(22): 3570-3575.

Jones, H. J. and L. de Cossart (1999). "Risk scoring in surgical patients." Br J Surg **86**(2): 149-157.

Jung, S. H., C. S. Yu, et al. (2008). "Risk factors and oncologic impact of anastomotic leakage after rectal cancer surgery." Dis Colon Rectum **51**(6): 902-908.

Kamangar, F., G. M. Dores, et al. (2006). "Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **24**(14): 2137-2150.

Kanazawa, H., H. Mitomi, et al. (2008). "Tumour budding at invasive margins and outcome in colorectal cancer." Colorectal Dis **10**(1): 41-47.

Karin, M. and F. R. Greten (2005). "NF-kappaB: linking inflammation and immunity to cancer development and progression." Nature reviews. Immunology **5**(10): 749-759.

Kaye, J. A. and L. N. Shulman (1992). "Screening program for colorectal cancer: effect on stage distribution." HMO practice / HMO Group **6**(3): 13-15.

Kenfield, S. A., M. J. Stampfer, et al. (2008). "Smoking and smoking cessation in relation to mortality in women." JAMA **299**(17): 2037-2047.

Khalid, U., A. Spiro, et al. (2007). "Symptoms and weight loss in patients with gastrointestinal and lung cancer at presentation." Support Care Cancer **15**(1): 39-46.

Khoo, C. K., C. J. Vickery, et al. (2007). "A prospective randomized controlled trial of multimodal perioperative management protocol in patients undergoing elective colorectal resection for cancer." Ann Surg **245**(6): 867-872.

Khuri, S. F., W. G. Henderson, et al. (2005). "Determinants of long-term survival after major surgery and the adverse effect of postoperative complications." Ann Surg **242**(3): 326-341; discussion 341-323.

Kikuchi, R., M. Takano, et al. (1995). "Management of early invasive colorectal cancer. Risk of recurrence and clinical guidelines." Dis Colon Rectum **38**(12): 1286-1295.

Kinzler, K. W. and B. Vogelstein (1998). "Landscaping the cancer terrain." Science **280**(5366): 1036-1037.

Klintrup, K., J. M. Makinen, et al. (2005). "Inflammation and prognosis in colorectal cancer." Eur J Cancer **41**(17): 2645-2654.

Knutsen, A., G. Adell, et al. (2006). "Inflammatory infiltration, fibrosis, necrosis and mucinous content in relation to clinicopathological and molecular factors in rectal cancers with or without preoperative radiotherapy." Oncol Rep **16**(2): 321-327.

Koike, Y., C. Miki, et al. (2008). "Preoperative C-reactive protein as a prognostic and therapeutic marker for colorectal cancer." J Surg Oncol **98**(7): 540-544.

Kolodner, R. D., J. D. Tytell, et al. (1999). "Germ-line msh6 mutations in colorectal cancer families." Cancer Res **59**(20): 5068-5074.

Koong, A. C., N. C. Denko, et al. (2000). "Candidate genes for the hypoxic tumor phenotype." Cancer Res **60**(4): 883-887.

Koprowski, H., M. Herlyn, et al. (1981). "Specific antigen in serum of patients with colon carcinoma." Science **212**(4490): 53-55.

Kotler, D. P. (2000). "Cachexia." Ann Intern Med **133**(8): 622-634.

Krasna, M. J., L. Flancbaum, et al. (1988). "Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance." Cancer **61**(5): 1018-1023.

Kressner, U., G. Lindmark, et al. (1996). "Immunohistological p53 staining is of limited value in the staging and prognostic prediction of colorectal cancer." Anticancer Res **16**(2): 951-957.

Kumar, S., E. Y. Chang, et al. (2009). "Combination of microsatellite instability and lymphocytic infiltrate as a prognostic indicator for adjuvant therapy in colon cancer." Arch Surg **144**(9): 835-840.

Kune, G. A., S. Kune, et al. (1988). "Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study." Cancer Res **48**(15): 4399-4404.

La Vecchia, C., E. Negri, et al. (1997). "Diabetes mellitus and colorectal cancer risk." Cancer Epidemiol Biomarkers Prev **6**(12): 1007-1010.

Laghi, L., P. Bianchi, et al. (2009). "CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastasis: a longitudinal study." Lancet Oncol **10**(9): 877-884.

Lakatos, P. L. and L. Lakatos (2008). "Risk for colorectal cancer in ulcerative colitis: changes, causes and management strategies." World J Gastroenterol **14**(25): 3937-3947.

Langer, C., T. Liersch, et al. (2003). "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 **18**(3): 222-229.

Larsson, S. C., E. Giovannucci, et al. (2005). "Diabetes and colorectal cancer incidence in the cohort of Swedish men." Diabetes Care **28**(7): 1805-1807.

Law, W. L. and K. W. Chu (2004). "Anterior resection for rectal cancer with mesorectal excision: a prospective evaluation of 622 patients." Ann Surg **240**(2): 260-268.

Le Marchand, L., L. R. Wilkens, et al. (1997). "Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer." Cancer Res **57**(21): 4787-4794.

Le Voyer, T. E., E. R. Sigurdson, et al. (2003). "Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089." J Clin Oncol **21**(15): 2912-2919.

Lee, W., D. Lee, et al. (2003). "Transanal endoscopic microsurgery and radical surgery for T1 and T2 rectal cancer." Surg Endosc **17**(8): 1283-1287.

Leung, E., A. M. Ferjani, et al. (2009). "Predicting post-operative mortality in patients undergoing colorectal surgery using P-POSSUM and CR-POSSUM scores: a prospective study." Int J Colorectal Dis **24**(12): 1459-1464.

Liebig, C., G. Ayala, et al. (2009). "Perineural invasion is an independent predictor of outcome in colorectal cancer." J Clin Oncol **27**(31): 5131-5137.

Limburg, P. J., K. E. Anderson, et al. (2005). "Diabetes mellitus and subsite-specific colorectal cancer risks in the Iowa Women's Health Study." Cancer Epidemiol Biomarkers Prev **14**(1): 133-137.

Liotta, L. A. and E. C. Kohn (2001). "The microenvironment of the tumour-host interface." Nature **411**(6835): 375-379.

Lloyd-Jones, D. M. and D. Levy (2003). "C-reactive protein in the prediction of cardiovascular events." N Engl J Med **348**(11): 1059-1061; author reply 1059-1061.

Lluis, J. M., F. Buricchi, et al. (2007). "Dual role of mitochondrial reactive oxygen species in hypoxia signaling: activation of nuclear factor-{kappa}B via c-SRC and oxidant-dependent cell death." Cancer research **67**(15): 7368-7377.

Locker, G. Y., S. Hamilton, et al. (2006). "ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer." J Clin Oncol **24**(33): 5313-5327.

Loddenkemper, C., M. Schernus, et al. (2006). "In situ analysis of FOXP3+ regulatory T cells in human colorectal cancer." J Transl Med **4**: 52.

Longo, W. E., K. S. Virgo, et al. (2000). "Risk factors for morbidity and mortality after colectomy for colon cancer." Dis Colon Rectum **43**(1): 83-91.

Loose, D. and C. Van de Wiele (2009). "The immune system and cancer." Cancer Biother Radiopharm **24**(3): 369-376.

Lynch, H. T. and A. de la Chapelle (1999). "Genetic susceptibility to non-polyposis colorectal cancer." J Med Genet **36**(11): 801-818.

Lynch, H. T., M. W. Shaw, et al. (1966). "Hereditary factors in cancer. Study of two large midwestern kindreds." Arch Intern Med **117**(2): 206-212.

Mainprize, K. S., N. J. Mortensen, et al. (2002). "Dukes' staging is poorly understood by doctors managing colorectal cancer." Ann R Coll Surg Engl **84**(1): 23-25.

Maltoni, M., A. Caraceni, et al. (2005). "Prognostic factors in advanced cancer patients: evidence-based clinical recommendations--a study by the Steering Committee of the European Association for Palliative Care." J Clin Oncol **23**(25): 6240-6248.

Mangram, A. J., T. C. Horan, et al. (1999). "Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee." Am J Infect Control **27**(2): 97-132; quiz 133-134; discussion 196.

Mantovani, A., P. Allavena, et al. (2008). "Cancer-related inflammation." Nature **454**(7203): 436-444.

Mantovani, A., S. Sozzani, et al. (2002). "Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes." Trends Immunol **23**(11): 549-555.

Marra, F., T. Steffen, et al. (2009). "Anastomotic leakage as a risk factor for the long-term outcome after curative resection of colon cancer." Eur J Surg Oncol **35**(10): 1060-1064.

Mayor, S. (2009). "Unravelling the secrets of ageing." BMJ **338**: a3024.

McArdle, C. S., D. C. McMillan, et al. (2005). "Impact of anastomotic leakage on long-term survival of patients undergoing curative resection for colorectal cancer." Br J Surg **92**(9): 1150-1154.

McDermott, F. T., E. S. Hughes, et al. (1984). "Influence of tumour differentiation on survival after resection for rectal cancer in a series of 1296 patients." Aust N Z J Surg **54**(1): 53-58.

McKeown-Eyssen, G. (1994). "Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk?" Cancer Epidemiol Biomarkers Prev **3**(8): 687-695.

McMichael, A. J. and J. D. Potter (1980). "Reproduction, endogenous and exogenous sex hormones, and colon cancer: a review and hypothesis." J Natl Cancer Inst **65**(6): 1201-1207.

McMillan, D. C. (2008). "An inflammation-based prognostic score and its role in the nutrition-based management of patients with cancer." Proc Nutr Soc **67**(3): 257-262.

McMillan, D. C. (2009). "Systemic inflammation, nutritional status and survival in patients with cancer." Curr Opin Clin Nutr Metab Care **12**(3): 223-226.

McMillan, D. C., K. Canna, et al. (2003). "Systemic inflammatory response predicts survival following curative resection of colorectal cancer." Br J Surg **90**(2): 215-219.

McMillan, D. C., J. E. Crozier, et al. (2007). "Evaluation of an inflammation-based prognostic score (GPS) in patients undergoing resection for colon and rectal cancer." Int J Colorectal Dis **22**(8): 881-886.

McMillan, D. C., M. M. Elahi, et al. (2001). "Measurement of the systemic inflammatory response predicts cancer-specific and non-cancer survival in patients with cancer." Nutr Cancer **41**(1-2): 64-69.

McMillan, D. C., T. Preston, et al. (1994). "Relationship between weight loss, reduction of body cell mass and inflammatory response in patients with cancer." Br J Surg **81**(7): 1011-1014.

McMillan, D. C., H. R. Scott, et al. (1998). "Longitudinal study of body cell mass depletion and the inflammatory response in cancer patients." Nutr Cancer **31**(2): 101-105.

Medzhitov, R. (2007). "Recognition of microorganisms and activation of the immune response." Nature **449**(7164): 819-826.

Meira, L. B., J. M. Bugni, et al. (2008). "DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice." J Clin Invest **118**(7): 2516-2525.

Menon, A. G., C. M. Janssen-van Rhijn, et al. (2004). "Immune system and prognosis in colorectal cancer: a detailed immunohistochemical analysis." Lab Invest **84**(4): 493-501.

Menon, K. V. and R. Farouk (2002). "An analysis of the accuracy of P-POSSUM scoring for mortality risk assessment after surgery for colorectal cancer." Colorectal Dis **4**(3): 197-200.

Metcalfe, M. S., M. G. Norwood, et al. (2005). "Unreasonable expectations in emergency colorectal cancer surgery." Colorectal Dis **7**(3): 275-278.

Meyerhardt, J. A., E. L. Giovannucci, et al. (2009). "Physical activity and male colorectal cancer survival." Arch Intern Med **169**(22): 2102-2108.

Miki, C., N. Konishi, et al. (2004). "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 **49**(6): 970-976.

Minsky, B. D., C. Mies, et al. (1988). "Resectable adenocarcinoma of the rectosigmoid and rectum. II. The influence of blood vessel invasion." Cancer **61**(7): 1417-1424.

Mitsiopoulos, N., R. N. Baumgartner, et al. (1998). "Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography." J Appl Physiol **85**(1): 115-122.

Mlecnik, B., M. Tosolini, et al. (2011). "Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction." J Clin Oncol **29**(6): 610-618.

Moertel, C. G., T. R. Fleming, et al. (1995). "Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report." Ann Intern Med **122**(5): 321-326.

Moertel, C. G., J. R. O'Fallon, et al. (1986). "The preoperative carcinoembryonic antigen test in the diagnosis, staging, and prognosis of colorectal cancer." Cancer **58**(3): 603-610.

Moghaddam, A. A., M. Woodward, et al. (2007). "Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events." Cancer Epidemiol Biomarkers Prev **16**(12): 2533-2547.

Morita, S., T. Nomura, et al. (2004). "Does serum CA19-9 play a practical role in the management of patients with colorectal cancer?" Dis Colon Rectum **47**(2): 227-232.

Morley, J. E., D. R. Thomas, et al. (2006). "Cachexia: pathophysiology and clinical relevance." Am J Clin Nutr **83**(4): 735-743.

Morris, E. J., N. J. Maughan, et al. (2007). "Who to treat with adjuvant therapy in Dukes B/stage II colorectal cancer? The need for high quality pathology." Gut **56**(10): 1419-1425.

Morris, R. and V. Carstairs (1991). "Which deprivation? A comparison of selected deprivation indexes." J Public Health Med **13**(4): 318-326.

Moskal, A., T. Norat, et al. (2007). "Alcohol intake and colorectal cancer risk: a dose-response meta-analysis of published cohort studies." Int J Cancer **120**(3): 664-671.

Moyes, L. H., E. F. Leitch, et al. (2009). "Preoperative systemic inflammation predicts postoperative infectious complications in patients undergoing curative resection for colorectal cancer." Br J Cancer **100**(8): 1236-1239.

Muir, C. S. and D. M. Parkin (1985). "The world cancer burden: prevent or perish." Br Med J (Clin Res Ed) **290**(6461): 5-6.

Mulcahy, H. E., M. Toner, et al. (1997). "Identifying stage B colorectal cancer patients at high risk of tumor recurrence and death." Dis Colon Rectum **40**(3): 326-331.

Munro, A. J., A. H. Bentley, et al. (2006). "Smoking compromises cause-specific survival in patients with operable colorectal cancer." Clin Oncol (R Coll Radiol) **18**(6): 436-440.

Mutch, M. G. (2007). "Molecular profiling and risk stratification of adenocarcinoma of the colon." J Surg Oncol **96**(8): 693-703.

Nagorsen, D., S. Voigt, et al. (2007). "Tumor-infiltrating macrophages and dendritic cells in human colorectal cancer: relation to local regulatory T cells, systemic T-cell response against tumor-associated antigens and survival." J Transl Med **5**: 62.

Nagtegaal, I. D., C. A. Marijnen, et al. (2001). "Local and distant recurrences in rectal cancer patients are predicted by the nonspecific immune response; specific immune response has only a systemic effect--a histopathological and immunohistochemical study." BMC Cancer **1**: 7.

Naito, Y., K. Saito, et al. (1998). "CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer." Cancer Res **58**(16): 3491-3494.

Neugut, A. I., J. S. Jacobson, et al. (1995). "Coronary artery disease and colorectal neoplasia." Dis Colon Rectum **38**(8): 873-877.

Nickelsen, T. N., T. Jorgensen, et al. (2005). "Lifestyle and 30-day complications to surgery for colorectal cancer." Acta Oncol **44**(3): 218-223.

Nielsen, H. J., U. Hansen, et al. (1999). "Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue." J Pathol **189**(4): 487-495.

O'Connell, J. B., M. A. Maggard, et al. (2004). "Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging." J Natl Cancer Inst **96**(19): 1420-1425.

O'Dwyer, P. J. and E. W. Martin, Jr. (1989). "Viable intraluminal tumour cells and local/regional tumour growth in experimental colon cancer." Ann R Coll Surg Engl **71**(1): 54-56.

O'Gorman, P., D. C. McMillan, et al. (2000). "Prognostic factors in advanced gastrointestinal cancer patients with weight loss." Nutr Cancer **37**(1): 36-40.

Oberg, A., S. Samii, et al. (2002). "Different occurrence of CD8+, CD45R0+, and CD68+ immune cells in regional lymph node metastases from colorectal cancer as potential prognostic predictors." Int J Colorectal Dis **17**(1): 25-29.

Ogino, S., K. Nosho, et al. (2009). "Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype." Clin Cancer Res **15**(20): 6412-6420.

Orlacchio, A., O. Schillaci, et al. (2009). "Role of PET/CT in the detection of liver metastases from colorectal cancer." Radiol Med **114**(4): 571-585.

Ouchi, K., T. Sugawara, et al. (1996). "Histologic features and clinical significance of venous invasion in colorectal carcinoma with hepatic metastasis." Cancer **78**(11): 2313-2317.

Pages, F., A. Berger, et al. (2005). "Effector memory T cells, early metastasis, and survival in colorectal cancer." N Engl J Med **353**(25): 2654-2666.

Park, Y., D. J. Hunter, et al. (2005). "Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies." JAMA **294**(22): 2849-2857.

Parkin, D. M., P. Pisani, et al. (1999). "Estimates of the worldwide incidence of 25 major cancers in 1990." Int J Cancer **80**(6): 827-841.

Peleg, II, H. T. Maibach, et al. (1994). "Aspirin and nonsteroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer." Arch Intern Med **154**(4): 394-399.

Peng, P. D., M. G. van Vledder, et al. (2011). "Sarcopenia negatively impacts short-term outcomes in patients undergoing hepatic resection for colorectal liver metastasis." HPB (Oxford) **13**(7): 439-446.

Perry, G. S., T. E. Byers, et al. (1995). "The validity of self-reports of past body weights by U.S. adults." Epidemiology **6**(1): 61-66.

Peschaud, F., S. Benoist, et al. (2008). "The ratio of metastatic to examined lymph nodes is a powerful independent prognostic factor in rectal cancer." Ann Surg **248**(6): 1067-1073.

Petersen, V. C., K. J. Baxter, et al. (2002). "Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer." Gut **51**(1): 65-69.

Pichard, C., U. G. Kyle, et al. (2004). "Nutritional assessment: lean body mass depletion at hospital admission is associated with an increased length of stay." Am J Clin Nutr **79**(4): 613-618.

Pollard, J. W. (2004). "Tumour-educated macrophages promote tumour progression and metastasis." Nat Rev Cancer **4**(1): 71-78.

Pollheimer, M. J., P. Kornprat, et al. (2010). "Tumor necrosis is a new promising prognostic factor in colorectal cancer." Hum Pathol **41**(12): 1749-1757.

Ponz de Leon, M., P. Benatti, et al. (2004). "Aetiology of colorectal cancer and relevance of monogenic inheritance." Gut **53**(1): 115-122.

Poon, J. T., B. Chan, et al. (2005). "Evaluation of P-POSSUM in surgery for obstructing colorectal cancer and correlation of the predicted mortality with different surgical options." Dis Colon Rectum **48**(3): 493-498.

Popat, S., R. Hubner, et al. (2005). "Systematic review of microsatellite instability and colorectal cancer prognosis." J Clin Oncol **23**(3): 609-618.

Potter, J. D., M. L. Slattery, et al. (1993). "Colon cancer: a review of the epidemiology." Epidemiol Rev **15**(2): 499-545.

Poynter, J. N., S. B. Gruber, et al. (2005). "Statins and the risk of colorectal cancer." The New England journal of medicine **352**(21): 2184-2192.

Poynter, J. N., K. D. Siegmund, et al. (2008). "Molecular characterization of MSI-H colorectal cancer by MLHI promoter methylation, immunohistochemistry, and mismatch repair germline mutation screening." Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology **17**(11): 3208-3215.

Prado, C. M., V. E. Baracos, et al. (2007). "Body composition as an independent determinant of 5-fluorouracil-based chemotherapy toxicity." Clin Cancer Res **13**(11): 3264-3268.

Prado, C. M., J. R. Lieffers, et al. (2008). "Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study." Lancet Oncol **9**(7): 629-635.

Prall, F., T. Duhrkop, et al. (2004). "Prognostic role of CD8+ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability." Hum Pathol **35**(7): 808-816.

Pricolo, V. E., S. D. Finkelstein, et al. (1996). "Prognostic value of TP53 and K-ras-2 mutational analysis in stage III carcinoma of the colon." Am J Surg **171**(1): 41-46.

Proctor, M. J., D. S. Morrison, et al. (2011). "An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study." Br J Cancer **104**(4): 726-734.

Prytherch, D. R., M. S. Whiteley, et al. (1998). "POSSUM and Portsmouth POSSUM for predicting mortality. Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity." Br J Surg **85**(9): 1217-1220.

Quirke, P., G. T. Williams, et al. (2007). "The future of the TNM staging system in colorectal cancer: time for a debate?" Lancet Oncol **8**(7): 651-657.

Rao, C. V., H. L. Newmark, et al. (2002). "Chemopreventive effect of farnesol and lanosterol on colon carcinogenesis." Cancer detection and prevention **26**(6): 419-425.

Ravitch, M. M. (1989). "Subjectivity in decision making: common problems and limitations." World J Surg **13**(3): 281-286.

Reiter, W., P. Stieber, et al. (2000). "Multivariate analysis of the prognostic value of CEA and CA 19-9 serum levels in colorectal cancer." Anticancer Res **20**(6D): 5195-5198.

Rex, D. K. (1995). "Colonoscopy: a review of its yield for cancers and adenomas by indication." Am J Gastroenterol **90**(3): 353-365.

Rex, D. K., E. Y. Rahmani, et al. (1997). "Relative sensitivity of colonoscopy and barium enema for detection of colorectal cancer in clinical practice." Gastroenterology **112**(1): 17-23.

Reza, M. M., J. A. Blasco, et al. (2006). "Systematic review of laparoscopic versus open surgery for colorectal cancer." Br J Surg **93**(8): 921-928.

Ribic, C. M., D. J. Sargent, et al. (2003). "Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer." N Engl J Med **349**(3): 247-257.

Richards, C. H., J. J. Platt, et al. (2011). "The impact of perioperative risk, tumor pathology and surgical complications on disease recurrence following potentially curative resection of colorectal cancer." Ann Surg **254**(1): 83-89.

Ridker, P. M., J. E. Buring, et al. (2003). "C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women." Circulation **107**(3): 391-397.

Rodel, C., P. Martus, et al. (2005). "Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer." J Clin Oncol **23**(34): 8688-8696.

Rosenberg, R., J. Friederichs, et al. (2008). "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 **248**(6): 968-978.

Ross, A., C. Rusnak, et al. (1999). "Recurrence and survival after surgical management of rectal cancer." Am J Surg **177**(5): 392-395.

Ross, P. J., S. Ashley, et al. (2004). "Do patients with weight loss have a worse outcome when undergoing chemotherapy for lung cancers?" Br J Cancer **90**(10): 1905-1911.

Ross, R. (1999). "Atherosclerosis is an inflammatory disease." Am Heart J **138**(5 Pt 2): S419-420.

Roxburgh, C. S. and A. K. Foulis (2011). "The prognostic benefits of routine staining with elastica to increase detection of venous invasion in colorectal cancer specimens." Journal of clinical pathology **64**(12): 1142.

Roxburgh, C. S. and D. C. McMillan (2010). "Role of systemic inflammatory response in predicting survival in patients with primary operable cancer." Future Oncol **6**(1): 149-163.

Roxburgh, C. S. and D. C. McMillan (2011). "The role of the in situ local inflammatory response in predicting recurrence and survival in patients with primary operable colorectal cancer." Cancer Treat Rev.

Roxburgh, C. S., J. M. Salmond, et al. (2009). "Comparison of the prognostic value of inflammation-based pathologic and biochemical criteria in patients undergoing potentially curative resection for colorectal cancer." Ann Surg **249**(5): 788-793.

Roxburgh, C. S., J. M. Salmond, et al. (2009). "Tumour inflammatory infiltrate predicts survival following curative resection for node-negative colorectal cancer." Eur J Cancer **45**(12): 2138-2145.

Roxburgh, C. S., A. M. Wallace, et al. (2010). "Comparison of the prognostic value of tumour- and patient-related factors in patients undergoing potentially curative surgery for colon cancer." Colorectal Dis **12**(10): 987-994.

Russell, E. M., J. Bruce, et al. (2003). "Systematic review of the quality of surgical mortality monitoring." Br J Surg **90**(5): 527-532.

Sagar, P. M., M. N. Hartley, et al. (1994). "Comparative audit of colorectal resection with the POSSUM scoring system." Br J Surg **81**(10): 1492-1494.

Saijo, Y., N. Kiyota, et al. (2004). "Relationship between C-reactive protein and visceral adipose tissue in healthy Japanese subjects." Diabetes Obes Metab **6**(4): 249-258.

Salama, P., M. Phillips, et al. (2009). "Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer." J Clin Oncol **27**(2): 186-192.

Samad, A. K., R. S. Taylor, et al. (2005). "A meta-analysis of the association of physical activity with reduced risk of colorectal cancer." Colorectal Dis **7**(3): 204-213.

Sandel, M. H., A. R. Dadabayev, et al. (2005). "Prognostic value of tumor-infiltrating dendritic cells in colorectal cancer: role of maturation status and intratumoral localization." Clin Cancer Res **11**(7): 2576-2582.

Sandhu, M. S., I. R. White, et al. (2001). "Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach." Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology **10**(5): 439-446.

Santarelli, R. L., F. Pierre, et al. (2008). "Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence." Nutr Cancer **60**(2): 131-144.

Sauer, R., H. Becker, et al. (2004). "Preoperative versus postoperative chemoradiotherapy for rectal cancer." N Engl J Med **351**(17): 1731-1740.

Scholefield, J. H. (2000). "ABC of colorectal cancer: screening." BMJ **321**(7267): 1004-1006.

Scholefield, J. H., S. Moss, et al. (2002). "Effect of faecal occult blood screening on mortality from colorectal cancer: results from a randomised controlled trial." Gut **50**(6): 840-844.

Schreiber, T. H. and E. R. Podack (2009). "A critical analysis of the tumour immunosurveillance controversy for 3-MCA-induced sarcomas." Br J Cancer **101**(3): 381-386.

Scott, N., P. Sagar, et al. (1991). "p53 in colorectal cancer: clinicopathological correlation and prognostic significance." Br J Cancer **63**(2): 317-319.

Selby, J. V., G. D. Friedman, et al. (1992). "A case-control study of screening sigmoidoscopy and mortality from colorectal cancer." The New England journal of medicine **326**(10): 653-657.

Senagore, A. J., A. J. Warmuth, et al. (2004). "POSSUM, p-POSSUM, and Cr-POSSUM: implementation issues in a United States health care system for prediction of outcome for colon cancer resection." Dis Colon Rectum **47**(9): 1435-1441.

Sengupta, S., C. M. Lohse, et al. (2005). "Histologic coagulative tumor necrosis as a prognostic indicator of renal cell carcinoma aggressiveness." Cancer **104**(3): 511-520.

Shankar, A., J. J. Wang, et al. (2006). "Association between circulating white blood cell count and cancer mortality: a population-based cohort study." Arch Intern Med **166**(2): 188-194.

Shankaran, V., H. Ikeda, et al. (2001). "IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity." Nature **410**(6832): 1107-1111.

Sharma, R., M. Zucknick, et al. (2008). "Systemic inflammatory response predicts prognosis in patients with advanced-stage colorectal cancer." Clin Colorectal Cancer **7**(5): 331-337.

Shepherd, N. A., K. J. Baxter, et al. (1997). "The prognostic importance of peritoneal involvement in colonic cancer: a prospective evaluation." Gastroenterology **112**(4): 1096-1102.

Siddiqui, A. A., H. Nazario, et al. (2009). "For patients with colorectal cancer, the long-term use of statins is associated with better clinical outcomes." Dig Dis Sci **54**(6): 1307-1311.

Sin, D. D. and S. F. Man (2003). "Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular diseases? The potential role of systemic inflammation in chronic obstructive pulmonary disease." Circulation **107**(11): 1514-1519.

Sinicrope, F. A., R. L. Rego, et al. (2009). "Intraepithelial effector (CD3+)/regulatory (FoxP3+) T-cell ratio predicts a clinical outcome of human colon carcinoma." Gastroenterology **137**(4): 1270-1279.

Siveen, K. S. and G. Kuttan (2009). "Role of macrophages in tumour progression." Immunol Lett **123**(2): 97-102.

Slattery, M. L., S. L. Edwards, et al. (1999). "Lifestyle and colon cancer: an assessment of factors associated with risk." Am J Epidemiol **150**(8): 869-877.

Slattery, M. L., J. D. Potter, et al. (1997). "Tobacco use and colon cancer." Int J Cancer **70**(3): 259-264.

Slim, K., Y. Panis, et al. (2006). "Predicting postoperative mortality in patients undergoing colorectal surgery." World J Surg **30**(1): 100-106.

Smith, R. A., P. Ghaneh, et al. (2008). "Prognosis of resected ampullary adenocarcinoma by preoperative serum CA19-9 levels and platelet-lymphocyte ratio." J Gastrointest Surg **12**(8): 1422-1428.

Solinas, G., G. Germano, et al. (2009). "Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation." J Leukoc Biol **86**(5): 1065-1073.

Soo, K. C., R. L. Carter, et al. (1986). "Prognostic implications of perineural spread in squamous carcinomas of the head and neck." Laryngoscope **96**(10): 1145-1148.

Sorensen, L. T., T. Jorgensen, et al. (1999). "Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery." Br J Surg **86**(7): 927-931.

Spivak, J. L. (2005). "The anaemia of cancer: death by a thousand cuts." Nature reviews. Cancer **5**(7): 543-555.

Staton, C. A., A. S. Chetwood, et al. (2007). "The angiogenic switch occurs at the adenoma stage of the adenoma carcinoma sequence in colorectal cancer." Gut **56**(10): 1426-1432.

Stemmermann, G. N., L. K. Heilbrun, et al. (1986). "Adenomatous polyps and atherosclerosis: an autopsy study of Japanese men in Hawaii." Int J Cancer **38**(6): 789-794.

Stemmermann, G. N., A. M. Nomura, et al. (1984). "Dietary fat and the risk of colorectal cancer." Cancer Res **44**(10): 4633-4637.

Sternberg, A., M. Amar, et al. (2002). "Conclusions from a study of venous invasion in stage IV colorectal adenocarcinoma." J Clin Pathol **55**(1): 17-21.

Stewart, C. J., M. Morris, et al. (2007). "Identification of serosal invasion and extramural venous invasion on review of Dukes' stage B colonic carcinomas and correlation with survival." Histopathology **51**(3): 372-378.

Stunkard, A. J. and J. M. Albaum (1981). "The accuracy of self-reported weights." Am J Clin Nutr **34**(8): 1593-1599.

Svennevig, J. L., O. C. Lunde, et al. (1984). "Lymphoid infiltration and prognosis in colorectal carcinoma." Br J Cancer **49**(3): 375-377.

Swinson, D. E., J. L. Jones, et al. (2002). "Tumour necrosis is an independent prognostic marker in non-small cell lung cancer: correlation with biological variables." Lung Cancer **37**(3): 235-240.

Tan, S. Y., Y. Fan, et al. (2005). "Prognostic significance of cell infiltrations of immunosurveillance in colorectal cancer." World J Gastroenterol **11**(8): 1210-1214.

Tanaka, K., H. Shimada, et al. (2004). "Outcome after simultaneous colorectal and hepatic resection for colorectal cancer with synchronous metastases." Surgery **136**(3): 650-659.

Tekkis, P. P., J. D. Poloniecki, et al. (2003). "Operative mortality in colorectal cancer: prospective national study." BMJ **327**(7425): 1196-1201.

Tekkis, P. P., D. R. Prytherch, et al. (2004). "Development of a dedicated risk-adjustment scoring system for colorectal surgery (colorectal POSSUM)." Br J Surg **91**(9): 1174-1182.

Thibault, R., L. Genton, et al. (2012). "Body composition: Why, when and for who?" Clin Nutr.

Thibault, R. and C. Pichard (2012). "The evaluation of body composition: a useful tool for clinical practice." Ann Nutr Metab **60**(1): 6-16.

Thibodeau, S. N., A. J. French, et al. (1998). "Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1." Cancer Res **58**(8): 1713-1718.

Thomas, G. D., M. F. Dixon, et al. (1983). "Observer variation in the histological grading of rectal carcinoma." Journal of clinical pathology **36**(4): 385-391.

Thompson, M. R., K. G. Flashman, et al. (2008). "Flexible sigmoidoscopy and whole colonic imaging in the diagnosis of cancer in patients with colorectal symptoms." Br J Surg **95**(9): 1140-1146.

Thompson, M. R., R. Perera, et al. (2007). "Predictive value of common symptom combinations in diagnosing colorectal cancer." Br J Surg **94**(10): 1260-1265.

Thomson, D. M., J. Krupey, et al. (1969). "The radioimmunoassay of circulating carcinoembryonic antigen of the human digestive system." Proc Natl Acad Sci U S A **64**(1): 161-167.

Thun, M. J., M. M. Namboodiri, et al. (1993). "Aspirin use and risk of fatal cancer." Cancer Res **53**(6): 1322-1327.

Trayhurn, P. and I. S. Wood (2004). "Adipokines: inflammation and the pleiotropic role of white adipose tissue." Br J Nutr **92**(3): 347-355.

Triantafillidis, J. K., G. Nasioulas, et al. (2009). "Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies." Anticancer Res **29**(7): 2727-2737.

Tricot, G. (2000). "New insights into role of microenvironment in multiple myeloma." Lancet **355**(9200): 248-250.

Tsilidis, K. K., C. Branchini, et al. (2008). "C-reactive protein and colorectal cancer risk: a systematic review of prospective studies." Int J Cancer **123**(5): 1133-1140.

Twelves, C., A. Wong, et al. (2005). "Capecitabine as adjuvant treatment for stage III colon cancer." N Engl J Med **352**(26): 2696-2704.

Ugolini, G., G. Rosati, et al. (2009). "An easy-to-use solution for clinical audit in colorectal cancer surgery." Surgery **145**(1): 86-92.

Umar, A., C. R. Boland, et al. (2004). "Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability." J Natl Cancer Inst **96**(4): 261-268.

Umar, A., J. I. Risinger, et al. (2004). "Testing guidelines for hereditary non-polyposis colorectal cancer." Nat Rev Cancer **4**(2): 153-158.

Vakkila, J. and M. T. Lotze (2004). "Inflammation and necrosis promote tumour growth." Nat Rev Immunol **4**(8): 641-648.

Valenti, V., J. L. Hernandez-Lizoain, et al. (2009). "Analysis of POSSUM score and postoperative morbidity in patients with rectal cancer undergoing surgery." Langenbecks Arch Surg **394**(1): 55-63.

van Rijnsoever, M., F. Grieu, et al. (2002). "Characterisation of colorectal cancers showing hypermethylation at multiple CpG islands." Gut **51**(6): 797-802.

van Vledder, M. G., S. Levolger, et al. (2012). "Body composition and outcome in patients undergoing resection of colorectal liver metastases." Br J Surg **99**(4): 550-557.

Vasen, H. F., J. P. Mecklin, et al. (1991). "The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC)." Dis Colon Rectum **34**(5): 424-425.

Vasen, H. F., P. Watson, et al. (1999). "New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC." Gastroenterology **116**(6): 1453-1456.

Visser, M., L. M. Bouter, et al. (1999). "Elevated C-reactive protein levels in overweight and obese adults." JAMA **282**(22): 2131-2135.

Vivanco, I. and C. L. Sawyers (2002). "The phosphatidylinositol 3-Kinase AKT pathway in human cancer." Nat Rev Cancer **2**(7): 489-501.

Vogelstein, B., E. R. Fearon, et al. (1988). "Genetic alterations during colorectal-tumor development." N Engl J Med **319**(9): 525-532.

Walker, K. G., S. W. Bell, et al. (2004). "Anastomotic leakage is predictive of diminished survival after potentially curative resection for colorectal cancer." Ann Surg **240**(2): 255-259.

Walsh, S. R., E. J. Cook, et al. (2005). "Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer." J Surg Oncol **91**(3): 181-184.

Wang, J. M., O. Chertov, et al. (1998). "Purification and identification of chemokines potentially involved in kidney-specific metastasis by a murine lymphoma variant: induction of migration and NFkappaB activation." Int J Cancer **75**(6): 900-907.

Weinstein, P. S., M. Skinner, et al. (1984). "Acute-phase proteins or tumour markers: the role of SAA, SAP, CRP and CEA as indicators of metastasis in a broad spectrum of neoplastic diseases." Scand J Immunol **19**(3): 193-198.

Weisenberger, D. J., K. D. Siegmund, et al. (2006). "CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer." Nat Genet **38**(7): 787-793.

White, E., E. J. Jacobs, et al. (1996). "Physical activity in relation to colon cancer in middle-aged men and women." Am J Epidemiol **144**(1): 42-50.

Whiteley, M. S., D. R. Prytherch, et al. (1996). "An evaluation of the POSSUM surgical scoring system." Br J Surg **83**(6): 812-815.

Whiteside, T. L. (2008). "The tumor microenvironment and its role in promoting tumor growth." Oncogene **27**(45): 5904-5912.

Wolin, K. Y., I. M. Lee, et al. (2007). "Leisure-time physical activity patterns and risk of colon cancer in women." Int J Cancer **121**(12): 2776-2781.

Wolmark, N., B. Fisher, et al. (1988). "Postoperative adjuvant chemotherapy or BCG for colon cancer: results from NSABP protocol C-01." J Natl Cancer Inst **80**(1): 30-36.

Wolmark, N., H. S. Wieand, et al. (2000). "Randomized trial of postoperative adjuvant chemotherapy with or without radiotherapy for carcinoma of the rectum: National Surgical Adjuvant Breast and Bowel Project Protocol R-02." J Natl Cancer Inst **92**(5): 388-396.

Worthley, D. L., V. L. Whitehall, et al. (2007). "Colorectal carcinogenesis: road maps to cancer." World J Gastroenterol **13**(28): 3784-3791.

Wouters, E. F. (2005). "Local and systemic inflammation in chronic obstructive pulmonary disease." Proc Am Thorac Soc **2**(1): 26-33.

Wu, A. H., A. Paganini-Hill, et al. (1987). "Alcohol, physical activity and other risk factors for colorectal cancer: a prospective study." Br J Cancer **55**(6): 687-694.

Wu, A. W., J. Gu, et al. (2003). "Role of COX-2 in carcinogenesis of colorectal cancer and its relationship with tumor biological characteristics and patients' prognosis." World J Gastroenterol **9**(9): 1990-1994.

Wynford-Thomas, D. (1992). "P53 in tumour pathology: can we trust immunocytochemistry?" J Pathol **166**(4): 329-330.

Yamaguchi, A., Y. Kurosaka, et al. (1992). "Expression of p53 protein in colorectal cancer and its relationship to short-term prognosis." Cancer **70**(12): 2778-2784.

Zhu, X. Y., E. Daghini, et al. (2008). "Disparate effects of simvastatin on angiogenesis during hypoxia and inflammation." Life sciences **83**(23-24): 801-809.

Zinkernagel, A. S., R. S. Johnson, et al. (2007). "Hypoxia inducible factor (HIF) function in innate immunity and infection." J Mol Med (Berl) **85**(12): 1339-1346.

Zlobec, I., K. Baker, et al. (2008). "Two-marker protein profile predicts poor prognosis in patients with early rectal cancer." Br J Cancer **99**(10): 1712-1717.

Zlobec, I. and A. Lugli (2009). "Invasive front of colorectal cancer: dynamic interface of pro-/anti-tumor factors." World J Gastroenterol **15**(47): 5898-5906.

Zorcolo, L., L. Covotta, et al. (2003). "Toward lowering morbidity, mortality, and stoma formation in emergency colorectal surgery: the role of specialization." Dis Colon Rectum **46**(11): 1461-1467; discussion 1467-1468.

Zou, W. (2006). "Regulatory T cells, tumour immunity and immunotherapy." Nat Rev Immunol **6**(4): 295-307.

APPENDIX 1

Immunohistochemistry protocol using CD3 (Vector Labs, code VP-RM01)
Performed in Beatson Oncology laboratory, Glasgow

	Time
Place sections in Xylene	5 minutes
Place sections in Ethanol	1 minute
Place sections in Ethanol	1 minute
Place sections in 70% Ethanol	1 minute
Wash sections in running tap water	
Heat Induced Epitope retrieval – pH 6 Na Citrate buffer	
2 minutes at full pressure in 800W microwave *	
Leave sections in buffer to cool	20 minutes
Wash sections in 10mM Tris buffered Tween (TbT) pH7.5	5 minutes(x2)
Block endogenous Peroxidase (Dako EnVision)	5 minutes
Wash sections in TbT	5 minutes(x2)
Apply Vector Labs CD 3 (1/100 @ room temperature)	45 minutes
Wash sections in TbT	5 minutes(x2)
Apply secondary antibody (Dako EnVision)	40 minutes
Wash sections in TbT	5 minutes(x2)
Apply 3,3'-Diaminobenzidine tetrahydrochloride	10 minutes
Terminate reaction with deionised water	1 minute
Gills haematoxylin	5 minutes
Wash sections in deionised water	30 seconds
1% Acid alcohol	2 dips
Wash sections in deionised water	30 seconds
Scotts tap water substitute	1 minute
Wash sections in deionised water	30 seconds
Dehydrate, clear and mount	

Materials Used

Reagent	**Company**	**Code**
CD 3 Rabbit Monoclonal	Vector Labs	VP-RM01
Primary antibody diluent	Dako	S2022
Retrieval buffer 10mM ph 6	Lab vision	S2369
EnVision Rabbit Kit	Dako	K4011
Tris buffered tween (TbT) - S3306	Dako	S3306

*Other heat retrieval methods such as water baths, automated retrieval (retrieval temperature should be set at 98 ºC) modules are also suitable but should be optimised For the technique before proceeding.

APPENDIX 2

Immunohistochemistry protocol using CD8 (DakoCytomation, code M7103)
Performed in Western Infirmary laboratory, Glasgow

Dewax and Rehydrate

- Dewax in Xylene 2 x 3 mins
- Rehydrate in 100% alcohol 2 x 2 mins
- 90% alcohol 2 mins
- 70% alcohol 2 mins
- Rinse in water

Antigen Retrieval

- Make Tris EDTA buffer pH8
  - 0.55g sodium EDTA
  - 0.825g Tris
  - Dissolve in 1.5 litres of dH_20 in pressure cooker
- Microwave on full power 13.5 minutes
- Add slides and lid and microwave for 2 mins to bring up pressure
- Microwave for 5mins under pressure
- Leave to cool for 40mins

Staining

- Treat with 3% H_2O_2 (40mls H_2O_2 + 360mls dH2O) for 10 mins on a stirrer
- Rinse in running water
- Ring sections with Dako pen to create a barrier
- Incubate in blocking solution: 5% Horse serum 50ul/ml TBS for 20mins at 25^0C
- Blot serum from sections
- Incubate in primary antibody o/n at 4^0C (1:100)
- Wash 2 x TBS for 5mins
- Incubate in Envision for 30mins at room temperature
- Wash 2 x TBS for 5mins
- Incubate in DAB substrate until colour develops 2-10mins
- Wash in running water for 10 minutes

Counterstain

- Haematoxylin for 45 secs
- Rinse in running water
- Blue with Scotts tap water substitute 45 secs
- Rinse in running water

Dehydrate and mount

- 70% alcohol 1 min
- 90% alcohol 1 min
- 100% alcohol 2 x 1 min
- Xylene 2 x 1 min
- Mount in DPX

APPENDIX 3

Immunohistochemistry protocol using CD45 (Dako, code M0724)
Performed in Beatson Oncology laboratory, Glasgow

	Time
Place sections in Xylene	5 minutes
Place sections in Ethanol	1 minute
Place sections in Ethanol	1 minute
Place sections in 70% Ethanol	1 minute
Wash sections in running tap water	
Heat Induced Epitope retrieval – pH 6 Na Citrate buffer	
2 minutes at full pressure in 800W microwave *	
Leave sections in buffer to cool	20 minutes
Wash sections in 10mM Tris buffered Tween (TbT) pH7.5	5 minutes(x2)
Block endogenous Peroxidase (Dako EnVision)	5 minutes
Wash sections in TbT	5 minutes(x2)
Apply Vector Labs CD 3 (1/100 @ room temperature)	45 minutes
Wash sections in TbT	5 minutes(x2)
Apply secondary antibody (Dako EnVision)	40 minutes
Wash sections in TbT	5 minutes(x2)
Apply 3,3'-Diaminobenzidine tetrahydrochloride	10 minutes
Terminate reaction with deionised water	1 minute
Gills haematoxylin	5 minutes
Wash sections in deionised water	30 seconds
1% Acid alcohol	2 dips
Wash sections in deionised water	30 seconds
Scotts tap water substitute	1 minute
Wash sections in deionised water	30 seconds
Dehydrate, clear and mount	

Materials Used

Reagent	**Company**	**Code**
CD45 Mouse Monoclonal	Dako	M0724
Primary antibody diluent	Dako	S2022
Retrieval buffer 10mM ph 6	Thermo	S2369
EnVision Rabbit Kit	Dako	K4011
Tris buffered tween (TbT)	Dako	S3306

*Other heat retrieval methods such as water baths, automated retrieval (retrieval temperature should be set at 98 ºC) modules are also suitable but should be optimised for the technique before proceeding.

APPENDIX 4

Immunohistochemistry protocol using FOXP3$^+$ (Abcam, code 20034)
Performed in Western Infirmary Laboratory, Glasgow

Dewax and Rehydrate

- Dewax in Xylene 2 x 3 mins
- Rehydrate in 100% alcohol 2 x 2 mins
- 90% alcohol 2 mins
- 70% alcohol 2 mins
- Rinse in water

Antigen Retrieval

- Make Tris EDTA buffer pH8
  - 0.55g sodium EDTA
  - 0.825g Tris
  - Dissolve in 1.5 litres of dH_20 in pressure cooker
- Microwave on full power 13.5 minutes
- Add slides and lid and microwave for 2 mins to bring up pressure
- Microwave for 5mins under pressure
- Leave to cool for 40mins

Staining

- Treat with 3% H_2O_2 (40mls H_2O_2 + 360mls dH2O) for 10 mins on a stirrer
- Rinse in running water
- Ring sections with Dako pen to create a barrier
- Incubate in blocking solution: 5% Horse serum 50ul/ml TBS for 20mins at 25^0C
- Blot serum from sections
- Incubate in primary antibody o/n at 4^0C (1:200)
- Wash 2 x TBS for 5mins
- Incubate in Envision for 30mins at room temperature
- Wash 2 x TBS for 5mins
- Incubate in DAB substrate until colour develops 2-10mins
- Wash in running water for 10 minutes

Counterstain

- Haematoxylin for 45 secs
- Rinse in running water
- Blue with Scotts tap water substitute 45 secs
- Rinse in running water

Dehydrate and mount

- 70% alcohol 1 min
- 90% alcohol 1 min
- 100% alcohol 2 x 1 min
- Xylene 2 x 1 min
- Mount in DPX