AN INVESTIGATION INTO THE RELATIONSHIPS BETWEEN HOST AND TUMOUR RELATED FACTORS AND THEIR INFLUENCE ON SURVIVAL IN PATIENTS WITH COLORECTAL CANCER

BY

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ABSTRACT

Colorectal cancer is the second commonest cause of cancer death in the western world. In addition to tumour factors such as depth of invasion, lymph node involvement and venous invasion it is increasingly recognised that host factors, are important determinants of survival. In particular the host local and systemic inflammatory responses are stage independent predictors of survival in operable disease. The present thesis further examines the prognostic importance of host and tumour factors in colorectal cancer, specifically:

1. An examination of the prognostic importance of venous invasion (detected using elastica stains) in colorectal cancer.
2. Detailed analysis of the determinants (including age, comorbidity and deprivation) of the systemic inflammatory response and their relationship with survival.
3. The application and validation of a prognostic score providing a measure of the local inflammatory response in colorectal cancer.
4. Detailed analysis of the determinants (including all white cells, lymphocytes and macrophages) of the local inflammatory response and their relationship with survival.
5. The inter-relationships between the local and systemic inflammatory responses in colorectal cancer specifically: early stage disease (node negative) and in patients receiving adjuvant chemotherapy.
6. Mediators (including immunological parameters and vitamin antioxidants) of the local and systemic inflammatory responses and their relationship with survival.

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DECLARATION

The work presented in this thesis was undertaken during a period of research between 2007 and 2010 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 West of Scotland.

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

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DEDICATION

To my wife, who has provided never-ending help, support and patience during the period of research and the writing of this thesis.

SUMMARY

Colorectal cancer is the second commonest cause of cancer death in the western world. Even with modern day treatments, overall survival is still poor; approximately half of those undergoing curative resection survive 5 years. Currently prediction of outcome and consequent allocation of adjuvant treatment is mainly determined by clinico-pathological criteria. These criteria are mainly high-risk tumour characteristics including extent of local spread and nodal status, which form the basis of the Dukes' and TNM staging systems, in addition to other characteristics such as venous invasion and serosal involvement.

However it is increasingly apparent that a number of host-related factors influence cancer outcome. In particular, a host systemic inflammatory response is a poor prognostic factor. The Glasgow Prognostic Score (mGPS) (composed of C-reactive protein and albumin measures), as a measure of the systemic inflammatory response has been obseved to strongly predict cancer outcome in a variety of cohorts.

It is also increasingly recognized that the presence of a host local inflammatory response around the colorectal tumour is predictive of improved cancer outcomes. A variety of different measures of local tumour inflammation have been proposed but most have largely failed to gain widespread acceptance due to lack of inter-observer reproducibility. The Klintrup-Makinen score of local tumour inflammation has recently been developed as a simple prognostic measure in colorectal cancer scoring all white cell types at the invasive margin of the tumour. However, this score has not yet been validated in another cohort.

The present thesis seeks to explore the relationships between tumour and host characteristics and their influence on survival in colorectal cancer. In particular the relationships between the mGPS and tumour characteristics will be explored. In addition a detailed analysis of the inter-relationships between the local and the systemic inflammatory responses will be performed.

Chapter 3.0 examines the prognostic value of improved detection of venous invasion in colorectal cancer when elastica staining is used routinely. For the first time, it was

demonstrated that increasing the detection rate of venous invasion from 18 to 58% was associated with an improved predictive value of this important high-risk tumour characteristic. This was observed in two consecutive cohorts in addition to a single cohort in which both traditional H&E staining and elastica H&E stainined were applied.

In chapter 4.0 the prognostic value of the mGPS is compared with other proposed measures of the systemic inflammatory response including the neutrophil:lymphocyte ratio and platelet:lymphocytes ratio. The mGPS was observed to be the strongest predictor of survival in comparison to these other scores.

Chapters 5.0 and 6.0 examined the relationships between the systemic inflammatory response (as evidenced by elevated mGPS) and tumour characteristics in colorectal cancer. The mGPS was independently prognostic for cancer specific survival when compared with high-risk 'tumour based' scores including the lymph node ratio and the Petersen Index. Furthermore the combination of these host and tumour based scores could be used to further stratify patients at risk of cancer recurrence and death.

In Chapter 7.0 an evaluation of potential host characteristics that may drive the presence of systemic inflammatory response was performed. The relationships between the mGPS and age, comorbidity, body mass index, smoking status and deprvation were explored. The mGPS was only weakly associated with comorbid disease and as a result it was concluded burden comorbidity does not explain the relationship between the mGPS and survival. The mGPS, in addition to age and comorbidity were all independent prognostic host factors in colorectal cancer.

In Chapter 8.0 the Klintrup-Makinen measure of local tumour inflammation was validated as an important prognostic measure in colorectal cancer for the first time. This observation was repeated in a cohort of low-risk node-negative patients. In Chapter 9.0 the inter-relationships between local and systemic inflammation were examined. Both the local (Klintrup-Makinen Score) and the systemic (mGPS) inflammatory responses were independent prognostic factors. Using these inflammation based criteria provides further stratification for risk of cancer death within Stage II and III disease.

In Chapters 10.0 and 11.0 the inter-relationships between local and systemic inflammation and firstly circulating immune factors (cytokines, immunoglobulins, differential white cells etc.) and secondly local tumour immune factors (intra-tumoural CD4+, CD8+, CD68+ and Ki67 index etc.) were explored. Low intratumoural CD4+ counts were related to high grade systemic inflammation and low grade local tumour inflammation highlighting the potential pivotal role of T helper cells in directing tumour-host interactions. It was also apparent that the presence of systemic inflammatory response was associated with increasing T stage, whilst the local inflammatory response was related to low T stage. It was hypothesized that the local response may degrade as the tumour grows with the subsequent development of systemic inflammation perhaps reflecting host-tumour immune incompetence. The mGPS and Klintrup-Makinen scores were still the strongest measure of each response in predicting survival.

Chapter 12.0 evaluated the relationships between a range of fat-soluble vitaims (vitamin anti-oxidants, Vitamin D) in addition to meaures of lipid peroxidation and the local and systemic inflammatory responses. Low levels of several vitamin anti-oxidants including vitamin D were associated with high-grade systemic inflammation. The carotenoid lycopene was related to low-grade local inflammation and high-grade systemic inflammation.

Chapter 13.0 examined the progostic value of host and tumour factors in a cohort of colon cancer patients who received adjuvant chemotherapy. Traditional measures of prognosis in these patient included in the Adjuvant! and Numeracy calculators were not observed to have strong prognostic value. The mGPS as a measure of systemic inflammation was the only independent prognostic factor. It is hypothesized that this observation may be explained by alterations in hepatic drug metabolism in the presence of high-grade systemic inflammation.

1.0 INTRODUCTION

1.1 EPIDEMIOLOGY OF COLORECTAL CANCER

Colorectal cancer is one of the most common types of cancer. Worldwide it is estimated that 1.23 million cases of colorectal cancer were diagnosed in 2008 (1). Colorectal cancer is the third commonest cancer in men with 663,000 cases and second commonest cancer in women with 570,000 cases. The disease is most common in developed countries in particular North America, Western Europe, Japan and Australia (1). The lowest rates are seen in Africa and South-Central Asia. Worldwide, the disease accounts for an estimated 608,000 deaths, 8% of all cancer deaths making it the fourth commonest cause of cancer death (1).

In the United Kingdom, colorectal cancer is the third commonest cancer following breast and lung cancer. After lung cancer, it is the second commonest cause of cancer death. In 2007, 38,608 diagnoses of colorectal cancer were made (2). This comprised 24,274 colon cancers and 14,334 rectal cancers. Of the total number of cases, 17,143 were males and 14,375 were females. The number of colon cancers is similar across both sexes, however rectal cancer is commoner in men. There is a slight geographical variation in incidence across the UK and Ireland. The highest rates are seen in Scotland and Ireland and the lowest rates are seen in London and Eastern England. The crude rate per 100,000 persons for colorectal cancer across the entire United Kingdom is 63.3, compared with Scotland's incidence of 71.8 (2, 3). While the disease is more common in urbanized areas, in comparison to other common cancer types (lung, head and neck and oesophago-gastric) there is little variation between the least deprived and most deprived areas (4, 5).

Colorectal cancer is a disease of old age, with 84% of cases occurring in those over 60 years old. Under the age of 50 years old the incidence is similar in both males and females. Between 50 and 80 years colorectal cancer incidence is higher in men and beyond 80 years the highest incidence is seen in females due to the larger population at risk (2).

In the United Kingdom, the trends for colorectal cancer incidence in males and females differ. Male colorectal cancer rates increased by 1% per year between 1979-1999, and have decreased slightly since. Female rates have remained unchanged over

this time(2). In contrast to trends in incidence, colorectal cancer mortality has fallen steadily across all age groups and sexes since the early 1990s. In the 10 years up to 2008, mortality fell by 13%. The largest improvements were in the 40-54 year age groupings for men and women between 55 and 79. In the United Kingdom in 2008, 16,259 patients died from colorectal cancer (8,758 men and 7,501 women).

1.2 AETIOLOGY OF COLORECTAL CANCER

Colorectal cancer is thought to develop gradually over a period of time through the sequential accumulation of genetic alterations. The majority of cases (80%) are sporadic, with genetic and environmental factors playing an important role. Approximately 20% of patients with colorectal cancer will have a family history of cancer in a first-degree relative, however an identified inherited genetic alteration is present in only 5% of cases (6, 7).

1.2.1 Genetic Factors

At present, there are thought to be two major pathways in colorectal carcinogenesis. One pathway occurs due to chromosomal instability and allelic losses (the adenoma-carcinoma sequence) resulting in sporadic colorectal cancer. Carcinogenesis occurs due accumulation of multiple genetic alterations over time due to environmental and lifestyle factors. The second occurs in 15-20% of all colorectal cancers and involves microsatellite instability**.**

1.2.1.1 Inherited syndromes

Inherited syndromes in which colorectal cancer risk is increased are categorized based on the presence of high numbers of adenomatous polyps, low numbers of adenomatous polyps or presence of hamartomatous polyps. These include adenomatous polyposis syndromes (Familial adenomatous polyposis and MYH-associated polyposis), non-polyposis syndrome (Hereditary non-polyposis colorectal cancer (HNPCC)) and hamartomatous polyposis syndromes (Peutz Jeghers Syndrome, Juvenile polyposis, Cowden Disease) (7).

1.2.1.2 Hereditary non-polyposis colorectal cancer (HNPCC) and Microsatellite Instability

HNPCC (or Lynch syndrome) is the most common form of familial colorectal cancer accounting for at least 50% of cases or hereditary disease and 2-4% of all colorectal cancer cases (7, 8). HNPCC is an autosomal dominant syndrome and whilst it is characterized by early onset of colorectal cancer, the median age of diagnosis with HNPCC is 60 years (9). Lifetime risk of developing cancer varies between individuals but overall lifetime risk for colorectal cancer is 50-60% (6). Apart from an earlier age of diagnosis, HNPCC cancers have a right sided predominance, higher frequency of

synchronous and metachronous disease (10) and are associated with extra colonic malignancies (see below).

HNPCC tumours display certain clinicopathological features in comparison to sporadic colorectal cancer. This stems from a separate molecular phenotype known as microsatellite instability due to inadequacies in DNA mismatch repair. Colorectal cancers in HNPCC tend to arise in the proximal colon, are poorly differentiated and are associated with increased frequency of local inflammatory reaction around the tumour termed 'Crohns like reaction' alongside an abundance of tumour infiltrating lymphocytes (11-14).

Microsatellites are repetitive sequences of DNA randomly distributed throughout the genome. Microsatellite instability is caused by mutations in the genes that are involved in DNA repair (mismatch repair genes). This leads to base-pair mismatches during DNA replication. To grade microsatellite instability, five standardized and validated microsatellites are assessed (D2S123, D5S346, D17S250, BAT-25 and BAT-26). Tumours are graded high frequency microsatellite instability (MSI-H) if two or more markers are unstable, and low frequency microsatellite instability (MSI-L) if one is unstable (15).

To date mutations in four mismatch repair genes MLH1, MLH2, MLH 6 and PMS2 can give rise to HNPCC and the MSI-H phenotype (16, 17). Most HNPCC cancers arise from MLH1 and MLH2 mutations (7). It is thought predisposed individuals have one inactivated mismatch repair gene and the second copy is lost as a somatic mutation. Of note, 10-15% of sporadic cancers are also classed MSI-H. However rather than high frequency microsatellite instability developing due to mutations, as seen in HNPCC, in sporadic MSI-H colorectal cancer an epigenetic phenomenon leads to mismatch repair deficiency. In most cases the MLH-1 gene has been silenced by hypermethylation of the MLH1 genes promoter region (18).

HNPCC is usually diagnosed after a thorough family history or by demonstration of pathogenic genetic mutations. To aid identification of at risk individuals, HNPCC was defined by the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC) in 1991 with the Amsterdam criteria (19). The

Amsterdam II criteria are the updated criteria including extracolonic malignancies as well as colorectal cancer (20):

The Amsterdam II criteria

1. ≥3 relatives with HNPCC associated cancer (colorectal, endometrial, stomach, ovary, ureter or renal pelvis, brain, small bowel, hepatobiliary, sebaceous tumours).
2. One affected individual is a first degree relative of two other relatives.
3. Two or more generations affected.
4. One of more of the cancers was diagnosed before 50 years of age.
5. FAP has been excluded.
6. Tumours should be verified by pathological examination.

As the causative genetic mutations in mismatch repair genes are well described (17, 21), fulfillment of the Amsterdam criteria is not required to diagnose HNPCC. With genetic testing widely available, the utilization of guidelines (ICG-HNPCC Revised Bethesda Guidelines 2004)(22), which aim to detect germline mutation carriers, can identify up to 80% of individuals (23).

The Revised Bethesda Guidelines

1. Colorectal cancer in a patient <50yrs of age
2. Synchronous, metachronous colorectal or other HNPCC associated tumours (as outlined in the Amsterdam criteria) regardless of age.
3. Colorectal cancer with the MSI-H histology in a patient <60yrs of age.
4. Colorectal cancer diagnosed in one or more first degree relatives with an HNPCC-related tumour, with one cancer diagnosed <50yrs.
5. Colorectal cancer diagnosed in two or more first or second degree relatives with HNPCC-related tumours regardless of age.

1.2.1.3 Familial Adenomatous Polyposis (FAP)

FAP accounts for less that 1% of all colorectal cancers and is associated with the development of hundreds to thousands of polyps (7). It is an autosomal dominant syndrome associated with duodenal polyps and other extracolonic manifestations including osteomas and dermoid cysts. Extra colonic tumours are important as periampullary cancers and desmoid tumours are the leading cause of death after

prophylactic colectomy (24). The underlying abnormality is a germline mutation of the APC tumour suppressor gene on chromosome 5q (25).

A second, less severe, predisposition to colorectal polyposis and carcinogenisis is associated with mutations in the MYH gene (MYH associated polyposis) (7).

1.2.1.4 Hamartomatous Polyposis Syndromes

These rare syndromes include Peutz Jeghers Syndrome (PJS), juvenile polyposis syndrome (JPS) and Cowden disease. PJS is autosomal dominant, characterized by peri-oral pigmentation and Peutz-Jeghers type hamartomatous polyps throughout the gastrointestinal tract. (6). 50% are due to underlying autosomal dominant mutations in the STK11 gene (7). The lifetime risk for colorectal cancer is 30%.

JPS is an autosomal dominant condition characterized in 50% by mutations in either of the SMAD4 and BMPR1A genes (7). In JPS colonic polyps develop from an early age. The lifetime risk for colorectal cancer is 60%.

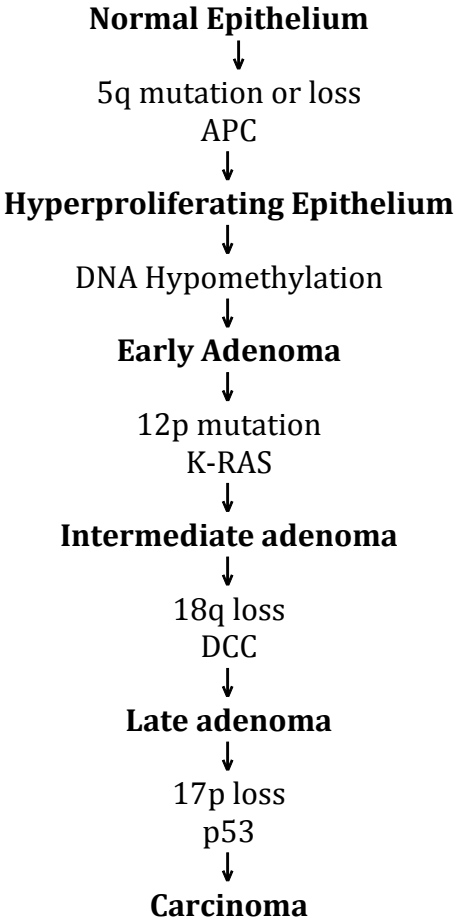
Cowden Disease is autosomal dominant characterized by germline mutations of the PTEN gene in most cases. It is characterised by facial trichelmmoma, oral papillomas, multinodular goitre and intestinal hamartonas (6, 7). Colon cancer develops in up to 10%.

1.2.1.5 Sporadic Colorectal Cancer and the adenoma-carcinoma sequence

Based on histological and epidemiological studies in the 1970s, it was recognized that the majority of colorectal carcinomas were likely to originate from pre-malignant adenomatous polyps. For example, the distribution of polyps is similar to that of carcinomas, co-existent adenomata are commonly located in the vicinity of a carcinoma, large adenomata often contain areas of carcinoma and the presence of large adenomas increases risk of subsequent carcinoma development (26, 27). Also the geographical prevalence of adenomas is similar to carcinomas (28) and age distribution curves for adenomatous disease peak at around 5-10 years prior to the peak for carcinomas suggesting that malignant transformation within adenomata may occur in a similar time frame (29).

Fearon and Vogelstein proposed the original multistep model for colorectal carcinogenesis in 1988 (30). The accumulation of series of genetic alterations leads to transformation from benign adenomatous polyps to malignant disease. In sporadic

colorectal cancer, such genetic changes are likely to occur due to environmental and lifestyle factors. The sequence of progression involves somatic mutations in proto-oncogenes and tumour suppressor genes. Initial inactivation of the adenomatous polyposis coli (APC) gene leads to adenoma development from normal mucosa. APC mutations are found in 60% of adenomas and carcinomas (Powel 1992). Subsequent mutations in K-ras and genetic alterations to genes on 18q resulted in growth and progression of the adenoma and finally adenoma carcinoma transition was mediated through the biallelic loss or inactivation of p53 gene. It is appreciated that chromosomal instability and loss of heterozygosity plays an important role in the whole process. The model proposed initially over 20 years ago is now thought only to apply only in a proportion of spontaneous colorectal cancers. In fact the carcinogenesis in sporadic colorectal cancer is far more complex process with multiple mutations in oncogenes (including myc, ras, src, erB2) and tumour suppressor genes (including p53, DCC, APC) (31, 32). It is emphasized that mutation profiles vary considerably between colorectal tumours.



Adenoma carcinoma sequence, adapted from Fearon and Vogelstein 1990 (33)

1.2.1.6 Hyperplastic polyps and the serrated adenoma pathway

Recently the view that all colorectal adenocarcinomas develop from conventional adenomas or through the HNPCC–mutator pathway has been challenged by the recognized hyperplastic polyp-serrated adenoma-serrated adenocarcinoma pathway (34-37). Precursor lesions are hyperplastic, serrated polyps that include hyperplastic aberrant crypt foci, conventional hyperplastic polyps, serrated adenomas and sessile serrated adenomas (37, 38). It is the sessile serrated adenoma in particular which is thought to lead to serrated adenocarinoma. This pathway is thought to account for 7.5% of colorectal cancers (37). Serrated adenocarcinomas have are characterized by prominent mucin, abundant eosinophilic cytoplasm, absence of necrosis, early BRAF mutations, excess CpG island methylation with silencing of mismatch repair genes and resultant higher rates of microsatellite instability (36, 37).

1.2.2 Environmental and Host/ Lifestyle Factors

Given 80% of colorectal cancers arise through the accumulation of sporadic mutations, environmental and host/lifestyle factors are particularly important in colorectal cancer development and progression. The aetiological role of lifestyle and environment is highlighted by the fact there is a 15-fold difference in age standardized incidence rates between different geographical regions of the world (39). Furthermore, migrants from low incidence regions who move to high incidence regions adopt the risk of the higher risk area within a generation (40). The disease is predominantly seen in Westernised countries indicating Western lifestyles harbor certain risk factors for its development (41).

1.2.2.1 The role of dietary factors in colorectal cancer development

Fibre

Burkitt first described a link between colorectal cancer and low intake of dietary fibre in 1971. This was based on the low incidence the disease in an African population with a high fibre intake. Burkitt suggested fibre depletion and resultant colonic stasis encourages concentration of carcinogens within the bowel prolonging mucosal contact. The hypothesis was supported by epidemiological studies in the 1970s and 1980s (42-44), although the correlation was less strong when adjusting for red meat and fat intake. Since then, the role of dietary fibre has been debated with confounding results reported by several large prospective studies (45-47). Two studies with large cohorts of over 85,000, and over 10 years of follow up; one from the USA and a second from Japan failed to demonstrate any protective effect from dietary fibre in preventing colorectal cancer (46, 47). However in the largest study to date examining diet and cancer risk, the European prospective Investigation of Cancer and Nutrition (48), in a cohort of 519, 978 individuals, a 40% reduction in colorectal cancer risk was observed between the highest and lowest quintiles of fibre intake. In a meta-analysis of 725,628 participants in 13 studies, an inverse relationship between high fibre diets and development of colorectal cancer was reported, but this was not apparent after accounting for other dietary factors (49). Finally, interventional studies in which dietary fibre is increased to prevent colorectal polyp recurrence after polypectomy have failed to detect any differences in

recurrence rates between treatment and control groups (50-52). Therefore, the role of dietary fibre in colorectal cancer development is not fully understood.

Red Meat and Animal Fat

Colorectal cancer risk is increased by the high consumption of red meat or processed meat. A recent meta-analysis of 15 studies examining the association between red meat and colorectal cancer found positive associations in all fifteen (53). The association between rectal cancer and red meat consumption was strongest. Cooking red meat at high temperatures may result in the formation of heterocyclic amines or polycyclic hydrocarbons which are potential carcinogens for colorectal cancer (54). Alternatively, haem iron present in red meat may be the aetiological factor. Haem iron is thought to increase cell proliferation and incidence of aberrant crypt foci in colonic mucosa. It also increases faecal concentrations of carcinogens such as N-nitroso compounds (55, 56).

In the 1980s several epidemiological studies reported associations between high intakes of animal fat and colorectal cancer (57, 58). In 1990, Willett et al. reported in a large cohort of almost 90,000 females that high animal fat intake was associated with colorectal cancer risk (59). Since then, several reports have failed to distinguish animal fat as an independent risk factor from total energy intake (60-62). A report from the American Institute for Cancer Research in 2007 concluded that there is a limited-suggestive association of increased colorectal cancer risk with high animal fat foods (63).

Other dietary constituents

Higher intakes of fruit and vegetables (64, 65) are thought to protect against colorectal cancer. Carotenoids (α and β-carotene, lycopene and lutein) and vitamin anti-oxidants (vitamins A, C and E) are present in high levels in these foods. La Vecchia et al. conduced a case control study with 1953 colorectal cancer patients and 4154 controls (66). They reported a protective effect with Vitamin C and carotenes. When combined with high calcium and Vitamin D intake, risk of colorectal cancer was further reduced. Two studies by Slattery and Nkondjock reported that the carotenoid, lutein was of particular importance in reducing risk. Murtaugh et al. reported high vitamin E and lycopene were protective (67-70). However the literature is inconsistent for carotenoids and anti-oxidants. A Canadian study of

56,837 women did not identify any protective effects from these agents (69). More recently, a pooled analysis of 11 cohort studies reported that carotenoids did not alter colorectal cancer risk (71).

Other dietary constituents linked to increased colorectal cancer risk include low intakes of calcium, phosphorus and vitamin D (72-74). In a large prospective study of US women, high vitamin D and calcium intake was related to reduced colorectal polyp incidence (74). Potential protective effects include the action of calcium in binding bile and fatty acids within the bowel lumen. Calcium and vitamin D are also thought to increase apoptosis and inhibit proliferation of colonic epithelium (74).

In comparison to red meats, Willett and colleagues reported diets with a higher intake of fish were observed to confer a reduced risk of colorectal cancer (59). The protective effect from diets high in fish has been attributed to increased consumption of fatty acids (e.g. omega-3 fatty acid) found in high concentrations in fish oils (75, 76). These fish oils are thought to have anti-inflammatory activity, inhibiting generation of cyclo-oxygenase-2 mediated pro-inflammatory cytokines, as well as anti-carcinogenic effects by reducing cell proliferation (77).

High intakes of refined carbohydrates (e.g. sucrose) may increase the risk of colorectal cancer, however high sugar intake is closely associated with other dietary and lifestyle factors that modulate colorectal cancer risk (60, 78). Studies by Slattery (1997) and Levi (2002) reported colorectal cancer risk was increased by diets with a high glycaemic index or glycaemic load (79-81).

Smoking

Cigarette smoke contains a variety of recognized carcinogens including polycyclic aromatic hydrocarbons, nitrosamines and aromatic amines. Colonic mucosa is exposed to these carcinogens through direct ingestion and via the circulation. A heavy smoking history has been associated with increased risk of colorectal cancer in a number of studies (82-84).

Alcohol

Heavy alcohol intake is also associated with increased risk of colorectal cancer at an earlier age (85-87). Metabolites of alcohol (e.g. acetaldehyde) may be carcinogenic and may generate free radical oxygen species (88). Furthermore, heavy intake is associated with a chronic systemic inflammatory response this may influence cancer

risk (see below) (89). Individuals with high alcohol intake are likely to have poorer dietary intake of protective nutrients and fibre (63). Colorectal cancer was recently classed 'an alcohol-related malignancy' by the International Agency for Research on Cancer (90).

1.2.2.2 The role of host factors in colorectal cancer development

Age

Old age is a risk factor for colorectal cancer. 80% of cases occur in patients aged over 60 (2). Increasing age allows greater exposure to risk factors and an increased time to accumulate genetic mutations. Old age is associated with higher levels of telomere attrition (91) and age related DNA methylation changes (92) which may predispose to genetic alterations.

The metabolic syndrome, insulin resistance and obesity

The metabolic syndrome is a combination of medical conditions recognized to increase risk of benign conditions including cardiovascular disease and diabetes. It is composed of obesity, insulin resistance, hyperglycaemia, hypertension and dyslipidaemia (93). Several epidemiological studies have reported associations between the metabolic syndrome and an increased risk of colorectal cancer (94-96). It is not clear whether one of the factors that comprise the metabolic syndrome is of particular importance compared with an accumulation of factors. Of the components of the metabolic syndrome assessed, Sturmer reported in a cohort of 22,071 men, of which 494 developed colorectal cancers, that obesity and diabetes were important risk factors (94). This risk is increased in diabetic men (97, 98). Diabetes is also associated with shorter cancer survival in treated colorectal cancer (99, 100). Insulin resistance results in high levels of circulating insulin, which stimulates insulin like growth factor (IGF-1). Insulin can act as a growth factor for colorectal mucosal and carcinoma cell lines and IGF-1 may act as a carcinogen on colonic mucosa and inhibit apoptosis (98, 101, 102).

Obesity and high energy intake is a risk factor for colorectal cancer (60, 103). From a recent meta-analysis the pooled estimate from 26 studies indicated a body mass index $\geq$30kg/m^2 confers a 40% increased risk of developing colorectal cancer (104). The risk is higher in men and central obesity is associated with highest risk. Mechanisms are poorly understood but it is suggested adipocytes produce pro-

inflammatory cytokines resulting in a chronic systemic inflammatory response predisposing to cancer (105). Alternatively, leptin produced by adipocytes may promote carcinogenesis (106). Obesity is also associated with several other confounding factors including comorbidity, insulin resistance, sedentary lifestyle, high fat and poor dietary habits.

Cardiovascular Disease

In recent years, several studies have demonstrated associations between coronary artery disease and the diagnosis of colorectal adenoma and carcinoma (107-109). It is suggested that heart disease and colorectal cancer share similar risk factors including diabetes, smoking, hyperlipidaemia, sedentary lifestyle and a high fat, low fibre diet (107). Furthermore the role of systemic inflammatory response is an important determinant of outcome in both conditions (see below). Chan et al reported colorectal neoplasms were present in 34% of patients with coronary artery disease who underwent colonoscopy following diagnosis of heart disease (n=206). This compared with 21% in the control group (107).

Sedentary lifestyle

Increasing physical activity and avoidance of a sedentary lifestyle has been associated with reduction in colorectal cancer risk (110-114). In the most physically active individuals, reported reduction in risk is between 20-70% (115). This effect is reported to be independent of diet, obesity and other confounding factors. Suggested mechanisms include modification in endogenous sex hormones and growth factors, enhanced immune function and reduction in body fat content. Exercise also lowers insulin, glucose and triglycerides and raises HDL cholesterol, which may contribute to the risk modification.

Endogenous sex hormones

Colorectal cancer risk is reduced in women who have been prescribed hormone replacement therapy (HRT) (116). The Women's Health Initiative (WHI) trial randomized over 16,000 patients to either HRT or placebo. A 37% lower risk of colorectal cancer was observed in patients who received exogenous oestrogen and progestin (117). It is hypothesized that exogenous hormones may reduce heptatic

synthesis of harmful proteins (insulin, IGF-1, low density lipoprotein) in addition to possible anti-proliferative effects on colonic mucosal cells (118).

Inflammatory Bowel Disease

Longstanding colonic inflammation is associated with increased risk of colorectal cancer. Inflammatory bowel disease (Crohn's disease and Ulcerative colitis) sufferers may have a five fold risk compared with age matched controls (119). The risk is associated with extent and duration of inflammation. Colorectal cancer is reported to account for 10-15% of deaths in patients with inflammatory bowel disease (120). The age at diagnosis is earlier than for sporadic colorectal cancer. In a meta-analysis by Eaden et al., the mean age of diagnosis was 43 years (121). In addition to extent and duration of inflammation from a young age, risk factors for cancer include family history of sporadic colorectal cancer and the presence of primary sclerosing cholangitis. The risk in ulcerative colitis is estimated to be 2% after 10years, 8% after 20 years and 18% after 30 years with the disease (121, 122). If colonic inflammation is present in Crohn's disease, the risks and are reported to be similar to ulcerative colitis (123). Chronic inflammation is thought to drive carcinogenesis in IBD, and similar molecular pathways to sporadic colorectal cancer are described, although the timing and frequency of genetic alterations vary (122).

Systemic Inflammatory Response

Colorectal cancer risk is increased by the presence of a chronic low-grade systemic inflammatory response (124, 125). In a large nested case control study of 22,887 patients followed for 11 years, higher baseline C-reactive protein concentrations were associated with subsequent cancer development (124). The strongest association was for colonic cancer. Similar findings were reported in another large study from Finland. In the same study, elevated C-reactive protein was associated with increasing body weight, smoking, lower omega-3 fatty acids levels, lower serum carotenoids and a history of coronary heart disease (125). In addition to increased cancer risk, a low-grade systemic inflammatory response has also been associated with development and progression of a range of benign conditions including obesity, diabetes, the metabolic syndrome, hypertension, atheroma formation and prediction of coronary events (126-134). Inflammation is thought to promote carcinogenesis. From laboratory-based studies, pro-inflammatory cytokines are reported to damage

DNA as well as stimulating cell proliferation and angiogenisis and inhibiting apoptosis (135, 136). Consistent with such a hypothesis is the fact that anti-inflammatory drugs modulate colorectal cancer risk (see below).

Non-steroidal anti-inflammatory drugs and Aspirin

There is good evidence that the use of non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin is associated with a reduced risk of colorectal adenoma and cancer development (137-141). Over 30 years ago, increased prostaglandin concentrations were observed in colorectal tumours in comparison with normal colonic mucosa (142). NSAIDs and aspirin reduced prostaglandin synthesis and over the following decade, several studies demonstrated the efficacy of these drugs including aspirin, sulindac and indomethacin in reducing colorectal adenoma/adenocarcinoma formation in animals and humans (143-147). Interventional studies followed in the 1990s and 2000s. One systematic review identified 9 randomised controlled trials in which sulindac, aspirin or celecoxib were studied. From the combined results, these drugs reduced polyp recurrence up to three years after polypectomy (HR 0.77). In FAP patients, a proportional reduction in the number of adenomas of 12-44% was observed (148).

The mechanisms underlying the reduction in colorectal cancer risk are unclear. NSAIDs and Aspirin act on the cyclo-oxygenase (COX) enzymes. These enzymes catalyze the reaction that produces prostaglandins from arachidonic acid. Prostaglandins are important mediators of inflammatory responses. There are two isomers of cyco-oxygenase, COX-1 and COX-2. COX-1 is expressed in most tissues and mediates the physiological effects of prostaglandins. COX-2 is not present in most tissues but is elevated in colorectal adenomas and cancers (149). Risk modification may occur due to modulation of the systemic inflammatory response. In addition, COX-2 may contribute to cancer development through influences on apoptosis, attachment, invasion and angiogenesis (150, 151). Three large trials examined chemoprevention with COX-2 inhibitors (152-154). Unfortunately all three closed early due to concerns regarding cardiovascular events in the groups receiving the active medication in two studies. Despite early closure, all three reported reductions in adenoma recurrence.

In summary, good evidence exists confirming the protective effects of these drugs in reducing colorectal adenoma/carcinoma risk. However, controversy still exists as to the chemopreventitive role of NSAIDs and aspirin in colorectal cancer. Concerns regarding side-effect profiles have limited their routine use to date.

1.2.3 Summary – Aetiology of colorectal cancer

Colorectal cancer development is a complex interplay between environmental factors and host factors. Environmental factors are predominantly associated with Westernised lifestyles, including diets low in fibre, vitamin anti-oxidants, carotenoids and micronutrients, diets high in red meat and animal fat, smoking and high alcohol intake. Host factors include genetic alterations that accumulate leading to cancer development, however it is increasingly apparent that accumulation of such mutations is influenced by the individual host's health including age, comorbidity (inflammatory bowel disease, obesity, sedentary lifestyle, cardiovascular disease and the metabolic syndrome) and the presence of a pre-morbid systemic inflammatory response. Of interest, the presence of a systemic inflammatory response has been associated with most of these environmental/ host factors also related to colorectal cancer risk. This observation suggests some of these risk factors may modulate cancer development through a common pathway, which is also associated with the generation of a systemic inflammatory response.

1.3 INVESTIGATION AND MANAGEMENT OF COLORECTAL CANCER

Colorectal cancer presentation varies in accordance with tumour site. Tumours or the sigmoid colon and rectum usually present with a combination of alteration in bowel habit (usually increased frequency of stools) and bleeding per rectum (155). Although rectal bleeding alone without change in bowel habit is associated with a low risk of having cancer, over 60% of patients with colorectal cancer have this sole symptom (156). Tumours proximal to the sigmoid colon are more likely to present as an emergency with obstruction, the presence of an abdominal mass or iron deficiency anaemia (155).

Diagnostic work up and choice of investigations will depend on the method of presentation. In the emergency setting, staging investigations are dependent on clinical urgency and the timing of surgery. In the elective setting, histological diagnosis should be achieved prior to surgery for all rectal cancer and where possible for colonic cancers (ACPGBI guidelines 2007)(157). Disease staging is performed in all cases and neoadjuvant therapies considered where applicable.

Approximately 15% of patients presenting with colorectal cancer will have advanced disease (2). If potentially curative resection is not an option, such patients are managed with palliative treatments. For example, local resection or creation of defunctioning colostomy may be considered for symptom palliation. Alternatively oncological palliation with chemotherapy or radiotherapy may be prescribed at the discretion of the treating oncologist. The text below will focus on the management of elective primary operable disease.

1.3.1 Investigation and Diagnosis of colorectal cancer

1.3.1.1 Flexible Sigmoidoscopy and Colonoscopy

Flexible sigmoidoscopy allows direct visualization of the distal 60cm of bowel enabling diagnosis of up to 80% of colorectal cancers (158). Full bowel preparation is often avoided, as an enema to clear the distal bowel will usually suffice. If colorectal cancer is diagnosed, then full colonic assessment is indicated, in most cases with completion colonoscopy. Synchronous bowel lesions are present in 4-5%.

Colonoscopy is the gold standard examination of the colon and rectum (158). The technique allows full visualization of the rectum and colon, enabling biopsies to be performed. Therapeutic interventions such as the removal of pre-cancerous adenomata can also be performed. Where barium enema or CT colonography is inconclusive, colonoscopy is the most appropriate investigation (159). Complete colonoscopy to the caecum can be performed in up to 90% of cases with a perforation rate of 1 in 1000 (160). The test requires full bowel preparation and patient sedation and is therefore more expensive than sigmoidoscopy (158).

1.3.1.2 Barium Enema

Double contrast barium enema also allows visualization of the whole colon and rectum. This test requires bowel preparation but is cheaper than colonoscopy (158). Other bowel pathology such as diverticular disease can cloud the interpretation of films and as a result it is not unusual for equivocal reports to be issued requiring further colonic assessment (157). As diverticular disease is common in the rectum and sigmoid, flexible sigmoiodoscopy should compliment this investigation. Given that the barium enema is of diagnostic value only, direct visualization to permit biopsy is required where cancer is diagnosed.

1.3.1.3 CT Colonography

CT colonography is a relatively new technique in which helical or multi-slice CT data is used to generate a volumetric display of the entire colon. Compared with colonoscopy, CT colonography is reported to have a similar diagnostic yield for colorectal cancer and has been examined as a potential screening investigation (161). CT colonography has several advantages over other investigations being non-invasive, requiring no sedation and relatively fast (scanning can be completed in a shorter time usually around 5 minutes) (162). Where left colonic tumours have been diagnosed with flexible sigmoidoscopy, CT colonography enables complete colonic assessment in addition to simultaneous staging assessment for extracolonic disease (157).

1.3.1.4 Pre-operative Staging and MRI

Prior to potentially curative surgery, staging to exclude distant disease is performed with focus on the liver and lungs. Chest X-ray or CT scan is performed to exclude

pulmonary metastases. Ultrasound or CT scanning can exclude liver metastases. It is recommended all patients should undergo full staging CT scan of the chest abdomen and pelvis to assess extent of disease prior to surgery. This should be normal practice unless the test will have no bearing on patient management (e.g. patients presenting as an emergency with peritonitis) (157).

Current Royal College of Radiologist guidelines recommend all rectal cancers should undergo high resolution MRI pre-operatively to assess the circumferential resection margin and exclude pelvic lymph node involvement (163). This investigation provides morphological information useful to the surgeon in operative planning as well as staging information used by clinical oncologists in decisions on the provision of neoadjuvant therapies.

1.3.2 Treatment of primary operable colorectal cancer

1.3.2.1 Surgery

Surgery remains the sole curative treatment for colorectal cancer. In the elective setting, where histology and pre-operative staging confirm localised primary operable disease, en bloc resection of colon or rectum and draining lymph nodes is performed as an open procedure or with laparoscopic assistance. The aim of curative resection is to remove all macroscopic disease present with an adequate margin of normal tissue. The nature of the resection is dependent on tumour site and blood supply. Very small T1 tumours may be removed by local excision using snare diathermy at colonoscopy. Segmental resection is performed for colonic tumours. Tumours of the right colon are resected by right hemicolectomy. Tumours of the transverse colon and splenic flexure are removed by extended right hemicolectomy as this is thought to be safer than local segmental resection (157). Tumours of the left colon are removed by left hemicolectomy. Tumours in the lower two thirds of the rectum should undergo total mesorectal excision (TME) either as part of an anterior resection or abdominoperineal resection as the technique significantly reduces local disease recurrence (164). Recently, more radical resections have been advocated for very low rectal tumours. Extralevator abdominoperineal resection is reported to provide an oncologically superior specimen with lower rates of circumferential

margin involvement than conventional AP resection (165). Similarly complete mesocolic excision for colonic tumours is reported to provide an oncologically superior specimen with higher number of lymph nodes harvested and reduced recurence (166).

1.3.2.2 Neoadjuvant chemo-radiotherapy

The aim of pre-operative or neoadjuvant treatment with chemo-radiotherapy is to improve local control of resectable rectal cancer. Radiotherapy can reduce tumour bulk, optimizing the likelihood of margin free resection, therefore reducing local recurrence. Such treatment has an important role in rectal cancer, where the locally advanced tumours are commonly fixed due to the natural confines of the pelvis. A Cochrane review including results from 19 studies reported a significant reduction in local recurrence with pre-operative radiotherapy. This is further optimized with the addition of pre-operative chemotherapy (167). Current guidance states patients with resectable rectal cancer should be considered for neoadjuvant therapy after multidisciplinary team discussion in cases where pre-operative MRI staging suggests stage II or Stage II disease (T3/4 or N1/2). Radiotherapy should be considered post-operatively in patients who have risk factors for recurrence such as venous invasion, tumour at the circumferential margin or mesorectal lymph node involvement (157).

1.3.2.3 Adjuvant Chemotherapy

There is good evidence from meta-analyses of numerous multicentre studies that adjuvant chemotherapy is of proven benefit in improving survival for patients with Stage III (node positive) colon and rectal cancer. Standard regimens are 5-fluorouracil modulated by folinic acid given for up to 6 months following curative resection. Such regimens are associated with a 10-15% improvement in survival in stage III disease (168, 169). Combination therapy with oxaliplatin, irenotecan, cetuximab or bevacizumab and 5-FU based regimens are reported to offer superior survival benefit compared with 5-FU alone in patients fit enough for such treatment (169-173).

The survival benefit from adjuvant chemotherapy in stage II disease is smaller. Some studies including the IMPACT B2 study included 1,116 patients with stage B2 colon cancer report no significant improvement in survival with adjuvant chemotherapy

(174). The UK-based QUASAR 1 study reported only a modest 4% improvement in overall survival. Treatment with single agent chemotherapy was deemed to be cost effective, particularly in the under 70s (175). Given the modest treatment benefits reported, there remains considerable interest in identifying which patients with Stage II disease may benefit the most from adjuvant treatment.

In a systematic review of the literature, The American Society of Clinical Oncology recommended against routine administration of adjuvant chemotherapy in node negative disease (176). Current recommendations are that patients with node positive disease in addition to node negative disease with high-risk features should be considered for adjuvant treatment by an oncologist (157, 176). However, across all Stage II/ III colorectal cancer patients, the absolute number of patients benefiting from treatment is small and adjuvant chemotherapy is not without toxicity. Several web-based prognostic models exist to guide clinicians in prescription of adjuvant therapy, however these models lack prospective validation in direct comparison with other established measures of risk stratification post-surgery (Numeracy and Adjuvant!).

1.4 DISEASE PROGRESSION AND PROGNOSIS IN COLORECTAL CANCER

With an apparently curative resection, survival rates are still poor. Approximately 50%-60% will suffer recurrence and die before 5 years (177, 178). Following diagnosis and resection, it is increasingly recognised that disease progression and survival in colorectal cancer is determined by a wide variety of factors including both tumour and host characteristics. Tumour characteristics include pathological features, molecular markers or mutations associated with early recurrence and poorer cancer specific survival. Host characteristics include age, comorbidity, tumour immune cell infiltrate and the presence of a host systemic inflammatory response.

1.4.1 TUMOUR CHARACTERISTICS AND PROGNOSIS IN COLORECTAL CANCER

1.4.1.1 Dukes' Stage

In 1932, Dukes' published classification of rectal cancer (179). His classification was based on knowledge that local and lymphatic spread were important determinants of outcome. Tumours were classified as those not penetrating the bowel wall without lymph node metastases (A), tumour growth through the bowel wall without lymph node metastases (B) and the presence of lymph node metastases (C). The classification was subsequently modified with the division of the stage C category into C1; involvement of regional lymph nodes close to the rectal wall or C2; extensive involvement of lymph nodes involving nodes at the point of ligature of the blood vessels and was also validated in colon cancer (180). In 1967, Dukes' stage D was used to describe the presence of distant metastases to liver, lung or bone (181). The Dukes' staging system was a useful indicator of survival and in 1958, Dukes' and Bussey reported 5 year survival rates of 81%, 64% and 27% for A, B and C disease respectively (182). The Dukes' system has undergone several modifications based on the observations that outcomes vary within the different staging categories. Kirlin and colleagues recommended stage A is categorised as tumours involving the mucosa only and that stage B is divided into B1, lesions extending into the muscularis propria and B2, lesions penetrating the muscularis propria (180). Astler and Coller introduced the use of C1 for tumours limited to the bowel wall with lymph node involvement and C2 for tumours extending through all layers of the bowel wall with involved nodes (183).

1.4.1.2 TNM stage

The Dukes' staging system has been superseded by the more precise tumour, nodes and metastases (TNM) system. The TNM system was originally described in 1968 and initially included the separation of colonic and rectal cancers (184). This system was not employed widely until in 1977, the International Union against Cancer (UICC) recommended the TNM system in colorectal cancer including a pre-treatment TNM stage and a post-resection pTNM stage (185, 186). TNM staging has similar categories to the Dukes' system but provides a more accurate description of tumour depth and nodal involvement with T stage describing assessment of size and depth of penetration, N stage the extent of nodal involvement and M stage distant metastases. The objectives of the standardised TNM staging system are to aid planning of treatment, indicate likely prognosis, allow the assessment of effects of treatment and to enable help with the exchange of information between different centres (187). The TNM system is revised every few years to ensure up to date evidence is incorporated. The most recent revision is the 6th edition with TNM 7 due to be published in 2009. In the UK, the Royal College of Pathologists currently recommends the 5th edition of TNM stage. This stance is based on an apparent lack of evidence for amendments in the 6th and 7th editions (188, 189). Examples of previous controversial changes to the TNM system for which the evidence base has been disputed include the addition of the N3 category in TNM 4 for lymph node involvement by the presence of tumour along a named artery (subsequently removed in TNM 5); the 3mm rule in TNM 5 stating any mesocolic tumour deposit >3mm in size should be thought of an involved lymph node even if no other lymph node involvement is seen (subsequently removed in TNM 6); new classifications for venous invasion in TNM 6 including V1 category for microscopic venous invasion and V2 for macroscopic venous invasion (189). Furthermore, continual changes to the TNM system do not assist in standardisation of entry criteria to clinical trials, many of which recruit over a 5-10 year period (189). The 5th edition of the TNM system currently used in the UK is shown in Table 1.1. The relationship between TNM stage and Dukes' stage in addition to 5 year survival for the major TNM and Dukes' categories is shown in Table 1.2.

Both Dukes' and TNM staging systems are entirely reliant on adequate surgical resection and skilled pathological processing and assessment. Of paramount

importance is a standardised and accurate pathological dissection and sectioning to enable T and N stage to be assessed. For example, a higher number of paraffin embedded tumour blocks will increase the likelihood of the pathologist detecting submucosal, extracolonic or peritoneal invasion and therefore a greater number of blocks sectioned is likely to result in a higher, more accurate report for T stage (189). Accurate reporting of N stage is of particular importance following tumour resection, given that N1/N2 patients should receive adjuvant chemotherapy in line with current treatment guidelines (173). Assessment of N stage is reliant on adequate number of lymph nodes harvested for assessment (190). The number of lymph nodes harvested depends on a range of factors including patient age and disease stage, in addition to surgical and pathological factors such as site (right sided tumours have higher node yields) and amount of tissue resected (191). Low lymph node counts can under-stage colorectal cancer and as a result, low lymph node yields are recognised as high risk features which may even prompt provision of adjuvant therapy in clinical trials involving stage II disease (192). As a result, lymph node number has become a standard for assessment of pathological quality. Current guidelines from the Royal College of Pathologists in the UK state a minimum of 12 nodes should be examined to allow accurate staging (188). Fewer than 10 nodes examined in stage II/Dukes' B colorectal cancer is considered a high risk factor and has even been included as one of the inclusion criteria for some clinical trials of adjuvant therapy (e.g. SCOT trial) (193).

Table 1.1 TNM classification of colorectal tumours

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 into subserosa or non-peritonealised pericolic/ perirectal tissues
pT4	Tumour directly invades other organs (pT4a) and/or involves the 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

TNM classification (adapted form RCPath colorectal cancer dataset 2007)

Table 1.2 TNM stage, Dukes' stage, incidence and survival

TNM stage	TNM classification	Dukes' classification	% of all cases	5 year survival
Stage 0	Tis, N0, M0			
Stage I	T1-2, N0, M0	A	13.3%	93.2%
Stage IIA	T3, N0, M0	B	36.8%	77.0%
Stage IIB	T4, N0, M0	B		
Stage IIIA	T1-2, N1, M0	C	35.7%	47.7%
Stage IIIB	T3-4, N1, M0	C		
Stage IIIC	Any T, N2, M0	C		
Stage IV	Any T, Any N, M1	D	14.2%	6.6%

Adapted from the TNM 5th edition and CRUK, Cancer statistics website

The Dukes' and TNM staging are also undermined by observations that survival rates can vary considerably within each staging category. For example, patients with stage II disease with high-risk tumour characteristics (e.g. presence of venous invasion, tumour perforation, surgical margin involvement) have a poorer survival compared with Stage III disease with single node involvement (194). Such observations have prompted calls for the inclusion of more detailed high-risk characteristics in routine reporting of colorectal cancer. High-risk pathological characteristics are those deemed predictive of local or systemic tumour recurrence following resection. As a result, a variety of more sophisticated pathological assessments have been developed with the aim of further stratifying the individual TNM or Dukes' categories (e.g. stage II and III disease). Examples of these pathological scores include the Petersen Index, the lymph node ratio in addition to the Jass criteria.

1.4.1.3 High-risk pathological characteristics

Venous invasion

Venous invasion is defined as tumour present within an epithelial lined space that is either surrounded by a rim of muscle or contains red blood cells (188). Venous invasion is recognised as an important high-risk feature in colorectal tumours (195-201). There is widespread agreement that the presence of venous invasion is associated with an increased risk of the future development of distant metastases (particularly hepatic) and cancer related death (198, 200, 202, 203). As an indication

of the importance of venous invasion in colorectal cancer, the minimum data set requirements, as set by The Royal College of Pathologists, state that frequency of detection for venous invasion should be at least 25% (188). However, the prevalence of venous invasion in published studies has ranged from 10-90%, highlighting the subjective nature of pathologist-based assessment of this important feature (195-201). This variation was demonstrated by a recent Australian study in which 82 Dukes' B colorectal cancer patients were initially reported to have no evidence of either venous invasion or serosal involvement in their tumours. However, on review by an expert pathologist, serosal involvement and/or venous invasion were identified in 32% of tumours, and these characteristics conferred a poorer survival (203). Methods to improve the detection of venous invasion to provide a more standardized assessment have therefore been sought. One such method is the use of elastica stains to aid detection. Elastica stains highlight elastin fibres present in the adventitia of blood vessels and aid pathological detection of tumour within these vessels. Inoue and colleagues (1992) (204) studied the association between the presence of venous invasion and the subsequent development of distant haematogenous metastases in colorectal cancer patients. This association was improved when venous invasion was assessed on elastica stained sections compared to H&E sections. Such stains are not widely employed and are not presently recommended by the Royal College of Pathologists.

Serosal/ Peritoneal Involvement

Serosal or Peritoneal involvement is defined as tumour breaching of the serosa with tumour cells visible either on the peritoneal surface or free in the peritoneal cavity (205). Local peritoneal involvement is an important high-risk pathological characteristic and is of particular importance in colonic tumours in comparison to rectal tumours (205, 206). Serosal involvement is reported to predict local recurrence often reflecting large, bulky tumours with extensive local spread (206). In common with all pathological characteristics, the detection of serosal involvement is also dependent on accurate tumour processing and pathological assessment. Stewart and colleagues highlighted the variation in detection when they demonstrated presence of serosal involvement and or venous invasion in 32% of tumours deemed to have absence of these features following review by an expert pathologist. The

Royal College of Pathologists colorectal dataset also recommends detection of serosal involvement is a marker of pathological quality stating the frequency of detected serosal involvement should be at least 20% for colonic cancers and 10% for rectal cancers (188).

Tumour Perforation

Perforation is defined by the Royal College of Pathologists as a macroscopically visible defect through the tumour, such that the bowel lumen is in communication with the external surface of the intact resection specimen (188). This is a recognized high-risk feature predictive of local recurrence and poor survival (195, 207). Petersen and colleagues demonstrated the prognostic significance of tumour perforation in 2002 (195). They reported a hazard ratio of 9.43 (3.28-27.05) for the relationship between tumour perforation and shorted cancer specific survival in Dukes' B/ Stage II Disease.

Margin Involvement

Surgical margin involvement indicates inadequate resection of tumour tissue and is a recognized indicator of likely local recurrence and poor survival (195, 208, 209). This feature is more common in rectal cancer, particularly following abdominperineal resection in comparison to anterior resection (165).

Tumour differentiation or grade

Poorly differentiated tumours are reported to have a poorer prognosis (195, 210, 211). Tumour differentiation is graded well (low grade), moderate (average grade) or poor differentiation (high grade) and is based entirely on subjective pathological assessment (211). Criteria considered by the Royal College of Pathologists to indicate poor differentiation include either irregularly folded, distorted and often small tubules or the absence of any tubular formation. The relationship with survival is not as strong as the above characteristics and some published studies have not reported any relationship with survival (212). This may in part relate to the subjective nature of assessment.

Perineural invasion

Perineural invasion is associated with poorer outcome in colorectal cancer. Most published work has identified perineural invasion as a high-risk feature in rectal

cancer (198, 199, 213, 214). The rectum is surrounded by a dense network of autonomic nerves and invasion along these nerves is an important indicator of local recurrence and poor long term survival (214). Despite such reports, the Royal College of Pathologists do not currently recommend the assessment of perineural invasion as either a core or non-core data item.

1.4.1.4 Petersen Index

Petersen and colleagues developed a prognostic index based on observations that Dukes' B/Stage II disease encompasses a wide variety of different tumours from tumours that only just penetrate the muscularis to bulky T4 tumours with extensive extramural venous invasion. In an analysis of 268 Stage II tumours, a range of pathological characteristics was examined to determine which were most important for predicting disease recurrence. The resulting Petersen Index is based on four 'high risk' pathological characteristics: peritoneal or serosal involvement, venous spread (both submucosal and extramural), spread to involve a surgical margin, and perforation through the tumour (195). Patients have a worse outcome when more of these features are present. Intra or extramural vascular invasion, peritoneal or serosal involvement and surgical margin involvement are allocated a score of 1. Tumour perforation is allocated a score of 2. The PI considered low risk where the total score is 0 or 1 and high risk from 2 to 5. In patients with Stage II (T3/T4 N0) disease, Petersen Index scores or 0, 1, 2 and >2 were associated with 5 year survival rates of 94%, 78%, 54% and 30% respectively. The Petersen Index has subsequently been validated as a prognostic score that stratifies patients with Stage II disease by risk of recurrence and who may benefit from adjuvant chemotherapy (194).

1.4.1.5 Lymph Node Ratio

In Dukes' C, or node positive (N1/2) colorectal cancer, increased quality of surgery and pathology and therefore increased lymph node retrieval has given rise to the examination of the ratio of metastatic lymph nodes to lymph nodes examined. This has been termed the lymph node ratio (LNR) and, within the above tumour staging systems patients with a high LNR have a worse outcome. The LNR is reported to provide more accurate prediction of outcome than N stage (215, 216). The lymph node ratio is calculated by dividing the number of metastatic lymph nodes identified by the total number of lymph nodes sampled and is reported to provide superior

prognostic information to N stage alone. Numerous thresholds for the LNR have been published (215-217). As yet there has been no general agreement on thresholds of the LNR to be used in routine clinical practice. Even the most recent published reports by Rosenberg and Peschaud use cut offs of 0.17, 0.41, 0.69 and 0.07 and 0.2 respectively (218, 219). Clearly, without widely accepted defined thresholds the widespread clinical use of the LNR will be hampered.

1.4.1.6 Jass classification

Based on observations that additional factors other than local spread and lymph node involvement were strongly associated with survival and recurrence, Jass sought to refine clinico-pathological staging of colorectal cancer in the late 1980s. It was observed that presence or absence of lymphocytic infiltration was of particular prognostic importance, potentially representative of the host immune response to the tumour (220). In addition, Jass reported in a series of publications that tumour margin characteristics (infiltrating or expanding), tumour growth beyond the bowel wall and increasing nodal involvement were the tumour characteristics with strongest prognostic significance (221, 222). Jass described this new prognostic classification based on these four characteristics in 1987 (Figure 1). At the time, the Jass classification was reported to stratify patient into four different risk groups more evenly than the Dukes' system.

The main additions to clinical assessment included in the Jass classification were the additions of peritumoural lymphocytic infiltrate and tumour margin characteristics. A pronounced peritumoural lymphocytic infiltrate is associated with good outcome. This describes the stromal/ inflammatory response at the tumours' invasive edge. A specific and important feature of this response is the presence of a loose connective tissue lamina or cap (resembling lamina propria) at the deepest point of tumour penetration. The tumour glands are often heavily infiltrated by neutrophils but lymphocytes are not necessarily present in large numbers: it is the connective tissue stroma, which is the most important feature. An infiltrating tumour margin is a high-risk margin characteristic. Specific features of a diffusely infiltrating tumour margin include 'streaming dissection' of tumour cells through the muscularis propria, or dissection of mesenteric adipose tissue by small glands or irregular clusters of cords of cells on microscopic examination. The presence of infiltrating growth pattern

appears to be similar to the phenomenon known as de-differentiation, where there is a dissociation of tumour cells at the invasive front allowing these cells to migrate away from the main tumour, which may represent a morphological assessment of 'tumour aggressiveness' (223). This feature has also recently been described as 'tumour budding' where there is a transition from glandular structures to single cells or clusters of up to four cells the invasive margin of colorectal tumours (224).

The Jass classification, in particular the addition of lymphocytic infiltrate and tumour margin characteristics are entirely reliant on skilled assessment by a trained pathologist. Any staging system is dependent on accurate collection of data to provide reproducible and comparable results. While Jass and colleagues reported high inter-observer agreement amongst specialist, trained pathologists, similar results were not obtained elsewhere (225-227). The subjective nature of these assessments led to problems with reproducibility, particularly with the assessment of lymphocytic infiltrate, and as a result the classification has not been adopted widely (188, 228).

Despite the failure of the Jass classification to gain widespread acceptance in routine reporting of colorectal cancer, there is continued interest in the concept of peri-tumoural or intra-tumoural immune/inflammatory response in colorectal cancer. A large body of literature has confirmed the prognostic value of a range of tumour infiltrating immune cells or a pronounced local inflammatory response. This feature is thought to be representative of host immune competences and as a result is discussed in more detail in the following section, "Host characteristics and prognosis in colorectal cancer".

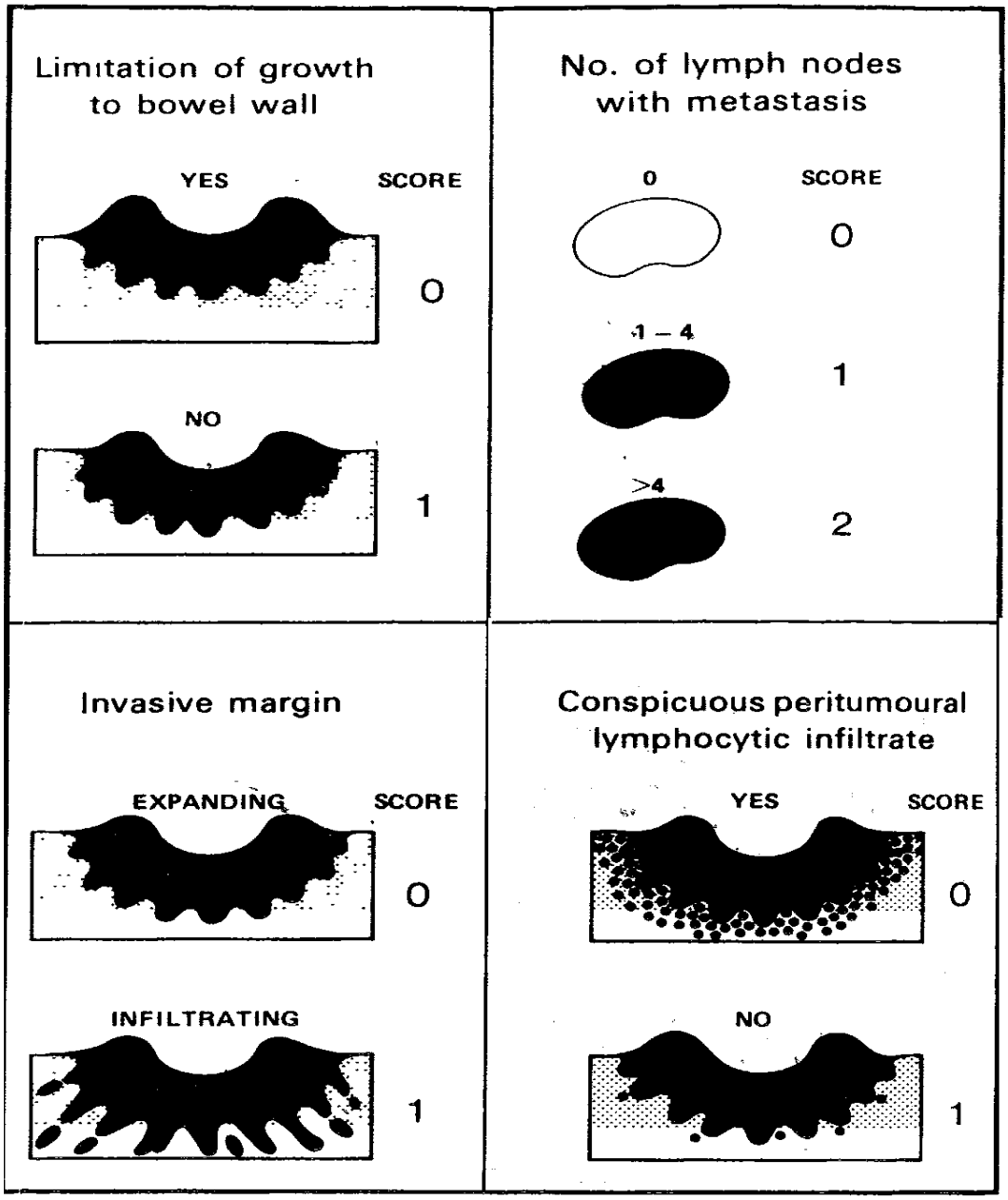


Figure 1.1: Scoring system for pathological variables selected by virtue of independent relationship with survival (adapted from Jass et al, 1987)

Table 1.3: 5 year survival with the Jass classification (adapted from Jass et al, 1987)

Group	Score	5 year survival
I	0-1	96%
II	2	85%
III	3	67%
IV	4-5	27%

1.4.1.7 Molecular markers

In addition to the above pathological characteristics, a wide range of molecular markers are reported to confer prognostic value in colorectal cancer. Graziano and Cascinu categorised these markers into six useful groupings; cell proliferation indices (Ki-67, Mib-1, proliferating cell nuclear antigen (PCNA)); markers of angiogenisis (vascular count, vascular endothelial growth factor); oncogenes/ tumour suppressor genes (p53, K-ras, Deleted in colorectal cancer (DCC), Bcl-2, c-erbB2); DNA repair (microsatellite instability); markers of invasion/metastasis (plasminogen-related molecules, matrix metalloproteinases); and biochemical markers (CEA, CA-19-9, thymidylate synthase) (229). The most well known molecular markers are discussed below.

Proliferation Indices

Immunohistochemistry using antibodies to proteins associated with cellular proliferation (PCNA, Ki-67, Mib-1) has enabled development of proliferation indices. The Ki-67 antigen is expressed in all phases of the cell cycle except in resting cells (G_0). The prognostic role of Ki-67 in colorectal cancer is not clear. Several groups have reported associations with recurrence and survival in colorectal cancer (230, 231) whilst others have been unable to reproduce these results (232-234). Of interest, Allegra in 2002 and Garrity in 2004 reported high proliferation indices assessed using Ki-67 were associated with improved response following adjuvant chemotherapy (232, 235). However, published evidence does not yet support the routine assessment of tumour proliferation in colorectal cancer.

Angiogenesis

Formation of new blood vessels or angiogenesis is important for tumour growth and metastases. This process is dependent on local growth factors, of which vascular endothelial growth factor (VEGF) is most important (236). VEGF expression is increased in advanced colorectal cancer (237) and when quantified, higher levels of VEGF expression are associated with poorer cancer outcome (234, 238, 239). Serum VEGF measured pre-operatively has been related to long-term survival by one group (240), however these results were not repeated by others (241, 242). The anti-VEGF monoclonal antibody bevacizumab is reported to confer therapeutic benefit in advanced colorectal cancer irrespective of VEGF expression (243). Other methods of assessment of angiogenesis include the immunohistochemical assessment of microvessel density.

Oncogenes and tumour suppressor genes

p53 is a tumour suppressor gene located on 17p13.1. p53 is the most commonly altered gene in human cancers. p53 encodes a the transcription factor, p53. In response to cellular stress, p53 activates transcriptional targets causing cell cycle arrest, apoptosis, differentiation and senescence (244). In this way, p53 prevents the accumulation of cellular damage that results in carcinogenisis. p53 status has been studied extensively in colorectal cancer. The methodology used to detect alterations in p53 is reported to impact results. For example, molecular analysis is thought to be more accurate than immunohistochemical analysis in determining mutation status for p53. A recent review identified 35 studies in which mutations in p53 were associated with poorer outcome in colorectal cancer and 24 studies in which colorectal cancer outcome was unaffected by p53 mutations (245). Such heterogeneity in results do not support routine assessment of p53 status in colorectal cancer.

K-ras is a proto-oncogene belonging to the RAS family. K-ras encodes a plasma membrane protein involved in the transduction of external stimuli to the intracellular effector molecules. Alterations in K-ras lead to increased, unregulated cellular proliferation (246)(Bos JL 1989 cancer). The RASCAL I and II studies reported mutations in K-ras are associated with reduced survival following curative resections for colorectal cancer (247, 248). Other reports have not observed strong prognostic

value for K-ras (249). As a result, the 2006 ASCO guidelines for the use of tumour markers in colorectal cancer do not recommend the use of K-ras mutations for screening, staging or surveillance (250).

The Deleted in Colorectal Cancer gene (DCC) is a tumour suppressor gene located on chromosome 18q. Mutations in this gene lead to impaired contacts between cells, contributing to tumour growth and invasion (251). Interpretation of the literature is problematic given many authors have reported results for loss of heterozygosity (LOH) of 18q. Several other tumour suppressor genes in addition to DCC are present on 18q (blc-2 and DPC4). Despite this, there is consistent evidence from a number of studies that LOH at 18q is associated with more aggressive colorectal cancers with early recurrence and reduced survival (252-255).

Microsatellite instability

The role of defective DNA mismatch repair and resulting microsatellite instability in colorectal cancer development and prognosis is discussed in more depth elsewhere. High frequency microsatellite instability is associated with a specific colorectal cancer phenotype with right-sided preponderance and increased frequency of tumour infiltrating immune cells. Such tumours are reported to have an improved prognosis in comparison to low frequency MSI or MSI stable tumours (7, 256).

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein encoded by the proto-oncogene c-erbB-1, involved in cellular adhesion, proliferation, apoptosis and metastasis (257). Galizia and colleagues reported that following curative resection, EGFR+ve tumours had a greater than ten-fold risk of recurrence and death from cancer (258). Cetuximab, an anti-EGFR antibody is a promising targeted agent for EGFR +ve tumours.

Carcino-embryonic antigen (CEA)

Carcino embryonic antigen (CEA) was first associated with colon cancer in 1965. (259). It is an intracellular glycoprotein that is not present in normal colonic mucosa, but is seen in up to 90% of colorectal cancers. Therefore CEA has been used in clinical practice as tumour marker. In addition to colorectal cancer, small elevations in CEA can also occur in smokers and patients with liver cirrhosis, peptic ulceration

or ulcerative colitis (245). Therefore, CEA lacks sufficient sensitivity for use in population-based screening. The prognostic role of CEA measured prior to surgery has previously been studied. Several studies have reported associations between elevated CEA and post-operative cancer specific survival (260-262). Others have not reproduced these results (263) and therefore the role of pre-operative CEA measurement is not currently recommended to routinely stratify risk of recurrence. In contrast CEA measurement is often performed routinely following surgical resection. Firstly, a failure of CEA to normalise may suggest occult disease. Monitoring CEA during clinical follow-up may detect early small elevations above baseline, which may be indicative of disease recurrence (264). Early detection of recurrence may enable potentially curative therapy to be administered. CEA is also measured in metastastic disease and can provide oncologists with evidence of progression or regression in response to treatment. However the economic value of routine CEA monitoring has been questioned given a lack of evidence that survival can be improved (265).

Carbohydrate Antigen 19-9

Carbohydrate antigen 19-9 (CA-19-9) is an adhesion molecule that plays a role in tumour progression. Elevations occur in colorectal and pancreatic cancers as well as benign biliary tract diseases (266, 267). Several authors have reported independent prognostic value of CA-19-9 in pre-operative colorectal cancer patients (268-270), however the 2006 ASCO recommendations on the use of tumour markers in colorectal cancer do not currently recommend routine CA-19-9 measurement in any area of colorectal cancer management (250).

Thymidylate synthase

Thymidylate synthase (TS) is a rate-limiting enzyme involved in DNA synthesis. TS is also the main intracellular target of 5-fluorouracil. Previously it was reported that high levels of TS correlated with poor response to chemotherapy in addition to reduced survival (271-273). However, more recently, published studies have not supported the routine assessment of TS expression in colorectal cancer reporting little prognostic value (232, 274). Heterogeneous results may relate to inconsistent methodology in assessment of TS and TS is not routinely assessed in colorectal cancer (245).

1.4.1.8 Summary: Tumour characteristics and colorectal cancer prognosis

In addition to tumour stage (TNM), a variety of colorectal cancer characteristics are of established prognostic value. Of these characteristics, venous invasion, serosal involvement, differentiation, tumour perforation, resection margin status in addition to T stage and N stage are core data items in the 2007 Royal College of Pathologists Colorectal cancer dataset based on established prognostic value. Non-core data items include margin characteristics or tumour budding and the presence of tumour infiltrating lymphocytes. These non-core characteristics have established prognostic value but so far lack of inter-observer agreement has hindered their widespread use in routine clinical assessment. It is also apparent that pathological characteristics are dependent on accurate pathological processing and reporting. Characteristics such as venous invasion vary widely in different cohorts. Methods to improve detection of venous invasion include elastica staining, however to date the prognostic value of venous invasion detected using elastica stains is not established.

A variety of molecular tumour characteristics have been reported to have prognostic value but none are recommended for routine assessment in clinical practice by either the American Society of Clinical Oncologists (ASCO), the Association of Coloproctologists of Great Britain and Ireland (ACPGBI) or the Royal College of Pathologists (RCPath dataset) (157, 188, 250). At present there is little clinical benefit from this extra molecular information in planning further treatment. The exception is CEA, which is currently recommended by ASCO for post-operative surveillance for the first 3 years following surgery.

1.4.2 HOST CHARACTERISTICS AND PROGNOSIS IN COLORECTAL CANCER

It is increasingly recognized that host factors play an important role in cancer progression and survival. For example, increasing age and burden of comorbidity relate to poorer survival in colorectal cancer (99, 275). In addition the presence or absence of local or systemic inflammatory responses is of prognostic importance. These responses have been associated with alterations in local and systemic immune cell concentrations and may reflect host anti-tumour immune competence (276-278). The host immune response is made up of innate and adaptive components (279). At present it is not clear how the presence or absence of local or systemic inflammation relates to these components.

1.4.2.1 The host immune response

The human immune system functions to protect individuals from foreign pathogens. The immune response is composed of two broad categories; innate immunity (natural) and adaptive immunity (acquired). The innate response provides an immediate "front line", non-specific response against pathogens. It comprises defence mechanisms present prior to pathogen exposure and includes the acute phase inflammatory response. Adaptive immunity is an antigen–specific response requiring the recognition of non-self antigens with antigen presentation. It develops after the innate immune response, enabling a stronger, more focussed response and development of immunological memory (279). As discussed below, there is good evidence that components of the innate and adaptive immune responses play a role in determining colorectal cancer outcome.

1.4.2.2 Innate and adaptive immunity

Innate immunity

In addition to natural epithelialised barriers, (e.g. skin and mucosa), innate immunity is composed of cellular components and humoral components. The major cellular components of the innate immune response are phagocytic cells (neutrophils and macrophages), degranulating cells (eosinophils, basophils and mast cells) and natural killer (NK) cells. Phagocytic cells are attracted to sites of acute infection or tissue injury and detect microbes using pattern recognition molecules (PRMs). Microbes are ingested through phagocytosis in order to undertake intracellular destruction.

The process is more effective if prior opsonisation has occurred with antibody or complement to facilitate receptor recognition by the phagocyte (279).

The innate immune response contributes to the adaptive immune response as macrophages and dendritic cells are antigen-presenting cells. After phagocytosis, and antigen processing, these cells migrate to lymphoid tissues to present antigen to T lymphocytes (279). Complimentary to this process is the acute phase response resulting in inflammation, which increases lymph flow and therefore the number of antigen presenting cells reaching lymphoid tissue.

The complement system is the major humoral component of the innate immune response augmenting the cellular response described above. This is a plasma protein cascade comprising over 20 glycoproteins which converge through two pathways, the classical and alternative pathways to activate the same central component (C3) (280). A final common pathway results in the formation of the "membrane attack complex" (MAC). The MAC is a transmembrane channel capable of cell destruction through osmotic lysis. Additional functions of complement include chemo-attraction and opsonisation of pathogens facilitating phagocytosis. Other natural opsonins involved in innate immune responses include manna binding lectin (MBL) and C-reactive protein, both of which coat pathogens for phagocytosis and activate complement.

Adaptive immunity

The adaptive immune response consists of lymphocytes and their products, including antibodies (279, 281). It is a highly specific antigen dependent response, which develops slowly enabling immunological memory. Lymphocyte clones develop expressing antigen specific receptors. B cell receptors (immunoglobulins) bind extracellular molecules. T cell receptors bind peptide fragments bound to MHC molecules on cell surfaces. Thereafter antigen specific lymphocytes proliferate developing into effector cells. A subset will develop into long-living memory cells enabling future proliferation if the antigen is encountered again (281, 282).

Adaptive immunity is divided into humoral and cell mediated immunity (279). Whilst T helper cells play an important directional role in each type of adaptive immune response, bone marrow derived B cells are the effector cells of the humoral immune response and thymus-derived cytotoxic T cells are the effector cells for the cell

mediated immune response. Terminally differentiated B cells are called plasma cells which make antibody (secreted immunoglobulin). Antibodies bind extracellular pathogens leading to their destruction. Antibody specific cytotoxic T cells kill pathogen-infected cells and therefore provide protection against intracellular pathogens. In addition helper T cells also activate macrophages to kill intracellular pathogens also termed cell-mediated immunity.

Immunoglobulins

Antibodies or immunoglobulins are produced by activated plasma cells. The binding of antibody to pathogen alone does not lead to destruction of the pathogen. Antibodies harness innate effector mechanisms in order to achieve their goal. The Fc portion of the immunoglobulin attracts and activates complement and antibodies opsonise pathogens facilitating the killing by phagocytes. Antibodies also have protective roles, mainly in neutralising toxins and organisms by binding to cell surface receptors altering cell function.

1.4.2.3 Control of the immune response

It is the presence of antigen that drives ongoing immune responses. However the nature of the antigen including the amount and sequence of peptide presented by antigen presenting cells influences the differentiation of CD4 T cells into effector subsets. Large amounts of peptide stimulate Th1 responses whereas those binding weakly stimulate Th2 responses. Th1 cytokines are crucial in the activation of cell-mediated immunity and macrophages and Th2 activate B cells. If one CD4 subset develops first it can suppress the development of the other subset. However most of the time there is a mixed response.

The development of T regulatory cells in the mucosal immune system induces tolerance, actively suppressing antigen specific responses following re challenge.

1.4.2.4 The immune response and cancer

Over forty years ago Burnet proposed that as part of cancer immunosurveillance the immune system acts to recognize and removed transformed cells (283). Transformed cells have genetic differences distinguishing them from other host cells. Antigenic changes on the cell surface can be recognised by the host immune system. These cell surface antigens can be tumour specific (not present on normal cells) or tumour

associated antigens (present in high numbers in tumour cells but also present on some normal cells) (284). Immune surveillance is the immune systems ability to detect tumour cells and destroy them. Anti-tumour responses may therefore develop in response to these antigens. The concept of immune surveillance has been contested but is thought to be evidenced by the fact immunocompromise is associated with increased development of malignancy (285, 286). Where tumours are able to evade the host immune response, this may be due to the weak immunogenicity of tumour cells, mutations resulting in loss of antigen expression or tumour induced immunosuppression (286, 287). Tumour induced immunosuppression may relate to local production of immunosuppressive growth factors or cytokines (TGF β, IL-10) which may suppress innate or adaptive immune cell responses (286).

1.4.3 SYSTEMIC INFLAMMATION AND THE ACUTE PHASE PROTEIN RESPONSE

Inflammation is a reaction to tissue injury (ischaemia, necrosis, trauma, hypoxia or cancer) or infection. Acute inflammation may be followed by resolution, or may become chronic if the stimulus persists. A myriad of potential stimuli exist including prostaglandins, and leukotrienes released from damaged cells in addition to pro-inflammatory cytokines (IL-1, IL-6, TNF-α) released primarily by phagocytes. These pro-inflammatory factors act on target cells to induce a cascade of mediators that contribute to the initiation and maintenance of an inflammatory response. The acute phase response is characterized by local and systemic changes in vasculature, metabolism and plasma protein composition. As an early response, inflammation acts to promote initiation of immune responses, facilitating an influx of neutrophils, complement and antibodies.

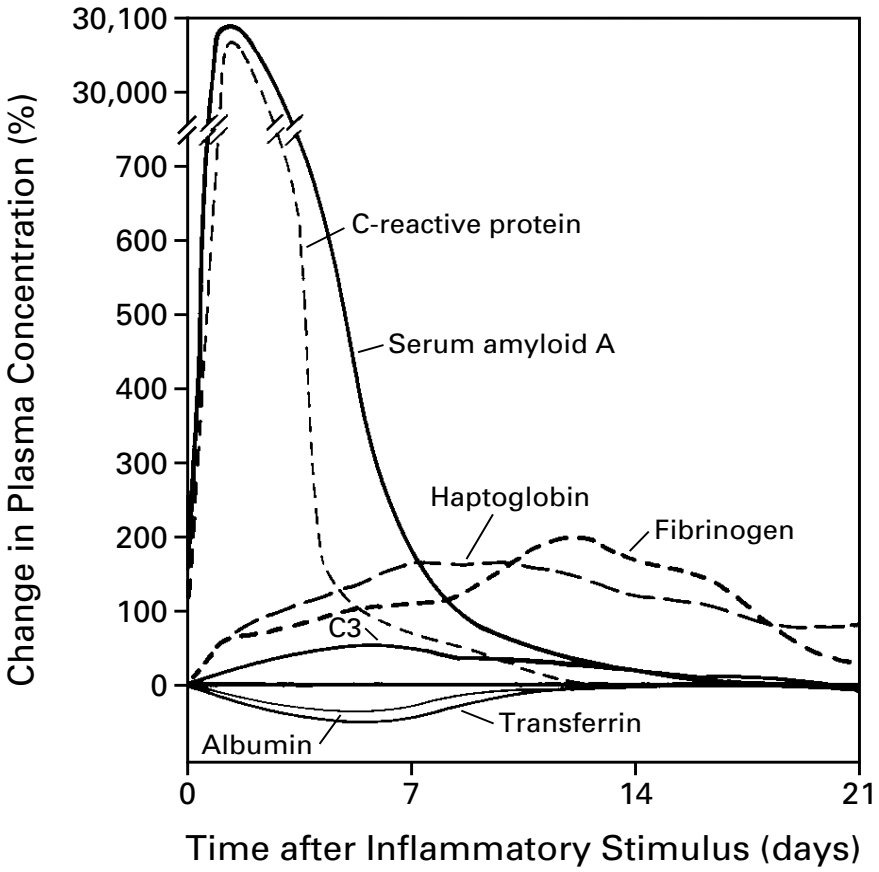


Figure 1.2: Characteristic patterns of change in plasma concentrations of acute phase proteins following moderate inflammatory stimulus (adapter from Gabay and Kushner, 1999)

Acute phase proteins are a class of proteins synthesized in the liver whose concentration increases or decreases in the presence of acute and chronic inflammation. The definition of an acute phase protein is one whose concentration changes by at least 25% in the presence of an inflammatory disorder (288). Table 1.5 shows some examples of the wide variety of acute phase proteins. Although the liver is central to the development of an acute phase protein response, a large number of metabolic alterations occur across several organ systems resulting in behavioral, psychological, biochemical and nutritional changes (289) (Table 3). Pro-inflammatory cytokines, in particular, the hepatocyte stimulating interleukin-6 released from a range of sources are believed to mediate the acute phase response (290). The magnitude of change in concentration for individual acute phase proteins varies considerably (Figure 2). Two highly sensitive markers of inflammation, C-reactive protein and serum amyloid A have particularly marked changes in

concentration above baseline whereas other acute phase reactants such as fibrinogen and transferring demonstrate less marked alterations.

The presence of a chronic acute phase protein response results in several important implications for a wide range of body systems (Table 3). Of particular significance to the patient are alterations in hepatic protein production, haemopoetic changes, metabolic changes and alterations in the hypothalamic-pituitary-adrenal axis. In the cancer patient undergoing treatment for their disease, the presence of an acute phase response is therefore likely to modify several responses to treatment. For example, alterations in metabolism are likely to confound attempts to improve patient nutrition; haemopoetic and immune changes may modify post-operative wound healing and risk of developing complications. Alterations in hepatic function and metabolism may modify drug metabolism.

Table 1.4: Multisystem effects of the acute phase response (adapted from Gabay and Kushner 1999) (289)

Neuroendocrine changes
Fever, somnolescence and anorexia
Increased secretion of corticotrophin-releasing hormone, corticotrophin and cortisol
Increased arginine vasopressin
Reduced production of insulin like growth factor-1
Increased secretion of adrenal catecholamines
Haemopoetic changes
Anaemia of chronic disease
Leukocytosis
Thrombocytosis
Metabolic changes
Loss of lean muscle and negative nitrogen balance
Reduced gluconeogenesis
Osteoporosis
Increased hepatic lipogenesis
Increased lipolysis in adipose tissue
Reduced lipoprotein lipase activity in muscle and adipose tissue
Cachexia
Hepatic changes
↑metallothioncin
↑inducible nitric oxide synthase
↑heme oxygenase
tissue inhibitor of metalloproteinase-1
Alterations in non-protein plasma constituents
↓Zinc
↑copper
↓iron
↑retinol
↑glutathione

Table 1.5: Acute phase proteins. (adapted from Gabay and Kushner, 1999) (289)

Proteins whose concentration increases

Complement system

- C3
- C4
- C9
- Factor B
- C1 inhibitor
- Mannose binding lectin

Coagulation and fibrinolytic system

- Fibrinogen
- Plasminogen
- Tissue plasminogen activator
- Urokinase
- Protein S
- Vitronectin
- Plasminogen activator inhibitor-1

Antiproteases

- α_1- Protease inhibitor
- α_1- antichymotrypsin
- Pancreatic secretory trypsin inhibitor
- Inter-α-trypsin inhibitors

Transport proteins

- Caeruloplasmin
- Haptoglobin
- Hemopexin

Participants in inflammatory responses

- Sectreted Phospholipase A
- Lipopolysaccharide-binding protein
- Interleukin-1 receptor antagonist
- Granulocyte colony stimulating factor

Others

- C-reactive protein
- Serum amyloid A
- Fibronectin
- α_1-Acid glycoprotein
- Ferritin
- Angiotensinogen

Proteins whose concentrations decrease

- Albumin
- Transferrin
- Transthyrectin
- Insulin growth factor 1
- Factor XII
- Alpha fetoprotein
- Thyroxine binding globulin
- α_2- HS glycoprotein

1.4.3.1 The systemic inflammatory response and cancer progression

In the last decade or so it has become clear that disease progression in cancer is dependent on a complex interaction of the characteristics of both the tumour and host characteristics, in particular the presence of a host systemic inflammatory response (291-293). Indeed, there has been substantial evidence in advanced cancer, and more recently in localised disease, that host factors such as weight loss, performance status and a host systemic inflammatory response are also important indicators of outcome, independent of tumour stage (294-296). In particular, systemic inflammation, as evidenced by an elevated C-reactive protein, is associated with increased weight loss and poorer performance status and therefore may be an important aetiological factor in the nutritional and functional decline of the advanced cancer patient (297-302). More recently, it has become clear that measures of the systemic inflammatory response, compared with weight loss and performance status, are superior prognostic factors independent of tumour related factors in advanced cancer (296). This has resulted in a measure of the systemic inflammatory response in the cancer patient being included in the definition of cancer cachexia (296, 303, 304). More recently attention has moved towards earlier stage, localised disease. The prognostic value of the systemic inflammatory response measured prior to surgery in operable colorectal disease is now established (Tables 1.7 and 1.8).

In healthy individuals, inflammation is usually short-lived with endogenous anti-inflammatory mechanisms bringing eventual resolution. The presence of a systemic inflammatory response in cancer bears similarities with that of chronic inflammatory diseases where there is an imbalance of pro and anti-inflammatory mechanisms. Endogenous anti-inflammatory mechanisms mediated through interleukin-10 (IL-10), transforming growth factor (TGF) -β, prostaglandins and lipoxins function to regulate inflammation normally (305). When these mechanisms are impaired there may be a predisposition to development of malignancy. For example, the TGF-β mediated suppression of pro-inflammatory cytokines such as IL-6 has been seen to inhibit tumour growth in mice (306). Deletion of the IL-10 gene in mice also leads to spontaneous inflammatory bowel disease and subsequent development of colorectal cancer (307).

1.4.3.2 Measurement of the systemic inflammatory response

Clinically, the commonest reported measures of the systemic inflammatory response in cancer patients are biochemical or haematological markers. These are namely, an elevated C-reactive protein concentration or increased white cell, neutrophil and platelet counts. Hypoalbuminaemia is also recognised to be part of the systemic inflammatory response (289). Indeed, recent definitions of cancer cachexia include elevation of C-reactive protein and hypoalbuminaemia (303, 304). Relationships between individual components of the systemic inflammatory response and cancer specific survival have been repeatedly observed. Moreover, combinations of such factors have been used to derive simple inflammation based prognostic scores. The Glasgow Prognostic Score combines circulating C-reactive protein and albumin concentrations (308) (Table 1). The neutrophil lymphocyte ratio combines circulating neutrophil and lymphocyte counts (309) (Table 1) and the platelet lymphocyte ratio combines circulating platelet and lymphocyte counts (310) (Table 1.6). The prognostic value and the clinical use of such markers of the systemic inflammatory response in operable colorectal cancer is examined below.

Table 1.6: Systemic inflammation based scoring systems

The Glasgow Prognostic Score (GPS, 3)	**Score**
C-reactive protein ≤10mg/l and albumin ≥35g/l	0
C-reactive protein ≤10mg/l and albumin <35g/l	0
C-reactive protein >10mg/l	1
C-reactive protein >10mg/l and albumin <35g/l	2
Neutrophil lymphocyte ratio	
Neutrophil count : lymphocyte count <5:1	0
Neutrophil count : lymphocyte count ≥5:1	1
Platelet lymphocyte ratio	
Platelet count : lymphocyte count <150:1	0
Platelet count : lymphocyte count 150-300:1	1
Platelet count : lymphocyte count >300:1	2

1.4.3.3 Prognostic value of the pre-operative systemic inflammatory response in resectable colorectal cancer

Although reports exist in numerous tumour types (including pancreatic, gastro-oesophageal, renal, prostate, ovarian, lung, breast and hepatobiliary cancers (311-317), the value of the systemic inflammatory response has been most extensively examined in colorectal cancer (Table 1.7) (309, 318-331). Following initial observations in the 1980-90s (332-334), an elevated pre-operative C-reactive protein was reported to predict early recurrence and death from colorectal cancer (319, 328, 331, 335). Also, three separate studies reported that pre-operative hypoalbuminaemia was associated with earlier tumour recurrence and death following potentially curative resection for colon and rectal cancer (318, 326, 327). In the above studies varying thresholds for the circulating concentrations of C-reactive protein and albumin concentrations were reported (Table 1.7). However, a

C-reactive protein concentration >10mg/L and an albumin concentration of <35g/l are consistently reported to indicate poorer colorectal cancer outcome (Table 1.7).

The prognostic value of the Glasgow Prognostic Score (based on C-reactive protein and albumin) was originally reported in non-small cell lung cancer in 2003 (336). In the study by Forrest and colleagues, elevated C-reactive protein and hypoalbuminaemia were both given scores of 1 and the cumulative score related to outcome. In 2007, McMillan and colleagues reported the prognostic value of the pre-operative GPS in 316 patients with primary operable colorectal cancer (308). In their study, the Glasgow Prognostic Score was modified based on evidence that cancer specific survival was not related to hypo-albuminaemia alone (Table 1.7). A GPS of 0 was associated with a 3-year cancer survival rate of 95% compared with a GPS of 2, which conferred a 3-year survival rate of 61%. When Stage II or Dukes' B patients were analysed separately, patients with a GPS of 0 had a 3-year cancer survival rate of 99% and those with a GPS of 2 had a survival rate of 72%. The GPS has subsequently been validated by a Japanese group who reported the GPS to be superior to carcinoembryonic antigen, CA 19-9 and other tumour markers in predicting survival (325).

Walsh and co-workers (2005) (309) examined the prognostic value of another inflammation based score, the neutrophil lymphocyte ratio in primary operable colorectal cancer patients. They reported the NLR >5 was related to poorer cancer specific survival, but the score was not independent of tumour stage (Table 1.7).

Table 1.7: Prognostic value of the pre-operative systemic inflammatory response (SIR) in resectable colorectal cancer

Study	Year	Patients	Measure of SIR	Comments
Goransson et al	1996	62	CRP and Albumin	Elevated CRP (>10mg/l) and low Albumin (<37g/l) predict inoperability and recurrence.
Longo et al	1998	591	Albumin	Low serum albumin an independent predictor of survival in rectal cancer.
Heys et al	1998	431	Albumin	Low albumin (25-34 and <25g/l) an independent predictor of survival.
Nozoe et al	1998	120	CRP	Elevated CRP (>8mg/l) predicts survival.
Longo et al	2000	5853	Albumin	Low albumin an independent predictor of survival in colon cancer.
Nielsen et al	2000	594	CRP	Elevated CRP (>11mg/l) level predicts survival outcome.
Wigmore et al	2000	202	CRP and Albumin	Elevated CRP (>10mg/l) an independent predictor of survival in Dukes' C/ D disease. Hypo-albuminaemia (<35g/l) an independent predictor in all stages.
McMillan et al	2003	174	CRP	Pre and post-op elevated CRP (>10mg/l) independently predict survival
Chung et al	2003	172	CRP	Elevated CRP (>8mg/l) not an independent predictor of survival
Miki et al	2004	71	CRP	Elevated CRP (>10mg/l) predicts survival.
Kandemir et al	2005	198	Platelet count	Thrombocytosis (>400x10^9) an independent predictor of survival in node negative disease.
Nikiteas et al	2005	74	CRP	Elevation of CRP (≥7mg/l) predicts survival.
Walsh et al	2005	230	NLR	NLR (>5) predicts survival but not stage independent.
Cengiz et al	2006	99	Albumin	Low serum albumin (<35g/l) an independent predictor of survival.
Ishizuka et al	2007	315	GPS	GPS an independent predictor of survival.
McMillan et al	2007	316	GPS	GPS an independent predictor of overall and cancer specific survival.
Leitch et al	2007	149	GPS	GPS superior to cellular components of systemic inflammatory response in predicting survival.

1.4.3.4 Prognostic value of the pre-operative systemic inflammatory response in resectable colorectal liver metastases

With the establishment of the prognostic importance of the systemic inflammatory response in primary operable disease, there has been interest in its prognostic value following curative resection of colorectal liver metastases (Table 1.8) (317, 337-342). Four separate studies from the same centre (317, 339, 341, 342) described the prognostic value of a variety of markers of the pre-operative systemic inflammatory response. These include an elevated C-reactive protein, hypoalbuminaemia, elevated circulating neutrophil count, and the neutrophil lymphocyte ratio in patients undergoing resection of colorectal liver metastases (Table 1.8). Malik et al. reported that in 560 patients, an "inflammatory response to the tumour (IRT)" characterised by an elevated pre-operative C-reactive protein >10 mg/l or NLR $\geq$5 was an independent predictor of survival and superior to the established Memorial Sloan Kettering Clinical Risk Score (341). The authors constructed their own prognostic score based on the IRT and the presence of 8 or more liver metastases, the only other independent predictor of cancer survival identified within their cohort. A later study from the same centre reported that the most important predictors of survival following liver resection were surgical margin involvement and the presence of a systemic inflammatory response as evidenced by C-reactive protein >10mg/l or the NLR (317). Further studies have reported relationships with poorer cancer survival and a variety of different markers of the systemic inflammatory response prior to surgery for colorectal liver metastases. These include the neutrophil lymphocyte ratio (338)(63) and peripheral blood monocyte counts (340).

In summary, a variety of different markers of the systemic inflammatory response have been examined in patients undergoing surgery for both localised and advanced colorectal cancer. Several scoring systems have been proposed including the Glasgow Prognostic Score, the neutrophil lymphocyte ratio and the platelet lymphocytes ratio. To date, a comprehensive evaluation of these different scoring systems in a single cohort of patients undergoing potentially curative resection for colorectal cancer has not been performed.

Table 1.8: Prognostic value of the pre-operative systemic inflammatory response (SIR) in resectable colorectal liver metastases

Study	Year	Patients	Measure of SIR	Comments
Tartter et al	1987	42	Lymphocyte count, alkaline phosphatase	Elevated peripheral lymphocyte count and alkaline phosphatase predict survival in colorectal cancer patients with synchronous liver metastases.
Kishi et al	2000	200	NLR	NLR (>5) independently predicts survival in patients treated with chemotherapy followed by resection.
Wong et al	2007	170	CRP	Elevated CRP (>10mg/l) predicts survival.
Sasaki et al	2007	97	Monocyte count	Elevated monocyte count ($>3x10^9$) an independent predictor of survival.
Malik et al	2007	687	CRP or NLR	Number of metastases and the inflammatory response to the tumour (CRP >10mg/l or NLR >5) were independent predictors of survival.
Halazun et al	2008	440	NLR	NLR (>5) a predictor of recurrence and poor survival.
Gomez et al	2008	705	CRP or NLR	Elevated CRP (>10mg/l) or NLR (>5) predicts survival.

1.4.3.5 Basis of the systemic inflammatory response in colorectal cancer

The underlying basis or stimulus of a systemic inflammatory response in cancer patients is not known. It may simply reflect a non-specific inflammatory response secondary to tumour hypoxia and necrosis or local tissue damage. However, apoptosis does not elicit an inflammatory immune response being is a relatively 'clean' form of cell death (343). Currently it is not clear whether the development and maintenance of a systemic inflammatory response is driven by local tumour inflammation. However, as discussed below, the systemic inflammatory response is associated with a poor prognosis whereas the presence of a local inflammatory response is related to good outcome. These relationships would also suggest tumour inflammation is not the principal driver of the systemic inflammatory response. Alternatively, the systemic inflammatory response may reflect immune competencies of the host disabling effective anti-tumour responses, in addition to promoting tumour growth. For example in localised colorectal cancer there is evidence that the systemic inflammatory response as evidenced by the elevation of C-reactive protein is associated with compromised cell-mediated immunity such as lymphocytopenia and impaired T lymphocytic response within the tumour (276, 344, 345) and increased activation of components of innate immune system, including complement and macrophage function (291, 345). Such activation of macrophages is of particular interest as there is a close association with neovascularisation (a cardinal feature of a chronic inflammatory response) of the tumour and blood borne dissemination of cancer cells (346). Also, pro-inflammatory cytokines and growth factors released as part of the systemic inflammatory response may promote and maintain tumour growth (291, 347).

A systemic inflammatory response may also persist due to imbalances in innate anti-inflammatory mechanisms. One important group of powerful endogenous anti-inflammatories is the glucocorticoids. They act to downregulate production of pro-inflammatory cytokines from monocytes and macrophages and in some cases induce apoptosis of cells recruited by inflammation (348). They also promote production of anti-inflammatory cytokines such as IL-10 from macrophages. Their release from the adrenal cortex is stimulated by adreno-corticotrophic hormone (ACTH) produced in the anterior pituitary gland. ACTH release is in-turn regulated by corticotrophin

releasing hormone for the hypothalamus so forming the hypothalamic-pituitary-adrenal axis (348).

Of interest the systemic inflammatory response is associated development and progression of a rage of benign conditions (Section 1.2.2.2). One further hypothesis could be that the systemic inflammatory response in cancer patients is simply reflective of comorbidity burden.

1.4.4 THE PROGNOSTIC VALUE OF THE LOCAL INFLAMMATORY RESPONSE IN COLORECTAL CANCER – TUMOUR INFILTRATING IMMUNE CELLS

Given that the systemic inflammatory response plays a role in colorectal cancer outcome, it is of interest that the local tumour inflammatory response is also important. Studies dating back to the 1970s have reported that a predominant tumour inflammatory cell response is recognised to confer an improved clinical outcome (Table 1.9). This local inflammatory reaction seen in and around tumours is thought to represent the in-situ immune response by the host in reaction to the tumour. Since these early reports over thirty years ago, there has been great interest in establishing the cellular composition of immune cell infiltrates in and around colorectal tumours. To date numerous studies have examined the prognostic role of in situ intratumoural or peritumoural immune cells involved in both innate and adaptive immune responses (Tables 1.9-1.21). It is generally apparent that an increasing number or density of immune cells in and around the tumour is associated with improved clinical outcome in colorectal cancer. The evidence is particularly robust for tumour infiltrating T lymphocytes and their various subsets as well as tumour associated macrophages. Recently, groups have focused on individual cell types in prediction of outcome. It is clear that multiple different cell types may have prognostic value. The following section reviews the evidence for the local inflammatory cell reaction and tumour infiltrating immune cells in predicting outcome in patients with primary operable colorectal cancer.

1.4.4.1 Microsatellite Instability and local inflammatory response

Although the majority of colorectal cancer arises through sporadic chromosomal alterations a smaller proportion arise through high frequency microsatellite instability. These comprise almost all cases of hereditary non-polyposis colorectal cancer (HNPCC) and 15% of spontaneous colorectal cancers (349). MSI-H tumours have a unique clinical phenotype. They are predominantly right sided, poorly differentiated and associated with a less aggressive clinical course (350). Of relevance here, it is recognised that MSI-H tumours are characterised by more abundant intratumoural and peritumoural lymphocytic infiltrates (11, 351). Importantly, to date, several groups have reported the prognostic effects of tumour

infiltrating lymphocytes are independent of microsatellite instability status (352, 353).

1.4.4.2 The prognostic value of generalised lymphocytic infiltrate in primary operable colorectal cancer.

Thirty-three published studies have reported a pronounced lymphocytic or inflammatory cell infiltrate in and around the tumour is associated with an improved prognosis in primary operable colorectal cancer (Table 1.9 and Table 1.10) (220, 222, 277, 329, 354-383). Since an early report in gastric cancer (McCarty et al) (384), a number of studies between 1967 and 1985 consistently demonstrated that high densities of infiltrating lymphocytes or inflammatory cells primarily at the tumour periphery but also the tumour centre conferred improved colorectal cancer survival when assessed on Haematoxylin and Eosin staining alone (354-363).

In 1986-87, two published studies by Jass and colleagues described the semi-quantitative assessment of peritumoural lymphocytic infiltrate in rectal cancer. This feature was an independent prognostic factor on multivariate analysis and formed part of a novel prognostic model (220, 225). The Jass criteria have been subsequently validated in both colon and rectal cancer as an important stage independent determinant of cancer specific survival (369-372, 375-377, 382).

In the early 1990s, Graham and Appelman described the presence of a Crohn's like reaction at the tumour's invasive margin consisting of discrete lymphoid aggregates, some with germinal centres (366). Such reactions were associated with lymphocytic tumour infiltrates as well as right-sided tumours and conferred improved long-term survival (366, 369, 372). Subsequently Crohn's like reaction in addition to lymphocytic infiltrate has been recognised to be a predominant feature of microsatellite unstable tumours (12, 385, 386).

Over the past 10 years, further methods for simple semi quantitative assessment of lymphocytic/ inflammatory cell reaction have been developed by several groups including Nagtegaal et al and Klintrup et al (277, 377). Importantly, the Nagtegaal et al method of assessment was validated by Gao and colleagues (381).

Finally, one study by Morris and colleagues reported a high-grade tumour lymphocytic infiltration was associated with increased survival benefit from adjuvant chemotherapy (387).

1.4.4.3 The prognostic value of tumour infiltrating lymphocytes in primary operable colorectal cancer.

T lymphocytes

More recently, the ability to identify lymphocyte subsets by immunohistochemistry has led to renewed interest in the relationship between the tumour inflammatory infiltrate and outcome. T lymphocytes are effector cells of the adaptive immune response. Almost all T-lymphocytes express CD3 and therefore the CD3+ antibody is used as a generic T-lymphocyte marker. Also, there is the helper T-lymphocyte that expresses CD4 and CD4 is expressed on 60% of mature T-lymphocytes. These helper T-lymphocytes require the processing and presentation of antigen in association with MHC class II molecules by antigen presenting cells and coordinate and assist other immune cells. Helper T-lymphocytes may be further subdivided by patterns of cytokine expression. The Th1 CD4 T-lymphocytes are known to produce interleukin-2, interferon γ and tumour necrosis factor enhancing macrophage function, cellular immunity and synthesis of opsonising and complement fixing antibodies. The Th2 CD4 T-lymphocytes are known to enhance Immunoglobulin G mediated responses and the activation of eosinophils through the actions of interleukin-4, interleukin-5 and interleukin-13. A further small subset of T-lymphocytes expressing surface molecules common to conventional T-lymphocytes and NK cells are called NK-T-lymphocytes. The functional significance of the NK-T cell is not well defined.

T lymphocytes (CD3+ expression)

To date, six published studies have examined the relationship between CD3+ stained T cells and outcome in primary operable colorectal cancer (Table 1.9 and Table 1.11) (352, 377, 388-391). Four of these studies have described survival relationships for CD3+ assessment alone or in combination with other immune cell markers in operable colorectal cancer (352, 377, 388, 389, 391). The first such study was that of Nagtegaal and colleagues (2001) who performed a quantitative immunohistochemical analysis in 160 rectal cancer patients (377). Higher CD3+ counts related to lower T and N stage. Low peritumoural and intratumoural CD3+

counts related significantly to development of distant metastases. Although low intratumoural CD3+ was a predictor of poorer survival, this was not independent of peritumoural eosinophil counts or TNM stage.

In 109 right-sided colonic tumours, immunohistochemical analysis was performed along with analysis for microsatellite instability by Guidiboni et al (352). Although microsatellite unstable tumours had higher counts of intra-epithelial CD3+ cells, the authors reported that high CD3+ counts were related to better survival in both microsatellite stable and unstable tumours.

Galon and colleagues also reported in a large cohort of 415 colorectal cancer patients that high CD3+ counts at the tumour periphery and tumour centre was associated with reduced recurrence and improved survival (391). Combining the analysis of these two tumour regions improved prediction of survival. Furthermore the authors reported that if CD3+ and CD45RO (memory T cells) were both low then a very poor prognosis could be expected. Such assessment of different immune cell types in different tumour locations was reported to be superior to TNM stage in predicting outcome.

Two studies did not report a significant survival relationship with CD3+ counts in colorectal cancer (388, 390). However in the study by Takemoto et al., the authors report a trend towards significance commenting that the groupings used may have contributed to a non-significant result (388).

Non cytotoxic T lymphocytes – Helper T cells (CD4+ expression)

Helper T cells don't have a specific effector function but coordinate and assist other immune cells. Helper T cells are further subdivided by patterns of cytokine expression. The Th1 CD4 cells produce interleukin-2, interferon γ and tumour necrosis factor enhancing macrophage function, cellular immunity and synthesis of opsonising and complement fixing antibodies. The Th2 CD4 cells enhance Immunoglobulin G mediated responses and the activation of eosinophils through the actions of interleukin-4, interleukin-5 and interleukin-13.

Five studies reporting relationships between CD4+ counts and colorectal cancer survival were identified (Table 1.9 and Table 1.12) (320, 377, 388, 392, 393).

Nagtegaal et al reported that low intratumoural CD4+ counts were associated with increased depth of invasion and increased local recurrence in 160 colorectal cancer patients (377). Ali et al. also reported an association between reduced CD4+ infiltrate and local recurrence in 80 patients (392). The only previous report with a significant association between CD4+ and survival was by Canna et al (2005) who described an improved survival in 147 patients with increasing general intratumoural CD4+ infiltrate (320). In addition to assessment of CD4+ infiltrates alone, previous work has highlighted the importance of an increasing CD8+:CD4+ ratio within tumours (assessed using flow cytometry of dissociated tumour cells) as a predictor of improved oncological outcome (394).

Non cytotoxic T lymphocytes – T regulatory cells (FOXP3+ expression)

T regulatory Cells (Tregs) are a heterogeneous group of T cells that play a role in the modulation of immune responses to foreign and self-antigenic material. The transcription factor FOXP3 is thought to be the most sensitive Treg cell marker. One small study in reported 2006 reported that FOXP3+ expression was not related to colorectal cancer outcome (395) (Table 1.9 and Table 1.13).

Non cytotoxic T lymphocytes – Memory T cells (CD45RO+ expression)

Memory T cells

The number of T cells reactive to a given antigen increases dramatically after priming then falls to a level 100-1000 fold higher than prior to priming. These long lived memory T cells express genes which control cell survival and have distinct surface molecule expression and distinct responses to stimuli. Memory CD8+ T cells are more sensitive to stimulation than naïve T cells and can be reactivated and become cytotoxic again after 24-48 hours. Memory CD4 cells have similar characteristics to effector CD4 T cells however require additional re-stimulation before acting on target cells. The main changes in cell surface molecule expression include loss of L seletin, increased CD44 expression and a change in the CD45 isoform from CD45RA to CD45RO. After re-exposure to antigen on an APC, memory T cells become armed effector T cells.

Between 2002 and 2007 three studies were identified in which CD45RO+ stained memory T lymphocytes were related to survival in primary operable colorectal

cancer (Table Table 1.9 and 1.14) (282, 391, 396). In all three of these studies increasing densities of CD45RO+ cells at both the tumour centre and invasive margin was associated with improved survival or reduced metastatic potential (282).

Cytotoxic T lymphocytes (CD8+ expression)

Of the various cell types assessed using immumohistochemistry, the most studied is the CD8+ T lymphocyte. Seventeen studies were identified between 1998-2007 in which the prognostic role of in situ CD8+ T cells was examined (Table 1.9 and Table 1.15) (320, 352, 353, 377, 379, 388, 389, 391-400). In 12 studies, increasing CD8+ T cell infiltrates at the tumour margin or tumour centre were significantly related to improved survival (352, 353, 377, 379, 391, 393, 394, 396-400). Five studies were identified which were contradictory (320, 388, 389, 392, 395), however trends toward improved survival were observed in two of these studies (320, 389). In three of the studies CD8+ infiltrates were independent prognostic factors irrespective of mismatch repair status (352, 399), however two further studies (353) reported significant survival relationships for CD8+ infiltrates in MMR proficient tumours only.

The first study to report the value of CD8+ infiltrations in colorectal cancer was that by Naito et al., in 1998 in which 139 patients with mature follow-up had a semi quantitative assessment of CD8+ cells within the cancer cell nests and stroma (397). On multivariate analysis cancer cell nest CD8+ was an independent factor. In a large study of 415 primary operable colorectal cancers, high CD8+ counts at both the tumour margin and tumour centre were related to improved survival (391). Combining the two counts improved the accuracy of prediction of outcome and was reported to be superior to TNM stage. The authors reported this in three independent cohorts.

Several studies reported that the prognostic value of CD8+ cells was increased by combining the analysis which that of another cell type or the characteristics of the tumour margin. Other cell types within or around the tumour which when combined with CD8+ assessment improved the prognostic value of included CD4+ cells and CD68+ cells (394, 398). A low ratio of CD8+ to CD4+ T cells was reported to correlate with survival by Diederichsen et al (394).

In the report by Prall and colleagues, in which 152 patients with stage III disease were studied, the positive effect on long-term survival associated with high CD8+ infiltration was most marked in the patients who received adjuvant chemotherapy (399).

Other cytotoxic lymphocytes - Natural Killer Cells (CD56+, CD57+, Vα24+ and CD161 expression)

Natural Killer cells have the same morphology as lymphocytes but do not bear T cell or B cell cell surface markers (CD3+ and CD4+). They make up 10-15% of circulating lymphocytes. They are not antigen specific or phagocytic and exert their effects through degranulation with release of toxins in response to viral infected cells and tumour cells. They are recognised by the cell surface molecules CD16 and CD56. NK cells can kill without cytokine activation but do proliferate in response to IL-2 stimulus. NK cells kill target cells through binding Fas on target cells to induce programmed cell death or apoptosis. They can also insert pores into target cells using perforin, which then polymerises on the cell surface of the target cell. These pores enable entry of proteolytic enzymes and encourage osmotic lysis of the target cell. CD16 is the Fc receptor for IgG and this enables NK cells to recognise and lyse IgG coated cells, a process known as antibody dependent cell mediated cytotoxicty.

Natural killer cells are a subset of T lymphocytes with cytoxic capacity. Four studies were identified in which the relationship between intratumoural natural killer cells and survival was assessed (Table 1.9 and Table 1.16) (370, 377, 393, 401). Two studies (370, 393) reported that extensive intratumoural NK cell infiltrates assessed with CD57 stains were independent prognostic factors. NK T cells stained using the V α24+ monoclonal antibody were also independent predictors of survival in the study by Tachibana et al (401).

The study by Nagtegaal et al. assessed NK infiltration with CD56 staining and whilst not related to survival, a relationship between higher NK cell infiltration and reduced recurrence was observed (377). Of note, in the study by Menon et al., CD56+ infiltrates were not prognostic whilst CD57+ staining was (393).

1.4.4.4 The prognostic value of tumour infiltrating B lymphocytes (CD20+ expression) in primary operable colorectal cancer

Mature B-lymphocytes develop from precursors originating from bone marrow and comprise 10-20% of the circulating lymphocytes population. B cells interact with antigens once they express surface immunoglobulin. They recognise antigen via the B lymphocyte antigen-receptor complex (BCR). The BCR antigen-binding component of the BCR comprises membrane bound forms of immunoglobulin D and immunoglobulin M and associated polypeptides (Igα and Igβ). On antigenic stimulation, the polypeptides transmit signals leading to B cell proliferation and differentiation. However as with the TCR, a second signal is also required for the BCR. The secondary signal comes from antibody specific T cells. This dependence on co stimulation with T cells prevents B cells from self-activating. The antigen binding with the B cell leads to uptake of antigen into the cell, processing into peptides and repackaging with antigen presenting MHC class II on the B cell surface. This brings antigen specific T cells into proximity along with B cells responding to the same antigen, a process known as linked recognition. Helper T cells interact with CD40 expressed on B cells and once activated, secrete cytokines, which drive B cell differentiation and proliferation (IL-4, TGF-β and IFN-γ).

B cells are play a predominant role in the humoral immune response and are responsible for both antigen presentation as well as antibody production. One study was identified which examined the role of B cell infiltrates in colorectal cancer and survival (Table 1.9 and Table 1.17) (389). A trend towards improved survival was observed for higher peri and intra-tumoural CD20+ counts although this was not significant. Further studies are therefore required to establish the prognostic value of intra-tumoural B cells in primary operable colorectal cancer.

1.4.4.5 The prognostic value of tumour associated macrophages CD68+ and CD133+ expression) in primary operable colorectal cancer

Monocytes circulate within the blood becoming tissue macrophages when they enter tissues. They migrate in response to chemotactic stimuli, phagocytose and destroy foreign pathogens or act as antigen presenting cells. Although they are cells of the innate immune response, macrophages play a key role in directing immune responses through secretion of over 100 factors including cytokines including tumour necrosis

factors α and β and interleukins (IL-1, IL-8 and IL-10) in addition to prostaglandins. One of the early roles of the macrophage is to release cytokines which initiate inflammation

Twelve previous studies have examined the role of tumour infiltrating macrophages in colorectal cancer (Table 1.9 and Table 1.18) (241, 277, 377, 383, 389, 390, 396, 398, 402-405). Eight of these studies; (241, 277, 377, 383, 390, 396, 402, 404) reported significant relationships between macrophages either at the tumour margin or tumour centre and survival. Five studies reported the strongest associations with survival were observed when macrophages at the invasive front were assessed (277, 383, 390, 402). Four studies of modest study size; 117 (Baeten et al), 94 (Funada et al), 40 (Nagorsen) and 22 (Inoue et al) reported no relationships between CD68+ and survival (389, 398, 403, 405). Forssell and colleagues (383) described a scoring method for CD68+ hotspots at the invasive margin. In 446 patients, increasing grade of macrophage infiltration at the tumour margin was independently associated with surival in colonic cancers and correlated with absence of metastases.

1.4.4.6 The prognostic value of tumour infiltrating polymorpho-nuclear cells/ Neutrophils (Elastase and CD16+ expression) in primary operable colorectal cancer

Neutrophils are cells of the innate immune system often involved in the early non-specific immune response. Four studies were identified in which the prognostic value of tumour infiltrating neutrophils were examined (Table 1.9 and Table 1.19) (277, 329, 377, 389). Higher neutrophilic infiltrates were associated with improved survival in three studies (277, 329, 389) along with other immune cell types including mast cells, eosinophils and lymphocytes. In the fourth study, neutrophil infiltrates were associated with reduced local recurrence but not improved survival (377).

In the study by Baeten and colleagues 117 patients with 12 year follow were examined (389). In addition to CD3 (T lymphocyte), CD 45 (Pan leukocyte), CD 8 (cytotoxic lymphocyte), and CD68 (Macrophages) staining, CD16 (polymorphonuclear neutrophils) were assessed. Significant survival relationships were observed for intratumoural or stromal CD16 and CD3+ cells. In addition to the study by Nagtegaal and colleagues (377), Klintrup et al. (277) reported increased

neutrophil grade at the invasive margin was an important prognostic factor although both studies reported a positive prognostic value of multiple other cell types concluding that a more general non-specific immune reaction was important.

1.4.4.7 The prognostic value of tumour infiltrating mast cells (CC1 an AA1 expression) in primary operable colorectal cancer

Six studies were identified in which the relationship between mast cell infiltration and survival in colorectal cancer was examined (Table 1.9 and Table 1.20) (329, 377, 404, 406-408). In five of these studies (329, 377, 404, 407, 408) high mast cell infiltration was related to improved survival. In the study by Pretlow and colleagues (1983), mast cell infiltration did not relate to survival, however, the maximum follow up post surgery was only 18 months (406). In the studies by Neilsen (n=564) (329) and Nagtegaal (n=160) (377), other intratumoural cell types such as lymphocytes, macrophages eosinophils and neutrophils were also examined. In both studies, high mast cell infiltrates were independently associated with improved survival. The study by Nagtegaal reported results for mast cells at the invasive margin (377). The other five studies reported results for intratumoural mast cell infiltrates.

1.4.4.8 The prognostic value of tumour infiltrating dendritic cells (CD1a, CD83, S100 and CD208 expression) in primary operable colorectal cancer

Dendritic cells are potent antigen presenting cells which play roles in both the innate and adaptive immune responses. Six published studies assessing the prognostic value of intra-tumoural dendritic cells were identified (403, 405, 409-412) (Table 1.9 and Table 1.21). Four studies observed improved survival or lower recurrence with higher dendritic cell counts or densities (403, 405, 409, 410). Conversely one study by Sandel et al., reported both increasing CD1a stained dendritic cells at the tumour margin and increasing CD208 cells in the tumour epithelium were associated with poorer survival (412). A further study observed no association with survival when dendritic cells were assessed at the margin or within the tumour stroma or epithelium (411). Further work is therefore required to establish the role of dendritic cell infiltrates in colorectal cancer.

1.4.4.9 The prognostic value of tumour infiltrating eosinophils in primary operable colorectal cancer

Eosinopils are not phagocytic but release cytotoxic granules in response to foreign pathogens. Six studies have examined the relationship between intratumoural eosinophils and survival in primary operable colorectal cancer between 1983 and 2005 (Table 1.9 and Table 1.22) (277, 329, 377, 406, 407, 413). All of these studies assessed eosinophillic infiltrates in haematoxylin and eosin stained specimens alone. In five of these studies (329, 377, 406, 407, 413), high eosinophil counts were significantly related to improved survival. In three studies eosinophil count was a stage independent indicator of survival (329, 377, 413). In four of these positive studies intratumoural eosinophils were related to survival, but in the study by Nagtegaal, peritumoural eosinophils were strongly related to cancer outcome (377). Klintrup and colleagues did not report a significant relationship with survival but did observe a trend towards better survival for peritumoural eosinophils (277).

1.4.4.10 Summary – The local inflammatory response

In summary there is good evidence that a pronounced inflammatory cell or immune cell reaction in and around colorectal tumours confers a good outcome (Table 1.9). Whilst the prognostic value of a generalized lymphocytic infiltrate or non-specific peritumoural inflammatory response is strongly related to survival based on 34 different studies, it is also apparent from more detailed analysis that that most individual immune cell types relate to recurrence and cancer specific survival. The evidence for the latter is particularly strong for T lymphocytes including cytotoxic T cells (CD8+) and other subgroups including memory T cells (CD45RO+) indicating the adaptive anti-tumour immune response plays a key role in determining cancer progression. However, in addition, inflammatory cell types characteristically associated with innate immune responses, for example macrophages, dendritic cells and neutrophils appear to have a role in predicting cancer outcomes.

Taken together, the type, density and location of tumour inflammatory cell infiltrate appear to be particularly pertinent to recurrence and survival in patients with colorectal cancer. Unfortunately, much of the work carried out has not compared different methods of tumour inflammatory cell infiltrate assessment. As a consequence, despite the large amount of work carried out there remains uncertainty

as to which parameters of tumour inflammatory cell infiltrate are most closely associated with cancer outcome. Therefore, in order to establish routine clinical utility there is now a need to rationalise this prognostic information, published over a 40 year period, into a standardized assessment of tumour inflammatory cell infiltrate in patients with colorectal cancer. A logical starting point for such rationalization would be to use a general assessment of the tumour inflammatory infiltrate such as the Jass or Klintrup-Makinen criteria and to compare the prognostic value of other tumour inflammatory infiltrate assessments with this standard. Finally, such standardization will also have the potential to inform novel therapeutic interventions.

At present it is not clear which of the inflammatory responses, local or systemic is most important in determining cancer progression. Furthermore, it is not known whether the two responses are directly or indirectly related. Given the paradoxical prognostic value of high-grade systemic inflammation compared with high-grade local inflammation, one hypothesis would be that there is a clear inverse relationship between the two.

Table 1.9: Summary table: Studies reporting the relationships between the local inflammatory response and survival in colorectal cancer.

Measure of Local Inflammatory Response	Total Number of studies	Number of studies reporting significant survival relationship	Number of studies reporting no survival relationship
Peritumoural lymphocytic infiltrate	33	29	4
T lymphocytic infiltrates			
CD3+ expression	6	4	2
CD4+ expression	5	1	4
FOXP3+ expression	1	0	1
CD45RO+ expression	3	3	0
CD8+ expression	17	12	5
Natural Killer cell infiltrates (CD56+, CD57+, Vα24+ and CD161+)	4	3	1
B lymphocytic infiltrates (CD20+)	1	0	1
Tumour infiltrating macrophages (CD68+ and CD133+)	12	8	4
Neutrophilic infiltrates (CD16+ and elastase)	4	3	1
Mast cell infiltrates (CC1 and AA1)	6	5	1
Dendritic cell infiltrates (CD1a, CD83, S100 and CD208)	6	4	2
Eopsinophilic infiltrates	6	5	1
All meaures of local inflammatory response	**104**	**77**	**27**

Table 1.10: The prognostic value of a generalised lymphocytic/ inflammatory cell infiltrate in primary operable colorectal cancer.

Author	Year	N	Tumours	Assessment	Location	Comments
Spratt et al	1967	1137	Stage I-IV	Inflammatory reaction around the tumour	Peritumoural	First report of intense peritumoural inflammatory reaction associated with improved survival
Murray et al	1975	148	Colonic resections	Inflammatory cell reaction	Peritumoural	Intense local inflammatory reaction associated with improved survival.
Zamcheck et al	1975	40	Colonic resections	Lymphocytic and plasma cell infiltration	Intratumoural Peritumoural	Intense lymphocytic infiltrate associated with improved survival.
Watt et al	1978	48	Stage II-III	Quantitative lymphocyte number	Invasive margin	Higher lymphocyte numbers present in Dukes' B compared with Dukes' C disease.
House et al	1979	107	Stage I-III	Quantitative peritumoural lymphocyte numbers	Invasive margin	Lymphocytic immune reaction associated with improved 3-year survival.
Thynne et al	1980	92	Stage III colon	Immune reaction	Invasive margin	Immune reaction not associated with survival.
De Mascarel et al	1981	82	Stage I-III	Lympho-plasmocytic infiltration	Tumour margin Tumour centre	Intense lympho-plasmocytic infiltration associated with improved survival.
Xhou et al	1983	1226	Stage I-IV	Semi-quantitative lymphocytic infiltration	Tumour margin Tumour centre	Intense lymphocytic infiltration associated with improved survival.
Svennevig et al	1984	100	Stage II	Quantitative mononuclear cells	Peritumoural Intratumoural	Higher lymphoid infiltrates associated with improved 5 year survival.
Carlon et al	1985	124	Stage I-III rectal	Semi-quantitative lymphocytic infiltration	Tumour margin Tumour centre	Intense lymphocytic infiltration associated with improved survival.
Jass et al	1986	447	Rectal resections	Semi-quantitative lymphocytic infiltration	Tumour margin	Intense lymphocytic infiltrate was associated with improved 10-year survival.
Jass et al	1987	379	Rectal resections	Lymphocytic infiltrate	Tumour margin	Intense lymphocytic infiltrate was a stage independent factor in rectal cancer and a factor in a novel prognostic model.

Halvorsen et al	1989	534	Colorectal resections	Semi-quantitative inflammatory cell reaction	Invasive margin	Intense peritumoural inflammation was a stage independent predictor of improved survival.
Adachi et al	1989	117	Colorectal resections	Semi-quantitative inflammatory cell reaction	Tumour margin	Intense peritumoural inflammatory reaction associated with 10-year survival.
Graham et al	1990	100	Stage I-III	Crohn's like reaction	Tumour margin	Crohn's like reaction associated with improved survival
Di Giorgio et al	1992	361	Stage I-II	Peritumoural lymphocytic infiltrate	Tumour margin	Intense lymphocytic infiltrate associated with earlier stage disease and improved 10-year survival.
Kubota et al	1992	100	Stage I-IV	Semi-quantitative lymphocytic infiltrate	Invasive margin	Intense lymphocytic infiltrate was a stage independent predictor of improved survival.
Harrison et al	1994	385	Stage I-III rectal cancer	Semi-quantitative Jass criteria and Crohns like reaction	Invasive margin	Intense Jass peritumoural lyphocytic infiltrate and crohns like reaction were associated with improved survival. Only Crohns like reaction was an independent prognostic factor.
Coca et al	1997	157	Stage I-III	Semi-quantitative Jass criteria	Intra-tumoural	Peritumoural lymphocytic infiltrate was a stage independent prognostic factor.
Cianchi et al	1997	235	Stage I-III	Semi-quantitative Jass criteria	Invasive margin	No association with survival.
Adams et al	1997	42	Stage I-IV	Semi-quantitative Jass criteria and Crohn's like reaction	Invasive margin	Jass criteria and Crohn's like reaction associated with improved survival.
Ropponen et al	1997	276	Stage I-IV	Semi-quantitative tumour infiltrating lymphocytes	Tumour margin Tumour centre	Increased tumour infiltrating lymphocytes associated with earlier stage and improved survival.
Diaz et al	1998	292	Stage II-III	Semi-quantitative lymphocytic infiltrate	Tumour margin	Intense lymphocytic infiltrate was a stage independent predictor of improved survival.

Kelly et al	1999	125	Stage I-IV	Semi-quantitative Jass criteria	Invasive margin	Intense peritumoural infiltrate was associated with improved survival.
Nielsen et al	1999	584	Stage I-IV	Computerised quantitative white cells, lymphocytes, neutrophils, plasma cells and eosinophils	Submucosal region	Intense infiltrate of white cells, lymphocytes, neutrophils and plasma cells all associated with improved survival.
Murphy et al	2000	415	Stage II	Semi-quantitative Jass criteria and Crohn's like reaction	Invasive margin	Intense lymphocytic infiltrate and Crohn's like reaction associated with improved survival in Stage II disease.
Nagtegaal et al	2001	1416	Stage I-IV rectal cancer	Semi-quantitative Jass criteria, eosinophils and all cell types in 160 patients.	Invasive margin	Intense lymphocytic infiltrate was associated with improved outcomes (recurrence and survival) a higher survival and lower recurrence. Intense eosinophilic was associated with improved survival.
Cianchi et al	2002	84	Stage I-II rectal cancer	Semi-quantitative lymphocytic infiltrate	Invasive margin	Lymphocytic infiltrate was not associated with survival.
Chiba et al	2004	371	Stage I-IV	Semi-quantitative lymphocytic infiltrate. Quantitative assessment of CD8+ T-lymphocytes	Invasive margin Cancer cell nests	Intense CD8+ T-lymphocytes in cancer nests associated with peritumoural lymphocytic infiltrate and associated with improved survival.
Buckowitz et al	2005	120	Stage I-IV HNPCC	Semi-quantitative peritumoural lymphoid reaction/ Crohn's like reaction	Invasive margin	Microsatellite unstable tumours were associated with a Crohn's like reaction (CLR). CLR and MSI-H were associated with earlier stage disease and improved survival.

Klintrup et al	2005	386	Stage I-IV	Semi-quantitative inflammatory cell reaction Lymphocyte, eosinophil, neutrophil, macrophage and Crohn's like reaction	Invasive margin Tumour centre	Intense lymphocytic infiltrate in tumour centre associated with survival. At the invasive margin, intense inflammatory infiltrate, neutrophil, lymphocyte and macrophage infiltrate were all associated with improved survival but only intensive inflammatory infiltrate at the invasive margin was independent factor.
Gao et al	2005	301	Stage I-IV	Semi-quantitative inflammatory cell reaction (similar to Nagtegaal and Jass methods).	Invasive margin Tumour centre	Intense inflammatory cell reaction at both the tumour margin and centre were associated with MSI and associated with improved survival.
Szynglarewicz et al	2007	45	Stage II-III rectal cancer	Semi-quantitative Jass criteria	Invasive margin	Intense lymphocytic infiltrate was associated with improved survival in univariate but not mulitivariate analysis.
Forssell et al	2007	446	Stage I-IV	Semi-quantitative CD68+ T-lymphocyte and lymphocytic infiltrate	Invasive margin	Intense macrophage infiltrate associated with early stage and lymphocytic infiltrate with survival.

Table 1.11: The prognostic value of tumour infiltrating CD3+ T cells in primary operable colorectal cancer.

Author	Year	N	Tumours	Analysis	Location	Conclusion
Nagtegaal et al	2001	160	Stage I-III rectal cancer	Semi-quantitative Jass criteria, eosinophils and all cell types including CD3 in 160 patients.	Invasive margin Intratumoural	High CD3+ counts were related to lower T stage and N stage. High intratumoural CD3+ counts related to survival on univariate analysis.
Guidoboni et al	2001	109	Stage II-III right sided colon cancers	Quantitative assessment	Tumour epithelium	Higher counts of intra-epithelial cells expressing CD-3+, CD8+ and granzyme B were stage independent predictors of improved survival, independent of MSI status.
Takemoto et al	2004	125	Stage II-III	Quantitative assessment of CD3, CD4 and CD8 staining	Intra-tumour cell Tumour stroma	Higher stromal and tumour cell infiltrating lymphocytes were associated with better survival although non-significant.
Baeten et al	2006	117	Stage I-IV	Quantitative assessment in 4 random fields at each site	Peritumoural Intra-tumoural Tumour stroma	High intratumoural and stromal CD3+ counts related to improved survival on univariate analysis.
Lackner et al	2004	70	Stage II-III	Quantitative assessment of CD3 and CD68	Invasive margin Tumour centre	CD3 counts were not related to survival.
Galon et al	2006	415	Stage I-III	Quantitative scoring using tissue micro arrays with an image analysis workstation	Invasive margin Tumour centre	Patients with high CD3+ densities at invasive margin (IM) and tumour centre (CT) had reduced recurrence and improved survival. If CD45RO and CD3+ were low, prognosis was very poor (shown in 3 cohorts).

Table 1.12: The prognostic value of tumour infiltrating CD4+ T cells in primary operable colorectal cancer.

Author	Year	N	Tumours	Analysis	Location	Conclusion
Nagtegaal et al	2001	160	Stage I-III rectal cancer	Semi-quantitative Jass criteria, eosinophils and all cell types including CD4 in 160 patients.	Invasive margin Intratumoural	Low CD4+ cell counts correlated with increased depth of invasion. Intratumoural CD4+ counts related to reduced local recurrence but was not related to survival.
Diederichsen et al	2003	70	Stage I-IV	Quantitative assessment using flow cytometry for CD4 and CD8	Flow cytometry of dissociated tumour tissue)	Increasing CD8:CD4+ ratio was an independent predictor of improved survival.
Takamoto et al	2004	125	Stage II-III	Quantitative assessment of CD3, CD4 and CD8.	Intra-tumour cell Tumour stroma	Higher stromal and tumour cell infiltrating lymphocytes were associated with better survival although non-significant.
Ali et al	2004	80	Stage II-III	Quantitative analysis	Intratumoural	Low intratumoural CD4+ count was associated with early recurrence.
Menon et al	2004	93	Stage II-III	Quantitative assessment of CD4, CD8, CD56 and CD57 intra tumourally. Semi-quantitative for stroma and margin – according to Naito criteria	Intra-tumoural Tumour stroma Invasive margin	CD4+ cell counts were not related to survival in any of the regions assessed.
Canna et al	2005	147	Stage I-III	Quantitative analysis	Intratumoural	Reduced tumour CD4+ infiltrates were related to poorer survival (not independent of stage or systemic inflammation)

Table 1.13: The prognostic value of FOXP3+ T regulatory cells in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Loddenkemper et al	2006	40	Stage I-IV	Quantitative assessment of tumour sections and normal colonic tissue	Tumour epithelium Tumour stroma Normal colon	FOXP3+ expression was not related to survival. A weak survival relationship was seen only after stage correction in 'post hoc' analysis (P=0.04). No survival relationships seen for CD8+/Treg ratio.

Table 1.14: The prognostic value of effector memory T cells (CD45RO+) in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Oberg et al	2002	93	Stage III	Quantitative assessment of CD 45RO+, CD8+, CD68+	Lymph node metastases	Higher counts for CD8, CD45RO and CD68 all led to a better cancer specific survival. CD45RO correlated with CD8 and CD68.
Pages et al	2005	415	Stage I-IV	Semi-quantitative assessment of immune cell infiltrates using (scored weak/mod/strong). Quantitative analysis of cell densities using tissue microarrays and image analysis workstation.	Invasive margin Tumour centre	Strong immune cell infiltrates were associated with fewer signs of metastatic potential. High densities of CD45RO+ cells had better survival and fewer indicators of early metastatic potential. CD45RO+ status was a stage independent prognostic factor.
Galon et al	2006	415	Stage I-III	Quantitative scoring using tissue micro arrays with an image analysis workstation	Invasive margin Tumour centre	Patients with high CD45RO+ densities at invasive margin (IM) and tumour centre (CT) had reduced recurrence and improved survival. If CD45RO and CD3+ were low, prognosis was very poor (shown in 3 cohorts).

Table 1.15: The prognostic role of tumour infiltrating CD8+ T cells in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Naito et al	1998	139	Stage I-IV	Semi-quantitative analysis (none/mild/moderate/ severe)	Invasive margin Cancer stroma Cancer cell nests	On multivariate analysis, cancer cell nest CD8+ count was a stage independent prognostic factor.
Nagtegaal et al	2001	160	Stage I-III rectal cancer	Semi-quantitative assessment Jass criteria and eosinophilic infiltrates. CD8 staining assessed in 160 patients.	Invasive margin Intratumoural	High intratumoural and peritumoural CD8+ counts were related to reduced development of distant metastases and improved survival on univariate analysis.
Guidoboni et al	2001	109	Stage II-III right sided colon cancers	Quantitative assessment	Tumour epithelium	Higher counts of intra-epithelial cells expressing CD-3+, CD8+ and granzyme B were stage independent predictors of improved survival independent of MSI status.
Oberg et al	2002	93	Stage III	Quantitative assessment of CD45RO+, CD8+, CD68+	Lymph node metastases	Higher counts for CD8, CD45RO and CD68 all led to a better cancer specific survival.
Funada et al	2003	97	Stage I-III	Quantitative assessment of the area of most abundant infiltration at invasive margin	Invasive margin	CD8+ alone was related to improved survival.
Diederichsen	2003	70	Stage I-IV	Quantitative assessment using flow cytometry for CD4 and CD8	Flow cytometry of dissociated tumour tissue	Increasing CD8:CD4+ ratio was an independent predictor of improved survival.
Ali et al	2004	80	Stage II-III	Quantitative analysis with 30 fields	Intratumoural	CD8+ counts did not relate to recurrence.
Takamoto et al	2004	125	Stage II-III	Quantitative assessment of CD3, CD4 and CD8.	Intra-tumour cell Tumour stroma	Higher stromal and tumour cell infiltrating lymphocytes were associated with better survival although non-significant. Intra-tumoural CD8+ related to less lympho-vascular invasion.

Menon et al	2004	93	Stage II-III	Quantitative assessment of CD4, CD8, CD56 and CD57 intra tumourally. Semi-quantitative for stroma and margin – (Naito criteria)	Intra-tumoural Tumour stroma Invasive margin	High tumour margin CD8+ and CD57+ cell counts were stage independent prognostic factors.
Prall et al	2004	152	Stage III	Quantitative assessment of CD8+	Intratumoural	High CD8+ density was an independent prognostic factor for better survival (most pronounced in patients receiving adjuvant chemotherapy). Independent of MSI status.
Baeten et al	2004	117	Stage I-IV	Quantitative assessment	Peritumoural Intra-tumoural Tumour stroma	High intratumoural CD8+ related to survival but not significant.
Chiba et al	2004	371	Stage I-IV	Semi-quantitative assessment of peritumoural lymphocytic infiltrate. Quantitative assessment of CD8+ in cancer cell nests	Invasive margin Cancer cell nests	CD8+ related to presence of peritumoural lymphocytic infiltrate (PTL) and early stage. PTL and high intra-epithelial CD8+ count related to survival. CD8+ was a stage independent factor.
Canna et al	2005	147	Stage I-III	Quantitative analysis	Intratumoural	CD8+ showed a trend toward improved survival but not significant.
Loddenkemper et al	2006	40	Stage I-IV	Quantitative assessment of tumour sections and normal colonic tissue	Tumour epithelium Tumour stroma Normal colon	CD8+ weakly related to better survival in all areas however not significant. No survival relationships seen for CD8+/Treg ratio.
Oshikiri et al	2006	146	Stage I-IV	Semi-quantitative analysis, using techniques described by Naito et al (none/mild/moderate/ severe)	Tumour stroma Cancer cell nest	Low CD8+ expression was associated with poor survival on multivariate analysis.

Galon et al	2006	415	Stage I-III	Quantitative scoring using tissue micro arrays with an image analysis workstation	Invasive margin Tumour centre	Patients with high CD8+ densities at invasive margin (IM) and tumour centre (CT) had reduced recurrence and improved survival. Combining the analysis from the CT and IM improved accuracy of prediction (shown in 3 independent cohorts).
Baker et al	2007	1420	Stage I-III	Quantitative assessment using tissue micro arrays	Intraepithelial	For both MMR proficient and deficient tumours higher infiltrate of CD8+ was associated with favourable prognostic features. A survival advantage was only seen in MMR proficient tumours.

Table 1.16: The prognostic value of tumour infiltrating natural killer cells in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Coca et al	1997	157	Stage I-III	Quantitative assessment of CD57 staining	Intra-tumoural	Extensive NK cell infiltrate related to improved disease free and overall survival on multivariate analysis.
Nagtegaal et al	2001	160	Stage I-III rectal cancers	Semi-quantitative assessment Jass criteria and eosinophilic infiltrates. Cellular composition assessed quantitatively in 160 patients using CD56.	Invasive margin Intratumoural	Intratumoural natural killer cells were related to reduced local recurrence.
Menon et al	2004	93	Stage II-III	Quantitative assessment of CD4, CD8, CD56 and CD57 intra tumourally – semi-quantitative for stroma and for margin – according to Naito criteria	Intra-tumoural Tumour stroma Invasive margin	High tumour margin CD8+ and CD57+ cell counts were stage independent prognostic factors.
Tachibana et al	2005	103	Stage I-III	Quantitative assessment using monoclonal antibodies TCR-V α24, TCR-Vβ11, CD56, CD57 and CD69 and CD161	Intratumoural	An increased intratumoural V α24+ NKT cell count was an independent prognostic factor for survival.

Table 1.17: The prognostic value of B-lymphocytes in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Baeten et al	2004	117	Stage I-IV	Quantitative assessment	Peritumoural Intra-tumoural Tumour stroma	High intratumoural CD20+ counts showed a trend towards improved survival but did not reach significance on univariate analysis

Table 1.18: The prognostic value of tumour associated macrophages in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Nagtegaal et al	2001	160	Stage I-III rectal cancers	Semi-quantitative Jass criteria, eosinophils and all quantitative assessment of CD68 staining in 160 patients.	Invasive margin Intratumoural	Intratumoural macrophages were associated with reduced recurrence and improved survival.
Oberg et al	2002	93	Stage III	Quantitative assessment of CD45RO+, CD8+, CD68+.	Regional lymph node metastases	Higher CD8, CD45RO and CD68 all led to improved survival. CD68 was not related to survival in patients receiving adjuvant treatment.
Nakayama et al	2002	30	Stage I-IV	Quantitative assessment of CD68 stained cells	Invasive Front Tumour stroma	Higher CD68 counts at the invasive front were associated with reduced recurrence.
Khorana et al	2003	131	Stage II-III	Quantitative assessment of CD68 staining	Tumour stroma Tumour epithelium	Higher CD68 counts were related to survival but were not independent of other factors on multivariate analysis.
Funada et al	2003	97	Stage I-III	Quantitative assessment of the area of most abundant infiltration at the invasive margin	Invasive margin	Low macrophage infiltration correlated with earlier stage. Patients with both high CD8+ and CD68+ infiltrates had an excellent survival. CD68 alone not related to survival.
Lackner et al	2004	70	Stage II-III	Semi-quantitative assessment of CD3 and CD68	Tumour margin Tumour centre	Higher CD68 count at the invasive margin was independently related to survival. CD68 in the tumour centre did not.
Baeten et al	2004	117	Stage I-IV	Quantitative assessment of CD68 staining	Peritumoural Intra-tumoural Tumour stroma	No survival relationships seen for CD68+ counts in any area

Inoue et al	2005	22	Stage I-III	Semi-quantitative assessment of CD68 and CD83 staining	Tumour stroma	No significant relationship between CD68 density and survival observed
Tan et al	2005	60	Stage I-IV	Quantitative assessment using CD68	Intratumoural	Low CD68 infiltration related to higher T stage, N stage and distant metastases. High CD68 counts were associated with improved survival. A combination of a high macrophage and mast cell count is an excellent prognostic factor.
Klintrup et al	2005	386	Stage I-IV	Semi-quantitative inflammatory cell reaction Lymphocyte, eosinophil, neutrophil, macrophage and Crohn's like reaction CD 68 staining undertaken in 20 cases.	Invasive margin Tumour centre	At the invasive margin, overall inflammation, increased neutrophil grade, lymphocytes and macrophages were all related to improved survival. The other cell types lost significance when macrophages were removed implying a strong correlation. Macrophages were significant prognostic factors when the other cell types were removed.
Forssell et al	2007	446	Stage I-IV	Semi-quantitative assessment if CD68+ cell staining and peritumoural lymphocytic infiltrate at the invasive front	Invasive margin	High macrophage counts correlated with early stage and presence of a peritumoural lymphocytic infiltrate. High CD68+ count was an independent prognostic factor.
Nagorsen et al	2007	40	Stage I-IV	Quantitative assessment of 9 markers of dendritic cells and macrophages including CD68 and CD163	Tumour stoma Tumour epithelium	High stromal CD163 macrophages associated with improved survival. CD68 counts were not related to survival.

Table 1.19: The prognostic role of neutrophils /polymorphonuclear cells in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Nielsen et al	1999	584	Stage I-IV	Computerised quantitative white cells, lymphocytes, neutrophils, plasma cells and eosinophils	Submucosal region	Total white cells, lymphocytes, neutrophils and plasma cells were all significant on univariate survival analysis.
Nagtegaal et al	2001	160	Stage I-III rectal cancers	Semi-quantitative Jass criteria, eosinophils and all cell types in 160 patients. Quantitative assessment in 160 patients using elastase staining.	Invasive margin Intratumoural	Peritumoural neutrophils related to reduced local recurrence. No other prognostic relationships observed.
Baeten et al	2004	117	Stage I-IV	Quantitative assessment	Peritumoural Intra-tumoural Tumour stroma	High intratumoural CD16+ counts related to improved survival.
Klintrup et al	2005	386	Stage I-IV	Semi-quantitative inflammatory cell reaction Lymphocyte, eosinophil, neutrophil, macrophage and Crohn's like reaction.	Invasive margin Tumour centre	In addition to an increase in overall inflammation including infiltrating lymphocytes and macrophages, higher neutrophil grade was related to improved survival when assessed at the invasive margin.

Table 1.20: The prognostic value of intratumoural mast cells in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Pretlow et al	1983	67	Stage I-III	Quantitative assessment using Giemsa staining to aid counting of mast cells and eosinophils	Tumour centre	Mast cell counts not related survival in this study with a maximum of 18 months follow up post surgery.
Fisher et al	1989	331	Stage I-III rectal cancer	Quantitative assessment of intratumoural eosinophils and mast cells	Intratumoural	High mast cell counts were stage independent predictors of improved survival.
Nielsen et al	1999	584	Stage I-IV	Computerised quantitative white cells, lymphocytes, neutrophils, plasma cells and eosinophils in H&E stained tissues and AA1 staining for mast cell tryptase separately.	Submucosal region	High eosinophils and mast cells counts were stage independent prognostic factors. Total white cells, lymphocytes, neutrophils and plasma cells were all significant on univariate survival analysis.
Nagtegaal et al	2001	160	Stage I-III rectal cancers	Semi-quantitative assessment for lymphocytic infiltrate (Jass criteria) and eosinophilic infiltrates. Quantitative assessment in 160 patients using AA1 antibodies.	Invasive margin Intratumoural	Peritumoural mast cells were an independent predictor of reduced local and distant recurrence. Higher peritumoural mast cell counts related to improved survival on univariate analysis only.
Acikalin et al	2005	60	Stage I-IV	Quantitative assessment using Giemsa blue staining	Intratumoural	Mast cells counts were related to poorer survival on univariate but not multivariate analysis.
Tan et al	2005	60	Stage I-IV	Quantitative assessment using CC1 and AA1 antibodies	Intratumoural Normal colon	Low mast cell infiltration related to higher T stage, N stage and distant mets. High mast cell counts were associated with improved survival.

Table 1.21: The prognostic value of intratumoural dendritic cells in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Ambe et al	1989	121	Stage I-IV	Quantitative assessment of S-100 staining	Invasive margin Tumour centre	Patients with high S-100 counts had longer survival. High S-100 counts related to lymphocytic infiltrate.
Nakayama et al	2003	30	Stage I-IV	Quantitative assessment of S100 staining	Tumour periphery	S100 counts were lower with increasing T stage, lymph node involvement and hepatic metastases. Low S100 counts were related to early recurrence and poor survival.
Dadabayev et al	2004	104	Stage II and III	Quantitative assessment of S-100 staining	Invasive margin Tumour stroma Tumour epithelium	No significant relationship was observed for S100 counts and survival at any location.
Inoue et al	2005	22	Stage I-III	Semi quantitative assessment of CD68 and CD83 staining	Tumour stroma	Increasing CD83 density related to improved survival
Sandel et al	2005	104	Stage II and III	Quantitative assessment a range of dendritic cell markers including CD1a and CD208	Invasive margin Tumour stroma Tumour epithelium	Higher CD208 counts in tumour epithelium and CD1a at the invasive related to shorter survival. Both were independent factors on multivariate analysis.
Nagorsen et al	2007	40	Stage I-IV	Quantitative assessment of 9 markers of dendritic cells and macrophages including S-100, CD1a, CD11c, CD123	Tumour stroma Tumour epithelium	High stromal and epithelial S100 dendritic cell infiltration was associated with significantly better survival.

Table 1.22: The prognostic value of tumour infiltrating eosinophils in primary operable colorectal cancer

Author	Year	N	Tumours	Analysis	Location	Conclusion
Pretlow et al	1983	67	Stage I-III	Quantitative assessment using Giemsa staining	Tumour centre	High eosinophil counts were related to absence of lymph node metastases and improved survival
Fisher et al	1989	331	Stage I –III rectal cancers	Quantitative assessment of intratumoural eosinophils and mast cells	Intratumoural	High eosinophil counts related to improved survival but not stage independent.
Nielsen et al	1999	584	Stage I-IV	Computerised quantitative assessment. Cell types counted included white cells, lymphocytes, neutrophils, plasma cells and eosinophils in H&E stained tissues.	Submucosal region	High eosinophils and mast cells counts were stage independent prognostic factors. Total white cells, lymphocytes, neutrophils and plasma cells were all significant on univariate survival analysis.
Fernandez-Acenero et al	2000	126	Stage I-III	Quantitative assessment using H&E stained specimens alone.	Intratumoural	High eosinophil counts are a stage independent predictor of improved survival.
Nagtegaal et al	2001	1416	Stage I-IV	Semi-quantitative assessment Jass criteria and eosinophilic infiltrates. Cellular composition assessed with immunohistochemistry in 160 patients.	Invasive margin Intratumoural	High peritumoural eosinophils are a stage independent predictor of reduced recurrence and improved survival.
Klintrup et al	2005	386	Stage I-III	Semi-quantitative inflammatory cell reaction Lymphocyte, eosinophil, neutrophil, macrophage and Crohn's like reaction	Invasive margin Tumour centre	Eosinophilic infiltrates at the invasive margin showed a trend towards better survival but this was not significant.

2.0 SUMMARY AND AIMS

2.1 SUMMARY

Colorectal cancer is the second commonest cause of cancer death in Europe and North America. Even with modern treatments, almost half of patients who undergo curative surgery die before 5 years. It is increasingly recognised that disease progression and survival in colorectal cancer is determined by a range of factors including both host and tumour characteristics.

A variety of tumour characteristics have been associated with recurrence and poorer cancer specific survival. These include pathological features and molecular markers. In routine clinical practice conventionally only high-risk pathological characteristics are considered when planning treatment and follow up. In addition to TNM stage, high-risk features include venous invasion, serosal involvement, margin involvement and tumour perforation. However, It is also apparent that the frequency of reporting for some of these characteristics varies considerably in the published literature. This is especially true for venous invasion. Indeed, several methods to optimise the reporting of venous invasion have been described. The clinical implications of such attempts to improve the reporting of venous invasion have not yet been explored. For example, it is not clear whether increased identification of venous invasion will improve the predictive value of venous invasion for disease recurrence and survival.

Host characteristics, in particular the systemic inflammatory response, are important determinants of outcome in colorectal cancer. Elevated markers of systemic inflammation are consistently reported to confer a poorer outcome independent of tumour stage. A variety of scores have been proposed to measure the systemic inflammatory response in cancer patients including the Glasgow Prognostic Score, the neutrophil lymphocyte ratio and the platelet lymphocyte ratio. It is unclear which of these scores in most predictive of outcome in primary operable colorectal cancer. The prognostic value of each of these scores in addition to other haematological or biochemical markers of the systemic inflammatory response has not previously been examined in a single cohort. Furthermore, it is not clear whether the systemic

inflammatory response predicts survival independent of more detailed high-risk pathological characteristics including the Petersen Index or the lymph node ratio.

In addition to the host systemic inflammatory response, it is also apparent that the presence or absence of a local inflammatory response is also a determinant of colorectal cancer outcome. The local inflammatory response may represent the host anti-tumour immune response and where present, high-grade local inflammation is associated with improved colorectal cancer survival. At present, no standardised method of assessment of the local tumour inflammatory response exists. Most previous studies have examined individual immune cell types using immunohistochemistry. Of the other methods of assessment, the Jass classification has been criticised due to apparent subjectivity and difficulty reproducing results. Several groups have proposed simpler methods of assessment but such scores have not been validated (e.g. Klintrup et al.). Furthermore, it remains to be seen whether assessment of the local inflammatory cell response provides additional prognostic information to modern pathological assessment of high-risk tumour characteristics including the Petersen Index.

The underlying basis of the systemic inflammatory response has not been elucidated. The presence of a systemic inflammatory response has been associated with a range of benign conditions including cardiovascular disease. Increasing burden of comorbidity is related to poorer colorectal cancer outcome and one hypothesis is that high grade systemic inflammation reflects overall host burden of comorbidity. A second hypothesis is that a high-grade systemic inflammatory response is related to local tumour inflammation. These hypotheses have not previously been tested.

The potential relationships between the local and systemic inflammatory responses in colorectal cancer are unknown. There are a number of circulating and local tumour factors previously associated with colorectal cancer survival that may relate to both local and systemic inflammatory responses. For example, a variety of circulating factors involved in immune responses including cytokines, immunoglobulins and immune cells may link the local and systemic inflammation. Furthermore, local tissue factors including tumour infiltrating immune cells (e.g. helper and cytotoxic T cells, macrophages), tumour proliferation indices (Ki-67) may

link the two inflammatory responses. Finally, these responses may relate to important vitamin anti-oxidants and other micronutrients including carotenoids and vitamin D.

In current clinical practice treatment decisions are based on tumour characteristics with little consideration given to host characteristics other than age or comorbidity. In the allocation of adjuvant chemotherapy, prognostic scores such as the Adjuvant! or Numeracy online models have been proposed, however these models lack prospective validation in other cohorts. Furthermore it is not clear whether host characteristics including the local and systemic inflammatory responses may offer additional important prognostic information following provision of adjuvant chemotherapy.

With the introduction of bowel screening, it is likely that clinicians will treat an increasing proportion of early stage or node negative disease. At present controversy exists with regard to the allocation of adjuvant chemotherapy to such patients. Currently, those with high-risk tumour characteristics are offered treatment. However with the optimisation of pathological reporting of venous invasion, it is not clear which factors including tumour and host characteristics are most important in the modern day treatment of node negative colorectal cancer.

2.2 AIMS

In order to examine the above areas of uncertainty, in patients undergoing surgery for colorectal cancer, studies were carried out:

1. To examine the impact of elastica staining on the value of venous invasion as a predictor of cancer specific survival (including in node negative disease).

2. To perform a comprehensive comparison of the prognostic value of tumour and patient factors including the Glasgow Prognostic Score, neutrophil lymphocyte ratio and platelet lymphocyte ratio

3. To compare the prognostic value of the tumour-based (Petersen Index) and inflammation-based (Glasgow Prognostic Score) scoring systems.

4. To compare the prognostic value of lymph nodes sampled, N stage, lymph node ratio and the systemic inflammatory response.

5. To examine the relationships between pre-operative comorbidity assessed with four separate comorbidity scores (Charlson comorbidity index, NIA/NCI comorbidity index, ACE-27 comorbidity index and Lee cardiac risk index) and the systemic inflammatory response (mGPS).

6. To examine the prognostic value of a simple assessment of the tumour inflammatory infiltrate (Klintrup-Makinen Score) in addition to routinely reported tumour pathological criteria including the Petersen Index.

7. To examine the relationships between the local inflammatory response (Jass and Klintrup-Makinen criteria) and systemic inflammatory response (GPS criteria) and to compare their prognostic value.

8. To examine relationships between tumour stage, systemic and local inflammatory responses and circulating immunological parameters, including cytokines, circulating immune cells and immunoglobulins.

9. To examine the relationships between the local and systemic inflammatory responses, tissue factors including immunohistochemical assessment for tumour infiltrating immune cells (CD3, CD4, CD8 and CD68), the Ki-67 proliferative index, and survival.

10. To examine the relationship between TNM stage, the systemic and local inflammatory responses and circulating vitamins A, D, E, carotenoids, and malondialdehyde.

11. To examine the prognostic value of tumour and patient related factors currently included in the standard Numeracy and Adjuvant! models together with measures of the local and systemic inflammatory responses in patients receiving adjuvant 5-FU based chemotherapy.

3.0 ELASTICA STAINING FOR VENOUS INVASION RESULTS IN SUPERIOR PREDICTION OF CANCER SPECIFIC SURVIVAL IN COLORECTAL CANCER.

3.1 Introduction

Colorectal cancer is the second most common cause of cancer death in Western Europe and North America. Overall survival is poor; even in those who undergo resection with curative intent only half survive five years (177, 414). Currently, prediction of outcome and consequent allocation of adjuvant treatment is mainly determined by clinico-pathological criteria. The main factors are the extent of local spread and lymph node status, the basis of the Dukes' and TNM staging systems (182, 188). Other important pathologically determined 'high-risk' criteria which provide stage-independent prognostic information following colorectal cancer resection have also been identified (195).

In particular, venous invasion is recognised to be an important high-risk feature in colorectal tumours (195-201). Indeed, the minimum data set requirements, as set by the Royal College of Pathologists, state that the frequency of detection for venous invasion should be at least 25% (188). However the prevalence of venous invasion in published studies has ranged from 10-90% (195-201). This variation was highlighted by a recent Australian study in which 82 Dukes' B colorectal cancer patients were initially reported to have no evidence of either venous invasion or serosal involvement in their tumours. However, on review by an expert pathologist, serosal involvement and/or venous invasion were identified in 32% of tumours, and persons with these characteristics in their malignancy had a poorer survival (203).

In our institution, within the context of a colorectal multidisciplinary team meeting, the pathology slides and reports have been reviewed by the attending pathologist (Alan Foulis) since 2001. It became apparent that there was satisfactory agreement among departmental pathologists over the content of the pathology reports, with the exception of the presence or absence of venous invasion. In an attempt to resolve the disparity over venous invasion in particular cases, elastica stains were used to provide more objective judgment. Subsequently, a retrospective review of 75

randomly selected colorectal cancer specimens was performed within the department by a senior pathologist, a trainee pathologist and a medical student (415). The prevalence of both intramural and extramural venous invasion combined was 27% in the freshly cut Haematoxylin and Eosin (H&E) sections and 57% in the elastica-stained sections. Inoue and colleagues (1992) (204) studied the association between the presence of venous invasion and the subsequent development of distant haematogenous metastases in colorectal cancer patients. This association was improved when venous invasion was assessed on elastica stained sections compared to H&E sections. In the light of this evidence, departmental policy changed during 2002 and a protocol was adopted whereby all tumour blocks had 2 serial sections stained by H&E and elastica H&E, respectively.

In the present study two cohorts of patients, operated on between 1997-2001 and 2003-2006, were selected. In the first cohort (cohort1) venous invasion had been assessed in the pathology reports using only H&E stained sections. In the second cohort (cohort 2) both H&E and elastica H&E stained sections had been examined to determine venous invasion. The aim of the present study was to examine the impact of elastica staining on the value of venous invasion by tumour as a predictor of cancer specific 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 abdominal computed tomography, were considered to have undergone potentially curative resection between January 1997 and March 2006 in a single surgical unit at the Royal Infirmary, Glasgow were included in the study. These were consecutive, elective patients entered prospectively into a maintained database. Patients who received neo-adjuvant chemo-radiotherapy, had recurrent or metastatic disease and patients who died within 30 days of surgery were excluded from the analysis. The tumours were staged using conventional Dukes' and TNM classifications (182, 188).

Throughout the study period, dissection of the specimens and selection of a minimum of four tumour blocks was done in accordance with Royal College of Pathologists guidelines (188) and did not differ between the 1997-2001 and 2003-2006 cohorts. Dissection of the specimens was performed by the majority of departmental consultant pathologists, or any of a number of rotating trainee pathologists, under consultant supervision. Tumours were cut transversely to the line of the bowel and a minimum of 4 blocks of tumour selected. The tumour sampling process was similar throughout the entire time period of the study (1997-2006). The average number of blocks did not vary significantly between the two time periods and was based on RCPath guidelines and departmental policy. At least one block was taken to show the presence or absence of serosal involvement, where appropriate. Other blocks were taken to show maximum penetration of tumour through the bowel wall.

Between 1997-2001 (cohort 1), 194 resected specimens were stained with H&E only and between 2003-2006 (cohort 2), two serial sections of all tumour blocks of 225 similar specimens were stained with elastica H&E and H&E respectively. All were routinely reported by departmental pathologists. Since the year 2002 represented a transition between the 2 staining protocols in the pathology department, cases from this year were excluded from the analysis.

In the study of Vass et al 2004 from our hospital, colorectal cancer specimens from 75 patients undergoing surgery between 1997 and 2000 had been studied (415). Fifty-three of these operations had been done with curative intent. In this retrospective

investigation, cases had been examined by H&E alone and, on a separate occasion, by H&E and elastica H&E, and the presence or absence of venous invasion noted. This cohort of 53 patients (cohort 3) has been included in the present study to allow a direct comparison of the prognostic predictive power of the two staining techniques on a single group of patients.

Routine pathological reporting of venous invasion in colorectal cancer cases was carried out according to the Royal College of Pathologists guidelines and defined as 'tumour present within an endothelium lined space that is either surrounded by a rim of muscle or contains red blood cells' and detected either intramurally or extramurally (188). When elastica and H&E stains were applied venous invasion was further defined as tumour seen within a vessel with elastic fibres in its adventitia. Sections were assessed for both the presence of extramural venous invasion and intramural venous invasion (invasion of veins of the submucosa and within the muscularis propria). With the exception of cohort 3 all pathology data for the present study were taken from pathology reports issued at the time of resection. The sections were not reviewed. In the study by Vass et al it (415) was shown that a plateau was reached in the prevalence of observed venous invasion when the number of tumour blocks stained by elastica was 4-5. This was used as the justification for staining all the tumour blocks that had been sampled.

The Petersen Index was derived from scores allocated to four selected pathological variables present in a tumour specimen. Intra or extramural vascular invasion, peritoneal involvement and surgical margin involvement were each allocated a score of 1. Tumour perforation was allocated a score of 2. The Petersen Index is considered low risk when the total score is 0 or 1 and high risk when 2 to 5 (195).

The provision of adjuvant treatment was at the discretion of the oncologist managing the patient following the multi-disciplinary team assessment. All biochemical and pathological results, as well as the patients' physiological status and age, were available to the oncologist when making such decisions on adjuvant treatment.

In our institution, patients undergo regular follow-up (3 months, 6 months and then yearly to five years) with yearly computed tomography scanning and regular colonoscopic surveillance until 5 years post surgery. Information on date and cause

of death was checked with that received by the cancer registration system and the Registrar General (Scotland).

Elastica H&E method

Following treatment of sections with acidified potassium permanganate for 5 minutes, sections were rinsed in water and treated with 1% oxalic acid until colourless. They were then rinsed in water, rinsed in methanol and stained overnight with Miller's elastica. After this they were rinsed in water and counterstained by H&E. Figure 1 shows a tumour section stained with H&E alone (Figure 3) and a serial section stained with elastica H&E (Figure 4).

Statistics

The study was approved by the Research Ethics Committee, Glasgow Royal Infirmary. Grouping of the variables was carried out using standard thresholds. Univariate and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. Follow up was censored at 36 months post surgery to enable direct comparison between the two groups. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.05. Survival curves were constructed using the Kaplan–Meier method with the log-rank test. Deaths up to April 1st 2009 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

3.3 Results

Four hundred and nineteen patients who underwent elective resection for colorectal cancer between 1997-2006 were studied. One hundred and ninety-four underwent surgery prior to the introduction of elastica staining in 2002 (cohort 1). Two hundred and twenty-five underwent surgery between 2003 and 2006 (cohort 2). Overall, the majority of patients were over 65 years (68%), male (56%) and had colonic cancers (64%). The median number of lymph nodes sampled was 13 (0-52). The majority had node negative disease (58%) and had a reported lymph node sample of 12 or more nodes (61%). Overall, 22% received adjuvant chemotherapy.

In those patients who underwent surgery for colorectal cancer between 1997 and 2001, the median number of lymph nodes were 13 (0-41); for Dukes' B tumours it was 13 (1-41) and for Dukes' C tumours 14 (5-34). In those patients who underwent surgery between 2003 and 2006, the median number of lymph nodes sampled was 13 (1-52); for Dukes' B tumours it was13 (1-52) and Dukes' C tumours 14 (5-32).

The individual clinico-pathological characteristics of the patient in cohorts 1 and 2 are shown in Table 3.1. There were no significant differences in terms of age, sex, tumour site, Dukes' stage, number of lymph nodes sampled or the provision of adjuvant chemotherapy between the patient cohorts (Table 3.1). Also there were no differences between the number of cancer and non-cancer deaths at 3 years and the 3 year survival analyses were similar in the two patient cohorts. In contrast, there was more frequent identification of venous invasion ($p<0.001$), peritoneal involvement and perineural invasion in the latter 2003-2006 cohort (Table 3.1).

In the 1997-2001 group, the minimum follow-up was 85 months; the median follow-up of the survivors was 112 months. During this period, 61 patients died from their cancer and a further 46 patients died of intercurrent disease. At 36 months post-surgery, 46 patients had died, 33 from cancer. In the 2003-2006 cohort, the minimum follow-up was 36 months; the median follow-up of the survivors was 56 months. During this period, 50 patients died of their cancer and a further 22 patients died of intercurrent disease. At 36 months post-surgery, 52 patients had died, 35 from cancer. No patients were lost to follow up in either cohort.

The univariate analysis for individual clinico-pathological variables and their relationship with 3-year cancer specific survival in patients undergoing potentially curative resection for colorectal cancer is shown in Table 3.2. In patients treated between 1997-2001 (prior to the introduction of routine elastica staining; cohort 1), T stage (P<0.05), N stage (P<0.001), poor tumour differentiation (P<0.05), peritoneal involvement (P<0.01), margin involvement (P<0.005), tumour perforation (P<0.05), and a high risk Petersen Index (P<0.005) were significantly related to poorer cancer specific survival. In patients treated between 2003-2006 (following the introduction of routine elastica staining; cohort 2), T stage (P<0.001), N stage (P<0.001), poor tumour differentiation (P<0.01), venous invasion (P<0.001), peritoneal involvement (P<0.001), margin involvement (P<0.001) and a high risk Petersen index (P<0.001) were significantly related to poorer cancer specific survival (Table 3.2). The provision of adjuvant chemotherapy was similar in both cohorts (23% and 22%) and was not associated with a significant survival benefit in either cohort (Cohort 1, P=0.697, Cohort 2, P=0.325).

The 3-year cancer specific survival rate, associated with the absence of venous invasion was 84% in cohort 1 compared with 96% in cohort 2 (P<0.01, Figure 3.2.1). In contrast, the 3-year cancer specific survival rate, associated with the absence of lymph node invasion was 93% in cohort 1 compared with 90% in cohort 2 (P=0.470, Figure 3.2.2). The 3-year cancer specific survival rate, associated with the presence of venous invasion was 77% in cohort 1 compared with 75% in cohort 2 (P=0.865, Figure 3.3.1). Also, the 3-year cancer specific survival rate, associated with the presence of lymph node invasion was 66% in cohort 1 compared with 75% in cohort 2 (P=0.196, Figure 3.3.2).

Receiver operator characteristics (ROC) analysis was undertaken to assess the prognostic significance of venous invasion in each cohort. Using cancer specific mortality as an endpoint, the area under the receiver operator curve was 0.59 (95% CI 0.50-0.68, P=0.040) for the presence of venous invasion between 1997-2001 and 0.68 (95% CI 0.60-0.76, P<0.001) for the presence of venous invasion between 2003-2006.

The inter-relationships between the pathological criteria are shown in Table 3.3. In the 1997-2001 H&E alone cohort increasing T stage was associated with the presence of peritoneal involvement (P<0.001), and perineural invasion (P<0.01). The presence of peritoneal involvement was associated with poor tumour differentiation (P<0.01) and perineural invasion (P<0.005). Venous invasion was not associated with any of the pathological criteria measured in the H&E alone cohort (Table 3.3).

In the 2003-2006 elastica staining group the presence of venous invasion was associated with increasing T stage (P<0.001), the presence of lymph node involvement (P<0.001), peritoneal involvement (P<0.001), and perineural invasion (P<0.05). Increasing T stage was associated with presence of lymph node involvement (P<0.001), peritoneal involvement (P<0.001) and perineural invasion (P<0.05). Lymph node involvement was associated with the presence of peritoneal involvement (P<0.001) and perineural invasion (P<0.001). Peritoneal involvement was associated with perineural invasion (P<0.01; Table 3.3).

The multivariate analysis of the individual pathological variables and cancer specific survival in the elastica stained Cohort 2 is shown in Table 3.4. To assess independent prognostic significance of the individual pathological variables, the Petersen Index was excluded from this analysis, as it is a cumulative score. On multivariate analysis, T stage (Hazard Ratio (HR) 1.85, 95% CI 1.08-3.19, P=0.026), N stage (HR 1.46, 95% CI 1.00-2.12, P=0.048), venous invasion (HR 3.81, 95% CI 1.68-9.25, P=0.002) and surgical margin involvement (HR 3.95, 95% CI 1.64-8.87, P=0.001) were independently related to cancer specific survival (Table 3.4).

Within the elastica stained cohort, the relationship between individual pathological variables and cancer specific survival in node negative and node positive disease were examined. The results, including 3-year cancer specific survival rates, are shown in Table 3.5. In node negative disease, following the introduction of routine elastica staining, T stage (P<0.05), venous invasion (P<0.05), peritoneal involvement (P<0.001), margin involvement (P<0.05), and a high risk Petersen Index (P<0.001) were significantly associated with poor cancer specific survival. In node positive disease, poor tumour differentiation (P<0.005), venous invasion (P<0.005), margin involvement (P<0.001), tumour perforation (P<0.05) and a high risk Petersen Index

(P<0.01) were significantly associated with poor cancer specific survival. The 3-year cancer specific survival rates for absence and presence of venous invasion was 96% and 85% respectively in node negative disease and 96% and 66% respectively for node positive disease (Figures 3.4 and 3.5).

A direct comparison between the two staining methods, H&E alone and elastica staining, was examined in 53 patients undergoing potentially curative surgery between 1997-2000 (cohort 3). Overall, the majority of patients were over 65 years (77%), male (59%) and had colonic cancers (52%). The median number of lymph nodes sampled was 13 (2-41). The majority had node negative disease (59%). In this cohort, the minimum follow-up was 112 months; the median follow-up of the survivors was 131 months. During this period, 25 patients died of their cancer and a further 9 patients died of intercurrent disease. No patients were lost to follow up in this cohort.

ROC analysis was undertaken to assess the prognostic significance of venous invasion using the two methods in this small cohort of 53 patients. Using cancer specific mortality as an endpoint, the area under the receiver operator curve was 0.58 (95% CI 0.43-0.74, P=0.293) for the presence of venous invasion using the H&E method and 0.74 (95% CI 0.60-0.88, P=0.003) for the presence of venous invasion using the elastica method. Of the 53 patients, 23 patients had no evidence of venous invasion by either staining method (group 1), 22 patients had venous invasion by elastica staining alone (group 2) and 8 patients had venous invasion identified using both staining methods (group 3). The 5-year survival rates were 77%, 59% and 29% for groups 1, 2 and 3 respectively (P<0.001).

3.4 Discussion

The results of the present study show that the increase in the detection of venous invasion by elastica staining compared with H&E staining alone is associated with improved prediction of cancer specific survival in patients undergoing potentially curative resection for colorectal cancer. In particular, the absence of venous invasion using the elastica stain was associated with an increase in cancer specific survival rates. This relationship was seen in the comparison of two consecutive cohorts (in both node negative and node positive disease) and in a direct comparison of stains in a single cohort (cohort 3). The results of the first 2 cohorts reflect the "real world" utility of elastica staining, since the data given here were generated at the time of surgical reporting. Results were taken from pathology reports with no consideration given to quantity or site of venous invasion (intra or extramural). Despite these generalisations, an improvement in prediction of cancer specific survival was observed. Therefore, we believe that this is good evidence that elastica staining should be incorporated into the routine pathological assessment of venous invasion in patients undergoing potentially curative resection for colorectal cancer.

Two basic approaches have been previously reported to increase the sensitivity of the detection of venous invasion in colorectal cancer. One involves the prospective examination of large numbers of blocks of tumour specimens by specialist pathologists. For example, Talbot and colleagues (1981) (197) examined an average of 5 blocks of tumour, taken both transversely as well as tangential to the outer aspect of the muscularis propria, to maximise the number of vein sections observed. If venous invasion was not observed they examined up to 8 additional blocks. They seldom used elastica stains and found venous invasion in 52% of cases, which is similar to the prevalence found in our elastica stained cohort. The second approach involves the use of elastica stains. For example, Minsky found venous invasion in 48% of rectal cancers and 42% of colon cancers when these stains were used. The figures for observations made on the H&E sections of the same cases were 8% and 7% respectively (416, 417). Both these approaches were combined in a prospective study of 426 colorectal cancer resections in which a single pathologist dissected, blocked out and sectioned the entire tumour (199). Elastica stains were used routinely. Venous invasion was found in 83 % of Dukes' B and 93% of Dukes' C

cancers. Although both approaches may be used to increase the detection of venous invasion, analysis of routinely performed elastica stained sections (readily analysed at low power magnification) on a recommended minimum number of tumour blocks (188) is likely to be more readily incorporated into diagnostic pathology services.

There is widespread agreement that the presence of venous invasion is associated with an increased risk of the future development of distant metastases (particularly hepatic) and cancer related death (198, 200, 201, 203). The sensitivity of the method used to detect venous invasion is likely to impact on the specificity of the prediction. If the sensitivity is very low many patients with no observed venous invasion will develop metastases and not survive. This was seen in the study of Morris et al (2007) (194) in which venous invasion was detected in 15% of cases, and the five year survival for patients without venous invasion was 70% for both colon and rectal cancer patients. On the other hand if the sensitivity is very high (87% with venous invasion) the opposite may occur, and the small number of patients with no venous invasion cannot be separated prognostically from those with minimal venous invasion (199).

In the present study elastica staining increased the identification of venous invasion to 58% of consecutive cases in cohort 2. This detection rate is similar to the 54% previously reported from our institution in 75 colorectal cancer specimens (415). Also, in the last 3 years (2007-2009) in our department, the detection rate of venous invasion using elastica has remained at 54%, showing that the present more objective method of assessing venous invasion results in consistent reporting. The present study sets a benchmark for the future reporting of venous invasion in patients with primary operable colorectal cancer, as the sensitivity of the method used in the elastica stained cohort appears close to the optimum in terms of predicting cancer specific survival.

The present study also showed that there was an increase in the reporting of peritoneal involvement and perineural invasion in the later time periods. Therefore, it is likely that there was a general improvement in the quality of reporting of these important pathological characteristics. Nevertheless, this would not explain the three-fold increase in the reporting of venous invasion. There was no increase in the

lymph node harvest between the 2 cohorts (median lymph node yield was 13), which is another measure of pathological quality. In addition, the prognostic value of lymph node invasion over the two consecutive cohorts was not significantly different. In the study of Vass and colleagues (415), review of freshly cut and stained sections of all tumour blocks, stained with H&E, by an experienced pathologist did not increase the rate of detection of venous invasion, whereas examination of elastica stained sections by the same pathologist doubled the rate of observed venous invasion. I would conclude that only the introduction of routine elastic staining could explain the three-fold increase in the reporting of venous invasion in the second cohort. One of the strengths of this study is that in neither cohort were the slides reviewed by an 'expert'. The results reflect a 'real world' situation where the same group of pathologists prospectively reported the presence of venous invasion to the best of their ability in both cohorts. The only difference in practice was the introduction of the elastica stain for the second cohort.

In the H&E alone cohort, no significant relationships were observed between venous invasion and other pathological variables such as T stage, nodal involvement, peritoneal involvement and perineural invasion (Table 3). In contrast, the elastica stained cohort showed that venous invasion was significantly associated with these high risk pathological features. Therefore, the results from the elastica staining, in addition to giving prognostic prominence to venous invasion, suggest that venous invasion is a process pivotal to tumour growth and invasion in colorectal cancer.

In the elastica stained cohort, the 3-year cancer specific survival rates for absence and presence of venous invasion was 96% and 85% respectively in node negative disease and 96% and 66% respectively for node positive disease. These results emphasise the importance of venous invasion compared with nodal positivity in determining cancer outcome. The excellent survival rates reported in patients without venous invasion provide further evidence that venous invasion is likely to indicate a risk of haematogenous micrometastases being present at the time of surgery. Also, the findings in the elastica stained cohort suggest that modern surgical and oncological techniques are capable of providing an excellent prognosis for those patients whose tumours do not exhibit significant venous invasion, whatever their Dukes' stage.

Despite a significant increase in reported venous invasion rates, the provision of adjuvant chemotherapy was similar in both cohorts (23% and 22%). This probably reflects that throughout the entire study period the decision on provision of adjuvant chemotherapy made at the multidisciplinary meetings was based primarily on nodal status of the resected tumour. On the basis of the present results, where elastica staining identified patients at high risk of dying from their disease, it may be that the provision of adjuvant chemotherapy should be increased in these patients. However, definitive studies in a randomised controlled trial setting are required to confirm such clinical utility.

The present study is unique in its design and findings. It is the first prospective study to report the prognostic implications of a change in practice in the method used to detect venous invasion in colorectal cancer. It shows that examination of an elastica stained section from every tumour block resulted in both a significantly increased prevalence of observed venous invasion and an improved prediction of cancer specific survival. Currently, neither the guidelines of the The Royal College of Pathologists nor those of The College of American Pathologists recommend this simple change of practice (188, 418) but the results presented here suggest that this protocol should be instituted widely.

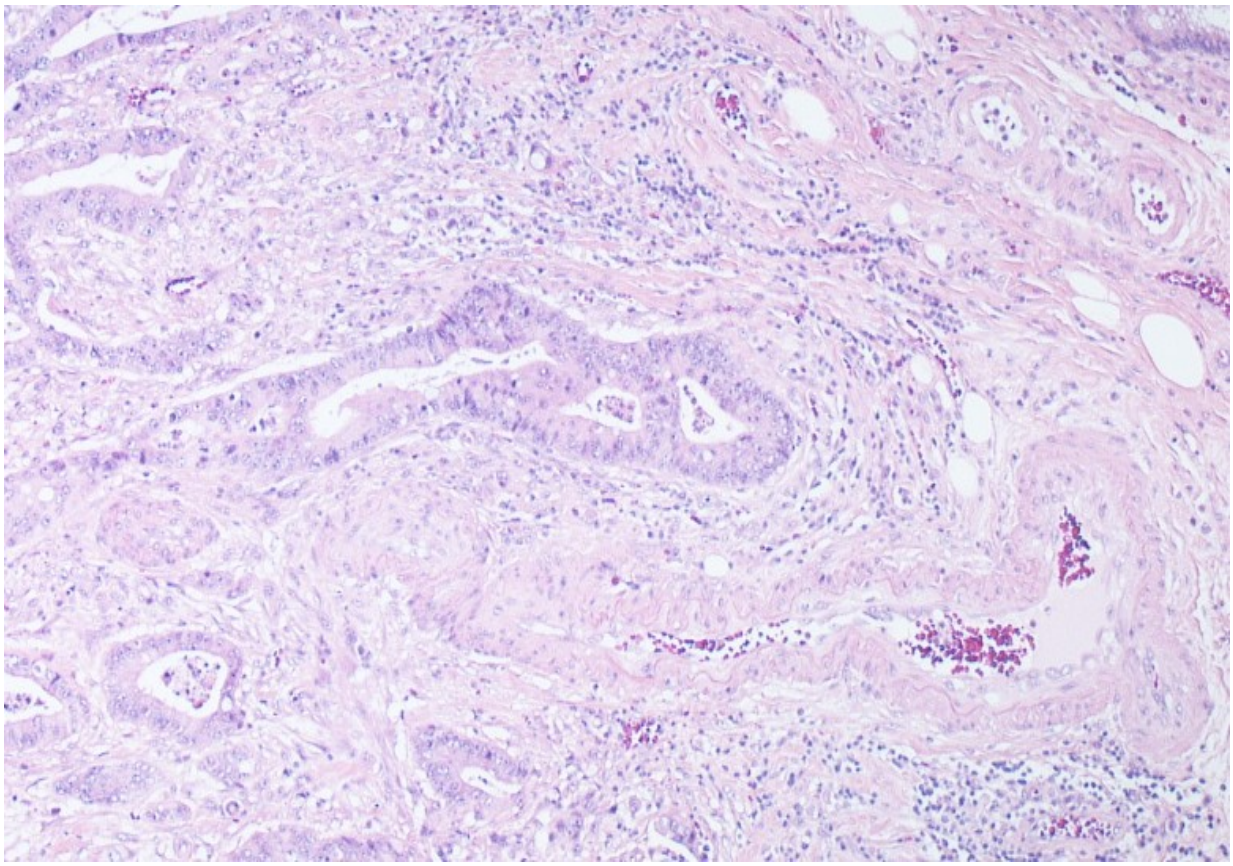


Figure 3.1: H&E staining of extramural fat of a pT3 adenocarcinoma of colon at 40x magnification.

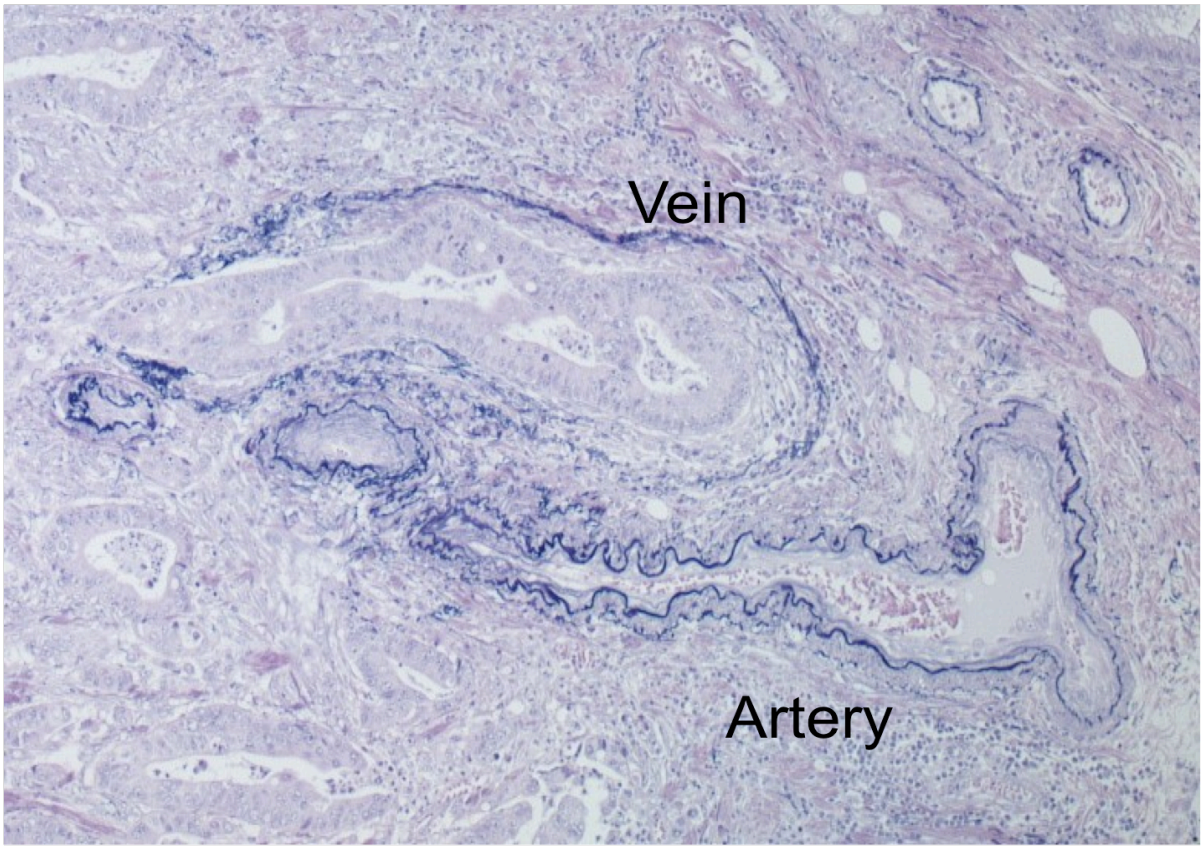
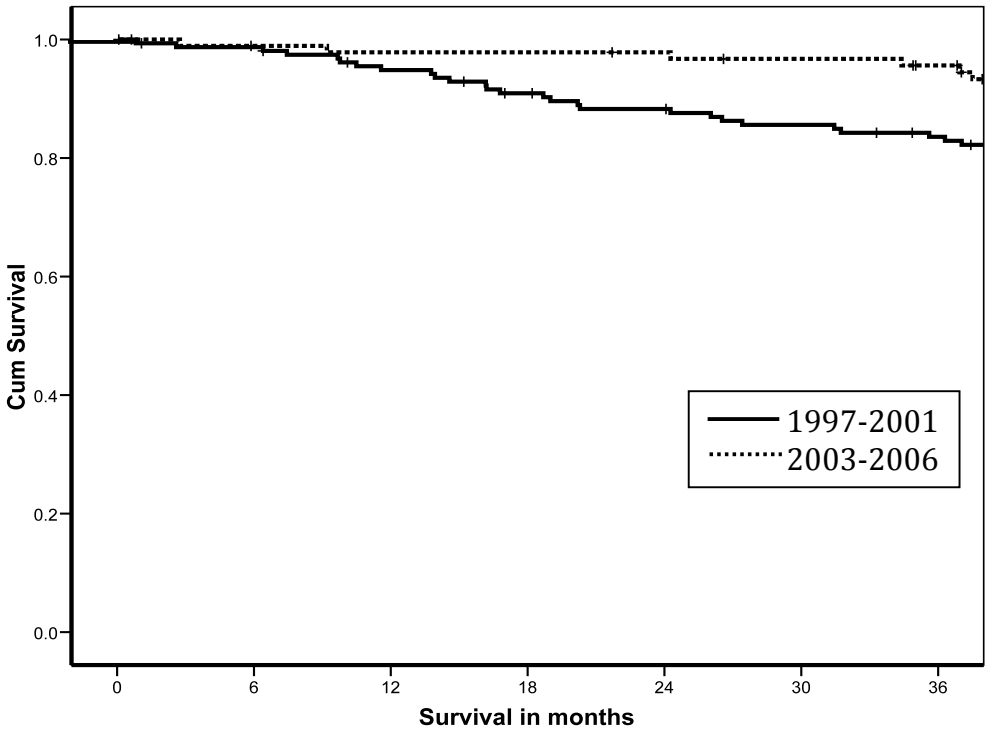


Figure 3.2: H&E elastica staining of extramural fat of a pT3 adenocarcinoma of colon at 40x magnification.

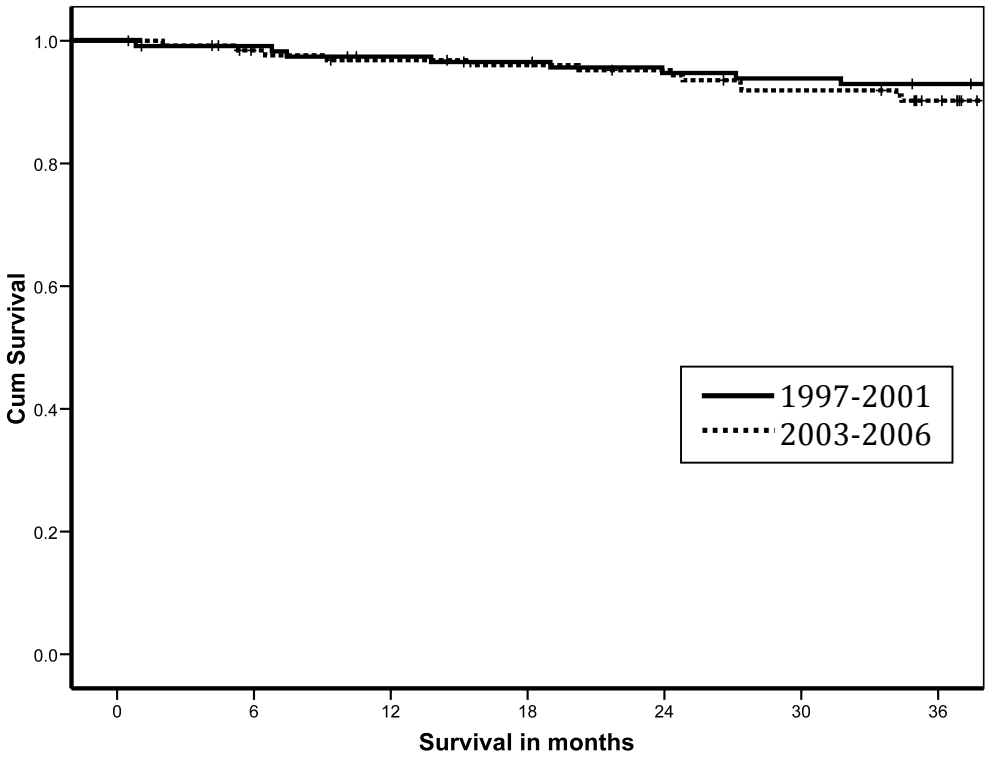
Figure 3.3: The relationship between the absence of venous invasion and survival in colorectal cancer patients treated between 1997-2001 and 2003-2006 (P=0.005).



Number at risk

1997-2001	158	154	146	138	133	128	123
2003-2006	94	91	90	90	89	87	84

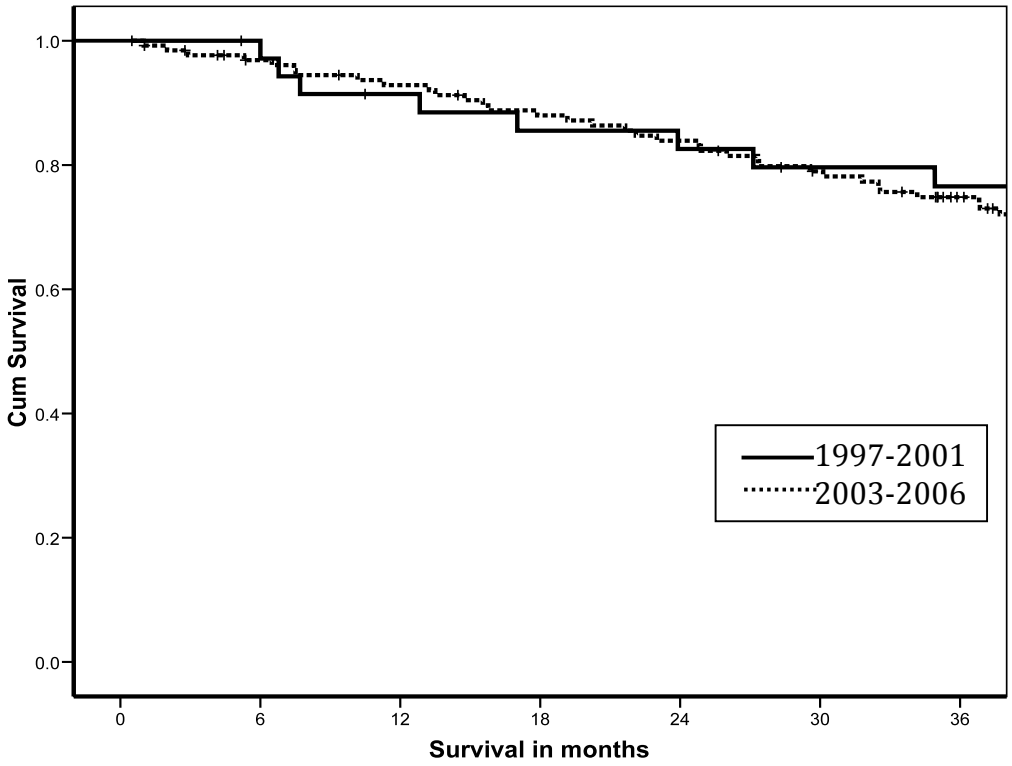
Figure 3.4: The relationship between the absence of lymph node involvement and survival in colorectal cancer patients treated between 1997-2001 and 2003-2006 (P=0.470).



Number at risk

1997-2001	117	115	111	109	106	105	103
2003-2006	130	123	120	118	116	111	104

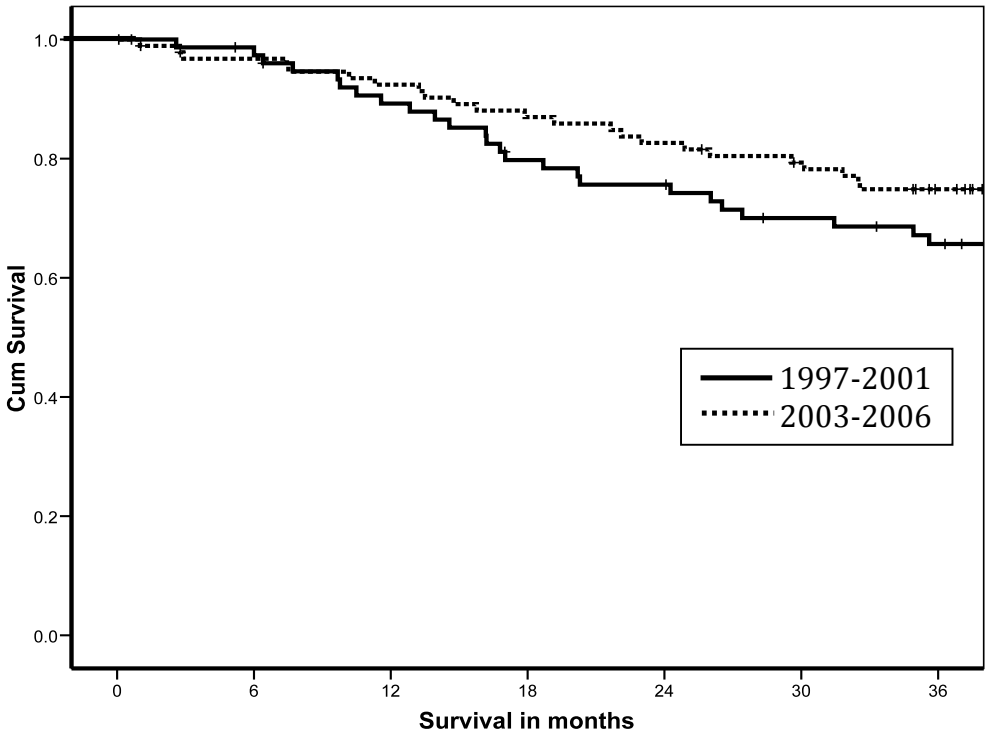
Figure 3.5: The relationship between the presence of venous invasion and survival in colorectal cancer patients treated between 1997-2001 and 2003-2006 (P=0.865)



Number at risk

1997-2001	36	35	31	29	28	26	25
2003-2006	131	121	115	108	103	95	83

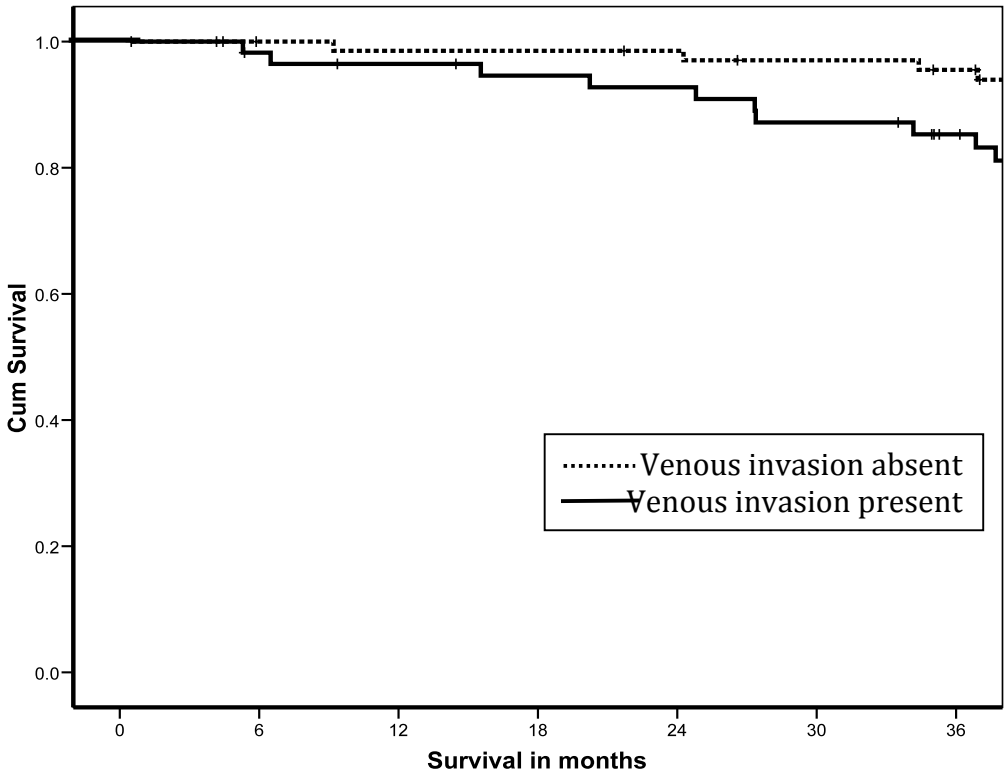
Figure 3.6: The relationship between the presence of lymph node involvement and survival in colorectal cancer patients treated between 1997-2001 and 2003-2006 (P=0.196).



Number at risk

1997-2001	77	74	66	58	55	49	45
2003-2006	95	89	85	80	76	71	63

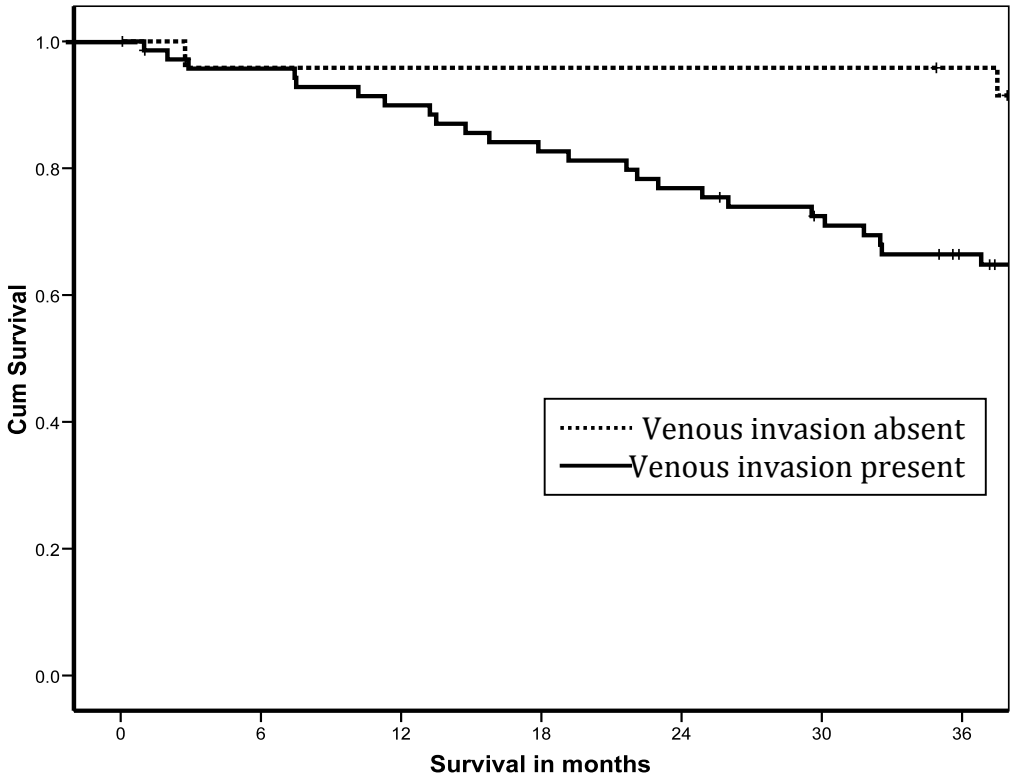
Figure 3.7: The relationship between the presence or absence of venous invasion (VI) and survival in node negative disease (2003-2006) (P=0.014).



Number at risk

VI Absent	69	68	67	67	66	64	62
VI Present	60	55	53	51	50	47	42

Figure 3.8: The relationship between the presence or absence of venous invasion (VI) and survival in node positive disease (2003-2006) (P=0.001).



Number at risk

VI Absent	25	23	23	23	23	23	22
VI Present	71	66	62	57	53	48	41

Table 3.1: Clinico-pathological characteristics of patients undergoing curative resection for colorectal cancer between 1997-2001 and 2003-2006. Comparisons between groups with Chi square (X^2).

		Cohort 1 1997-2001 H&E alone	**Cohort 2 2003-2006 Elastica H&E**	
		Patients n=194 (%)	**Patients n=225 (%)**	**P value**
Age	<65 years	59 (30)	77 (34)	
	65-74years	61 (32)	79 (35)	
	>75years	74 (38)	67 (31)	0.158
Sex	Female	87 (45)	97 (43)	
	Male	107 (55)	128 (57)	0.722
Site	Colon	128 (66)	141 (63)	
	Rectum	66 (34)	84 (37)	0.481
TNM Stage	I	9 (4)	29 (13)	
	II	108 (56)	100 (44)	
	III	77 (40)	96 (43)	0.439
Lymph nodes sampled	<12	116 (60)	141 (63)	
	≥12	78 (40)	84 (37)	0.548
Adjuvant therapy	No	150 (77)	176 (78)	
	Yes	44 (23)	49 (22)	0.825
Tumour Differentiation				
	Mod-well	177 (91)	199 (88)	
	Poor	17 (8)	26 (12)	0.340
Venous Invasion	Absent	158 (81)	94 (42)	
	Present	36 (18)	131 (58)	<0.001
Peritoneal Involvement	Absent	143 (74)	144 (64)	
	Present	51 (26)	81 (36)	0.033
Surgical Margin Involvement				
	Absent	188 (97)	214 (95)	
	Present	6 (3)	11 (5)	0.353
Tumour perforation	Absent	190 (98)	220 (98)	
	Present	4 (2)	5 (2)	0.910
Perineural Invasion	Absent	186 (96)	200 (89)	
	Present	8 (4)	25 (11)	0.008
Petersen Index	Low risk	179 (92)	160 (71)	
	High risk	15 (8)	65 (29)	<0.001
Alive at 3yrs		148 (76)	173 (77)	
Dead at 3 years	Cancer	33 (17)	35 (16)	
	Non cancer	13 (7)	17 (7)	0.965

Table 3.2: The relationship between pathological characteristics and 3 year cancer specific survival in patients undergoing curative resection for colorectal cancer from 1997-2001 (n=194) and between 2003-2006 (N=225): Univariate survival analysis censored at 3 years.

		Cohort 1 1997-2001			Cohort 2 2003-2006		
		Patients N=194 (%)	3year survival % (SE)	P value (log rank)	Patients N=225 (%)	3 year survival % (SE)	P value (log rank)
T stage	1	3 (1)	100 (0)		9 (4)	100 (0)	
	2	11 (6)	100 (3)		25 (11)	100 (3)	
	3	128 (66)	86 (3)		115 (51)	87 (3)	
	4	52 (27)	70 (6)	0.043	26 (34)	71 (5)	0.001
N stage	0	117 (60)	93 (2)		130 (58)	90 (3)	
	1	56 (29)	72 (6)		67 (30)	81 (5)	
	2	21 (11)	49 (11)	<0.001	28 (12)	59 (9)	<0.001
Lymph nodes sampled	<12	116	82 (4)		141 (63)	83 (3)	
	≥12	78	83 (4)	0.765	84 (37)	85 (4)	0.694
Tumour Differentiation							
	Mod-well	177 (91)	84 (3)		199 (88)	86 (3)	
	Poor	17 (8)	60 (14)	0.019	26 (12)	68 (9)	0.008
Venous Invasion	Absent	158 (81)	84 (3)		94 (42)	96 (2)	
	Present	36 (18)	77 (7)	0.320	131 (58)	75 (4)	<0.001
Peritoneal Involvement							
	Absent	143 (74)	87 (3)		144 (64)	91 (2)	
	Present	51 (26)	70 (7)	0.009	81 (36)	70 (5)	<0.001
Surgical Margin Involvement							
	Absent	188 (97)	83 (3)		214 (95)	86 (2)	
	Present	6 (3)	40 (22)	0.003	11 (5)	44 (15)	<0.001
Tumour perforation	Absent	190 (98)	83 (3)		220 (98)	84 (3)	
	Present	4 (2)	50 (25)	0.032	5 (2)	60 (22)	0.064
Perineural Invasion	Absent	186 (96)	83 (3)		200 (89)	86 (3)	
	Present	8 (4)	63 (17)	0.112	25 (11)	68 (9)	0.069
Petersen Index	Low risk	179 (92)	84 (3)		160 (71)	91 (2)	
	High risk	15 (8)	57 (135)	0.003	65 (29)	67 (6)	<0.001

Table 3.3: The inter-relationships between the individual pathological variables in the two different cohorts; 1997-2001 (H&E staining alone) and 2003-2006 (with elastica H&E staining)

Cohort 1 1997-2001 (H&E alone)	T stage 1/ 2/ 3/ 4	Lymph Node Inv No/ Yes	Differentiation Mod-well/ Poor	Peritoneal Inv No/ Yes	Perineural Inv No/ Yes
Venous Invasion No/Yes	0.278	0.307	0.582	0.520	0.160
T stage 1/ 2 / 3/ 4		0.209	0.212	<0.001	0.006
Lymph node involvement No/Yes			0.092	0.212	0.179
Tumour Differentiation Mod-well/ Poor				0.009	0.098
Peritoneal Involvement No/ Yes					0.001
Cohort 2 2003-2006 (Elastica H&E)	T stage 1/ 2/ 3/ 4	Lymph Node Inv No/ Yes	Differentiation Mod-well/ Poor	Peritoneal Inv No/ Yes	Perineural Inv No/ Yes
Venous Invasion No/Yes	<0.001	<0.001	0.099	<0.001	<0.001
T stage 1/ 2 / 3/ 4		<0.001	0.169	<0.001	0.010
Lymph node involvement No/Yes			0.991	<0.001	<0.001
Tumour Differentiation Mod-well/ Poor				0.260	0.165
Peritoneal Involvement No/ Yes					0.008

Table 3.4: Multivariate analysis for cancer specific survival for individual pathological characteristics in the 2003-2006 elastica stained cohort.

Cohort 2 Elastica and H&E staining 2003-2006		**Multivariate Analysis**	
		Hazard Ratio (95% CI)	**P-value**
T stage	1		
	2		
	3		
	4	1.85 (1.08-3.19)	0.026
N stage	0		
	1		
	2	1.46 (1.00-2.12)	0.048
Differentiation	Mod-well		
	Poor		0.083
Venous Invasion	Absent		
	Present	3.81 (1.68-9.25)	0.002
Peritoneal Involvement	Absent		
	Present		0.505
Surgical Margin Involvement			
	Absent		
	Present	3.95 (1.64-8.87)	0.001
Tumour perforation	Absent		
	Present		0.738
Perineural Invasion	Absent		
	Present		0.126

Table 3.5: The relationship between the individual pathological variables and 3-year cancer specific survival (%) in patients undergoing curative resection for node negative and node positive colorectal cancer in the elastica stained cohort 2003-2006

Cohort 2 Elastica H&E 2003-2006	Node negative disease			Node positive disease		
	Patients N=129 (%)	3year survival % (SE)	P value (log rank)	Patients N=96 (%)	3 year survival % (SE)	P value (log rank)
T stage 1/ 2	29 (23)	100 (0)		5 (5)	100 (0)	
3/ 4	100 (77)	88 (3)	0.026	91 (95)	72 (5)	0.094
Lymph nodes sampled <12	53 (41)	90 (4)		31 (32)	73 (8)	
≥12	76 (59)	92 (4)	0.661	65 (68)	74 (6)	0.981
Tumour Differentiation						
Mod-well	115 (89)	91 (3)		84 (88)	79 (5)	
Poor	14 (11)	93 (7)	0.498	12 (12)	36 (15)	0.002
Venous Invasion Absent	69 (53)	96 (3)		25 (26)	96 (4)	
Present	60 (47)	85 (5)	0.014	71 (74)	66 (6)	0.001
Peritoneal Involvement						
Absent	97 (75)	96 (2)		47 (49)	82 (6)	
Present	32 (25)	76 (8)	<0.001	49 (51)	66 (7)	0.013
Surgical Margin Involvement						
Absent	123 (95)	92 (3)		91 (95)	77 (5)	
Present	6 (5)	63 (21)	0.012	5 (5)	20 (18)	<0.001
Tumour perforation Absent	127 (98)	95 (3)		93 (97)	77 (5)	
Present	2 (2)	100 (0)	0.584	3 (3)	33 (27)	0.013
Perineural Invasion Absent	124 (96)	91 (3)		76 (79)	77 (5)	
Present	5 (4)	80 (18)	0.069	20 (21)	65 (11)	0.120
Petersen Index Low risk	106 (82)	95 (2)		54 (56)	82 (5)	
High risk	23 (18)	72 (10)	<0.001	42 (44)	64 (8)	0.008

4.0 COMPARISON OF THE PROGNOSTIC VALUE OF TUMOUR AND PATIENT RELATED FACTORS IN PATIENTS UNDERGOING CURATIVE SURGERY FOR COLON CANCER

4.1 Introduction:

Following curative resection for colon cancer, pathological analysis for tumour related factors guides prognosis and provision of adjuvant therapy. A variety of high risk features including tumour stage, nodal status, the ratio of metastatic to examined lymph nodes and presence or absence of venous invasion are considered to be important in planning adjuvant therapy and follow-up (176, 194, 419-421).

However, it is also now recognised that cancer outcomes are not solely determined by tumour-related factors but also by patient-related factors (292, 422). Indeed, the presence of a pre-operative systemic inflammatory response, as evidenced by a simple objective score (modified Glasgow Prognostic Score (mGPS) based on circulating levels of two acute phase proteins, C-reactive protein and albumin, is independently associated with poor cancer outcomes in patients undergoing surgery for colon and rectal cancer (278, 308, 325).

The acute phase protein response is only one aspect of the systemic inflammatory response (289). Previous work has also identified a significant relationship between cellular components of the pre-operative systemic inflammatory response including white cell, neutrophil, lymphocyte, monocyte and platelet counts and cancer survival in patients colorectal cancer (278, 322, 340, 423-426). Also, the combination of these cellular components such as the neutrophil-lymphocyte ratio and the platelet-lymphocyte ratio have been proposed to have prognostic value (309, 310, 342).

The systemic inflammatory response, as evidenced by the mGPS, also appears to be associated with a number of routine biochemical parameters, in particular alkaline phosphatase and γ-glutamyl transferase (427). Therefore, it of interest that alkaline phosphatase, aspartate transaminase and γ-glutamyl transferase have been reported to have prognostic value in patients undergoing surgery for colon and rectal cancer (428-433).

To date, there has been no comprehensive comparison of the prognostic value of tumour and patient-related factors, including the systemic inflammatory response. The aim of the present study was to examine the relationship between tumour and patient related factors, including the mGPS, and cancer specific survival in patients under going potentially curative surgery for colon cancer.

4.2 Materials and methods

Patients with histologically proven colon cancer who, on the basis of laparotomy findings and pre-operative abdominal computed tomography, were considered to have undergone potentially curative resection between January 1997 and July 2005 in a single surgical unit, were included in the study. Patients were identified from a prospectively maintained colorectal cancer database. Exclusion criteria were: (i) emergency surgery (ii) death within 30 days of surgery (iii) clinical evidence of infection or other inflammatory conditions such as inflammatory bowel disease or rheumatoid arthritis. Tumours were staged using the conventional Dukes' classification (182).

Pathological details were obtained from reports issued following tumour resection. The lymph node ratio was calculated by dividing the number of metastatic lymph nodes identified by the total number of lymph nodes sampled. In the present study, cut offs of 0.25 and 0.5 were used to stratify patients as high or low risk within the Stage III or node positive patients as previously described (217, 434). Routine laboratory measurements for haemoglobin, white cell, neutrophil, lymphocyte, platelet counts, bilirubin, aspartate transaminase, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase, adjusted calcium, globulins, albumin and C-reactive protein concentration prior to surgery were recorded.

The calcium concentrations were adjusted for albumin using the formula: adjusted calcium = measured calcium + (43-measured albumin) x 0.07. Coefficient of variation for these methods, over the range of measurement, was less than 10% as established by routine quality control procedures.

The GPS was constructed as previously described (336). Briefly, patients with both an elevated C-reactive protein (>10mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of 2. Patients in whom only one of these biochemical abnormalities was present were allocated a score of 1. Patients in whom neither of these abnormalities was present were allocated a score of 0. Recently, however, this has been modified based on evidence that hypoalbuminaemia, in patients with colorectal cancer without an elevated C-reactive protein concentration, had no significant association with cancer specific survival. Therefore, patients with an elevated C-

reactive protein were assigned a modified GPS score (mGPS) of 1 or 2 depending on the absence or presence of hypoalbuminaemia (308).

The provision of adjuvant chemotherapy following surgery was at the discretion of the medical or clinical oncologists present at multi-disciplinary assessment. All clinical and pathological data, including co-morbidities, were available to the oncologist in making these decisions and the treatment offered was based on the treatment guidelines for colon cancer at that time.

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

Statistics

Grouping of the variables was carried out using standard thresholds for laboratory parameters (278, 296, 435-437). The relationships between the mGPS and other variables were analysed using the Mantel–Haenszel (X^2) test for trend as appropriate. Deaths up to August 2008 were included in the analysis. Univariate survival analysis was performed using the Kaplan–Meier method with the log-rank test. Multivariate survival analysis, including all significant covariates was performed using a stepwise backward procedure to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.05. Because of the number of statistical comparisons, a *P* value of ≤ 0.01 was considered to be significant. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

4.3 Results

Two hundred and eighty seven patients undergoing elective potentially curative resection for colon cancer were studied. All pathological data and criteria for the mGPS were available in all 287 patients. Biochemistry including full liver function testing was available for 224 patients. Pre-operative haematology results were available for 167 patients.

The tumour characteristics and relationships with overall and cancer specific survival are shown in Table 4.1. The majority of patients had Dukes' A/B disease (59%), moderate to well differentiated tumours (86%), had no evidence of vascular invasion (65%), no evidence of peritoneal involvement (71%), clear surgical margins (90%), no evidence of tumour perforation (96%), no evidence of perineural invasion (93%), a lymph node ratio of 0 (59%) and 12 or more lymph nodes sampled (63%). Median number of lymph nodes sampled was 14 (range 2-52). Sixty patients (21%) received adjuvant chemotherapy (Table 4.1).

The patient related characteristics and relationships with overall and cancer specific survival are shown in Table 4.2. The majority of patients were 65 years or older (70%), male (54%). The majority of patients had pre-operative total white cell counts (59%), neutrophil counts (82%), lymphocyte counts (92%) and platelet counts (73%) in the normal range. Therefore, the majority of patients had a neutrophil/ lymphocyte ratio (77%) and platelet/ lymphocyte ratio (76%) within the normal range (Table 2). The majority of patients had a pre-operative bilirubin (96%), aspartate transaminase (99%), alanine transaminase (99%), γ-glutamyl transferase (79%) and alkaline phosphatase (55%) within the normal range. The majority of patients had globulin (94%), adjusted calcium (97%) and mGPS (57%) within the normal range (Table 4.2).

The median follow-up for survivors was 65 months (minimum 36 months). Over this period one hundred and twenty-five patients died, eighty-one from their cancer. On univariate survival analysis of tumour-related factors, Dukes' stage ($P<0.001$), extramural vascular invasion ($P<0.001$), peritoneal involvement ($P<0.01$), margin involvement ($P<0.001$) and increasing lymph node ratio ($P<0.001$) were significantly related to cancer specific survival (Table 4.1).

On univariate survival analysis of patient-related characteristics, age ($P<0.001$), white cell count ($P<0.01$) and the mGPS ($P<0.001$) were significantly related to cancer specific survival (Table 4.2).

On multivariate analysis of significant tumour and patient related factors, Dukes' stage (HR 3.01, 95% CI 1.50-6.06, P=0.002), extramural vascular invasion (HR 3.16, 95% CI 1.53-6.58, P=0.002), and the mGPS (HR 1.96, 95% CI 1.19-3.21, P=0.008) were independently related to cancer specific survival (Table 4.3).

The relationships between an increasing mGPS and patient related factors are shown in Table 4.4. An increased mGPS was associated with increased age ($P<0.001$), lower haemoglobin ($P<0.001$), increased white cell ($P\leq0.001$), neutrophil ($P\leq0.001$) and platelet ($P\leq0.001$) counts and increased neutrophil/lymphocyte ratio $\geq$5:1 ($P<0.001$) and increased alkaline phosphatase ($P\leq0.001$).

4.4 Discussion

In the present study, in addition to Dukes' stage, the most important tumour-related factors associated with cancer specific survival was extramural vascular invasion. These results are consistent with current guidelines, which identify patients undergoing potentially curative resection for colon cancer at high risk of recurrence (176, 194, 419-421).

In the present study, the mGPS was the most important patient-related factor associated with cancer specific survival. These results are consistent with previous studies confirming the role of the mGPS in primary operable colon and rectal cancer (308, 325). Ishizuka and colleagues (325) have called for the 'worldwide adoption of the GPS for postoperative prognostication of patients with colon and rectal cancer".

Recently, in addition to the Glasgow Prognostic Score, a variety of inflammation-based scores have been developed to predict cancer specific survival in patients with primary operable gastrointestinal cancer, including the neutrophil/ lymphocyte ratio and the platelet lymphocyte ratio (309, 310, 342). In the present study, neither the neutrophil/ lymphocyte ratio nor the platelet/ lymphocyte ratio were significantly associated with cancer specific survival. Therefore, these new scores based on the cellular components of the systemic inflammatory response cannot be recommended for routine use in predicting survival in patients undergoing potentially curative resection for colon cancer.

In the present study, in addition to the cellular components of the systemic inflammatory response, alkaline phosphatase was directly associated with the mGPS. These results are consistent with those previously reported in patients with advanced lung and gastrointestinal cancer (427). Given that circulating concentrations of enzymes primarily reflect that synthesised by the liver in response to systemic inflammation, this increased functional requirement may be important for regulating other enzyme activity in the liver. For example, it has recently been reported that cytochrome P450 3A4 activity is reduced as part of the systemic inflammatory response in patients with advanced cancer (438). This mechanism may account for the observation that a raised mGPS is associated with a poor tolerance to chemotherapy in patients with colorectal cancer (Chapter 13.0) (439).

Irrespective of the mechanisms involved the results of this study indicate that, along with its prognostic value, the mGPS is associated with a cluster of cellular and biochemical changes in patients undergoing potentially curative resection for colon cancer.

In the present study, neither CEA or CA-19-9, proposed tumour markers were measured prior to surgery. There is some evidence that the combination of pre-operative serum CEA and CA-19-9 have independent prognostic value in patients undergoing resection for colorectal cancer (440). However, Ishizuka and coworkers (325) recently reported that, compared with tumour markers such as CEA, CA 19-9 and CA 72-4, the mGPS had superior prognostic value. Further work is required to determine whether a combination of tumour markers offers prognostic value in addition to pathological staging and the mGPS.

The results of the present study add further evidence to the importance of the systemic inflammatory response and the prognostic value of the mGPS in patients with colon cancer (278, 308, 325). Therefore, the mGPS has the potential to aid detection of early tumour recurrence following surgery. In contrast, the role of the mGPS in predicting response to neoadjuvant or adjuvant chemotherapy is less clear. There are some recent reports from other centres that suggest that the mGPS might be useful in predicting response to chemotherapy (325, 439, 441). This is of particular interest given a recent report from the UK National Confidential Enquiry into Patient Outcome and Death (NCEPOD) which concluded that chemotherapy had probably hastened or caused death, in over a quarter of patients who died within 30 days of receiving treatment (442). They suggest therefore, that greater caution be used in prescribing chemotherapy in very sick patients (442). However, they do not suggest how this problem might be avoided or how the very sick patients are to be identified. Therefore, the mGPS has also the potential to guide the selection of chemotherapy for colorectal cancer patients. However, the impact of using the mGPS as a therapeutic target has as yet not been explored.

In summary, both tumour-related and patient-related factors are important predictors of survival in patients undergoing potentially curative resection of colon cancer. In addition to tumour stage, and vascular invasion, the systemic

inflammatory response, as evidenced by the mGPS, should be included in the routine clinical assessment, planning of treatment and the stratification of randomized trials of patients with colon cancer.

Table 4.1: The relationship between tumour related factors and overall survival and cancer specific survival in patients undergoing potentially curative resection for colon cancer

			Overall survival		Cancer Specific Survival	
		Patients n=287 (%)	**5 year survival % (SE)**	**P value**	**5 year survival %(SE)**	**P value**
Dukes' Stage	A	19 (7)	88 (4)		85 (8)	
	B	149 (52)	68 (5)		85 (4)	
	C	119 (41)	48 (5)	<0.001	65 (5)	<0.001
Differentiation						
	Mod-well	248 (86)	64 (3)		78 (3)	
	Poor	39 (13)	43 (8)	0.017	70 (9)	0.171
Extramural vascular invasion	Absent	188 (65)	69 (4)		84 (3)	
	Present	19 (35)	45 (5)	<0.001	62 (6)	<0.001
Peritoneal involvement						
	Absent	205 (71)	65 (4)		83 (3)	
	Present	82 (29)	52 (6)	0.021	62 (7)	0.006
Margin involvement						
	Absent	259 (90)	64(3)		79 (3)	
	Present	28 (10)	32 (9)	<0.001	33 (16)	<0.001
Tumour perforation						
	Absent	276 (96)	62 (3)		78 (3)	
	Present	11 (4)	27 (13)	0.002	20 (24)	0.018
Perineural invasion						
	Absent	268 (93)	62 (3)		81 (3)	
	Present	19 (7)	39 (12)	0.113	46 (11)	0.042
Lymph nodes sampled						
	≥12	180 (63)	63 (4)		80 (4)	
	<12	107 (37)	57 (5)	0.288	70 (6)	0.848
Lymph Node Ratio	0	166 (59)	70 (4)		86 (3)	
	0.01-0.24	78 (27)	51 (6)		60 (6)	
	0.25-0.49	32 (11)	59 (9)		61 (9)	
	≥0.5	11 (4)	9 (9)	<0.001	21 (13)	<0.001
Adjuvant chemotherapy						
	Yes	60 (21)	58 (3)		80 (3)	
	No	227 (79)	71 (6)	0.059	69 (7)	0.972

Table 4.2: The relationship between patient related factors and overall survival and cancer specific survival in patients undergoing potentially curative resection for colon cancer.

		Overall Survival		Cancer Specific Survival	
	Patients n=287 %	**5 year survival % (SE)**	**P value**	**5 year survival % (SE)**	**P value**
Age <65 years	85 (30)	82 (4)		86 (4)	
65-74years	87 (30)	66 (5)		75 (5)	
>75years	115 (40)	42 (5)	<0.001	62 (5)	<0.001
Sex Female	133 (46)	58 (4)		74 (4)	
Male	154 (54)	63 (4)	0.474	73 (4)	0.866
Haemoglobin					
≥13g/d (men) ≥11.5g/dl (women)	66 (40)	66 (6)		79 (5)	
<13g/dl (men) <11.5g/dl (women)	101 (60)	61 (5)	0.555	78 (4)	0.671
White cell count <8.5x10^9	98 (59)	67 (4)		82 (4)	
8.5-11x10^9	45 (27)	69 (7)		82 (6)	
>11x10^9	24 (14)	32 (9)	0.001	56 (10)	0.004
Neutrophil count <7.5x10^9	137 (82)	66 (4)		81 (4)	
≥7.5x10^9	30 (18)	47 (10)	0.055	66 (9)	0.051
Lymphocyte count >3.0x10^9	10 (6)	38 (14)		55 (17)	
1.0-3.0x10^9	143 (86)	66 (5)		81 (4)	
<1.0x10^9	14 (8)	69 (8)	0.035	70 (12)	0.160
Platelet count <400 x10^9	122 (73)	65 (5)		81 (4)	
≥400 x10^9	45 (27)	57 (8)	0.084	70 (7)	0.020
Neutrophil/ lymphocyte ratio					
<5:1	129 (77)	67 (5)		82 (4)	
≥5:1	38 (23)	48 (8)	0.047	66 (8)	0.056
Platelet/ lymphocyte ratio					
≤150:1	40 (24)	62 (8)		74 (8)	
>150:1	127 (76)	63 (5)	0.611	80 (4)	0.719
Bilirubin ≤22 µmol/L	215 (96)	61 (4)		74 (3)	
>22 µmol/L	9 (4)	40 (18)	0.379	74 (16)	0.910
Aspartate transaminase ≤50 U/L	223 (99)	60 (4)		74 (3)	
>50 U/L	1 (1)	100 (0)	0.502	100 (0)	0.591
Alanine transaminase ≤50 U/L	223 (99)	60 (4)		74 (3)	
>50 U/L	1 (1)	100 (0)	0.497	100 (0)	0.591
Alkaline phosphatase ≤ 200 U/L	123 (55)	55 (5)		80 (4)	
> 200 U/L	101 (45)	45 (5)	0.037	67 (5)	0.060
γ-glutamyl transferase					
<55U/L (men), <35U/L (females)	175 (79)	63 (4)		76 (3)	
≥55U/L (men), ≥35U/L (females)	50 (21)	53 (7)	0.054	70 (7)	0.324
Globulin ≥22g/L	207 (94)	60 (4)		75 (3)	
<22g/L	14 (6)	62 (14)	0.999	73 (14)	0.756
Calcium Adjusted					
>2.5mmol/L	190 (97)	61 (4)		74 (3)	
≤2.5mmol/L	6 (3)	50 (20)	0.418	82 (16)	0.874
mGlasgow Prognostic Score					
Low Risk (0)	143 (57)	74 (4)		83 (3)	
Intermediate (1)	102 (33)	56 (5)		70 (5)	
High Risk (2)	42 (10)	28 (7)	<0.001	46 (9)	<0.001

Table 4.3: Tumour and patient related factors and relationship with cancer specific survival in colon cancer. Multivariate analysis of significant variables (P<0.01).

		Overall Survival		Cancer Specific Survival	
Tumour Related Factors	**(n=287)**	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Dukes' Stage (A/ B/ C)	19/ 149/ 119	1.51 (0.93-2.48)	0.099	3.01 (1.50-6.06)	0.002
Extramural Vascular Invasion (absent/ present)	188/ 19	2.01 (1.14-3.55)	0.016	3.16 (1.53-6.58)	0.002
Peritoneal Involvement (absent/ present)	205/ 82		0.424		0.262
Margin Involvement (absent/ present)	259/ 28	2.25 (0.93-5.45)	0.072		0.410
Lymph node ratio (0/ 0.01-0.24/ 0.25-0.49/ $\geq$0.5)	166/ 78/ 32/ 11		0.869		0.832
Patient Related Factors					
Age (<65/ 65-75/ >75years)	85/ 87/ 115	1.91 (1.34-2.72)	<0.001		0.110
White Cell Count (<8.5/ 8.5-11 >11x10^9)	98/ 45/ 24		0.607		0.878
mGPS (0/ 1/ 2)	143/ 102/ 42	1.73 (1.18-2.55)	0.005	1.96 (1.19-3.21)	0.008

Table 4.4: The relationship between an inflammation based prognostic score (mGlasgow Prognostic Score) and other patient- related factors in colon cancer patients.

	mGPS 0 n=143	**mGPS 1 n=102**	**mGPS 2 n=42**	**P value**
Age Group <65/ 65-74/ >75years	51/ 49/ 43	27/ 48/ 27	7/ 10/ 25	<0.001
Sex Male/ Female	65/ 78	47/ 55	21/ 21	0.646
Haemoglobin ≥13g/d (men), ≥11.5g/dl (women)/ <13g/dl (men) <11.5g/dl (women)	46/ 36	16/ 49	4/ 16	<0.001
White cell count <8.5x10^9/ 8.5-11x10^9/ >11x10^9	60/ 17/ 5	29/ 21/ 15	9/ 7/ 4	0.001
Neutrophil count <7.5x10^9/ ≥7.5x10^9	76/ 6	47/ 18	14/ 6	0.001
Lymphocyte count <1x10^9/1-3x10^9/>3x10^9	6/ 73/ 3	4/ 54/ 7	0/ 16/ 4	0.012
Platelet count <400 x10^9/ ≥400 x10^9	71/ 11	39/ 26	12/ 8	0.001
Neutrophil-lymphocyte ratio <5:1/ ≥5:1	76/ 6	44/ 21	9/ 11	<0.001
Platelet-lymphocyte ratio ≤150:1/ >150:1	23/ 59	12/ 53	5/ 15	0.408
Bilirubin ≤22 µmol/L/ >22 µmol/L	100/ 5	84/ 3	31/ 1	0.610
Aspartase transaminase ≤50 U/L/ >50 U/L	105/ 0	86/ 1	32/ 0	0.647
Alanine transaminase ≤50 U/L/ >50 U/L	105/ 0	86/ 1	32/ 0	0.647
Alkaline phosphatase ≤ 200 U/L/ > 200 U/L	69/ 36	43/ 44	11/ 21	0.001
γ-Glutamyl transferase <55U/L (men), <35U/L in females/ ≥55U/L (men), ≥35U/L in females	86/ 20	68/ 19	21/11	0.094
Globulin ≥22/ <22g/L	96/ 7	82/ 4	29/ 3	0.847
Calcium Adjusted >2.5/ ≤2.5mmol/L	84/ 4	77/ 0	29/ 2	0.884

5.0 COMPARISON OF TUMOUR-BASED (PETERSEN INDEX) AND INFLAMMATION BASED (GLASGOW PROGNOSTIC SCORE) SCORING SYSTEMS IN PATIENTS UNDERGOING CURATIVE RESECTION FOR COLORECTAL CANCER.

5.1 Introduction

Whilst Dukes' stage is widely used to predict outcome in colon cancer, it is also recognized that the survival of patients within the staging categories is variable, particularly those with Dukes' B or T3/4 N0 tumours. There is particular interest in identifying sub groups of patients with either Dukes' stage B disease or Dukes' C with only 1 positive node, who may be at relatively high or low risk respectively of developing recurrent cancer and therefore may or may not benefit from adjuvant chemotherapy (194).

Consequently, considerable effort has been directed at refining prognostic criteria. For example, numerous molecular-based factors have been evaluated (229). Clinically useful factors should be routinely available, well standardised and validated in a variety of different patient cohorts. However, few molecular-based factors satisfy these criteria and have been incorporated into routine clinical practice. There remains a continuing need to identify clinically relevant factors that would improve the prediction of survival in patients undergoing potentially curative surgery for colon cancer.

A score based on four routinely reported pathological criteria (vascular invasion, peritoneal involvement, margin involvement and tumour perforation), the Petersen Index (PI) has been reported to predict cancer-specific outcome in Dukes' B colon cancer (195). More recently, the PI has been validated as a prognostic score in patients undergoing potentially curative resection for both Dukes' B and C cancer of the colon and rectum (194). Similarly, an inflammation-based score, based on two routinely measured acute phase proteins (C-reactive protein and albumin), the Glasgow Prognostic Score (mGPS) has been reported to predict cancer-specific outcome in Dukes' B colon cancer (308). The GPS has recently been validated as a prognostic score in patients undergoing potentially curative resection for both Dukes'

B and C cancer of the colon (325). To date, the relationship between the PI and the GPS has not been examined. Moreover, the application of both scores to a single cohort of colon cancer patients has not previously been undertaken.

The aim of the present study was to compare the prognostic value of the tumour-based (Petersen Index) and inflammation-based (mGlasgow Prognostic Score) scoring systems in patients undergoing resection for colon cancer.

5.2 Materials and methods

Patients with histologically proven colon cancer who underwent potentially curative resection between January 1997 and July 2005 were identified from a prospectively collected database with similar exclusion criteria to those in Chapter 4.0 (Methods 4.2). Tumours were staged using conventional Dukes' classification (182).

The Petersen Index was constructed from the scores allocated to the four selected pathological variables present in a 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 where the score is between 0 and 1 and high risk between 2 and 5 (194, 195).

Blood samples were taken for routine laboratory measurements of albumin and C-reactive protein measurement prior to surgery. This is standard practice in all cancer patients in our institution. The coefficient of variation for these methods, over the range of measurement, was less than 5% as established by routine quality control procedures. The GPS was constructed as previously described (Chapter 4.0, Methods 4.2) (336), (308).

The provision of adjuvant treatment was at the discretion of the oncologist managing the patient following the multi-disciplinary team assessment. Therefore, all biochemical and pathological results as well as patient comorbidities were available to the oncologist in making such decisions on adjuvant treatment.

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

Statistics

Grouping of the variables was carried out using standard thresholds. Univariate survival analysis was performed using the Kaplan–Meier method with the log-rank test. Multivariate survival analysis and calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding

P-value had to be greater than 0.05. Deaths up to August 1st 2008 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

5.3 Results

Baseline clinico-pathological characteristics and the relationship with 5-year survival rate of the patients (n= 244) who underwent curative surgery for colon cancer are shown in Table 5.1. The majority of patients were 65 or older (73%), were male (52%), and had Dukes' stage A/B disease (59%). Fifty-six (23%) patients received adjuvant chemotherapy. Median number of lymph nodes sampled was 14 (range 3-52) for Dukes' B tumours and 14 (range 3-34) for Dukes' C tumours. The majority of patients had no evidence of vascular invasion (67%), peritoneal involvement (74%), resection margin involvement (91%) and tumour perforation (97%) and had a low risk Petersen Index (87%). The majority of patients had C-reactive protein (51%) and albumin (83%) concentrations in the normal range and a normal mGPS (51%). Of the 40 patients with hypoalbuminaemia, 31 (78%) had an elevated C-reactive protein concentration.

The minimum follow-up was 36 months; the median follow-up of the survivors was 67 months. No patients were lost to follow up. During this period 68 patients died of their cancer and a further 41 patients died of intercurrent disease. Univariate survival analysis for cancer-specific and overall survival of baseline clinico-pathological characteristics are shown in Table 5.1. On univariate survival analysis of individual variables, age ($P<0.001$), Dukes' stage ($P<0.001$), vascular invasion ($P<0.001$), peritoneal involvement ($P<0.001$), resection margin involvement ($P<0.001$), tumour perforation ($P<0.005$) C-reactive protein ($P<0.001$) and albumin ($P<0.05$) were associated significantly with overall survival. Both PI ($P<0.001$) and mGPS ($P<0.001$) were associated significantly with overall survival (Table 5.1).

On univariate survival analysis of individual variables, age ($P<0.001$), Dukes' stage ($P<0.001$), vascular invasion ($P<0.001$), peritoneal involvement ($P<0.001$), resection margin involvement ($P<0.001$), tumour perforation ($P<0.005$) C-reactive protein ($P<0.001$) and albumin ($P<0.005$) were associated significantly with cancer specific survival. On multivariate analysis of these significant variables age (HR, 1.80, 95% CI, 1.30–2.49, $P<0.001$), Dukes' stage (HR, 3.14, 95% CI, 1.82-5.40, $P<0.001$), vascular invasion (HR, 2.18, 95% CI, 1.25-3.82, $P=0.006$), C-reactive protein (HR, 2.09, 95% CI, 1.20-3.65, $P=0.010$) and albumin (HR, 2.33, 95% CI, 1.30-4.17, $P=0.004$) were

associated independently with cancer specific survival. On multivariate analysis of age, Dukes' stage, PI and mGPS, age (HR, 1.74, 95% CI, 1.27-2.39, P=0.001), Dukes' stage (HR, 3.63, 95% CI, 2.13-6.18, P<0.001), PI (HR, 2.05, 95% CI, 1.19-3.56, P=0.010) and mGPS (HR, 2.34, 95% CI, 1.65-3.31, P<0.001) were associated independently with cancer specific survival.

Multivariate survival analysis in patients with Dukes' stage B and stage C disease is shown in Table 5.2. In those patients with Dukes' B stage disease, age (P<0.05), PI (P<0.001) and mGPS (P<0.01) were associated independently with cancer specific survival. In those patients with Dukes' C stage disease, age (P<0.05) and mGPS (P<0.001) were associated independently with cancer specific survival.

The relationships between the PI and mGPS and cancer-specific survival in Dukes' B and C colon cancer are shown in Figures 11 and 12 and Figures 13 and 14 respectively. The three-year cancer specific survival rate for patients with low risk PI and Dukes' B stage disease was 98%, 92% and 82% for mGPS of 0, 1 and 2 respectively (p<0.05; Table 5.3). The three-year cancer-specific survival rate in all patients with Dukes' C stage disease and a low risk PI was 84%, 46% and 10% for an mGPS of 0, 1 and 2 respectively (p<0.001).

5.4 Discussion

The PI was initially reported in Dukes' B colon cancers (195). To date only one other study has validated the PI as a prognostic score in Dukes' B and C colon cancer as well as rectal cancer (194). The results of the present study further validate the PI in a different population of patients undergoing potentially curative resection for colon cancer. Of the 244 colon cancer patients included in this study, only 17% were classified as having a high risk PI. The present PI high-risk population among Dukes' B cases was 9%; smaller than the 29% of colon cancer cases originally reported by Petersen (195), but is more comparable with the recent study by Morris and colleagues (194) who also reported 9% of Dukes' B colon cancers and rectal cancers as having a high risk PI.

The basis of these differences in the classification of high risk PI between the studies is unclear. However, it may reflect differences in case mix or variability in reporting those factors that form the PI and discriminate between Dukes' B and C cases. In the Petersen study of Dukes' B colon cancers the prevalence of venous invasion (extra and intramural) was 30%; in Morris' paper of Dukes' B and C patients with both colon and rectal cancers the prevalence of extramural venous was 14% and in the present paper of colon cancers venous invasion was seen in 33% of colonic resections. In the 3 studies peritoneal involvement was seen in 42% (195), 14% (194) and 26% (present study). The number of lymph nodes can affect the Dukes' staging and the mean number of lymph nodes harvested was 21, 11 and 14 in the 3 studies.

In spite of these drawbacks in pathology reports both the present study and that of Morris and co-workers highlight the prognostic value of the PI. In particular, given that the results of both studies were drawn from cases dissected and reported by a number of pathologists, including trainee pathologists, they are likely to be representative of 'real world' pathology reporting used to inform multi-disciplinary team meetings of high risk patients with colon cancer. Therefore, I would recommend the PI should be reported routinely in patients having undergone resection for Dukes' B colon cancer, for whom it was designed, where the hazard ratio for survival in our study was approximately 10.

The present study shows for the first time that both tumour-based (PI) and inflammation-based (mGPS) scoring systems have independent prognostic value in patients undergoing potentially curative resection for colon cancer. While the PI measurement is subject to variation in reporting, the pre-operative mGPS, based on standard reliable laboratory measurements, is objective and therefore there is likely to be little variation in reporting.

It is of interest to consider how these results might be combined in a clinical context. At present, patients with Dukes' C tumours are offered adjuvant chemotherapy and those with Dukes' A tumours are not. All the relevant studies concur that the PI identifies Dukes' B patients who are at high risk and arguably these patients should also be offered chemotherapy. Morris has shown that patients with single node positive Dukes' C tumours had a better prognosis than patients with Dukes' B tumours with a PI of 1 or more. In the present study, among patients with Dukes' B tumours and a low risk PI, a high-risk mGPS indicated a statistically significant poorer prognosis when compared to patients with pathologically similar tumours who had a low risk mGPS (Table 5.3). Such high-risk patients may therefore be thought to benefit from adjuvant chemotherapy.

The utility of the PI in predicting response to chemotherapy is not, to our knowledge, known. In contrast, there is evidence that suggests an elevated C-reactive protein of the mGPS not only identifies those patients which are increased risk of recurrent disease but also those patients who are likely to benefit from adjuvant chemotherapy (Chapter 13.0). Therefore, on the basis of the evidence available the mGPS should be included, together with the PI, in the post-operative multi-disciplinary assessment of patients with primary operable colon cancer and the stratification of patients entering randomised trials of adjuvant chemotherapy.

The basis of the independent relationship between an elevated mGPS prior to surgery and poor long-term cancer specific survival in patients with primary operable colon cancer is not clear. A plausible explanation is that an elevated mGPS may reflect compromised cell mediated immunity since an elevated C-reactive protein and hypoalbuminaemia are associated with lymphocytopenia (344) and an impaired T-lymphocytic response in the tumour (276). Furthermore, the presence of an elevated

C-reactive protein concentration and hypoalbuminaemia have also been shown to be associated with upregulation of components of innate immune system, including complement and macrophage function (291, 345). In addition, it is known that as part of the systemic inflammatory response, there is a release of pro-inflammatory cytokines and growth factors, which may promote tumour growth (347, 443). Therefore, the mGPS may reflect host responses that impact prognosis in colon cancer whereas the PI might be considered to provide prognostic information on tumour behaviour.

In summary, the results of the present study validate the use of the Petersen Index in predicting cancer specific survival in patients undergoing elective potentially curative for Dukes' B colon cancer. Furthermore the results indicate that the mGPS further stratifies those patients with Dukes' B and single node positive Dukes' C cancers, classified as low risk by the PI. The PI and the mGPS scoring systems could therefore be combined at a multidisciplinary meeting to identify those patients with colorectal cancer who have undergone potentially curative surgery but who are at high risk of cancer related death.

Table 5.1. Clinico-pathological characteristics in patients undergoing potentially curative resection for colon cancer: Univariate survival analysis.

	Patients 244 (%)	Overall 3-year survival % (SE)	P-value	Cancer 3-year survival % (SE)	P-value
Age <65 years	65 (27)	95 (3)		95 (3)	
65-74years	72 (29)	76 (5)		84 (4)	
>75years	107 (44)	61 (5)	<0.001	68 (5)	<0.001
Sex Female	118 (48)	70 (4)		79 (4)	
Male	126 (52)	79 (4)	0.416	81 (4)	0.832
Dukes' Stage A	18 (7)	94 (5)		100 (0)	
B	127 (52)	85 (3)		90 (3)	
C	99 (41)	58 (5)	0.001	63 (5)	<0.001
Adjuvant therapy no	88 (77)	74 (3)		81 (3)	
yes	56 (23)	77 (6)	0.028	77 (6)	0.906
Date of Surgery 1997-2001	116 (48)	77 (4)		81 (4)	
2002-2005	128 (52)	73 (4)	0.514	79 (4)	0.773
Pathological Characteristics					
Vascular invasion no	163 (67)	83 (3)		86 (3)	
yes	81 (33)	58 (5)	<0.001	67 (5)	<0.001
Peritoneal involvement no	180 (74)	81 (3)		86 (3)	
yes	64 (26)	51 (6)	0.001	64 (6)	<0.001
Margin involvement no	221 (91)	76 (3)		82 (3)	
yes	23 (9)	57 (10)	<0.001	60 (10)	<0.001
Tumour Perforation no	308 (98)	75 (3)		81 (3)	
yes	6 (2)	50 (25)	0.001	50 (25)	0.002
Biochemical Characteristics					
C-reactive protein ≤10mg/l	125 (51)	86 (4)		92 (3)	
>10mg/l	119 (49)	63 (4)	<0.001	68 (4)	<0.001
Albumin ≥35g/l	204 (83)	78 (3)		83 (3)	
<35g/l	40 (17)	58 (8)	0.001	67 (8)	0.004
Petersen Index					
Low Risk	203 (87)	79 (3)		84 (3)	
High Risk	41 (13)	54 (8)	<0.001	61 (8)	<0.001
mGlasgow Prognostic Score					
Low Risk (0)	125 (51)	86 (3)		92 (3)	
Intermediate (1)	88 (36)	68 (5)		72 (5)	
High Risk (2)	31 (13)	48 (9)	<0.001	57 (9)	<0.001

Table 5.2. Clinico-pathological characteristics and 3 year cancer specific survival in patients undergoing potentially curative resection for Dukes' B and Dukes' C colon cancer: Multivariate survival analysis.

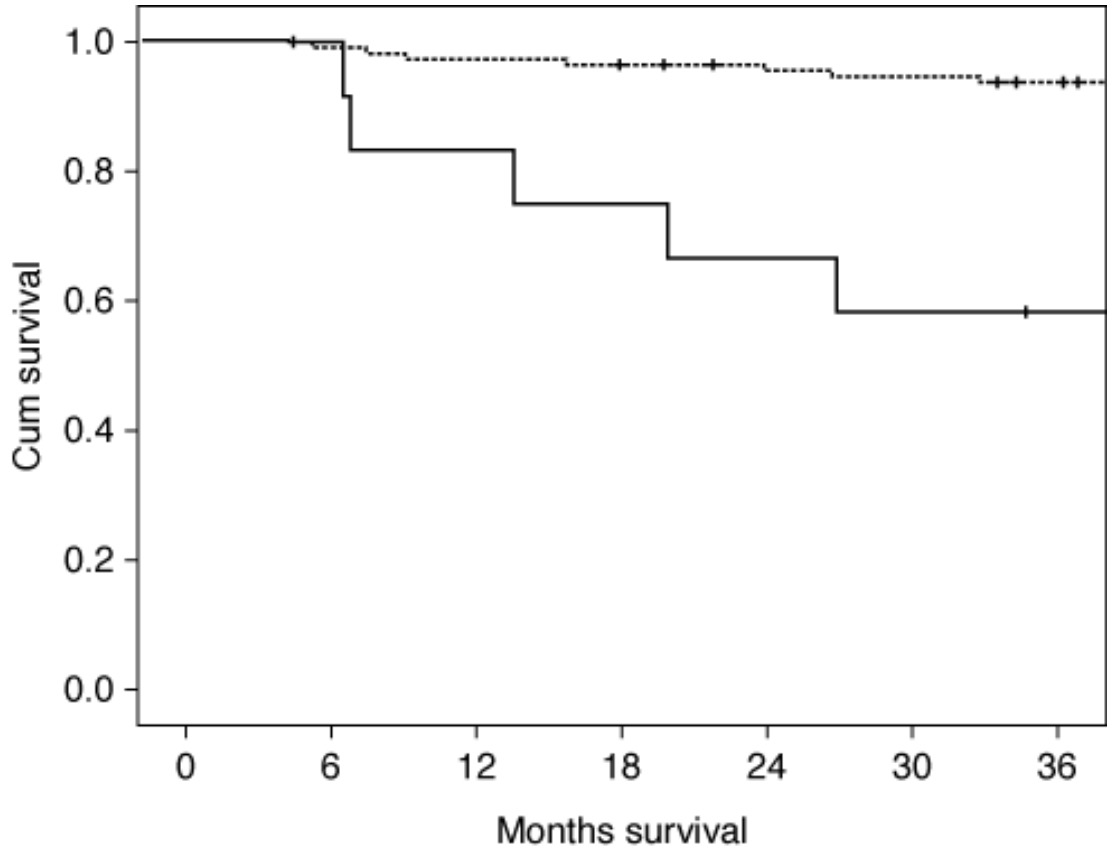
Dukes' B/ Stage II	**n=127 (%)**	**3 yr Survival % (SE)**	**Hazard ratio (95% CI)**	**P-value**
Age <65yr	37 (29)	100 (0)		
65-74yr	40 (31)	92 (4)		
>75yr	50 (40)	81 (6)	1.87 (1.05-3.34)	0.034
Sex Female	61 (48)	90 (4)		
Male	66 (62)	91 (4)	0.99 (0.39-2.51)	0.984
Adjuvant therapy No	111 (87)	92 (3)		
Yes	16 (13)	81 (10)	0.98 (0.22-4.44)	0.979
Petersen Index Low Risk	115 (91)	94 (2)		
High Risk	12 (9)	58 (14)	9.61 (3.27-28.26)	<0.001
mGlasgow Prognostic Score Low 0	62 (49)	97 (2)		
Intermediate 1	47 (37)	87 (5)		
High Risk 2	18 (14)	78 (10)	2.15 (1.19-3.87)	0.010
Dukes' C/ Stage III	**n=99 (%)**	**3 yr Survival % (SE)**	**Hazard ratio (95% CI)**	**P-value**
Age <65yr	25 (25)	88 (6)		
65-74yr	25 (25)	66 (10)		
>75yr	49 (50)	48 (7)	1.72 (1.08-2.75)	0.022
Sex Female	49 (49)	62 (7)		
Male	50 (51)	64 (7)	1.25 (0.68-2.31)	0.477
Adjuvant therapy No	59 (60)	54 (7)		
Yes	40 (40)	75 (7)	0.92 (0.42-2.00)	0.832
Petersen Index Low Risk	70 (71)	63 (6)		
High Risk	29 (29)	63 (9)	1.16 (0.60-2.24)	0.655
mGlasgow Prognostic Score Low 0	50 (51)	83 (6)		
Intermediate 1	37 (32)	51 (8)		
High Risk 2	12 (17)	24 (13)	2.80 (1.80-4.44)	<0.001

Table 5.3. The relationship between the low risk Petersen Index, and the mGlasgow Prognostic Score with 3-year survival (%) in patients undergoing potentially curative resection for Dukes' B, single node positive Dukes' C and Dukes' C colon cancer.

Dukes' B/ Stage II	**Petersen Index Low Risk (n=115)**	**Petersen Index High Risk (n=12)**
mGlasgow Prognostic Score		
Low Risk (0)	98% (n=56)	82% (n=6)
Intermediate (1)	92% (n=42)	40% (n=5)*
High Risk (2)	82% (n=17)**	0% (n=1)**
mGlasgow Prognostic Score (0-2)	94% (n=115)	58% (n=12)
Dukes' C/ Stage III	**Petersen Index Low Risk (n=70)**	**Petersen Index High Risk (n=29)**
mGlasgow Prognostic Score		
Low Risk (0)	84% (n=39)	76% (n=11)
Intermediate (1)	46% (n=24)***	62% (n=13)
High Risk (2)	10% (n=7)****	40% (n=5)***
mGlasgow Prognostic Score (0-2)	63% (n=70)	63% (n=29)

*p<0.1, **p<0.05, ***p<0.01, ****p<0.001: Association between increasing mGPS and cancer-specific survival on univariate analysis.

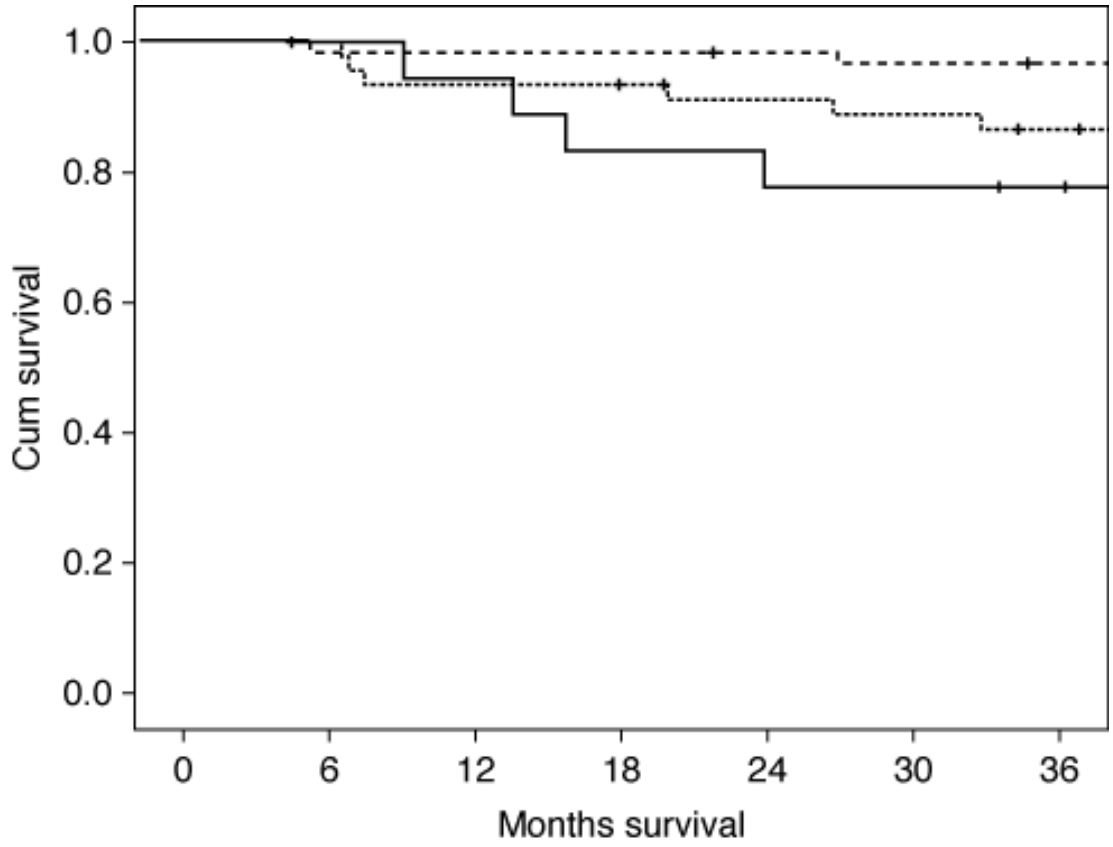
Figure 5.1: The relationship between low and high risk Petersen Index (from top to bottom) and cancer specific survival in Dukes' B colon cancer patients (P<0.001).



Numbers at risk

PI Low risk	115	113	111	109	106	105	102
PI High risk	12	12	10	9	8	7	6

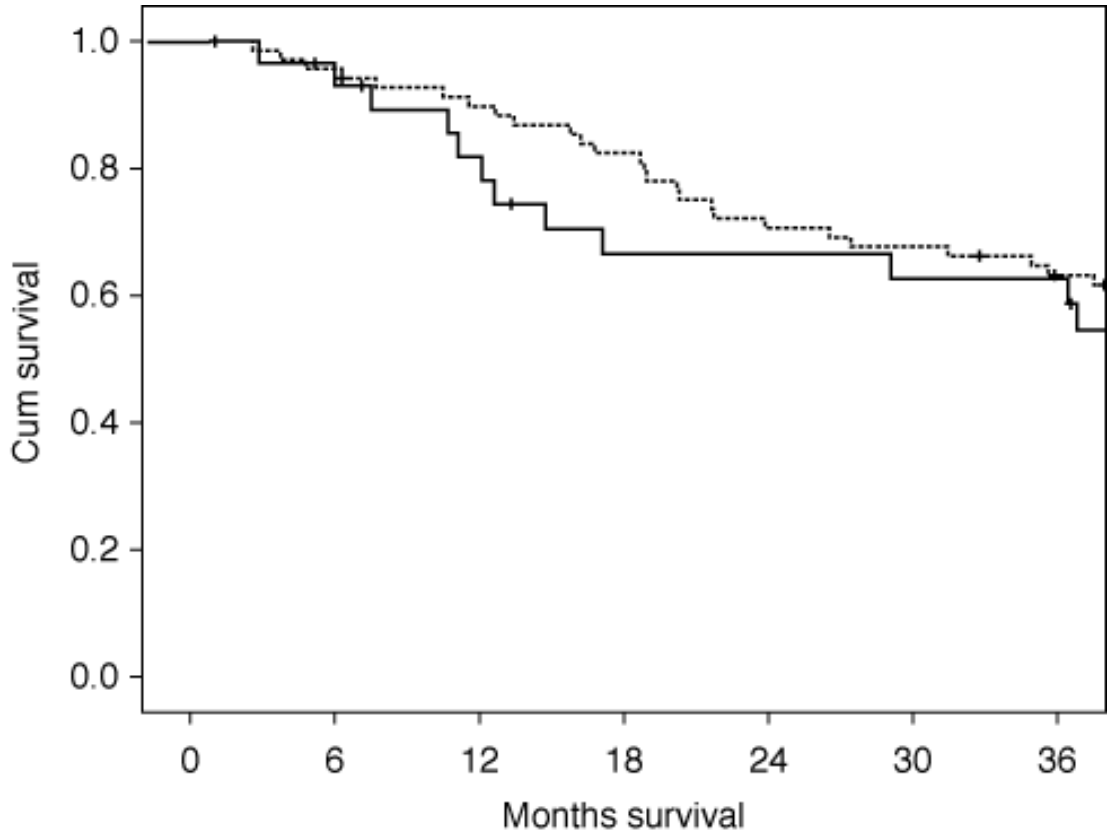
Figure 5.2: The relationship between increasing mGPS (from the top to bottom) and cancer specific survival in Dukes' B colon cancer patients (P<0.05).



Numbers at risk

mGPS 0	62	61	61	61	60	59	58
mGPS 1	47	46	43	42	40	39	37
mGPS 2	18	18	17	15	14	14	13

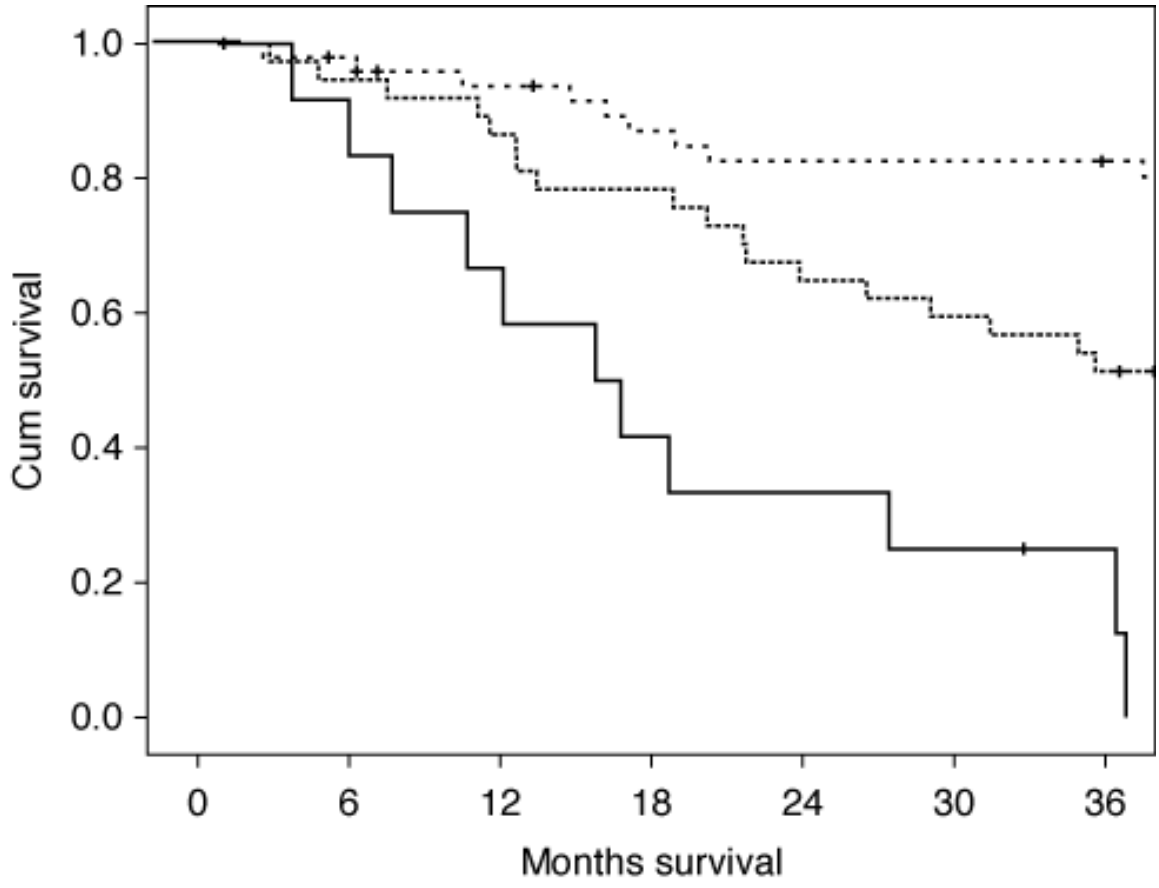
Figure 4: The relationship between low and high risk Petersen Index (from top to bottom) and cancer specific survival in Dukes' C colon cancer patients (P=0.195).



Numbers at risk

PI Low risk	115	113	111	109	106	105	102
PI High risk	12	12	10	9	8	7	6

Figure 5.4: The relationship between increasing mGPS (from the top to bottom) and cancer specific survival in Dukes' C colon cancer patients (P<0.001).



Numbers at risk

mGPS 0	50	47	43	39	37	37	36
mGPS 1	37	35	32	29	24	22	19
mGPS 2	12	11	8	5	4	3	2

6.0 COMPARISON OF THE PROGNOSTIC VALUE OF LYMPH NODES SAMPLED, N STAGE, THE LYMPH NODE RATIO AND THE SYSTEMIC INFLAMMATORY RESPONSE IN PATIENTS UNDERGOING CURATIVE RESECTION FOR COLORECTAL CANCER.

6.1 Introduction

Tumour stage, in terms of the extent of local spread and lymph node status, has long been recognised to predict outcome. This forms the basis of the Dukes' and TNM staging systems (182, 188). Both Dukes' and TNM staging systems are widely used in colon and rectal cancer but it is recognised that survival of patients within the staging categories is variable. Indeed, certain subgroups of patients with stage III disease have a better prognosis than patients with stage II disease. As a result other prognostic models in addition to TNM have been developed.

In Dukes' B or T3/4 N0 colorectal cancer, the Petersen Index (PI) has been validated as a prognostic score that stratifies patients with Stage II disease by risk of recurrence and who may benefit from adjuvant chemotherapy (194, 195). This PI is based on the presence of so called 'high risk' pathological characteristics other than nodal status, including peritoneal or serosal involvement, venous spread (both submucosal and extramural), spread to involve a surgical margin, and perforation through the tumour. Within the above tumour staging systems patients have a worse outcome when more of these features are present.

In Dukes' C, or node positive (N1/2) colorectal cancer, increased quality of surgery and pathology and therefore increased lymph node retrieval has given rise to the examination of the ratio of metastatic lymph nodes to lymph nodes examined. This has been termed the lymph node ratio (LNR) and, within the above tumour staging systems patients with a high LNR have a worse outcome (215, 216). However, it is also recognised that the constituents used to form the LNR including the number of positive and negative lymph nodes and the total lymph node count are of prognostic significance (444, 445). Therefore it is unclear whether the LNR provides additional prognostic information independent of N stage, positive and negative lymph node

counts or even total lymph node count.

It is increasingly recognised that in addition to factors intrinsic to the tumour such as pathological characteristics, host factors such the presence of a systemic inflammatory response predict poor outcome in patients with colorectal cancer. An inflammation-based score, based on two routinely measured acute phase proteins (C-reactive protein and albumin), the mGlasgow Prognostic Score (GPS) has been shown to predict cancer-specific survival independent of Dukes' and TNM stage (308, 325) and superior to other markers of the systemic inflammatory response (278) in patients undergoing potentially curative resection for colorectal cancer. More recently, it has been reported that the GPS can predict cancer-specific survival independent of the tumour characteristics of the PI in node negative disease (Chapter 5.0) in patients undergoing potentially curative resection for colon cancer. However, whether the GPS offers prognostic value independent of lymph node counts including the LNR in both colon and rectal cancer has not been previously examined.

The aim of the present study was to compare the prognostic value of lymph nodes sampled including positive and negative lymph node counts, N stage, lymph node ratio and the systemic inflammatory response of patients undergoing curative resection for colon and rectal cancer.

6.2 Materials and Methods

Patients with histologically proven colorectal cancer cancer who underwent potentially curative resection between 1997 and 2007 were identified from a prospectively collected database with similar exclusion criteria to those in Chapter 4.0. Tumours were staged using the conventional Tumour, Node and Metastases classification (from the 5th edition UICC and according to the Royal College of Pathologists Dataset 2007) (188). Pathological data were taken from the pathology reports issued at the time of resection.

The lymph node ratio is calculated by dividing the number of metastatic lymph nodes identified by the total number of lymph nodes sampled. A variety of thresholds have been examined in the past. In the present study, cut offs of 0.05, 0.2 and 0.4 were used to stratify patients as high or low risk within the Stage III or node positive patients as previously described (215, 446).

Blood samples were taken for routine laboratory measurements of albumin and C-reactive protein measurement prior to surgery. The mGPS was constructed as previously described (Chapter 4.0, Methods 4.2).

The provision of adjuvant treatment was at the discretion of the oncologist managing the patient following the multi-disciplinary team assessment. Therefore, all biochemical and pathological results as well as patient co-morbidities were available to the oncologist in making such decisions on adjuvant treatment.

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

Statistics

Grouping of the variables was carried out using standard thresholds. Univariate survival analysis was performed using the Kaplan–Meier method with the log-rank test. Multivariate survival analysis and calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.05. Deaths up to March 1st 2010 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

6.3 Results

Between January 1997 and February 2007, 540 patients underwent elective resection for colorectal cancer at Glasgow Royal Infirmary. The median number of lymph nodes sampled overall was 14 (1-52). 348 patients (64%) had 12 or more lymph nodes retrieved from the pathology specimen.

Of the 540 patients who underwent curative resection for colorectal cancer, the majority were 65 or older (74%), male (55%), and had TNM stage I/II disease (57%). One hundred and eighteen (22%) patients received adjuvant chemotherapy. The majority had a LNR of <0.05 (59%). In 22% the LNR was 0.05-0.19, 11% had a LNR between 0.2-0.39 and 8% a LNR of 0.4 or more. The majority of patients had C-reactive protein (56%) and albumin (80%) concentrations in the normal range and a normal mGPS (56%). Of the 106 patients with hypoalbuminaemia, 76 (71%) had an elevated C-reactive protein concentration.

The minimum follow-up was 37 months; the median follow-up of the survivors was 77 months. No patients were lost to follow up. During this period 156 patients died of their cancer and a further 102 patients died of intercurrent disease. The univariate survival analysis for lymph nodes sampled including, positive and negative lymph node counts, N stage, lymph node ratio and the systemic inflammatory response in patients undergoing curative resection for colorectal cancer (n=540) are shown in Table 6.1.

On univariate analysis in all patients (n=540), number of positive lymph nodes (P<0.001), number of negative lymph nodes (P<0.001), the LNR as a continuous (P<0.001) and categorical variable (P<0.001), N stage (P<0.001) and the mGPS (P<0.001) were associated significantly with cancer specific survival (Table 2). On multivariate analysis of these significant variables, positive lymph nodes (HR 1.16, 95% CI 1.11-1.21, P<0.001), negative lymph nodes (HR 0.96, 95%CI 0.94-0.99, P=0.004), N stage (HR 2.06, 95%CI 1.68-2.52, P<0.001), and the mGPS (HR 1.82, 95%CI 1.47-2.24, P<0.001) were all independently associated with cancer specific survival (Table 6.1).

Table 6.2 shows the univariate and multivariate survival analysis in colon cancer (n=348) On univariate analysis, the number of positive lymph nodes (P<0.001), number of negative lymph nodes (P<0.05), the LNR as a continuous (P<0.001) and categorical variable (P<0.001), N stage (P<0.001) and the mGPS (P<0.001) were associated significantly with cancer specific survival (Table 2). On multivariate analysis of these significant variables, positive lymph nodes (HR 1.18, 95%CI 1.12-1.24, P<0.001), N stage (HR 2.14, 95%CI 1.68-2.74, P<0.001), and the mGPS (HR 1.73, 95%CI 1.35-2.26, P<0.001) were all independently associated with cancer specific survival (Table 6.2).

Table 6.3 shows the univariate and multivariate survival analysis in rectal cancer (n=192) On univariate analysis the number of positive lymph nodes (P<0.001), number of negative lymph nodes (P<0.001), the LNR as a continuous (P<0.001) and categorical variable (P<0.001), N stage (P<0.001) and the mGPS (P<0.001) were associated significantly with cancer specific survival (Table 2). On multivariate analysis of these significant variables, positive lymph nodes (HR 1.17, 95% CI 1.09-2.53, P=0.084), negative lymph nodes (HR 0.92, 95%CI 0.88-0.97, P=0.001), LNR as a categorical variable (HR 1.67, 95%CI 1.30-2.15, P<0.001), and the mGPS (HR 1.85, 95%CI 1.26-2.15, P=0.002) were all independently associated with cancer specific survival (Table 6.3).

The relationship between clinico-pathological characteristics, the total nodes sampled, positive and lymph node counts and the lymph node ratio in patients undergoing potentially curative resection for colon cancer and rectal cancer are shown in tables 6.4 and 6.5. In colon cancer, increasing age was associated with higher lymph node sample (P<0.01). Increasing TNM stage was associated with higher lymph node sample (P<0.005), higher number of positive lymph nodes (P<0.001), higher N stage (P<0.001) and increasing LNR (P<0.001). Increasing T stage was associated with higher lymph node sample (P<0.05), higher number of positive lymph nodes (P<0.001), higher N stage (P<0.001) and increasing LNR (P<0.001). No significant differences were observed between these variables and time period of surgery, patient sex or the mGPS (Table 6.4).

In rectal cancer, increasing TNM stage was associated with higher number of positive lymph nodes ($P<0.001$), higher N stage ($P<0.001$) and increasing LNR ($P<0.001$). Increasing T stage was associated with higher lymph node sample ($P<0.05$), higher number of positive lymph nodes ($P<0.005$), higher N stage ($P<0.005$) and increasing LNR ($P<0.005$). Increasing mGPS was associated with higher number of lymph nodes sampled in rectal cancer ($P<0.001$). No significant differences were observed between these variables and age, time period of surgery or patient sex (Table 6.5).

6.4 Discussion

The results of the present study confirm the importance of adequate lymph node retrieval and accurate pathological assessment of lymph nodes in colorectal cancer. Indeed, the presence of lymph node involvement in colorectal cancer has an established prognostic role and is used to guide allocation of adjuvant therapy (182, 419). More recently the lymph node ratio has been reported to provide additional prognostic information in addition to tumour stage including lymph node status (216, 218, 446). The present study examined the role of the LNR with previously validated thresholds, in addition to other measures of lymph node assessment. The results presented here confirm the prognostic significance of the positive and negative lymph node counts in addition to the lymph node ratio and N stage on univariate analysis. However, the lymph node ratio lost significance on multivariate analysis. In all patients the positive and negative lymph node counts and N stage were independent measures of lymph node involvement. When tumour site was considered separately, the LNR measured as a categorical variable obtained independent prognostic significance in rectal cancer only. These results suggest that the LNR requires further evaluation before its use is recommended in routine clinical practice.

In the present study the prognostic value of the mGPS was consistently independent of lymph node status, including the LNR in both colon and rectal cancer. Therefore the pre-operative mGPS could be used in addition to established measures of lymph node assessment to stratify patients with colon and rectal cancer by risk of cancer death.

In the present study, the overall median number of lymph nodes sampled was 14. This is in line with the Royal College of Pathologists Guidelines (188). Adequate lymph node retrieval is essential for accurate prognostication in colorectal cancer (188, 447, 448). Factors associated with higher lymph node yield in the present study were increasing TNM stage and T stage. The reasons for such an observation are not clear, however inadequate lymph node yields may under-stage patients placing them in a lower TNM category. Increasing age was also associated with lower lymph node harvest in colon cancer. This observation is in keeping with previous reports (447-449). Reasons for this observation are not clear but may potentially reflect lower radicality of surgery in the very elderly and symptomatic patients. Of interest,

increasing mGPS was significantly related to lower lymph node retrieval in rectal cancer. The basis of this observation is unclear given all patients underwent total mesorectal excision in the present cohort. Importantly, the mGPS was not related to increased nodal involvement. One possibility is that an increasing mGPS may relate to increasing burden of comorbid disease and old age (Chapter 7.0). In elderly patients with significant comorbidity, the threshold for a radical lymphadenectomy may be higher.

These results would not support the use of the LNR routinely; however as yet there has been no general agreement on the LNR thresholds. Even recently published reports by Rosenberg and Peschaud use cut offs of 0.17, 0.41, 0.69 and 0.07 and 0.2 respectively (218, 219). Clearly, without widely accepted defined thresholds the widespread clinical use of the LNR will be hampered. The present study has validated the cut-offs of 0.05, 0.2 and 0.4 in patients undergoing an elective potentially curative resection for colon cancer in agreement with recent reports (215, 446). The variation in thresholds reported in the published literature may reflect the clear tension that exists between N stage, positive and negative lymph node counts and the lymph node ratio in attaining independent prognostic significance in colorectal cancer.

Given the observed prognostic importance of positive and negative lymph node counts in addition to the lymph node ratio, several criteria must be fulfilled in clinical practice. Firstly, that a high quality surgical lymphadenectomy is performed and secondly, that the subsequent pathological lymph node retrieval is optimal. The results from the present study represent a consecutive case series from a single centre, the Glasgow Royal Infirmary. A variety of surgeons and pathologists including training grade doctors performed the surgery or undertook the pathological analysis. Therefore, these results are reflective of 'real world' surgery and pathological reporting in a busy teaching hospital in the United Kingdom. The use of single institution data provides a degree of standardisation of surgical and pathological techniques over the time period studied.

The mGlasgow Prognostic Score, a simple objective and standardised measure of the systemic inflammatory response measured prior to surgery, has been shown to have prognostic value, independent of tumour stage, in a variety of common solid tumours

(450). Recently, the pre-operative mGPS has been shown to have prognostic value in node negative colon cancer, independent of high-risk pathological criteria (Petersen Index) (Chapter 5.0). The results of the present study indicate that pre-operative mGPS has prognostic value in addition to positive and negative lymph node counts and, to a lesser extent, the use of the LNR in predicting cancer-specific survival in patients undergoing elective potentially curative for colorectal cancer. Taken together these results support the routine use of the mGPS in addition to accurate lymph node evaluation for prognostication and trial stratification in colon and rectal cancer.

Table 6.1: Clinico-pathological variables and cancer specific survival of patients undergoing curative resection for colorectal cancer (n=540). Univariate and multivariate analysis.

Continuous variable	**Univariate analysis**	**Multivariate Analysis**	
	P value	**HR (95% CI)**	**P value**
Total lymph nodes	0.130		
Positive lymph nodes	<0.001	1.16 (1.11-1.21)	<0.001
Negative lymph nodes	<0.001	0.96 (0.94-0.99)	0.004
Lymph node ratio	<0.001		0.238
Categorical variable			
Lymph Node Ratio <0.05/ 0.05-0.19/ 0.20-0.39/ 0.4-1.0	<0.001		0.371
N stage 0/1/2	<0.001	2.06 (1.68-2.52)	<0.001
mGPS 0/ 1/ 2	<0.001	1.82 (1.47-2.24)	<0.001

Table 6.2: Clinico-pathological variables and cancer specific survival of patients undergoing curative resection for colon cancer (n=348). Univariate and multivariate analysis.

Continuous variable	Univariate analysis	Multivariate Analysis	
	P value	**HR (95% CI)**	**P value**
Total lymph nodes	0.639		
Positive lymph nodes	<0.001	1.18 (1.12-1.24)	<0.001
Negative lymph nodes	0.011		0.513
Lymph node ratio	<0.001		0.454
Categorical variable			
Lymph Node Ratio <0.05/ 0.05-0.19/ 0.20-0.39/ 0.4-1.0	<0.001		0.371
N stage 0/1/2	<0.001	2.14 (1.68-2.74)	<0.001
mGPS 0/ 1/ 2	<0.001	1.73 (1.35-2.26)	<0.001

Table 6.3: Clinico-pathological variables and cancer specific survival of patients undergoing curative resection for rectal cancer (n=192). Univariate and multivariate analysis.

Continuous variable	Univariate analysis	Multivariate Analysis	
	P value	**HR (95% CI)**	**P value**
Total lymph nodes	0.053		
Positive lymph nodes	<0.001	1.17 (1.09-2.53)	0.084
Negative lymph nodes	<0.001	0.92 (0.88-0.97)	0.001
Lymph node ratio	<0.001		0.595
Categorical variable			
Lymph Node Ratio <0.05/ 0.05-0.19/ 0.20-0.39/ 0.4-1.0	<0.001	1.67 (1.30-2.15)	<0.001
N stage 0/1/2	<0.001		0.801
mGPS 0/ 1/ 2	0.001	1.85 (1.26-2.15)	0.002

Table 6.4: The relationship between clinico-pathological characteristics, the total nodes sampled, positive lymph node counts and the lymph node ratio in patients undergoing potentially curative resection for colon cancer (n=348).

	Total LN sampled mean +/-SD	P value	Positive LN mean+/-SD	P value	N stage mean+/-SD	P value	Lymph node ratio mean+/-SD	P value
Age <65 years	16.4+/-7.2		1.3+/-2.7		0.5+/-0.6		0.09+/-0.16	
65-74years	14.3+/-6.8		1.5+/-3.2		0.5+/-0.6		0.10+/-0.18	
>75years	13.7+/-6.0	0.009	1.3+/-2.0	0.269	0.6+/-0.7	0.309	0.10+/-0.18	0.281
Sex Female	14.5+/-6.8		1.3+/-2.7		0.5+/-0.6		0.10+/-0.17	
Male	14.9+/-7.0	0.310	1.4+/-2.6	0.786	0.5+/-0.7	0.798	0.10+/-0.18	0.796
Timespan 1997-2002	14.5+/-6.3		0.9+/-1.6		0.4+/-0.6		0.08+/-0.15	
2002-2006	15.0+/-6.6	0.642	1.7+/-3.2	0.060	0.6+/-0.7	0.066	0.12+/-0.20	0.873
Stage TNM I	11.6+/-6.0		-		-		-	
TNM II	15.4+/-7.0		-		-		-	
TNM III	14.5+/-5.6	0.003	3.2+/-3.3	<0.001	1.3+/-0.4	<0.001	0.24+/-0.21	<0.001
T stage 1	12.5+/-10.4		-		-		-	
2	12.6+/-5.5		0.2+/-0.6		0.1+/-0.4		0.02+/-0.03	
3	15.1+/-6.8		0.9+/-1.7		0.4/-0.6		0.07+/-0.15	
4	14.8+/-5.8	0.010	2.3+/-3.6	<0.001	0.8+/-0.7	<0.001	0.16+/-0.22	<0.001
mGPS 0	14.0+/-5.6		1.2+/-2.3		0.5+/-0.7		0.09+/-0.17	
1	15.4+/-6.9		1.7+/-3.2		0.6+/-0.7		0.12+/-0.20	
2	16.4+/-7.6	0.150	1.3+/-2.4	0.388	0.5+/-0.7	0.455	0.07+/-0.13	0.377

Table 6.5: The relationship between clinico-pathological characteristics, the total nodes sampled, positive lymph node counts and the lymph node ratio in patients undergoing potentially curative resection for rectal cancer (n=192).

	Total LN sampled mean +/-SD	P value	Positive LN mean+/-SD	P value	N stage mean+/-SD	P value	Lymph node ratio mean+/-SD	P value
Age <65 years	16.7+/-7.5		1.9+/-2.8		0.8+/-0.8		0.10+/-0.15	
65-74years	16.0+/-8.1		1.5+/-3.0		0.6+/-0.7		0.10+/-0.19	
>75years	13.3+/-6.5	0.309	1.0+/-1.5	0.684	0.5+/-0.6	0.505	0.08+/-0.14	0.843
Sex Female	16.0+/-8.4		1.5+/-2.7		0.6+/-0.7		0.10+/-0.18	
Male	15.3+/-7.0	0.729	1.5+/-2.7	0.842	0.6+/-0.7	0.712	0.10+/-0.15	0.798
Timespan 1997-2002	16.1+/-8.6		1.3+/-2.0		0.6+/-0.8		0.09+/-0.15	
2002-2006	15.3+/-6.8	0.928	1.7+/-3.0	0.738	0.6+/-0.7	0.516	0.10+/-0.17	0.674
Stage TNM I	12.4+/-7.2		-		-		-	
TNM II	15.1+/-8.1		-		-		-	
TNM III	16.9+/-7.0	0.057	3.2+/-3.1	<0.001	1.3+/-0.5	<0.001	0.21+/-0.18	<0.001
T stage 1	8.9+/-2.4		0.8+/-1.2		0.4+/-0.5		0.08+/-0.13	
2	13.9+/-6.8		0.9+/-1.8		0.4+/-0.7		0.07+/-0.13	
3	16.3+/-7.9		1.3+/-2.3		0.6+/-0.7		0.09+/-0.16	
4	16.5+/-7.1	0.011	2.7+/-4.0	0.002	0.9+/-0.8	0.003	0.15+/-0.18	0.003
mGPS 0	15.3+/-7.0		1.5+/-2.8		0.6+/-0.7		0.10+/-0.17	
1	18.2+/-8.9		1.5+/-2.1		0.7+/-0.7		0.08+/-0.11	
2	11.0+/-5.4	0.001	1.7+/-2.8	0.505	0.6+/-0.9	0.488	0.14+/-0.23	0.731

7.0 THE RELATIONSHIP BETWEEN PRE-OPERATIVE COMORBIDITY, THE SYSTEMIC INFLAMMATORY RESPONSE AND SURVIVAL IN PATIENTS UNDERGOING CURATIVE RESECTION FOR COLORECTAL CANCER.

7.1 Introduction

It is increasingly recognised that disease progression and cancer specific survival in colorectal cancer patients is not solely determined by the intrinsic characteristics of the tumour but also by host characteristics and in particular the systemic inflammatory response. Since the initial report (308), more than 20 studies have reported markers of the systemic inflammatory response (in particular C-reactive protein, albumin and their combination the mGlasgow Prognostic Score; mGPS), predict cancer specific survival in patients undergoing surgery for colorectal cancer and this is independent of tumour stage and other high risk pathological features (325, 451).

The basis of this relationship with survival is not clear and it is not known which host characteristics are associated with an elevated mGPS in cancer patients represents. It is therefore of interest that a systemic inflammatory response had been reported to predict cardiac events (126, 128, 452) and is associated with patient-related factors such as obesity (129, 130), diabetes (131-133) and smoking (453). One hypothesis, therefore, is that a pre-operative systemic inflammatory response, as evidenced by the mGPS, reflects in part the pre-existence of co-morbid disease. Indeed, validated measures of comorbid disease such as an elevated Charlson comorbidity index, National Institute on Aging and National Cancer Institute (NIA/NCI) Comorbidity Index and the Adult Comorbidity Evaluation-27 (ACE-27) have been reported to be associated with poorer long term survival in patients undergoing surgery for colorectal cancer (99, 454-456). However, it remains to be established whether such comorbidity measures are associated with the mGPS and whether comorbid disease can account for the relationship between the systemic inflammatory response (mGPS) and cancer specific survival.

The aim of the present study was to examine the relationships between pre-operative comorbidity assessed with four separate comorbidity scores (Charlson comorbidity index, NIA/NCI comorbidity index, ACE-27 comorbidity index and Lee cardiac risk index), the systemic inflammatory response (mGPS) and survival in patients undergoing curative resection for colorectal cancer.

7.2 Materials and Methods

Patients with histologically proven colon cancer who underwent potentially curative resection between January 1997 and December 2005 were identified from a prospectively collected database with similar exclusion criteria to those in Chapter 4.0. Patients were included where case notes were available for retrieval and data abstraction. Tumours were staged using the conventional Tumour, Node and Metastases classification (from the 5th edition and according to the Royal College of Pathologists Dataset 2007) (188). Other high-risk pathological characteristics were scored using the Petersen Index. This is a prognostic index validated in TNM stage II and III colorectal cancer (Chapter 5.2 Methods section) (194, 195).

Prospectively collected data included patient demographics, pathological characteristics of the tumour, biochemistry results to construct the mGlasgow Prognostic Score and allocated treatment including provision of adjuvant chemotherapy. The medical notes were then reviewed and data on patient comorbidity abstracted retrospectively. The case notes included anaesthetic assessment, medical and nursing notes, discharge summary as well as other clinical follow-up letters and previous drug prescriptions. Pre-operative patient height and weight was documented where available. Data was collected for post-operative complications both infective and non-infective. Infective complications included wound infection, intra-abdominal abscess, anastomotic leak, pneumonia and septicaemia. Non-infective complications included cardiac events encompassing cerebrovascular accidents, dysrythmias, acute coronary syndromes, acute myocardial infarction and pulmonary embolism. The criteria used to define post-operative complications in this cohort of patients have been described previously (457).

Only comorbid medical conditions present prior to surgery for colorectal cancer were included. On the basis of burden of comorbidity, patients were classified by four separate comorbidity scores; the Charlson Comorbidity Index (CCI), the National Institute for Aging and National Cancer Institute Index (NIA/NCI), the Adult Comorbidity Evaluation-27 (ACE-27) and the Lee Cardiac Risk Index (LCRI). The development and construction of these scores is described elsewhere (458-463). Briefly, the CCI is based on 19 conditions, which are weighted depending on risk of mortality. The sum of these weighted comorbidities categorises patients into

standard groupings as previously described (456, 458, 460). The NIA/NCI index is a constructed from a list of non-weighted conditions, which also includes smoking status and psychiatric conditions: the full list is available elsewhere (99, 459). Patients are categorised by number of comorbidities according to previously set thresholds (99, 456). The ACE-27 index categorises patients by grade of comorbidity as none, mild, moderate and severe based on 27 conditions from 12 different physiological systems or categories (461, 462, 464). The Lee cardiac risk index categories patients by risk of cardiovascular mortality based on six separate variables associated with increased cardiovascular risk (463, 465).

Extent of deprivation was defined using the Carstairs Deprivation Index (466), which was originally developed from census data in the 1980s and is based on individual postal code of residence at time of surgery. It is composed of four indicators of deprivation (car ownership, overcrowded housing, Registrar General social class and male unemployment). It has subsequently been constructed based on 2001 census data (467) and has been validated for use within central Scotland (468).

Blood samples were taken for routine laboratory measurements of C-reactive protein and albumin prior to surgery. The construction of the Glasgow Prognostic score is described in Chapter 4.0 (Methods 4.2) (308)(40).

Individual patients' suitability for adjuvant chemotherapy was determined post operatively at a multi-disciplinary team meeting including surgical, oncological, radiological, pathological and nursing input. Selected patients with TNM stage III disease and high-risk TNM stage II disease were referred to a consultant oncologist for adjuvant 5-FU based chemotherapy. In all cases the final decision to prescribe adjuvant therapy was at the discretion of the treating oncologist. Patients received regular follow-up (3 months, 6 months and then yearly to five years) with yearly CT scanning and regular colonoscopic surveillance until 5 years post surgery. Information on date and cause of death was checked with that received by the cancer registration system and the Registrar General (Scotland). The study was approved by the Research Ethics Committee, Royal Infirmary, Glasgow.

Statistics

Grouping of the variables was carried out using standard thresholds. Univariate survival analysis and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.05. Deaths up to April 2009 were included. Inter-relationships between variables were assessed using contingency table analysis with the chi-squared test for trend as appropriate. A P-value less than 0.05 was taken as significant. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

7.3 Results

Baseline clinico-pathological characteristics and the relationship with 5-year survival rate of the patients (n= 302) who underwent curative surgery for colorectal cancer are shown in Table 7.1. Most patients were 65 or older (66%), male (54%), had surgery for colonic tumours (64%), TNM stage I/II disease (60%), a low risk Petersen Index (83%) and underwent surgery between 2002-2005 (54%). Seventy-one (23%) patients received adjuvant chemotherapy. Most patients had C-reactive protein (62%) and albumin (87%) concentrations in the normal range and a normal mGPS (62%). Of the 39 patients with hypoalbuminaemia, 29 (75%) had an elevated C-reactive protein.

The majority of patients for whom heights and weights were available had a body mass index between 18-30 (69%). Most patients had a smoking history, either current or previously (59%). One hundred and four patients (34%) had a post-operative complication. Eighty-two of these were infective complications. The majority of patients' postcodes were classes within the two highest deprivation categories (54%). When the four comorbidity indices were constructed, most patients had a low burden of comorbidity. The ACE-27 index categorised most as none/mild (64%), for the Charlson Index most scored <2 (78%), for NIA/NCI most had fewer than four comorbidities (70%) and most were scored as 0-1 by the Lee Cardiac Risk Index (89%) (Table 7.1).

The minimum follow-up was 45 months; the median follow-up for survivors was 74 months. During this period 85 patients died from cancer and a further 50 patients died of intercurrent disease. For all 302 patients, the univariate survival analysis and 5 year survival rates for cancer specific and overall survival for individual clinico-pathological characteristics are shown in Table 7.1. On univariate analysis for cancer specific survival of individual variables, age ($P<0.05$), TNM stage ($P<0.001$), high risk Petersen Index ($P<0.001$), ACE-27 comorbidity index ($P<0.005$), Charlson Index ($P<0.1$), Lee Cardiac Risk Index ($P<0.005$) and the mGPS ($P<0.001$) were significant (Table 1). On univariate analysis for overall survival, age ($P<0.001$), TNM stage ($P<0.005$), high risk Petersen Index ($P<0.001$), the presence of a post operative infective complication ($P<0.01$), ACE-27 comorbidity index ($P<0.001$), Charlson Index

(P<0.001), Lee Cardiac Risk Index (P<0.005), the NIA/NCI index (P<0.1) and the mGPS (P<0.001) were significant.

For all 302 patients the multivariate analysis of significant variables for both cancer specific and overall survival is shown in Table 7.2. On multivariate analysis for cancer specific survival, age (HR 1.31, 95%CI 1.00-1.71, P=0.047), TNM stage (HR 2.28, 95%CI 1.49-3.49, P<0.001), high risk Petersen Index (HR 2.40, 95% CI 1.48-3.91, P<0.001), Lee Cardiac Risk Index (HR 1.35, 95% CI 1.05-1.75, P=0.021) and the mGPS (HR 1.81, 95% CI 1.32-2.48, P<0.001) were independently significant (Table 7.2).

When age was excluded from the analysis for cancer specific survival, TNM stage (HR 2.28, 95% CI 1.49-3.50, P<0.001), high risk Petersen Index (HR 2.37, 95%CI 1.46-3.84, P<0.001), Lee Cardiac Risk Index (HR 1.40, 95% CI 1.09-1.81, P=0.008) and the mGPS (HR 1.86, 95%CI 1.36-2.55, P<0.001) remained independently significant. When the mGPS was excluded from the analysis, age (HR 1.37, 95% CI 1.05-1.80, P=0.022), TNM stage (HR 2.01, 95% CI 1.35-3.00. P=0.001), high risk Petersen Index (HR 2.49, 95% CI 1.54-4.04, P<0.001) and the Lee Cardiac Risk Index (HR 1.38, 95%CI 1.07-1.79, P=0.014) remained independently associated with cancer specific survival.

On multivariate analysis for overall survival, age (HR 1.58, 95%CI 1.27-1.96, P<0.001), TNM stage (HR 1.72, 95%CI 1.26-2.34, P=0.001), high risk Petersen Index (HR 1.92, 95% CI 1.28-2.87, P=0.002), post-operative infective complications (HR 1.78, 95% CI 1.25-2.05, P=0.002), ACE-27 (HR 1.28, 95% CI 1.06-1.54, P=0.010) and the mGPS (HR 1.60, 95% CI 1.25-2.05, P<0.001) were independently significant (Table 7.2).

When age was excluded from the analysis for overall survival, TNM stage (HR 1.71, 95% CI 1.25-2.33, P=0.001), high risk Petersen Index (HR 1.89, 95%CI 1.26-2.82, P=0.002), post-operative infective complication (HR 1.61, 95% CI 1.12-2.33, P=0.011), ACE-27 (HR 1.33, 95% CI 1.11-1.59, P=0.002) and the mGPS (HR 1.64, 95%CI 1.28-2.10, P<0.001) remained independently significant. When the mGPS was excluded from the analysis, age (HR 1.62, 95% CI 1.30-2.02, P<0.001), TNM stage (HR 1.58, 95% CI 1.18-2.11, P=0.002), high risk Petersen Index (HR 1.95, 95% CI 1.30-2.91, P=0.001), post-operative infective complications (HR 1.82, 95% CI 1.26-2.62,

P=0.001) and ACE-27 (HR 1.29, 95% CI 1.07-1.54, P=0.007) remained independently associated with overall survival.

The inter-relationships between age, TNM stage, Petersen Index, BMI, smoking history, presence of post operative complications, the four different comorbidity indices and the mGPS are shown in Table 7.3. Increasing age was significantly related to higher comorbidity burden assessed with ACE-27 (P<0.001), Charlson index (P<0.005) and Lee cardiac risk index (P<0.005). Increasing age was also significantly related to a higher mGPS (P<0.005). Increasing BMI was significantly related to higher comorbidity indices assessed with the CCI (P<0.005), ACE-27 (P<0.01) and NCI/NIA (P<0.001) scores. A positive smoking history was significantly related to increasing burden of comorbidity assessed by all 4 indices (P<0.005). Higher levels of deprivation were related to high levels of comorbidity assessed with the CCI, ACE-27 and NCI/NIA comorbidity indices (P<0.05). Post-operative complications were related to higher comorbidity burden assessed by all four comorbidity indices (P<0.05). Post-operative infective complications were associated with a smoking history, higher levels of deprivation and increased burden of comorbidity measured by the NCI/NIA index only. Deprivation was weakly associated with the mGPS (P=0.070). The four individual comorbidity indices were all closely related (P<0.001). The mGPS was associated with a higher burden of comorbidity assessed using the ACE-27 index (P=0.065), the Charlson Index (P=0.016), Lee cardiac risk index (P=0.095) and the NIA/NCI index (P=0.084) (Table 7.3).

7.4 Discussion

The results of the present study show that pre-operative measures of comorbid disease, such as the Charlson Comorbidity Index, Adult Comorbidity Evaluation-27, and the Lee Cardiac Risk Index, were significantly associated with cancer specific survival in patients undergoing curative colorectal cancer resection. However, when considered with age, TNM stage and the mGPS, all comorbidity measures except the Lee Cardiac Risk Index no longer achieved statistical significance. Furthermore, all comorbidity measures were associated with the systemic inflammatory response, in particular, the Charlson Index. However, the results of the present study indicate that, using the above measures, generalised comorbidity, in particular that measured by the LCRI, does not fully explain the relationship between the systemic inflammatory response and poorer cancer specific survival in patients undergoing potentially curative resection for colorectal cancer.

The ACE-27, CCI and NCI/ NIA indices have previously been observed to predict cancer specific and overall survival in colorectal cancer (99, 454-456). In the present study the Lee Cardiac Risk index was also examined and found to be the only one of the four comorbidity indices to independently predict cancer survival. Each of the four comorbidity indices included in the present study reflect different aspects of comorbidity. The Lee Risk index is validated as a predictor of peri-operative cardiovascular morbidity and mortality (463, 465). It is constructed from 6 simple variables: high risk surgery, history of coronary artery disease, history of congestive cardiac failure, history of cerebrovascular disease, diabetes mellitus treated with insulin and pre-operative serum creatinine >2mg/dl. This differs from the other indices which include significantly higher number of conditions such as the CCI or ACE-27 which are based on 19 and 27 conditions respectively (458-461).

The basis of the independent relationship between an increased Lee Cardiac Risk Index and cancer survival is not clear. However, it is of interest that Chan and coworkers reported a two-fold greater incidence of colorectal cancer in patients with coronary heart disease (469). They suggested that this was due to similar shared risk factors such as metabolic syndrome or smoking history and that both colorectal neoplasm and coronary artery disease develop through the mechanism of chronic inflammation. In the present study the Lee Cardiac Risk Index was indeed

significantly associated with smoking history but was only weakly associated with systemic inflammation as evidenced by the mGPS. Therefore, although cardiac disease appears to be an independent determinant of colorectal cancer outcome it does not appears that this is being predominantly mediated through a systemic inflammatory response.

The inter-relationships between the host factors of age, deprivation, comorbidity and the systemic inflammatory response are likely to be complex, but in the present study both comorbidity and the systemic inflammatory response were significantly associated with increasing age. However, although age weakened the prognostic value of the comorbidity measures this was not the case with the mGPS highlighting the importance of the systemic inflammatory response in determining cancer outcome.

In the present study patient comorbidity did not fully explain the relationship between the systemic inflammatory response, as evidenced by the mGlasgow Prognostic Score, and poorer cancer specific survival. This would suggest a more complex basis. Indeed, in addition to comorbid disease, the presence of a systemic inflammatory response is recognised to be associated with cancer cachexia, compromised cell mediated immunity and upregulation of growth factors and angiogenesis, all of which may contribute to tumour growth and dissemination and poorer cancer specific survival (422).

At present clinicians treating cancer patients make decisions based on an individual patient's fitness and comorbidity. However, in the present study, with the exception of the Lee Cardiac Risk Index, the relationship between high comorbidity index scores and poor survival appears to primarily reflect increasing age. Of course a heavy burden of comorbidity, as well as age, may have an indirect impact on the host physiological reserve including anti-tumour immune competences. Work is required to establish the survival benefits of strategies whereby both the host and tumour factors are staged and managed in patients with colorectal cancer.

There are a number of important implications of the results of the present study. Firstly, that measures of comorbidity not only are associated with poor short term outcomes but also long term survival, in particular the Lee Cardiac Risk index, in

patients undergoing curative resection for colorectal cancer. If this is confirmed in other large studies clinicians should consider incorporating a measure of cardiac function such as the Lee Cardiac Risk Index in the routine pre-operative staging of patients undergoing surgery for colorectal cancer. Secondly, that the mGPS although associated with measures of comorbidity, has additional independent prognostic value not encapsulated in the present comorbidity scores. Therefore, the results of the present study confirm the clinical utility of the pre-operative measurement of the mGPS. This would suggest that it will be a valuable tool for patient stratification within large-scale prospective trials of anti-cancer therapies and may form the basis of new anti-inflammatory therapies in patients undergoing curative resection of colorectal cancer.

With reference to the latter implication it is of interest that the systemic inflammatory response (as evidenced by elevated C-reactive protein) has recently been reported to predict development of cardiovascular disease including hypertension and atheroma formation and place patients at higher risk of cardiac events (126-128). Such patients are also at higher risk of developing type II diabetes and the metabolic syndrome (129-133, 470). Indeed, there is some evidence that the use of statins which reduce the systemic inflammatory response and improve cardiovascular disease may improve outcomes in patients with colorectal cancer (471). Therefore, although the tumour and its response to therapy form the mainstay of current cancer treatment it may be that novel "host-related" targets for oncological therapy will become of considerable importance. These may include attenuation of comorbid conditions and the host systemic inflammatory response. In any case, there is a now need for clinicians to not only accurately stage the tumour but also to accurately stage the patient.

From the above and given the limitations of the present comorbidity measures further work investigating more direct measures of organ function such as physiological POSSUM will be required to exclude the possibility that comorbidity is responsible for the relationship between the systemic inflammatory response and survival in patients undergoing potentially curative resection for colorectal cancer.

In summary, comorbidity does not fully explain the relationship between the mGPS and cancer specific survival in colorectal cancer patients. Furthermore, comorbidity, in particular that measured by the LCRI, is an important independent indicator of cancer survival.

Table 7.1: Clinico-pathological characteristics in patients undergoing curative resection for colorectal cancer: Univariate survival analysis.

	Patients 302 (%)	Cancer 5-year survival % (SE)	P-value	Overall 5-year survival % (SE)	P-value
Age <65 years	102 (34)	79 (4)		71 (5)	
65-74years	105 (35)	73 (5)		65 (5)	
>75years	95 (31)	61 (6)	0.037	39 (5)	<0.001
Sex Female	138 (46)	76 (4)		61 (4)	
Male	164 (54)	68 (4)	0.122	58 (4)	0.197
Site Colon	192 (64)	73 (3)		62 (4)	
Rectum	110 (36)	69 (5)	0.866	54 (5)	0.592
TNM Stage I	35 (12)	88 (5)		77 (8)	
II	145 (48)	83 (4)		66 (4)	
III	122 (40)	55 (6)	<0.001	46 (5)	0.002
Petersen Index Low risk	250 (83)	81 (3)		69 (3)	
High risk	52 (17)	51 (7)	<0.001	42 (7)	<0.001
Adjuvant therapy no	231 (77)	75 (3)		61 (3)	
yes	71 (23)	63 (6)	0.110	54 (6)	0.508
Date of Surgery 1997-2001	140 (46)	78 (4)		61 (4)	
2002-2005	162 (54)	73 (4)	0.288	58 (4)	0.333
Body Mass Index* <18	11 (4)	51 (16)		45 (15)	
18-25	112 (37)	70 (5)		60 (5)	
25-30	98 (32)	74 (5)		63 (5)	
>30	41 (14)	77 (7)	0.436	66 (9)	0.711
Smoking history No	124 (41)	74 (4)		67 (4)	
(current or previous) Yes	178 (59)	70 (4)	0.824	54 (4)	0.067
Carstairs Deprivation Index 1-2	12 (4)	92 (8)		83 (11)	
3-5	127 (42)	74 (4)		61 (5)	
6-7	163 (54)	68 (4)	0.250	56 (4)	0.142
Post-operative complication No	198 (66)	62 (5)		45 (5)	
Yes	104 (34)	58 (7)	0.256	43 (6)	0.099
Post-op infective complication No	220 (37)	62 (4)		46 (4)	
Yes	82 (27)	58 (8)	0.151	39 (7)	0.009
ACE-27 comorbidity index None	93 (31)	74 (6)		63 (5)	
Mild	101 (33)	82 (4)		70 (5)	
Moderate	83 (27)	66 (5)		52 (6)	
Severe	26 (9)	39 (10)	0.001	22 (8)	<0.001
Charlson comorbidity index 0	156 (52)	74 (4)		62 (4)	
1	80 (26)	72 (6)		58 (6)	
2-3	58 (19)	70 (7)		62 (7)	
≥4	9 (3)	59 (18)	0.070	0 (0)	<0.001
Lee Cardiac Risk Index 0	207 (68)	77 (3)		64 (3)	
1	65 (21)	65 (7)		54 (7)	
2	26 (9)	55 (10)		42 (10)	
≥3	5 (2)	13 (17)	0.001	0 (0)	0.001
NCI/NIA comorbidities index 0-1	110 (36)	73 (5)		65 (5)	
2-3	102 (34)	76 (5)		60 (5)	
4-5	64 (21)	69 (6)		56 (6)	
≥6	27 (9)	48 (13)	0.473	34 (11)	0.080
mGlasgow Prognostic Score 0	188 (62)	77 (3)		67 (4)	
1	85 (28)	69 (6)		53 (6)	
2	29 (10)	45 (11)	<0.001	29 (9)	<0.001

* Body mass index available in 262/ 302 patients

Table 7.2: Clinico-pathological characteristics and relationship with cancer specific and overall survival in patients following curative resection for colorectal cancer: Multivariate survival analysis of significant variables from Table 7.1.

	Cancer-specific survival		**Overall survival**	
	Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Age <65yr/ 65-74/ >74	1.31 (1.00-1.71)	0.047	1.58 (1.27-1.96)	<0.001
TNM Stage I/ II/ III	2.28 (1.49-3.49)	<0.001	1.72 (1.26-2.34)	0.001
Petersen Index Low/ High risk	2.40 (1.48-3.91)	<0.001	1.92 (1.28-2.87)	0.002
Post op Infective Complications No/ Yes		-	1.78 (1.25-2.05)	0.002
ACE-27 comorbidity index None/ Mild/ Moderate/ Severe		0.127	1.28 (1.06-1.54)	0.008
Charlson comorbidity index 0/ 1/ 2-3/ $\geq$4		-		0.164
Lee Cardiac Risk Index 0/ 1/ 2/ $\geq$3	1.35 (1.05-1.75)	0.021		0.772
mGlasgow Prognostic Score 0/ 1/ 2	1.81 (1.32-2.48)	<0.001	1.60 (1.25-2.05)	<0.001

Table 7.3: The inter-relationships between TNM stage and host factors such as deprivation, smoking comorbidity and the mGPS in patients undergoing curative resection for colorectal cancer (n=302).

	TNM	PI	BMI	Smoking	Deprivation	Post op complication	Post op infective complication	ACE-27 CI	Charlson CI	Lee Cardiac Risk Index	NCI/NIA CI	mGPS
Age <65yr/ 65-74/ >74	0.913	0.969	0.116	0.676	0.419	0.951	0.329	<0.001	0.002	0.002	0.054	0.005
TNM Stage I/ II/ III		<0.001	0.953	0.595	0.163	0.252	0.156	0.673	0.600	0.054	0.699	0.583
Petersen Index (PI) Low risk/ High risk			0.315	0.627	0.694	0.322	0.095	0.090	0.649	0.124	0.467	0.752
Body Mass Index <18/ 18-25/ 25-30/ >30				0.592	0.012	0.082	0.362	0.006	0.004	0.144	<0.001	0.387
Smoking history None/ Current or previous					0.121	0.223	0.036	<0.001	<0.001	0.003	<0.001	0.462
Carstairs Deprivation Index 1-2, 3-5, 6-7						0.077	0.039	0.016	0.037	0.101	0.049	0.070
Post op Complications No/ Yes							-	0.006	0.015	0.049	0.002	0.616
Post op Infective Complications No/ Yes								0.086	0.256	0.343	0.047	0.092
ACE-27 comorbidity index None/ Mild/ Moderate/ Severe									<0.001	<0.001	<0.001	0.065
Charlson comorbidity index 1/ 2-3/ ≥4										<0.001	<0.001	0.016
Lee Cardiac Risk Index 0/ 1/ 2/ ≥3											<0.001	0.095
NCI/NIA comorbidities index 0-1/ 2-3/ 4-5/ ≥6												0.084

8.0 TUMOUR INFLAMMATORY INFILTRATE PREDICTS SURVIVAL FOLLOWING CURATIVE RESECTION FOR NODE-NEGATIVE COLORECTAL CANCER

8.1 Introduction

Tumour stage, in terms of the extent of local spread and lymph node status, has long been recognised to predict outcome in colorectal cancer. This is the basis of the Dukes' and TNM staging systems which have four main categories (182, 188). However some stages, e.g. Stage II (T3/4 N0, Dukes' B) include patients with a wide range of clinical outcomes. Additional clinically applicable prognostic features have therefore been sought. Pathologically determined criteria which provide independent prognostic information following resection of node-negative colorectal cancer have been identified by Shepherd's group (195). These are: peritoneal involvement, venous spread (both submucosal and extramural), spread to involve a surgical margin, and perforation through the tumour. The so-called Petersen prognostic index (PI) combines these four factors in a simple cumulative scoring system. Patients have a worse outcome when more of these features are present. Thus in lymph node negative disease, a group which makes up 40-50% of surgical resections for colorectal cancer (308, 472), 5 year survival of 75-90% is reported; however, where high risk pathological features are present survival is similar to node positive disease (473, 474). The Petersen Index can select patients at high risk of tumour recurrence who may benefit from adjuvant treatment and is validated in Dukes' B and C (single positive node) colorectal cancer (194, 195).

Since the first study by Jass and colleagues (222), the presence of a pronounced tumour inflammatory infiltrate has also been recognised as an important determinant of good outcome following potentially curative resection for colorectal cancer (276, 329, 373, 391). More recently, Galon and co-workers (391) concluded that the type and density of immune cells in and around the tumour were a primary determinant of tumour progression. However, assessment of the tumour inflammatory infiltrate is not routinely performed: for example, presence of tumour infiltrating lymphocytes is listed as non-core data within the RCPath guidelines (188). This is largely because its assessment has been perceived to be more subject to inter-observer variation than

the standard staging and Petersen parameters (227, 475). Recently, however, Klintrup, Makinen and colleagues described a simplified method for structured scoring of the inflammatory reaction at the tumour invasive edge (277). This includes all white cell types and results in a binary score of low-grade or high-grade.

The aim of the present study was to examine the prognostic value of a simple assessment of the tumour inflammatory infiltrate in addition to routinely reported tumour pathological criteria including the Petersen Index in patients undergoing curative resection for node negative colorectal cancer. Furthermore the prognostic value of inflammatory infiltrate and tumour margin characteristics in node negative disease will be examined.

8.2 Materials and methods

Patients with histologically proven colon cancer who underwent potentially curative resection between January 1997 and June 2004 were identified from a prospectively collected database with similar exclusion criteria to those in Chapter 4.0. Patients receiving pre-operative radiotherapy were excluded from the study since radiotherapy has been reported to evoke an inflammatory response (476, 477). Patients who died within 30 days of surgery were excluded from the analysis. The tumours were staged using the conventional Tumour, Node and Metastases classification (from the 5th edition and according to the Royal College of Pathologists Dataset 2007) (188). All other pathological data were taken from the pathology reports issued at the time of resection.

The routine haematoxylin and eosin slides were retrieved from the pathology archives. A minimum of three slides per specimen were selected from the deepest area of tumour invasion and scored according to both Jass (222) and Klintrup (277) criteria. The Klintrup-Makinen method is based on the deepest point of invasion identified from the 3 slides and this provides the overall score for the specimen.

Jass scoring of slides was carried out as described previously (222, 227). Briefly, the term "peritumoural lymphocytic infiltrate" was applied to the stromal response at the tumours' invasive edge. A specific and important feature of this response is the presence of a loose connective tissue lamina or cap (resembling lamina propria) at the deepest point of tumour penetration. The tumour glands are often heavily infiltrated by neutrophils but lymphocytes are not necessarily present in large numbers: it is the connective tissue stroma which is the most important feature. The tumours were scored on a 2-point scale as peritumoural infiltrate either present or absent.

Klintrup-Makinen scoring of slides was carried out as described previously (277). Briefly, tumours were scored according to a 4 point score. Scores were based on appearances at the deepest area of tumour invasion. A score of 0 indicated there was no increase in inflammatory cells at the deepest point of the tumour's invasive margin; score 1 denoted a mild and patchy increase in inflammatory cells; score 2 denoted a prominent inflammatory reaction forming a band at the invasive margin

with some evidence of destruction of cancer cell islands and score 3 denoted a florid cup-like inflammatory infiltrate at the invasive edge with frequent destruction of cancer cell islands. These scores were then subsequently classified as low-grade (scores 0 and 1) or high-grade (scores 2 and 3) (Figures 15 and 16 (Low grade) and 17 and 18 (High grade)).

Assessment of tumour margin characteristics was also undertaken in accordance with Jass criteria (222, 227). Briefly, specific features of a diffusely infiltrating tumour margin include 'streaming dissection' of tumour cells through the muscularis propria, or dissection of mesenteric adipose tissue by small glands or irregular clusters of cords of cells on microscopic examination.

A total of 100 tumour specimens were scored independently by 2 observers (Campbell Roxburgh and Jonathan Salmond), who were blinded to patient outcome, to confirm consistency of scoring. Training was provided by a consultant pathologist (Karin Oien). The inter-observer intraclass correlation coefficients (ICCC) provides a measure of interobserver agreement. The ICCCs for assessment of peritumoural inflammatory cell infiltrate were: 0.71 for Jass and 0.81 for Klintrup-Makinen. For assessment of tumour margin characteristics (expanding or infiltrating), the ICCC was 0.83 (ICCC values of ≥ 0.6 are considered acceptable and >0.7 is considered good). CSDR then scored all slides (n=200) and these data were used in the analysis.

The Petersen Index (PI) was generated from scores allocated to the four selected pathological variables present in a tumour specimen. A full description of the construction and calculation of the PI is found in Chapter 5.0 (Methods 5.2) (194, 195).

In the Royal Infirmary, patients undergo regular follow-up (3 months, 6 months and then yearly to five years) with yearly CT scanning and regular colonoscopic surveillance until 5 years post surgery. Information on date and cause of death was checked with that received by the cancer registration system and the Registrar General (Scotland). The study was approved by the Research Ethics Committee, Royal Infirmary, Glasgow.

Statistics

Grouping of the variables was carried out using standard thresholds. Univariate survival analysis and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.1. Deaths up to August 2008 were included in the analysis. Inter-relationships between variables were assessed using contingency table analysis with the chi-squared test for trend as appropriate. Because of the number of statistical correlations, a P value of <0.01 was considered to be significant. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

8.3 Results

Baseline clinico-pathological characteristics and relationship with cancer specific survival of the patients with node negative disease (n= 200) who underwent curative surgery for colorectal cancer are shown in Table 7.1. The majority of patients were 65 or older (68%), were male (55%), and had colon cancer (66%). Eighteen (9%) patients received adjuvant chemotherapy. Median number of lymph nodes sampled was 14 (range 1-41) for colonic tumours and 12 (range 1-41) for rectal tumours. 62% of patients had twelve or more lymph nodes harvested (Table 8.1). Overall the mean number of lymph nodes sampled was 14, the frequency of extramural venous invasion was 24% and serosal involvement was 22%, in line with current audit standards set by the Royal College of Pathologists (188).

On routine pathological analysis, the minority of patients had evidence of the 'high-risk' pathological features including poor tumour differentiation (9%), extramural vascular invasion (24%), peritoneal involvement (22%), margin involvement (8%), tumour perforation (3%) or perineural invasion (8%). The minority of patients were thus classed as low risk by the Petersen Index (11%). On assessment by Jass' criteria, 61% (n=121) had tumour margins classed as expanding and the majority (74%, n=148) had no peritumoural inflammatory infiltrate. On assessment using the Klintrup-Makinen criteria, 59% (n=118) patients were given scores of 0 or 1 (low grade inflammation) and 41% (n=82) were given scores of 2 or 3 (high grade inflammation).

The minimum follow-up was 43 months; the median follow-up of the survivors was 73 months. No patients were lost to follow up. During this period 76 patients died, 36 of their cancer. On univariate survival analysis of individual variables, age ($P<0.01$), fewer than 12 nodes sampled ($P<0.1$), vascular invasion ($P<0.01$), peritoneal involvement ($P<0.1$), tumour perforation ($P=0.05$), presence of an infiltrating invasive tumour margin ($P<0.05$) and absent or low grade peritumoural inflammatory infiltrate score assessed by Klintrup's criteria ($P<0.05$) were associated significantly with cancer-specific survival (Table 8.1).

On multivariate survival analysis of significant variables for cancer-specific survival, age (HR 2.26, 95% CI 1.42-3.61, P=0.001), high risk Petersen Index (HR 2.45, 95% CI

1.08-5.45, P=0.032), perineural invasion (HR 3.62, 95% CI 1.48-8.89, P=0.005) and a low-grade peritumoural inflammatory infiltrate assessed by the Klintrup-Makinen criteria (HR 2.61, 95% CI 1.19-5.77, P=0.017) were all independently associated with cancer specific survival (Table 8.1).

179 patients with node-negative colorectal cancer were classed as low risk by the Petersen Index. In this group, on univariate survival analysis of individual variables, age (P<0.001), poor tumour differentiation, (P<0.1), perineural invasion (P<0.05), presence of an infiltrating invasive margin (P<0.1) and absent or low grade peritumoural inflammatory infiltrate score assessed by the Klintrup-Makinen criteria (P<0.05) were associated with cancer-specific survival (Table 8.2).

On multivariate survival analysis for cancer-specific survival in the low risk Petersen Index group, age (HR 2.31, 95% CI 1.37-3.89, P=0.002), perineural invasion (HR 3.55, 95% CI 1.20-10.55, P=0.022) and a low-grade peritumoural inflammatory infiltrate assessed by the Klintrup-Makinen criteria (HR 3.04, 95% CI 1.22-7.58, P=0.017) were all independently associated with cancer specific survival (Table 8.2).

When those patients who received adjuvant chemotherapy were removed the from analysis, age (P<0.1), less than 12 nodes sampled (P<0.1), poor tumour differentiation (P<0.05), vascular invasion (P<0.05), a high risk Petersen Index (P<0.1), perineural invasion (P<0.005), an infiltrating tumour margin (P<0.1), and low-grade peritumoural inflammatory infiltrate assessed by the Klintrup-Makinen criteria (P<0.05) were associated with cancer specific survival on univariate analysis. On multivariate analysis of these significant variables in patients who did not receive adjuvant chemotherapy, only age (HR 1.81, 95% CI 1.14-2.88, P=0.011) and low-grade peritumoural inflammatory infiltrate assessed by Klintrup-Makinen criteria (HR 2.46, 95% CI 1.10-5.48, P=0.028) were independently associated with cancer specific survival.

The inter-relationships between the individual pathological criteria in the low risk Petersen Index group (n=179) are shown in Table 8.3. Increasing T stage was directly related to the presence of peritoneal involvement (P<0.001), an infiltrating tumour growth pattern and a low-grade peritumoural infiltrate assessed by both Jass (P<0.01) and Klintrup-Makinen criteria (P<0.01). A lymph node sample less than 12

was directly related to the presence of peritoneal involvement (P<0.01). The presence of vascular invasion was directly associated with presence of perineural invasion (P<0.001). Peritoneal involvement was directly associated with an infiltrating tumour growth pattern (P<0.01). The presence of an infiltrating tumour growth pattern was associated with a low-grade inflammatory cell infiltrate assessed with Jass (P=0.001) and Klintrup-Makinen criteria (P<0.001). Both Jass and Klintrup-Makinen criteria were directly associated (P<0.001)

8.4 Discussion

Following potentially curative resection for colorectal cancer there are a number of pathological features which guide treatment. Principal among these is nodal disease which indicates a high likelihood of disease recurrence and the need for adjuvant therapy (182, 419). In patients with no evidence of nodal disease, other pathological criteria have been proposed by Shepherd's group which identify patients at high risk of recurrence and which are now routinely reported (195). These include vascular invasion, peritoneal involvement, margin involvement and tumour perforation. These criteria have been combined in a score, the Petersen Index (PI), which reliably predicts poorer cancer-specific outcome in Dukes' B colon cancer (195). These findings have recently been validated in colorectal cancer by Quirke's group (194).

In the present study of patients with node-negative colorectal cancer I have confirmed the finding that a high risk PI is associated with poorer cancer specific survival. However, the majority of patients (89%) were identified by the PI as low risk. Therefore it was of interest that the presence of a low-grade tumour inflammatory cell infiltrate (Klintrup-Makinen criteria) was associated with an approximately 3-fold poorer cancer specific survival in these low-risk patients. In the present study, tumour inflammatory cell infiltrate assessed using Jass criteria was also examined. However, in terms of predicting cancer specific survival, the Klintrup-Makinen criteria were superior to that of Jass. Taken together these results would suggest that tumour inflammatory cell infiltrate, in particular using Klintrup-Makinen criteria, should be considered for inclusion in routine pathological reporting of colorectal cancer.

In the present study it was interest that both Jass and Klintrup-Makinen criteria were inversely associated with T stage and an infiltrating tumour margin. The presence of infiltrating growth pattern appears to be similar to the phenomenon known as de-differentiation, where there is a dissociation of tumour cells at the invasive front allowing these cells to migrate away from the main tumour, which may represent a morphological assessment of 'tumour aggressiveness' (478). This feature has also recently been described as 'tumour budding' where there is a transition from glandular structures to single cells or clusters of up to four cells the invasive margin of colorectal tumours (224). A high-grade local immune response may represent

effective host cellular immune responses preventing the invasive margin from developing an infiltrating or budding appearance. These results are consistent with the detailed analysis of the tumour immune response in patients with colorectal cancer by Nielsen and co-workers (329) and Galon and co-workers (391). Furthermore, the results of the present study are consistent with the concept that the type, density, and location of a variety of immune cells, and not an individual immune cell type, is an important independent determinant of cancer specific survival in patients with colorectal cancer.

The Klintrup-Makinen method used in the present study differs from previous methods of assessing immune cells within the tumour as it is a structured assessment of all white cell types at the invasive margin. The technique can be applied to routine H&E specimens with no special staining required. Previous assessments of peritumoural inflammation have been criticised for the subjective nature of the scoring methodology (475). In the present study the simple scoring method described by Klintrup and Makinen was applied with relative ease and, as the low inter-observer variation demonstrates, it is reproducible. In the present study, Klintrup and Makinen's scoring was undertaken using the original 4-point method, subsequently modified to a 2-point score (high and low grade) (Figures 1 and 2). This methodology was similar to the original Klintrup et al study and I would recommend a similar method be applied when classifying tumours in future studies.

With the introduction of whole population screening for colorectal cancer, earlier diagnosis should result in a higher proportion of early stage or node negative disease being treated by clinicians. Therefore, the identification of high-risk pathological features in this cohort will be important in guiding provision of adjuvant therapy. The results of the present study have demonstrated that in addition to existing routinely reported pathological features, other criteria such as low-grade peritumoural inflammation and the presence of an infiltrating tumour margin (which are currently listed in the Royal College of Pathologists guidelines but as non-core data items) are significantly related to poor survival. These criteria may therefore provide additional prognostic information, which could be used to guide multidisciplinary teams on treatment decisions. Currently, it is not known whether patients with pathological features such as low-grade inflammatory cell infiltrate

would benefit adjuvant systemic treatment; however the same is true of other criteria such as vascular invasion and poor tumour differentiation (176, 474). Which patients might benefit from adjuvant therapy may become clearer as large adjuvant chemotherapy trials publish further results.

In summary, assessment of peritumoural inflammatory cell infiltrate provides independent prognostic information in node negative disease and should therefore be considered for future inclusion in routine pathological reporting of colorectal cancer.

Figure 8.1: Low grade or absent inflammatory cell infiltrate at the tumours invasive margin. Staining: H&E, Magnification 20x

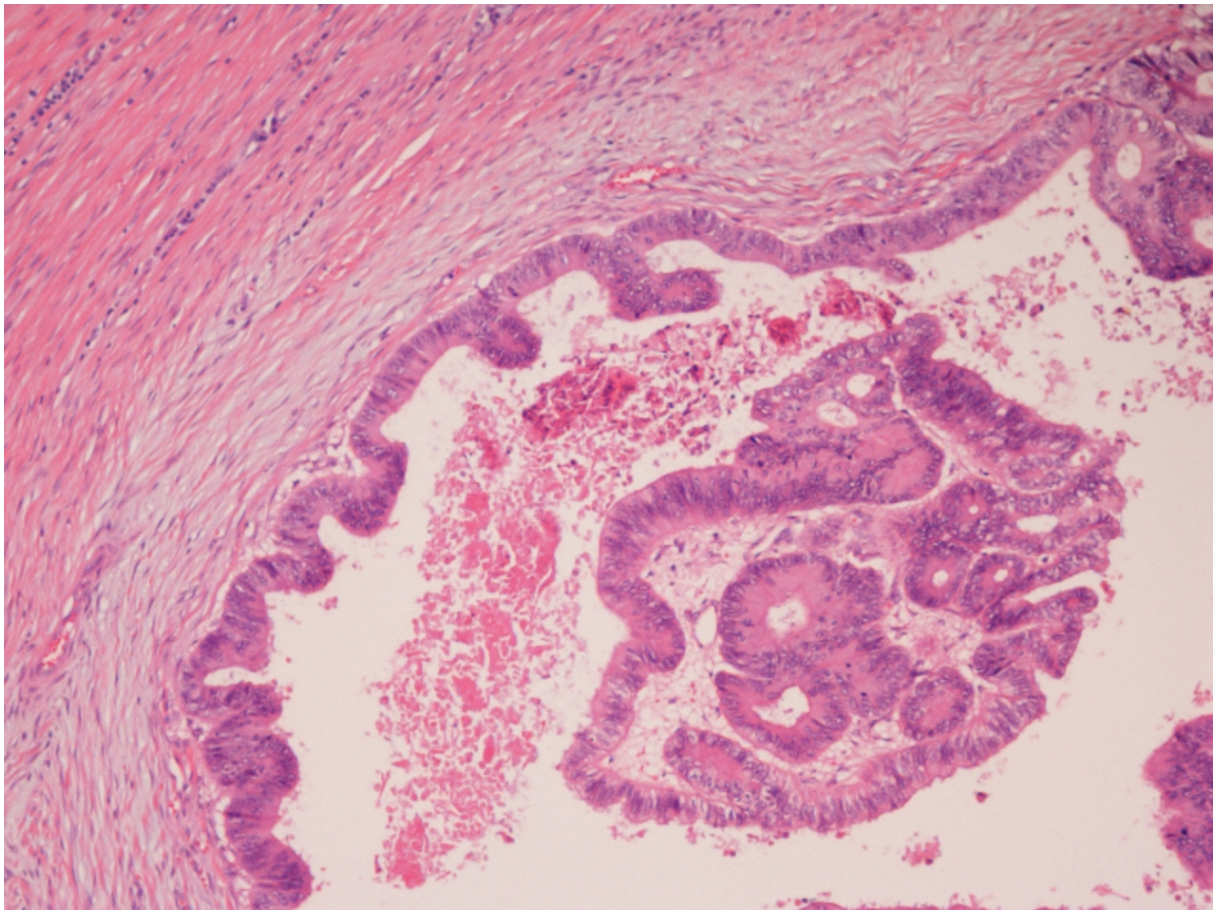


Figure 8.2: Low grade or absent inflammatory cell infiltrate at the tumour's invasive margin. Staining H&E, Magnification 20x

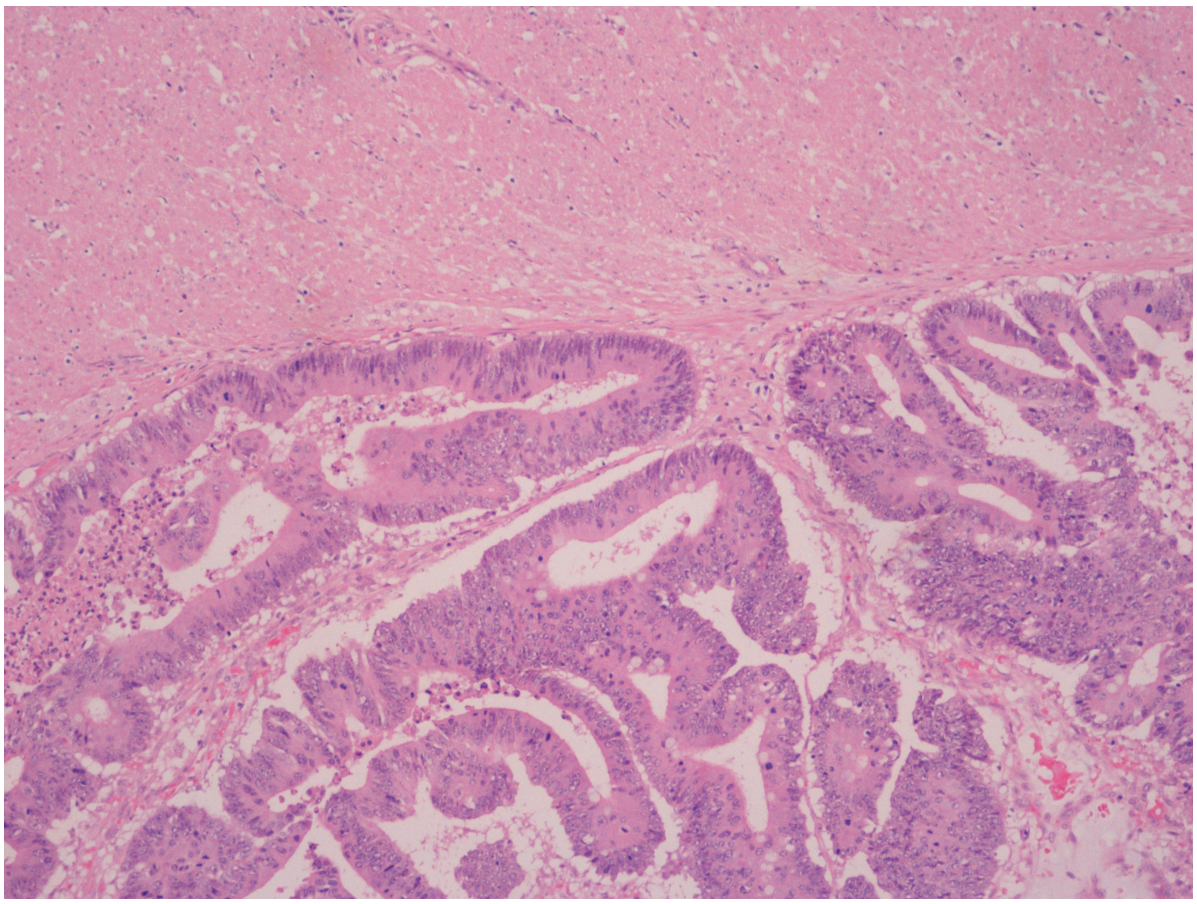


Figure 8.3: High grade inflammatory cell infiltrate at the tumours invasive margin. Staining: H&E, Magnification 40x.

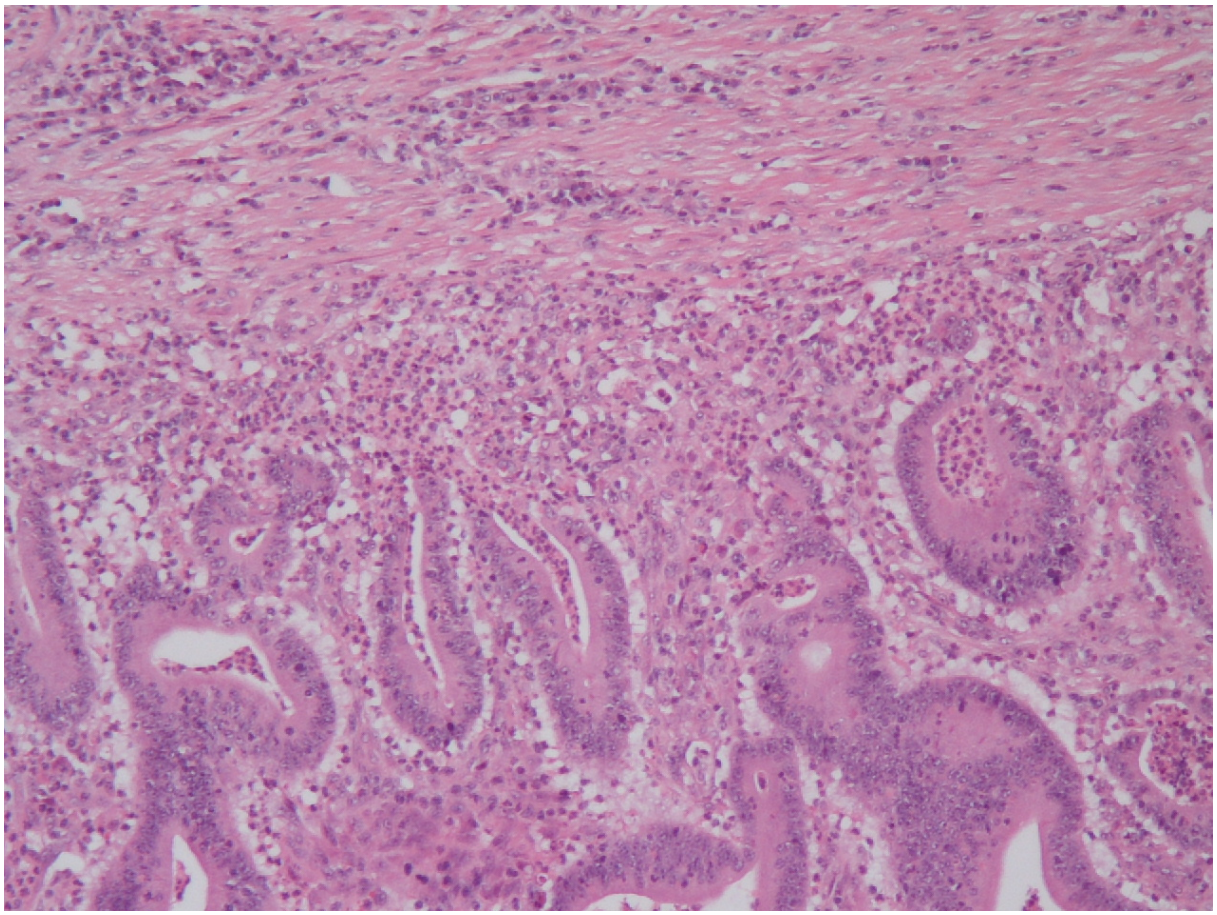


Figure 8.4: High-Grade inflammatory cell infiltrate at the tumour's invasive margin. Staining: H&E, Magnification: 40x.

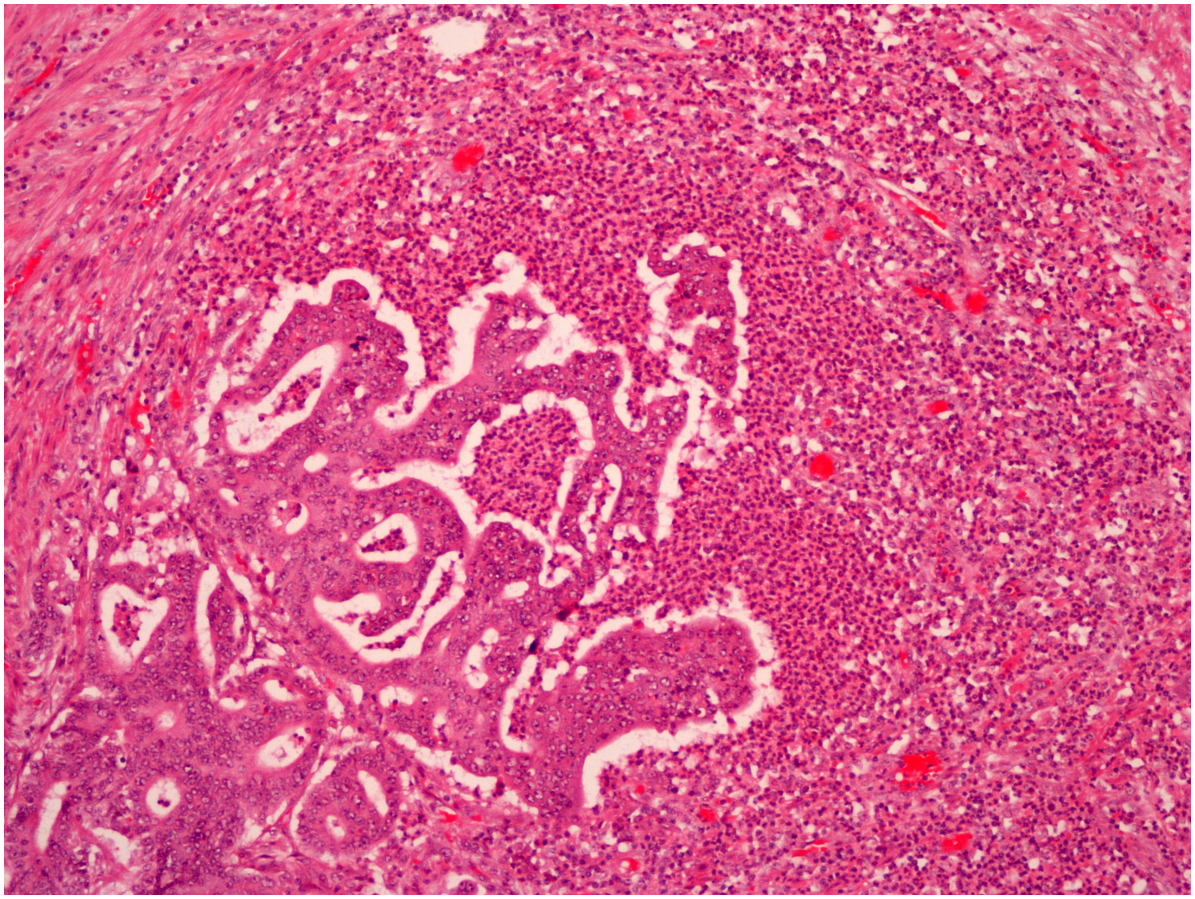


Table 8.1: The relationship between clinico-pathological variables and cancer specific survival in patients undergoing curative surgery for node-negative colorectal cancer.

	Patients n=200 (%)	Univariate Analysis HR (95% CI)	P-value	Multivariate Analysis HR (95% CI)	P-value
Age <65 years	64 (32)				
65-74years	70 (35)				
>75years	66 (33)	1.92 (1.24-2.98)	0.004	2.26 (1.42-3.61)	0.001
Sex female	90 (45)				
Male	110 (55)	0.86 (0.45-1.66)	0.659		
Site Colon	132 (66)				
Rectum	68 (34)	0.75 (0.36-1.55)	0.437		
Number of nodes harvested					
≥12	123 (62)				
<12	77 (38)	1.88 (0.97-3.62)	0.060		0.134
T Stage T1	7 (4)				
T2	22 (11)				
T3	126 (63)				
T4	45 (22)	1.48 (0.87-2.52)	0.152		
Adjuvant therapy no	182 (91)				
yes	18 (9)	1.42 (0.50-4.03)	0.507		
Differentiation Mod-well	183 (91)				
Poor	17 (9)	2.17 (0.84-5.61)	0.110		
Vascular invasion no	152 (76)				
yes	48 (24)	2.63 (1.34-5.17)	0.005		
Peritoneal involvement					
no	157 (78)				
yes	43 (22)	1.81 (0.90-3.61)	0.095		
Margin involvement no	186 (92)				
yes	14 (8)	0.84 (0.20-3.50)	0.811		
Tumour Perforation no	195 (97)				
yes	5 (3)	4.23 (1.00-17.89)	0.050		
Petersen Index Low Risk	179 (89)				
High Risk	21 (11)	3.36 (1.52-7.46)	0.003	2.45 (1.08-5.45)	0.032
Perineural Invasion no	185 (92)				
yes	15 (8)	3.63 (1.59-8.29)	0.002	3.62 (1.48-8.89)	0.005
Invasive Margin					
Expanding	121 (61)				
Infiltrating	79 (39)	1.95 (1.01-3.76)	0.046		0.407
Peritumoural Infiltrate (Jass criteria)					
Cap-like yes	52 (26)				
Cap-like no	148 (74)	2.00 (0.83-4.80)	0.122		
Peritumoural Infiltrate (Klintrup-Makinen)					
High grade inflammation	82 (41)				
Low grade inflammation	118 (59)	2.65 (1.21-5.82)	0.015	2.61(1.19-5.77)	0.017

Table 8.2: The relationship between clinico-pathological variables and cancer specific survival in patients with low risk Petersen Index following curative surgery for node-negative colorectal cancer.

	Patients n=179 (%)	**Univariate Analysis HR (95% CI)**	**P-value**	**Multivariate Analysis HR (95% CI)**	**P-value**
Age <65 years	57 (32)				
65-74years	63 (35)				
>75years	59 (33)	2.01 (1.21-3.33)	0.005	2.31 (1.37-3.89)	0.002
Sex female	77 (43)				
Male	102 (57)	0.96 (0.45-2.02)	0.907		
Number of nodes harvested					
≥12	109 (61)				
<12	70 (39)	1.80 (0.86-3.79)	0.121		
T Stage T1	7 (4)				
T2	22 (12)				
T3	125 (70)				
T4	25 (14)	1.04 (0.57-1.88)	0.908		
Adjuvant therapy No	166 (93)				
Yes	13 (7)	0.46 (0.06-3.40)	0.487		
Differentiation Mod-well	165 (92)				
Poor	14 (8)	2.51 (0.86-7.32)	0.091		0.198
Vascular invasion no	146 (82)				
yes	33 (18)	1.46 (0.59-3.62)	0.409		
Peritoneal involvement					
no	154 (74)				
yes	25 (26)	1.15 (0.44-3.03)	0.776		
Margin involvement no	176 (93)				
yes	3 (7)	1.87 (0.25-13.94)	0.540		
Tumour Perforation no	179 (97)				
yes	0 (3)	-	-		
Perineural Invasion no	169 (94)				
yes	10 (6)	3.39 (1.18-9.79)	0.024	3.55 (1.20-10.55)	0.022
Invasive Margin					
Expanding	111 (62)				
Infiltrating	68 (38)	2.07 (0.98-4.36)	0.055		0.246
Peritumoural Infiltrate (Jass criteria)					
Cap-like yes	48 (27)				
Cap-like no	131 (73)	2.40 (0.83-6.93)	0.105		
Peritumoural Infiltrate (Klintrup-Makinen)					
High grade inflammation	76 (43)				
Low grade inflammation	103 (57)	2.83 (1.15-6.98)	0.024	3.04 (1.22-7.58)	0.017

Table 8.3: Inter-relationships between pathological variables in patients with a low risk Petersen Index following potentially curative resection for node-negative colon cancer (n=179).

	Node sample (<12/ >12)	**Diff** (mod-well/ poor)	**VI** (no/ yes)	**Perit Inv** (no / yes)	**Margin Inv** (no / yes)	**Perineur Inv** (no / yes)	**Tumour Margin** (exp/inf)	**Jass criteria** (Yes/ No)	**Klintrup Makinen** (Yes/ No)
T stage (1/ 2/ 3/ 4)	0.943	0.218	0.993	<0.001	0.868	0.846	0.002	0.001	0.004
Node sample (>12/<12)		0.386	0.124	0.006	0.837	0.545	0.044	0.339	0.017
Differentiation (poor/ mod/well)			0.310	0.403	0.612	0.141	0.697	0.637	0.975
Vascular invasion (yes/ no)				0.011	0.408	<0.001	0.170	0.095	0.434
Perit. involvement (yes/ no)					0.483	0.572	0.004	0.022	0.015
Margin Involved (yes/ no)						0.672	0.867	0.292	0.135
Perineural inv. (yes/ no)							0.422	0.218	0.140
Tumour margin (exp/ infiltrating)								0.001	<0.001
Inflammatory cell infiltrate present (Jass criteria) (yes/ no)									<0.001

9.0 COMPARISON OF THE PROGNOSTIC VALUE OF INFLAMMATION BASED PATHOLOGICAL AND BIOCHEMICAL CRITERIA IN PATIENTS UNDERGOING CURATIVE RESECTION FOR COLON AND RECTAL CANCER

9.1 Introduction

It has long been recognised that disease progression in cancer patients is not solely determined by the local characteristics of the tumour but also by the systemic host response. Indeed, there is increasing evidence that both local and systemic inflammatory responses play an important role in the progression of a variety of common solid tumours (292, 479).

In terms of the localised tumour, there is good evidence that in patients with colorectal cancer, the presence of a pronounced lymphocytic infiltrate around the infiltrating tumour, on simple H&E staining of sections, is associated with improved survival (222, 329, 373). Furthermore, Galon and co-workers have provided further persuasive evidence that the type, density and location of immune cells in colorectal tumours may provide prognostic information superior to that of tumour staging (391).

Klintrup and co-workers have simplified the subjective measurement of the tumour inflammatory infiltrate by including all white blood cell types and classifying the inflammatory infiltrate as either low or high grade (277). They showed that a high-grade inflammatory infiltrate was associated with improved survival in patients undergoing potentially curative resection of node negative colorectal cancer.

In terms of systemic inflammation, there is now good evidence that the presence of a systemic inflammatory response, as evidenced by a simple objective score (Glasgow Prognostic Score, GPS), based on elevated circulating concentrations of C-reactive protein and hypoalbuminaemia, is independently associated with poor outcome in patients with colorectal cancer (278, 308, 325).

It is also of interest that previous studies have reported that a high-grade local inflammatory cell response is more commonly present in rectal tumours (277)

whereas an elevated systemic inflammatory response is more commonly present in colonic tumours (308). In addition, the original description of the prognostic value of tumour margin inflammatory cell reaction was initially made in rectal cancer (222). Therefore, in terms of local and systemic inflammatory responses and survival it is not clear whether such relationships are similar in colon and rectal tumours.

The aim of the present study was to examine the inter-relationships between these local subjective pathological (Jass and Klintrup criteria) and systemic objective biochemical (GPS criteria) inflammatory scores and to compare their prognostic value in patients undergoing potentially curative resection for colon and rectal cancer.

9.2 Materials and methods

Patients with histologically proven colon cancer who underwent potentially curative resection between 1997 and 2007 were identified from a prospectively collected database with similar exclusion criteria to those in Chapter 4.0. The tumours were staged using conventional TNM classification (182).

The routine haematoxylin and eosin slides were retrieved from the pathology archives. A minimum of three slides from the deepest area of tumour invasion were selected and scored according to both Jass (222) and Klintrup-Makinen (277) criteria. Jass scoring of slides were carried out as described previously (222, 227). Klintrup-Makinen scoring of slides were carried out as described previously (277). The methodology for these scores is described in detail in Chapter 8.0 (Methods 8.2). A total of 197 tumour specimens were scored independently by 2 observers (Campbell Roxburgh and Jonathan Salmond), who were blinded to patient outcome, to confirm consistency of scoring. The inter-observers intraclass correlation coefficients (ICCC) were as follow; Jass =0.71 and Klintrup-Makinen =0.81 (ICCC values ≥ 0.6 were considered acceptable). Campbell Roxburgh scored all slides (n=385) and these data were used in the analysis.

Blood samples were taken for routine laboratory measurements of albumin, C-reactive protein and differential white cell count measurement prior to surgery. The GPS was constructed as previously described (336), (308) (Chapter 4.0, Methods 4.2).

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

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). Grouping of the variables was carried out using standard thresholds. Univariate and multivariate survival analysis and calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.05. Deaths from colorectal cancer up to April 2009 were included in the analysis. Inter-relationships between variables were assessed using contingency table analysis with the chi-squared test for trend as appropriate.

9.3 Results

Three hundred and eighty five patients undergoing potentially curative resection for colorectal cancer between 1997 and 2007 were studied. Two hundred and forty-five patients (64%) underwent surgery for colonic tumours and one hundred and forty patients (36%) had surgery for rectal tumours. The majority of patients were 65 or older (65%), were male (55%) and had TNM stage I/II disease (55%). Median number of lymph nodes sampled was 14 (range 1-41) for TNM stage II tumours and 14 (range 3-34) for TNM stage III tumours. One hundred and sixty seven patients (43%) had an elevated C-reactive protein concentration (>10 mg/l), and 65 patients (17%) had hypoalbuminaemia (<35g/l). Of the 50 patients with hypoalbuminaemia, 45 (69%) had an elevated C-reactive protein. The majority of tumours had no evidence of peritumoural inflammatory infiltrate using Klintrup-Makinen (66%) or Jass criteria (76%). One hundred and ten patients (29%) received adjuvant therapy.

The inter-relationships between inflammation-based pathological and biochemical criteria were examined in a subgroup of 287 patients. This is shown in Table 9.1. Old age was associated with a greater proportion of females ($P<0.01$), colonic tumours ($P<0.01$) and elevated mGPS ($P<0.01$). Increasing TNM stage was associated with less peritumoural infiltrate (Jass criteria $P<0.001$, Klintrup-Makinen criteria $P<0.01$). Increased mGPS was associated with increased circulating white cell ($P<0.01$) and neutrophil ($P<0.01$) counts and low lymphocyte counts ($P<0.01$). Increased circulating white cell count was associated with increased neutrophil count ($P<0.001$) and low grade peritumoural infiltrate ($P<0.05$, Klintrup-Makinen criteria). Jass and Klintrup-Makinen criteria for peritumoural infiltrate were directly associated ($P<0.001$).

The individual clinicopathological characteristics for patients undergoing surgery for colon and rectal cancer are shown in Table 9.2. Patients with colon cancers were older ($P<0.05$) had higher T Stage ($P<0.001$) and mGPS ($P\leq0.001$) compared with rectal cancers. The proportions of patients with a high-grade tumour inflammatory cell infiltrate were similar in colon and rectal cancers.

The minimum follow-up was 25 months; the median follow-up of the survivors was 71 months. No patients were lost to follow up. During this period, 105 patients died

of their cancer and a further 64 patients died of intercurrent disease. The relationship between clinical, pathological and biochemical characteristics and cancer specific survival in patients undergoing potentially curative resection for colon and rectal cancer is shown in Table 9.3. On univariate survival analysis in colon cancer patients, age ($P<0.01$), TNM stage ($P<0.001$), mGPS ($P\leq0.001$) and a low grade or absent peritumoural inflammatory cell infiltrate assessed by Klintrup-Makinen criteria ($P\leq0.001$) were associated significantly with cancer-specific survival (Table 9.3). On univariate survival analysis in rectal cancer patients, TNM stage ($P<0.05$), mGPS ($P<0.05$) and a low grade or absent peritumoural inflammatory cell infiltrate assessed by Klintrup-Makinen criteria ($P<0.01$) were associated significantly with cancer-specific survival (Table 9.3).

On multivariate survival analysis in colon cancer patients, TNM stage (HR 2.73, 95% CI 1.51-4.91, $P\leq0.001$), mGPS (HR 1.56, 95% CI 1.03-2.38.60, $P<0.05$), and Klintrup-Makinen criteria for inflammatory cell infiltrate (HR 2.12, 95% CI 1.05-4.30, $P<0.05$) were independently associated with cancer specific survival (Table 9.4). On multivariate survival analysis in rectal cancer patients, mGPS (HR 1.76, 95% CI 1.00-3.10, $P<0.05$), and Klintrup-Makinen criteria for inflammatory cell infiltrate (HR 5.74, 95% CI 1.34-15.60, $P<0.05$) were independently associated with cancer specific survival (Table 9.4).

The relationship between TNM stage, local (Klintrup-Makinen) and systemic inflammatory (mGPS) responses and 3-year cancer specific survival rates (%) in patients undergoing potentially curative resection for colorectal cancer is shown in Table 9.5. In TNM stage II disease, patients with a high and low-grade tumour inflammatory cell infiltrate had a 3-year cancer specific survival of 97% and 88% respectively. In these patients with a low-grade inflammatory cell infiltrate the 3-year cancer specific survival were 95%, 82% and 68% for a mGPS of 0, 1 and 2 respectively. In TNM stage III disease, patients with a high and low-grade tumour inflammatory cell infiltrate had a 3-year cancer specific survival of 85% and 70% respectively. In these patients with a low grade inflammatory cell infiltrate the 3-year cancer specific survival were 78%, 60% and 60% for a mGPS of 0, 1 and 2 respectively (Table 9.5).

9.4 Discussion

The present study, to our knowledge, shows for the first time the inter-relationships between the preoperative systemic inflammatory response (GPS criteria), peritumoural inflammatory infiltrate (Jass and Klintrup-Makinen criteria) and cancer specific survival in patients undergoing potentially curative resection for colorectal cancer. In the present study, a low-grade peritumoural infiltrate, measured by the Klintrup-Makinen criteria, was associated with increased TNM stage and increased circulating total white cell count and neutrophil count. Furthermore, peritumoural infiltrate (Klintrup-Makinen criteria) was independently associated with cancer specific survival.

The results of the present study are consistent with those reported by Klintrup, Makinen and co-workers (277). In a study of 374 patients (229 with node-negative disease) who underwent surgery between 1986 and 1996, a significant relationship was observed between low-grade inflammatory infiltrate at the invasive margin and poor survival. In the same study, in addition to a grading all white cell counts at the invasive margin, increased neutrophils, lymphocytes and macrophages were all correlated with an improved 5-year survival.

In contrast, the systemic inflammatory response, measured by mGPS criteria, was not associated with TNM stage nor peritumoural infiltrate (Jass or Klintrup-Makinen criteria), but was associated with increased circulating total white cell count, increased neutrophil count and decreased circulating lymphocyte count. The mGPS was also independently associated with cancer specific survival in patients undergoing potentially curative resection for colorectal cancer. Taken together the results of the present study suggest that both low peritumoural infiltrate (Klintrup-Makinen criteria) and increased systemic inflammation (mGPS criteria) may be linked through the cell mediated immune system; both acquired (lymphocytes) and innate immune (neutrophils) cells.

The results of the present study also show for the first time that, in patients with colon and rectal cancer, pathological (Klintrup-Makinen) and biochemical (mGPS) measures of the inflammatory response independently predict cancer specific survival following potentially curative resection. Such routinely available

inflammatory measures offer a new approach to staging the biologic phenotype of the tumour and together with tumour staging offer a more sophisticated and accurate approach to outcome prediction in patients with primary operable colon and rectal cancer.

For example, in the present study the 3-year survival rate for TNM stage II and III colorectal cancer was 91% and 73% respectively. However, within the TNM stage II disease, 3-year survival rate varied between 100% and 68% depending on the Klintrup-Makinen and Glasgow Prognostic Scores. Similarly, within the TNM stage III disease, 3-year survival rate varied between 97% and 60% depending on the Klintrup-Makinen and Glasgow Prognostic Scores. Therefore, I believe such measures should be considered for incorporation into routine staging of primary operable colon and rectal cancer.

In the present study it was of interest that, on multivariate survival analysis, although the hazard ratios for the mGPS were similar in both colon (HR 1.56) and rectal (HR 1.76) cancers, the corresponding hazard ratios for Klintrup-Makinen criteria were 2.12 and 5.74 respectively. The basis of the increased hazard ratio for the Klintrup-Makinen score in rectal cancer is not clear. However, it may be that the local inflammatory response is better at controlling tumour dissemination in rectal cancer. Indeed, there were fewer T4 stage rectal tumours compared with distribution in colonic tumours. This might explain why Jass' observation that tumour inflammatory cell infiltration had prognostic value was initially made in rectal cancer (222).

The results of the present study suggest low peritumoural infiltrate (Klintrup-Makinen criteria) and increased systemic inflammation (mGPS criteria) may potentially be linked by the cell-mediated immune system. However, the relationships between the inflammatory responses are likely to be more complex reflecting a continuous interplay between the tumour and host. For example, a high-grade local inflammatory response may represent an adequate host immune defence preventing tumour spread. It is clear that systemic inflammatory markers, such as C-reactive protein, play a pivotal role in the tumour–host relationship, its elevation reflecting compromised cell-mediated immunity as it is associated with lymphocytopenia and impaired T lymphocytic response within the tumour (276,

344). In addition, elevated C-reactive protein and hypoalbuminaemia have also been shown to be associated with upregulation of components of innate immune system, including complement and macrophage function (291, 345). As part of the systemic inflammatory response, there is a release of pro-inflammatory cytokines and growth factors which may promote and maintain tumour growth (347) (443). Taken together, the apparent inverse relationship between markers of the systemic inflammatory response and the local inflammatory response are likely to reflect imbalances in the innate and adaptive immune systems compromising effective host-tumour immune responses. As tumour growth continues, there may then be a switch from a local to a systemic inflammatory response reflecting a change in anti-tumour immune competence.

Extrinsic pathways that may be associated with cancer related inflammation include nutritional and functional decline, immune dysfunction and tumour angiogenesis, growth and dissemination (480). Recently, it has also been proposed that there are also intrinsic pathways involved in cancer related inflammation, such as the induction of genetic instability by inflammatory mediators, leading to the accumulation of genetic alterations in cancer cells and progressive tumour growth and dissemination. Indeed, a recent review proposes that cancer related inflammation represents 'the seventh hallmark of cancer' (480).

There are a number of factors that might result in the presence of a pre-operative systemic inflammatory response as evidenced by the mGPS (i.e. C-reactive protein and albumin). These include emergency presentation (481), and clinical evidence of infection and other chronic inflammatory conditions. In the present study, patients with such factors were excluded from the analysis to obviate confounding results.

The results of the present study are consistent with previous studies in that where a high-grade local inflammatory response exists, a variety of innate and adaptive immune cells are associated with long-term survival (222, 277, 329, 373, 391). Also, the results of the present study suggest that the systemic inflammatory response is associated with changes in the type, density and location of immune cells in colorectal tumours type. Further detailed investigation of this relationship may result in a

better understanding of the loss immune control in patients with primary operable colorectal cancer.

Whilst both pathological (Klintrup-Makinen criteria) and biochemical (mGPS) measures of the inflammatory response had independent prognostic value it is more likely that the mGPS could be adopted for the routine clinical assessment of the inflammatory response alongside tumour staging because the mGPS is simple, objective, internationally well standardised and can be measured pre-operatively, compared with the Klintrup-Makinen criteria. The systemic inflammatory response is also recognised to be a precursor to progressive involuntary loss of weight and lean tissue, both of which are key factors in determining the survival of the cancer patient. Moreover, the mGPS, can be used, in addition to traditional risk factors, to stratify colorectal cancer patients into specific follow-up (278, 325) and perhaps treatment regimes (482).

With the increasing evidence that host or immune responses are important prognostic indicators in addition to TNM stage, a variety of prognostic scores based on the presence of the systemic inflammatory response have been described (308, 309, 341, 483). Recently, Iimura and coworkers (484) have developed and validated a combined model of TNM stage and C-reactive protein, termed TNM-C for predicting outcome in patients undergoing surgery for renal cancer. The results of the present study indicate that a similar model based on TNM stage, Klintrup-Makinen and mGPS scores would be of value in patients undergoing potentially curative surgery for colorectal cancer.

In summary, the results of the present study show that both local and systemic inflammatory responses are important independent predictors of survival in patients undergoing potentially curative surgery for colon and rectal cancer. These scores combined with TNM stage improve the prediction of survival in these patients.

Table 9.1. Clinico-pathological characteristics of patients undergoing curative resection for colon cancer and rectal cancer.

		Colon cancer	Rectal cancer	
		n=245 (%)	**n=140 (%)**	**P value**
Age	<65 yrs	81 (33)	53 (38)	
	65-74yrs	74 (30)	55 (39)	
	>75yrs	90 (37)	32 (23)	0.031
Sex	Female	117 (48)	57 (41)	
	Male	128 (52)	83 (59)	0.182
T Stage	T1	2 (1)	8 (5)	
	T2	11 (4)	19 (14)	
	T3	140 (57)	84 (60)	
	T4	92 (38)	29 (21)	<0.001
N stage	N0	139 (57)	72 (52)	
	N1	81 (33)	48 (34)	
	N2	25 (10)	20 (14)	0.201
TNM Stage	I	11 (5)	18 (13)	
	II	128 (52)	54 (39)	
	III	106 (43)	68 (48)	0.642
mGPS	Low Risk (0)	122 (50)	96 (69)	
	Intermediate (1)	89 (36)	33 (23)	
	High Risk (2)	34 (14)	11 (8)	0.001
Klintrup-Makinen criteria				
	High grade inflammation	82 (34)	47 (34)	
	Low grade inflammation	163 (66)	93 (66)	0.984
Adjuvant therapy	No	175 (71)	100 (71)	
	Yes	70 (29)	40 (29)	1.00

Table 9.2: Inter-relationships between the inflammation based pathological and biochemical criteria in patients undergoing potentially curative resection for colorectal cancer (n=287).

	Sex (F/ M)	**Site** (C/ R)	**TNM Stage** (I/II/III)	**mGPS** (0-2)	**WCC** (<8.5/8.5-11/>11)	**Neutrophils** (<7.5/ ≥7.5)	**Lymphocytes** (<1/1-3/>3)	**Jass criteria** (Yes/ No)	**Klintrup Makinen** (Yes/ No)
Age (<65/ 65-74/ >75yrs)	0.008	0.004	0.724	0.004	0.484	0.966	0.298	0.329	0.983
Sex (Female/ Male)		0.124	0.628	0.205	0.401	0.513	0.149	0.834	0.641
Tumour site (Colon/ Rectum)			0.813	0.082	0.303	0.438	0.070	0.448	0.443
TNM stage (I/II/III)				0.871	0.197	0.271	0.960	<0.001	0.001
mGPS (0-2)					0.003	0.001	0.005	0.128	0.626
White Cell Count (<8.5/ 8.5-11/ >11x10^9/L)						<0.001	0.135	0.164	0.012
Neutrophils (<7.5/ ≥7.5x10^9/L)							0.684	0.241	0.067
Lymphocytes (<1/ 1-3/ >3 x10^9/L)								0.173	0.412
Peritumoural Infiltrate (Jass criteria) Cap-like (Yes/ No)									<0.001

Table 9.3. The relationship between clinical, pathological and biochemical characteristics and cancer specific survival in patients undergoing potentially curative resection for cancer of the colon (n=245) and rectum (N=140): Univariate survival analysis

		Colon cancer		Rectal cancer	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio (95% CI)**	**P-value**
Age	<65 years				
	65-74years				
	>75years	1.57 (1.19-2.07)	0.002	1.15 (0.72-1.85)	0.563
Sex	Female				
	Male	0.99 (0.63-1.55)	0.946	0.81 (0.39-1.66)	0.557
TNM Stage	I				
	II				
	III	2.76 (1.77-4.32)	<0.001	2.02 (1.10-3.72)	0.024
mGPS	Low risk (0)				
	Intermediate (1)				
	High risk (2)	1.65 (1.22-2.24)	0.001	1.77 (1.11-2.83)	0.017
Klintrup-Makinen criteria					
	High grade inflammation				
	Low grade inflammation	2.79 (1.53-5.07)	0.001	5.74 (1.74-18.99)	0.004
Adjuvant therapy	No				
	Yes	1.01 (0.61-1.67)	0.967	1.43 (0.65-3.13)	0.374

Table 9.4. The relationship between clinical, pathological and biochemical characteristics and cancer specific survival in patients undergoing potentially curative resection for cancer of the colon (n=245) and rectum (n=140): Multivariate survival analysis.

		Colon cancer		Rectal cancer	
		Hazard Ratio (95% CI)	**P-value**	**Hazard Ratio (95% CI)**	**P-value**
Age	<65 years				
	65-74years				
	>75years		0.258		0.274
Sex	Female				
	Male		0.207		0.630
TNM Stage	I				
	II				
	III	2.73 (1.51-4.91)	0.001		0.275
mGPS	Low risk (0)				
	Intermediate (1)				
	High risk (2)	1.56 (1.03-2.38)	0.038	1.76 (1.00-3.10)	0.033
Klintrup-Makinen criteria					
	High grade inflammation				
	Low grade inflammation	2.12 (1.05-4.30)	0.037	5.74 (1.34-15.60)	0.015
Adjuvant therapy	No				
	Yes		0.259		0.774

Table 9.5. The relationship between TNM stage, local (Klintrup) and systemic inflammatory (mGPS) responses and 3-year cancer specific survival rates (%) in patients undergoing potentially curative resection for colorectal cancer (n=366).

	TNM Stage II (n=182)				**TNM Stage III (n=174)**		
	Klintrup-Makinen High Grade	Klintrup-Makinen Low Grade	Klintrup-Makinen Low/ High Grade		Klintrup-Makinen High Grade	Klintrup-Makinen Low Grade	Klintrup-Makinen Low/ High Grade
mGPS 0	100% (n=34)	95% (n=65)	97% (n=99)		97% (n=26)	78% (n=71)	84% (n=97)
mGPS 1	94% (n=21)	82% (n=36)	87% (n=57)		77% (n=14)	60% (n=45)	64% (n=59)
mGPS 2	92% (n=13)	68% (n=13)	80% (n=26)		67% (n=3)	60% (n=15)	61% (n=18)
mGPS 0-2	97% (n=68)	88% (n=114)	91% (n=182)		85% (n=43)	70% (n=131)	73% (n=174)

10.0 THE RELATIONSHIP BETWEEN TNM STAGE, THE LOCAL AND SYSTEMIC INFLAMMATORY RESPONSES AND CIRCULATING IMMUNOLOGICAL PARAMETERS IN PATIENTS UNDERGOING CURATIVE RESECTION FOR COLORECTAL CANCER

10.1 Introduction

Besides intrinsic tumour characteristics, there is increasing evidence that disease progression and survival in cancer is also determined by the presence or absence of a host systemic and local inflammatory responses. This relationship is particularly well established in colorectal cancer with over 20 studies reporting that markers of the systemic inflammatory response, in particular C-reactive protein, albumin and their combination the Glasgow Prognostic Score predict survival independent of tumour stage (Chapter 1.0). Also, there is increasing evidence that the presence of a high grade inflammatory or immune cell infiltrate in and around colorectal tumours predict survival independent of tumour stage (277, 391)(Chapter 1.0).

Although these systemic and local inflammatory response appear to have independent prognostic value in patients with colorectal cancer (Chapter 9.0), it is likely that they are linked. However, the factors that may link systemic and local inflammatory responses in patients with colorectal cancer have not been examined in detail.

One hypothesis is that the systemic and local inflammatory responses reflect circulating immunological factors. For example, circulating cytokines and growth factors have both stimulant and suppressive actions on immunological function (485-487).

Therefore, the aim of the present study was to examine relationships between tumour stage, systemic and local inflammatory responses and circulating immunological parameters in patients undergoing curative resection for colorectal cancer.

10.2 Materials and Methods

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and pre-operative abdominal computed tomography, were considered to have undergone an elective potentially curative resection between April 2004 and July 2009 were included in this prospective study. All operations took place within a single surgical unit at Glasgow Royal Infirmary. Tumours were staged using the conventional tumour node metastasis (TNM) staging system (188). Patients with conditions known to evoke a systemic inflammatory response either acute or chronic were excluded. These were namely (i) pre-operative chemo-radiotherapy (ii) clinical evidence of active pre-operative infection or (iii) chronic active inflammatory diseases such as rheumatoid arthritis.

Blood samples were collected, prior to surgery for routine laboratory analysis of full blood count, white cell and lymphocyte counts, immunoglobulins, albumin and C-reactive protein. Immunoglobulin A, Immunoglobulin G and Immunoglobulin M assays were performed using a standard immunoturbidometric procedure and specific antibodies to these immunoglobulins (Architect, Abbott Laboratories, IL, USA). Briefly, the insoluble immune complexes between the immunoglobulin and its specific antibody is measured as turbidity. Therefore, the concentration of the immunoglobulin in the sample is measured as a direct function of turbidity. Each immunoglobulin is first incubated with a buffer containing TRIS, polyethylene glycol and sodium azide prior to performing the test. The coefficient of variation for the laboratory measurements was less that 10% as established by routine quality control procedures.

A pre-operative blood sample was also taken for analysis of circulating T-lymphocyte subset populations. The T-lymphocyte subsets were analysed on a FACScanto flow cytometry (BD Bioscience, Oxford, UK) equipped with a 488-nm argon laser and a 635-nm red diode laser using FACScanto software. The monoclonal antibodies used were CD3 FITC / CD4 PE / CD3 PenCPCy 5.5 / CD8 APC and CD3 FITC / CD19 PE / CD16+ 56 PenCPCy 5.5 / CD45 APC (BD Bioscience Oxford, UK). Absolute counting was performed on a single platform using TruCOUNT tubes.

The modified Glasgow Prognostic Score was constructed as previously described based on routine pre-operative blood tests (27) (Chapter 4). In addition to routine tests patients were also asked whether they would consent to further samples to be taken for the purposes of the present study. Each patient was provided with an information sheet and those who agreed to participate signed a consent form. These extra blood samples were, centrifuged, and the serum stored at –80^{o}C prior to analysis of interleukin-6, interleukin-10 and vascular endothelial growth factor (VEGF). Circulating concentrations of IL-6, IL-10 and VEGF were measured using commercially available human colorimetric enzyme linked immunosorbent assays (Quantikine ELISA, R&D Systems, Europe Ltd, Abingdon, UK). The minimum detectable concentrations were 2 pg/ml for interleukin-6, 4pg/ml for interleukin-10 and 9pg/ml for VEGF. Inter- and intra-assay variability was less than 10% for the three assays. Cytokine concentrations below the threshold of sensitivity of the respective assays were expressed as equal to this threshold.

The local inflammatory cell reaction at the tumours invasive edge was assessed using the method first described by Klintrup and Makinen (277). This is described in detail in chapter 8.0.

The study design, information sheets and patient consent forms were approved by the Research Ethics Committee at Glasgow Royal Infirmary.

Statistics

Grouping of the variables was carried out using standard thresholds for laboratory parameters. Immunoglobulins were grouped by standard laboratory reference ranges. Components of the white cell count were grouped as previously described (278). Grouping of IL-6 and IL-10 was carried out using standard thresholds as previously described (488). Grouping of VEGF was carried out using tertiles as no standard threshold has been described. IL-6, IL-10 and VEGF concentrations below the threshold of sensitivity of the respective assays were expressed as equal to this threshold. Data is also presented as median and range. The relationships between the groups of patients was carried out using Mantel–Haenszel (X^2) test for trend and the Kruskal Wallis test as appropriate. Univariate survival analysis and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's

proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. Deaths up to October 2010 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

10.3 Results

One hundred and eighteen patients who underwent potentially curative resection for colorectal cancer between April 2004 and July 2009 were included in the study. Most patients were 65 or older (66%), male (51%), TNM stage I/II disease (62%). Most patients had C-reactive protein (68%) and albumin (72%) concentrations in the normal range and a normal mGPS (68%). Of the 33 patients with hypoalbuminaemia, 16 (48%) had an elevated C-reactive protein.

Most patients had interleukin-6 concentrations greater than 4pg/ml (60%) and interleukin-10 concentrations greater than 10pg/l (52%). The majority of patients had total white cell counts (71%), neutrophil counts (89%) lymphocyte counts (82%) and platelet counts (87%) in the normal range. Most had levels of immunoglobulin A (84%), immunoglobulin G (98%) and immunoglobulin M (96%) within the normal laboratory reference range. Also, the majority of patients had CD3+ lymphocyte (74%), CD 4+lymphocyte (74%) and CD 8+ lymphocyte (74%) subpopulation and CD 19+lymphocyte (82%) counts in the normal range. On assessment of Klintrup-Makinen scoring to assess the local tumour inflammatory cell response, the majority of patients were considered to have a low-grade inflammatory cell infiltrate (55%).

Table 10.1 shows the relationships between TNM stage, circulating immunological parameters and tumour characteristics in patients undergoing curative resection for colorectal cancer. Increasing TNM stage was related to increasing platelet count as a categorical variable ($P<0.01$) and increasing IL-6 as both a continuous ($P<0.05$) and categorical ($P<0.01$) variable, vascular invasion ($P<0.01$) and the Klintrup-Makinen inflammatory cell infiltrate score ($P<0.01$).

Table 10.2 shows the relationships between the systemic inflammatory response (mGlasgow Prognostic Score), circulating immunological parameters and tumour characteristics patients undergoing curative resection for colorectal cancer. An increasing mGPS was related to increasing white cell count as a continuous ($P<0.05$) and categorical variable ($P<0.005$), neutrophil count as a continuous ($P<0.001$) and categorical variable ($P<0.05$), platelet count as a continuous ($P<0.001$) and categorical variable ($P<0.01$), IgG count as a categorical variable ($P<0.05$), CD3+ cell count as a categorical variable ($P<0.05$), CD8+ cell count as a continuous ($P<0.05$) and

categorical variable (P<0.05), IL-6 count as a continuous (P<0.001) and categorical variable (P<0.001), IL-10 count as a continuous (P<0.01) and categorical variable (P<0.05) and T stage (P<0.01).

Table 10.3 shows the relationships between the local inflammatory response (Klintrup-Makinen score), circulating immunological parameters and tumour characteristics patients undergoing curative resection for colorectal cancer. A low grade inflammatory cell response around the tumour was related to a lower white cell count as a continuous variable (P<0.05), lower circulating CD4+ cell count as a continuous variable (P<0.05), lower circulating CD19+ cell count as a continuous variable (P<0.01), lower circulating VEGF as a categorical variable (P<0.05), increasing T stage (P<0.05) and increasing N stage (P<0.05).

10.4 Discussion

The results of the present study show clearly that a number of circulating immunological factors were associated with tumour stage, systemic and local inflammatory responses. In particular, T-stage appeared to be associated with elevated circulating interleukin-6 concentrations, an elevated mGPS and a low-grade Klintrup-Makinen inflammatory cell infiltrate. However, circulating interleukin-6 concentrations were not associated with Klintrup-Makinen inflammatory cell infiltrate. Therefore, these results would suggest that some circulating immunological factor(s) other than interleukin-6 link tumour stage, systemic and local inflammatory responses.

In the present study an elevated mGPS was associated with a number of elevated circulating immunological factors including white cell and platelet counts and the pro and anti-inflammatory cytokines interleukin-6 and interleukin-10. Weaker direct relationships with immunoglobulin G, circulating CD3+ T cells and CD8+ T cells were also observed. These results are consistent with previous observations that the GPS accurately reflects many aspects of the systemic inflammatory response (289, 427, 489).

In the present study a low-grade Klintrup-Makinen score was associated with a number of circulating immunological factors. Weak relationships with lower white cell count, CD4+ and CD19+ counts, and lower VEGF were observed. The basis of these observations is unclear and worthy of further investigation. However, it would be important to know what aspect of the tumour inflammatory infiltrate, that is type, density and location, was most closely associated with cancer specific survival in order to correctly determine the most important circulating immunological factors.

Interleukin-6 is recognised to be a key regulator of hepatic production of acute phase proteins such as C-reactive protein (289). Although not as well established as C-reactive protein or the GPS a prognostic indicator in patients with colorectal cancer (450), circulating interleukin-6 has been reported to predict cancer incidence and cancer survival in large population based studies (490, 491). Furthermore, interleukin-6 has been shown to have prognostic value in established colorectal cancer (323, 492-494). It has been hypothesised that much of this survival

relationship could be explained through production of IL-6 by tumour cells or immune cells in and around the tumour (495, 496).

Interleukin-10 has been proposed to be a key anti-inflammatory cytokine involved in Th2 immune responses (497, 498). In the present study an elevated interleukin-10 was directly associated with the mGPS but not the Klintrup-Makinen inflammatory cell infiltrate. However, elevated circulating concentrations of interleukin-10 have been reported to predict poorer response to treatment, recurrence following surgery and survival in colorectal cancer (499, 500).

To our knowledge, there has been no direct comparison of the prognostic value of the circulating factors interleukin-6, interleukin-10, C-reactive protein and the mGPS. In the present small study with a minimum follow-up of 14 months and 18 cancer deaths only C-reactive protein (p=0.019) and the mGPS (p=0.063) had prognostic value. Interleukin -6 (p=0.769) and interleukin-10 (p=0.633) did not. On comparison using the Cox proportional hazard model only the mGPS (HR1.74, 95%CI 0.96-3.13, p=0.068) had independent prognostic value. Clearly, this comparison should be carried out in larger series with more follow-up. However, the present results would suggest the combination of C-reactive protein and albumin as a measure of the systemic inflammatory response is more closely related to cancer specific survival than interleukin-6 and interleukin-10.

The present study therefore shows that, in particular, systemic inflammatory cell responses relate to abnormalities in circulating immunological factors. At present, it is unclear at which factors degrade the local inflammatory or immune cell infiltrate. Such a degradation in local immune response is clearly associated with T stage and the development of an infiltrating tumour margin (Chapter 8.0). Also, it is not clear which factors may drive the development of systemic inflammation as the tumour depth increases. Further work is required to establish which characteristics developing as T stage increases that may link local and systemic inflammatory response. One such possibility is tumour necrosis, a recognised prognostic feature in colorectal cancer that could be expected to relate to both local and systemic inflammatory responses (501).

In summary the results of the present study confirmed that the systemic inflammatory response was associated with an upregulation of circulating immunological factors. No circulating immune factor provided a strong relationship between local and systemic inflammatory responses. A significant relationship was observed between depth of invasion and both the local and systemic inflammatory responses. Further work is required to establish whether local tumour immune factors more strongly link these responses.

Table 10.1: The relationship between the TNM stage, circulating immunological parameters and tumour characteristics in patients undergoing curative resection for colorectal cancer (n=118). *median (range)

	TNM stage			
	TNM I (n= 18)	**TNM II (n= 55)**	**TNM III (n= 45)**	**P value**
Blood				
White cell count (10^9/l)*	7.8 (3.6-13.5)	7.2 (4.7-16.1)	7.4 (4.1-15.5)	0.282
White cell count (<8.5/8.5-11.5/>11.5 x10^9/l)	13/4/1	39/10/6	32/9/4	0.886
Neutrophils (10^9/l)*	4.4 (2.4-11.2)	5.8 (2.4-12.7)	6.15 (1.8-13.1)	0.293
Neutrophils (<7.5/ >7.5x10^9/l)	18/0	48/7	39/6	0.202
Lymphocytes (10^9/l)	1.35 (0.5-2.2)	1.9 (1.0-2.8)	1.9 (1.0-2.2)	0.834
Lymphocytes (≥1/ <1 10^9/l)	3/15/0	9/46/0	7/36/2	0.529
Platelets (x10^6/L)	219 (205-234)	254 (167-583)	348 (271-602)	0.625
Platelets (≤400/ >400 x10^6/L)	18/0	49/6	36/9	0.027
Immunoglobulin A (g/L)*	2.8 (1.1-4.5)	2.3 (0.8-5.7)	2.1 (1.2-4.1)	0.432
Immunoglobulin A (<4/ ≥4g/L)	5/2	19/4	23/3	0.282
Immunoglobulin G (g/L)*	9.7 (8.0-11.0)	9.3 (6.0-15.3)	9.2 (5.0-12.6)	0.378
Immunoglobulin G (<16/ ≥16g/L)	7/0	21/1	24/0	0.644
Immunoglobulin M (g/L)*	1.0 (0.9-1.1)	0.5 (0.3-1.3)	0.9 (0.6-1.4)	0.911
Immunoglobulin M (<2/ ≥2g/L)	7/0	22/1	23/1	0.701
CD3+ (10^9/l)*	1.12 (0.57-1.83)	1.45 (0.57-1.78)	0.99 (0.52-1.61)	0.138
CD3+ (≥0.96/ <0.96 10^9/l)	8/3	18/10	20/3	0.212
CD4+ (10^9/l)*	0.52 (0.46-0.98)	0.76 (0.28-1.73)	0.78 (0.34-1.80)	0.999
CD4+ (≥0.54/ <0.54 10^9/l)	7/4	19/9	20/3	0.099
CD8+ (10^9/l)*	0.23 (0.07-0.91)	0.38 (0.05-1.59)	0.54 (0.16-1.64)	0.233
CD8+ (≥0.27/ <0.27 10^9/l)	7/4	21/7	18/5	0.688
CD19+ (10^9/l)*	0.30 (0.05-0.45)	0.19 (0.03-0.72)	0.20 (0.07-1.49)	0.705
CD19+ (≥0.12/ <0.12 10^9/l)	10/1	22/6	19/4	0.688
Interleukin-6 (pg/ml)*	2.82 (1.0-32.1)	4.63 (0.8-23.6)	5.51 (2-252.6)	0.021
Interleukin-6 (≤4/ >4pg/ml)	11/7	24/31	12/33	0.008
Interleukin-10 (pg/ ml)*	10.0 (7.6-27.2)	9.9 (5.7-25.8)	10.7 (6.6-218.5)	0.142
Interleukin-10 (≤10/ >10pg/ml)	7/8	27/22	14/23	0.316
VEGF (pg/ml)*	128 (39-520)	102 (10-373)	106 (7-309)	0.862
VEGF (tertiles)	5/3/8	18/13/18	12/18/9	0.303
Tumour				
T stage (1/2/3/4)	7/11/0/0	0/0/50/5	0/2/24/19	
N stage (0/1/2)	18/0/0	55/0/0	0/30/15	
Venous invasion (absent/present)	11/7	25/30	11/34	0.004
Inflammatory cell infiltrate (Klintrup-Makinen High/Low)	13/5	23/27	13/29	0.004

Immunoglobulin results available for 56/118 patients

Circulating T lymphocyte results available for 62/118 patients

Inflammatory cell infiltrate scoring available for 110/118

Table 10.2: The relationship between the systemic inflammatory response, circulating immunological parameters and tumour characteristics in patients undergoing curative resection for colorectal cancer (n=118). *median (range)

	mGlasgow Prognostic Score			
	mGPS = 0 (n= 80)	**mGPS = 1 (n= 22)**	**mGPS= 2 (n= 16)**	**P value**
Blood				
White cell count (10^9/l)*	7.2 (3.6-15.5)	8.2 (4.7-16.1)	8.85 (4.2-16)	0.028
White cell count (<8.5/8.5-11.5/>11.5 x10^9/l)	65/10/5	13/6/3	6/7/3	0.001
Neutrophils (10^9/l)*	4.4 (2.4-11.2)	5.8 (2.4-12.7)	6.15 (1.8-13.1)	<0.001
Neutrophils (<7.5/ >7.5x10^9/l)	75/5	17/5	13/3	0.040
Lymphocytes (10^9/l)	1.55 (0.5-6.1)	1.3 (0.5-2.9)	1.6 (0.6-2.7)	0.422
Lymphocytes (≥1/ <1 10^9/l)	13/65/2	5/17/0	1/15/0	0.803
Platelets (x10^6/L)	257 (115-620)	320 (173-610)	380 (147-811)	<0.001
Platelets (≤400/ >400 x10^6/L)	75/5	17/5	11/5	0.002
Immunoglobulin A (g/L)*	2.1 (0.8-4.5)	2.9 (1.0-5.7)	2.7 (2.2-6.4)	0.119
Immunoglobulin A (<4/ ≥4g/L)	36/5	7/2	4/2	0.158
Immunoglobulin G (g/L)*	10.1 (3.6-15.8)	9.3 (5.9-15.3)	11.0 (7.5-16.0)	0.466
Immunoglobulin G (<16/ ≥16g/L)	39/0	9/0	4/1	0.011
Immunoglobulin M (g/L)*	0.7 (0.2-2.0)	0.7 (0.3-1.3)	1.1 (0.7-3.0)	0.083
Immunoglobulin M (<2/ ≥2g/L)	38/1	9/0	5/1	0.198
CD3+ (10^9/l)*	1.13(0.39-3.34)	1.34 (0.61-2.33)	1.33 (0.99-2.34)	0.424
CD3+ (≥0.96/ <0.96 10^9/l)	27/14	13/2	6/0	0.031
CD4+ (10^9/l)*	0.75(0.28-1.80)	0.73 (0.47-1.73)	0.63 (0.48-1.47)	0.893
CD4+ (≥0.54/ <0.54 10^9/l)	29/12	13/2	4/2	0.674
CD8+ (10^9/l)*	0.34(0.05-1.64)	0.49 (0.16-0.85)	0.67 (0.52-1.00)	0.031
CD8+ (≥0.27/ <0.27 10^9/l)	27/14	13/2	6/0	0.031
CD19+ (10^9/l)*	0.19 (0.3-1.46)	0.19 (0.07-0.27)	0.27 (0.07-0.43)	0.519
CD19+ (≥0.12/ <0.12 10^9/l)	33/8	13/2	5/1	0.694
Interleukin-6 (pg/ml)*	3.49 (1-23.61)	5.66 (0.8-252)	12.57 (2-32)	<0.001
Interleukin-6 (≤4/ >4pg/ml)	74/6	16/6	3/13	<0.001
Interleukin-10 (pg/ ml)*	9.61 (5.68-218)	10.72 (7.14-32)	12.17 (9.68-33)	0.005
Interleukin-10 (≤10/ >10pg/ml)	37/32	8/11	3/10	0.039
VEGF (pg/ml)*	97 (7-520)	127 (50-381)	132 (27-373)	0.068
VEGF (tertiles)	27/23/21	5/7/8	3/4/6	0.129
Tumour				
T stage (1/2/3/4)	7/11/51/11	0/2/14/6	0/1/8/6	0.005
N stage (0/1/2)	50/20/10	13/7/2	10/3/2	0.885
Venous invasion (absent/present)	34/46	6/16	7/9	0.695
Inflammatory cell infiltrate (Klintrup-Makinen High/Low)	38/40	7/13	4/8	0.194

Immunoglobulin results available for 56/118 patients

Circulating T lymphocyte results available for 62/118 patients

Inflammatory cell infiltrate scoring available for 110/118

Table 10.3: The relationship between the local inflammatory cell response (Klintrup-Makinen criteria), interleukin-6 and interleukin-10, serum immunoglobulins and lymphocyte subpopulations in patients with colorectal cancer (n=110). *median (range)

	Tumour Inflammatory Cell Infiltrate		
	High Grade (n= 49)	**Low Grade (n= 61)**	**P value**
Blood			
White cell count (10^9/l)*	7.6 (4.9-15.5)	7.2 (3.6-16.1)	0.035
White cell count (<8.5/8.5-11.5/>11.5 x10^9/l)	32/12/5	47/10/4	0.204
Neutrophils (10^9/l)*	5.0 (2.4-11.2)	4.4 (2.4-13.1)	0.179
Neutrophils (<7.5/ >7.5x10^9/l)	44/5	55/6	0.949
Lymphocytes (10^9/l)	1.7 (0.5-6.1)	1.5 (0.6-3.0)	0.455
Lymphocytes (≥1/ <1 10^9/l)	8/39/2	9/52/0	0.740
Platelets (x10^6/L)	246 (167-570)	331 (204-602)	0.354
Platelets (≤400/ >400 x10^6/L)	45/4	52/9	0.289
Immunoglobulin A (g/L)*	1.45 (0.8-5.67)	2.32 (1.38-4.05)	0.244
Immunoglobulin A (<4/ ≥4g/L)	17/4	28/4	0.519
Immunoglobulin G (g/L)*	9.37 (5.04-15.31)	9.32 (6-12.57)	0.542
Immunoglobulin G (<16/ ≥16g/L)	20/0	29/1	0.414
Immunoglobulin M (g/L)*	0.96 (0.42-1.43)	0.72 (0.25-1.05)	0.111
Immunoglobulin M (<2/ ≥2g/L)	21/0	28/2	0.232
CD3+ (10^9/l)*	1.30 (0.57-1.83)	1.13 (0.52-1.76)	0.120
CD3+ (≥0.96/ <0.96 10^9/l)	23/4	21/12	0.063
CD4+ (10^9/l)*	0.85 (0.40-1.80)	0.58 (0.28-1.73)	0.023
CD4+ (≥0.54/ <0.54 10^9/l)	23/4	21/12	0.063
CD8+ (10^9/l)*	0.48 (0.07-0.93)	0.24 (0.16-0.85)	0.231
CD8+ (≥0.27/ <0.27 10^9/l)	22/5	22/11	0.200
CD19+ (10^9/l)*	0.20 (0.05-0.71)	0.15 (0.08-0.37)	0.001
CD19+ (≥0.12/ <0.12 10^9/l)	24/3	25/8	0.195
Interleukin-6 (pg/ml)*	3.28 (2-11.4)	4.77 (2-12.4)	0.167
Interleukin-6 (≤4/ >4pg/ml)	23/26	22/39	0.251
Interleukin-10 (pg/ ml)*	9.97 (5.7-218.5)	10.51 (6.5-34.6)	0.866
Interleukin-10 (≤10/ >10pg/ml)	22/22	23/29	0.754
VEGF (pg/ml)*	117 (11-520)	104 (3-309)	0.081
VEGF (tertiles)	11/14/20	21/20/13	0.034
Tumour			
T stage (1/2/3/4)	4/10/30/5	3/4/39/15	0.016
N stage (0/1/2)	36/10/3	32/18/11	0.016
Venous invasion (absent/ present)	17/32	25/36	0.502

Immunoglobulin results available for 56/118 patients

Circulating T lymphocyte results available for 62/118 patients

Inflammatory cell infiltrate scoring available for 110/118

11.0 THE RELATIONSHIPS BETWEEN THE LOCAL AND SYSTEMIC INFLAMMATORY RESPONSES , TUMOUR INFILTRATING IMMUNE CELLS AND TUMOUR PROLIFERATIVE ACTIVITY IN COLORECTAL CANCER

11.1 Introduction

It is increasingly recognised that disease progression in colorectal cancer is not solely determined by characteristics of the tumour but also by host characteristics. Host characteristics recognized to play and important role in determining cancer specific survival include patient age, burden of comorbidity and the host local and systemic inflammatory responses (99, 277, 325, 456).

In terms of the systemic inflammatory response, there is now good evidence from over 20 published studies, that elevated markers of the systemic inflammatory response predict poorer cancer specific survival in operable colorectal cancer (Chapter 1.0). McMillan and colleagues have simplified the measurement of the systemic inflammatory response in cancer patients with the development of an objective score (Glasgow prognostic score, GPS), based on elevated circulating concentrations of C-reactive protein and hypoalbuminaemia (308). The GPS has been validated in several different cohorts of colorectal cancer patients and other tumour types as an independent predictor of poor outcome in patients with colorectal cancer (325) (Chapter 1.0).

In terms of the local inflammatory response, there is persuasive evidence that a pronounced lymphocytic infiltrate in and around the infiltrating tumour identified on routine pathology is associated with improved cancer- specific survival (222, 277, 282). Furthermore, Galon and colleagues, using more sophisticated techniques, reported that the type, density, and location of immune cells in colorectal tumours can provide prognostic information superior to that of existing tumour staging (391). Klintrup and colleagues simplified the assessment of inflammatory cell infiltrate with a robust binary score of high grade or low grade inflammatory infiltrates (277). This score has now been validated in Stage II and II colorectal cancer (Chapters 8.0 and 9.0). However, it is unclear which particular tumour infiltrating immune cells the

Klintrup-Makinen score reflects. It is also unclear whether the Klintrup-Makinen score is reflective of many different cell types or is over-reliant on a single cell type for prognostic value.

Multiple cell types have previously been examined in colorectal cancer. These include the T lymphocytes (CD3+ cells), including the helper (CD4+) and cytotoxic (CD8+) subsets, and tumour associated macrophages (CD68+) all of which have previously been reported to confer prognostic value (276, 277, 377, 383, 391, 397, 502).

The aim of the present study was to examine the relationships between the local and systemic inflammatory responses, tissue factors and survival in patients undergoing curative resection for colorectal cancer. These tissue factors include routinely assessed pathological features in addition to immunohistochemical assessment for tumour infiltrating immune cells (CD4, CD8 and CD68). As well as immunostaining for immune cells, the Ki-67 proliferative index was also assessed.

11.2 Materials and Methods

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and pre-operative abdominal computed tomography, were considered to have undergone an elective potentially curative resection between January 1997 and January 2004 were included in this prospective study. Patients with histologically confirmed stage II and III colorectal cancer were included. Tumours were staged using the conventional tumour node metastasis (TNM) staging system (RCPath 2007) and pathological details were taken from the reports issued at the time of resection. Exclusion criteria were similar to those in previous chapters (Chapter 4.0).

Measurement of the local and systemic inflammatory responses

The local inflammatory cell reaction at the tumour's invasive edge was assessed using the method first described by Klintrup and Makinen (Klintrup et al 2003). This method is described in detail in chapter 8.0 (Methods 8.2).

The Glasgow Prognostic Score was constructed as previously described (Chapter 4.0, Methods 4.2) (308).

Tumour margin characteristics

Assessment of tumour margin characteristics was also undertaken in accordance with Jass criteria (222, 227). Margins were graded as expanding or infiltrating in nature. Briefly, specific features of a diffusely infiltrating tumour margin include 'streaming dissection' of tumour cells through the muscularis propria, or dissection of mesenteric adipose tissue by small glands or irregular clusters of cords of cells on microscopic examination.

Immunohistochemistry and analysis

Blocks from the primary tumour were fixed in 10% buffered formalin in saline and embedded in paraffin wax. One representative block per tumour was selected for each patient in the present study. 4μm slides were cut from each block and mounted on slides coated with aminopropyltriethoxysilane and placed in an oven at 56 ^{o}C for 40mins. Slides were then dewaxed in xylene for 4 minutes twice and rehydrated through graded alcohols then rinsed with water.

The following monoclonal antibodies were used: CD4 (Vector, Peterborough, UK) at a dilution of 1:50, CD8+ (Dako, Cambridgeshire, UK) at a dilution of 1:100, CD68 (Dako, Cambridgeshire, UK) at a dilution of 1 in 200, Ki-67 (Dako, Cambridgeshire, UK) at dilution of 1 in 200.

Antigen retrieval for CD4, CD8, CD68 and Ki-67 was performed by microwaving in Tris EDTA buffer solution (0.555g Sodium EDTA, 0.825g Trisma base in 1.5L distilled water; pH=8) for 5 minutes in a microwave under full pressure in a plastic pressure cooker.

Following antigen retrieval, sections were placed in the Dako Autostainer in which they were first incubated with blocking serum (5% normal goat serum in Tris buffered saline (TBS)) for 20 minutes prior to application of the primary antibody (CD4, CD8, CD68 or Ki-67) at the desired dilution at room temperature for 30 minutes. Sections were then rinsed in TBS and endogenous peroxidase was blocked by incubation with ChemMate Dako Peroxidase blocking solution (Dako S2023) for 5 minutes. A further wash with TBS was performed prior to application of ChemMate Dakao Envision for 30 minutes. The sections were rinsed a=gain with TBS prior to application of 3'3' diaminobenzidine (DAB) for 10 minutes at room temperature. A wash with water was performed before counterstaining.

Tissues were counterstained with haematoxylin for 15 seconds and washed with water, followed by Scotts water for a further 30 seconds before a further wash with water. Copper enhancement was undertaken for a further 5 minutes before a final wash in water. Finally the slides were dehydrated through graded alcohols 70%, 90% and 100% and then xylene. Each section was cleared, mounted with Petrex and slide covers applied.

Quantitative analysis of CD4+, CD8+ and CD68+ tumour infiltrating immune cells

A quantitative analysis of tumour immune cells positive for CD4, CD8 and CD68 was performed using point counting (503) with a random sampling technique. With this technique the volume occupied by any given component (volume density) is expressed as a percentage of the total volume of the tissue. A 100-point ocular grid is

used at 400x magnification and 30 fields were counted per case for each immunopositive cell type. Only fields within the tumour were counted, including tumour cell nests and stroma. Normal tissue was not included in the analysis.

Semi-quantitative analysis of CD68

A semi-quantitative analysis of CD 68 immunostaining at the invasive margin of the tumour was performed using the methods described previously by Forssell and colleagues (383). Briefly immunostaining was graded on 7-10 fields along the tumours invasive margin, using a four point score as 1. none/weak, 2. moderate, 3. strong/robust or 4. massive. Weak or moderate infiltration were either totally negative for cells or included a few scatted positive cells. A moderate grade was given where CD68 staining was continuous along the invasive margin but was not more than one cell layer. A strong/robust grade indicated a continuous CD68 infiltrate of two to three cell layers away from the margin. To be classed as grade 4 (massive) several cell layers of CD68 immunostained cells were seen along the tumour front in all field viewed. Forssell also reported the prognostic value of CD68+ hotspots, defined as the infiltration grade of the two highest fields and evaluated using the same 4-point score as above.

Quantitative Analysis of Ki-67

The percentages of Ki-67 reactive cells were evaluated at 400x magnification by scoring a minimum of 1000 tumour cells in randomly selected fields. For each case, two different counts were performed and the highest score was chosen as the corresponding index. Assessment of all indexes was carried out blindly by two independent observers counting every case twice and blinded to outcome (Khalid Canna and Mustapha Hilmy).

The study design, information sheets and patient consent forms were approved by the Research Ethics Committee at Glasgow Royal Infirmary.

Statistics

Grouping of the variables was carried out using standard thresholds for laboratory parameters. For the purpose of analysis, T lymphoctyte subpopulations, tumour

associated macrophages and the Ki-67 proliferative index were grouped by tertiles. CD68 counts assessed semi-quantitatively at the invasive margin and as hot spots were grouped using the same four-point scale described by Forssell and colleagues (383). The relationships between variables were analyzed using the Mantel–Haenszel (X^2) test for trend. Univariate survival analysis and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. Deaths up to October 2010 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

11.3 Results

Between January 1997 and January 2004, 133 patients underwent elective resection for stage II and II colorectal cancer. The majority of patients were over 65 years (68%), male (53%), underwent surgery for colon cancer (30%), had TNM stage II disease (62%). On routine pathological analysis the majority of patients' tumours were T stage 3 (71%), N stage 0 (62%), had no evidence of venous invasion (80%), no peritoneal involvement (78%), no tumour perforation (98%), no perineural involvement (91%), underwent margin free resections (94%). The majority of tumours were categorised as having expanding margins (54%) and were scored as low grade Klintrup-Makinen inflammatory cell infiltrate (70%). Most tumours had non/mild or moderate CD68+ infiltration at the invasive margin according to Forssell's criteria (51%). When scored for CD68+ hot spots most tumours were scored strong or massive (56%). Of the other variables assessed with immunohistochemistry, the median score for CD4+ was 27 (range 1-107), the median score for CD8+ was 32 (range 7-189), the median score for CD68+ was 9.23 (1-23) and the median score for Ki-67 was 0.72 (0.32-0.96)

The minimum follow up was 89 months; the median follow up for survivors was 127 months. During this period 71 patients died, 44 from cancer. On univariate analysis, age ($P<0.01$), the Glasgow Prognostic Score ($P<0.001$), TNM stage ($P<0.005$), T stage ($P<0.05$), N stage ($P<0.005$), venous invasion ($P<0.005$), tumour perforation ($P<0.05$), perineural invasion ($P<0.05$), infiltrating tumour margin ($P<0.005$), Klintrup-Makinen inflammatory cell infiltrate ($P<0.005$), increasing Ki-67 tumour proliferative index ($P<0.05$) were all related to cancer specific survival (Table 11.1). On multivariate analysis, age (HR 1.96, 95% CI 1.33-2.93, P=0.001), mGPS (HR 2.02, 95%CI 1.37-2.99, P<0.001), TNM stage (HR 8 21, 95%CI 1.99-33.83, P=0.004), T stage (HR 2.06, 95%CI 1.09-3.88, P=0.026), venous invasion (HR 2.71, 95%CI 1.07-4.39, P=0.031), tumour margin characteristics (HR 2.17, 95%CI 1.07-4.39, P=0.031) and the Klintrup-Makinen inflammatory cell infiltrate (HR 2.88, 95%CI 1.08-7.65, P=0.004) were independently related to cancer specific survival (Table 11.1).

Table 11.2 shows the inter-relationships between tumour stage, clinicopathological factors, tumour infiltrating immune cells and tumour proliferative activity in patients undergoing curative resection of stage II and III colorectal cancer. Increasing TNM

stage related to increasing N stage (P<0.001) and infiltrating tumour margin (P<0.05) and the Klintrup-Makinen score (P=0.053).

Table 11.3 shows the inter-relationships between the systemic inflammatory response (mGlasgow Prognostic Score, clinicopathological factors, tumour infiltrating immune cells and tumour proliferative activity in patients undergoing curative resection of stage II and III colorectal cancer. Increasing mGPS was related to increasing age (P<0.05), reduced CD4+ tumour infiltrate (P<0.05), increasing CD68+ infiltrate at the tumour margin when assessed for CD68+ hotspot (P<0.05) and increasing Ki-67 tumour proliferative index (P<0.05).

Table 11.4 shows the inter-relationships between the local inflammatory response (Klintrup-Makinen Score), clinicopathological factors, tumour infiltrating immune cells and tumour proliferative activity in patients undergoing curative resection of stage II and III colorectal cancer. Low grade inflammatory cell infiltrate was related to the presence of lymph node metastases (P<0.05), an infiltrating tumour margin (P<0.001), reduced tumour infiltration of CD4+ cells and reduced CD68+ at the tumour margin (P<0.01) (similar results obtained for CD68+ hotspots at the tumour margin, P<0.005). A weak relationship was seen between low-grade Klintrup-Makinen score and low CD8+ infiltration (P<0.1).

11.4 Discussion

The results of the present study show that both the systemic and local inflammatory responses were associated with characteristic alterations in immune cell infiltrates in patients undergoing potentially curative resection for colorectal cancer. Specifically, an elevated mGPS and a low Klintrup-Makinen grade were associated with lower tumour CD4+ T-lymphocytic infiltrate. Also, an elevated mGPS was associated with a higher tumour CD68+ macrophage infiltrate where as a low Klintrup-Makinen grade was associated with a lower tumour CD68+ macrophage infiltrate. Taken together these results would suggest that CD4 T-lymphocytes may play an important role in coordinating systemic and local inflammatory responses in these patients.

It was however of interest that the prognostic value of tumour CD4 T-lymphocytic infiltrate was relatively weak compared with mGPS and Klintrup-Makinen grade. The role of tumour infiltrating CD4+ cells in colorectal cancer has previously been examined in other cohorts (377, 388, 392, 393) (Table 1.11). Nagtegaal et al reported that low intratumoural CD4+ counts were associated with increased depth of invasion and increased local recurrence in 160 colorectal cancer patients (377). Ali et al. also reported an association between reduced CD4+ infiltrate and local recurrence in 80 patients (392). Taken together these studies would suggest that further investigation of a more specific measure of CD4+ lymphocytic activity would be of considerable interest. Indeed, there has been increased interest in recent years in other forms of non-cytotoxic T lymphocytic infiltrates related to cancer specific survival, including T regulatory cells (FOXP3+) and in situ memory T cells (CD45RO+) (391, 396, 504-506). Further work is required to confirm whether these tumour inflammatory infiltrate measures link systemic and local inflammatory responses and whether these measure have similar or superior prognostic value. Irrespective, taken together, these observations highlight the role of the non-cytotoxic T lymphocytes that express CD4+ antigens in coordinating immune responses in colorectal cancer.

In the present study, tumour CD8+ T-lymphocytic infiltrate was not related to survival. However, to date, of the 17 studies which have reported the relationships between CD8+ cell infiltration and survival, 12 studies (Table 1.14) have reported an association between intra-tumoural or tumour margin CD8+ infiltrates and survival. It is not clear why this association was not observed in the present study. A plausible

explanation is that its prognostic value is related to a specific location. Nevertheless, using an unbiased point counting method it had inferior prognostic value compared with tumour CD4+ T-lymphocytic infiltrate.

To date, 12 previous studies have reported the role of tumour infiltrating macrophages in colorectal cancer (Table 1.17). Eight of these studies reported significant relationships between macrophages either at the tumour margin or tumour centre and survival. The remaining studies were contradictory. The present study employed similar methodology for CD68+ assessment as the studies by Forssell and Zhou but no relationships with survival were observed (383, 507). Therefore, the prognostic value of tumour infiltrating macrophages in patients with colorectal cancer remains uncertain.

From the work presented in this study and previously (Chapter 8.0), it is apparent that a high-grade local inflammatory cell response is associated with reduced T and N stage and the absence of an infiltrating tumour margin. The present study also reports that CD4+, CD8+ and CD68+ cells are also present in and around the tumour in increased numbers. These cell types are involved in the innate and adaptive immune responses and indicate the local inflammatory response when present represents a more focused or targeted host anti-tumour defence, that may prevent tumour growth and the development of an infiltrating tumour margin. Other authors have described this phenomenon as an ongoing battle between host and tumour, even developing a prognostic score based on this 'pro-tumour:anti tumour' appearance at the invasive margin (based on CD8+ infiltrates and tumour budding) (508). On the basis of the present results it may be that prognostic score based on tumour CD4+ T-lymphocytic infiltrate and tumour budding would be of more value.

As reported here and in previous work (Chapters 4.0, 5.0, 9.0 and 10.0), an increasing mGPS is associated with increasing tumour depth, alterations in circulating components of the innate and adaptive immune response, upregulation of both pro and anti-inflammatory cytokines, and reduced T lymphocyte infiltration within the tumour. All of which suggest represented an impaired or disorganised immune response. In the present study it is also apparent that increased tumour proliferation (Ki-67 index) was associated with an elevated mGPS. Therefore, it may be that an

elevated mGPS reflects this conflict between host and tumour in which the host's immune defences have been overwhelmed or a deteriorating in a similar phenomenon to that described by Lugli and Zlobec (508). At present it is unclear whether normalisation of the mGPS would be associated with an increase in the tumour inflammatory infiltrate, in particular CD4+ T-lymphocytes, and whether this would be associated with an improvement in cancer outcomes.

In summary, the present study reports that an elevated mGPS and a low Klintrup-Makinen grade were associated with lower tumour CD4+ T-lymphocytic infiltrate. Also, an elevated mGPS was associated with a higher tumour CD68+ macrophage infiltrate where as a low Klintrup-Makinen grade was associated with a lower tumour CD68+ macrophage infiltrate. Compared with mGPS and Klintrup-Makinen grade tumour CD4+ T-lymphocytic infiltrate had weak prognostic value in patients with primary operable colorectal cancer.

Table 11.1: The relationship between the local and systemic inflammatory responses, tumour infiltrating immune cells and tumour proliferative activity in patients undergoing curative resection for colorectal cancer (n=133) *median.

	Univariate survival analysis		Multivariate survival analysis	
	Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Age <65/ 65-74/ >75years	1.67 (1.15-2.45)	0.007	1.96 (1.33-2.93)	0.001
Sex Male/ Female		0.145		
mGlasgow Prognostic Score 0/1/2	2.14 (1.50-3.05)	<0.001	2.02 (1.37-2.99)	<0.001
TNM stage (II/III)	2.81 (1.55-5.12)	0.001	8.21 (1.99-33.83)	0.004
Venous Invasion Absent/ Present	2.63 (1.40-4.92)	0.003	2.71 (1.40-5.22)	0.003
Differentiation Mod-well/ Poor		0.730		
Peritoneal Involvement Absent/ Present		0.125		
Margin Involvement Absent/ Present		0.607		
Tumour Perforation Absent/ Present	4.75 (1.13-20.08)	0.034		0.088
Perineural Invasion Absent/ Present	2.42 (1.08-5.45)	0.032		0.274
Tumour margin Expanding/ Infiltrating	2.67 (1.44-4.97)	0.002	2.17 (1.07-4.39)	0.031
Klintrup Makinen inflammatory infiltrate High grade/low grade	4.14 (1.63-10.50)	0.003	2.88 (1.08-7.65)	0.034
% Tumour associated helper T lymphocytes CD4+ Tertiles (13/22/56)		0.089		
% Tumour associated cytotoxic T lymphocytes CD8+ Tertiles (19/32/58)*		0.213		
% Tumour associated macrophages CD68+ Tertiles (5.8/9.2/15.3)*		0.821		
CD68 tumour front (- / + / ++ / +++)		0.268		
CD68 Hotspot at tumour front (- / + / ++ / +++)		0.149		
Ki-67 labelling index Tertiles (52/72/91)*	1.54 (1.05-2.26)	0.026		0.217

Table 11.2: The relationship between the tumour stage, clinico-pathological factors, tumour infiltrating immune cells and tumour proliferative activity in patients undergoing curative resection for Stage II and III colorectal cancer (n=133) *median

TNM stage			
	TNM II n=82	**TNM III n=51**	**P value**
Age <65/ 65-74/ >75years	22/30/30	21/12/18	0.290
Sex Male/ Female	38/44	25/26	0.764
mGlasgow Prognostic Score 0/1/2	54/18/10	29/13/9	0.270
T stage 1/ 2/ 3/ 4	0/1/61/20	1/2/34/14	0.697
N stage 0/ 1/ 2	82/0/0	0/40/11	<0.001
Venous Invasion Absent/ Present	67/15	40/11	0.644
Differentiation Mod-well/ Poor	77/5	44/7	0.137
Peritoneal Involvement Absent/ Present	64/18	40/11	0.959
Margin Involvement Absent/ Present	76/6	49/2	0.425
Tumour Perforation Absent/ Present	80/2	50/1	0.857
Perineural Invasion Absent/ Present	77/5	44/7	0.137
Tumour margin Expanding/ Infiltrating	50/32	22/29	0.046
Klintrup Makinen inflammatory infiltrate High grade/low grade	29/53	10/41	0.053
% Tumour CD4+ lymphocytes Tertiles (13/22/56)*	26/31/25	18/14/19	0.827
% Tumour CD8+ lymphocytes Tertiles (19/32/58)*	25/30/27	17/16/18	0.974
% Tumour associated macrophages CD68+ Tertiles (5.8/9.2/15.3)*	28/24/26	12/15/16	0.450
CD68 tumour front (- / + / ++ / +++)	17/23/23/15	8/14/16/5	0.821
CD68 Hotspot at tumour front (- / + / ++ / +++)	19/33/13/13	7/17/13/6	0.464
Ki-67 labelling index Tertiles (52/72/91)*	27/30/25	16/16/19	0.565

Table 11.3: The relationship between the systemic inflammatory response, clinicopathological factors, tumour infiltrating immune cells and tumour proliferative activity in patients undergoing curative resection for colorectal cancer. (n=133) *median

mGlasgow Prognostic Score				
	mGPS 0 n=83	**mGPS 1 n=31**	**mGPS 2 n=19**	**P value**
Age <65/ 65-74/ >75years	30/31/22	8/7/16	5/4/10	0.028
Sex Male/ Female	38/45	15/16	10/9	0.584
T stage 1/ 2/ 3/ 4	1/1/62/19	0/2/20/9	0/0/13/6	0.360
N stage 0/ 1/ 2	54/23/6	18/10/3	10/7/2	0.285
Venous Invasion Absent/ Present	65/18	27/4	15/4	0.658
Differentiation Mod-well/ Poor	76/7	29/2	16/3	0.465
Peritoneal Involvement Absent/ Present	65/18	25/6	14/5	0.785
Margin Involvement Absent/ Present	78/5	29/2	18/1	0.940
Tumour Perforation Absent/ Present	82/1	30/1	18/1	0.251
Perineural Invasion Absent/ Present	74/9	30/1	17/2	0.614
Tumour margin Expanding/ Infiltrating	43/40	18/13	11/8	0.531
Klintrup Makinen inflammatory infiltrate High grade/low grade	25/58	9/22	5/14	0.749
% Tumour CD4+ lymphocytes Tertiles (13/22/56)*	23/27/33	12/11/8	9/7/3	0.020
% Tumour CD8+ lymphocytes Tertiles (19/32/58)*	26/27/30	10/14/7	6/5/8	0.935
% Tumour associated macrophages CD68+ Tertiles (5.8/9.2/15.3)*	24/26/26	11/8/8	5/5/8	0.771
CD68 tumour front (- / + / ++ / +++)	15/28/24/9	4/6/10/7	6/3/5/4	0.469
CD68 Hotspot at tumour front (- / + / ++ / +++)	20/19/34/15	3/10/6/8	3/6/5/4	0.015
Ki-67 labelling index Tertiles (52/72/91)*	31/30/22	10/9/12	2/7/10	0.011

Table 11.4: The relationship between the local tumour inflammatory response, clinicopathological factors, tumour infiltrating immune cells and tumour proliferative activity in patients undergoing curative resection for colorectal cancer. (n=133) *median

Inflammatory cell infiltrate (Klintrup Makinen Score)			
	High grade (n=39)	**Low grade (n=94)**	**P value**
Age <65/ 65-74/ >75years	10/15/14	33/27/34	0.561
Sex Male/ Female	17/22	46/48	0.575
T stage 1/ 2/ 3/ 4	1/2/29/7	0/1/66/27	0.041
N stage 0/ 1/ 2	29/9/1	53/31/10	0.034
Venous Invasion Absent/ Present	33/6	74/20	0.437
Differentiation Mod-well/ Poor	35/7	86/8	0.750
Peritoneal Involvement Absent/ Present	33/6	71/23	0.250
Margin Involvement Absent/ Present	38/1	87/7	0.283
Tumour Perforation Absent/ Present	38/1	92/2	0.878
Perineural Invasion Absent/ Present	38/1	83/11	0.095
Tumour margin Expanding/ Infiltrating	32/7	40/54	<0.001
% Tumour CD4+ lymphocytes Tertiles (13/22/56)*	9/11/19	35/34/25	0.020
% Tumour CD8+ lymphocytes Tertiles (19/32/58)*	9/13/17	33/33/28	0.095
% Tumour associated macrophages CD68+ Tertiles (5.8/9.2/15.3)*	9/11/12	31/28/30	0.538
CD68 tumour front (- / + / ++ / +++)	2/6/15/9	23/31/24/11	0.001
CD68 Hotspot at tumour front (- / + / ++ / +++)	2/14/7/9	23/36/19/10	0.006
Ki-67 labelling index Tertiles (52/72/91)*	12/15/12	31/31/32	0.945

12.0 THE RELATIONSHIP BETWEEN TNM STAGE, THE SYSTEMIC AND LOCAL INFLAMMATORY RESPONSES AND CIRCULATING VITAMINS A, D, E, CAROTENOIDS, AND LIPID PEROXIDATION IN PATIENTS UNDERGOING CURATIVE RESECTION FOR COLORECTAL CANCER

12.1 Introduction

It is now recognized that disease progression and survival in colorectal cancer is not solely determined by characteristics of the tumour but also by host factors in particular, the presence or absence host systemic or local inflammatory responses. The inter-relationships between the systemic and local inflammatory responses are likely to be complex, however both high grade systemic inflammation and low grade tumour inflammation are likely to reflect immune disturbances within the host representing a failure of the host immune system to coordinate an effective anti-tumour defence. One hypothesis is that a high-grade systemic inflammation and low-grade tumour immune cell infiltration are reflective of a state of chronic oxidative stress. The state of oxidative stress can result from either an excess of free radical production or a depletion of anti-oxidant defences (509, 510). Indeed, oxidative stress has been reported to play an important role in cancer development and progression partly as a result of oxidative damage to DNA and generation of pro-inflammatory cytokines (511-513). Furthermore, oxidative stress has been related to the upregulation of components of the systemic inflammatory response including the mGlasgow Prognostic Score (513) in addition to impaired intratumoural immune cell function (514, 515). Oxidative stress is defined as a state in which the level of toxic reactive oxygen intermediates overwhelms the endogenous anti-oxidant defences of the host. These lipid soluble anti-oxidant defences include vitamin A and E and the carotenoids.

It is also of interest that another fat soluble vitamin, Vitamin D (25-hydroxyvitamin D, 25-OHD) has also been reported to play an important role in colorectal cancer progression and survival (516). Furthermore, a recent meta-analysis showed a consistent inverse relationship between vitamin D status and the risk of colorectal cancer (517). The basis of these observations are not clear. One alternative

explanation is that, similar to other fat-soluble vitamins, plasma concentrations of 25-OHD fall as part of a chronic inflammatory response (518, 519) which is a recognised precursor to cancer development as well as an important determinant of survival.

The aim of the present study was to examine the relationship between TNM stage, the systemic and local inflammatory responses and circulating vitamins A, D, E, carotenoids, and malondialdehyde in patients undergoing curative resection for colorectal cancer.

12.2 Materials and Methods

Patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and pre-operative abdominal computed tomography, were considered to have undergone an elective potentially curative resection between June 2004 and July 2009 were included in this prospective study. Tumours were staged using the conventional tumour node metastasis (TNM) staging system (188) and pathological details were taken from the reports issued at the time of resection. Exclusion criteria were similar to those in previous chapters (Chapter 4.0, Methods section 4.2)

The Glasgow Prognostic Score was constructed as previously described (Chapter 4.0, Methods 4.2). In addition to routine pre-operative blood tests patients were also asked whether they would consent to further samples to be taken for the purposes of the present study. Each patient was provided with an information sheet and those who agreed to participate signed a consent form. At the time of participation, all patients were scheduled for elective curative resection of colorectal cancer. These extra blood samples were collected in EDTA tubes and following centrifuge at 2000g for 10mins, the separated plasma was stored in a vial at -70^{o}C prior to analysis of cholesterol, triglycerides, antioxidant vitamins A (retinol) vitamin D (25(OH)D), E (α-tocopherol) and the carotenoids (lutein, lycopene, α-carotene and β-carotene) and malondialdehyde (MDA) as a marker of lipid peroxidation.

Triglyceride and cholesterol concentrations were measured by enzymatic methods using an auto-analyser (Abbott Diagnotics, Abbott Park, IL). The inter-assay coefficient of variation was less than 6% for both methods. The cut-off points for normal healthy individuals in our laboratory were <2.2 nmol/L and <5.5 mmol/L respectively.

Plasma concentrations of retinol, α-tocopherol, lutein, lycopene, α-carotene and β-carotene were determined using a high performance liquid chromatography (HPLC) method (520). Briefly, plasma was deproteinized with alcohol containing internal standards and extraction of the analytes of interest was performed using hexane. Analysis was carried out using reversed-phase HPLC (5μm microbore, Phenomenex, Macclesfield, UK) and dual wavelength monitoring (Waters, MA). The limit of sensitivity for retinol and α-tocopherol was 0.3 and 3.0 μmol/L respectively. The

limit of sensitivity for lutein, lycopene, α-carotene and β-carotene was 10 μg/L. The inter-assay coefficient of variation was less than (% for all analytes over the sample concentration range. The 95% normal reference intervals for the above assays as established in our laboratory were as follows: retinol (1.0-2.8 μmol/L), α-tocopherol (14-39 μmol/L), lutein (82-202 μg/L), lycopene (100-300 μg/L), α-carotene (14-60 μg/L) and β-carotene (92-312 μg/L).

Measurement of plasma Vitamin D (25-OHD) incorporated an automated solid-phase extraction (SPE) procedure followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The lower limit of sensitivity was 4nmol/L. The intra-assay coefficient of variation was 10% over the concentration range of 22.5-120 nmol/L.

Malondialdehyde in plasma was measured as its MDA-TBA adduct, using reverse phase high HPLC with fluorometric detection as previously described (521). The intra-assay coefficient of variation was 9% over the sample concentration range. The 95% normal reference interval as established in our laboratory was 0.30-1.00 μmol/L.

The local inflammatory cell reaction at the tumours invasive edge was assessed using the method first described by Klintrup and Makinen (277). This method is described in detail in Chapter 8.0 (Methods 8.2).

The study design, information sheets and patient consent forms were approved by the Research Ethics Committee at Glasgow Royal Infirmary.

Statistics

Grouping of the variables was carried out using standard thresholds for laboratory parameters. Data is presented as median and range. Analytes below the threshold of sensitivity of the assay were expressed as equal to this threshold. The relationships between the groups of patients was carried out using Mantel–Haenszel (X^2) test for trend and the Kruskal Wallis test as appropriate. Univariate survival analysis and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship

with survival. Deaths up to October 2010 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

12.3 Results

One hundred and twenty six patients who underwent potentially curative resection for colorectal cancer between June 2004 and July 2009 were included in the study. Most patients were 65 or older (66%), male (52%), TNM stage I/II disease (62%). Most patients had C-reactive protein (68%) and albumin (74%) concentrations in the normal range and a normal mGPS (66%). Of the 33 patients with hypoalbuminaemia, 18 (54%) had an elevated C-reactive protein. On assessment of Klintrup-Makinen scoring to assess the local tumour inflammatory cell response, the majority of patients were considered to have a low grade inflammatory cell infiltrate (56%).

Most patients had pre-operative cholesterol concentrations less than 5.5 mmol/L (86%) and triglyceride levels less than 2.2 mmol/L (96%). Of the vitamin antioxidants, most patients had preoperative retinol concentrations greater than or equal to 1.0 μmol/L (92%), 25(OH)D concentrations greater than or equal to 30 nmol/L (55%), α-tocopherol concentrations greater than or equal to 14 μmol/L (94%), lutein concentrations greater than or equal to 82 μmol/L (52%), lycopene concentrations greater than or equal to 100 μmol/L (55%). Most patients had α-carotene concentrations greater than or equal to 14 μg/L (85%), β-carotene concentrations less than or equal to 92 μg/L (51%) and plasma MDA concentrations greater than 1.00 μmol/L (58%).

Table 12.1 shows the relationships between TNM stage and clinicopathological factors including retinol, 25(OH)D, α-tocopherol and MDA in patients undergoing curative resection for colorectal cancer. Increasing TNM stage was related to increasing T stage ($P<0.001$), N stage ($P<0.001$), the presence of venous invasion ($P<0.05$), low grade Klintrup-Makinen inflammatory cell infiltrate ($P<0.001$) and plasma MDA as a continuous variable ($p<0.05$).

Table 12.2 shows the relationships between the systemic inflammatory response (mGlasgow Prognostic Score), and clinicopathological factors including including retinol, 25(OH)D, α-tocopherol and MDA in patients undergoing curative resection for colorectal cancer. Increasing mGPS was related to increasing T stage ($P<0.05$), lower concentrations of circulating retinol as both a continuous ($P<0.01$) and categorical variable ($P<0.01$), lower concentrations of lutein as a categorical variable ($P<0.05$)

and lower concentrations of lycopene as both a continuous ($P<0.05$) and categorical variable ($P<0.01$).

Table 12.3 shows the relationships between the local inflammatory response (Klintrup-Makinen score), and clinicopathological factors including including retinol, 25(OH)D, α-tocopherol and MDA in patients undergoing curative resection for colorectal cancer. A low grade inflammatory cell response around the tumour was related increasing TNM stage ($P<0.001$), increasing T stage ($P<0.01$), increasing N stage ($P<0.005$), lower concentrations of lycopene as both a continuous ($P<0.005$) and categorical variable ($P<0.005$).

12.4 Discussion

The results of the present study confirm the inverse relationship between markers of the systemic inflammatory response and vitamins A, E and the carotenoids, previously reported in advanced cancer (513, 522-524) in patients undergoing potentially curative resection for colorectal cancer. Furthermore, they suggest that a similar relationship may also apply to vitamin D. It was also of interest that of the relationships examined between TNM stage, the systemic and local inflammatory responses and circulating vitamins A, D, E, carotenoids, and malondialdehyde only low circulating concentrations of lycopene were significantly associated with an elevated mGPS and a low grade Klintrup-Makinen score.

The basis of the relationship between lower circulating concentrations of the antioxidant lycopene and an elevated systemic inflammatory response and a low grade local inflammatory cell infiltrate is not clear. Both high-grade systemic inflammatory and low-grade local inflammatory cell responses are thought to represent compromised cell mediated immunity (276, 277, 345) (Chapter 9.0). Lycopene is a natural carotenoid present in a range of foods with particularly high levels in tomatoes and carrots. In recent years lycopene has been under investigation as a potential chemopreventative agent with most work performed in prostate cancer (525). The reported beneficial actions of lycopene include down regulation of the systemic inflammatory response through a reduction in generation of pro-inflammatory cytokines as well as modulation of various signalling pathways through inhibitory actions on Nuclear Factor Kappa B and mitogen activated protein kinase (MAPK) (526, 527). Other reported anti-tumour actions of lycopene include an increase in rate of apoptosis and a reduction in mutations in colorectal adenomas and adenocarcinomas (528, 529). One published trial in a cohort of colorectal adenoma patients observed that markers of inflammation and oxidative stress can be reduced with dietary supplementation of vitamin anti-oxidants (530), however it is not yet clear whether this may alter the progression of the disease. Taken together it is plausible that the above effects of lycopene, direct and indirect, may act to minimise the systemic inflammatory response and maintain a competent immune response in patients with colorectal cancer. Clearly, further work is therefore required to confirm the present unique observation and to test the hypothesis that active

supplementation of the antioxidant lycopene can influence the host immune response to, and disease progression in, colorectal cancer.

In the present study circulating vitamin D levels were not related to TNM stage or the Klintrup-Makinen inflammatory cell infiltrate or survival. However a weak relationship was observed for lower levels of Vitamin D with an increasing mGPS. Therefore, it is of interest that there have been a number of reports implicating low circulating vitamin D concentrations in the development and progression of colorectal cancer (516, 531-534).

To our knowledge, there has been no direct comparison of the prognostic value of the circulating vitamins A, D, E carotenoids and lipid peroxidation together with the local and systemic inflammatory responses in patients undergoing curative resection for colorectal cancer. In the present small study with a minimum follow-up of 15 months and 34 deaths (21 cancer deaths) only TNM stage (P=0.038, P=0.006), the mGPS (P=0.238, P=0.031) and Klintrup Makinen inflammatory cell infiltrate (P=0.018, P=0.009) were associated with overall or cancer specific survival respectively. As a continuous variable plasma retinol (P=0.249, P=0.373), alpha-tocopherol (P=0.717, P=0.953), 25(OH)D (P=0.564, P=0.327), lutein (P=0.146, P=0.384), lycopene (P=0.134, P=0.353) alpha- (P=0.306, P=0.346) and beta-carotene (P=0.299, P=0.482) and MDA (P=0.163, P=0.737) were not associated with overall and cancer specific survival respectively. Therefore, these unique results do not suggest a major role for these lipid soluble vitamins in disease progression in colorectal cancer. Also, a retrospective observational study of vitamin D supplementation in 2198 patients with cancer reported no effect of supplementation on plasma concentrations in patients with colorectal cancer (535). However, the more prospective studies should be carried out in larger cohorts with more follow-up. Irrespective, the present results would suggest that local and systemic measures of inflammation have more prognostic value than vitamins A, D, E and markers of oxidative stress.

In summary, the results of the present study show that vitamin D concentrations, similar to other lipid soluble vitamin, are significantly lowered in the presence of a systemic inflammatory response in patients with colorectal cancer. Also, of the relationships examined between TNM stage, the systemic and local inflammatory

responses and circulating vitamins A, D, E, carotenoids, and malondialdehyde only low circulating concentrations of lycopene were significantly associated with an elevated mGPS and a low grade Klintrup-Makinen score.

Table 12.1: The relationship between TNM stage and circulating vitamins A, D E, carotenoids, and lipid peroxidation in patients undergoing curative resection for colorectal cancer (n=126).

	TNM stage			
	TNM I n=19	**TNM II n=60**	**TNM III n=47**	**P value**
Age	69.8 (43-83)	68.5 (44-90)	69.1 (37-84)	0.932
<65/ 65-74/ >75years	6/6/7	24/26/10	13/22/12	0.848
Sex				
Male/ Female	8/11	27/33	25/22	0.344
T stage 1/ 2/ 3/ 4	7/12/0/0	0/2/56/6	0/2/25/20	<0.001
N stage 0/ 1/ 2	19/0/0	60/0/0	0/31/16	<0.001
Venous Invasion				
Absent/ Present	12/7	25/35	14/33	0.014
mGlasgow Prognostic Score 0/1/2	14/3/2	37/12/11	32/10/5	0.922
Klintrup Makinen inflammatory infiltrate				
High grade/low grade	14/5	29/31	12/35	<0.001
Cholesterol	3.7 (2.5-5.6)	3.9 (2.2-7.2)	4.6 (2.0-6.2)	0.345
<5.5/ ≥5.5 mmol/L	6/1	20/3	17/3	0.916
Triglycerides (mmol/L)*	1.1 (0.6-2.2)	1.2 (0.5-2.8)	1.0 (0.7-2.0)	0.513
<2.2/ ≥2.2mmol/L	6/1	22/1	20/0	0.114
Retinol (µmol)*	2.2 (1.3-3.7)	1.7 (0.3-16)	1.8 (0.4-4.0)	0.394
≥1.0/<1.0 µmol	18/1	57/3	41/6	0.186
Vitamin D (nmol/L)*	36 (28-97)	33 (20-84)	31 (9-98)	0.500
<25/ 25-75/ >75	2/6/4	5/18/17	3/20/11	0.673
α-tocopherol (µmol)*	27 (20-46)	27 (12-53)	26 (13-42)	0.915
≥14/<14 µmol	18/1	56/4	45/2	0.755
Lutein (µg/L)*	90 (50-237)	89 (21-607)	82 (22-267)	0.596
≥82/<82 µg/L	10/9	32/26	23/24	0.665
Lycopene (µmol)*	113 (29-435)	97 (11-376)	67 (13-385)	0.327
≥100/<100 µmol	10/8	28/30	17/29	0.138
α-carotene (µg/L)*	22 (13-151)	29 (3-128)	26 (9-137)	0.508
≥14/<14µg/L	11/1	38/8	30/5	0.816
β-carotene (µg/L)*	99 (40-256)	134 (15-862)	96 (17-463)	0.653
≥92/<92 µg/L	7/11	31/26	22/25	0.847
Plasma MDA (µmol)*	1.26 (1.0-2.8)	1.06 (0.4-2.0)	1.00 (0.4-2.6)	0.024
≤1.00/>1.00 µmol	1/8	12/13	11/12	0.123

*median and range

Pre-operative cholesterol and triglycerides results available in 50 patients
Pre-operative MDA results available in 57 patients
Pre-operative vitamin D results available in 86 patients

Table 12.2: The relationship between the systemic inflammatory response and circulating vitamins A, D E, carotenoids, and lipid peroxidation in patients undergoing curative resection for colorectal cancer (n=126).

	mGlasgow Prognostic Score			
	mGPS 0 n=83	**mGPS 1 n=25**	**mGPS 2 n=18**	**P value**
Age Group	68.0 (37-90)	71.0 (45-79)	71.8 (48-84)	0.541
<65/ 65-74/ >75years	33/28/22	5/18/2	5/8/5	0.540
Sex				
Male/ Female	36/47	11/14	13/5	0.054
Tumour Stage				
I/II/III	14/37/32	3/12/10	2/11/5	0.922
T stage 1/ 2/ 3/ 4	6/12/53/12	1/2/14/8	0/2/10/6	0.023
N stage 0/ 1/ 2	51/23/9	15/6/4	13/2/3	0.932
Venous Invasion				
Absent/ present	36/47	8/17	7/11	0.506
Klintrup Makinen inflammatory infiltrate				
High grade/low grade	38/55	10/15	7/11	0.521
Cholesterol	4.7 (2.4-7.2)	3.8 (2.0-5.7)	4.4 (2.4-5.4)	0.256
<5.5/ ≥5.5 mmol/L	28/6	12/1	3/0	0.261
Triglycerides (mmol/L)*	1.2 (0.5-2.8)	0.9 (0.6-1.5)	1.9 (0.8-2.0)	0.076
<2.2/ ≥2.2mmol/L	32/2	13/0	3/0	0.363
Retinol (µmol)*	1.9 (0.7-4.0)	1.5 (0.4-16)	1.3 (0.3-2.7)	0.008
≥1.0/<1.0 µmol	81/2	20/5	15/3	0.006
Vitamin D (nmol/L)*	46 (9-98)	33 (13-55)	17 (9-31)	0.064
<25/ 25-75/ >75	8/26/11	1/11/4	1/7/7	0.491
α-tocopherol (µmol)*	27.0 (12-48)	25.0 (9-53)	25.0 (12-37)	0.361
≥14/<14 µmol	80/3	23/2	16/2	0.167
Lutein (µg/L)*	89 (21-607)	77 (22-260)	70 (23-176)	0.327
≥82/<82 µg/L	48/34	11/13	6/12	0.039
Lycopene (µmol)*	111 (17-435)	60 (17-258)	52 (11-252)	0.023
≥100/<100 µmol	43/38	9/15	3/14	0.005
α-carotene (µg/L)*	29 (9-151)	18 (10-71)	23 (3-57)	0.469
≥14/<14µg/L	59/8	10/4	10/2	0.352
β-carotene (µg/L)*	119 (17-862)	98 (34-281)	110 (15-277)	0.470
≥92/<92 µg/L	42/38	10/15	8/9	0.454
Plasma MDA (µmol)*	1.09 (0.52-2.84)	1.00 (0.41-1.37)	0.85 (0.46-1.14)	0.640
≤1.00/>1.00 µmol	15/22	7/7	2/4	0.984

* median and range

Table 12.3: The relationship between the local inflammatory response and circulating vitamins A, D E, carotenoids, and lipid peroxidation in patients undergoing curative resection for colorectal cancer (n=126).

Inflammatory cell infiltrate			
	High grade n=55	**Low grade n=71**	**P value**
Age Group	71.5 (43-83)	71.4 (45-90)	0.281
<65/ 65-74/ >75years	24/18/13	19/36/16	0.242
Sex			
Male/ Female	25/30	35/36	0.670
Tumour Stage			
I/II/III	14/29/12	5/31/35	<0.001
T stage 1/ 2/ 3/ 4	4/12/32/7	3/4/45/19	0.007
N stage 0/ 1/ 2	49/9/3	36/22/13	0.002
Venous Invasion			
Absent/ present	19/36	32/39	0.234
Cholesterol	3.8 (2.4-5.8)	4.4 (2.0-7.2)	0.363
<5.5/ ≥5.5 mmol/L	15/1	28/6	0.283
Triglycerides (mmol/L)*	1.0 (0.6-2.1)	1.2 (0.5-2.8)	0.363
<2.2/ ≥2.2mmol/L	16/0	32/2	0.327
Retinol (µmol)*	1.8 (0.3-16.0)	1.8 (0.4-4.0)	0.859
≥1.0/<1.0 µmol	52/3	67/4	0.366
Vitamin D (nmol/L)*	32 (9-97)	33 (9-98)	0.682
<25/ 25-75/ >75	5/19/14	5/25/18	0.811
α-tocopherol (µmol)*	27 (12-46)	27 (13-53)	0.733
≥14/<14 µmol	52/3	67/4	0.965
Lutein (µg/L)*	87 (29-607)	84 (21-267)	0.893
≥82/<82 µg/L	29/25	36/34	0.802
Lycopene (µmol)*	120 (11-373)	65 (13-435)	0.003
≥100/<100 µmol	32/21	23/46	0.003
α-carotene (µg/L)*	29 (10-151)	27 (3-137)	0.782
≥14/<14µg/L	36/5	43/9	0.496
β-carotene (µg/L)*	123 (28-862)	105 (15-463)	0.360
≥92/<92 µg/L	28/24	32/38	0.376
Plasma MDA (µmol)*	1.05 (0.73-2.84)	1.07 (0.41-2.57)	0.857
≤1.00/>1.00 µmol	7/13	17/20	0.428

*median and range

13.0 ADJUVANT CHEMOTHERAPY FOR RESECTED COLON CANCER: COMPARISON OF THE PROGNOSTIC VALUES OF TUMOUR AND PATIENT RELATED FACTORS

13.1 Introduction

There is now robust level 1 evidence generated within a variety of large multi-center prospective randomized studies which demonstrate a survival benefit from the use of 5-fluorouracil based chemotherapy following surgery for colon cancer (169, 173, 419, 536). Such treatment is the standard of care for patients with TNM stage III disease or increasingly those considered high-risk TNM stage II patients (169, 419, 421, 536). Nevertheless, there remains considerable difficulty in identifying those patients who will derive specific benefit from such treatment. Although the relative reduction in recurrence is substantial, the absolute number of patients directly benefiting from adjuvant chemotherapy is small and such treatment is not without morbidity (421, 442).

To assist with such decisions, prognostic models (Numeracy and Adjuvant!) have been recently introduced (168, 537, 538). These models are based on a number of factors including age, T stage, number of positive lymph nodes and tumour grade for Numeracy plus gender, number of lymph nodes sampled and comorbidity for Adjuvant! (Table 1). Such models provide clinicians with a useful estimate of prognosis for adjuvant chemotherapy in colon cancer, however they lack prospective validation and the predicted outcomes with each index vary for similar patient scenarios entered (538).

There is good evidence that the presence of a pronounced lymphocytic infiltrate around the infiltrating tumour is associated with improved survival in patients with colon and rectal cancer (222, 329, 373). Using similar methods to the earlier work by Jass, Klintrup, Makinen and co-workers (222, 277) have simplified the subjective measurement of the tumour inflammatory infiltrate by including all white blood cell types and classifying the inflammatory infiltrate as either low or high grade. Studies have shown that a high-grade inflammatory infiltrate was associated with improved survival in patients undergoing potentially curative resection of node negative

colorectal cancer (277) (Chapter 8.0). However, the utility of the Jass or Klintrup-Makinen criteria in predicting the value of adjuvant chemotherapy in colon cancer is not, to our knowledge, known.

In contrast, the presence of a systemic inflammatory response, as evidenced by the combination of an elevated C- reactive protein and hypoalbuminaemia termed the Glasgow Prognostic score (mGPS), has been shown to be a useful prognostic factor in patients with colon cancer, independent of tumour stage and other high risk features including the Petersen Index (308, 325) (Chapter 5.0). It has also been reported that, in patients with advanced cancer, the GPS independently predicted survival in patients receiving chemotherapy for non-small cell lung cancer (539), gastro-oesophageal cancer (441, 540) and colon and rectal cancer (439, 541). Recently, Ishizuka and colleagues reported that the GPS was the most important prognostic factor in 112 patients who had undergone chemotherapy for advanced or recurrent unresectable colorectal cancer (542).

The aim of the present study was to examine the prognostic value of tumour and patient related factors currently included in the standard Numeracy and Adjuvant! models together with measures of the local and systemic inflammatory responses in patients receiving adjuvant 5-FU based chemotherapy for colon cancer.

13.2 Materials and methods

Patients with histologically proven colorectal cancer cancer who underwent potentially curative resection between 1997 and 2007 were identified from a prospectively collected database with similar exclusion criteria to those in Chapter 4.0. The tumours were staged using conventional TNM classification from the 5th edition UICC and according to the Royal College of Pathologists Dataset 2007 (188). All routinely reported pathology data was taken from the pathology reports issued following resection. Tumours were also graded by the Petersen Index, a pathological grading of high risk characteristics (195). The Petersen Index is described in detail in chapter 5.0.

Clinico-pathological variables entered into the Numeracy online calculator are available at www.mayoclinic.com/calcs. Criteria determined to be important in patients receiving colon cancer from a pooled analysis of trial patients were age, T stage (T1/2, T3, T4), lymph nodes involved (none, 1-4, 5+), and tumour grade (low or high) as shown in 13.1 (168). The Adjuvant! online calculator was developed following analysis of the US Surveillance, Epidemiology and End Results (SEER) tumour registry (537). In addition to the variables included in Numeracy, Adjuvant! also includes sex (male/female), number of examined lymph nodes (0, 1-3, 4-10, >10) and an assessment of comorbidity in addition to age, T stage, number of positive nodes and tumour grade. The full list of constituent variables included in the Adjuvant! Online calculator are shown in Table 13.1. The Adjuvant! calculator is available online at www.adjuvantonline.com/index.jsp. In the present study, data on comorbidity was abstracted retrospectively from clinical case records. To grade comorbidity we used the Adult Comorbidity Evaluation – 27 (ACE-27) index. This index categorises patients by grade of comorbid disease as none, mild, moderate or severe based on 27 different conditions from 12 physiological systems and is described in detail elsewhere (Chapter 7.0) (461, 462). This index has been applied to assess comorbid disease in colon cancer patients previously. The default setting for comorbid disease for Adjuvant! online is 'minor health problems' and where full comorbidity data was unavailable in the present cohort, those patients were grouped as 'mild comorbidity' for the ACE-27 index.

Tumour inflammatory cell infiltrate was assessed using the methods described by Klintrup and Makinen (277) and Jass (222). The methods are described in detail in chapter 8.0 (Methods 8.2).

The mGPS was constructed as previously described (Chapter 4.0, Methods 4.2).

Patients' suitability for adjuvant chemotherapy was determined following surgery in a multi-disciplinary team meeting including surgical, oncological, radiological, pathological and nursing input. Selected patients with TNM stage III disease and high-risk TNM stage II disease were referred to a consultant oncologist for adjuvant 5-FU based chemotherapy. The majority of patients received single agent fluoropyrimidine treatment, with the minority (22%) receiving combination therapy. All biochemical and pathological criteria with the exception of the Klintrup-Makinen criteria was available to the multidisciplinary team and treating oncologist.

Statistics

Grouping of the variables was carried out using standard thresholds. Comparisons between groups of patients were carried out using contingency table analysis (X^2) as appropriate. Univariate survival analysis with generation of survival curves was performed using the Kaplan–Meier method with the log-rank test. Univariate and multivariate survival analysis and calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. Deaths up to 31st April 2010 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

13.3 Results

Between January 1997- February 2007, 348 patients underwent potentially curative resection for colon cancer at Glasgow Royal Infirmary. Of these, 76 patients (22%) commenced adjuvant chemotherapy following their surgery. For all patients (n=348), most were 60 or older (82%), were male (54%), had TNM stage I/II disease with no lymph node involvement (57%) and were managed electively (85%). On assessment of inflammatory cell infiltrate, most tumours were categorised as low grade inflammation by the Klintrup-Makinen (65%) and Jass criteria (78%). Forty–eight per cent of patients had C-reactive protein levels in the normal range (≤10mg/L) and therefore 48% had a pre-operative mGPS of 0.

For all patients (n=348), the majority of patients had T3 tumours (56%), classed as low tumour grade (87%), >10 lymph nodes examined (68%) a low risk Petersen Index (76%). Most patients were categorised as none or mild comorbidity assessed with the ACE-27 comorbidity index (77%).

Baseline clinico-pathological characteristics of the patients who did not receive adjuvant chemotherapy (n= 272) compared with patients who did receive adjuvant chemotherapy (n= 76) following curative surgery for colon cancer are shown in Table 13.2. Patients were more likely to receive chemotherapy if they were younger ($P<0.001$), had greater lymph node involvement ($P<0.001$), higher T stage ($P<0.05$) and a high risk Petersen Index ($P<0.05$). Patients with a higher burden of comorbid disease were less likely to receive chemotherapy ($P<0.05$). There were no significant differences in the proportion of patients receiving chemotherapy according to sex, the time period of surgery (1997-2001 vs. 2002-2007) presentation (emergency vs. elective), number of examined nodes or tumour grade. The relative proportions of patients with low/ high-grade tumour inflammation and a mGPS of 0, 1 or 2 were not statistically different in the group not receiving chemotherapy compared with the group receiving adjuvant chemotherapy (Table 13.2). Compared with patients who did not receive adjuvant chemotherapy, more patients in the group who received adjuvant chemotherapy died from cancer than intercurrent disease ($P<0.05$).

For the 76 patients who received adjuvant chemotherapy, the minimum follow-up was 37 months; the median follow-up of the survivors was 78 months. No patients

were lost to follow up. During this period 30 patients died of their cancer and a further 3 patients died of intercurrent disease. The univariate cancer specific survival analysis for clinico-pathological characteristics is shown in Table 13.3. On univariate survival analysis of individual variables, age, sex, time period of surgery, emergency presentation, number of lymph nodes involved with tumour, number of examined nodes, tumour grade and comorbidity were not related to cancer survival in the patients receiving adjuvant chemotherapy. In contrast, a high risk Petersen Index ($P<0.05$) and increasing T stage ($P=0.053$) and an elevated mGPS ($P<0.005$, Figure 1) were associated significantly with poorer cancer specific survival. A weak relationship between low-grade inflammatory cell infiltrate ($P=0.071$) assessed with the Klintrup-Makinen criteria and poorer cancer specific survival was also observed. On multivariate analysis of significant variables, only the mGPS (HR= 3.24, 95% CI 1.45-7.27, $P=0.004$) was independently related to cancer specific survival (Table 13.3).

The univariate overall survival analysis for clinico-pathological characteristics is also shown in Table 13.3. On univariate survival analysis of individual variables, sex, time period of surgery, emergency presentation, number of lymph nodes involved with tumour, number of examined nodes, and tumour grade were not related to cancer survival in the patients receiving adjuvant chemotherapy. In contrast, a high risk Petersen Index ($P<0.05$), increasing T stage ($P<0.05$) and an elevated mGPS ($P<0.005$) were associated significantly with poorer overall survival. A weak relationship between age ($P=0.079$), higher burden of comorbidity ($P=0.063$) and a low-grade inflammatory cell infiltrate assessed with the Klintrup-Makinen criteria ($P=0.071$) and poorer overall survival was also observed. On multivariate analysis of significant variables, only the mGPS (HR= 3.23, 95% CI 1.49-7.01, $P=0.003$) was independently related to overall survival (Table 13.3).

The relationship between the mGPS (0 vs 1or 2) and individual clinico-pathological variables are shown in Table 13.4. An elevated mGPS was related to emergency presentation, date of surgery prior to 2001 ($P<0.05$), high risk Petersen ($P<0.01$) and increasing T stage ($P<0.05$). The mGPS was not related to age, sex, the local inflammatory cell infiltrate, number of lymph nodes involved or examined, tumour grade or comorbidity (Table 13.4).

13.4 Discussion

The results of the present study show that the mGPS is the most important prognostic factor in patients receiving adjuvant chemotherapy for colon cancer. These results are consistent with those of Ishizuka and colleagues (542) and indicate that the systemic inflammatory response, as evidenced by the mGPS, may have clinical role in predicting survival in patients receiving systemic 5-FU based chemotherapy for colon cancer, both in the advanced and adjuvant settings.

In the present study it was of interest that of the 7 factors included in Adjuvant! and Numeracy online calculators only T stage attained significance on univariate survival analysis but was not significant not on multivariate analysis. Similarly, the Petersen Index attained significance on univariate survival analysis but was not significant on multivariate analysis. These results would suggest these prognostic scores do not have a reliable clinical role in predicting survival in patients with colon cancer receiving systemic 5-FU based chemotherapy.

In the present study the prognostic value of the Klintrup-Makinen or Jass scores was not confirmed in patients receiving adjuvant chemotherapy. This was surprising since recent studies of patients undergoing potentially curative resection for colorectal cancer have consistently shown that the Klintrup-Makinen score for inflammatory cell infiltrate had independent prognostic value (277) (Chapters 8.0 and 9.0). Furthermore, Morris and colleagues (387) reported that, in 305 patients receiving adjuvant chemotherapy for colon cancer, a pronounced lymphocytic response on routine pathology was associated with significantly longer survival. One possible explanation for these apparently contradictory results is that the prognostic value of the Klintrup-Makinen or Jass scores differ according to tumour site. Indeed, it has recently been reported that the hazard ratio associated with the Klintrup-Makinen score in rectal cancer was more than twice that in colon cancer (Chapter 9.0). In addition the Jass assessment of peritumoural infiltrate was initially described in rectal cancer (222). It may therefore be the case that the Klintrup-Makinen and Jass scores may have a more important role in predicting outcome in patients undergoing adjuvant or neoadjuvant therapy for rectal cancer. Another possible explanation is that the nature of the tumour inflammatory infiltrate is important in determining its prognostic value. Indeed, Galon and colleagues have reported that a

quantitive analysis of the type and density of immune cells within colorectal tumours was superior to TNM stage in predicting cancer outcome (391, 543). Recently, this has led to a number of different assessments of tumour inflammatory infiltrate reporting to have independent prognostic value in patients with colorectal cancer (502, 544). Therefore, in order to provide a reliable pathological assessment with prognostic value in patients receiving chemotherapy for colon cancer, further work is required to define the optimal method of assessing inflammatory infiltrate.

A potential criticism of the results of the present study is that the dataset of patients receiving adjuvant chemotherapy was collected over a 10 year period. However, the standard systemic adjuvant chemotherapy over the entire study period has been 5-FU based either alone of in combination. In addition, only 22% of these patients received combination treatment during this time. In particular, despite the level 1 evidence that chemotherapy should be prescribed in node positive disease (169, 419, 536), only 52 of the 149 Stage III patients received this treatment, primarily due to age and comorbid disease. Potential selection bias of these patients could influence the results presented here, however there was no difference in the Glasgow Prognostic Score or Klintrup-Makinen score between those patients receiving adjuvant chemotherapy compared with those who did not.

The basis of the relationship between the systemic inflammatory response (mGPS) and poorer cancer specific survival, in patients receiving adjuvant chemotherapy for colon cancer, is not clear. In the present study an elevated mGPS was associated with emergency presentation, increasing T stage and high risk Petersen Index (Table 13.4). Such potential associations have been explored previously (481, 545) (Chapter 5.0). The prognostic value of the mGPS was found to persist independent of emergency presentation, Tumour size (T stage) and high risk tumour characteristics (Petersen Index). Therefore, these high risk features do not fully explain the prognostic value of the mGPS in patients with colon cancer. It may be that that the presence of a systemic inflammatory response is more likely to reflect the host's ability to mount a response to the tumour. For example, the mGPS has recently been shown to closely relate to patient physiology (546) (Chapter 7.0) and an independent predictor of poor cancer specific survival. More specifically, C-reactive protein and the mGPS are associated with a disordered cell mediated immune response such as neutrophilia,

lymphocytopenia and thrombophilia (345) (Chapters 4.0 and 9.0), an impaired T lymphocyte response within the tumour (Chapter 11.0) as well as increased activation of components of the innate immune response such as complement and macrophage function (291, 345). Also with reference to the use of adjuvant chemotherapy, reduced activity of cytochrome P450 and drug transporters have been associated with the presence of a systemic inflammatory response (547). Indeed, impaired drug clearance may lead to increased toxicity. Recent work has shown that the activity of the enzyme cytochrome P450 3A, which is involved in the metabolism of many chemotherapeutic drugs including taxanes, vina alkaloids, cyclophosphamides as well as the anti-metabolites, is compromised in patients with elevated pro-inflammatory markers such as interleukin-6 and C-reactive protein (548-550).

Given that the administration of systemic chemotherapy is not without risk (421, 442) there are considerable implications for current practice. There is now a need to undertake prospective studies aimed at investigating the role of the mGPS in predicting the efficacy of chemotherapy in patients with colon cancer. If this were shown to be the case it would also be important to determine whether anti-inflammatory treatment (e.g. steroids, NSAIDS) can normalize the mGPS and whether normalization of the mGPS will be associated with improvement in survival.

In summary, the results of the present study showed that the components of Numeracy and Adjuvant! models and the tumour inflammatory infiltrate had inferior prognostic value compared with that of the systemic inflammatory response, as evidenced by the mGPS, in patients receiving adjuvant chemotherapy for colon cancer.

Table 13.1: Clinico-pathological information entered into Numeracy and Adjuvant online prognostic models available at www.mayoclinic.com/calcs and www.adjuvantonline.com/index.jsp.

Numeracy criteria			Adjuvant! criteria	
Characteristic	**Options**		**Characteristic**	**Options**
Age	≤49 years 50-59years 60-69years ≥75years		Age	Entered as continuous variable
Number of positive Nodes	None 1-4 ≥ 5		Positive Nodes	0 1-3 4-10 >10
Depth of tumour (T)	T1/2 T3 T4		Depth of Invasion	T1 T2 T3 T4
Grade	Low High		Histologic Grade	Grade 1 Grade 2 Grade 3
			Examined nodes	0 1-3 4-10 >10
			Sex	Male Female
			Comorbidity	Perfect Health Minor problems Average for age Major problems (+10) Major problems (+20) Major problems (+30)

Table 13.2. Clinico-pathological characteristics in patients undergoing potentially curative resection for colon cancer and receiving/ not receiving adjuvant chemotherapy.

		No adjuvant chemotherapy N=272 (%)	Adjuvant chemotherapy N=76 (%)	P value
Age	≤49 years	12 (4)	10 (13)	
	50-59years	28 (10)	14 (19)	
	60-69years	67 (25)	26 (34)	
	≥75years	165 (61)	26 (34)	<0.001
Sex	Female	130 (48)	31 (41)	
	Male	142 (52)	45 (59)	0.280
Date of Surgery	1997-2001	99 (36)	26 (34)	
	2002-2007	173 (64)	56 (66)	0.726
Presentation	Elective	230 (85)	65 (86)	
	Emergency	42 (15)	11 (14)	0.836
Lymph nodes involved	None	176 (64)	24 (32)	
	1-4	76 (28)	45 (59)	
	5+	21 (8)	7 (9)	<0.001
Examined Nodes	0	0 (0)	0 (0)	
	1-3	5 (2)	1 (1)	
	4-10	84 (31)	22 (29)	
	>10	183 (67)	53 (70)	0.652
T category	T1/2	25 (9)	1 (1)	
	T3	152 (56)	42 (55)	
	T4	95 (35)	33 (43)	0.035
Grade	Low	248 (87)	64 (86)	
	High	34 (13)	12 (14)	0.455
Comorbidity	None	27 (10)	18 (24)	
	Mild	182 (67)	42 (55)	
	Moderate	45 (16)	13 (17)	
	Severe	18 (7)	3 (4)	0.046
Petersen Index	Low Risk	215 (79)	50 (66)	
	High Risk	57 (21)	26 (34)	0.017
Klintrup criteria*				
High grade inflammation		59 (34)	25 (34)	
Low grade inflammation		116 (66)	48 (66)	0.898
Jass Peritumoral infiltrate**				
Present		32 (18)	11 (22)	
Absent		142 (82)	39 (78)	0.334
mGlasgow Prognostic Score	0	132 (49)	34 (45)	
	1	87 (32)	33 (43)	
	2	53 (19)	9 (12)	0.694
Alive		138 (51)	43 (57)	
Dead	Cancer	81 (29)	30 (39)	
	Non-cancer	53 (20)	3 (4)	0.027

* Klintrup-Makinen scoring was available in 73 of the 76 patients who received adjuvant chemotherapy and 175 of the 272 patients who did not receive adjuvant chemotherapy.
**Jass Scoring was available in 50 of the 76 patients who received adjuvant chemotherapy and 174 of the 272 patients who did not receive adjuvant chemotherapy

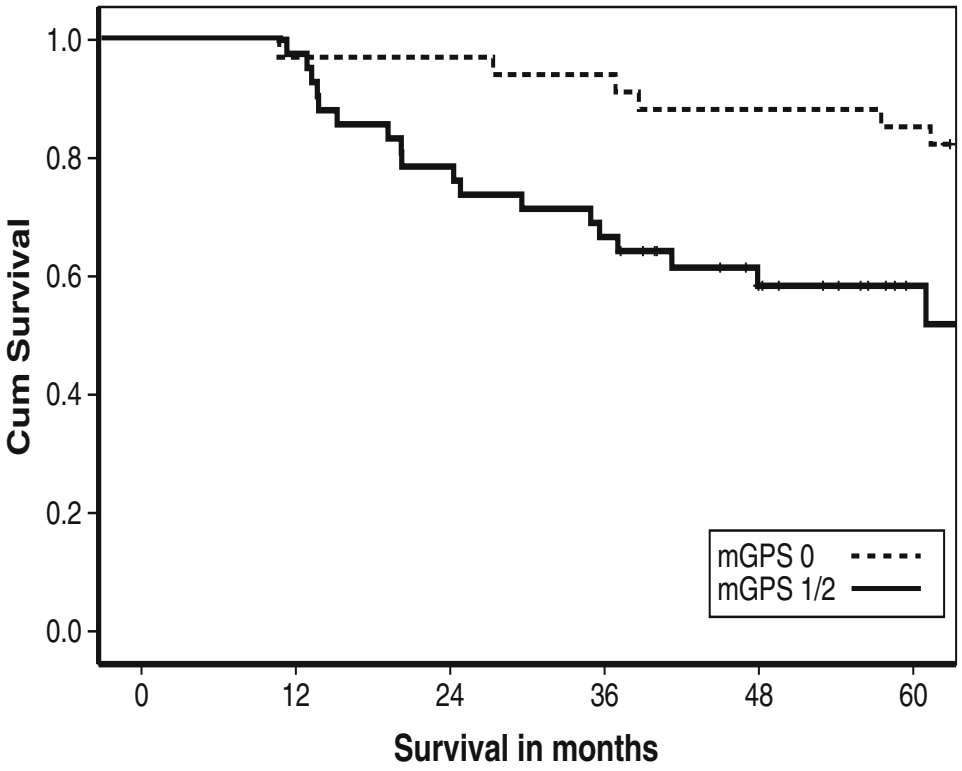
Table 13.3. The relationship between clinico-pathological characteristics and survival in patients receiving adjuvant chemotherapy following potentially curative resection for colon cancer (n=76). Univariate and multivariate survival analysis.

	Cancer specific survival				Overall Survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age								
≤49/ 50-59/ 60-69/ ≥70years	1.03 (0.73-1.46)	0.854			1.06 (0.76-1.48)	0.746		
Sex								
Female/ Male	0.99 (0.48-2.06)	0.986			1.04 (0.51-5.09)	0.922		
Date of surgery								
1997-2001/ 2002-200	1.27 (0.58-2.80)	0.550			1.24 (0.58-2.65)	0.577		
Presentation								
Elective/ Emergency	0.52 (0.12-2.21)	0.332			0.50 (0.12-2.12)	0.348		
Lymph nodes involved								
None/ 1-4/ 5+	1.38 (0.75-2.51)	0.300			1.12 (0.63-2.01)	0.698		
Examined Nodes								
0/ 1-3/ 4-10/ >10	1.46 (0.65-3.27)	0.358			1.11 (0.54-2.28)	0.785		
T category								
T1 or 2/ T3/ T4	2.01 (0.99-4.08)	0.053		0.623	2.23 (1.13-4.43)	0.021		0.355
Grade								
Low/ High	1.62 (0.66-3.98)	0.292			1.83 (0.79-4.24)	0.161		
Comorbidity								
None/ Mild/ Moderate/ Severe	1.38 (0.92-2.08)	0.113			1.44 (0.98-2.12)	0.063		
Petersen Index								
Low/ High Risk	2.23 (1.06-4.68)	0.034		0.506	2.28 (1.12-4.63)	0.023		0.611
Klintrup Inflammatory Infiltrate								
High/ Low grade	2.30 (0.93-5.59)	0.071			2.18 (0.94-5.06)	0.071		
Jass Peritumoral infiltrate								
Present/ Absent	2.36 (0.70-7.99)	0.153			1.94 (0.66-5071)	0.217		
mGlasgow Prognostic Score								
0/ 1 or 2	3.24 (1.45-7.27)	0.004	3.24 (1.45-7.27)	0.004	3.23 (1.49-7.01)	0.003	3.23 (1.49-7.01)	0.003

Table 13.4: The relationship between the mGlasgow Prognostic Score and clinico-pathological variables in patients receiving adjuvant chemotherapy. (n=76).

	mGPS 0 (n=34)	**mGPS = 1 or 2 (n=42)**	**P value**
Age			
≤49/ 50-59/ 60-69/ ≥70years	6/ 6/ 9/ 13	4/ 8/ 17/ 13	0.750
Sex			
Female/ Male	13/ 21	18/ 24	0.685
Date of surgery			
1997-2001/ 2002-2007	16/ 18	10/ 32	0.035
Presentation			
Elective/ Emergency	34/ 0	31/ 11	0.001
Lymph nodes involved			
None/ 1-4/ 5+	13/ 19/ 2	11/ 26/ 5	0.193
Examined Nodes			
0/ 1-3/ 4-10/ >10	0/ 0/ 12/ 22	0/ 1/ 10/ 31	0.557
T category			
T1 or 2/ T3/ T4	1/ 23/ 10	0/ 19/ 23	0.019
Grade			
Low/ High	31/ 3	33/ 9	0.137
Comorbidity			
None/ Mild/ Moderate/ Severe	13/ 13/ 6/ 2	5/ 29/ 7/ 1	0.293
Petersen Index			
Low/ High Risk	28/ 6	22/ 20	0.007
Klintrup Inflammatory Infiltrate			
High/ Low grade	13/ 21	12/ 27	0.505
Jass Peritumoural Infiltrate			
Present/ Absent	8/ 22	3/ 17	0.334

Figure 13.1: The relationship between an increasing Glasgow Prognostic Score from top to bottom (GPS 0 vs GPS 1 and 2) and cancer specific survival in patients who received adjuvant chemotherapy following curative resection for colorectal cancer (log rank P<0.005).



Number at risk						
mGPS 0	34	33	33	32	30	29
mGPS 1/2	42	41	33	28	18	9

14.0 CONCLUSIONS

At the outset of this body of work it was recognized that colorectal cancer progression is determined by a range of host and tumour related factors. These include the presence of both local and systemic inflammatory responses. Indeed, up to 2007, there were approximately 90 published reports relating cancer progression to the presence or absence of a systemic inflammatory response or local immune cell/inflammatory infiltrates in colorectal cancer (Chapter 1.0). Such inflammation based responses are thought to be host-derived given their components (biochemical and cellular) are important constituents of both innate and adaptive immune responses. However, their underlying basis is not clear. Potential drivers for an ongoing systemic inflammatory response were hypothesised to be either local tumour inflammation or host burden of comorbid disease.

In terms of the systemic inflammatory response, several groups reported its measurement using a variety of proposed prognostic scores. These included the neutrophil lymphocyte ratio, the platelet lymphocyte ratio and the Glasgow Prognostic Score. No previous work had examined these scores in a single cohort of colorectal cancer patients.

In terms of the local inflammatory response, several groups reported the prognostic value of generalised assessments of local inflammation including the Jass criteria and Klintrup-Makinen criteria. Furthermore, the prognostic value of tumour infiltration by individual immune cell types had also been reported. No previous work had examined the prognostic value of specific immune cell infiltrates in addition to measures of generalised local inflammation within a single cohort of colorectal cancer patients.

At the outset of this thesis, it was also unclear whether the host local and systemic inflammatory responses were in any way linked. No previous work had investigated potential relationships. The present thesis sought to investigate whether relationships existed between local and systemic inflammatory responses and circulating immune factors including cytokines, tissue infiltrating immune cells and

the Ki-67 proliferation index or measures of vitamin antioxidants, carotenoids and markers of lipid peroxidation.

As an extension to the above work Chapter 3.0 sought to further optimise an important tumour related characteristic, venous invasion. With elastica staining to aid identification of this characteristic, venous invasion was reported with increased frequency since it introduction. Furthermore, this increased frequency of reporting was associated with improved predictive value for cancer specific mortality when examined in a single cohort as well as two large consecutive cohorts of patients. This study is a unique and important study in colorectal cancer reporting, indicating for the first time that techniques used to provide a more objective assessment of venous invasion in colorectal cancer may improve prognostic accuracy. Furthermore, the more accurate assessment of venous invasion was associated with numerous other high risk pathological characteristics indicting venous invasion, as a tumour factor, is a key early feature of local and metastatic spread. It is hoped that the results of the study may inform or influence routine pathological practice to introduce more objective and accurate assessment for venous invasion. Potential future research should investigate whether the reporting of other high-risk tumour factors including serosal involvement or perineural invasion can be further optimised with pathological processing. Furthermore, the improved predictive value of venous invasion should prompt a reassessment of other reported prognostic scores such as the Petersen Index.

Chapter 4.0 and 5.0 examined the role of the systemic inflammation based, Glasgow Prognostic Score in colorectal cancer. The mGPS was observed to be superior to other measures of systemic inflammation including the neutrophil lymphocyte ratio and platelet lymphocyte ratio as well as other high-risk tumour characteristics in predicting cancer specific survival. Therefore, these results suggest future studies should use the mGPS as a measure of systemic inflammation to stratify patients by risk of recurrence and death. Furthermore, in Stage II disease the mGPS was an independent prognostic factor when compared with the Petersen index, a prognostic index designed to stratify patients at risk of recurrence and death. In Chapter 6.0 the prognostic role of lymph node counts (total, positive and negative), N stage and the lymph node ratio were examined. The mGPS was observed to predict survival

independent of N stage and the lymph node ratio. The results from Chapters 5.0 and 6.0 suggest that the mGPS could be used in addition to pathological criteria to more accurately inform clinicians of an individual patient's risk of recurrence following resection.

Chapter 7.0 sought to explore the relationships between the systemic inflammatory response and burden of comorbidity. Both the GPS and comorbidity were observed to independently predict cancer specific survival. Of interest the most accurate predictor of survival was the Lee Cardiovascular Risk Index. These results suggest cardiovascular health and colorectal cancer progression may be linked. For example, in terms of development and progression of cancer and heart disease, both share common risk factors. These include, dietary and lifestyle factors including high fat and low anti-oxidant intake, smoking and a sedentary lifestyle. Furthermore, these factors have all been related to the presence of a systemic inflammatory response. Therefore future work is required to investigate whether the attenuation of cardiovascular risk (smoking cessation, lipid lowering medications, anti-oxidant supplementation and exercise to improve cardiovascular health) and also the systemic inflammatory response (using aspirin or non-steroidal anti-inflammatory drugs) are potential therapeutic strategies, routinely employed in cardiovascular disease, that may also be efficacious in colorectal cancer.

In chapter 8.0, the Klintrup-Makinen inflammatory infiltrate score was successfully validated for the first time. The results suggested that this score was superior to older, more established measures of lymphocytic infiltrate such as the Jass score in predicting cancer specific survival in node negative disease. The Klintrup-Makinen criteria provided stage independent prognostic information and even predicted outcome in a cohort of node negative colorectal cancer patients deemed to have low risk of cancer recurrence (low risk Petersen Index). These results highlight the prognostic importance of the local inflammatory infiltrate in colorectal cancer. Furthermore the Klintrup-Makinen score provided a simple and reproducible measurement of the local inflammatory response. This validated scoring method enabled further examination of the relationships between the local and systemic inflammatory responses in the following chapters.

In chapter 9.0 the inter-relationships between biochemical and cellular components of the systemic inflammatory response (GPS, differential white cell counts) and the local inflammatory response (Klintrup-Makinen and Jass criteria) were assessed. Importantly, for the first time both the local and systemic inflammatory responses were observed to predict survival independent of stage. These results were observed in both colon and rectal cancers. Interestingly, despite the paradoxical prognostic effects (low grade systemic inflammation and high grade local inflammation are related to improved survival), no direct relationship was observed. The results did suggest the responses might be linked via cellular components of the immune response such as total white cell count and possibly neutrophils. Assessments of both local and systemic inflammatory responses could be applied in a single cohort to greatly improve the prediction of cancer specific survival. The following chapters examined the inter-relationships in greater detail.

In Chapter 10.0, a more detailed analysis of systemic immune and cytokine factors was performed. The aim of Chapter 10.0 was to evaluate whether the local and systemic inflammatory responses were associated with characteristic abnormalities in circulating cytokine, immune cell or immunoglobulin profiles. The presence of systemic inflammatory response was associated with elevations of both pro and anti-inflammatory cytokines (IL-6 and IL-10) in addition to elevated immune cells including total white cell and neutrophil counts and more specifically circulating CD8+ cells. The local inflammatory response was more weakly related to circulating CD4+ and CD19+cells and elevations in VEGF. However, Chapter 10.0 added to work in Chapter 8.0 reporting that a pronounced local inflammatory response is a more common feature in early T stage cancers whereas the systemic inflammatory response is a common feature in larger cancers (increasing T stage). A survival analysis was performed in these patients, however, a limited follow up and small patient numbers makes these results unreliable. Despite this, the mGPS was the strongest prognostic factor when compared with other circulating measures of the systemic inflammatory response.

In Chapter 11.0, a more detailed analysis of the local tissue immune cell infiltration and tumour proliferation was performed. The aim of Chapter 11.0 was to evaluate whether the local and systemic inflammatory cell responses were associated with

characteristic abnormalities in local tissue factors. The systemic inflammatory response as evidenced by the mGPS was related to increased tumour proliferation measured with the Ki-67 labeling index. Furthermore a high mGPS was associated with a lower infiltration of CD4+ cells and weakly with lower CD68+ at the invasive margin. The local inflammatory response was related to increased tumour infiltration by CD4+ and CD68+. This work confirms immune cells of both the adaptive and innate immune responses appear to have a role in colorectal cancer. Furthermore it is likely that intra-tumoural T helper cells play a key role in directing local tumour immune responses. The prognostic value of CD4, CD45RO and FOXP3+ non-cytotoxic T cells has previously been reported (Chapter 1.0). In this chapter, however, a generalised score for peritumoural inflammation was of superior prognostic value when compared with separate immune cell types. Given that almost all immune cell types including those of the innate and adaptive immune responses have previously been related to cancer outcome (Chapter 1.0), it is not surprising that a 'broad-brush' assessment of immune/ inflammatory cell infiltrate such as the Klintrup-Makinen score was most important. Therefore, these results suggest the Klintrup-Makinen score should be used as a comparison for future immune scores also reported to predict disease progression.

In Chapter 12.0 the relationships between both the local and systemic inflammatory responses and a variety of micronutrients all reported to play a role in colorectal cancer development and progression. These included vitamin antioxidants and carotenoids, measures of lipid peroxidation and Vitamin D. Although the follow up was limited, the strongest prognostic factors were the mGPS, Klintrup-Makinen criteria and TNM stage. The mGPS was associated with lower levels of vitamin antioxidants and also weakly with low vitamin D levels. This observation may partly explain the previously reported prognostic value of these micronutrients. Of particular interest, the antioxidant lycopene was strongly related to both upregulation of the systemic inflammatory response and down regulation of the local inflammatory response. This observation requires further validation, and if repeated, pilot studies should be performed to examine whether lycopene supplementation may confer therapeautic benefit. Of interest, lycopene is reported to have chemopreventative benefit in prostate cancer (525).

Chapter 13.0 sought to investigate the clinical utility of measurements of local and systemic inflammation (Klintrup-Makinen criteria and the GPS) in a cohort of patients who received adjuvant chemotherapy following surgical resection. Whilst neither score was related to the allocation of chemotherapy, the GPS was an independent predictor of survival in patients who received adjuvant treatment. The prognostic value of the local inflammatory response was not as strong in this cohort. This study sought to validate other high risk factors previously reported to guide clinicians in predicting outcomes with adjuvant chemotherapy. This included the Numeracy and Adjuvant! online criteria. A measure of systemic inflammation was the most important indicator of survival. Impaired drug metabolism in the presence of a systemic inflammatory response may provide a potential explanation for these results. Alternatively, 'inflamed' patients may have such a high risk of recurrence due to pre-existing occult metastatic spread or host immune incompetence, that adjuvant therapy may be ineffective. At the very least, the results from this chapter highlight the importance of the systemic inflammatory response and the lack of validated prognostic tools for use in the adjuvant setting. Moving forward, prospective trials need to collect data on the systemic inflammatory response to stratify patients more accurately. This is already the case for host characteristics such as age or comorbidity; two prognostic factors that have been further validated by work in the present thesis. The work presented here suggests the measure should be the Glasgow Prognostic Score. The concept of clinicians 'staging the host as well as the tumour' may make its way into clinical oncological practice in future years.

To conclude, the work presented in this thesis has further validated the Glasgow Prognostic Score as the most reliable measure of the systemic inflammatory response in colorectal cancer patients. This score predicts survival independent of tumour stage, high-risk tumour characteristics, the local inflammatory response, age, comorbidity or emergency presentation. An elevated mGPS reflects a wide range of biochemical and cellular characteristics associated with the systemic inflammatory response. This includes elevations in pro and anti-inflammatory cytokines suggesting an imbalance in control of inflammation. The mGPS weakly correlates with comorbidity and age but was related to increasing T stage and increased tumour proliferation. The mGPS does not directly relate to local immune cell/ inflammatory

infiltrate. Therefore the underlying basis of this systemic inflammatory response is as yet unclear.

Further work to establish the basis of the systemic inflammatory response is required. Given the observed relationships with T stage and increased tumour proliferation (Ki-67), it is also interesting that tumour necrosis has recently been proposed as a novel prognostic factor in colorectal cancer. Future work to investigate the relationships between local, systemic inflammatory responses and tumour necrosis is necessary. Given the reported associations with local inflammatory reactions present with microsatellite instability, further studies should investigate tumour genetics and their relationships with local and systemic inflammatory reactions. These include mismatch repair gene defects, common mutated genes implicated in cancer progression including APC, DCC, PTEN and p53 as well as profiling of inflammatory gene expression in tumour cells.

This thesis has validated a recently developed score for local tumour inflammation, the Klintrup-Makinen score. This score was effective in predicting cancer survival in a variety of settings. Of value, the score was easily reproducible and should form a standard for which other measures of tumour immune responses are assessed against. Over the coming years, refinement of scoring methods for tumour immune/ inflammatory cell infiltrates is necessary. Large-scale validation of previously proposed scores/ measures is required to guide which best predicts outcome. Scores other than the Klintrup Makinen criteria include the CD8+ budding index described by Lugli and Zlobec (508), the 'Immune Score' reported by Fridman, Pages and Galon et al (551) and individual scores for different immune cell types including the CD68+ counts reported by Forssell (383), CD8+ assessment reported by Naito (397) and measurement of FOXP3+ proposed by Salama et al (506). Increasingly there are calls for such measures to be added to the TNM staging system to guide prognosis in colorectal cancer (552). However, the clinical benefits of such scores are unclear. Firstly, based on these results, large scale prospective clinical trials in cancer patients should start to stratify patients using measures such as the Klintrup-Makinen score or mGPS to ascertain the effect of such scores on drug efficacy, treatment allocation and outcomes. Secondly, interventional studies are required to move these scores from research tools into routine clinical practice. Of interest, one study has previously

reported that tumour infiltrating lymphocytes may be increased with the prescription of anti-inflammatory drugs or aspirin (553). In addition these drugs may also act to down regulate the systemic inflammatory response. Such observations merit further investigation given the literature such drugs have generated in chemoprevention. More recently, it has been reported, in observational studies, that patients with colorectal cancer who are also prescribed low dose aspirin (75mg) have an improved cancer outcome (554). Such observations should prompt large-scale trials of such drugs in the adjuvant setting with stratification by inflammatory status (local and systemic).

From results presented in the present thesis, other potential therapeutic strategies worthy of further investigation that may down-regulate systemic inflammation, potentially improving tumour immune/inflammatory cell infiltrate include supplementation with antioxidants (e.g. lycopene) and medications aimed at reducing cardiovascular risk. In cardiovascular disease, studies have already demonstrated the risk of developing a cardiac event is lowered by prescription of aspirin or statins, both of which have anti-inflammatory effects lowering C-reactive protein.

To summarise, both local and systemic inflammatory responses are important determinants of outcome in colorectal cancer. This thesis has demonstrated the prognostic vale of two independent, robust, and reproducible scoring methods, the mGlasgow Prognostic Score and the Kintrup-Makinen criteria. Further work is required to assess whether modulation of such responses is possible and whether successful manipulation would be beneficial in terms of improving cancer outcomes.

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