AN INVESTIGATION OF THE RELATIONSHIP BETWEEN PATIENT PHYSIOLOGY, INFLAMMATORY RESPONSE AND OUTCOME IN PATIENTS WITH OESOPHAGEAL AND GASTRIC CANCER

by

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SUMMARY OF THE THESIS

Upper gastrointestinal tract cancers originating from oesophagus, oesophago-gastric junction or stomach constitute a major health problem around the world as well as in UK. Chapter 1 of the thesis describes the incidence, mortality, aetiology, staging, treatment and outcome of patients with oesophageal and gastric cancer. Each year approximately 8,000 people are diagnosed with oesophageal cancer and a similar number of people diagnosed with gastric cancer in UK. Early detection and surgery confers the greatest chance of long-term cure in oesophageal and gastric cancer. However, upper GI cancer surgery is associated with considerable morbidity and mortality. Postoperative mortality following gastro-oesophageal cancer resection is significant and has been reported as varying from 1%–23%. Therefore, preoperative surgical risk assessment is a vital part of modern surgical practice.

Pathological TNM staging by American Joint Committee on Cancer (AJCC) is an established factor in predicting long term survival following resection of oesophageal and gastric cancer. However, it is increasingly recognised that, not only the intrinsic properties of tumour cells determine tumour spread but also the host inflammatory response has a vital role. Indeed, the systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, prior to surgery, has previously been shown to have independent prognostic value in patients with resectable gastro-oesophageal cancer.

The overall aim of the thesis was to examine the inter-relationships between patient physiology, local and systemic inflammatory response and outcome (both short and long term), in patients undergoing resection for oesophageal and gastric cancer.

Chapter 2 compared the POSSUM (Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity) and mGPS (modified Glasgow prognostic score) models in prediction of post-operative outcome, both short and long term, in 121

patients undergoing resection of oesophago-gastric cancer. The results in this chapter demonstrated that, the POSSUM physiological score was an independent predictor of post-operative complications. On the other hand, elevated systemic inflammatory response as evidenced by the mGPS and not, patient physiology or post-operative complications, was independently associated with poor cancer specific survival. Therefore, results of the present study suggested that, pre-operative host related factors were important in determining both short and long term outcome following potentially curative resection of oesophago-gastric cancer.

Chapter 3 examined the value of serial daily post-operative markers of systemic inflammatory response such as white cell count, albumin and C-reactive protein concentrations in the prediction of post-operative infectious complications in 136 patients following resection of oesophago-gastric cancer. The results showed that the magnitude of the systemic inflammatory response, in particular C reactive protein, following resection of oesophago-gastric cancer was associated with the development of post operative complications, in particular surgical site infections and anastomotic leak. Furthermore, C reactive protein threshold of 180mg/l on post operative days 3 and 4 was shown to predict surgical site infection and anastomotic leak with good and very good diagnostic accuracy respectively.

Chapter 4 compared the prognostic value of selected markers of systemic inflammatory response such as white cell count, neutrophil count, lymphocyte count, neutrophil-lymphocyte ratio (NLR), platelet- lymphocyte ratio (PLR) and mGPS in 112 patients undergoing potentially curative resection of oesophageal cancer. The result demonstrated that, only mGPS was significantly associated with cancer specific survival and had prognostic value independent of pathological TNM stage. Therefore, an acute-phase protein-based prognostic score, the mGPS, was established to have the superior

predictive value, compared to cellular components of the systemic inflammatory response in predicting survival in oesophageal cancer.

Chapter 5 compared the prognostic value of selected markers of systemic inflammatory response such as white cell count, neutrophil count, lymphocyte count, neutrophil-lymphocyte ratio (NLR), platelet- lymphocyte ratio (PLR) and mGPS in 120 patients undergoing potentially curative resection of gastric cancer. The result showed that, only mGPS was significantly associated with cancer specific survival and had prognostic value independent of pathological TNM stage. Therefore, an acute-phase protein-based prognostic score, the mGPS, was established to have the superior predictive value, compared to cellular components of the systemic inflammatory response in predicting survival in gastric cancer.

Chapter 6 examined the relationship between tumour necrosis, tumour proliferation, local and systemic inflammatory response, microvessel density and survival in 98 patients undergoing potentially curative resection of oesophageal adenocarcinoma. The results suggested that, among the tissue based factors, tumour macrophage infiltration appeared to play a central role in the proliferative activity and coordination of the inflammatory cell infiltrate and was also independently associated with poor outcome in patients with oesophageal adenocarcinoma. There was no direct relationship between local and systemic inflammatory response, tumour necrosis and angiogenesis; suggesting that the mechanisms underlying the relationship between local and systemic inflammatory responses and cancer-specific survival are likely to be complex.

Chapter 7 examined the relationship between tumour necrosis, tumour proliferation, local and systemic inflammation, microvessel density and survival in 104 patients undergoing potentially curative resection of gastric cancer. The results suggested that, local peritumoural inflammatory infiltrate and intra tumoural necrosis appeared to

play a role in tumour proliferation and poor outcome in patients with gastric cancer. There was no direct relationship between local and systemic inflammatory responses, tumour necrosis and angiogenesis; suggesting that the mechanisms underlying the relationship between local and systemic inflammatory responses and cancer-specific survival are likely to be complex.

In summary, preoperative patient's physiological status (as measured by POSSUM model) has been shown to be a good predictor of short term outcome i.e. postoperative morbidity and mortality following resection of oesophago-gastric cancer. Moreover postoperative serial measurements of C-reactive protein have been shown to be a useful tool to predict infectious complications. An elevated systemic inflammatory response (as evidenced by the mGPS) preoperatively has been established to be a predictor of poor survival, independent of tumour stage in both oesophageal and gastric cancer. Therefore, these results suggest that measurement of the mGPS should be performed routinely as part of preoperative clinical staging, to improve stratification of patients and therefore enable clinicians to optimise the treatment of patients with oesophageal and gastric cancer.

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DECLARATION

I declare that the work presented in this thesis was carried out solely by me, as a clinical research fellow in the University Dept of Surgery, Royal Infirmary, Glasgow, except where indicated below:

Measurement of biochemical and haematological data was performed by the hospital laboratory service.

The selection of appropriate tissue-tumour sections and tissue blocks to create tissue micro array including appropriate markings was performed with the assistance of Dr James J. Going, Department of Pathology, Glasgow Royal Infirmary, Glasgow. Technical assistance was provided by Ms Clare Orange; Unit of Experimental Therapeutics, Institute of Cancer, College of MVLS University of Glasgow, Western Infirmary, Glasgow.

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Statistical analysis was performed with the assistance of Prof Donald C McMillan, University Dept of Surgery, Royal Infirmary, Glasgow.

DEDICATION

I lovingly dedicate this thesis to my wife Sagarika, for her presence, support and encouragement in each step of the way.

To my dear daughter Sinjini, who was born before completion of this thesis and brought us enormous joy and happiness.

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LIST OF PUBLICATIONS

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

1 Dutta S, Horgan PG, McMillan DC (2010) POSSUM and Its Related Models as Predictors of Postoperative Mortality and Morbidity in Patients Undergoing Surgery for Gastro-oesophageal Cancer: A Systematic Review. *World J Surg* 34: 2076-2082,

2 Dutta S, Al-Mrabt NM, Fullarton GM, Horgan PG, McMillan DC (2011) A Comparison of POSSUM and GPS Models in the Prediction of Post-operative Outcome in Patients Undergoing Oesophago-gastric Cancer Resection. *Ann Surg Oncol*, 18, (10), 2808-2817

3 Dutta S, Fullarton GM, Forshaw MJ, Horgan PG, McMillan DC (2011b) Persistent elevation of C-reactive protein following esophagogastric cancer resection as a predictor of postoperative surgical site infectious complications. *World J Surg* 35: 1017-1025,

4 Dutta S, Crumley AB, Fullarton GM, Horgan PG, McMillan DC (2011a) Comparison of the Prognostic Value of Tumour- and Patient-Related Factors in Patients Undergoing Potentially Curative Resection of Oesophageal Cancer. *World J Surg*; 35(8):1861-6.

5 Dutta S, Crumley AB, Fullarton GM, Horgan PG, McMillan DC (2012) Comparison of the prognostic value of tumour and patient related factors in patients undergoing potentially curative resection of gastric cancer. *Am J Surg*, 2012 Sep; 204 (3):294-9

6 Dutta S, Going JJ, Crumley AB, Mohammed Z, Orange C, Edwards J, Fullarton GM, Horgan PG, McMillan DC (2012) The relationship between tumour necrosis, tumour proliferation, local and systemic inflammation, microvessel density and survival in patients undergoing potentially curative resection of oesophageal adenocarcinoma. *Br J Cancer* 106: 702-710,

1 Chapter I: Introduction

1.1 Epidemiology of oesophageal and gastric cancer

1.1.1 Incidence and trends

Upper gastrointestinal tract cancers originating from oesophagus, oesophago-gastric junction or stomach constitute a major health problem around the world as well as in UK. Each year about 8,000 people are diagnosed with oesophageal cancer and a similar number of people diagnosed with gastric cancer in UK (Cancerstats, 2006; www.cancerresearchuk.org). Oesophageal cancer was the 6th most common cause of cancer death in UK and about 7,400 people died with oesophageal cancer in UK in the year of 2006. About 5,300 people died of stomach cancer in UK in the year of 2006 and it was the 7th most common cause of cancer death in UK (Cancerstats, 2006; www.cancerresearchuk.org).

It was estimated that in the year 2008, about 44,700 new cases of oesophageal cancer were diagnosed in Europe and 38,600 people died from the disease (Ferlay, Parkin & Steliarova-Foucher 2010). In 1970's stomach cancer was the most commonly diagnosed cancer worldwide, but it gradually declined, accounting for around 9% of all newly diagnosed cancer in the West (Parkin, Stjernsward & Muir 1984, Pisani et al. 1999, Parkin et al. 2005). It was estimated that 149,200 new cases of gastric cancer were diagnosed in Europe in 2008, with 116,600 deaths attributable to the disease in the same year (Ferlay, Parkin & Steliarova-Foucher 2010).

Oesophageal cancer is divided in two major histological subgroups i.e. squamous cell carcinoma and adenocarcinoma (Siewert, Ott 2007). There has been a significant change in the histological subtype and site of oesophageal and gastric cancers both in United States (Blot et al. 1991) and in Europe (Johnston, Reed 1991, Powell, McConkey 1990). There has been a sharp rise in oesophageal adenocarcinoma in all parts of the world

and in both sex (Brown, Devesa & Chow 2008). It has increased approximately by six fold in United States (Pohl, Welch 2005) in the last three decades. An epidemiological study on 22,759 patients with oesophageal cancer between 1975 and 2004 in USA showed 463% and 335% increase in the incidence of oesophageal adenocarcinoma in white men and women respectively (Brown, Devesa & Chow 2008). In northern European countries, in particular Scotland, adenocarcinoma of oesophagus is now the most common oesophageal cancer in male (Bosetti et al. 2008). Indeed, Scotland has the highest incidence of oesophageal adenocarcinoma in Europe (Anonymous 2002). On the other hand, the incidence of oesophageal squamous cell carcinoma has declined slightly in southern and western European countries, accounting for less than 30% of all oesophageal cancers in Western Europe and United States (Brown, Devesa & Chow 2008, Steevens et al. 2010). Within the stomach, there has been a significant change in the site of adenocarcinoma development with a noticeable increase of proximal and junctional tumours and sharp decline of distal gastric cancer incidence (Steevens et al. 2010, SEER 2010, Blot et al. 1991).

1.1.2 Age and Sex

Oesophageal and gastric cancers are predominantly diseases of old age and have male predominance (SEER 2010). In UK around 8 in 10 oesophageal cancers occur in people over 60 years of age. It has increased by 50 percent in men in UK over last 25 years. The male to female ratio of all types of oesophageal cancer is approximately 3:2 (CancerStats - Cancer Statistics for the UK 2010a). In case of gastric cancer, 95% of new cases are in people aged 50 and over. It is more common in men (CancerStats - Cancer Statistics for the UK 2010b).

1.1.3 Geography and Ethnicity

The incidence of oesophageal cancer has a very wide variation across the world. The high prevalence areas are oesophageal cancer belt in Asia (which stretches from eastern Turkey through north-eastern Iran, northern Afghanistan and southern Russia to northern China) (Parkin 2004), southern and eastern Africa and Northern France (Corley, Buffler 2001) (Pickens, Orringer 2003). Oesophageal squamous cell carcinomas are more common in the endemic areas as mentioned above. But the adenocarcinomas are more common in non endemic areas such as Western European countries and North America (Brown, Devesa & Chow 2008). The prevalence of gastric adenocarcinoma is high in East Asia, South America and Eastern European countries and the low in United States and Western European countries (Corley, Buffler 2001).

It has also been reported that there are wide variations in incidence of oesophageal and gastric cancer in different ethnic groups within a country. For example, in United States, the most recent figures (2003-2007), showed that the overall incidence of oesophageal cancer varies among different American ethnic groups. African Americans have the highest age adjusted rates (5.4 cases per 100,000), followed by Whites (4.6 per 100,000), Asians and Pacific islanders (2.4 per 100,000) (SEER 2010). Similar variation in the incidence among various ethnic groups is observed in gastric cancer. In USA, the incidence of gastric cancer in African Americans is twice that of white Americans (12 and 7 per 100,000 respectively). The rate in Hispanics is 12 per 100,000 but the highest incidence is found in people from the Pacific Islands (13.2 per 100,000) (SEER 2010).

1.1.4 Socio-economic factors

Higher incidence of oesophageal squamous cancer and gastric cancer has been shown to be linked with socio-economic deprivation in the UK (Anonymous 2002,

Brewster et al. 2000). In contrast, oesophageal adenocarcinoma has been linked to the most affluent socio-economic groups (Powell, McConkey 1990, Dutta Roy et al. 2005). Incidence of oesophageal cancer has increased in higher socio-economic class and the incidence of gastric cancer has decreased in the deprived group (Dutta Roy et al. 2005).

1.1.5 Mortality and Survival

Oesophageal and gastric cancer have demonstrated opposite trends in mortality over the last 30 years. Oesophageal cancer mortality rates have increased. In USA the rates have increased steadily between 1975 and 2007 from 3.7 to 4.3 per 100,000. On the other hand, mortality rates from gastric cancer have decreased rapidly during the same period in USA; from 8.5 per 100,000 to 3.6 deaths per 100,000 (SEER 2010).

Currently one year survival in UK is around 30% for oesophageal cancer patients (CancerStats - Cancer Statistics for the UK 2010a). In Scotland, age standardised five year survival for oesophageal cancer for the year 2003 -2007 in male and female was 10.1% and 15.5% respectively (ISD Scotland 2010).

Over the last 25 years, five-year relative survival rate for stomach cancer has trebbled in Britain, and is around 15% at present (CancerStats - Cancer Statistics for the UK 2010b). For gastric cancer, age standardised five year survival for the year 2003 -2007 in Scotland for male and female was 15.4% and 18% respectively (ISD Scotland 2010).

1.2 Aetiology of Oesophageal and Gastric cancer

1.2.1 Inheritance

A small proportion of oesophageal and gastric cancers are hereditary. Familial clustering of these tumours may be due to exposure to similar environmental factors. Inheritance certainly has a role in oesophageal squamous and adenocarcinoma albeit in a

small proportion (less than one percent in reported studies) (Lagergren et al. 2000, Hemminki, Jiang 2002). Mutations of the gene for the calcium-dependent cell-adhesion protein E-cadherin (CDH1) have been found in inherited or familial gastric cancer (Fitzgerald, Caldas 2004). Almost all of these inherited gastric cancers are early onset, autosomal dominant, diffuse gastric cancers (Caldas et al. 1999, Guilford et al. 1998, Huntsman et al. 2001). Hereditary diffuse gastric cancer syndrome (HDGC) is associated with a 70% lifetime risk of gastric cancer (Robertson, Jankowski 2008).

1.2.2 Lifestyle factors

1.2.2.1 Smoking

Tobacco smoking has been recognised as an important risk factor for squamous cell carcinoma of the oesophagus and the effect is about nine fold compared with age and sex matched controls (Brown et al. 2001, Gallus et al. 2003). Development of the oesophago-gastric junctional tumour and gastric cancer has also been linked to smoking but to a lesser extent (Engel et al. 2003). However, the role of smoking in oesophageal adenocarcinoma is not clear (Engel et al. 2003, Lagergren et al. 2000).

1.2.2.2 Alcohol

Oesophageal squamous cancer and gastric adenocarcinoma are strongly associated with heavy alcohol intake (Lagergren et al. 2000, Bagnardi et al. 2001). In contrast, oesophageal adeno-carcinoma and oesophago-gastric junctional tumours are not shown to be related to alcohol consumption (Engel et al. 2003, Lagergren et al. 2000, Gammon et al. 1997) and therefore increasing incidence of these cancers is unlikely to be alcohol related.

1.2.2.3 Diet

The role of dietary intake in development of the oesophageal and gastric cancer is not clear. A large case control study showed that, higher intake of animal based food increases the risk of adenocarcinoma of the oesophagus; where as plant-based food reduces the risk (Mayne et al. 2001). Use of green leafy vegetables, citrus fruits and dietary fibre reduces the risk of oesophageal cancer (Cheng et al. 1992, Cheng et al. 1995, Terry et al. 2001, Terry et al. 2001). Nitrosamines and their precursors (nitrate, nitrite and secondary amines) have been positively associated with development of oesophageal and gastric cancers (Mayne et al. 2001, Cheng et al. 1992). Studies have confirmed that nitrosative stress was created by the nitrate-derived nitric oxide generated within the lumen at the oesophago-gastric junction, where saliva encounters gastric acid and this may contribute to the high prevalence of mutagenesis at this site. Moreover luminal generation of nitric oxide from dietary nitrate via salivary nitrite is maximal at the gastroesophageal junction and cardia. This might explain the incidence of junctional cancer (Iijima et al. 2002, Iijima et al. 2003). On the other hand, antioxidants such as vitamin C, vitamin E and beta carotene are associated with reduced risk of these cancers (Terry et al. 2000, Serafini et al. 2002, Ekstrom et al. 2000). Diets with poor fruit and vegetable intake have been associated with oesophageal cancer in the USA (Engel et al. 2003) and gastric cancer in Brazil (Nishimoto et al. 2002).

1.2.2.4 Body mass index (BMI)

Higher BMI has been shown to be associated with an increased risk of oesophageal adenocarcinoma and carcinoma of the oesophago-gastric junction. Various independent population based studies have shown this positive association (Abnet et al. 2008, Chow et al. 1998b, Lagergren, Bergstrom & Nyren 1999). Moreover recent systematic review and meta-analysis by Kubo et al supported a positive correlation between high BMI and the

increased risk of oesophageal and possibly for oesophago-gastric junction adenocarcinoma. (Kubo, Corley 2006). There is some evidence of an inverse association of BMI to oesophageal squamous cell carcinoma (Engeland, Tretli & Bjorge 2004, Vaughan et al. 1995, Smith et al. 2008) but other population based studies found no evidence of association of increased BMI with gastric cancer or with squamous cancer of the oesophagus(Chow et al. 1998b, Lagergren, Bergstrom & Nyren 1999). More recently the link between obesity and the risk of cancer death was studied in a cohort of over 900,000 patients in the USA (Calle et al. 2003). They found that increasing BMI was associated with an increased risk of death in oesophageal and gastric cancer. The link between obesity and the oesophageal and gastric adenocarcinoma is complex (Corley 2007). There is evidence that the body fat can mediate cancer risk through intermediary hormones that modulate inflammation (Stoll 2002, Kaaks, Lukanova 2001, Schoen et al. 2002, Lagergren 2005). Adipose-associated polypeptides such as leptin, adiponectin, insulin like growth factors and ghrelin have a plausible role for modifying cancer risk at the cellular level (Bolukbas et al. 2004, Bub, Miyazaki & Iwamoto 2006, Lord 2006, de Martel et al. 2007). It is also reported that obesity induced inflammation might be an important link between obesity and cancer (van Kruijsdijk, van der Wall & Visseren 2009). Moreover, recently in a matched controlled cohort study by Beddy et al involving 194 patients with oesophageal adenocarcinoma, oesophageal squamous cell carcinoma and gastric adenocarcinoma and 90 controls, showed that proportion of visceral distribution of fat is significantly higher in patients with oesophageal or junctional adenocarcinoma compared to the other two groups (Beddy et al. 2010). Similarly, studies reported on the association between increased visceral fat particularly at oesophago-gastric junction with metaplasia and high grade dysplasia (Nelsen et al. 2012).

1.2.3 Gastro-oesophageal reflux

The relationship between gastro-oesophageal reflux disease and oesophago gastric junctional cancer has now been well established (Lagergren et al. 1999, Wu, Tseng & Bernstein 2003). The co-relation between gastro-oesophageal reflux and cancer is thought to be mediated through Barrett's metaplasia. Patients with Barrett's oesophagus have 30 to 60 times more risk of developing oesophageal adenocarcinoma compared to the general population (Cossentino, Wong 2003). It has also been reported that the risk of oesophageal cancer in patients with Barrett's oesophagus is significantly greater than in patients with long standing reflux symptoms in the absence of Barrett's metaplasia (Solaymani-Dodaran et al. 2004).

1.2.4 Helicobacter pylori

The role of Helicobacter pylori in gastric adenocarcinoma has been reported in several studies (Danesh 1999). A meta-analysis conducted by Eslick et al showed the increased risk of developing gastric adenocarcinoma in patients with H pylori infection (Eslick et al. 1999). A study by a Swedish group has reported that, infection by cytotoxin-associated gene A (cagA) strains of H pylori was primarily associated with the development of the gastric cancer (Ekstrom et al. 2001). Another meta-analysis by Huang and co-workers confirmed the association between H-pylori (in particular the cag A strain) and the elevated risk of gastric cancer (Huang et al. 2003). It has been shown however, that H pylori was associated with a reduced risk of developing oesophageal adenocarcinoma but an elevated risk of oesophageal squamous cancer (Chow et al. 1998a, Ye et al. 2004).

The association between H-Pylori infection and gastro-oesophageal junctional malignancy however is not clear. Previously mentioned studies did not show any definitive association (Danesh 1999, Eslick et al. 1999, Huang et al. 2003). But recently

some published reports indicated that there might be some association between H pylori infection and oesophageal or junctional adenocarcinoma (McColl, Watabe & Derakhshan 2008, de Martel et al. 2005). It has been reported that, the decrease in H pylori infection is associated with increased incidence of oesophageal and junctional adenocarcinoma (Clark 2003, Ye et al. 2004, Anderson et al. 2008). The plausible mechanism is that, H pylori infection may protect the lower oesophageal epithelial layer by inducing atrophic gastritis which in turn reduces the gastric acid secretion (McColl, Watabe & Derakhshan 2008).

1.2.5 Other predisposing conditions

A number of conditions have been identified as increased risk factors of developing oesophageal and gastric cancer. Tylosis is rare autosomal dominant skin disorder characterized by hyperkeratosis of the palms and soles. Late onset tylosis has been shown to be associated with oesophageal squamous cancer (Ellis et al. 1994, Stevens et al. 1996, Risk et al. 1999). Achalasia has been shown to increase risk of squmaous carcinoma and adenocarcinoma of oesophagus (Ribeiro et al. 1996, Sandler et al. 1995, Brucher et al. 2001, Ellis et al. 1997). It has also been reported that patients who had surgery for peptic ulcer disease had increased risk of developing oesophageal cancer (Lundegardh et al. 1994) and gastric cancer (Macintyre, O'Brien 1994). Pernicious anaemia or achlorhydria has been shown to be associated with elevated risk of developing gastric cancer as well as oesophageal squamous cell cancer (Ye, Nyren 2003). However, it is not clear, if there is any elevated risk of developing oesophageal adenocarcinoma associated with pernicious anaemia (Mellemkjaer et al. 1996).

1.3 Clinical presentation of oesophageal and gastric cancer

Generally, both oesophageal and gastric cancer present late at advanced stage. The diagnosis of patients with oesophageal and gastric cancer on basis of clinical features was recognised to be difficult, as correlation of symptoms with endoscopic findings were

known to be poor (Adang et al. 1996). Symptoms of oesophageal cancer are usually insidious. Dysphagia, weight loss, vomiting or regurgitation, pain, cough or hoarsness are the most common presenting symptoms. Gastric cancer, in particular in early stage, lacks specific symptoms. Vague epigastric discomfort, indigestion, pain, weight loss, anorexia, fatigue or vomiting may be the presenting symptoms for some of the patients. In UK, NICE (National Institute for Health and Clinical Excellence) guidance suggested the 'alarm symptoms' (such as progressive dysphagia, weight loss, anaemia, persistent vomiting and epigastric mass etc) for oesophago-gastric cancer that require urgent referral and or investigation (National Institute for Health and Clinical Excellence 2005). However, approximately 70% of patients with early gastric cancer have symptoms of uncomplicated dyspepsia with no associated anaemia, dysphagia or weight loss (Hallissey et al. 1990). It has also been reported recently that localised cancers can be missed if only patients with these alarm symptoms have been investigated (Bowrey et al. 2006).

1.4 Diagnosis of oesophageal and gastric cancer

Diagnosis of oesophageal and gastric cancer dependent on clinical features alone is unreliable (Meineche-Schmidt, Jorgensen 2002). Two modalities of investigations can be performed i.e. contrast swallow (barium swallow or barium meal) and upper GI endoscopy.

Endoscopy and biopsy remain the investigation of choice for diagnosis in upper GI cancer. About 10% malignant lesions are missed on first endoscopy (Bramble, Suvakovic & Hungin 2000, Yalamarthi et al. 2004). Lack of adequate biopsy specimen is one of the major reasons for the repeat endoscopy. Minimum of eight biopsies from the suspected lesions are recommended (The Scottish Intercollegiate Guidelines Network 2006). Barium studies are safe, but less sensitive than endoscopy in early cancers (Dooley et al. 1984) and

it cannot diagnose precancerous lesions (Arai, Yamada & Maruyama 2002). Therefore, barium studies are becoming less routinely carried out nowadays.

1.5 Clinico-pathological staging of oesophageal and gastric cancer

Clinical baseline staging provides useful information for the development of an initial therapeutic strategy and is associated with patient outcome. However, pathological staging is considered to be the most important prognostic factor in patients with oesophageal and gastric cancers.

In oesophageal cancer, two major staging systems are commonly used i.e. The Japanese Society for Esophageal Diseases (Japanese Society for Esophageal Diseases; In 2002) and The Tumour Node Metastasis (TNM) Criteria by the American Joint Committee on Cancer (AJCC) (Sobin, Wittekind & editors. April 2002) (Table 1-1). The AJCC system is most widely used in UK. In western countries, there are no screening programme for early detection of oesophageal cancer because of low incidence, the diagnosis is often made late in the disease course. At diagnosis, nearly 50% patients have cancer beyond loco-regional level. Moreover fewer than 60% patients with loco-regional disease can undergo a curative resection. Approximately 70-80% of resected specimens will have metastasis in the regional lymph nodes.

Adenocarcinoma of the oesophago-gastric junction has been classified by Sierwert et al into three types. This classification is purely based on anatomical location of the tumour (Siewert, Stein 1996). If the epicentre of the tumour or more than 66% of the tumour mass is located more than 1 cm above the anatomical oesophago-gastric junction, then the tumour is classified as Type I adenocarcinoma of the distal oesophagus. If the epicentre of the tumour or the tumour mass is located within 1cm proximal and 2 cm distal to the anatomical oesophago-gastric junction, this tumour is classified as Type II. If the epicentre of the tumour or more than 66% of the tumour mass is located more than 2 cm

distal to the anatomical oesophago-gastric junction, this tumour is classified as Type III (Siewert, Stein 1996). The classification was changed slightly in 2000 by the same group (Rudiger Siewert et al. 2000). Type I adenocarcinoma of the distal oesophagus was defined as tumour that arises from an area with specialised intestinal metaplasia of the oesophagus (i.e., Barrett oesophagus) and may infiltrate the oesophago-gastric junction from above. Type II, the true carcinoma of the cardia, arises immediately at the oesophago-gastric junction. And Type III or subcardial gastric carcinoma infiltrates the oesophago-gastric junction and distal oesophagus from below. There is no clear consensus about which Siewert type of junctional tumour is oesophageal or gastric cancer. In AJCC TNM staging system (6^{th} edition), it specifically states that 'if more than 50% of the tumour involves the oesophagus, the tumour is classified as oesophageal cancer, if less than 50% then it is classified as gastric cancer' (Sobin, Wittekind & editors. April 2002). In the present study, Type I and Type II lesions of the gastro-oesophageal junction were designated oesophageal cancers and Type III tumours as gastric cancer (Siewert, Stein 1996, Siewert, Stein 1998). Indeed this classification has been recognised by the British Society of Gastroenterology (Allum et al. 2002) and The Scottish Intercollegiate Guidelines Network for oesophageal and gastric cancer (The Scottish Intercollegiate Guidelines Network 2006).

In gastric cancer, two major classifications are currently used. The Japanese classification is elaborate and is based on anatomical involvement, particularly the lymph node stations or echelons (Japanese Gastric Cancer 1998). In western countries, AJCC (American Joint Committee on Cancer) and /or UICC (the International Union against cancer) staging system is primarily used (Roder et al. 1998) (Table 1-2). Approximately 50% of patients present in advanced stage of their disease at diagnosis and have poor outcome. Nearly 70-80% of all patients have involvement of regional lymphnode. The number of positive lymph node has a profound influence on survival (Karpeh et al. 2000).

Table 1-1: TNM staging in oesophageal cancer (6th edition)

Primary tumour (T)

TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
Tis Carcinoma in situ
T1 Tumour invades lamina propria or submucosa
T2 Tumour invades muscularis propria
T3 Tumour invades adventitia
T4 Tumour invades adjacent structures

Regional lymph nodes (N)

NX Regional lymph node cannot be assessed
N0 No regional lymph node metastasis
N1 Regional lymph node metastasis

Distant metastasis (M)

MX Presence of distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis
For tumours in the lower thoracic oesophagus:
M1a Metastasis in ceoliac lymph nodes

M1b Other distant metastasis
For tumours in the upper thoracic oesophagus:
M1a Metastasis in cervical lymph nodes
M1b Other distant metastasis
For tumours in the mid thoracic oesophagus:
M1a Not applicable
M1b Non-regional lymph node or other distant metastasis

Stage grouping

0	Tis	N0	M0
I	T1	N0	M0
IIa	T2	N0	M0
IIa	T3	N0	M0
IIb	T1	N1	M0
IIb	T2	N1	M0
III	T3	N1	M0
III	T4	Any N	M0
IV	Any T	Any N	M1
IVa	Any T	Any N	M1a
IVb	Any T	Any N	M1b

Table 1-2: TNM staging in gastric cancer (6th edition)

Primary tumour (T)

TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
Tis Carcinoma in situ
T1 Tumour invades lamina propria or submucosa
T2 Tumour invades muscularis propria or sub-serosa
T3 Tumour penetrates serosa without invasion of adjacent structures
T4 Tumour invades adjacent structures

Regional lymph nodes (N)

NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Metastasis in 1 to 6 regional lymph nodes
N2 Metastasis in 7 to 15 regional lymph nodes
N3 Metastasis in more than 15 regional lymph nodes

Distant metastasis (M)

MX Presence of distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis

Note: Involvement of retro-pancreatic, mesenteric and para-aortic lymph nodes, are classified as distant metastasis.

Stage grouping

0	**Tis**	**N0**	**M0**
Ia	T1	N0	M0
Ib	T1	N1	M0
Ib	T2	N0	M0
II	T1	N2	M0
II	T2	N1	M0
II	T3	N0	M0
IIIa	T2	N2	M0
IIIa	T3	N1	M0
IIIa	T4	N0	M0
IIIb	T3	N2	M0
IV	T4	N1, 2, 3	M0
IV	T1, 2, 3	N3	M0
IV	Any T	Any N	M

1.5.1 Staging modalities

The principle imaging modalities for staging oesophageal and gastric cancer are multidetector computed tomography (CT), endoscopic ultrasound (EUS), Positron emission tomography (PET) integrated with CT (PET-CT) and laparoscopy.

1.5.1.1 Endoscopy

For the purpose of proper staging of early (i.e. intra mucosal) oesophageal malignancy, endoscopy and endoscopic mucosal resection (EMR) are essential. EMR is primarily performed in case of Barrett's oesophagus with endoscopic evidence of mucosal irregularity and dysplasia. The depth of resection involves the submuosal layer. In a comparative study, Wani and colleagues reported that, in 88% samples of endoscopic mucosal resection had submucosa compared to only 1% samples of endoscopic biopsy, and the overall inter-observer agreement for the diagnosis of neoplasia was significantly higher for endoscopic mucosal resection specimens than biopsy specimens (Wani et al. 2010). It allows assessment not only of depth of penetration but also of degree of differentiation and vascular and lymphatic involvement. It is superior to endoscopic ultrasound in staging early T1 cancers (Curvers et al. 2008, Mino-Kenudson et al. 2007, Peters et al. 2008).

1.5.1.2 Computerised Tomography (CT)

Computerised Tomography is most commonly used as initial staging investigation in patients with oesophageal and gastric cancer. A spiral contrast enhanced scan with thin collimation (2.5 to 5 mm) is recommended (Allum et al. 2002). The study is generally performed with intravenous as well as oral contrast. CT scan has been reported to be very accurate in detecting distant metastasis. A patient in whom advanced disease has been identified with CT scan does not require any further investigation (The Scottish Intercollegiate Guidelines Network 2006). CT scan is unable to differentiate between

various layers of the oesophagus and therefore it may not be very useful to clarify T1 and T2 cancers. Moreover microscopic invasion of T3 tumour is not well visualised on CT scan (Saunders, Wolfman & Ott 1997) and therefore there is a risk of under staging of oesophageal cancer if it is only relied upon CT scan.

In gastric cancer, CT scan has been reported to be accurate in detecting distant metastasis but not in assessing the T and N stage (Blackshaw et al. 2003). Similar to oesophageal cancer, CT cannot differentiate between T1 and T2 lesions. About 80-88% patients with advanced disease (including T4) can be identified by CT scan (Minami et al. 1992, Cho et al. 1994, Davies et al. 1997).

PET-CT scan has a low detection rate because of the low tracer accumulation in diffuse and mucinous tumour type, that are frequent in gastric cancer (Stahl et al. 2003). Therefore, it has limited use in staging in patients with gastric cancer (Dassen et al. 2009) and requires further evaluation. PET scan has a significantly lower sensitivity compared to CT scan in detecting regional lymph node involvement (56% vs 78%), although it has an improved specificity (92% vs 62%) (Chen et al. 2005). Combined PET-CT imaging has been reported to be better than PET scan alone (Dassen et al. 2009, Lim et al. 2006). PET – CT has a significantly higher accuracy in preoperative staging (68%) than PET (47%) or CT (53%) alone (Rosenbaum et al. 2006). Therefore, PET-CT is currently advocated for preoperative staging of nodal status as well as distant metastasis particularly in oesophageal cancer (Chowdhury, Bradley & Gleeson 2008).

1.5.1.3 Endoscopic Ultrasound (EUS)

The ability to identify different layers of the oesophageal and stomach walls by EUS makes it superior investigation compared to CT scan in local staging of oesophageal

(Ziegler et al. 1991, Tio et al. 1989, Dittler, Siewert 1993a) as well as gastric (Dittler, Siewert 1993b, Ziegler et al. 1993) cancer. EUS guided fine needle aspiration cytology for potential nodal disease has been shown to improve the accuracy (Vazquez-Sequeiros et al. 2001).

In oesophageal cancer, EUS has very good accuracy in T staging and can differentiate mucosal and sub mucosal involvement (Thosani et al. 2012). It has also been reported that EUS is very useful in detecting regional lymph node metastasis with a sensitivity of 80% (75%-84%) and specificity of 70% (65%-75%) (van Vliet et al. 2008). Detection of caeliac lymph node metastasis by EUS has been reported to be better compared to abdominal ones. Therefore it has been advocated that, in oesophageal cancer, EUS and fine needle aspiration cytology should be used for better T and N staging (Quint, Bogot 2008).

In gastric cancer, EUS is indicated for assessing the depth of tumour invasion (Matsumoto et al. 2000). The accuracy of EUS for gastric cancer T staging ranges from 82% to 99% and for N staging from 58% to 65%. Sensitivity of detecting T stage in gastric cancer is reported to be higher in advanced disease i.e. EUS performs better to exclude T4 disease than T1 disease (Kwee, Kwee 2008, Puli et al. 2008). On the other hand, the accuracy of detecting nodal metastasis was reported to be variable, therefore the role of EUS in staging gastric cancer is yet to be defined.

Distant lymph node evaluation by EUS has been reported to be less than adequate (Tsendsuren, Jun & Mian 2006). Staging for metastatic disease using EUS alone is not satisfactory. Moreover EUS examination can be limited by a stricture forming lesion.

1.5.1.4 Laparoscopy

Laparoscopy is indicated to exclude peritoneal metastasis in oesophago-gastric and gastric cancer as CT scan has been reported to be less than adequate (Blackshaw et al. 2003, Clements, Bowrey & Havard 2004). De Graaf and colleagues have recently reported that additional staging information can be obtained by staging laparoscopy in 17.1% patients with distal oesophageal cancer, 17.2% patients with oesophago-gastric junctional tumour and 28 % patients with gastric cancer (de Graaf et al. 2007). Laparoscopic staging may not be necessary in patients with cancer of upper two thirds of oesophagus (de Graaf et al. 2007).

In gastric cancer, laparoscopic staging can be useful in detecting occult metastasis (Sarela et al. 2006). Blackshaw and co-workers have also showed the accuracy of laparoscopy in 258 consecutive patients with gastric cancer. Laparoscopic examination detected metastatic disease in 21 patients, in whom CT scanning failed to report the metastasis (Blackshaw et al. 2003). Limitations of laparoscopic staging include two dimensional evaluation and limited use in identification of hepatic metastasis and peri gastric lymph nodes. Indications for laparoscopic staging may vary among different institutions. In some, it is performed on patients intended to have curative treatment (surgery and or chemotherapy) whereas in some other institutions, laparoscopy is performed in patients intended to receive chemotherapy especially if there is consideration for addition of radiation.

Another potential benefit for staging laparoscopy is to obtain peritoneal fluid for cytology. Studies have shown that the peritoneal fluid cytology for malignant cells can help to improve staging (Abdalla, Pisters 2004). Positive peritoneal cytology is associated with poor prognosis and recurrence in gastric cancer (Bentrem et al. 2005) and oesophago-gastric junctional adenocarcinoma (Nath et al. 2008). Patients without distant metastasis

(M0) with positive peritoneal fluid cytology in gastric cancer are unlikely to benefit from resection alone (Burke et al. 1998). Moreover, a recent report suggested that clearing cytology positive gastric cancer with chemotherapy resulted in improved survival (Mezhir et al. 2010). Clearly, positive peritoneal fluid cytology has a role in staging gastric cancer and should be considered as M1, even in absence of visible peritoneal implants.

1.5.1.5 Bronchoscopy

Oesophageal cancer at or above the level of carina may invade the tracheo-bronchial tree and bronchoscopy may have a role in detecting this as well as obtaining biopsy. EUS may be adequate to detect tumour extension to the airway, but any uncertainty should warrant bronchoscopic examination (Omloo et al. 2008).

1.5.2 Pathological Stage and outcome

Patient outcome may be associated with the initial clinical stage, but the best correlation with survival is associated with pathological tumour stage (pTNM) following resection for gastro-oesophageal cancer (Lerut et al. 2004, Barchielli et al. 2001). Although the pathological T category has been shown to be independently associated with survival, within the TNM system, the presence of metastatic tumour in lymph nodes has been shown to be of primary importance (Karpeh et al. 2000). Moreover, the ratio of positive lymph nodes, to the resected lymph nodes has been demonstrated as a better measurement of extent of lymph node involvement and has been shown to have independent prognostic significance in oesophageal cancer (Mariette et al. 2008, Liu et al. 2009) and gastric cancer (Deng et al. 2009, Fukuda et al. 2009, Marchet et al. 2007).

In gastric cancer, there is no universally accepted guidance on the minimum number of lymph nodes necessary for accurate staging. But resection of at least 15 lymph nodes is recommended to reduce stage migration. Better survival in patients at any stage of

gastric cancer was reported to be related to the number of lymph nodes examined (Smith, Schwarz & Schwarz 2005).

1.6 Treatment of oesophageal and gastric cancer

The treatment strategy for patients with gastro-oesophageal cancer is dependent on TNM stage, presence of co-morbid conditions and patient choice. Patients can be classified using the above criteria into curative and palliative groups.

1.6.1 Curative treatments of oesophageal and gastric cancer

1.6.1.1 Endoscopic treatments

Endoscopic therapy has now become an essential part of the treatment of early oesophageal and gastric cancer. There are few treatment modalities available to treat dysplasia and early cancer such as endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD) and ablation techniques such as photo dynamic therapy (PDT), argon plasma coagulation (APC), laser (photo thermal) and radio frequency ablation (RFA). All patients who receive endoscopic treatment must have repeat surveillance endoscopy.

EMR is increasingly used for treatment of pre-cancerous lesions and early oesophageal cancer i.e. high grade dysplasia, T1cancer involving mucosa only (ASGE TECHNOLOGY COMMITTEE et al. 2008) as an alternative to surgery. Following resection of early Barrett's cancer and high grade dysplasia, mucosal ablation of the residual Barrett's tissue is required to decrease the risk of subsequent cancer development (Pech et al. 2008, Larghi et al. 2007, Seewald et al. 2003, Lopes et al. 2007, Ganz et al. 2008).

Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) have also been used in early gastric cancer as an alternative to gastrectomy. The criteria for endoscopic mucosal resection (EMR) are Tis or T1 differentiated tumours limited to mucosa ≤ 2 cm and not ulcerated (Soetikno et al. 2005, Ono et al. 2001) (Okines et al. 2010). Use of this technique is limited in western countries as the incidence of early gastric cancer is low. In some reported studies endoscopic submucosal dissection has been shown to be more effective than EMR in early gastric cancer (Oda et al. 2006) (Yahagi et al.). However, there are no randomised studies that compare these endoscopic techniques in treatment of gastric cancer and further evaluation is required.

1.6.1.2 Surgical resection

Oesophageal cancer:

Surgery is regarded as standard treatment in selected patients who have localised oesophageal tumour. Patients who are medically fit (i.e. medically able to tolerate major abdominal and/or thoracic surgery) and have TNM stage I to III disease are generally selected for surgery. T4 oesophageal tumours with involvement of heart, great vessels, trachea, distant non regional lymph node metastasis or metastasis to other solid organs are considered non resectable although advanced oesophageal tumours may be converted to potentially operable cancer following neo-adjuvant chemo-radiotherapy (Slater et al. 2001). There are various surgical approaches for oesophagectomy such as, Transhiatal, Transthoracic and Minimally invasive oesophagectomy (Ng, Vezeridis 2010). The optimal operative approach is determined by type, location of the tumour as well as patient factors and surgeon's experience. In a recent large population based study, it was reported that, there was no difference in long term survival between transhiatal and transthoracic oesophagectomy (Chang et al. 2008). There is no well designed randomised controlled trial to compare the long term outcome of minimally invasive oesophagectomy and open

resection. Lymph node resection should be performed in a standard extended (en-bloc) technique which will help in minimising staging error and reduce the chance of loco-regional recurrence. Number of lymph nodes removed has been reported to be an independent predictor of survival (Rizk et al. 2010) as is the ratio of the positive lymph node (Roder et al. 1994).

Gastric cancer:

Surgery is the principle treatment option for early gastric cancer. Complete resection with adequate margin (4 cm or greater) is advisable although the type of surgery (total versus subtotal gastrectomy) and the extent of lymphadenectomy remains a subject of controversy. Subtotal gastrectomy is suitable for distal gastric cancers and has a similar surgical outcome with fewer post operative complications compared to total gastrectomy (Bozzetti et al. 1999). Proximal gastrectomy or total gastrectomy is indicated for proximal/mid body gastric cancer. It is recommended that the distal, subtotal or total gastrectomy for T1 to T3 tumours. T4 tumours require enbloc resection with involved structures. A carcinoma is considered unresectable if there is evidence of peritoneal involvement, distant metastasis or locally advanced disease such as invasion or encasement of major blood vessels.

The role of laparoscopic approach in gastrectomies is still being investigated. It may have important advantages such as less blood loss, accelerated post operative recovery, less post operative pain, early return of normal bowel function when compared to open surgery (Reyes et al. 2001). Although a recent metaanalysis demonstrated that laparoscopic surgery was associated with longer operating time and reduced nodal harvest compared with open surgery (Memon et al. 2008). Clearly the role of laparoscopic approach needs to be further evaluated.

In gastric cancer, perigastric lymph nodes along the greater and lesser curvatures are grouped as N1. The nodes along the left gastric artery, common hepatic artery, coeliac artery and the splenic artery are grouped together as N2. Metastasis to more distant nodes such as para aortic group of lymph nodes (N3 and N4) is regarded as distant metastasis. D0 resection refers to incomplete resection of N1 lymphnodes. D1 resection includes N1group of lymph nodes completely. In D2 resection, the omental bursa along with the front leaf of the transverse mesocolon is removed along with N2 group of lymph nodes. For proximal gastric cancer, D2 dissection involves splenectomy. Reports have suggested that the more extensive lymph node dissection has influence on survival in patients with advanced gastric cancer (Schwarz, Smith 2007). However, various reports comparing D1 and D2 resection for gastric cancer have shown no survival benefit with extended lymph node dissection in gastric cancer (Bonenkamp et al. 1999, Cuschieri et al. 1999, Hartgrink et al. 2004, McCulloch et al. 2004). It has been argued that if the complication rate after D2 resection could be decreased then there might be a noticeable benefit in selected group of gastric cancer patients. A modified D2 lymphadenectomy without pancreatectomy and splenectomy has been suggested (Jansen et al. 2005) (Khatri, Douglass 2004) (Douglass et al. 2007). Indeed, D2 lymphadenectomy has shown survival benefit in various European studies (Degiuli et al. 2004a) (Degiuli et al. 2004b) (Sierra et al. 2003) (Edwards et al. 2004). Modified D2 lymphadenectomy (without pancreatectomy or splenectomy) is associated with low morbidity and mortality and therefore recommended (Schwarz, Smith 2007, Songun et al. 2010). So currently in UK, D2 lymphnode dissection is regarded as a standard procedure in specialised centres with appropriate surgical expertise and postoperative care, for patients considered medically fit for surgery . Resection of the spleen and pancreas is only indicated if there is direct invasion. Splenectomy is indicated for tumours of the proximal greater curve and gastric fundus; principally to remove splenic hilar nodes. Resection of adjacent organs is indicated when there is definite or suspected transmural invasion and the patient is fit for radical surgery (Okines et al. 2010).

1.6.2 Neo adjuvant, peri-operative and adjuvant therapy

Oesophageal cancer:

A recent meta-analysis on pre operative radiotherapy for patients with potentially resectable oesophageal cancer showed only a modest increase in overall survival (about 3-4% only). Therefore preoperative radiotherapy is not recommended for operable oesophageal cancer (Arnott et al. 2005). On the contrary, pre operative chemoradiation followed by surgery has been reported to offer survival benefit compared to surgery alone (Gebski et al. 2007). A large randomised controlled trial conducted by medical research council UK on pre-operative chemotherapy for patients with oesophageal cancer involving 802 patients. There was significant improval in survival in patients who received preoperative chemotherapy compared to surgery alone (Medical Research Council Oesophageal Cancer Working Group 2002, Allum et al. 2009) and is now considered as the gold standard in potentially curative oesophageal cancer patients. Post operative chemo-radiation in oesophageal cancer has been reported to have beneficial effect on survival (Bedard et al. 2001, Rice et al. 2003, Adelstein et al. 2009). However, a randomised controlled trial has failed to show any significant survival benefit of radiotherapy following oesophageal cancer resection (Teniere et al. 1991). Similarly in a recently published meta-analysis on adjuvant chemotherapy after oesophageal cancer surgery has not shown any improved outcome (Zhang et al. 2008).

The role of definitive chemoradiation has been reported in some studies particularly in oesophageal squamous cell carcinoma. Some European studies have reported at least equivalent survival in patient who received definitive chemoradiation compared to surgery (Stahl et al. 2005, Bedenne et al. 2007).

Gastric and oesophago gastric junctional cancer:

A UK MRC randomised trial (MAGIC trial) with three cycles of preoperative and post operative chemotherapy (i.e. peri-operative chemotherapy) with three cycles of epirubicin (E) 50 mg/m2, cisplatin (C) 60 mg/m2 and continuous intravenous infusion of 5-fluorouracil (F) 200 mg/m2/day (ECF) significantly improved 5-year survival from 23.0% with surgery alone to 36.3% (Cunningham et al. 2006). This perioperative chemotherapy regimen is most widely accepted in UK.

A landmark intergroup North American randomised trial demonstrated that, post operative chemoradiation with 5 fluorouracil/leucovorin before, during and after radiotherapy in 556 patients resulted in improved five year survival rate in gastric and oesophago gastric junctional adenocarcinoma (Macdonald et al. 2001). Therefore this treatment option has been widely accepted in the USA particularly for those patients who didn't receive preoperative chemotherapy. Moreover, another large non-randomized observational study suggested a potential clinical benefit from postoperative chemoradiation after optimal D2 dissection for gastric cancer (Kim et al. 2005).

In a metaanalysis post operative adjuvant chemotherapy in gastric cancer has demonstrated a small survival benefit (Liu et al. 2008). A large randomised phase III trial in Japan in 1059 patients evaluated the efficacy of oral fluoropyrimidine S-1 in stage II and stage III gastric cancer patients who underwent D2 resection (Sakuramoto et al. 2007). Overall survival was better in the S-1 group. There are no data available with oral fluoropyrimidine in the western population.

In patients with gastro-oesophageal junctional adenocarcinoma and tumour of gastric cardia, preoperative chemoradiation has shown some benefit in terms of complete pathological response and survival (Walsh et al. 1996) (Stahl et al. 2009). For localised adenocarcinoma of gastro-oesophageal junction or gastric cardia preoperative chemoradiation is the preferred approach in USA. However the role of neoadjuvant

chemoradiation in adenocarcinoma of gastro-oesophageal junction needs to be evaluated further preferably through large prospective randomised controlled trials.

In gastric cancer, although neoadjuvant chemoradiation has shown some benefit in terms of complete pathological response and better chance of radical surgery (Ajani et al. 2006), it remains experimental and its value has not been confirmed in a comparative study.

In summary, pre-operative chemotherapy is recognised as a standard of care for patients with mid and distal oesophageal cancer and also for oesophago-gastric junctional cancer. For localised squamous cell cancer of oesophagus definitive chemoradiation is advocated. Peri-operative chemotherapy for gastric cancer and oesophago-gastric junctional tumour (type I and type II adenocarcinoma) is now most widely used in UK.

1.6.3 Palliative treatment

Majority of oesophageal and gastric cancer present in the advanced stage and therefore require palliative treatment. Palliative chemotherapy has been shown to be benefitial compared to the best supportive care in advanced gastric cancer (Wagner et al. 2010, Cunningham et al. 2008, Pyrhonen et al. 1995, Murad et al. 1993, Glimelius et al. 1997). Similar findings were noted in inoperable oesophageal and oesophago-gastric junctional tumours as well (Shah, Schwartz 2004).

Until recently, the combination of chemotherapeutic regimen of ECF has been preferred in oesophageal, gastric and junctional cancer (Webb et al. 1997). Newer regimens containing capecitabine or oxaliplatin are reported to be equally effective (Cunningham et al. 2008). External beam radiotherapy can help in relieving symptoms of dysphagia in oesophageal cancer (Caspers et al. 1988, Cwikiel, Cwikiel & Albertsson 1996).

In gastric cancer,external beam radiotherapy (45-50.4 Gy) as a single modality has minimal effect in patients who has locally unresectable gastric cancer and does not have any effect on survival (Hazard, O'Connor & Scaife 2006). However, concurrent use of chemotherapeutic agents such as 5 fluorouracil along with radiotherapy in patients with locally unresectable gastric cancer has resulted in better survival (Moertel et al. 1969). A combination of chemotherapy is more effective than 5 fluorouracil alone (Wagner et al. 2010). Two drug regimen incorporating a platinum agent (i.e. cisplatin) and a fluoropyrimidine (i.e. 5 fluorouracil) is generally used. Adding an anthracycline can have significant benefit as a triple drug regimen. ECF (epirubicin, cisplatin and protracted infusion of 5 fluorouracil) among the most active and well tolerated regimen. Docetaxel is also considered as a first line drug in combination with cisplatin and 5 fluorouracil(as DCF) (Van Cutsem et al. 2006). Irinotecan incombination with5- fluorouracil/leucovorin has similar efficacy to 5-fluorouracil/cisplatin and therefore can be considered in a selected group of patients (Dank et al. 2008).

The over expression of vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER 2) has been associated with poor prognosis in patients with oesophageal and gastric cancer(Wagner, Moehler 2009, Gravalos, Jimeno 2008). In a recent randomised prospective trial, it has been demonstrated that the addition of trastuzumab with standared chemotherapy has improved overall survival in advanced gastric and gastro-oesophageal adenocarcinoma (Bang et al. 2010). All patients should have HER-2 status tested and Trastuzumab should be added to the standard chemotherapy agents in patients with HER-2 positive tumours. The use of bevacizumab (anti VEGFR antibody) and cetuximab (anti EGFR antibody) have been explored in clinical trials but remains experimental (Pinto et al. 2007, Pinto et al. 2009).

Palliative gastric resection should not be done unless patient is symptomatic and in that case lymph node resection is not required. A systematic review compared distal gastric/duodenal stent with gastro jejunostomy and has reported that there was no difference in technical success, late major complications and persisting symptoms. However patients with stents were reported to have better initial improvement of clinical symptoms and had less frequent early minor complications. Recurrent obstructive symptoms were more common after stent placement. Mean survival was higher with gastro jejunostomy. Therefore, gastric bypass with gastrojejunostomy to the proximal stomach (in case of distal obstructing tumour) instead of self expanding metal stent placement is a preferred method in symptomatic patients if surgery is feasible (Jeurnink et al. 2007).

Other palliative treatments options for oesophageal and gastric cancer include oesophageal intubation, stenting, dilatation, brachytherapy, laser therapy, photo dynamic therapy, argon plasma coagulation, injection therapy with ethanol with a variable success (Allum et al. 2002).

1.7 Determinants of short term outcome of oesophageal and gastric cancer following surgery

Early detection and surgery confers the greatest chance of long-term cure in oesophageal and gastric cancer. However, upper GI cancer surgery is associated with considerable morbidity and mortality. Postoperative mortality following gastro-oesophageal cancer resection is significant and has been reported as varying from 1%–23% (Koshy et al. 2004, Jamieson et al. 2004). Therefore preoperative surgical risk assessment is a vital part of modern surgical practice. This will allow a surgeon to anticipate the adverse events following the surgery; facilitate the informed consent process and surgical decision making. Nevertheless, the impact of the quality of surgery and post-operative

care in patients undergoing gastro-oesophageal resection, in particular for cancer, remains unclear due to variations in case mix.

Various surgical risk prediction models have been developed to objectively quantify the postoperative morbidity and mortality. Some of the common risk prediction models in surgery are APACHE III (Acute Physiology and Chronic Health Evaluation) (Knaus et al. 1991), POSSUM (Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity) (Copeland, Jones & Walters 1991) and ASA (The American Society of Anesthesiologist') physical status classification (Owens, Felts & Spitznagel 1978, Haynes, Lawler 1995). The ASA score is easy to use but it is not very precise and it does not consider surgical insult in predicting post operative outcome. The APACHE scoring system is quite complex and time consuming. Moreover all the parameters are not always easily obtainable, particularly outside that of the intensive care setting. Therefore, missing values in the data is a potential source of error in APACHE scoring system (Chandra, Mangam & Marzouk 2009). POSSUM scoring system was particularly developed as an audit tool to compare post operative outcome in general surgery as well as in various sub specialities. POSSUM and its other modifications are most widely used in UK due its reasonable ability to predict post operative outcome (Chandra, Mangam & Marzouk 2009).

1.7.1 Role of POSSUM and its related models as predictors of post-operative outcome in gastro-oesophageal cancer

In order to assess the impact of treatment in multidisciplinary teams and institutions reliable tools are required to assess the morbidity and mortality risks including the severity of the surgical insult. The POSSUM (Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity) model for predicting post-operative mortality

was developed by Copeland and co-workers (Copeland, Jones & Walters 1991) using cohorts of general surgical patients. This was subsequently revised to P-POSSUM by Whitelay and coworkers who reported that POSSUM over predicted post-operative mortality particularly in those who were at low risk (Whiteley et al. 1996). P-POSSUM used the same set of variables as POSSUM but had a different logistic regression equation. Attempts have been made to modify the POSSUM scoring system for specific surgical procedures. For example, V-POSSUM (Prytherch DR. Ridler BM. Beard JD. Earnshaw JJ. Audit and Research Committee,The Vascular Surgical Society of Great Britian and Ireland 2001) for vascular, RAAA-POSSUM (Prytherch, Sutton & Boyle 2001) for ruptured abdominal aortic aneurysm and CR-POSSUM (Tekkis et al. 2004b) for colorectal surgery. These risk assessment tools have been used to allow comparative audit of surgical mortality although they fail to address prediction of morbidity and mortality in individual patients.

POSSUM has been reported to be superior to the Acute Physiology And Chronic Health Evaluation (APACHE) II tool, for the prediction of postoperative death in patients undergoing major surgery in a high-dependency unit (Jones, Copeland & de Cossart 1992). More recently, this model has been adapted to a specialised model for the prediction of risk-adjusted postoperative mortality in oesophageal and upper gastrointestinal surgery (O-POSSUM) (Tekkis et al. 2004a). Both POSSUM and P-POSSUM scoring systems use a 12 factor physiological score and a 6 factor operative severity score. In O-POSSUM, the operative severity score is modified to exclude operative blood loss, number of procedures and peritoneal soiling. Age was regressed independently from POSSUM (Table 1-3).

It is therefore interesting to examine the use of POSSUM and its related models as predictors of post-operative mortality and morbidity in patients undergoing surgery for gastro-oesophageal cancer.

An online database search in Pubmed, Cochrane Database of Systematic Reviews, Database of abstracts of reviews, Cochrane Controlled Trials Register, EMBASE (the Excerpta Medica Database), was carried out from 1991 (year of the original POSSUM scoring system; Copeland, Jones & Walters 1991) to 2008. Key words used in search were as detailed in Table 1-4. Articles that examined POSSUM scoring (POSSUM/ P-POSSUM/ O-POSSUM) prospectively or retrospectively in gastro-oesophageal cancer surgery were examined as well as relevant references to obtain useful information.

In the studies examined, the predictive value (the observed: expected ratio (O/E) in each POSSUM model and the p-value for goodness-of-fit) and the discriminatory power (area under the curve from receiver operator characteristic curve) were recorded where given. The weighted O/E ratio was calculated and plotted using SPSS software (Statistical Package for the Social Sciences version 15·0, SPSS Inc., Chicago, IL, USA).

A total of 22 articles were identified. On close inspection 12 papers did not provide useful data (three papers had data published in other articles, four articles included colorectal and general surgical patients as well as gastro-oesophageal cancer patients, three articles incorporated only physiological components of POSSUM and two articles were not in English). This left 10 original studies to be included in the present review.

There were five studies of POSSUM on post-operative mortality in patients undergoing resection for gastro-oesophageal cancer (Zafirellis et al. 2002b, Bollschweiler et al. 2005, Lai et al. 2007, Otsuka et al. 2007, Lamb et al. 2008) (Table 1-5). Two studies were on oesophageal and three studies were on gastric cancer. In the individual studies POSSUM appeared to have poor predictive accuracy and showed significant lack of goodness of fit. In the pooled data of these studies the observed: expected ratio in oesophageal cancer resections varied from 0.37 to 0.66 and from 0.08 to 0.17 in gastric cancer. The weighted observed to expected ratio for 1189 patients was 0.37 (Figure 1-1).

There were six studies of P-POSSUM on post-operative mortality in patients undergoing resection for gastro-oesophageal cancer (Tekkis et al. 2004a, Lai et al. 2007, Lamb et al. 2008, Gocmen et al. 2004, Nagabhushan et al. 2007, Internullo et al. 2008) (Table 1-6). In the individual studies P-POSSUM appeared to have good predictive accuracy and showed significant goodness of fit in oesophageal cancer resections. In contrast P-POSSUM appeared to have poor predictive accuracy in gastric cancer. In the pooled data of these studies the observed: expected ratio in oesophageal cancer resections varied from 1.03 to 1.17, from 0.21 to 0.7 in gastric cancer and from 0.83 to 0.89 in gastro-oesophageal cancer. The weighted observed to expected ratio for 2314 patients was 0.83 (Figure 1-1).

There were five studies of O-POSSUM on post-operative mortality in patients undergoing resection for gastro-oesophageal cancer (Lai et al. 2007, Gocmen et al. 2004, Nagabhushan et al. 2007, Internullo et al. 2008, Lagarde et al. 2007) (Table 1-7). In a single study O-POSSUM appeared to have good predictive accuracy and showed significant goodness of fit in gastric cancer. In contrast, in three oesophageal studies as well as in one gastro-oesophageal study, O-POSSUM appeared to have poor predictive accuracy and showed significant lack of goodness of fit. In the pooled data of these studies the observed: expected ratio in oesophageal cancer resections varied from 0.29 to 0.51, 0.90 in gastric cancer and 0.65 in gastro-oesophageal cancer. The weighted observed to expected ratio for 1755 patients was 0.51 (Figure 1-1).

There were five studies of POSSUM on post-operative morbidity in patients undergoing resection for gastro-oesophageal cancer (Zafirellis et al. 2002b, Bollschweiler et al. 2005, Otsuka et al. 2007, Lamb et al. 2008, Sah et al. 2008a) (Table 1-8). In the individual studies POSSUM appeared to have good predictive accuracy and showed significant goodness of fit. In the pooled data of these studies the observed: expected ratio

in oesophageal cancer resections was 0.86 and from 0.40 to 0.99 in gastric cancer. The weighted observed to expected ratio for 1038 patients was 0.86.

In the present systematic review the POSSUM model of post-operative mortality prediction has clear limitations in patients undergoing surgery for gastro-oesophageal cancer. POSSUM significantly over predicted mortality in gastro-oesophageal cancer and more so in gastric compared with oesophageal resections. The poor predictive value of POSSUM in these patients may be due to the fact that the model was originally developed from data in general surgical patients. In patients with gastro-oesophageal cancer, surgery has a high operative severity score and therefore results in a higher risk prediction. In contrast, O-POSSUM was developed specifically for gastro-oesophageal surgery and appears to be good in predicting mortality following gastric cancer surgery but less so in oesophageal resections (however, this was based on only one gastric cancer study). Higher prediction of mortality in older patients (as age is included twice in this model) and exclusion of operative variables like blood loss may have played a role in the over prediction of the mortality. Lastly, P-POSSUM appeared to be the most useful risk prediction model in oesophageal resections but substantially over predicted the risk in gastric resections. Despite the varying degree of over prediction of mortality of the different POSSUM models all maintained good discriminatory power in gastro-oesophageal cancer. Therefore, the present systematic review confirms that subsequent revisions of POSSUM such as P-POSSUM and O-POSSUM have improved the reliability of the prediction of post-operative mortality in patients with gastro-oesophageal cancer.

The aim of the present study was to undertake a systematic review of the use POSSUM and its related models as predictors of post-operative mortality and morbidity in patients undergoing surgery for gastro-oesophageal cancer. However, a number of studies identified and included in the present review had gastric cancer patients alone. Also, these gastric cancer studies were heterogeneous in case mix. Therefore, inclusion of these

studies may be considered to be a confounding factor in the present systematic review. Nevertheless, there were also a number of studies identified and included in the present review that had oesophageal cancer patients alone and these were also considered separately and together with the gastric cancer studies. Therefore, the approach used in the present systematic review we believe was even handed and consistent with its aim.

It has long been recognised that a risk prediction model that could determine an individual patient's risk in a specific surgical operation would be very useful in preoperative assessment and consent process. This need has driven the development POSSUM and its related models. Nevertheless POSSUM and its related models have been used as an audit tool in different fields of surgery. In the present study all three POSSUM models over predicted post operative mortality in gastro-oesophageal cancer. This may be due to improvements in surgery and peri-operative care as part of an increasingly specialised and centralised upper gastrointestinal cancer service since each of the POSSUM models was originally developed.

It is of interest that although the POSSUM was introduced in 1991 the application of POSSUM , P-POSSUM and O-POSSUM in gastro-oesophageal cancer has only been reported in the last decade. Nevertheless, over this period it would appear that the POSSUM models increasingly overestimate mortality. A possible reason for this is the increasing use of minimally invasive operative techniques and better peri-operative care.

With the continuing reduction in mortality following gastro-oesophageal cancer resection there is now a need to accurately predict morbidity in these patients. It was of interest therefore that, compared with mortality, POSSUM appeared to better predict post operative morbidity. Indeed, attempts have been made to modify the POSSUM scoring system for predicting morbidity in upper gastrointestinal surgery (Otsuka et al. 2007). However, a deficiency in the current POSSUM models is that they are not based on a good

understanding of the patho-physiological process that results in morbidity and mortality following cancer surgery. Recently it has become clear that the systemic inflammatory response, as evidenced by an inflammation based prognostic score (Glasgow Prognostic Score, GPS), is strongly associated with long term survival of both inoperable (McMillan 2008) and operable (McMillan 2009) cancer patients. Given that the physiological component of POSSUM also predicts long-term survival following potentially curative colorectal (Brosens et al. 2006, Jenkins, O'Neill & Morran 2007) and pancreatic (de Castro et al. 2009) cancer it may be that the systemic inflammatory response will also be useful in post-operative morbidity and mortality. Indeed, Moyes and co-workers (Moyes et al. 2009) have recently shown that the GPS independently predicts post operative infective complications in these patients. Given that the GPS is much simpler (two factors) compared with physiological POSSUM (12 factors) further work is required to compare its value in predicting morbidity and mortality following cancer surgery.

In summary, the present systematic review shows that POSSUM, P-POSSUM and O-POSSUM over-estimate mortality in gastro-oesophageal cancer. Furthermore P-POSSUM appears to be the most useful of the POSSUM scoring systems in predicting post-operative mortality in patients undergoing surgery for gastro-oesophageal cancer. It is not clear which POSSUM scoring system best predicts post-operative morbidity in these patients.

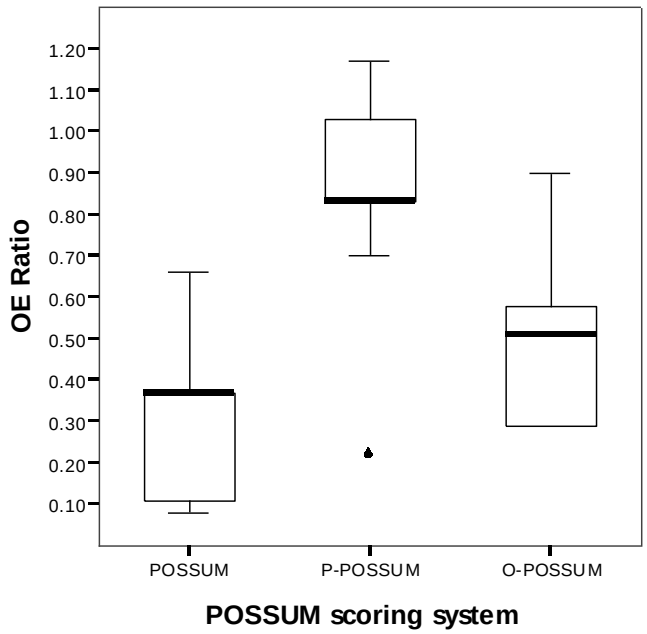

Figure 1-1: The weighted observed to expected mortality ratio (O:E Ratio) for POSSUM (n= 1189), P-POSSUM (n= 2314) and O-POSSUM (n=1755) from a systematic review of patients undergoing surgery for gastro-oesophageal cancer.

Table 1-3: Variables used in POSSUM, P-POSSUM and O-POSSUM equations.
Physiological Scores:

Score	1	2	4	8
Age (years)*	≤60	61-70	≥71	-
Cardiac Signs	Normal	Cardiac drugs/steroid	Oedema; warfarin. Borderline cardiomegaly on CXR	JVP Cardiomegaly on CXR
Respiratory signs	Normal	SOB on Exertion Mild COPD on CXR	SOB stairs Mod COPD on CXR	SOB rest fibrosis or consolidation on CXR
Systolic BP, mm Hg	110-130	131-170 100-109	≥171 90-99	≤89
Pulse, beats/min	50-80	81-100 40-49	101-120	≥121 ≤39
Glasgow Coma score	15	12-14	9-11	≤8
Urea nitrogen, mmol/L	<7.5	7.6-10	10.1-15	≥15.1
Na mEq/L	>136	131-135	126-130	≤125
K mEq/L	3.5-5	3.2-3.4 5.1-5.3	2.9-3.1 5.4-5.9	≤2.8 ≥6
Hb g/dl	13-16	11.5-12.9 16.1-17	10-11.4 17.1-18	≤9.9 ≥18.1
WCC x1012/L	4-10	10.1-20 3.1-3.9	≥20.1 ≤3	-
ECG	Normal		AF (60-90)	Any other changes

Operative Severity scores:

Score	1	2	4	8
Operative severity	Minor	Intermediate	Major	Major +
No. of procedures over 30 days†	1	2	>2	
Blood loss per operation, mL†	<100	101-500	501-999	>1000
Peritoneal contamination†	No	Serous blood (< 250 ml)	Local pus	Free Bowel content, pus or blood
Presence of malignancy	No	Primary only	Nodal metastases	Distant metastases
Mode of Surgery	Elective		Emergency resuscitation Possible. operation <24 h	Emergency, immediate operation <2 h

POSSUM equation (R1for mortality and R2 for morbidity):
$Log_eR1/(1-R1) = -7.04 + (0.13 \times$ Physiological Score) + (0.16 x Operative Severity Score).
$Log_eR2/(1-R2) = -5.91 + (0.16 \times$ Physiological Score) + (0.19 x Operative Severity Score).

P-POSSUM equation for mortality:
$Log_eR/(1-R) = -9.065 + (0.1692 \times$ Physiological Score) + (0.1550 x Operative Severity Score)

O-POSSUM calculation:
*Age was regressed independently from the Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM).
†Risk factors not used in scoring system specific for upper gastrointestinal surgery (O-POSSUM).

Table 1-4: Search terms and methods.

1 exp esophageal neoplasms/ or exp stomach neoplasms/
2 exp surgical procedures, operative/
3 1 and 2
4 exp esophageal neoplasms/su or exp stomach neoplasms/su
5 ((oesophag$ or esophag$ or gastric$ or gastro$ or stomach) adj (cancer$ or tumour$ or tumor$ or carcin$ or malignan$ or neoplas$ or oncolog$)).ti,ab.
6 (oesophagect$ or esophagect$ or surg$ or gastrect$ or resect$ or esophagogastrect$ or oesphagogastrect$).ti.
7 6 and 5
8 4 or 3 or 7
9 possum.ti,ab
10 8 and 9

Table 1-5: Studies of POSSUM on post-operative mortality in patients undergoing resection for gastro-oesophageal cancer

Study	Year (Country)	Patients (n)	Tumour type	POSSUM Predicted mortality (%)	POSSUM Observed mortality (%)	O:E mortality ratio	Comments
Zafirellis et al	2002 (UK)	204	Oesophageal	19.12	12.75	0.66	Overestimates risk p=0.002 AUC=0.62
Bollschweiler et al	2005 (Germany)	137	Gastric	21.17	3.65	0.17	Overestimates risk
Lai et al[a]	2007 (China)	545	Oesophageal	15	5.5	0.37	Overestimates risk p<0.001 AUC=0.776
Otsuka et al[a]	2007 (Japan)	123	Gastric	14.1	1.6	0.11	Overestimates risk
Lamb et al	2008 (UK)	180	Gastric	21.4	1.7	0.08	Overestimates risk p<0.001

Weighted O/E ratio for 1189 patients was 0.37.
Peri-operative mortality = Deaths within 30 days of surgery. AUC = area under ROC curve.
[a] in hospital mortality described. O:E is Observed to Expected ratio

Table 1-6: Studies of P-POSSUM on post-operative mortality in patients undergoing resection for gastro-oesophageal cancer

Study	Year (Country)	Patients (n)	Tumour type	P-POSSUM Predicted mortality (%)	P-POSSUM Observed mortality (%)	O:E mortality ratio	Comments
Gocmen et al	2004(Turkey)	126	Gastric	15.87	11.1	0.7	Overestimates risk p=0.002 AUC=0.703
Tekkis et al	2004(UK)	1042	Gastro- oesophageal	14.5	12	0.83	Overestimates risk p=0.001 AUC=0.743
Nagabhushan et al	2007 (UK)	313	Gastro- oesophageal	11.5	10.2	0.89	Overestimates risk p=0.019 AUC=0.68
Lai et al	2007 (China)	545	Oesophageal	4.7	5.5	1.17	p=0.814 AUC=0.776
Internullo et al[a]	2008 (Belgium)	108	Oesophageal	7.2	7.4	1.03	
Lamb et al	2008 (UK)	180	Gastric	7.8	1.7	0.21	Overestimates risk p=0.006

Weighted O/E ratio for 2314 patients was 0.83.
Peri-operative mortality = Deaths within 30 days of surgery. AUC = area under ROC curve.
[a] in hospital mortality described. O:E is Observed to Expected ratio

Table 1-7: Studies of O-POSSUM on post-operative mortality in patients undergoing resection for gastro-oesophageal cancer

Study	Year (Country)	Patients (n)	Tumour type	O-POSSUM Predicted mortality (%)	O-POSSUM Observed mortality (%)	O:E mortality ratio	Comments
Gocmen et al	2004 (Turkey)	126	Gastric	11.9	11.1	0.9	p=0.13 AUC=0.880 Overestimates risk
Nagabhushan et al	2007(UK)	313	Gastro- oesophageal	15.65	10.2	0.65	p=0.011 AUC=0.61 Overestimates risk
Lagarde et al[a]	2007 (Netherlands)	663	Oesophageal	12.4	3.6	0.29	p<0.001 AUC= 0.60
Lai et al	2007 (China)	545	Oesophageal	10.9	5.5	0.51	p=0.002 AUC=0.676
Internullo et al[a]	2008 (Belgium)	108	Oesophageal	15.1	7.4	0.49	Overestimates risk

Weighted O/E ratio for 1755 patients was 0.51.
Peri-operative mortality = Deaths within 30 days of surgery. AUC = area under ROC curve.
[a] in hospital mortality described. O:E is Observed to Expected ratio

Table 1-8: Studies of POSSUM on post-operative morbidity in patients undergoing resection for gastro-oesophageal cancer

Study	Year (Country)	Patients (n)	Tumour type	POSSUM Predicted morbidity (%)	POSSUM Observed morbidity (%)	O:E morbidity ratio	Comments
Zafirellis KD	2002 (UK)	204	Oesophageal	62.3	53.4	0.86	p<0.001 *AUC=0.55*
Bollschweiler E	2005 (Germany)	137	Gastric	65.7	34.3	0.52	
Otsuka Y	2007 (Japan)	123	Gastric	47.9	39.8	0.83	
Sah BK	2008 (China)	394	Gastric	45.5	45.2	0.99	p = 0.962
Lamb P	2008 (UK)	180	Gastric	67.2	26.6	0.40	p <0.0001

Weighted O/E ratio for 1038 patients was 0.86.
Peri-operative morbidity = Complication within 30 days of surgery. AUC = area under ROC curve. O:E is Observed to Expected ratio

1.7.2 Role of systemic inflammatory response

There has been a lot of improvement in surgery and peri-operative care in last couple of decades resulting in reduced mortality following oesophageal and gastric cancer resection. But post-operative morbidity remains a clinically significant problem (Zafirellis et al. 2002b, Sah et al. 2008b) and have been reported to vary between 40-80% (Hulscher et al. 2001, Lagarde et al. 2008, Sah et al. 2010). It has now become clear that post-operative infectious complications, in particular anastomotic leak, also compromise long term outcome in patients with colorectal cancer (McArdle, McMillan & Hole 2005, Marra et al. 2009, Jung et al. 2008, Law et al. 2007). Rizk et al have examined the impact of post operative complication on long term outcome following gastro-oesophageal cancer in 510 consecutive patients. Post operative complication was associated with increased length of stay, increased mortality and poorer overall survival (Rizk et al. 2004). Recently, similar results have also been reported by Tsujimoto et al in 1332 patients with gastric cancer. Patients with post operative infective complication had significant unfavourable outcome and poorer long term survival in patients who underwent resection for gastric cancer (Tsujimoto et al. 2009). Post-operative anastomotic leak contributes to significant proportion of post operative mortality in oesophageal cancer (Alanezi, Urschel 2004). Therefore, infectious complications, in particular the anastomotic leak, can be catastrophic for the patient, both in terms of long and short term outcomes. So, it is clear that early detection of post operative infectious complications may be of considerable clinical benefit.

Recently, it has been reported that biochemical markers of the systemic inflammatory response, in particular C reactive protein, provide an early indication of post operative infectious complications following gastrointestinal cancer surgery (Welsch et al. 2007, Welsch et al. 2008, Korner et al. 2009). Moreover Moyes et al have reported that pre operative Glasgow Prognostic Score (systemic inflammatory marker based scoring) is

useful in predicting post operative infectious complications in colorectal cancer (Moyes et al. 2009). There are very limited studies available to show the relationship between pre or post operative systemic inflammatory markers and post operative infectious complications. Deitmar and colleagues have reported that elevated post operative C reactive protein may predict anastomotic leak following oesophagectomy (Deitmar et al. 2009). But it is not clear what threshold concentration of C-reactive protein is a good predictor of post operative infectious complications following resection of oesophago-gastric cancer.

1.8 Tumour related determinants of long term survival following oesophageal and gastric cancer resection

1.8.1 Established pathological determinants

Pathological TNM staging by American Joint Committee on Cancer (AJCC) is the most important prognostic stratification of oesophageal and gastric cancer (Sobin, Wittekind & editors. April 2002) (Siewert et al. 2001, Siewert et al. 1998) as discussed above in chapter 1.5.2. Moreover, presence of lymph node metastasis (in particular the ratio of involved nodes to the total number of nodes removed and identified), positive resection margins (R1 resection) and poorly differentiated tumours are associated with poorer survival (Roder et al. 1994, Zafirellis et al. 2002a) (Bilici et al. 2010). However variable outcome following resection for oesophageal and gastric cancer in same stage demonstrates a need for other prognostic parameters (Khan et al. 2004). Therefore identification of alternative prognostic markers in oesophageal and gastric cancer may help to develop a precise prognostic classification.

1.8.2 Tumour proliferation

One of the hallmarks of cancer is its potential for uncontrolled proliferation. Immuno-histochemical assessment Ki67 protein is the most common method of assessing

tumour cell proliferation. Ki67 is a large protein, which is associated with dense fibrillary component of the nucleolus (Verheijen et al. 1989) in the cell . This protein undergoes changes during cell cycle, in particular during mitosis and certainly plays a very important role in cell proliferation (Brown, Gatter 2002). Although the exact role of Ki67 still to be defined, it has been examined as a prognostic indicator of various tumours (Brown, Gatter 2002).

Role of Ki67 has been extensively investigated in breast cancer. Number of studies has reported that the high proliferative activity i.e. high Ki67 expression was associated with poorer survival in breast cancer (Yerushalmi et al. 2010, Beck et al. 1995). Ki67 has also been reported to have a prognostic value in soft tissue tumour (Rohr et al. 1998, Stefanou et al. 1998), lung cancer (Scagliotti et al. 1993, Harpole et al. 1995), brain tumour (Enestrom et al. 1998, Wakimoto et al. 1996), cervical cancer (Garzetti et al. 1995, Ho, Hsu & Chiang 2000) and in colorectal cancer (Palmqvist et al. 1999, Kimura et al. 2000).

The role of Ki67 has been recognized in the process of carcinogenesis in oesophageal cancer (Jin, Zhang & Liu 2001) . There was a strong association of high Ki67 expression with dysplasia and neoplasia in barretts oesophagus (Polkowski et al. 1995, Xu et al. 2002, Kerkhof et al. 2008). On the other hand Chao et al reported that Ki67 might not play an important role in development of adenocarcinoma of oesophagus from barrett's metaplasia (Chao et al. 2008). Qi et al showed in tissue micro array made from paraffin embedded oesophageal squamous cell cancer tissues, Ki67 index was associated with high risk tumour features such as higher tumour grade (Qi et al. 2006). Oesophageal tumours with overexpression of Ki67 were reported to have better response following chemo-radiotherapy (Ressiot et al. 2008, Okuno et al. 1999, Takeuchi et al. 2003). Higher expression of intra tumoral Ki67 has been correlated with poorer survival in oesophageal cancer (Youssef et al. 1995, Imdahl et al. 2000).On the contrary Serbia et al have reported

in 150 oesophageal squamous cell cancer patients that Ki67 proliferation index does not provide any prognostic information (Sarbia et al. 1996).

In gastric cancer, high Ki67 expression was associated with aggressive tumour behaviour as well as poorer survival (Tzanakis et al. 2009, Chen et al. 2008). On the contrary, Ki67 was not associated with survival in a study conducted by Al-Moundhri et al in 121 patients with gastric cancer (Al-Moundhri et al. 2005) as well as by Muller et al in 418 patients with gastric cancer (Muller et al. 1996).

1.8.3 Tumour necrosis

Presence of intra tumoral necrosis has been reported as a marker of poor prognosis in various solid tumours. The role of tumour necrosis has extensively been studied in renal or transitional cell carcinoma and majority of them reported that the presence of tumour necrosis as a poor prognostic marker in respect to overall survival (Amtrup, Hansen & Thybo 1974, Roosen et al. 1994, Sabo et al. 2001) and cancer specific survival (Cheville et al. 2003, Frank et al. 2002) (Sengupta et al. 2005). It has also been implicated to enhanced risk of metastasis and disease recurrence (Brinker et al. 2000, Leibovich et al. 2003, Frank et al. 2003). Tumour necrosis has been associated with other aggressive tumour characteristics such as size, grade, pathological stage, higher tumour proliferation index (Ki-67), vascular invasion (Sengupta et al. 2005, Lam et al. 2005, Lee et al. 2006, Klatte et al. 2009). Tumour micro vessel density was inversely associated with intra tumoral necrosis in localised renal cell carcinoma (Sabo et al. 2001). The relationship between clinical parameter and the tumour necrosis has also been examined such as poor performance status (Lam et al. 2005), anaemia and body mass index (Sengupta et al. 2005). Sengupta et al also reported the co-relation between tumour necrosis and markers of inflammatory response i.e. elevated white cell count and erythrocyte sedimentation rate (Sengupta et al. 2005). Similar correlation has been reported in breast cancer. Tumour

necrosis was associated with tumour size, local extension and grade (Fisher et al. 1978), tumour recurrence (Gilchrist et al. 1993) and survival (Gilchrist et al. 1993, Marques et al. 1990, Shek, Godolphin 1988). Several other studies in breast cancer failed to associate between tumour necrosis and survival (Rilke et al. 1991, Kato et al. 1997, Leek et al. 1999, Lee et al. 2006). Leek et al examined the correlation between tumour necrosis, macrophage infiltration and microvessel density in 109 consecutive invasive breast cancer patients. The degree of tumour necrosis was correlated with macrophage infiltration and angiogenesis as well as other features of aggressive tumour behaviour i.e. high grade, larger size and low oestrogen receptor status (Leek et al. 1999). Therefore, they have suggested that the aggressive tumours outgrow their blood supply leading to intra tumoral hypoxia and subsequent necrosis. This in turn may attract macrophages which initiate the angiogenic process in the tumour. Carlomagno et al noted the association between peri-tumoral inflammatory cell infiltrate and tumour necrosis in 1457 patients with infiltrating ductal carcinoma of breast (Carlomagno et al. 1995). On the other hand, Lee AH el al has reported that there was no obvious correlation between tumour inflammatory infiltrate and tumour necrosis in invasive breast cancer (Lee et al. 2006). The role of tumour necrosis and a poor predictor of survival has been shown in other tumours such as in lung cancer (Eerola et al. 1999, Kessler et al. 1996, Swinson et al. 2002), colorectal cancer (Pollheimer et al. 2010, Gao et al. 2005, Svennevig et al. 1984) and other solid organ tumours (Llombart-Bosch et al. 1986, Muro-Cacho, Cantor & Morgan 2000, Hiraoka et al. 2010). Preoperative neo adjuvant radio therapy in rectal cancer has shown to increase the rumour necrosis (Knutsen, Adell & Sun 2006) but was not associated with survival.

It is now apparent that tumour necrosis has a definitive role to play in tumour biology and behaviour. Moreover it has been strongly associated with other aggressive tumour characteristics. To date, there is no published report on tumour necrosis in

oesophageal and gastric cancer, in particular as a prognostic marker or the interrelationship between inflammatory response and necrosis.

1.8.4 Microvessel density (CD34+)

Angiogenesis is a process by which new capillaries are formed from the endothelial cells. Intra tumoral angiogenesis is one of the key mechanisms by which the tumour maintains its growth and metastatic potential (Hasan, Byers & Jayson 2002, Sasano, Suzuki 2005). Several studies have reported a correlation between intra tumoral microvessel density and tumour angiogenesis (Hasan, Byers & Jayson 2002). Numerous methods are available for a quantitative assessment of intra tumoural microvessel density. Among these, anti CD34 monoclonal antibody which is an immune-histochemical endothelial marker, has been most frequently used because of its good sensitivity and reproducibility (Vieira et al. 2004) (da Silva et al. 2009).

Increased intra tumoral microvascular density was associated with long term survival in breast cancer (Hansen et al. 2000) (Heimann et al. 1996), non small cell lung cancer (Fontanini et al. 1997), malignant mesothelioma (Edwards et al. 2001), endometrial cancer (Guset et al. 2010) and in rectal cancer (Svagzdys et al. 2009).

Elpek el al has reported in 53 patients with oesophageal squamous cell cancer that, increased microvessel density in the tumour specimen was associated with poorer survival (Elpek et al. 2001). Whereas Nomiya and colleagues reported that high microvessel density was associated with aggressive tumour behaviour such as higher tumour proliferation (Ki67 index) and tumour infiltration in oesophageal cancer (Nomiya et al. 2004). In gastric cancer, Chen et al reported in 86 patients that, CD34 positive microvessel was associated with poorer survival as well as aggressive tumour behaviour (Chen et al. 2008). On the other hand some published reports have shown that there are poor association between

microvessel density and survival but it was related to the aggressive tumour behaviour in gastric cancer. Mao et al showed in 92 patients with gastric cancer using tissue micro array, that increased CD34 positive microvessel density was associated with poor histological types and metastasis (Mao et al. 2007). Similarly, increased microvessel density was significantly associated with lymph node metastasis and TNM stage but not survival (Da et al. 2008). Iordache et al also reported the association between the aggressive tumour behaviour and increased microvessel density (Iordache et al. 2010). Ohno et al reported the lack of prognostic significance of the CD34+ microvessel density in gastric cancer (Ohno et al. 2005).

1.9 Host related determinants of long term survival following oesophageal and gastric cancer resection

The key role of host related factors have long been identified in disease progression and outcome following resection of oesophageal and gastric cancer along with tumour related factors. Therefore a careful selection of patients for resection not only depends on tumour related factors (i.e. TNM stage etc) but also patient related factors such as co-morbidity and performance status. Poor performance status, co-morbidity or organ dysfunction have been reported to have a prognostic significance in oesophageal and gastric cancer (McCulloch et al. 2003, Bartels, Stein & Siewert 1998) (Chau et al. 2004). There is a lack of consensus in assessing pre operative co-morbidity prior to oesophago-gastric cancer resection. The assessment of performance status is however subjective in most of the cases. It has been reported that there is a significant difference in the assessment of performance status between oncologists, nurses and patients (Ando et al. 2001). Therefore it is imperative to develop objective and reproducible prognostic score that can reliably reflect post operative outcome following resection of oesophageal and gastric cancer.

1.9.1 Host immune response

The function of immune response is to protect from infection and or tissue destruction. There are two broad categories of immune response: innate and adaptive immune response. The innate immune system or native immunity is non-specific defence against microbes or antigen or foreign body. Whereas adaptive immunity, also called specific immunity, is stimulated by microbes or antigens. By convention, the term 'immune response' refers to adaptive immunity. The main components of the innate immune system are phagocytic cells, such as neutrophils, natural killer cells and macrophages, myeloid derived suppressor cells, dendritic cells and the complement proteins. Antigen stimulates the acute inflammatory response, with mobilisation of initially neutrophils, and subsequently macrophages, to engulf and destroy the microbes or antigen. The adaptive immune system can also be of two different kinds; cell mediated immunity and humoral immunity. Cell mediated immunity is responsible for defence against intracellular microbes and is controlled by CD4+ and CD8+ T lymphocytes from the thymus. Humoral immunity, in contrast, is responsible for defence against extracellular pathogens and is controlled by B lymphocytes, from bone marrow. This adaptive immunity is slower to evolve but characterised by long term memory.

Hanahan and Weinberg listed six alterations essential for malignant growth: self-sufficiency in growth signals, insensitivity to antigrowth signals, limitless replicative potential, ability to evade apoptosis, sustained angiogenesis, and ability to invade the tissues and metastasise (Hanahan, Weinberg 2000). An increasing body of research in the following years has suggested that host immune response against the tumour cells, in particular by T and B lymphocytes, macrophages, and natural killer cells might have a major role to play in tumour growth and metastasis. Indeed Colotta et al and colleagues have recently suggested the cancer-related inflammation as the seventh hallmark of cancer (Colotta et al. 2009). There is substantial evidence that indicates that the immune system

may contribute to both in tumour progression and inhibition (Dougan, Dranoff 2009). This dual role of the immune system in cancer pathogenesis indicates a complex interplay of innate and adaptive immune response in the tumour microenvironment. It is believed that the adaptive immune response helps to specifically detect and target the infectious agents, whereas innate immune system helps in wound healing and clearing the debris. This task of tissue housecleaning is carried out by macrophages and neutrophils. These cells are also believed to promote tumour progression (DeNardo, Andreu & Coussens 2010, Biswas, Mantovani 2010, Egeblad, Nakasone & Werb 2010).

1.9.2 Systemic inflammatory response

Inflammation as described above is a reaction in response to cellular injury or damage. Initiation, maintenance and termination of inflammatory process are highly complex. It can be initiated by trauma, infection, allergic reaction or malignancy. There are numerous cellular and humoral mediators that play key roles in inflammation process. It has also been recognised that there is a systemic component of inflammatory response apart from local inflammation which thought to be mediated by cytokines (Heinrich, Castell & Andus 1990, Kushner 1993). The systemic changes involving various organ system in the body is referred as “acute phase response” (Kushner 1993) (Table 1-9). Moreover, there are number of plasma proteins which undergo changes in their concentration during inflammatory process, termed as “acute phase proteins” (Gabay, Kushner 1999). However, it has been shown that a persistent response is seen in many patients, including those with malignant disease. It has therefore been suggested that the term “systemic inflammatory response” is used to reflect the sometimes chronic nature of the systemic response to inflammation.

Table 1-9: Systemic changes associated with the acute phase response.

Neuroendocrine changes	Fever, somnolence, and anorexia Increased secretion of corticotropin-releasing hormone, Decreased production of insulin-like growth factor I Increased adrenal secretion of catecholamines
Hematopoietic changes	Anemia of chronic disease Leukocytosis Thrombocytosis
Metabolic changes	Loss of muscle and negative nitrogen balance Decreased gluconeogenesis Increased hepatic lipogenesis Increased lipolysis in adipose tissue Decraesed lipoprotein lipase activity in muscle and adipose tissue Cachexia
Hepatic changes	Increased metallothionein, inducible nitric oxide synthase, tissue inhibitor of metalloproteinase-1 decreased phosphopyruvate carboxykinase activity
Changes in non protein plasma constituents	hypozincemia, hypoferremia, and hypercupremia increased plasma retinol and glutathione concentrations

1.9.2.1 Acute phase proteins

Acute phase protein as mentioned above has been defined as plasma proteins whose concentration in serum changes either way (increase or decrease) by at least 25 percent during any inflammatory process (Morley, Kushner 1982) (Table 1-10). The magnitude of the change of concentration varies significantly as well as the timing. For example there might be as much as 1000 fold increase of some acute phase proteins such as C reactive protein and serum amyloid A (Figure 1-2). Complement proteins or fibrinogen or haptoglobin exhibit a sustained but slow response to the inflammation. Amongst the acute phase proteins, C-reactive protein and albumin are the two most commonly measured in routine clinical practice, due to their well standardised assays and sensitivity.

Table 1-10: The major acute-phase proteins

Protein Types	**Increased**	**Decreased**
Complement system	C3,C4,C9, Factor B, C1 inhibitor, C4b binding protein, Mannose binding lectin	
Coagulation system	Fibrinogen, plasminogen, Tissue plasminogen activator, Urokinase, Protein S, Vitronectin, Plasminogen activator inhibitor 1	Factor XII
Antiproteases	α 1 protease inhibitor, α 1 antichymotrypsin, trypsin inhibitors,	
Transport proteins	Ceruloplasmin, Haptglobin, Hemopexin	Transferrin, Thyroxine binding globulin,
Participants in inflammatory response	Phospholipase A2, Lipopolysaccaride binding protein, Interleukin 1 receptor antagonist, Granulocyte colonystimulating factor	
Other	C- reactiveprotein, Serum amyloid A, α 1 acid glycoprotein, Fibronectin, ferritin, Angiotensinogen	Albumin, Alpha fetoprotein, Insulin like growth facctor 1

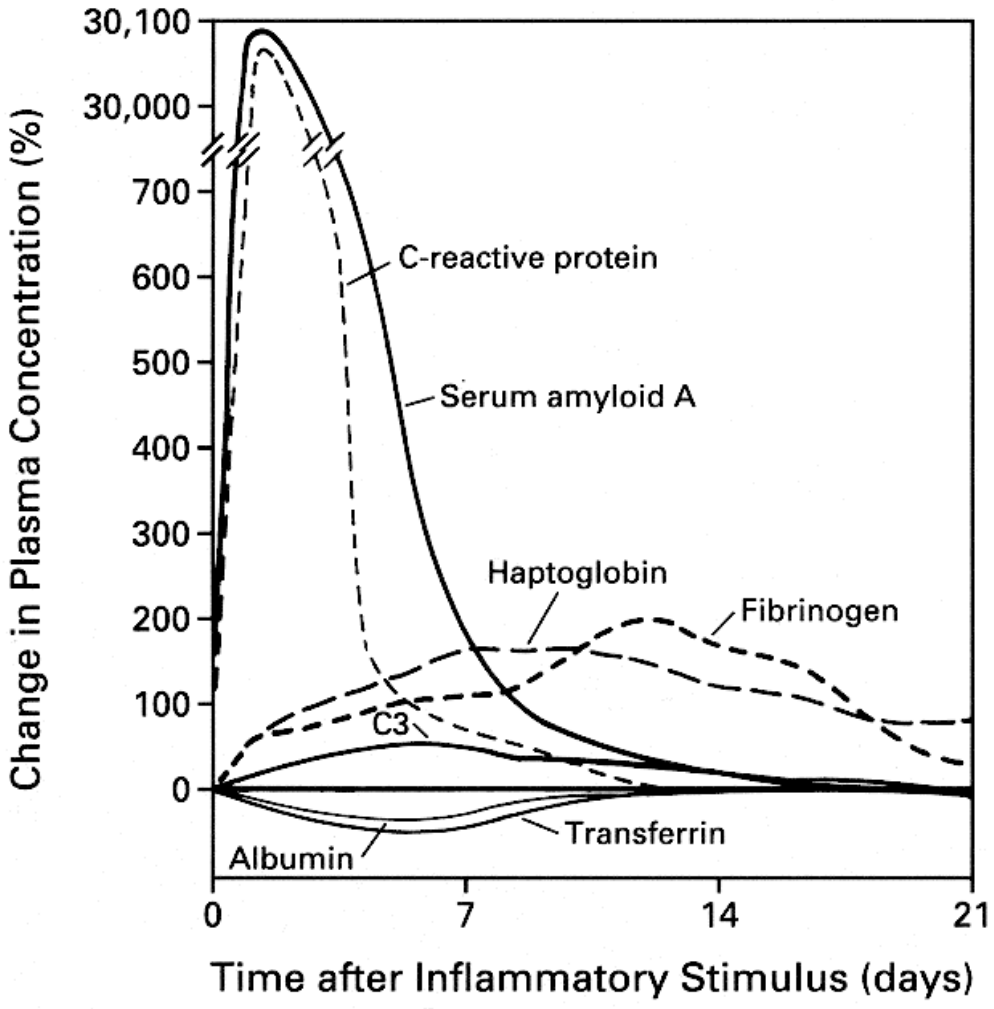

Figure 1-2: Characteristic patterns of change in plasma concentrations of some acute phase proteins after a moderate inflammatory stimulus (Gabay, Kushner 1999).

1.9.2.1.1 C- reactive protein

C-reactive protein is a non-specific, acute phase protein that was first described in 1930. It was named as C reactive protein because of its ability to bind to the C-polysaccharide in the pneumococcal cell wall (Tillett, Francis 1930). C reactive protein is produced in liver in response to a variety of inflammatory cytokines (i.e. interleukin 1, interleukin 6 or tumour necrosis factor alpha) (Du Clos 2000). C reactive protein works as a key component of the innate immune system in the body. It binds to phosphocholine and thus helps to identify some of the foreign pathogens as well as damaged cells (Volanakis JE 1997). It has been shown to activate the complement cascade, bind to phagocytic cells and act as an opsonin for various pathogens (Volanakis JE 1997). It also helps to produce inflammatory cytokines and other tissue factors by activating monocytes (Ballou, Lozanski 1992) (Du Clos 2000, Cermak et al. 1993). The synthesis of C reactive protein in hepatocytes starts rapidly (approximately within six hours) following a inflammation and reaches its peak concentrations at about 48 hours, with a doubling rate at every 8 hours (Kushner, Broder & Karp 1978) (Pepys, Hirschfield 2003). The level of C reactive protein rise is usually proportional to the tissue damage. Concentrations of C reactive protein can come down to normal level within 2-3 days of cessation of the inflammatory process (Kolb-Bachofen 1991). Due to its fast kinetics C reactive protein can provide adequate and useful information about the inflammatory process and therefore it has been a very useful marker in the monitoring of patients with inflammatory diseases (Werner et al. 2003, van Leeuwen, van Rijswijk 1994). The normal concentration of C reactive protein in human subjects may vary from less than 2 mg per litre to 10mg per litre. Therefore it has been suggestion that clinically significant rise of C reactive protein should be considered if the level is more than 10 mg per litre. (Morley, Kushner 1982).

1.9.2.1.2 Albumin

Albumin is a multigene family of protein and is the most abundant protein in the circulatory system. It is produced in the liver and has many physiological functions such as maintenance of colloid osmotic pressure, transporting various molecules in blood, free radical scavenging, platelet function inhibition and antithrombotic effect etc (He, Carter 1992). It has also been recognised that serum albumin behaves as a negative acute phase protein, as its concentration in serum decreases in inflammation (Don, Kaysen 2004). Hypoalbuminaemia also reflect a poor nutritional status (Don, Kaysen 2004, McMillan et al. 2001). Albumin has a long plasma half-life of around twenty days, compared with 19 hours for C-reactive protein. Changes in serum albumin concentration are easily detectable and it is routinely measured in clinical practice.

1.9.2.2 Cellular immunity

One of the main components of the adaptive immunity is cellular reaction involving circulating cells i.e. neutrophils, monocytes, eosinophils, lymphocytes, basophils and platelets. Leukocytosis is a common feature of inflammation and primarily because of accelerated release of cells from bone marrow mediated by cytokines, i.e interleukin 1 (IL 1) and tumour necrosis factor etc. These inflammatory mediators stimulate bone marrow stromal cells and T cells to produce colony stimulating factors (CSFs), which in turn stimulates proliferation and differentiation of bone marrow precursors of granulocytes (Kumar V, Abbas A K & Fausto N 2004).

1.9.2.3 Systemic inflammation and cancer survival

It has now been recognised that survival of patients with cancer not only depends on tumour related characteristics but also on host related factors. It is now known that cancer can induce inflammatory microenvironment (Colotta et al. 2009, Balkwill,

Mantovani 2001, Mantovani et al. 2008, Coussens, Werb 2002, Vakkila, Lotze 2004). The mechanism by which tumour initiates systemic inflammatory response is complex. One possible hypothesis is the tumour hypoxia and necrosis may initiate inflammatory process as apoptosis is a relatively clean form of programmed cell death and does not elicit inflammatory process (Thompson 1995) (Vakkila, Lotze 2004). Moreover, tumour cells can instigate the production of pro inflammatory cytokines, which in turn stimulates the production of acute phase proteins including C reactive proteins (Barber et al. 2000, Tisdale 1999).

An elevated systemic inflammatory response (as evidenced by an increased C-reactive protein concentration in blood as well as leucocytosis) has been found to be associated with a poor survival in patients with a variety of cancers independent of tumour stage. It has been reported that the elevated C reactive protein is a prognostic indicator in patients with colorectal (McMillan 2008), gastro-oesophageal (Crumley et al. 2006), pancreatic (Jamieson et al. 2005), renal (Karakiewicz et al. 2007), urinary bladder (Hilmy et al. 2005) and non small cell lung (Hara et al. 2007) cancer.

Nozoe et al reported in 262 patients with oesophagectomy for oesophageal cancer that, those who had elevated serum level of C reactive protein (>5 mg/l) had poorer survival in 1, 3 and 5 year follow up (Nozoe, Saeki & Sugimachi 2001). Similar findings were noted by Ikeda et al, who reported in 356 patients with oesophageal cancer that, elevated C reactive protein (>5mg/l) is a independent prognostic factor of survival (Ikeda et al. 2003). A different cut off value of C reactive protein (10 mg/l) was used by Shimada et al in patients with oesophageal squamous cell cancer and it was shown to be an independent factor of poor survival (Shimada et al. 2003). In case of gastric cancer, raised pre operative C reactive protein was reported to have independent prognostic value as well (Wang, Sun 2009)

Pre operative low serum albumin level has been reported to have independent prognostic value in colorectal cancer (Heys et al. 1998) (Cengiz et al. 2006, Longo et al. 1998) and breast cancer (Lis et al. 2003) (Al Murri et al. 2007). Lien et al looked at the role of pre operative hypoalbuminaemia in gastric cancer and its relationship with survival. It was found that pre operative low serum albumin (<35 g/dl) was associated with poorer survival in operable gastric cancer (Lien et al. 2004).

It is also of interest that in cancer related inflammation, as serum C reactive protein level rises, albumin level falls and this phenomenon is consistent in various types of tumour (McMillan 2008). Therefore a new inflammation based scoring system for cancer was devised and was called Glasgow Prognostic Score, which has subsequently been modifiedand called Modified Glasgow Prognostic Score (mGPS) (McMillan 2008). Patients with an elevated C-reactive protein (>10 mg/l) were assigned a modified GPS score (mGPS) of 1 or 2 depending on the absence or presence of hypo-albuminaemia (<35 g/l). Patients in whom neither of these abnormalities was present were allocated a score of 0. In a recent review, it has been reported that the Glasgow Prognostic Score has an independent prognostic value in breast, colorectal, gastro-oesophageal, renal, pancreatic and non small cell lung cancer (McMillan 2009). Glasgow Prognostic score prior to neo-adjuvant therapy has been reported to predict survival of oesophageal cancer (Kobayashi et al. 2008).

It has been reported that there are strong direct associations between obesity, cardiovascular co-morbidity and the magnitude of the systemic inflammatory response e.g elevated C-reactive protein concentrations in North American and European population (Choi, Joseph & Pilote 2013, Tzotzas, Evangelou & Kiortsis 2011, Kao, Shiesh & Wu 2006). With reference to the mGPS and its thresholds (C-reactive protein >10mg/l and albumin <35) factors such as obesity and co-morbidity have a relatively small effect with the exception of anaemia (Richards et al. 2010, Roxburgh et al. 2010). In contrast, tumour

necrosis is recognised to be associated with changes in the mGPS (Richards et al. 2012). Therefore, it is not likely that obesity and comorbidity per se confounded the interpretation of the results in the present thesis.

The role of elevated leucocytes and platelets as a marker of systemic inflammatory response and its prognostic significance in cancer is less clear. It has been reported that Neutrophil Lymphocyte Ratio (NLR) has independent prognostic value in non small cell lung cancer (Sarraf et al. 2009), colorectal cancer (Walsh et al. 2005) (Malik et al. 2007) (Kishi et al. 2009) (Halazun et al. 2008), hepatocellular cancer (Gomez et al. 2008a), cholangiocarcinoma (Gomez et al. 2008b) and ovarian cancer (Cho et al. 2009). Moreover high neutrophil count ($>6x10^9$) preoperatively has been shown to be of poor prognostic indicator in colorectal cancer (Neal et al. 2009).

Pre operative elevated platelet count ($>400x10^9$) was associated with poorer survival in non small cell lung cancer (Tomita et al. 2008), colorectal cancer (Kandemir et al. 2005), pancreatic cancer (Suzuki et al. 2004), hepatocellular cancer (Hashimoto et al. 2005), breast cancer (Taucher et al. 2003), ovarian cancer (Menczer et al. 1998) (Li et al. 2004) (Gungor et al. 2009) and renal cell cancer (Jacobsen et al. 2002, Inoue et al. 2004, Gogus et al. 2004). Platelet lymphocyte ratio (PLR) was also recognised as a prognostic marker in resectable pancreatic cancer (Smith et al. 2009).

Similarly pre operative lymphocyte count and monocyte count were also been shown to have a role in predicting survival in colorectal cancer (Tartter 1987)(Sasaki et al. 2007).

The relationship between blood leucocyte and platelet count and survival resection of oesophageal and gastric cancer is rather sparse. Shimada et al reported in 374 patients with oesophageal squamous cell cancer that pre operative thrombocytosis ($>293x10^9$) was

an independent predictor of survival as well as associated with poor tumour characteristics (Shimada et al. 2004). Similarly Ikeda et al looked into 369 consecutive patients with gastric cancer and found that preoperative elevated platelet count (>400x10^9) was associated with poorer survival (Ikeda et al. 2002).

1.9.3 Tumour associated macrophages

The most frequently found immune cells within the tumour microenvironment are tumour-associated macrophages (TAMs) and T cells. It is believed that the tumour-associated macrophage (TAM, CD68+) plays the most important role in the tumour related inflammatory response. However, also mast cells, neutrophils and even effectors of the adaptive immunity (especially in the form of antibodies) may activate inflammatory reactions that promote cancer progression (de Visser, Eichten & Coussens 2006, Galli, Grimbaldeston & Tsai 2008). In most studies, accumulation of TAM has been associated with the angiogenesis and poor prognosis (Mantovani et al. 2008, Balkwill, Charles & Mantovani 2005, Bingle, Brown & Lewis 2002, Pollard 2004). Pro tumourogenic property of TAM might be mediated by releasing cytokines, growth factors and matrix-degrading enzymes (Wyckoff et al. 2007, Mantovani et al. 2006, Condeelis, Pollard 2006) and various angiogenic factors (e.g. vascular endothelial growth factor (VEGF), platelet-derived growth factor and fibroblast growth factor) (Balkwill, Mantovani 2001, Mantovani et al. 2006, Lewis, Pollard 2006, Murdoch et al. 2008). It has also been reported that TAM accumulates in the hypoxic areas of tumour and hypoxia triggers a proangiogenic program in these cells (Murdoch et al. 2008). However increased macrophage infiltration in tumour also been reported as good prognostic indicator (Kim et al. 2008, Pupa et al. 1996, Shimura et al. 2000). This anti tumour immunity mediated by TAMs may be through presenting tumor antigens to T-cells and by tumourocidal activity (Mantovani 1994). Tumouricidal activity by the activated macrophages is exerted either by direct or indirect cytotoxicity.

Various toxic factors, such as TNFα, serine proteases, and reactive nitrogen intermediates are involved in this process (Bingle, Brown & Lewis 2002, Lewis, Pollard 2006).

Previous studies have reported that the increased TAMs in the tumour tissues in oesophageal squamous cell cancer are associated with tumour progression and poorer survival (Guo et al. 2007, Koide et al. 2002). Although, in another published report on oesophageal squamous cell carcinoma CD68+ macrophage in the tumour tissue was not associated with survival (Ishibashi et al. 2006)

In gastric cancer, Ishigami et al. reported that increased TAMs infiltration in tumour had more aggressive clinico-pathological characteristics and poorer prognosis (Ishigami et al. 2003). Similarly, Kawahara et al have also reported the poor prognostic value of tumour associated macrophages (CD68+) and also its association with microvessel density (Kawahara et al. 2010). But in contrast, Ohno et al has reported that the higher infiltration of the CD68+ macrophages in the tumour nests had a beneficial effect in terms of survival in gastric cancer (Ohno et al. 2003). Moreover they have also reported the close association between CD68+ macrophages and CD8+ T lymphocytes in gastric cancer (Ohno et al. 2003) and also with the microvessel density (Ohno et al. 2005). In another study involving tissue microarray in gastric cancer, CD68+ macrophages were not associated with survival (Haas et al. 2009).

The role of the tumour associated macrophages in oesophageal and gastric cancer needs to be investigated further.

1.9.4 Tumour infiltrating lymphocyte

The role of T lymphocyte in cancer immunity has long been recognised (DeNardo, Andreu & Coussens 2010). Mature T cells are divided into two major groups based on the T cell receptors (TCRs) they express: γδ and αβ. αβ T cells are further classified according

to their effector functions as CD8+ cytotoxic T cells (CTLs) and CD4+ helper T (Th) cells, which include Th1, Th2, Th17 and T regulatory (Treg) cells as well as natural killer T (NKT) cells. T lymphocyte can have tumour promoting as well as tumour suppressing effects (Smyth, Dunn & Schreiber 2006, DeNardo et al. 2009). Most tumour infiltrating lymphocytes (TILs) are cytotoxic T lymphocytes (CTLs), which are related to tumour specific immunity (Boon et al. 1994).

CD8+ T lymphocytes infiltration in tumour micro environment was correlated with better survival in colorectal cancer (Ohtani 2007, Galon et al. 2006, Pages et al. 2009), pancreatic cancer (Fukunaga et al. 2004), hepato cellular cancer (Gao et al. 2007), ovarian cancer (Sato et al. 2005, Zhang et al. 2003), cervical cancer (Piersma et al. 2007), renal cancer (Nakano et al. 2001), urothelial cancer (Sharma et al. 2007) and lung cancer (Kawai et al. 2008). It has been reported that, in melanoma, dense intratumoral, not peritumoral T cell infiltrates were correlated with improved survival (Clark et al. 1989, Clemente et al. 1996). Similar findings were reported in colorectal cancer as well (Naito et al. 1998).

In oesophageal squamous cell cancer higher CD8+ lymphocytes associated with better overall survival (Cho et al. 2003, Schumacher et al. 2001). Moreover, Schumacher et al reported that the intra tumoral CD8+ T lymphocyte infiltration is a good predictor of outcome than peri-tumoral infiltration (Schumacher et al. 2001). Also following chemo-radiotherapy in oesophageal squamous cell cancer, CD8+ T lymphocytes were predictive of long term outcome (Ashida et al. 2006). But in another study involving one hundred and thirty patients with oesophageal adenocarcinoma, CD8+ lymphocyte was not associated with survival (Zingg et al. 2010).

In gastric cancer tumour infiltrating lymphocytes have been reported to be associated with survival (Davessar et al. 1990, Ma et al. 1994, Songun et al. 1996). However Fukuda et al reported no association between TILs and survival in T3 gastric

cancer (Fukuda et al. 2002). More specifically, CD8+ T-lymphocytes have been reported to provide prognostic information in gastric cancer using tissue microarray (Lee et al. 2008). Similar findings were reported by Milasiene et al (Milasiene, Stratilatovas & Norkiene 2007). Moreover, the number of CD8+ T lymphocytes in cancer cell nests were predictive of survival (Ohno et al. 2002). In contrast, other reported studies in gastric cancer, CD8+ T lymphocyte was not associated with survival (Haas et al. 2009, Chiaravalli et al. 2006, Shen et al. 2010).

1.10 The role of Tissue Micro Array (TMA) in research of molecular markers

The tissue microarray (TMA) technology makes it possible to simultaneously analyse hundreds of tissue specimens for numerous molecular markers. It has also been shown that the TMA samples were quite useful to link up molecular changes in the tissue with the clinical endpoints (Torhorst et al. 2001, Kallioniemi et al. 2001).

Tissue Micro array has been used to assess tumour infiltrating lymphocytes in various other studies involving Hodgkin's lymphoma (Alvaro-Naranjo et al. 2005), prostrate (Karja et al. 2005), colorectal cancer (Pages et al. 2005, Zlobec et al. 2008a, Zlobec et al. 2008b), ovarian cancer (Callahan et al. 2008) and breast cancer (Bates et al. 2006).

Micro vessel density (CD34+) has also been assessed in TMA in various cancers such as breast, lung and hepato-cellular (Xu et al. 2008, Donnem et al. 2007, Chebib et al. 2007) and gastric cancers (Mao et al. 2007, Zheng et al. 2006). Similarly Ki-67 proliferation index was assessed in TMA in various cancers such as bladder cancer (Burger et al. 2007), breast cancer (Cheang et al. 2009, Park et al. 2005, Sapino et al. 2006), prostrate cancer, colorectal cancer (Hashimoto et al. 2006, Okon et al. 2006) and gastric cancer (Zheng et al. 2006).

1.11 Aims

The overall aim of the thesis was to examine the inter-relationships between patient physiology and systemic inflammatory response as well as local inflammatory response and outcome (both short term and long term), in patients undergoing resection for oesophageal and gastric cancer.

More specifically the aims of the thesis are:

To compare patients' pre operative physiological status (POSSUM scoring system) and systemic inflammatory response (modified Glasgow prognostic scoring model) in the prediction of post-operative outcome, both short term and long term, in patients undergoing resection of oesophago-gastric cancer. Also to examine the value of serial daily post-operative markers of systemic inflammatory response like white cell count, albumin and C-reactive protein concentrations in the prediction of post-operative complications in these patients.

To compare the prognostic value of selected markers of the systemic inflammatory response in patients undergoing potentially curative resection for oesophageal and gastric cancer.

To examine the relationship between local and systemic inflammatory responses and outcome in patients undergoing potentially curative resection for oesophageal and gastric cancer.

2 Chapter II: A comparison of POSSUM and GPS models in the prediction of post operative outcome in patients undergoing oesophago-gastric cancer resection.

2.1 Introduction

Despite improvements in surgery and peri-operative care resulting in reduced mortality for oesophago-gastric cancer resections, post-operative morbidity remains a clinically significant problem (Zafirellis et al. 2002b, Sah et al. 2008b) and have been reported to vary between 40-80% (Hulscher et al. 2001, Lagarde et al. 2008, Sah et al. 2010).

To objectively assess the impact of the patients' physiological condition on outcome following surgery Copeland and co-workers developed the Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM) (Copeland, Jones & Walters 1991) using cohorts of general surgical patients. This was subsequently revised to P-POSSUM (Portsmouth POSSUM) by Whitelay and co-workers who reported that POSSUM over predicted post-operative mortality, particularly in those who were at low risk (Whiteley et al. 1996). P-POSSUM used the same set of variables as POSSUM but had a different logistic regression equation. More recently, this model has been adapted to a specialised model for the prediction of risk-adjusted postoperative mortality in oesophageal and upper gastrointestinal surgery (O-POSSUM) (Tekkis et al. 2004a). Both POSSUM and P-POSSUM scoring systems use a 12 factor physiological score and a 6 factor operative severity score. In O-POSSUM, the operative severity score was modified to exclude operative blood loss, number of procedures and peritoneal soiling. Age was regressed independently from POSSUM. (Table 2-1). In terms of outcome following surgery for oesophago-gastric cancer various POSSUM models have been used with variable accuracy (Chapter 1).

Recently, it has become clear that the presence of a systemic inflammatory response underlies the recognised relationship between poor nutritional status and poor outcome in patients with oesophago-gastric cancer (Deans et al. 2009, Crumley et al. 2010b). Indeed, an elevated C-reactive protein has been shown to be related to increased incidence of peri-operative blood transfusion and general complication in oesophago-gastric cancer patients (Gockel et al. 2006). This has recently been refined to include the combination of C-reactive protein and albumin, termed the modified Glasgow prognostic score (mGPS) (McMillan 2009). Therefore, it is of interest that the mGPS has been shown to be associated with post-operative complications (Moyes et al. 2009) and long term survival (Ishizuka et al. 2007, McMillan et al. 2007) in patients undergoing potentially curative resection for colorectal cancer. Furthermore, both POSSUM and mGPS are significantly associated with long term survival in these patients (Brosens et al. 2006, Jenkins, O'Neill & Morran 2007, Richards et al. 2010).

With reference to oesophago-gastric cancer several studies have reported that the development of a post-operative complication after major surgery contributes not only to adverse short term outcome, but also to reduced long term survival (Rizk et al. 2004, Tsujimoto et al. 2009, Lagarde et al. 2008). Therefore, it may be that post-operative complications are simply a surrogate marker for underlying pre-operative host-related factors, and it is these that are the true determinants of survival. However, the relationship between pre-operative patient physiology, systemic inflammatory response, postoperative complications and survival has not been examined.

The aim of the present study, therefore, was to compare POSSUM and mGPS models in the prediction of post-operative outcome, both short term and long term, in patients undergoing resection of oesophago-gastric cancer.

2.2 Patients and Methods

All patients who underwent oesophago-gastric cancer resections in Glasgow Royal infirmary from January 2005 to May 2009 and had data for POSSUM, P-POSSUM and O-POSSUM and the mGPS models to be scored were included in the study. Data on patients' demographics, deprivation score, preoperative physiological score, ASA risks, operative details, and post-operative outcomes were collected from a comprehensive review of patient records.

The tumours were staged according to the tumour node metastasis (TNM) Criteria from the 6th edition of the International Union Against Cancer (UICC) Classification of the Malignant Tumours (Sobin, Wittekind & editors. April 2002). Tumours of the oesophago-gastric junction were further subdivided according to site, using the Siewert classification; Type I and II lesions of the oesophago-gastric junction were designated, as cancers of the oesophagus. Type III tumours of the cardia were designated gastric cancers (Siewert, Stein 1996).

POSSUM physiological score and operative severity score were calculated according to Copeland and co-workers (Copeland, Jones & Walters 1991). P-POSSUM and O-POSSUM were calculated as described by Whitelay and coworkers (Whiteley et al. 1996) and Tekkis et al (Tekkis et al. 2004a) respectively (2-1). Missing data (3%) was limited to "operative blood loss" and "evidence of peritoneal soiling" with missing values allocated a score of '1' (representing a normal result) as suggested by previous studies (Lai et al. 2007, Nagabhushan et al. 2007) .

The mGPS was constructed as previously described (McMillan 2008). An elevated C-reactive protein (>10 mg/l) were assigned a modified GPS score (mGPS) of 1 or 2 depending on the absence or presence of hypo-albuminaemia (<35g/l). Patients in whom neither of these abnormalities was present were allocated a score of 0.

The extent of deprivation was defined using the Carstairs deprivation index (Carstairs, Morris 1990). This is composed of four indicators of deprivation (car ownership, overcrowded housing, Registrar General social class and male unemployment) and has been validated for use within central Scotland (Hole, McArdle 2002). Deprivation scores were based on the postcode of the patients' residence at the time of surgery (www.isdscotland.org). For illustrative purposes, the results are presented by amalgamating the seven categories into three groups: affluent (categories 1 and 2), intermediate (categories 3–5) and deprived (categories 6 and 7). Furthermore, patients who drink more than permissive limits of alcohol (21 units and 14 units of alcohol per week for male and female respectively) have been categorised as alcohol excess (Morgan MY, Ritson B 2003). Patients were assigned to POSSUM physiology groups (score 11 – 14, 15 – 20, 21 – 30, > 30) and POSSUM operative groups (score ≤15 and > 15) according to previously published thresholds (Tekkis et al. 2004a, Richards et al. 2010). Patients were also categorised according to The American Society of Anesthesiologist' (ASA) physical status classification (1:A normally healthy patient; 2:A patient with mild systemic disease; 3: A patient with severe systemic disease that is not incapacitating; 4: A patient with an incapacitating systemic disease that is a constant threat to life) (Owens, Felts & Spitznagel 1978, Haynes, Lawler 1995)

Postoperative morbidity and mortality was defined as any complication or death occurring within 30 days of surgery as this metric was used in various risk adjusted predictive models (Internullo et al. 2008, Steyerberg et al. 2006, Daley et al. 1997). Data were collected from the case notes review and regional cancer death registry office. All cases of postoperative mortality were excluded from the subsequent survival analysis.

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

2.2.1 Statistical analysis

The risks were calculated for POSSUM and P-POSSUM on a calculation sheet (website: http://www.sfar.org). The O-POSSUM was obtained by introducing preoperative physiological variables and the surgical variables on the spreadsheet (website: http://www.risk prediction.org.uk). Post-operative morbidity was assessed over the first 30 days of surgery. Morbidity was documented on the basis of the definition by Copeland et al (Copeland, Jones & Walters 1991). However, only whether or not the patient had a complication (and not the number of complications that each patient had) was used in the analysis. Non-symptomatic or minor urinary tract infection was not recorded and therefore only included if complicated by sepsis. Similarly, post-operative mortality was defined as death during first 30 days of surgery, regardless of cause was recorded. The observed numbers of events (morbidity and mortality) were compared with expected/predicted numbers by all three scoring systems. Comparison of categorical variables was performed using binary logistic regression; variables significant on univariate analysis were progressed to a multivariate model.

Survival analysis was performed using Cox proportional hazards regression; variables significant on univariate analysis were progressed to a multivariate model. Deaths, excluding post-operative mortality, up to the end of August 2010 were included in the analysis. The results of the statistical tests were given, where appropriate, with the 95% confidence interval. The discriminatory power of the three scoring systems was examined by receiver–operator characteristic (ROC) curve analysis, using the area under the curve (AUC). Statistical analysis was performed using SPSS software (version 15.0 SPSS Inc, Chicago, USA).

2.3 Results

The clinico-pathological characteristics of the 121 patients have been summarised in Table 2-2. The majority of patients were over the age of 60 years (64%), male (72%), had an ASA of 1 or 2 (84%), were non smokers (66%), did not consume excess alcohol (65%), were not deprived (62%) and had mGPS of 0 (75%). The majority patients had physiology scores were less than 20 (75%) and had high operative scores (78%). The majority of patients had TNM stage I or II disease (64%). Similar numbers of patients underwent resection for oesophageal and gastric cancer and had neo-adjuvant therapy.

Overall fifty nine patients (49%) had post operative complications and five patients (4%) died within 30 days of surgery. Details of the complications are shown in Table 2-3. Complications were categorised into six grades according to the modified Clavien-Dindo classification (Dindo, Demartines & Clavien 2004). Briefly, these grades are as follows: no complications (grade 0); deviation from normal hospital course, but no need for medication or intervention e.g. minute anastomotic leakage, wound infections opened bedside, vocal cord paralysis, social/placement problems etc (grade 1); complications requiring drugs or blood transfusion e.g. pneumonia requiring antibiotics, atrial fibrillation requiring medication, blood transfusion (any: pre-, per-, or post-OP), wound infection requiring antibiotics, anastomotic leakage requiring somatostatin etc (grade 2); complications requiring interventional treatment e.g. pleural effusion requiring drainage, pneumothorax requiring drainage, chest drain for empyema, bronchoscopic lavage, endoscopic dilatation, electric reconversion atrial fibrillation or any surgical reintervention etc (grade 3); life threatening complications requiring ICU admittance e.g. single or muli organ failure (grade 4); and postoperative mortality (grade 5).

Post operative infection was the most common cause of morbidity in our study group and multiple complications in one case. In terms of complications, respiratory

complication (25%) and anastomotic leak (12%) were most common. The distribution of complications according to Cavien-Dindo classification was grade 1: 6 patients (5%), grade 2: 24 patients (20%), grade 3: 12 patients (10%), grade 4: 17 patients (14%) and grade 5: 5 patients (4%) (Table 2-3).

Predicted and observed morbidity in POSSUM risk categories are shown in Table 2-4. There were predicted complications in 72 patients (60%) giving an overall standardised morbidity ratio of 0.82 and ROC analysis for the POSSUM morbidity equation was (AUC 0.639, 95% CI 0.541 to 0.737, p=0.008) relatively poor discriminatory power. In particular, in the high risk group the standardised morbidity ratio was 0.73, the POSSUM morbidity equation substantially over predicted post-operative complications.

The relationship between clinico-pathological characteristics and development of post-operative complication is shown in Table 2-5. On univariate analysis, increased age ($p<0.05$), male sex ($p<0.05$), higher ASA score ($p<0.01$), current smoking ($p<0.10$), alcohol excess ($p<0.10$), elevated mGPS ($p<0.10$) and higher POSSUM physiological score ($p<0.01$) were associated with development of post operative complication. On multivariate analysis of these significant factors, male sex (HR 3.61, 95% CI 1.38-9.46, p=0.009) and POSSUM physiology score (HR 2.13, 95% CI 1.11-4.08, p=0.023) were independently associated with post operative morbidity.

The post-operative mortality rates predicted by the POSSUM, P-POSSUM and O-POSSUM equations compared to the observed mortality rate are shown in Table 2-6. The post-operative mortality rates predicted by POSSUM, P-POSSUM and O-POSSUM were 16.5, 5.8 and 9.9 per cent respectively giving a standardised mortality ratio of 0.25, 0.71 and 0.42. POSSUM and O-POSSUM over predicted mortality in all risk groups, whereas P-POSSUM over predicted mortality in low risk groups. ROC analysis for the P-POSSUM mortality equation was AUC 0.808, 95% CI 0.55 to 1.06, p=0.020. This showed relatively

good discriminatory power in predicting post operative mortality compared with POSSUM (AUC 0.759, 95% CI 0.48 to 1.04, p=0.051), and O-POSSUM (AUC 0.715, 95% CI 0.46 to 0.97, p=0.105).

The relationships between pre-operative clinico-pathological characteristics, post-operative complications and cancer specific survival are shown in Table 2-7. The median follow up for survivors was 31 months (range 15-65). During this period, 39 patients died from oesophago-gastric cancer and 5 patients died from other causes. On univariate survival analysis, male sex ($p<0.05$), elevated mGPS ($p<0.05$), POSSUM operative score ($p<0.05$) and increased TNM stage ($p<0.001$) were significantly associated with cancer specific survival. On multivariate survival analysis, mGPS (HR 1.96, 95% CI 1.09–3.54, p=0.025) and TNM stage (HR 2.21, 95% CI 1.44–3.38, $p<0.001$) remained independently associated with cancer specific survival (Table 2-7).

2.4 Discussion

In the present study, male sex and the POSSUM physiological score were independent predictors of post-operative complications in patients undergoing potentially curative resection of oesophago-gastric cancer resection. In contrast, the POSSUM operative score was not significantly associated with the development of post-operative complications. Moreover, the pre-operative systemic inflammatory response and hypoalbuminaemia, as evidenced by the mGPS, but not the patient physiology or the post-operative complications were independently associated with poorer cancer specific survival. Taken together, the results of the present study suggest that pre-operative host related factors are important in determining both short and long term outcome following potentially curative resection of oesophago-gastric cancer.

To date, there has been very little data on the role of systemic inflammatory markers in post-operative complications in patients undergoing potentially curative

resection for gastrointestinal cancer. Previously, Gockel and coworkers (2006) reported that an elevated C-reactive protein was associated with increased post-operative complications in patients undergoing surgery for oesophago-gastric cancer (Gockel et al. 2006). More recently, Moyes and coworkers reported that an elevated mGPS was associated with increased post-operative complications in patients undergoing surgery for colorectal cancer (Moyes et al. 2009). In the present study, compared with the POSSUM morbidity equation, the mGPS was weakly and not independently associated with the development of post-operative complications. Therefore, the results of the present study would suggest that patient physiology, compared with the systemic inflammatory response, is the major factor in determining post-operative complications.

In the present study, male sex was also associated with development of post operative complications. It might be due to the fact that majority (81%) of patients were male. Therefore it will be interesting to examine patients' physiological status and its relationship with development of post operative complications in a larger cohort. Indeed in a recent study in 1017 patients with gastric cancer it has been reported that, pre operative patients co morbidity is related to development of post operative complication not GPS (Kubota et al. 2012).

Clearly, a risk adjusted model with accurate prediction of post-operative morbidity and mortality is important in surgical audit. In the present study although the POSSUM morbidity equation appeared to predict post-operative complications well in low risk group, it appeared to overestimate post-operative complication in the high risk group. Nevertheless, the overall observed to expected ratio was 0.82, surprisingly good given that the morbidity equation was developed approximately 20 years ago and in a general surgical population. Indeed, the POSSUM physiology score performed better than ASA score in predicting post-operative complications in these patients. Furthermore, the results suggest that the physiology component of the POSSUM score, compared with the

operative component, has the strongest influence on the development of post-operative complications. However, given the fact that all patients had oesophago-gastric cancer resections and had similar operative severity score, predictive value of the POSSUM operative score may have been underestimated. Therefore, since post-operative morbidity is now much more common than post-operative mortality future work may be directed at optimising the POSSUM morbidity score.

In the present study although the POSSUM physiology score predicted post-operative complications, it was not significantly associated with cancer specific survival. These results are not consistent with previous studies in patients undergoing surgery for colorectal cancer reporting long term prognostic value (Brosens et al. 2006, Jenkins, O'Neill & Morran 2007). However, when recently, the prognostic value of the POSSUM physiology score and the mGPS in predicting cancer survival were compared in colorectal cancer it was clear that the mGPS had superior value (Richards et al. 2010). Therefore, the results of the present study suggest that the systemic inflammatory response, compared with patient physiology, is a major factor in determining long term survival.

The impact of neo-adjuvant therapy on the systemic inflammatory response is not clear. However, it has been previously reported that, elevated C reactive protein was an independent predictor of overall as well as disease free survival in patients with oesophageal cancer following neo-adjuvant therapy (Guillem, Triboulet 2005, Zingg et al. 2010). Further longitudinal studies are required to demonstrate effect of systemic chemotherapy in inflammatory response in particular with C reactive protein.

The routine clinical use of the mGPS has yet to be established. However, from the present and recent studies (Vashist et al. 2011) its potential role in the risk stratification of patients undergoing surgery for oesophageal cancer is clear since this procedure has a high potential for morbidity and mortality and recovery to pre-resectional functional status, if

ever reached, can take a year or more. Therefore, it can be concluded that 'Preoperative evaluation of the GPS may help to stratify oesophageal cancer patients to different risk profiles, which is essential in the era of customized therapy' (Vashist et al. 2011). Furthermore, with the increasing use of neoadjuvant therapy in oesophageal cancer prior to surgery and the consequent reduction in the value of pathological findings (as it may down stage the tumour), the value of the mGPS in clinical staging and predicting outcome may be of increasing importance in these patients. Clearly, further work is required to establish the role of the mGPS in patients undergoing surgery for gastric cancer.

There is now increasing evidence of the role of the systemic inflammatory response in predicting cancer survival in lung, gastrointestinal and renal cancers, independent of tumour stage (McMillan 2009, Roxburgh, McMillan 2010). More recently, in a study of almost 10,000 cancer patients, this has been extended to other tumour types (Proctor et al. 2011). Whilst the mGPS is predictive of cancer survival, TNM stage remains a significantly more powerful predictor oesophago-gastric cancer since on multivariate analysis the HR for mGPS is 1.96 compared with HR of 2.21 for TNM stage. Therefore, the results of the present study suggest an important role for mGPS (and therefore inflammation and hypoalbuminemia) in predicting cancer survival that can be considered in addition to TNM stage.

To date, following oesophago-gastric cancer resection, few studies have compared the different POSSUM models in predicting mortality (Lai et al. 2007, Luna et al. 2009). In the present study all three POSSUM models examined over predicted the post operative mortality rate. POSSUM and O-POSSUM over predicted in all risk groups. In contrast, P-POSSUM had the least over predicted mortality particularly in high risk group and showed a good discriminatory power. The relatively poor performance of the POSSUM and O-POSSUM models, compared with P-POSSUM, in the present study may have been due to small sample size. Nevertheless, the results of the present study are consistent with the

large majority of the literature in oesophago-gastric cancer (Chapter 1). In the present study the basis of the overestimation of post-operative mortality following oesophago-gastric cancer resection by all three models, although not certain, was likely to be due to improved post-operative care and centralisation of upper gastrointestinal cancer service since the prediction models were devised. Therefore, given that post-operative mortality following oesophago-gastric surgery is now considerably lower, it would be of interest to up-date and refine the existing POSSUM models to recapture its role as an effective audit tool in this surgical speciality.

In summary, the results of the present study show that, in patients undergoing surgery for oesophago-gastric cancer, the POSSUM physiology score, was useful in predicting the development of post-operative complications. Also, our results suggest that the mGPS was useful in predicting cancer specific survival. In contrast, post-operative complications were not independently associated with poorer cancer specific survival. Clearly, in the present study the relatively small numbers of patients and outcome events, especially mortality, limit the strength of these conclusions. Nevertheless, the results of the present study would suggest that pre-operative host related factors are important in determining both short and long term outcome following potentially curative resection of oesophago-gastric cancer.

Table 2-1: Variables used in POSSUM, P-POSSUM and O-POSSUM equations.

Physiological Score	**1**	**2**	**4**	**8**
Age (years)*	≤60	61-70	≥71	-
Cardiac Signs	Normal	Cardiac drugs/steroid	Oedema; warfarin. Borderline cardiomegaly on CXR	JVP Cardiomegaly on CXR
Respiratory signs	Normal	SOB on Exertion Mild COPD on CXR	SOB stairs Mod COPD on CXR	SOB rest fibrosis or consolidation on CXR
Systolic BP, mm Hg	110-130	131-170 100-109	≥171 90-99	≤89
Pulse, beats/min	50-80	81-100 40-49	101-120	≥121 ≤39
Glasgow Coma score	15	12-14	9-11	≤8
Urea nitrogen, mmol/L	<7.5	7.6-10	10.1-15	≥15.1
Na mEq/L	>136	131-135	126-130	≤125
K mEq/L	3.5-5	3.2-3.4 5.1-5.3	2.9-3.1 5.4-5.9	≤2.8 ≥6
Hb g/dl	13-16	11.5-12.9 16.1-17	10-11.4 17.1-18	≤9.9 ≥18.1
WCC x1012/L	4-10	10.1-20 3.1-3.9	≥20.1 ≤3	-
ECG	Normal		AF (60-90)	Any other changes
Operative Score	**1**	**2**	**4**	**8**
Operative severity	Minor	Intermediate	Major	Major +
No. of procedures over 30 days†	1	2	>2	
Blood loss per operation, mL†	<100	101-500	501-999	>1000
Peritoneal contamination†	No	Serous blood (< 250 ml)	Local pus	Free Bowel content, pus or blood
Presence of Malignancy	No	Primary only	Nodal metastases	Distant metastases
Mode of Surgery	Elective		Emergency resuscitation Possible. operation <24 h	Emergency, immediate operation <2 h

POSSUM equation (R1for mortality and R2 for morbidity):
$Log_eR1/(1-R1) = -7.04 + (0.13 \times$ Physiological Score) + (0.16 x Operative Severity Score).
$Log_eR2/(1-R2) = -5.91 + (0.16 \times$ Physiological Score) + (0.19 x Operative Severity Score).

P-POSSUM equation for mortality:
$Log_eR/(1-R) = -9.065 + (0.1692 \times$ Physiological Score) + (0.1550 x Operative Severity Score)

O-POSSUM calculation:
*Age was regressed independently from the Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM).
†Risk factors not used in scoring system specific for upper gastrointestinal surgery (O-POSSUM).

Table 2-2: The clinico-pathological and operative characteristics of patients who underwent potentially curative resection of oesophago-gastric cancer

Variables		Patients (n=121 (%))
Age	≤60	44 (36)
	61-70	48 (40)
	≥71	29 (24)
Sex	Female	34 (28)
	Male	87 (72)
ASA	1	20 (16)
	2	82 (68)
	3	18 (15)
	4	1 (1)
Smoking status	Non	80 (66)
	Ex or current	41 (34)
Alcohol excess	No	78 (65)
	Yes	43 (35)
Deprivation	1-2	18 (15)
	3-5	57 (47)
	6-7	46 (38)
mGPS	0	99 (82)
	1	16 (13)
	2	6 (5)
POSSUM physiology score	11-14	27 (22)
	15-20	64 (53)
	21-30	30 (25)
POSSUM operative score	≤15	26 (22)
	>15	95 (78)
POSSUM morbidity category	Low risk	64 (53)
	High risk	57 (47)
POSSUM mortality category	Low risk	87 (72)
	Medium risk	24 (20)
	High risk	10 (8)
P-POSSUM mortality category	Low risk	116 (96)
	Medium risk	5 (4)
O-POSSUM mortality category	Low risk	107 (88)
	Medium risk	14 (12)
TNM	Stage I	48 (40)
	Stage II	29 (24)
	Stage III	44 (36)
Tumour site	Oesophageal	61 (50)
	Gastric	60 (50)
Neoadjuvant therapy	No	54 (45)
	Yes	67 (55)
Adjuvant therapy	No	102 (85)
	Yes	19 (15)

Table 2-3: List of complications in patients underwent resection for oesophago-gastric cancer (n=59).

Complication types	Number of patients
Anastomotic leak	14
Other serious infections	9
Respiratory (pneumonia, insufficiency etc)	30
Cardiac complications, Liver and renal failure	14
Other complications (wound infection, urinary, ileus, post op confusion etc)	14
Post operative deaths (in hospital)	5
Clavien-Dindo classification*	
Grade 1	6
Grade 2	24
Grade 3	12
Grade 4	17
Grade 5	5

Values do not equal to 100% as several patients had more than one post operative complications.
*Clavien-Dindo classification (Dindo, Demartines & Clavien 2004)

Table 2-4: Predicted and Observed morbidity in POSSUM risk categories in patients who underwent potentially curative resection of oesophago-gastric cancer (n= 121).

	Predicted Risk (%)	Number of patients	Predicted morbidity	Observed morbidity	O:E
Low risk	≤30	9	2	1	
	31-40	16	6	5	
	41-50	21	10	10	
	51-60	18	10	11	
Total	≤60	64	28	27	0.96
High risk	61-70	22	14	10	
	71-80	18	14	11	
	81-90	11	10	7	
	91-100	6	6	4	
Total	>60	57	44	32	0.73
Overall	0-100	121	72	59	0.82

Table 2-5: The relationship between clinico-pathological characteristics and the development of post-operative complications; binary logistic regression analysis

Variables	Complications n=59 (%)	Univariate OR (95% CI)	P value	Multivariate OR (95% CI)	P value
Age ≤60 yrs	13 (22)				
61-70	30 (51)				
≥71	16 (27)	1.82 (1.12-2.95)	0.016		0.237
Sex Female	11 (19)				
Male	48 (81)	2.57 (1.12-5.92)	0.026	3.61 (1.38-9.46)	0.009
ASA 1	6 (10)				
2	39 (66)				
3	13 (22)				
4	1 (2)	2.54 (1.28-5.04)	0.008		0.106
Smoking status	34 (58)				
Non					
Ex or current	25 (42)	2.11 (0.98-4.56)	0.056		0.270
Alcohol excess	33 (56)				
No					
Yes	26 (44)	2.09 (0.98-4.45)	0.058		0.051
Deprivation 1-2	8 (14)				
3-5	25 (42)				
6-7	26 (44)	1.36 (0.80-2.29)	0.253		
mGPS 0	44 (75)				
1	11 (19)				
2	4 (7)	1.97 (0.94-4.14)	0.074		0.078
PPS 11-14	8 (14)				
15-20	31 (52)				
21-30	20(34)	2.18 (1.24-3.81)	0.006	2.13 (1.11-4.08)	0.023
POS ≤15	10 (17)				
>15	49 (83)	1.70 (0.70-4.14)	0.239		
TNM Stage I	23 (39)				
Stage II	18 (30)				
Stage III	18 (30)	0.88 (0.58-1.32)	0.525		
Tumour site					
Oesophageal	33 (56)				
Gastric	26 (44)	0.65 (0.32-1.33)	0.237		
Neoadjuvant No	23 (39)				
Yes	36 (61)	1.57 (0.76-3.22)	0.224		

PPS POSSUM physiology score, POS POSSUM operative score

Table 2-6: Predicted and Observed mortality in risk categories as defined by POSSUM, P-POSSUM and O-POSSUM mortality equations

	POSSUM					P-POSSUM				O-POSSUM			
	Predicted Risk (%)	Patients (n)	Predicted mortality	Observed mortality	O:E	Patients (n)	Predicted Mortality	Observed mortality	O:E	Patients (n)	Predicted mortality	Observed mortality	O:E
Low risk	0-10	43	3	1		107	4	2		75	4	1	
	10-20	44	6	0		9	1	1		32	5	2	
	≤20	87	9	1	0.11	116	5	3	0.6	107	9	3	0.33
Medium risk	21-40	24	6	2	0.33	5	2	2	1.0	14	3	2	0.67
High risk	>40	10	5	2	0.40	0	0	0		0	0	0	
Total	0-100	121	20	5	0.25	121	7	5	0.71	121	12	5	0.42

Table 2-7: The relationship between clinicopathological characteristics, post-operative complications and cancer-specific survival; Cox regression analysis (post-operative mortality excluded).

Variable	Univariate		Multivariate	
	HR (95% C.I.)	P-value	HR (95% C.I.)	P-value
Age (≤60/ 61-70/ >70 yrs)	0.96 (0.63-1.44)	0.827		
Sex (female/ male)	2.45 (1.03-5.85)	0.044		0.377
ASA (1/ 2/ 3/ 4)	1.04 (0.59-1.81)	0.902		
Smoking status (Non/ Ex or current)	0.61 (0.30-1.26)	0.181		
Alcohol excess (No/ yes)	1.11 (0.58-2.13)	0.757		
Deprivation (1-2/ 3-5/ 6-7)	0.98 (0.62-1.57)	0.937		
mGPS (0/ 1/ 2)	1.70 (1.01-2.86)	0.045	1.96 (1.09-3.54)	0.025
POSSUM physiology score (11-14/ 15-20/ 21-30)	1.04 (0.64-1.70)	0.881		
POSSUM operative score (≤15/ >15)	2.97 (1.05-8.31)	0.039		0.111
Post –operative Complication (No/ yes)	1.39 (0.74-2.61)	0.304		
TNM stage (I/ II/ III)	2.43 (1.61-3.69)	<0.001	2.21 (1.44-3.38)	<0.001
Tumour site (oesophageal/ gastric)	0.54 (0.29-1.04)	0.065		0.551
Neo-adjuvant (No/ yes)	0.60 (0.32-1.13)	0.116		
Adjuvant therapy (No/yes)	1.60 (0.73-3.48)	0.240		

3 Chapter III: Persistent elevation of C-reactive protein following oesophago-gastric cancer resection as a predictor of post operative surgical site infectious complications

3.1 Introduction

Despite improvements in surgery and peri-operative care resulting in reduced mortality for oesophago-gastric cancer resections, post operative morbidity remains a clinically significant problem (Zafirellis et al. 2002b, Sah et al. 2008b). In particular, post operative infectious complications remain a significant clinical problem and are the most common cause of peri-operative morbidity (Sah et al. 2010).

Such complications are in the main surgical site-specific infections (SSI) or remote site infections (RI) and have been reported to vary between 40-80% (Hulscher et al. 2001, Lagarde et al. 2008, Sah et al. 2010). An anastomotic leakage is the most serious SSI and has been reported to vary from 3-25% (Sah et al. 2010, Lorentz, Fok & Wong 1989, Hankins et al. 1989, Urschel 1995, Isguder et al. 2005, Lo et al. 2002, Lerut et al. 2002), and accounts for approximately 30-40% post operative deaths (Whooley et al. 2001, Griffin et al. 2001).

It has now become clear that post-operative infectious complications, in particular anastomotic leak, also compromise long term outcome in patients with colorectal cancer (McArdle, McMillan & Hole 2005, Marra et al. 2009, Jung et al. 2008). Recently, similar results have also been reported following both oesophageal (Lagarde et al. 2008) and gastric (Tsujimoto et al. 2009) resections. Therefore, infectious complications, in particular the anastomotic leak, can be catastrophic for the patient, both in terms of long and short term outcomes.

Currently, in patients who have undergone oesophago-gastric surgery an anastomotic leak is usually detected symptomatically or radiologically prior to re-

instituting normal feeding (approximately 8 days) and often when the patient has become critically ill (Griffin et al. 2001). Similarly, other infectious complications are only detected when the patient develops clinical symptoms. Clearly, when such infectious complications are detected at a late symptomatic stage they are likely to require major clinical intervention such as re-operation and or admission to intensive care. From the above, it is clear that early detection of post operative infectious complications may be of considerable clinical benefit.

Recently, it has been reported that biochemical markers of the systemic inflammatory response, in particular C reactive protein, provide an early indication of post operative infectious complications following gastrointestinal cancer surgery. Welsch and coworkers (2007), in 48 patients with infectious complications matched with 48 patient with no infectious complications undergoing surgery for rectal cancer, reported that increased C-reactive protein concentrations on postoperative day 3 were associated with infectious complications and the optimal threshold value was 140mg/l (Welsch et al. 2007). The same group reported, in 688 patients undergoing surgery for pancreatic disease, that increased C-reactive protein concentrations on postoperative day 4 were associated with infectious and the optimal threshold value was 140mg/l (Welsch et al. 2008). Korner and co-workers (2009) reported, in 231 patients undergoing surgery for colorectal cancer, that increased C-reactive protein concentrations on postoperative day 3 were associated with intra-abdominal infections with the threshold value being 190mg/l (Korner et al. 2009).

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

were associated with anastomotic leaks and the optimal threshold value was 125mg/l (Ortega-Deballon et al. 2010). Mackay and co-workers (2010) reported that, in 160 patients undergoing surgery for colorectal cancer, increased C-reactive protein concentrations on postoperative day 4 were associated with infectious complications and the optimal threshold value was 145mg/l (Mackay, Molloy & O'Dwyer 2010).

To date there is little information on the diagnostic value of post-operative C reactive protein concentrations in predicting post operative complications in patients undergoing oesophago-gastric cancer surgery. Deitmar and colleagues (2009) reported that, in 50 patients who developed an anastomotic leakage matched with 50 patient with no leak undergoing oesophagectomy, increased C-reactive protein concentrations on postoperative day 2 were associated with anastomotic leak and the optimal threshold value was 135mg/l (Deitmar et al. 2009).

Therefore, it is not clear what threshold concentration of C-reactive protein or what post-operative day is optimal for the detection of infectious complication in patients who have undergone surgery for oesophago-gastric cancer. The aim of the present study was to examine the value of serial daily post-operative markers of systemic inflammatory response like white cell count, albumin and C-reactive protein concentrations in the prediction of post-operative infectious complications in these patients.

3.2 Patients and Methods

3.2.1 Patients

Patients who underwent oesophago-gastric cancer resections in the Royal Infirmary, Glasgow, UK from January 2005 to August 2009 were included in the study. Patient characteristics were collected in a prospective surgical database. All patients were operated on electively with exception of three patients who underwent an emergency

procedure. The tumours were staged according to the tumour node metastasis (TNM) Criteria from the 6th edition of the International Union Against Cancer (UICC) Classification of the Malignant Tumours (Sobin, Wittekind & editors. April 2002). Tumours of the gastro-oesophageal junction were further subdivided according to site, using the Siewert classification; Type I and II lesions of the gastro-oesophageal junction were designated, as cancers of the oesophagus. Type III tumours of the cardia were designated gastric cancers (Siewert, Stein 1996).

Prior to surgery patients were assessed to establish their respiratory status. Normal respiratory status was considered as those patients without having shortness of breath on exertion or having no signs on chest X-ray. Pre-operatively the patients received DVT (deep venous thrombosis) prophylaxis and antibiotic prophylaxis. Patients with oesophageal cancer under went trans-hiatal or Ivor-lewis oesophagectomy and for gastric cancer partial or total gastrectomy. Patients with oesophago-gastric junctional tumour had Ivor-lewis oesophagectomy. All patients underwent potentially curative en-bloc resection with D2 lymph-adenectomy. Serial daily blood samples were taken for routine laboratory analysis of white cell count, albumin and C-reactive protein in the pre and post-operative period (days 1-7). Measurement of pre operative C-reactive protein was carried out in the few days prior to surgery as part of their routine work up, irrespective of whether or not they had received neoadjuvant chemotherapy. In the post-operative period patients had a daily clinical assessment and additional investigations were carried out as clinically indicated. All patients had anastomotic drains post-operatively which were left in position until radiological evidence of anastomotic integrity was confirmed.

Post-operative complications were recorded according to the recognised POSSUM definitions (Copeland, Jones & Walters 1991). Infectious complications were classified as surgical site infections (SSI) and remote infections (RI). For example, a surgical site infection included wound infection, anastomotic leak or intra-abdominal collection that

occurred following surgery and was associated specifically with the surgical procedure. An anastomotic leak was confirmed by radiology (i.e., contrast enhanced multi-detector CT scan or conventional radiology with water soluble contrast), endoscopy, or during surgical exploration. With reference to remote infections, an example would be pneumonia that occurred at sites not directly associated with the surgical procedure. Non-symptomatic or minor urinary tract infection were not recorded and therefore only recorded if complicated by sepsis.

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

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

3.2.2 Methods

The white cell count (WCC) (reference range 4–10×109/L) was analyzed using a hematological blood analyzer (Advia 120, Bayer, or CellDyn, Abbott). Serum concentrations of albumin (normal range 35-50 g/L) and C-reactive protein (normal range 0-10 mg/L) were measured by a BCG dye-binding method and turbidometric assay, respectively, using an auto-analyser (Abbott Diagnostics, Abbott Park, IL). The coefficient of variation for these methods, over the range of measurement, was less than 10% as established by routine quality control procedures.

3.2.3 Statistics

Data are presented as median and range. Data from different time periods were tested for statistical significance using the Friedman test and where appropriate comparisons of data from different time periods were carried out using the Wilcoxon signed rank test. The diagnostic accuracy of white cell count, C-reactive protein and albumin was assessed by ROC (Receiver Operator Curve) curve analysis (Robertson, Zweig 1981, Zweig, Campbell 1993, Soreide 2009). The area under the ROC curve (AUC) is a direct measure of the diagnostic accuracy of the test. An AUC value greater than >0.50 indicates the ability of a test to significantly discriminate between positive and negative cases with regard to the classification variable (e.g., presence or absence of disease). A test with an AUC greater than 0.80 was considered as having a high diagnostic accuracy and indicates that at least 80% of the patients with the disease were classified correctly. A P value <0.05 (two-sided tests) was considered significant. Statistical analysis was performed using SPSS version 15.0 for Windows (SPSS Inc, Chicago, IL).

3.3 Results

One hundred and thirty six patients underwent oesophago-gastric cancer resection during the study period. The relationship between patient characteristics and the development of post operative infectious and non-infectious complications is shown in Table 3-1. The majority of patients were under the age of 65 years (51%), were male (73%), had an ASA of 1 or 2 (85%), normal respiratory status (84%), normal heart rate (70%) and were not deprived (64%). The majority of patients had a pre-operative white cell count (94%), albumin (86%) and C-reactive protein concentration (82%) within the normal range.

Similar numbers of patients underwent resection for oesophageal and gastric cancer. Of the 136 patients studied 54 (40%) developed infectious complications and 13

(10%) developed non-infectious complications in post operative period. Of the thirteen non-infectious complications; there were four patients who developed cardio-vascular complications, three developed respiratory failure without infection and six developed other complications (Table 3-1). In those patients who developed complications they were more likely to be male ($p<0.05$), had a higher ASA score ($p<0.05$) and had abnormal respiratory status pre-operatively ($p<0.05$). The development of post-operative complications was also related with the type of the operation ($p<0.05$).

The relationship between circulating white cell count, albumin and C reactive protein concentrations and infectious and non-infectious complications in the first seven post operative days are shown in 3- 2. Compared with those patients who did not develop complications the white cell count was significantly higher on post-operative day 4 ($p<0.05$), day 5 ($p<0.01$), day 6 ($p<0.01$), and day 7 ($p<0.05$) in those patients who developed infectious and non-infectious complications. Similarly, albumin was significantly lower on post-operative day 5 ($p<0.01$), day 6 ($p<0.01$), and day 7 ($p<0.01$) in those patients who developed infectious and non-infectious complications. In contrast C reactive protein was significantly higher on post-operative day 1 ($p<0.05$), day 3 ($p<0.01$) day 4 ($p<0.01$), day 5 ($p<0.01$), day 6 ($p<0.01$), and day 7 ($p<0.01$) in those patients who developed infectious and non-infectious complications.

Of the 54 infectious complications 28 were surgical site infections and 26 were remote site infections. Of the 28 surgical site infections seventeen patients developed an anastomotic leak by the median post op day 6 (range 2-13). A further five patients developed wound infection and six patients developed a deep infection (intra abdominal or intra thoracic) or collection. Of the 26 remote site infections twenty-one developed a chest infection, three developed septicaemia, one developed urinary tract infection and one developed clostridium difficile diarrhoea

The relationship between circulating white cell count, albumin and C reactive protein concentrations and surgical site and remote infectious complications in the first seven post operative days are shown in Table 3-3. Compared with those patients who did not develop complications the white cell count was significantly higher on post operative day 5 ($p<0.01$), day 6 ($p<0.01$), and day 7 ($p<0.001$) in those patients who developed surgical site infectious complications. Similarly, the white cell count was significantly higher on post-operative day 4 ($p<0.05$), day 5 ($p<0.001$), day 6 ($p<0.001$), and day 7 ($p<0.01$) in those patients who developed remote site infectious complications (Figure 3-1).

Compared with those patients who did not develop complications albumin was significantly lower on post operative day 3 ($p<0.05$), day 4 ($p<0.01$), day 5 ($p<0.001$), day 6 ($p<0.001$), and day 7 ($p<0.001$) in those patients who developed surgical site infectious complications. Similarly, albumin was significantly lower on post-operative day 2 ($p<0.05$), day 3 ($p<0.05$) day 4 ($p<0.05$), day 5 ($p<0.01$), day 6 ($p<0.001$), and day 7 ($p<0.001$) in those patients who developed remote site infectious complications (Figure 3-2).

Compared with those patients who did not develop complications C-reactive protein was significantly higher on post operative day 1 ($p<0.01$), day 2 ($p<0.01$), day 3 ($p<0.001$), day 4 ($p<0.001$), day 5 ($p<0.001$), day 6 ($p<0.001$), and day 7 ($p<0.001$) in those patients who developed surgical site infectious complications. Similarly, C-reactive protein was significantly higher on post-operative day 5 ($p<0.01$), day 6 ($p<0.001$), and day 7 ($p<0.001$) in those patients who developed remote site infectious complications (Figure 3-3).

Compared with those patients who did not develop anastomotic leaks (n=119), white cell count was not significantly different to those who developed anastomotic leak

(n=17, Figure 3-4). In contrast, C-reactive protein was significantly higher from post operative day 3 onwards in those patients who developed anastomotic leaks (Figure 3-5).

When the relationship between peri-operative concentration of C-reactive protein and the development of the post-operative complications were examined only in those patients who underwent a thoracotomy (n=57), day 3 and day 4 C-reactive protein concentrations were associated with the development of post-operative infectious complications (p=0.072 and p=0.012 respectively).

In order to establish a threshold for the relationship between C reactive protein and the development of infectious complications following surgery for oesophago-gastric cancer Receiver Operator Curves (ROC) were plotted for post operative days 3 and 4 (Figure 3-6 and 3-7). On post operative day 3 a threshold of 180 mg/l was associated with the development of a SSI, providing a sensitivity of 71%, specificity of 65%, and a diagnostic accuracy of 0.736 (95% CI, 0.623–0.848; p <0.001). For remote site infections the diagnostic accuracy of a C reactive protein threshold of 180 mg/l on post op day 3 was 0.463 (95% CI, 0.350–0.577; p =0.567).

On post operative day 4 a threshold of 180 mg/l was associated with the development of a SSI, providing a sensitivity of 61%, specificity of 86%, and a diagnostic accuracy of 0.788 (95% CI, 0.680–0.895; p <0.001). For remote site infections the diagnostic accuracy of a C reactive protein threshold of 180 mg/l on post op day 4 was 0.502 (95% CI, 0.375–0.629; p=0.977).

On post operative day 3, a threshold of 180 mg/l was associated with the development of an anastomotic leak, providing a sensitivity of 82%, specificity of 63%, and a diagnostic accuracy of 0.808 (95% CI, 0.683–0.933; P<0.001). On post operative day 4, a threshold of 180 mg/l was associated with the development of an anastomotic

leak, providing a sensitivity of 71%, specificity of 83%, and a diagnostic accuracy of 0.857 (95% CI, 0.751–0.963; P<0.001).

3.4 Discussion

The results of the present study show that the magnitude of the systemic inflammatory response, in particular C reactive protein, following resection for oesophago-gastric cancer is associated with the development of post operative complications, in particular surgical site infections and anastomotic leakage. Furthermore, a C reactive protein threshold of 180mg/l on post operative days 3 and 4 was shown to predict SSI and anastomotic leak with good and very good diagnostic accuracy respectively. When those patients who underwent a thoracotomy alone were examined day 3 and day 4 C-reactive protein predicted the development of infectious complications.

In the present study the pre-operative C-reactive concentrations was not significantly associated with the development of the post-operative complications (p=0.186). This is in contrast to previous studies in patients undergoing resection for colorectal cancers (Moyes et al. 2009). However, they examined the pre-operative mGPS (modified Glasgow Prognostic Score) (McMillan 2008), a combination of C-reactive protein and albumin, in 455 patients. In the present study in 136 patients undergoing resection for oesophago-gastric cancer the pre operative mGPS was more strongly associated with the development of the post-operative complication (p=0.106). Therefore, the present study may have been underpowered to detect an association between the pre-operative systemic inflammatory response and the development of the post operative complications.

The results of the present study are consistent with previous studies which have highlighted the superiority of post operative concentration of C reactive protein, compared with white cell count in predicting the development of the infectious complications.

However these studies have suggested a variety of C reactive protein thresholds in different post operative days to predict the likelihood of developing a surgical site infectious complications (Welsch et al. 2007, Welsch et al. 2008, Korner et al. 2009, Deitmar et al. 2009, Ortega-Deballon et al. 2010, Mackay, Molloy & O'Dwyer 2010). When these threshold values (including the present study) in different post operative days were applied to the data of the present study (Table 3-4), on day 3 there was variation in the sensitivity between 64% and 89% and specificity between 38% to 67% in predicting surgical site infection. Also, on day 4 there was variation in the sensitivity predicting surgical site infection between 61% and 79% and specificity between 55% to 86%. Therefore depending on the threshold value of the C reactive protein used and the post operative day chosen there is a considerable variation in the predictive value of the C reactive protein.

Nevertheless, it was of interest that the sensitivity and the specificity of the C reactive protein thresholds established in previous studies both on day 3 and day 4 were similar to that in the present study. Furthermore, that the sensitivity and specificity of the C reactive protein threshold were consistently better on day 4. These results have a number of important implications. Firstly, that clinical monitoring of daily C reactive protein concentrations provides some prior warning of the development of a surgical site infectious complication, in particular an anastomotic leak. We would suggest that a C reactive protein concentration between 170mg/l and 190mg/l on post operative day 3 and 4 is indicative of the development of a surgical site infectious complication following oesophago-gastric cancer resection. Given that, both in the present and previous studies, the average time for the development of an anastomotic leak is between post operative day 6 and 8, daily monitoring of C reactive protein concentrations may substantially improve the early detection of an anastomotic leak and perhaps its subsequent treatment. Clearly, if this was proven to be the case then this would be the major contribution to reducing the

post operative morbidity and mortality associated with resection of oesophago-gastric cancer.

It was of interest that the post-operative changes of C-reactive protein were significantly associated with the development of infectious complications, in particular anastomotic leaks, whereas changes of white cell count were not. The reasons for the discrepancy in their relative prognostic value is not clear. Usually, a rise in circulating C-reactive protein concentrations considered as a result, rather than a cause, of an infectious complication. However, it may be that C-reactive protein is more sensitive to the presence of infection. Indeed, C-reactive protein has an important role in innate immunity as an early defence against infection, assisting complement-binding to foreign and damaged cells and enhancing phagocytosis by macrophages. More recently, through activation of complement and interaction with Fcgamma receptors, C-reactive protein has been shown to provide a link between the innate and adaptive immune systems (Peisajovich et al. 2008, Coventry et al. 2009, Du Clos, Mold 2004, Sander et al. 2010). Furthermore, With increasing concentrations of C-reactive protein there is a depression of T-lymphocyte function (Fietta et al. 2009, Wichmann et al. 2005). Also, with increasing C-reactive protein there is an increase in the stress response and the degree of hyperglycaemia (Motoyama et al. 2010) and that post-operative hyperglycaemia has recently been shown to be an important factor associated with the promotion of bacterial growth and the development of post-operative infectious complications (Ramos et al. 2008, Ambiru et al. 2008). Therefore, in addition to giving advance notice of a clinical infection, it may also play an important direct role in modulating the post-operative immune function of patients with oesophago-gastric cancer.

In present study, although the non-infectious complications were 4 times less common than the infectious complications, it was of interest that there were elevated post operative C reactive protein concentrations in patients with non infectious complications as

well. Therefore, this should be considered when the specificity of C reactive protein for infectious complications and anastomotic leakage is interpreted.

In summary, early identification of post-operative infectious complications in patients undergoing oesophageal or gastric resection for cancer is crucial in order to provide adequate therapeutic interventions. The present study shows that post-operative CRP measurements on days 3 and 4 predicts surgical site infectious complications, in particular an anastomotic leak, following resection for oesophago-gastric cancer.

Table 3-1: Characteristics of oesophago-gastric cancer patients with and without postoperative complications

	No Complications (n=69)	Infectious Complications (n=54)	Non-infectious Complications (n=13)	P value
Age (<65/ 65-74/ ≥75 years)	37/25/7	26/21/7	6/6/1	0.597
Sex (Male/ Female)	44/25	45/9	10/3	0.046
ASA (1/2/3/4)	14/49/6/0	5/37/11/1	1/9/3/0	0.014
Respiratory status (Normal/ Abnormal)	61/8	43/11	8/5	0.019
Heart rate (50-80/ >80 or <50)	49/20	38/16	8/5	0.595
Deprivation (1-2/3-5/6-7)[a]	11/37/21	5/27/22	3/4/6	0.411
Tumour site (Oesophageal/ Gastric)	30/39	32/22	8/5	0.164
Type of operations Partial gastrectomy/ Total gastrectomy/ Trans-hiatal oesophagectomy/ Ivor-lewis oesophagectomy	16/20/12/21	7/11/6/30	2/1/4/6	0.016
Operative blood loss (101-500/ 501-999/ ≥1000 ml)	29/20/20	6/30/18	5/5/3	0.213
TNM stage (1/2/3/4)	27/14/27/1	18/22/12/2	6/3/3/1	0.621
Neoadjuvant therapy (Yes/No)	36/33	36/18	8/5	0.261
Pre-operative white cell count (<8.5/ 8.5–11/>11x10^9/l)	57/ 9/ 3	44/ 6/ 4	10/ 2/ 1	0.541
Pre-operative albumin (≥35/ <35 g/l)	60/ 9	48/ 6	9/ 4	0.291
Pre-operative C-reactive protein (≤10/ >10 mg/l)	60/ 9	42/ 12	10/ 3	0.186

[a]Individual deprivation categories were used in the statistical analysis

Table 3-2:The relationship between serial post-operative values of white cell count, albumin and C-reactive protein and the development of infectious and non-infectious complications.

	No complications (n=69)	Infectious complications (n=54)	P value[a]	Non-infectious complications (n=13)	P value[a]
Pre op WCC	6.2 (1.6-15.6)	7.0 (2.2-17.9)	0.283	5.5 (2.2-11.5)	0.485
WCC day 1	10.2 (4.3-17.9)	11.3 (5.6-25.9)	0.103	11.3 (5.4-14.9)	0.969
WCC day 2	10.9 (6.4-20.9)	11.7 (2.2-32.7)	0.326	11.9 (7-18.7)	0.172
WCC day 3	9.1 (4.5-18.4)	9.6 (1.9-22.6)	0.293	12.0 (5.5-20.2)	0.137
WCC day 4	7.6 (3.8-17.5)	9.2 (2.6-18.5)	0.022	9.7 (3.8-15.8)	0.045
WCC day 5	6.7 (3.9-16)	10.3 (3.5-19.6)	<0.001	9.2 (4.8-17.1)	0.003
WCC day 6	7.4 (4.4-15)	11.5 (5.3-21.7)	<0.001	9.7 (5.9-21.7)	0.008
WCC day 7	8.1 (3.9-16.6)	12.9 (3.5-34.9)	<0.001	10.7 (5.7-20.7)	0.015
Pre op Alb	40 (20-47)	39 (22-49)	0.630	39 (15-32)	0.844
Alb day 1	23 (15-32)	23 (13-31)	0.265	26 (19-33)	0.579
Alb day 2	25 (16-33)	23 (15-29)	0.033	22 (18-31)	0.265
Alb day 3	24 (16-33)	22 (14-29)	0.008	23 (16-31)	0.214
Alb day 4	25 (16-37)	22 (13-30)	<0.001	23 (15-29)	0.077
Alb day 5	26 (18-37)	21 (11-34)	<0.001	21 (16-31)	0.004
Alb day 6	27 (18-40)	22 (10-33)	<0.001	22 (13-34)	0.002
Alb day 7	28 (17-38)	21 (10-34)	<0.001	22 (11-36)	0.001
Pre op CRP	4.5 (1-77)	3.6 (1-85)	0.515	6 (1-31)	0.361
CRP day 1	114 (10-214)	140.5 (25-306)	0.010	160 (65-332)	0.038
CRP day 2	196.5 (53-328)	216 (95-350)	0.077	268 (90-366)	0.008
CRP day 3	152.5 (42-298)	183.5 (61-392)	0.006	295 (46-369)	0.001
CRP day 4	108 (37-226)	163.5 (40-393)	<0.001	229 (20-300)	0.002
CRP day 5	80.5 (18-293)	196.5 (25-321)	<0.001	167 (14-341)	0.006
CRP day 6	66 (16-312)	181 (16-395)	<0.001	144 (11-316)	0.003
CRP day 7	55 (13-293)	179.5 (12-429)	<0.001	140 (19-344)	0.004

Median (range), WCC white cell count, Alb albumin, CRP C reactive protein.
[a] compared with no complications.

Table 3-3:The relationship between serial post-operative values of white cell count, albumin and C-reactive protein and the development of surgical site and remote infectious complication

		Infectious complication (n=54)			
	No complications (n=69)	Surgical site infection (n=28)	P value[a]	Remote infection (n=26)	P value[a]
Pre op WCC	6.2 (1.6-15.6)	6.2 (2.2-17.9)	0.729	7 (2.6-13.7)	0.164
WCC day 1	10.2 (4.3-17.9)	11.8 (7-25.9)	0.016	10.8 (5.6-19.1)	0.874
WCC day 2	10.9 (6.4-20.9)	11.8 (6.5-32.7)	0.208	11.2 (2.2-20.5)	0.761
WCC day 3	9.1 (4.5-18.4)	9.4 (4.4-22.6)	0.363	10.2 (1.9-16.9)	0.441
WCC day 4	7.6 (3.8-17.5)	8.7 (4.1-18.5)	0.183	9.9 (2.6-16.4)	0.017
WCC day 5	6.7 (3.9-16)	9.0 (3.5-17.9)	0.007	12.0 (4.7-19.6)	<0.001
WCC day 6	7.4 (4.4-15)	9.7 (5.3-21.7)	0.006	12.4 (6.6-20.5)	<0.001
WCC day 7	8.1 (3.9-16.6)	12.9 (6.8-34.9)	<0.001	13.5 (3.5-32.7)	0.002
Pre op Alb	40 (20-47)	39 (34-47)	0.925	40 (22-49)	0.373
Alb day 1	23 (15-32)	24 (13-31)	0.740	23 (14-28)	0.139
Alb day 2	25 (16-33)	24 (16-29)	0.225	23 (15-26)	0.027
Alb day 3	24 (16-33)	22 (16-29)	0.028	22 (14-29)	0.044
Alb day 4	25 (16-37)	21 (15-29)	0.002	22 (13-30)	0.012
Alb day 5	26 (18-37)	21 (14-30)	<0.001	21 (11-34)	0.001
Alb day 6	27 (18-40)	22 (13-31)	<0.001	22 (10-33)	<0.001
Alb day 7	28 (17-38)	23 (11-33)	<0.001	21 (10-34)	<0.001
Pre op CRP	4.5 (1-77)	3.7 (1-85)	0.346	3.2 (1-71)	0.936
CRP day 1	114 (10-214)	146 (25-306)	0.007	139 (25-241)	0.153
CRP day 2	196.5 (53-328)	221 (95-350)	0.007	196 (106-310)	0.930
CRP day 3	152.5 (42-298)	219 (61-392)	<0.001	160 (69-316)	0.562
CRP day 4	108 (37-226)	212 (42-393)	<0.001	143 (40-328)	0.171
CRP day 5	80.5 (18-293)	217 (33-321)	<0.001	133 (25-313)	0.008
CRP day 6	66 (16-312)	189 (27-333)	<0.001	126 (16-395)	<0.001
CRP day 7	55 (13-293)	196 (18-378)	<0.001	139 (12-429)	<0.001

Median (range)
WCC white cell count, Alb albumin, CRP C reactive protein.
[a] compared with no complications.

Table 3-4: Comparison of different threshold values of C- reactive protein in predicting various infective complications in the study cohort.
OG: Oesophago-gastric; O: oesophageal; CR: colorectal; R: rectal and panc: pancreatic. POD: post operative day; CRP C reactive protein.

					Analysis of CRP threshold in the present study (n=136)					
Study	Type	Patients (n)	POD	CRP Threshold	Infectious complications		Surgical site infection		Anastomotic leakage	
					Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Deitmar S et al	O	50	2	135	89	13	93	17	100	17
Kørner H et al	CR	231	3	190	46	65	64	67	77	66
Welsch T et al	R	48	3	140	78	39	89	38	94	36
This study	OG	136	3	180	52	64	71	65	82	63
MacKay G et al	CR	160	4	145	61	71	75	66	88	66
Welsch T et al	Panc	688	4	140	63	67	75	62	88	61
Ortega-Deballon et al	CR	133	4	125	65	57	79	55	88	53
This study	OG	136	4	180	43	90	61	86	71	83

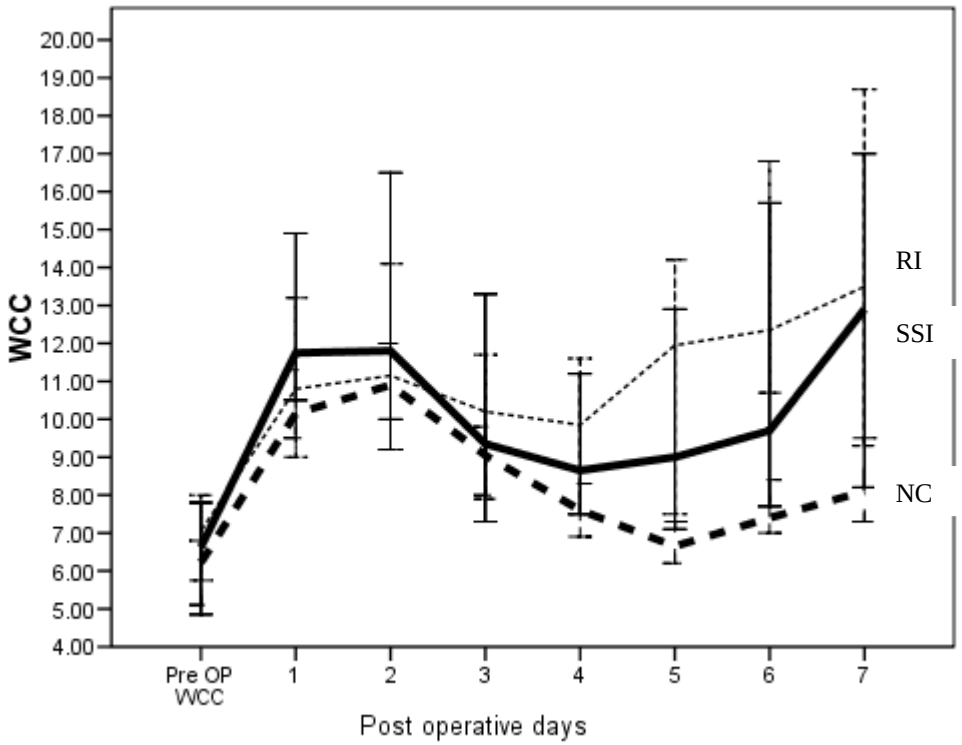

Figure 3-1: The perioperative changes in white cell count (10^9/ l) in patients with surgical site infection (SSI), remote site infection (RI) and no complication (NC).

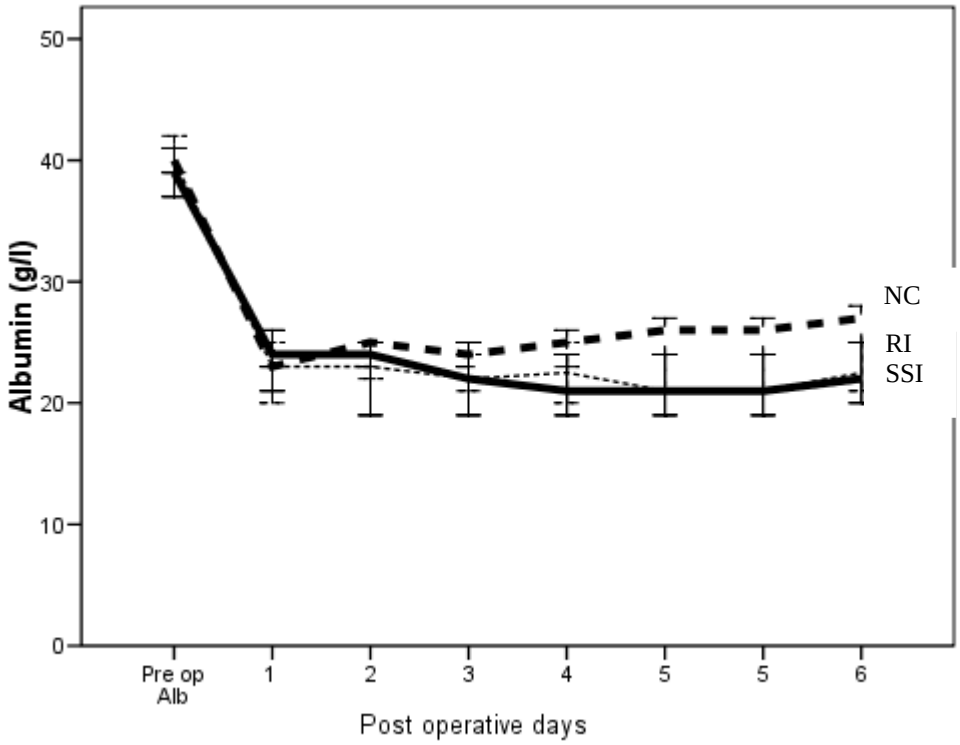

Figure 3-2: The peri-operative changes in albumin in patients with surgical site infection (SSI), remote site infection (RI) and no complication (NC).

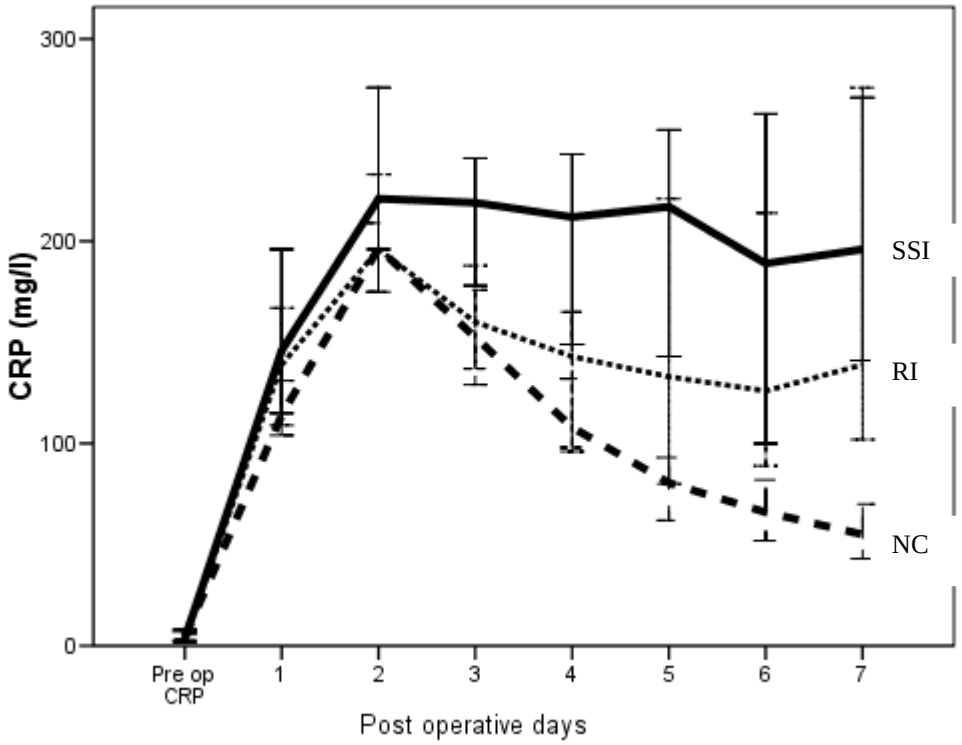

Figure 3-3: The peri-operative changes in C-reactive protein in patients with surgical site infection (SSI), remote site infection (RI) and no complication (NC).

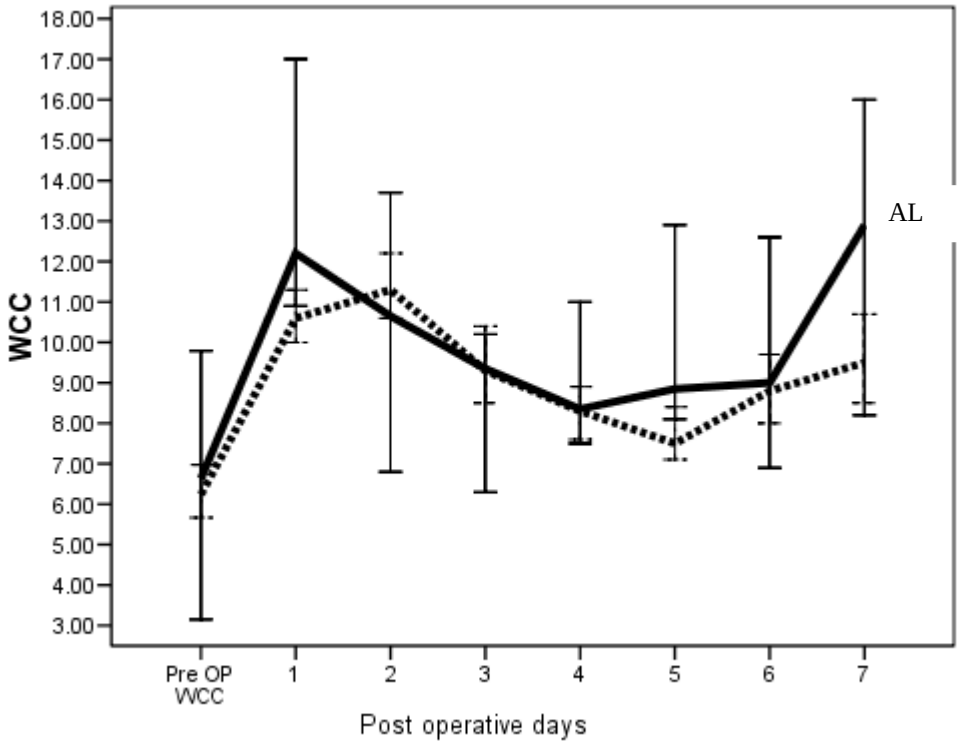

Figure 3-4: The peri-operative changes in white cell count (10^9/ l) in patients with anastomotic leaks versus patients with out anastomotic leak.

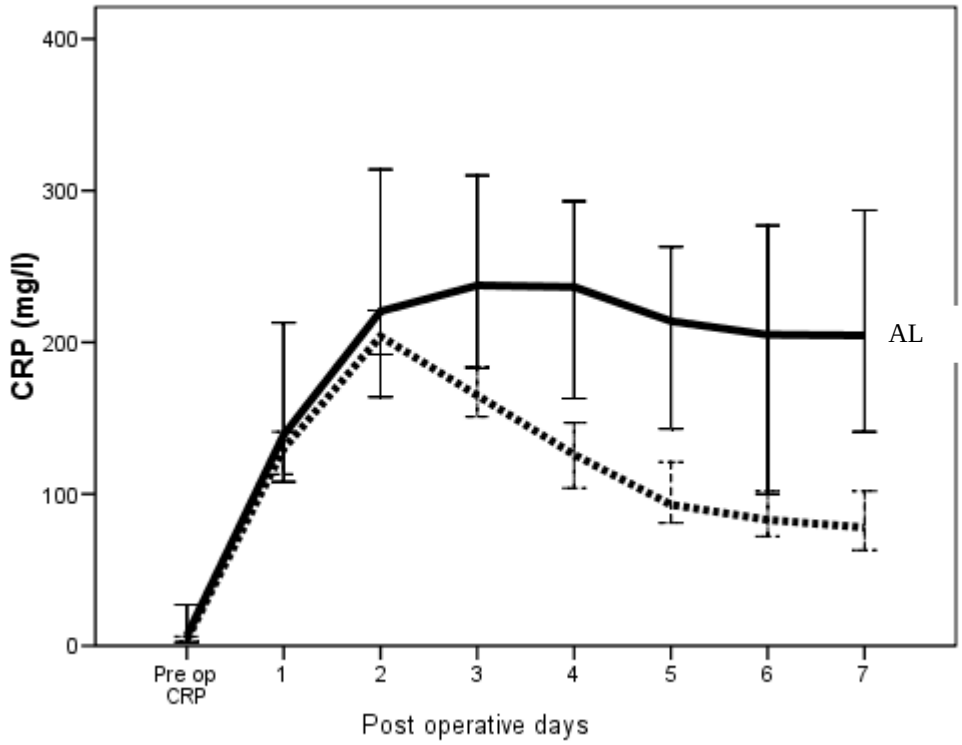

Figure 3-5: The peri-operative changes in C-reactive protein patients with anastomotic leaks versus patients with out anastomotic leak.

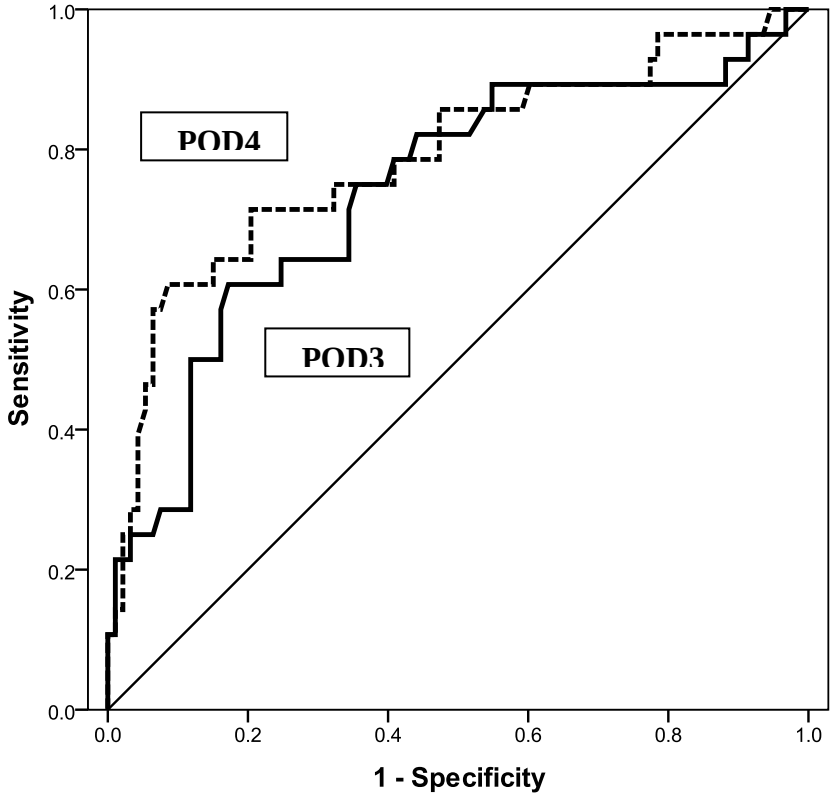

Figure 3-6: Diagnostic accuracy of CRP with regard to surgical site infections after oesophago-gastric cancer resection. Comparison of ROC curves for post operative day (POD) 3 and 4 with respective AUC values of 0.736 and 0.788.

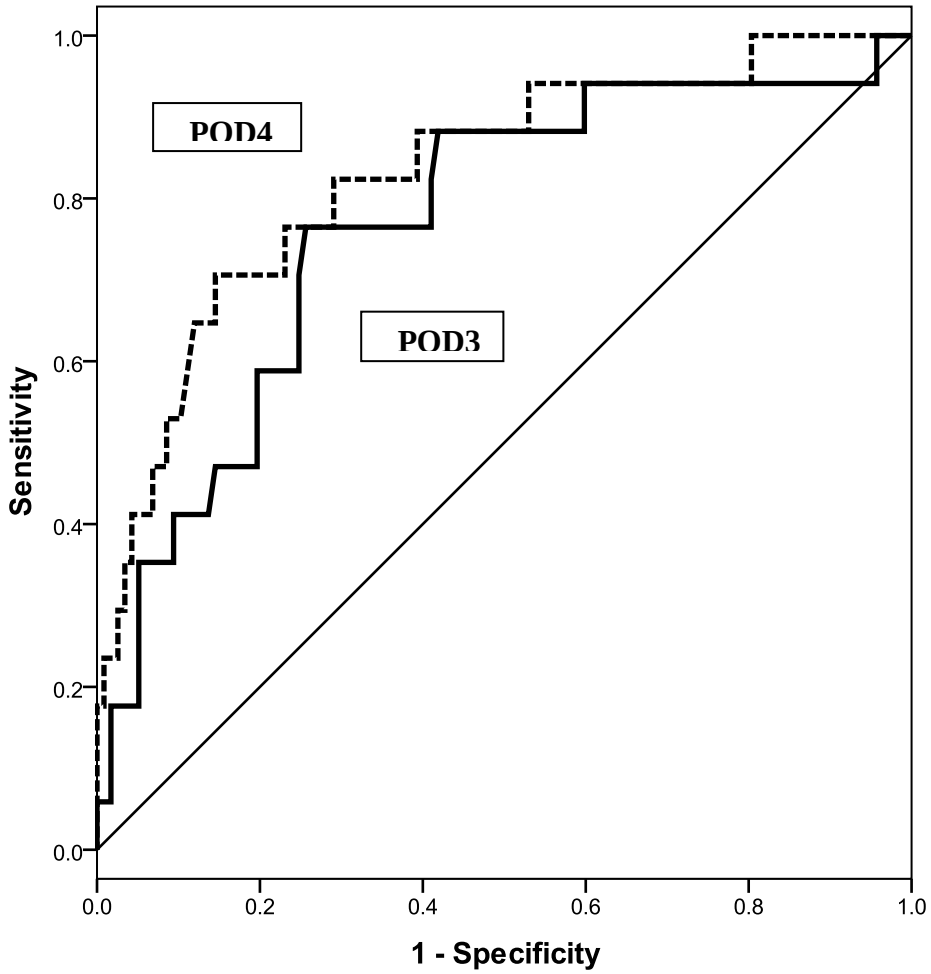

Figure 3-7: Diagnostic accuracy of CRP with regard to development of the anastomotic leaks after oesophago-gastric cancer resection. Comparison of ROC curves for post operative day (POD) 3 and 4 with respective AUC values of 0.808 and 0.857.

4 Chapter IV: Comparison of the prognostic value of tumour and patient related factors in patients undergoing potentially curative resection of oesophageal cancer

4.1 Introduction

Following curative resection for oesophageal cancer, pathological analysis for tumour related factors guides' prognosis. A variety of high risk features including tumour stage, resection margin, nodal status, the ratio of metastatic to examined lymph nodes are considered to be important (Liu et al. 2009, Roder et al. 1994, Hsu et al. 2009, Saha et al. 2009).

It is now increasingly recognised that the outcome for patients with cancer is not only determined by tumour related factors but also by host related factors, in particular the systemic inflammatory response (McMillan 2009, Roxburgh, McMillan 2010). With reference to oesophageal cancer an elevated C-reactive protein concentration prior to surgery has been reported to have independent prognostic value predicting poor survival (Nozoe, Saeki & Sugimachi 2001, Ikeda et al. 2003, Shimada et al. 2003, Gockel et al. 2006, Crumley et al. 2006). An elevated platelet count has also been shown to have a similar independent prognostic value (Shimada et al. 2004) in oesophageal cancer patients.

From such work a number of systemic inflammation based prognostic scores have been proposed to simplify and standardise the measurement of the systemic inflammatory response in routine clinical practice. These include the modified Glasgow Prognostic Score (mGPS), the Neutrophil Lymphocyte Ratio (NLR) and the Platelet Lymphocyte Ratio (PLR) all of which have been reported to be independent predictors of survival in gastrointestinal cancer (McMillan 2009). Recently, Crumley and co-workers (Crumley et al. 2010a) reported that the mGPS improved the pre treatment clinical staging of patients who presented with gastro-oesophageal cancer thus aiding clinical decision making in these difficult to treat patients. In terms of pathological staging in patients who have

undergone potentially curative surgery the relative prognostic value of the mGPS, NLR and PLR is not clear. More specifically, it is not clear which of these scores best predicts survival in oesophageal cancer.

Therefore, the aim of the present study was to compare the prognostic value of selected markers of the systemic inflammatory response in patients undergoing potentially curative resection for oesophageal cancer.

4.2 Patients and Methods

One hundred and twelve patients undergoing potentially curative resection for oesophageal carcinoma (including type I and II tumours of the gastro-oesophageal junction (Siewert, Stein 1996) in the upper GI surgical unit, Glasgow Royal Infirmary, between 1996 and 2008 were included in the study. Patients had laboratory measurement of white cell, neutrophil, lymphocyte and platelet counts, albumin and C-reactive protein in the few days prior to surgery as part of their routine work up, irrespective of whether or not they had received neoadjuvant chemotherapy. Clinical staging was performed primarily by radiological investigation using CT scan in the period 1996- 2006, using CT scan with endoscopic ultrasound in the period 2006-2007 and using CT scan with endoscopic ultrasound and PET scan in the period 2007 to present. From the period of 2005 to present all patients also underwent staging laparoscopy.

The tumours were staged according to the tumour node metastasis (TNM) Criteria from the 6th edition of the International Union Against Cancer (UICC) Classification of the Malignant Tumours (Sobin, Wittekind & editors. April 2002). Tumours of the gastro-oesophageal junction were further subdivided according to site, using the Siewert classification; Type I and II lesions of the gastro-oesophageal junction were designated, as cancers of the oesophagus. Type III tumours of the cardia were designated gastric cancers (Siewert, Stein 1996) and were therefore excluded from the study.

All patients underwent potentially curative en-bloc resection with lymph-adenectomy (median 18, range 6–44 nodes resected) and survived at least 30 days following surgery. Thirty four patients received neo-adjuvant chemotherapy, mostly in last two years of the study period. Oesophageal cancer patients and Type I gastro-oesophageal junctional tumour received 2 cycles of pre operative cisplatin and 5-flurouracil as in the MRC OE-O2 study (Medical Research Council Oesophageal Cancer Working Group 2002). Type II gastro-oesophageal junctional tumour was treated with 3 cycles of ECF (Epirubicin, Cisplatin and 5-Flurouracil).

Patients who had clinical evidence of infection or other inflammatory condition (e.g. vasculitis, connective tissue disorders, rheumatological conditions) were excluded from the study.

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

The coefficient of variation for the routine laboratory measurements of absolute white cell, neutrophil, lymphocyte, and platelet counts, albumin and C-reactive protein, over the range of measurement, was less than 10% as established by routine quality control procedures. The limit of detection of the assay was a C-reactive protein concentration lower than 6mg/l. A C-reactive protein concentration of greater than 10mg/l was considered to indicate the presence of a systemic inflammatory response (McMillan et al. 2001)

The mGPS was constructed as previously described (McMillan 2008). An elevated C-reactive protein (>10 mg/l) were assigned a modified GPS score (mGPS) of 1 or 2

depending on the absence or presence of hypo-albuminaemia (<35g/l). Patients in whom neither of these abnormalities was present were allocated a score of 0.

4.2.1 Statistics

The laboratory variables were grouped using standard thresholds (Ikeda et al. 2002, McMillan et al. 2001a, Maltoni et al. 2005, Yamanaka et al. 2007). Survival analysis of the group variables was performed using the Cox proportional hazard model. Deaths up to the end of February 2010 were included in the analysis. Multivariate survival analysis, including all significant covariates ($P \leq 0.05$ to account for multiple comparisons) 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. The relationships between the mGPS and other variables were analysed using the Mantel–Haenszel (X^2) test for trend as appropriate. Analysis was performed using SPSS software (SPSS Inc., Chicago IL, USA).

4.3 Results

The relationship between clinico-pathological characteristics, systemic inflammatory response and survival in patients with oesophageal cancer is shown in Table 4-1. Overall, the majority of patients were less than the age of 65years old (61%), male (76%) and had adenocarcinoma (78%). The majority of patients had TNM stage II or III (81%) disease. The majority of patients had white cell (93%), neutrophil (89%), lymphocyte (91%), and platelet (96%) counts in the normal range. Of the 112 patients who underwent a curative resection for oesophageal cancer, only 13 (12%) patients had a mGPS of 1 (Table 4-1).

Majority of patients had curative surgery (n=111) except for one patient who had pathological T4 disease. The median follow up of the survivors was 55 months and the minimum was 15 months. During this period 52 (46%) patients died of their cancer and 7 (6%) patients died of non-cancer causes. On univariate analysis only TNM stage (p=0.001), Tumour differentiation (p=0.001), positive to total lymph node ratio (LNR) (p<0.001), adjuvant therapy (p=0.012) and mGPS (p=0.001) were significantly associated with cancer specific survival (Table 4-1). On multivariate analysis, LNR (HR 2.87, 95% CI 1.99 - 4.15, p<0.001, Figure 4-1) and mGPS (HR 4.31, 95% CI 2.20 - 8.45, p<0.001, Figure 4-2) retained independent significance (Table 4-1). When the lymph node ratio was removed from the model TNM stage (HR 2.18, 95% CI 1.40-3.39, p=0.001) retained independent prognostic value. In our study only 25 patients had squamous cell carcinoma and 87 patients had adenocarcinoma. Only 5 patients had positive mGPS score in squamous cell carcinoma group and 8 patients had positive mGPS in adenocarcinoma group (Table 4-2). When patients with adenocarcinomas (n=87, 8 patients with a mGPS of 1) underwent multivariate survival analysis, only LNR (HR 3.01, 95% CI 1.98-4.57; p<0.001) and mGPS (HR 3.72, 95% CI 1.67-8.31; p = 0.001) were independently associated with cancer specific survival.

The relationship between the mGPS and clinicopathological characteristics in oesophageal cancer patients is shown in Table 4-2. An increase in the mGPS was associated with high white cell count (p<0.05). Patients with mGPS 0 had mean survival of 83 months and patients with mGPS 1/ 2 had mean survival of 31 months (p=0.001).

4.4 Discussion

In the present study a comparison of the relationship of a number of cellular components of the systemic inflammatory response and cancer specific survival was examined in patients undergoing potentially curative resection for oesophageal cancer. Of

the inflammation based prognostic scores, the mGPS, NLR and PLR, only mGPS was significantly associated with cancer specific survival and had prognostic value independent of pathological TNM stage.

These results are consistent with the recent report of Rashid and co-workers (2010) that, in 294 patients undergoing oesophageal cancer resection, the pre operative neutrophil lymphocyte ratio did not offer useful prognostic value (Rashid et al. 2010). Moreover these results are consistent with previous work that compared the prognostic value of the mGPS and NLR in patients undergoing potentially curative resection for colorectal cancer (Leitch et al. 2007). Therefore, it would appear that the systemic inflammation-based prognostic scores based on the acute-phase proteins, C-reactive protein and albumin, are superior to those based on the cellular components of the white cell count, specifically neutrophils, lymphocyte and platelets. Taken together this would suggest that mGPS has superior prognostic value in gastrointestinal cancer and should be used in preference to cellular markers of the systemic inflammatory response.

The basis of the relationship between the systemic inflammatory response and poorer cancer survival in patients with oesophageal cancer is not clear. However, the components of the mGPS are recognised to be associated with cancer cachexia (McMillan 2008, Morley, Thomas & Wilson 2006, Fearon et al. 2006), compromised cell-mediated immunity (Du Clos, Mold 2004, Canna et al. 2005) and upregulation of growth factors and angiogenesis (de Jong et al. 2004, Krzystek-Korpacka et al. 2008). Therefore, the mechanisms underlying the relationship between systemic inflammatory responses and cancer specific survival are likely to be complex. These include extrinsic pathways such as nutritional and functional decline, immune dysfunction and tumour angiogenesis, growth and dissemination. More specifically, recent work in operable colorectal cancer has pointed to a strong relationship between poor patient physiology (Richards et al. 2010) increased cormorbidity (Roxburgh et al. 2010) and an increased mGPS. Therefore, it may

be that an elevated mGPS, prior to surgery, in patients with oesophageal cancer reflects poor patient physiology and comorbidity. Further studies in oesophageal cancer are required to define these relationships. 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 (Colotta et al. 2009).

Irrespective of the mechanisms involved, there is now evidence that the mGPS is useful in the clinical staging (Crumley et al. 2010a) of oesophageal cancer, predicting the response to neoadjuvant chemotherapy (Kobayashi et al. 2008) and, in the present study, predicting the response to surgery. However, the clinical use of the mGPS is as yet not clear. Surgery for oesophageal cancer has a high potential for morbidity and mortality and recovery to pre-resectional functional status, if ever reached, can take a year or more. Therefore, choosing patients with the highest potential for cure is extremely important. With reference to how the mGPS might fit into current pre-operative assessment of these patients this remains problematical since currently most surgeons would not deny a patients surgery where their tumour was localised. Very recently, Vashist and coworkers have published similar findings (Vashist et al. 2011). They concluded that 'Preoperative evaluation of the GPS may help to stratify oesophageal cancer patients to different risk profiles, which is essential in the era of customized therapy'. We also concur with this view that the mGPS should be evaluated prior to surgery and should be used in the risk stratification of patients with oesophageal cancer and could be incorporated into future prospective randomised trials of surgery in oesophageal cancer. Furthermore, with the increasing use of neoadjuvant therapy in oesophageal cancer prior to surgery and the

consequent reduction in the value of pathological findings, the value of the mGPS in clinical staging and predicting outcome will be of increasing importance in these patients.

In the present study the positive to total lymph node ratio was also independently associated with poorer cancer specific survival. Moreover the positive to total lymph node ratio had superior prognostic value compared to TNM stage. These results are consistent with recent studies that this ratio is superior to simply assessing nodal status in predicting cancer outcome in patients undergoing curative resection of oesophageal cancer (Mariette et al. 2008, Hsu et al. 2009, Dhar et al. 2007, Greenstein et al. 2008). Further work is required to establish whether positive to total lymph node ratio can be used routinely to supplant current TNM staging in these patients.

In summary, an acute-phase protein-based prognostic score, the mGPS, appears to be a superior predictor of cancer survival compared with cellular components of the systemic inflammatory response in patients undergoing potentially curative resection for oesophageal cancer.

Table 4-1: The relationship between clinico-pathological characteristics, the systemic inflammatory response and cancer specific survival in patients undergoing potentially curative resection for oesophageal cancer.

		Univariate analysis		Multivariate analysis	
	Patients (n=112)	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Tumour related factors					
TNM Stage (I/II/III/IV)	20/39/52/1	2.15 (1.39-3.3)	0.001		0.558
Tumour type (Adeno/ Squamous)	87/25	0.97 (0.51-1.86)	0.937		
Tumour differentiation (well-mod/poor)	68/44	2.44 (1.41-4.22)	0.001		0.161
Resection Margin (R0/R1)	86/26	1.67 (0.92-3.01)	0.091		
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	41/43/28	2.65 (1.83-3.83)	<0.001	2.87 (1.99-4.15)	<0.001
Neo-adjuvant therapy (yes/no)	34/78	0.87 (0.44-1.71)	0.677		
Adjuvant therapy (yes/no)	17/95	0.40 (0.20-0.82)	0.012		0.817
Patient related factors					
Age (<65/65 –74/≥75 years)	68/38/6	0.99 (0.63-1.55)	0.948		
Sex (male/female)	85/27	0.69 (0.35-1.38)	0.292		
White cell count (<8.5/ 8.5–11/ >11x10^9 l^{-1})	84/20/8	1.35 (0.89-2.10)	0.165		
Neutrophil count (<7.5/≥7.5x10^9 l^{-1})	100/12	1.59 (0.75-3.39)	0.231		
Lymphocyte count (>3.0/1.0 – 3.0/<1.0x10^9 l^{-1})	4/98/10	0.44 (0.14-1.35)	0.151		
Platelet count (<400/≥400x10^3 l^{-1})	107/5	0.72 (0.17-2.95)	0.644		
Neutrophil/lymphocyte ratio (<2.5/2.5-5/>5)	63/34/15	1.08 (0.75-1.56)	0.686		
Platelet/lymphocyte ratio (<150/150-300/>300)	60/44/8	0.94 (0.60-1.48)	0.781		
mGPS (0/1/2)	99/13/0	3.10 (1.61-5.95)	0.001	4.31 (2.20-8.45)	<0.001

Table 4-2: The relationship between the mGPS and clinicopathological characteristics and cancer specific survival in patients undergoing potentially curative resection for oesophageal cancer (n= 112).

	mGPS 0 (n= 99)	mGPS 1/2 (n= 13)	P-value
Tumour related factors			
TNM Stage (I/ II/ III/ IV)	19/ 32/ 47/ 1	1/ 7/ 5/ 0	0.984
Tumour (Adeno/ Squamous)	79/ 20	8/ 5	0.139
Tumour differentiation (well-mod/poor)	63/ 36	5/ 8	0.082
Resection Margin (R0/R1)	76/ 23	10/ 3	0.990
Total node retrieved (<15/≥15)	30/69	5/8	0.540
No of positive node (0/1-2/3-6/>6)	34/28/24/13	7/3/2/1	0.204
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	34/ 39/ 26	7/ 4/ 2	0.187
Neo-adjuvant therapy (yes/no)	31/ 68	3/ 10	0.546
Adjuvant therapy (yes/no)	14/ 85	3/ 10	0.401
Patient related factors			
Age (<65/ 65–74/ ≥75 years)	62/ 32/ 5	6/ 6/ 1	0.278
Sex (male/ female)	76/ 23	9/ 4	0.552
White cell count (<8.5/ 8.5–11/ >11x10^9 l^{-1})	77/ 17/ 5	7/ 3/ 3	0.018
Neutrophil count (<7.5/ ≥7.5x10^9 l^{-1})	90/ 9	10/ 3	0.127
Lymphocyte count (>3.0/ 1.0 – 3.0/ <1.0x10^9 l^{-1})	3/ 87/ 9	1/ 11/ 1	0.558

Platelet count (<400/ ≥400x10^3 l^{-1})	94/ 5	13/ 0	0.409
Neutrophil/lymphocyte ratio (<2.5/ 2.5-5/ >5)	57/ 31/ 11	6/ 3/ 4	0.143
Platelet/lymphocyte ratio (<150/ 150-300/ >300)	52/ 40/ 7	8/ 4/ 1	0.651
Survival in months (mean, 95% CI)	82.7 (70.3-95.1)	30.3 (15.5-45.1)	0.001*

* Mantel-Cox Log Rank test

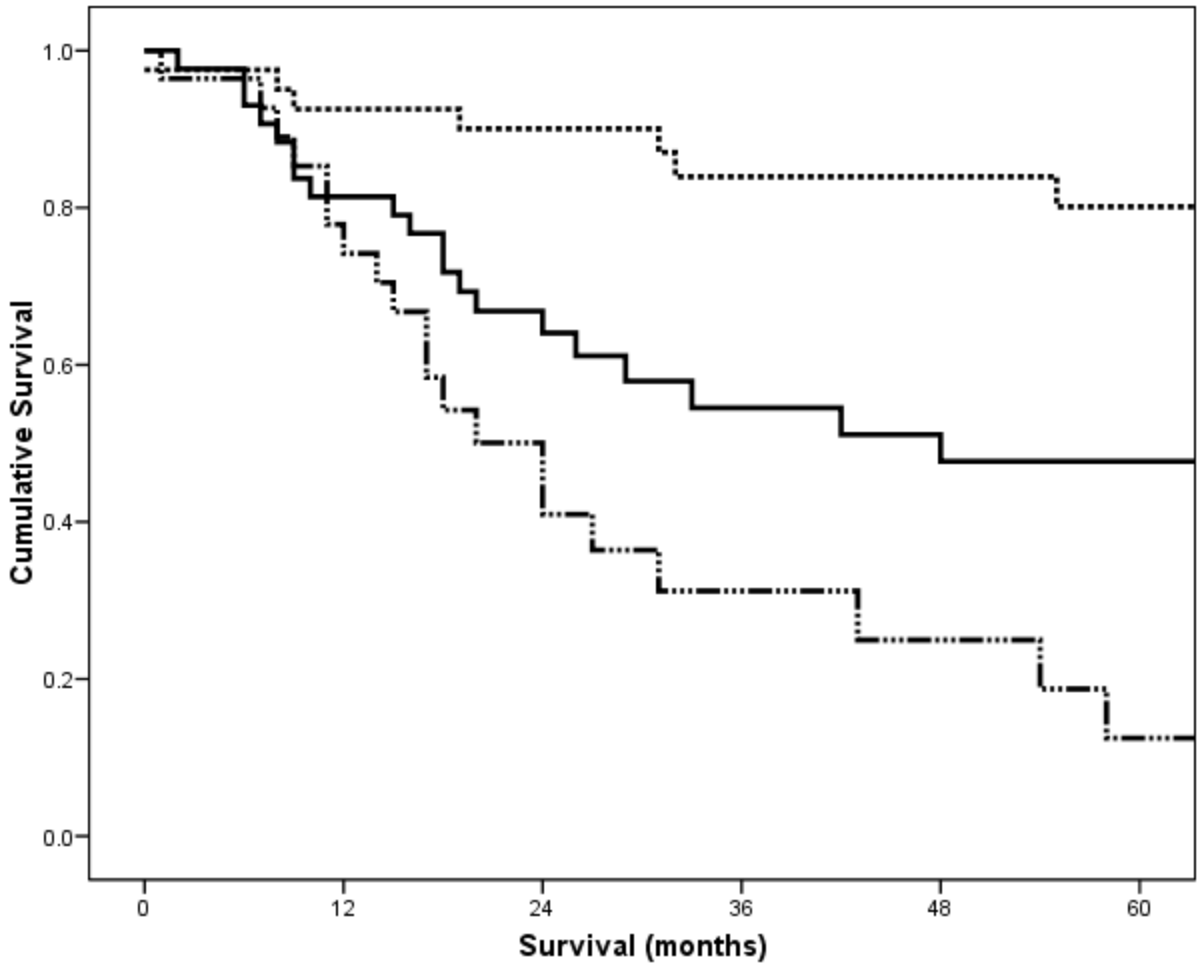

Figure 4-1: The relationship between the positive to total lymph node ratio ((0, ≤ 0.2 and >0.2 from top to bottom) and cancer-specific survival in patients undergoing surgery for oesophageal cancer.

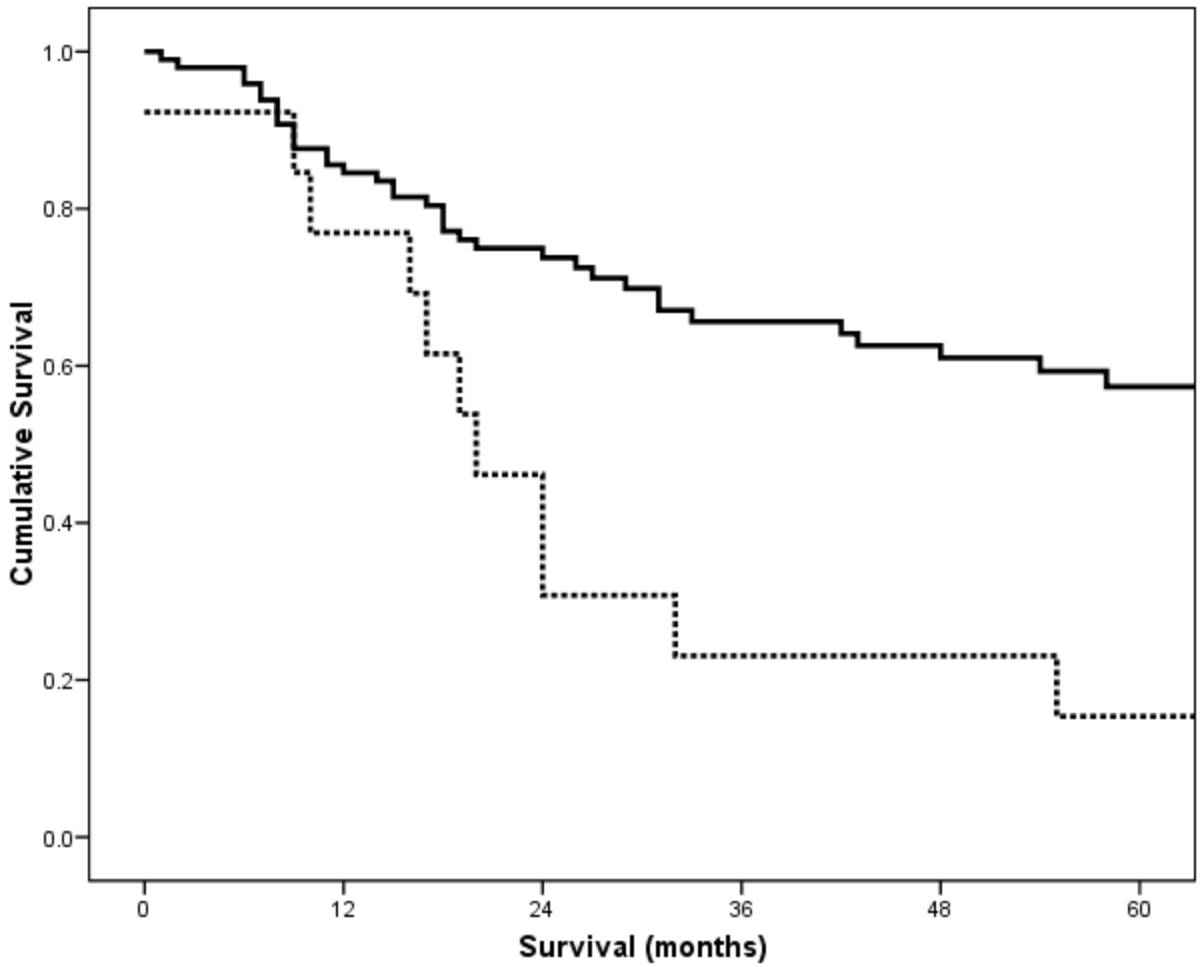

Figure 4-2: The relationship between mGPS (0 and 1 from top to bottom) and cancer-specific survival in patients undergoing surgery for oesophageal cancer.

5 Chapter V: Comparison of the prognostic value of tumour and patient related factors in patients undergoing potentially curative resection of gastric cancer

5.1 Introduction

Following curative resection for gastric cancer, pathological analysis of tumour related factors guide prognosis and treatment. A variety of high risk features including tumour stage, resection margin and nodal status are considered to be important in determining cancer recurrence and survival (Siewert et al. 1998, Setala et al. 1996, Wang et al. 2009).

It is now increasingly recognised that the outcome for patients with cancer is not only determined by tumour related factors but also by host related factors, in particular the systemic inflammatory response (McMillan 2009, Roxburgh, McMillan 2010). With reference to the gastric cancer an elevated C-reactive protein concentration prior to surgery has been reported to have independent prognostic value (Wang, Sun 2009, Chang et al. 2010).

From such work a number of systemic inflammation based prognostic scores have been proposed to simplify and standardise the measurement of the systemic inflammatory response in routine clinical practice. These include the modified Glasgow Prognostic Score (mGPS), the Neutrophil Lymphocyte Ratio (NLR) and the Platelet Lymphocyte Ratio (PLR) all of which have been reported to be independent predictors of survival in gastrointestinal cancer (McMillan 2009).

Recently, Crumley and co-workers (Crumley et al. 2010a) reported that the mGPS improved the pre treatment clinical staging of patients who presented with gastro-oesophageal cancer thus aiding clinical decision making in these difficult to treat patients. More specifically Nozoe and coworkers have recently reported that the mGPS has

prognostic value in operable gastric cancer (Nozoe et al. 2011). An elevated platelet count (Ikeda et al. 2002) and neutrophil lymphocyte ratio (Mohri et al. 2010) have also been shown to have prognostic value in patients with gastric cancer.

In terms of patients who have undergone potentially curative surgery the relative prognostic value of the mGPS, NLR and PLR is not clear. More specifically, it is not clear which of these scores best predicts survival in gastric cancer. Therefore, the aim of the present study was to compare the prognostic value of selected markers of the systemic inflammatory response together with tumour related factors in patients undergoing potentially curative resection for gastric cancer.

5.2 Patients and Methods

One hundred and twenty patients undergoing potentially curative resection of gastric cancer (including type III tumours of the gastro-oesophageal junction (Siewert, Stein 1996)) in the upper GI surgical unit, Glasgow Royal Infirmary between January 1996 and May 2009 were included in the study. Patients had laboratory measurements of white cell, neutrophil, lymphocyte and platelet counts, albumin and C-reactive protein in the few days prior to surgery as part of their routine work up, irrespective of whether or not they had received neo-adjuvant chemotherapy.

The tumours were staged according to the tumour node metastasis (TNM) Criteria from the 6th edition of the International Union Against Cancer (UICC) Classification of the Malignant Tumours (Sobin, Wittekind & editors. April 2002). Tumours of the gastro-oesophageal junction were further subdivided according to site, using the Siewert classification; Type I and II lesions of the gastro-oesophageal junction were designated, as cancers of the oesophagus and were therefore excluded from the study. Type III tumours of the cardia were designated as gastric cancers (Siewert, Stein 1996). Furthermore patients

only with TNM stage I to III tumours were considered amenable to curative surgical resection and included in the study.

All patients underwent potentially curative en-bloc resection with majority of them had D2 lymph-adenectomy and survived at least 30 days following surgery. Forty six patients received neo-adjuvant chemotherapy, mostly in last two years of study period. Gastric cancer patients (including type III gastro-oesophageal junctional tumour patients) received 3 cycles of pre operative and post-operative epirubicin, cisplatin and 5-flurouracil (ECF) (the MRC Adjuvant Gastric Infusional Chemotherapy; MAGIC trial) (Cunningham et al. 2006).

Patients who had clinical evidence of infection or other inflammatory condition (e.g. vasculitis, connective tissue disorders, rheumatological conditions) were excluded from the study.

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

The coefficient of variation for the routine laboratory measurements of absolute white cell, neutrophil, lymphocyte, and platelet counts, albumin and C-reactive protein, over the range of measurement, was less than 10% as established by routine quality control procedures. The limit of detection of the assay was a C-reactive protein concentration lower than 6 mg/l. A C-reactive protein concentration of greater than 10 mg/l was considered to indicate the presence of a systemic inflammatory response (McMillan et al. 2001)

The mGPS was constructed as previously described (McMillan 2008). Patients with an elevated C-reactive protein (>10 mg/l) were assigned a modified GPS score (mGPS) of

1 or 2 depending on the absence or presence of hypo-albuminaemia (<35 g/l). Patients in whom neither of these abnormalities was present were allocated a score of 0.

5.2.1 Statistics

The laboratory variables were grouped using standard thresholds (Ikeda et al. 2002, McMillan et al. 2001, Maltoni et al. 2005, Yamanaka et al. 2007). Survival analysis of the group variables was performed using the Cox proportional hazard model. Deaths up to the end of January 2011 were included in the analysis. Multivariate survival analysis, including all significant covariates ($P \leq 0.05$) 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. The relationships between the mGPS and other variables were analysed using the Mantel–Haenszel (X^2) test for trend as appropriate. Analysis was performed using SPSS software (SPSS Inc., Chicago IL, USA).

5.3 Results

The relationship between clinico-pathological characteristics, systemic inflammatory response and survival in patients with gastric cancer is shown in Table 5-1. Overall, the majority of patients were older than 65 years (59%) and male (65%). The majority of patients had white cell (93%), neutrophil (94%), lymphocyte (94%) and platelet (93%) counts in the normal range. Of the 120 patients who underwent a curative resection for gastric cancer, only 18 (15%) patients had mGPS 1 and 5 (4%) had mGPS of 2.

The median follow up of the survivors was 55 months and the minimum was 17 months. During this period 44 (37%) patients died of their cancer and 7 (6%) patients died of non cancer causes. On univariate survival analysis only TNM stage ($p<0.001$), tumour

differentiation ($p<0.01$), resection margin ($p<0.05$), positive to total lymph node ratio ($p<0.001$) and mGPS ($p<0.001$) were significantly associated with cancer specific survival (Table 5-1). On multivariate analysis, positive to total lymph node ratio (HR 2.29, 95% CI 1.57- 3.33, $p<0.001$, Figure 5-1) and mGPS (HR 2.23, 95% CI 1.40-3.54, $p=0.001$, Figure 5-2) retained independent significance (Table 5-1).

The relationship between the mGPS and clinico-pathological characteristics in gastric cancer patients is shown in Table 5-2. An increase in the mGPS was associated with higher neutrophil lymphocyte ratio ($p<0.05$). Patients with mGPS 0 had mean survival of 116.4 months and patients with mGPS 1 and 2 had mean survival of 37.7 and 21.2 months respectively ($p<0.001$).

5.4 Discussion

In the present study a comparison of the relationship of a number of cellular components of the systemic inflammatory response and cancer specific survival was examined in patients undergoing potentially curative resection for gastric cancer. Of the inflammation based prognostic scores, the mGPS, NLR and PLR, only mGPS was significantly associated with cancer specific survival and had prognostic value independent of pathological TNM stage.

In the present study when those patients who did not receive pre-operative chemotherapy were compared to those who did received pre-operative chemotherapy, the latter group were more likely to have had higher TNM stage ($P<0.001$), a higher positive to total lymph node ratio ($p=0.001$) and a positive resection margin ($p<0.05$). In contrast, the patient related factors and survival were similar between the groups (separate analysis performed, result not shown). Given that pre-operative chemotherapy has been used more often in recent years, the period of follow-up was shorter in those patients who received neo-adjuvant therapy. Therefore, in the present study it is as yet unclear whether neo-adjuvant chemotherapy impacts on survival in patients undergoing gastric cancer surgery. Indeed, there are various conflicting reports on benefit of neo-adjuvant chemotherapy in gastric cancer (Janunger, Hafstrom & Glimelius 2002, Earle et al. 2002)

The results of the present study are in apparent contradiction to the recent study of Mohri and colleagues (2010) who reported that the NLR was superior to the individual components of the mGPS, namely C-reactive protein and albumin(Mohri et al. 2010). However, they used a non-standard threshold for C-reactive protein (<3/ >3mg/l), a fact that was noted in the invited comment on the paper of Mohri et al, specifically “Therefore, a minimal elevation in C-reactive protein might have a less significant impact on outcome than a definitive elevation” (Yamashita, Katai 2010). This has recently also been noted by

Nozoe and coworkers (Nozoe et al. 2011). Therefore, the results of the present study confirm the above suspicion and show that the combination C-reactive protein and albumin, using the established thresholds in the mGPS, has superior prognostic value to that of the NLR in patients undergoing potentially curative resection of gastric cancer. They also agree with previous work that compared the prognostic value of the mGPS and NLR in patients undergoing potentially curative resection for oesophageal (Chapter 4) and colorectal cancer (Leitch et al. 2007). In the present study the number of patients with an mGPS score of 2 and at most risk of increased recurrence and poor survival was small. However, the very poor outcome of those patients with a mGPS of 2 are in accord with a larger series of patients undergoing surgery for oesophageal cancer (Vashist et al. 2011) and would suggest that a mGPS of 2 in patients undergoing surgery for gastric cancer is of clinical importance.

The above, taken together, would support the contention that the mGPS based on the acute-phase proteins, C-reactive protein and albumin, is superior to those based on the cellular components of the white cell count, specifically neutrophils, lymphocyte and platelets. It is our opinion that the mGPS should be used in preference to cellular markers of the systemic inflammatory response in determining likely outcome in patients undergoing surgery for gastrointestinal cancer.

The basis of the relationship between the systemic inflammatory response and poorer cancer survival in patients with gastric cancer is not clear. However, the components of the mGPS are recognised to be associated with cancer cachexia and loss of lean tissue (McMillan 2008, Morley, Thomas & Wilson 2006, Fearon et al. 2006), compromised cell-mediated immunity (Du Clos, Mold 2004, Canna et al. 2005) and upregulation of growth factors and angiogenesis (de Jong et al. 2004, Krzystek-Korpacka et al. 2008).

Therefore, the mechanisms underlying the relationship between systemic inflammatory responses and cancer specific survival are likely to be complex. These include extrinsic pathways such as nutritional and functional decline, immune dysfunction and tumour angiogenesis, growth and dissemination. More specifically, recent work in operable colorectal cancer has pointed to a strong relationship between poor patient physiology (Richards et al. 2010) increased comorbidity (Roxburgh et al. 2010) and an increased mGPS. Therefore, it may be that an elevated mGPS, prior to surgery, in patients with gastric cancer reflects poor patient physiology and co-morbidity. Indeed, Kerem and coworkers (2008) reported that the mGPS was associated with weight loss and adipokines (Kerem et al. 2008).

Irrespective of the mechanisms involved, there is now evidence that the mGPS or its component proteins are useful in the clinical staging (Crumley et al. 2010a) of gastric cancer, predicting the response to adjuvant chemotherapy (Hashimoto et al. 2010) and, in the present study, predicting the response to surgery.

However, the clinical use of the mGPS in patients with primary operable gastric cancer is as yet not clear. It is of interest that the mGPS gives a pre-operative risk assessment whereas the lymph node ratio gives a post-operative risk assessment. Although an elevated pre-operative mGPS would not preclude surgery it would identify the patient at high risk of recurrence and therefore would point to more careful tumour staging and follow-up of the patient and the need for neo and/ or adjuvant treatment (Clarke et al. 2011)".

The positive to total lymph node ratio was also independently associated with poorer cancer specific survival in the present study. Moreover the positive to total lymph node ratio had superior prognostic value compared to TNM stage. These results are consistent with recent studies that this ratio is superior to simply assessing nodal status in

predicting cancer outcome in patients undergoing curative resection of gastric cancer (Deng et al. 2009, Fukuda et al. 2009, Marchet et al. 2007). Further work is required to establish whether positive to total lymph node ratio can be used routinely to supplant current TNM staging in these patients.

In summary, an acute-phase protein-based prognostic score, the mGPS, was a superior predictor of cancer survival compared with cellular components of the systemic inflammatory response in patients undergoing potentially curative resection for gastric cancer.

Table 5-1: The relationship between clinico-pathological characteristics, the systemic inflammatory response and cancer specific survival in patients undergoing potentially curative resection for gastric cancer.

		Univariate analysis		Multivariate analysis	
	Patients (n=120)	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Tumour related factors					
TNM Stage (I/II/III)	56/27/37	2.30 (1.60-3.31)	<0.001		0.209
Tumour differentiation (well-mod/poor)	69/51	2.33 (1.27-4.29)	0.006		0.210
Resection Margin (R0/R1)	106/14	2.46 (1.07-5.66)	0.034		0.943
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	67/32/21	2.37(1.64-3.42)	<0.001	2.29 (1.57-3.33)	<0.001
Neo-adjuvant therapy (yes/no)	46/74	1.59 (0.77-3.29)	0.208		
Patient related factors					
Age (<65/65 –74/≥75 years)	49/51/20	1.38 (0.92-2.09)	0.124		
Sex (male/female)	78/42	1.07 (0.57-2.00)	0.830		
White cell count (<8.5/ 8.5–11/ >11x10^9 l^{-1})	87/25/8	0.99 (0.61-1.62)	0.973		
Neutrophil count (<7.5/≥7.5x10^9 l^{-1})	113/7	0.60 (0.14-2.48)	0.477		
Lymphocyte count (>3.0/1.0 – 3.0/<1.0x10^9 l^{-1})	5/108/7	0.71 (0.27-1.87)	0.489		
Platelet count (<400/≥400x10^3 l^{-1})	111/9	0.50 (0.12-2.08)	0.343		
Neutrophil/lymphocyte ratio (<2.5/2.5-5/>5)	64/47/9	1.19 (0.76-1.87)	0.454		
Platelet/lymphocyte ratio (<150/150-300/>300)	61/53/6	0.83 (0.49-1.40)	0.483		
mGPS (0/1/2)	97/18/5	2.40 (1.53-3.77)	<0.001	2.23 (1.40-3.54)	0.001

Table 5-2: The relationship between the mGPS and clinicopathological characteristics and cancer specific survival in patients undergoing potentially curative resection for gastric cancer (n= 120).

	mGPS 0 (n= 97)	mGPS 1 (n= 18)	mGPS 2 (n=5)	P-value
Tumour related factors				
TNM Stage (I/ II/ III)	47/23/27	6/4/8	3/0/2	0.363
Tumour differentiation (well-mod/poor)	58/39/97	8/10	3/2	0.451
Resection Margin (R0/R1)	87/10	15/3	4/1	0.338
Total node retrieved (<15/≥15)	36/61	4/14	¼	0.182
No of positive node (0/1-2/3-6/>6)	56/17/17/7	8/4/3/3	3/0/1/1	0.255
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	56/52/16	8/6/4	3/1/1	0.526
Neo-adjuvant therapy (yes/no)	38/59	5/13	3/2	0.922
Patient related factors				
Age (<65/ 65–74/ ≥75 years)	42/38/17	6/10/2	1/3/1	0.494
Sex (male/ female)	65/32	12/6	¼	0.108
White cell count (<8.5/ 8.5–11/ >11x10^9 l^{-1})	72/20/5	12/4/2	3/1/1	0.189
Neutrophil count (<7.5/ ≥7.5x10^9 l^{-1})	92/5	16/2	5/0	0.781
Lymphocyte count (>3.0/ 1.0 – 3.0/ <1.0x10^9 l^{-1})	3/99/5	1/15/2	1/4/0	0.410
Platelet count (<400/ ≥400x10^3 l^{-1})	91/6	15/3	5/0	0.544
Neutrophil/lymphocyte ratio (<2.5/ 2.5-5/ >5)	56/38/3	5/7/6	3/2/0	0.028
Platelet/lymphocyte ratio (<150/ 150-300/ >300)	49/46/2	9/5/4	3/2/0	0.581
Survival in months (mean, 95% CI)	116.4 (99.4-133.4)	37.7 (24.6-50.7)	21.2 (9.0-33.4)	<0.001*

* Mantel-Cox Log Rank test

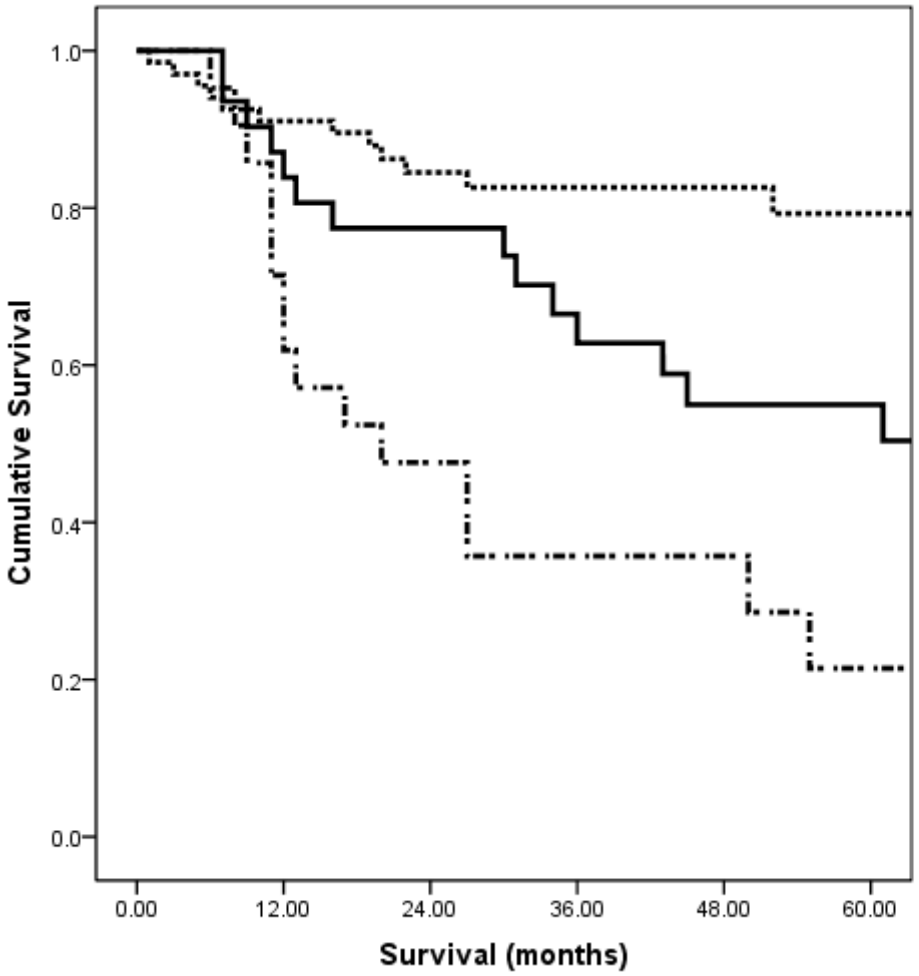

Figure 5-1: The relationship between positive to total lymph node ratio ((0, ≤ 0.2 and >0.2 from top to bottom) and cancer-specific survival in patients undergoing surgery for gastric cancer.

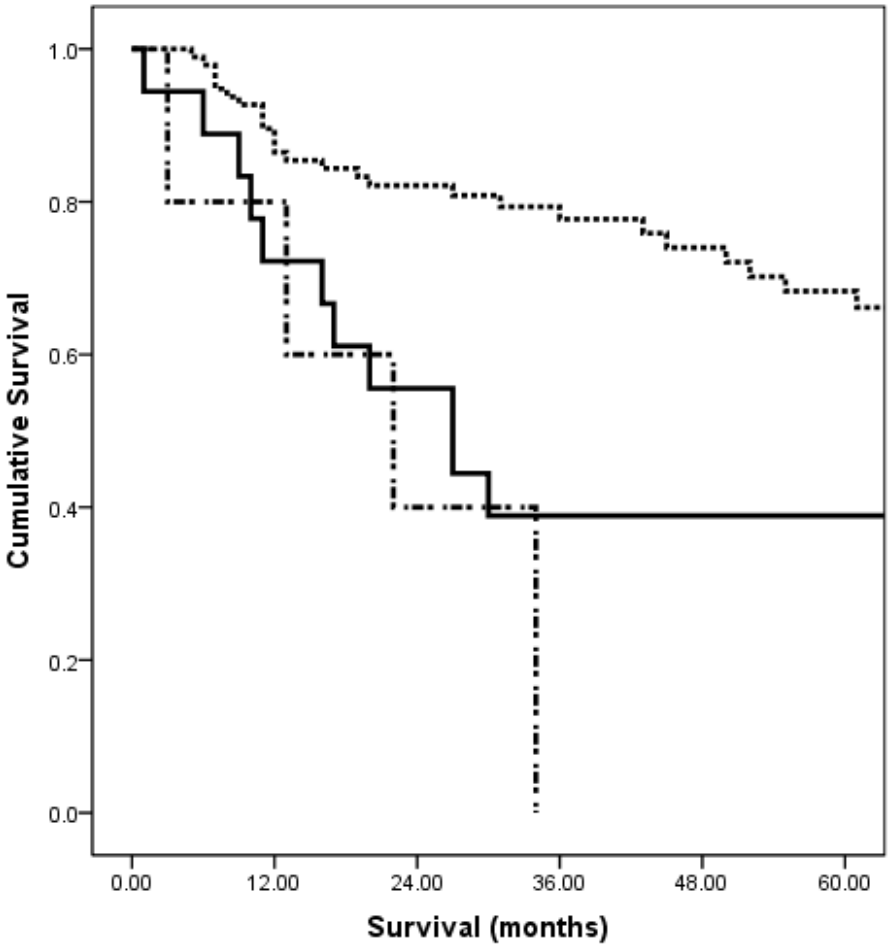

Figure 5-2: The relationship between mGPS (0, 1 and 2 from top to bottom) and cancer-specific survival in patients undergoing surgery for gastric cancer.

6 Chapter VI: The relationship between tumour necrosis, tumour proliferation, local and systemic inflammation, microvessel density and survival in patients undergoing potentially curative resection of oesophageal adenocarcinoma

6.1 Introduction

It is increasingly recognised that outcomes for patients with cancer are determined by host as well as tumour-related factors. Host-related factors include local and systemic inflammatory responses (McMillan 2009, Roxburgh, McMillan 2010, Hanahan, Weinberg 2011, Colotta et al. 2009) and an increased systemic inflammatory response prior to surgery is an independent prognostic factor of survival following resection of oesophageal cancer (Nozoe, Saeki & Sugimachi 2001, Ikeda et al. 2003, Shimada et al. 2003, Gockel et al. 2006). For example, on a comprehensive examination of the prognostic value of both tumour and patient related factors, only mGPS and positive to total lymph node ratio (LNR) were independent predictors of cancer specific survival in oesophageal cancer (Chapter 4).

With reference to the local inflammatory response, a pronounced tumour inflammatory cell infiltrate of gastro-oesophageal carcinomas (assessed on haematoxylin and eosin stained sections) has been reported to be associated with improved survival (Ma et al. 1994). It is of interest therefore, that Klintrup, Makinen and colleagues reported a simplified subjective assessment of the inflammatory infiltrate at the invasive margin of colorectal cancer, including all inflammatory cell types and classifying the infiltrate as low or high grade, had independent prognostic value (Klintrup et al. 2005). This method, in addition to being validated in an independent cohort of colorectal cancer patients (Roxburgh et al. 2009a, Roxburgh et al. 2009b), has also been validated in patients with gastro-oesophageal cancer (Crumley et al. 2011).

More specifically, CD8+ T-lymphocytes have been reported to provide prognostic information in oesophageal cancer (Cho et al. 2003, Schumacher et al. 2001). Also, tumour associated macrophages (CD68+) may have prognostic value in oesophageal cancer (Guo et al. 2007, Koide et al. 2002). Therefore, it would appear that the type, density and location of tumour inflammatory cells are important in determining cancer outcome in these patients. However, to date the few reports e.g. Crumley and coworkers (2011) have been in heterogeneous cohorts of patients including esophageal, junctional, and gastric sites and squamous and adeno carcinomas. Therefore, the prognostic value of measures of the local inflammatory response in patients with oesophageal cancer, in particular in adenocarcinoma, remains to be established.

The basis of the relationship between the tumour and local and systemic inflammatory responses and outcome is not clear. However, a plausible hypothesis is that rapidly proliferating tumours outgrow their blood supply becoming hypoxic and necrotic thereby stimulating both local and systemic inflammatory responses and angiogenesis that, in turn, promote tumour progression and metastases (Vakkila, Lotze 2004, Degenhardt et al. 2006). Indeed, it has been reported that high tumour proliferative activity is associated with poorer survival in oesophageal cancer (Imdahl et al. 2000). Also, Elpek et al and colleagues have reported that CD34+ positive intra tumoral microvessel density was associated with poorer survival in oesophageal squamous cell cancer (Elpek et al. 2001). Finally, consistent with the above hypothesis is the report that tumour proliferative activity was associated with tumour angiogenesis in oesophageal squamous cell cancer (Nomiya et al. 2004, Li et al. 2008).

Although, the histological evidence of tumour necrosis is recognised to be associated with decreased survival in other gastrointestinal malignancies such as pancreas and colorectal cancers (Hiraoka et al. 2010, Pollheimer et al. 2010, Richards et al. 2011).

To our knowledge the prognostic of value of tumour necrosis in patients with oesophageal cancer, either squamous or adeno carcinoma, has not been previously reported.

The aim of the present study was to examine the relationship between tumour necrosis, tumour proliferation, local and systemic inflammatory responses and microvessel density and survival in patients undergoing potentially curative resection of oesophageal adenocarcinoma.

6.2 Patients and Methods

One hundred and twenty one patients undergoing potentially curative resection for oesophageal carcinoma at Glasgow Royal Infirmary between January 1996 and May 2009 were included.

Carcinomas were staged according to the tumour node metastasis (TNM) criteria, 6th edition of the International Union Against Cancer (UICC) Classification (Sobin, Wittekind & editors. April 2002). Tumours of the gastro-oesophageal junction were further subdivided by site, using the Siewert classification; Type I and II lesions of the gastro-oesophageal junction were designated oesophageal cancers and included in this study, while type III tumours of the cardia were designated gastric cancer and therefore excluded (Siewert, Stein 1996). Only TNM stage I - III tumours were considered amenable to curative surgical resection and included in the study.

All patients underwent potentially curative en-bloc lymph-adenectomy and survived at least 30 days following surgery. Forty-seven patients received neo-adjuvant chemotherapy, mostly in last three years of the study period. Oesophageal cancer patients and Type I gastro-oesophageal junctional tumour received 2 cycles of pre-operative cisplatin and 5-flurouracil as in the MRC OE-O2 study (Medical Research Council

Oesophageal Cancer Working Group 2002). Type II gastro-oesophageal junctional tumour was treated with 3 cycles of ECF (Epirubicin, Cisplatin and 5-Flurouracil).

This study was approved by the Research Ethics Committee of Glasgow Royal Infirmary.

6.2.1 Biochemical measurements

The coefficient of variation for laboratory measurements of albumin and C-reactive protein, over the range of measurement, was less than 10% as established by laboratory quality control procedures. The limit of detection of the assay was a C-reactive protein concentration lower than 6mg/l. A C-reactive protein concentration greater than 10mg/l was considered to indicate the presence of a systemic inflammatory response (McMillan et al. 2001)

The mGPS was constructed as previously described (McMillan 2008). An elevated C-reactive protein (>10 mg/l) were assigned a modified GPS score (mGPS) of 1 or 2 depending on the absence or presence of hypo-albuminaemia (<35g/l). Patients in whom neither abnormality was present were allocated an mGPS score 0.

6.2.2 Assessment of tumour necrosis

The same routine haematoxylin and eosin slides from the resected tumour specimens were used to evaluate tumour necrosis over the entire area of invasive carcinoma available. The scoring method for evaluating necrosis was adapted from a previously published protocol (Ikpatt, Ndoma-Egba & Collan 2002) where it was subjectively graded into three categories using complete haematoxylin and eosin-stained histological sections. Score 0, absent (no confluent necrosis at all, i.e. only single-cell death (apoptosis) identifiable. Score 1, mild: confluent areas of invasive carcinoma cell necrosis in fewer than 25% of x40 fields. Score 2, moderate: confluent areas of invasive

carcinoma cell necrosis in 25-50% of x40 fields. Score 3, extensive: Confluent areas of invasive carcinoma cell necrosis in >50% of x40 fields. These scores were aggregated as low grade (scores 0 and 1) or high grade (scores 2 and 3) (Figures 6-3 and 6-4 respectively). Confluent necrosis was defined as areas of definite death of small or large foci of carcinoma cells with some or all of the following features: condensation, darker staining, fragmentation or total loss of tumour cell nuclei; increased cytoplasmic eosinophilia, loss of cytological detail, granular eosinophilic debris, occasionally with calcification. All cases were scored independently by two observers (SD, JG) blinded to clinical outcomes. In case of disagreement in the score, an agreed score was determined by revision of the specimen by both of the observers together in a double headed microscope.

6.2.3 Assessment of Tumour Inflammatory infiltrate

The routine haematoxylin and eosin slides from the resected tumour specimens were retrieved from the pathology archive and scored as described by Klintrup and colleagues (Klintrup et al. 2005). Tumours were scored based on the appearance at the deepest area of tumour invasion on a four point score. A score of 0 indicated that there was no increase in the inflammatory cells at the deepest point of the tumours invasive margin; score 1 denoted a mild and patchy increase in the inflammatory cells; score 2 denoted a prominent inflammatory reaction forming a continuous 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 6-1 and 6-2 respectively). All cases were scored independently by two observers (SD or AC and JG). Observers were blinded to the clinical outcome of the patient. The inter-observer intraclass correlation coefficient for tumour inflammatory infiltrate was 0.81.

6.2.4 Tissue micro array (TMA) construction:

The routine haematoxylin and eosin slides of the resected tumour specimens along with corresponding paraffin blocks were retrieved from the pathology archive for all patients in our study group. A minimum of three representative areas of tumour were defined by the researcher (SD) and the pathologist (JG). Tissue micro arrays were then constructed in triplicate cores 0.6-mm in diameter from each tumour which were placed in separate TMA blocks (Beecher Scientific, Silver Spring, Maryland , USA) as previously described (Kononen et al. 1998). Sections 2.5 µm thick from each TMA block were mounted on silanised glass slides. These sections were used to perform immunohistochemistry for CD8+ T cells, CD68+ (tumour-associated macrophages), Ki-67 (tumour proliferative index) and CD34+ (for microvessel density) (Figure 6-5; A to D respectively). This was performed in the Unit of Experimental Therapeutics, Institute of Cancer, College of MVLS University of Glasgow, Western Infirmary, Glasgow.

6.2.5 Immunohistochemistry

Immunohistochemistry of TMA slides was performed using the Dako Envision method (Dako, Cambridgeshire, UK). The primary antibody for CD8+ was monoclonal mouse anti-human CD8+, Clone CD8+/144B (DAKO, Glostrup, Denmark) at a dilution of 1:100 (overnight incubation) and for CD68+ was monoclonal mouse anti-human CD68+, Clone PG-M1 (DAKO, Glostrup, Denmark) at a dilution of 1:200 (1 hour incubation). The primary antibody for Ki-67 was monoclonal mouse anti-human Ki-67, Clone MIB-1(DAKO, Glostrup, Denmark) at a dilution of 1:50 (overnight incubation) and for CD34+ was monoclonal mouse anti-human, CD34+ Class II, Clone QBEnd 10 (DAKO, Glostrup, Denmark) at a dilution of 1:150 (30 minutes incubation).

Cores were dewaxed and rehydrated. Antigen retrieval was performed by keeping the slides in Tris EDTA buffer (pH 8), in pressure cooker for 5 minutes. Endogenous

peroxidise was blocked by incubation in 3% hydrogen peroxide for 10 minutes. The cores were then incubated with the normal horse serum at dilution 1:20 for 20 minutes at 25ºC to block non- specific binding sites. Respectively primary antibody was added in appropriate concentrations. Sites of binding were detected using the Envision technique (DAKO code K5007, Glostrup, Denmark) with DAB (3-3´ diaminobenzidine, Vector code SK 4001, USA), a chromogenic substrate, according to the manufacturer's instruction. Cores were counterstained with haematoxylin, dehydrated and mounted with DPX. Appropriate positive controls were included in each run and negative controls were omission of the primary antibody.

6.2.6 Morphometry

For automated image analysis of digitised slides were accessed through the Slidepath Image Analysis system and evaluated with the program's nuclear (for Ki-67), cytoplasmic (for CD68+) and membranous (for CD8+) scoring algorithm.

Individual TMA cores were identified, annotated on the scanned image and associated with TMA map entries. Individual nuclei stained with haematoxylin and/or polymerised diaminobenzidine are identified by a thresholding and segmentation algorithm which outlines nuclei and separates touching nuclei. Nuclear size (area) limits can be specified to accept or reject individual nuclei to be quantified. Staining for Ki-67 in each nucleus was classified as positive or negative based on the threshold specified by the observer. Pseudo-colours (red/ orange/ yellow/ blue) display these staining intensity measurements for individual nuclei, allowing thresholds to be chosen appropriately. These thresholds were chosen using a sample of TMA cores from the whole cohort and once chosen were used for analysis over the entire patient cohort without further adjustment.

For CD68+, intracellular and positive pixel detection algorithm was used and for the CD8+, algorithm for thin cell membrane was used. Each TMA cores were crosschecked

by one of the authors (SD) to detect any obvious error. Moreover, at least 25% of the cases were manually scored to produce inter-observer reproducibility. The inter-observer intraclass correlation coefficient values for Ki67, CD68+ and CD8+ were 0.93, 0.87 and 0.74 respectively.

To assess intra tumoural micro-vessel density, immuno-histochemical staining for CD34+ was performed. Quantitative assessment of CD34+ was performed by manual counting of CD34+ positive endothelial cells or cluster regardless of whether a vessel lumen was seen in each of the TMA cores. All cores were counted by one of us (SD) and at least 25% of the cases were counted by a second observer (ZM) and interclass correlation coefficient for CD34+ was 0.83.

6.2.7 Statistical analysis

Survival analysis of the group variables was performed using the Cox proportional hazard model including deaths up to the end of May 2011. Multivariate survival analysis, including all covariates with a P value of ≤ 0.1 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. The relationships between the mGPS and other variables were analysed using the Mantel–Haenszel (X^2) test for trend as appropriate. Analysis was performed using SPSS software (SPSS Inc., Chicago IL, USA).

6.3 Results

One hundred and twenty one patients with oesophageal cancer were included in our study (Table 6-1). Overall, the majority of patients were male (81%), less than 65 years of age (60%) and had an mGPS of 0 (87%). Most of the patients had pTNM stage II or III (82%), adenocarcinoma (81%), well to moderately differentiated tumour (59%), no

resection margin involvement (79%), lymph node involvement (72%) and had high grade tumour necrosis (53%). Twenty seven patients had high grade peri-tumour inflammatory infiltrate according to Klintrup-Makinen criteria (22%). The median values for CD8+, CD68+ and Ki-67 were 4.7%, 14.5% and 20.3% respectively and for CD34+ the median value was 40. The majority did not receive either neoadjuvant (61%) or adjuvant (85%) therapy.

The relationship between tumour type (adeno and squamous carcinomas) and clinico-pathological characteristics are shown in Table 6-1. Patients with oesophageal adenocarcinoma were more likely to be male ($p<0.001$), had higher infiltration of CD8+ ($p<0.001$) and CD68+ ($p<0.01$), had higher TNM stage ($p<0.05$), had neo-adjuvant therapy ($p<0.01$), and as well as had higher CD34+ positive micro-vessel compared with squamous cell carcinoma ($p<0.001$, Table 6-1). There was no significant difference in the degree of tumour necrosis.

Due to the limited number of patients with squamous cancer the relationship between clinic-pathological factors and cancer specific survival was examined only in those patients with oesophageal adenocarcinomas (n=98, Tables 6-2). In patients with oesophageal adenocarcinoma, the median follow up of survivors was 45 months with a minimum of 22 months. During this period 49 (50%) patients died of their cancer and 4 (4%) patients died of non-cancer causes. In patients with oesophageal squamous cell carcinoma, median follow up of survivors was 90 months with a minimum of 23 months. During this period 11 (48%) patients died of their cancer and 4 (17%) patients died of non-cancer causes.

On univariate analysis, only age ($p<0.05$), mGPS ($p<0.01$), TNM stage ($p\leq0.001$), tumour differentiation ($p\leq0.001$), resection margin ($p<0.10$), LNR ($p<0.001$), Klintrup-Makinen score ($p<0.05$), CD8+ ($p<0.05$), CD68+ ($p<0.10$) and Ki-67 ($p<0.05$) were

significantly associated with cancer specific survival. On multivariate analysis, age (HR 1.93, 95% CI 1.23 – 3.04, p=0.004), mGPS (HR 2.91, 95% CI 1.51-5.62, p=0.001), LNR (HR 2.38, 95% CI 1.60-3.52, p<0.001) and CD68+ (HR 1.49, 95% CI 1.02-2.18, p=0.041, Figure 6-6) retained independent significance (Table 6-2).

The relationship between the mGPS and clinicopathological characteristics in patients undergoing potentially curative resection for oesophageal adenocarcinoma is shown in Table 6-3. There was no association between mGPS and clinic-pathological charectristics in this cohort.

Interrelationships between clinical and pathological charecteristics are shown in Table 6-4. Male sex was associated with poor tumour differentiation (p<0.01). TNM stage was directly associated with poor tumour differentiation (p<0.01), positive resection margin (R1) (p<0.001) and LNR(p<0.001). Poorly differentiated oesophageal adenocarcinoma was directly associated with a LNR (p<0.001), inversely with the necrosis score (p<0.05) and directly with CD68+ infiltration (p<0.05). Positive resection margin (R1) was directly associated with a LNR(p<0.01) and inversely with CD8+ infiltration (p<0.01). A LNR was directly associated with the Klintrup-Makinen score (p<0.01). The Klintrup-Makinen score was directly associated with CD8+ infiltration (p<0.01). Tumour necrosis was not significantly associated with any tumour measure other than the degree of differentiation. Tumour CD8+ infiltrate was directly associated with CD68+ infiltration (p<0.01), CD34+ (p<0.05) and neo-adjuvant therapy (p<0.05). Tumour CD68+ infiltration of the oesophageal adenocarcinoma was directly associated with the Ki-67 proliferation index (p<0.001) as well as neo-adjuvant therapy (p<0.01). The tumour Ki-67 proliferation index was directly associated with CD34+ micro-vessel density (p=0.05) and neo-adjuvant therapy (p<0.001).

Interrelationships between clinical and pathological charecteristics of patents who did not receive neo-adjuvant chemotherapy in oesophageal adenocarcinoma are shown in Table 6-5 (n=53). TNM stage was directly associated with poor tumour differentiation ($p<0.05$), positive resection margin (R1) ($p=0.001$) and LNR($p<0.001$). Poorly differentiated oesophageal adenocarcinoma was directly associated with a LNR ($p=0.001$) and directly with CD68+ infiltration ($p<0.05$). Positive resection margin (R1) was directly associated with a LNR($p<0.05$) and inversely with CD8+ infiltration ($p<0.05$). A LNR was directly associated with the Klintrup-Makinen score ($p<0.01$) and inversely with CD8+ infiltration($p<0.05$) . The Klintrup-Makinen score was directly associated with CD8+ infiltration ($p<0.05$).

The relationship between clinic-pathological factors and cancer specific survival was examined also in a subgroup of oesophageal adenocarcinoma patients who did not receive neo-adjuvant therapy (n=53, Tables 6-6). On univariate analysis, only age ($p<0.05$), mGPS ($p\leq0.001$), TNM stage ($p<0.01$), tumour differentiation ($p<0.05$), resection margin ($p<0.05$), LNR ($p<0.001$), Klintrup-Makinen score ($p<0.05$), and CD68+ ($p<0.10$) were significantly associated with cancer specific survival. On multivariate analysis, age (HR 2.62, 95% CI 1.27 – 5.39, $p=0.009$), mGPS (HR 12.71, 95% CI 4.15-38.94, $p<0.001$), LNR (HR 3.18, 95% CI 1.77-5.72, $p<0.001$) and CD68+ (HR 1.88, 95% CI 1.12-3.15, $p=0.017$) retained independent significance. (Table 6-6).

When multivariate survival analysis was carried out in those patients with stage III adenocarcinoma alone (n=50), only age (HR 1.90, 95%CI 1.14-3.17, $p= 0.014$) and mGPS (HR 2.91, 95%CI 1.12-7.58, $p= 0.029$) were independently associated with cancer specific survival. None of the markers of inflammatory cell infiltrate, including CD68+ were associated with cancer specific survival.

6.4 Discussion

The results of the present study show that, in patients with oesophageal cancer, the extent of the inflammatory infiltrate and angiogenesis was greater in adenocarcinoma compared with squamous carcinoma. Furthermore, within the adenocarcinoma cohort, although tumour necrosis was not significantly associated with proliferative activity, inflammatory cell infiltrate and angiogenesis, tumour proliferative activity was directly associated with the extent of macrophage infiltration. Also, the generalised inflammatory and cytotoxic T-lymphocyte infiltrate were inversely associated with the LNR and resection margin respectively. Moreover, patients with neo-adjuvant therapy had more intra tumoral inflammatory cell infiltrate as well as had higher proliferative activity. When the prognostic value of such tissue factors were examined, only the tumour macrophage infiltration retained significance. Taken together these results would suggest that tumour necrosis does not link local and systemic inflammatory responses but that the tumour inflammatory infiltrate, in particular macrophages, play a role in the control of tumour progression and dissemination in patients with oesophageal adenocarcinoma.

The present report of tumour necrosis in oesophageal adenocarcinoma is to our knowledge unique in the literature and therefore further work is required to establish whether it has prognostic value and relationship with patient and tumour related factors in oesophageal cancer. Furthermore, the basis of the differences in the inflammatory cells and microvessel density in squamous and adenocarcinomas is not clear. However, it may reflect more aggressive nature of squamous cell carcinoma (Siewert et al. 2001, Stein et al. 2005). Irrespective, the results do emphasise the heterogeneous nature of oesophageal cancer and may have been a significant confounding factor in previous studies.

Given that pre-operative neo-adjuvant chemotherapy might influence the some of the clinicopathological characteristics examined in the present study, a subgroup analysis

was performed on patients who did not receive neoadjuvant therapy. Indeed, when compared with the no neoadjuvant therapy group, the neoadjuvant therapy group had higher Ki-67 proliferation index ($p<0.001$) and higher densities of both CD8+ T-lymphocytes ($p= 0.016$) and CD68+ macrophages ($p= 0.004$). In contrast, in the no neoadjuvant therapy group alone, the association between higher Ki-67 proliferation index and densities of both CD8+ T-lymphocytes and CD68+ macrophages was not significant. The basis of this apparent contradiction is not clear however it may suggest that neoadjuvant therapy does influence the relationship between Ki-67 proliferation index and densities of both CD8+ T-lymphocytes and CD68+ macrophages.

The role of Ki67 in oesophageal cancer is also not well established. It has been reported to predict the complete response in oesophageal cancer following chemo-radio therapy (Ressiot et al. 2008, Takeuchi et al. 2003). However, there are some conflicting reports on the predictive value of Ki67 on survival in oesophageal squamous cell cancer (Youssef et al. 1995, Imdahl et al. 2000, Sarbia et al. 1996). In the present study, a high proliferation index in oesophageal adenocarcinoma was associated with poor outcome (on univariate survival analysis) and was also associated with intra-tumoural macrophage infiltration (CD68+) and micro-vessel density (CD34+).

With reference to the tissue factors examined, the results of the present study are consistent with previous literature. Cho and colleagues have reported the prognostic value of CD8+ lymphocyte in 122 patients with oesophageal squamous cell carcinoma(Cho et al. 2003). Similarly Tsuchikawa and coworkers reported the prognostic significance of CD8+ lymphocytes in 98 patrients with oesophageal squamous cell carcinoma. (Tsuchikawa et al. 2011). In contrast, in another study involving 130 patients with oesophageal adenocarcinoma, CD8+ lymphocyte was not associated with survival (Zingg et al. 2010). Moreover, tumour associated macrophages (CD68+) had been reported to have independent prognostic value in oesophageal cancer (Guo et al. 2007, Koide et al. 2002).

Indeed, Koide and coworkers reported, in 56 patients, that the CD68+ macrophage infiltrate was associated with tumour proliferation index (Ki67) and disease progression in oesophageal squamous cell cancer (Koide et al. 2002) Also, Guo and coworkers (2007) reported that, 137 patients with oesophageal squamous carcinoma, a high tumour macrophage infiltrate and a low lymphocytic infiltrate were associated with poor outcome. However, that the high tumour macrophage infiltrate had superior prognostic value. In the present study, the relationships between tumour necrosis, tumour proliferation, intra tumoral inflammatory cell infiltrates and microvessel density and survival in patients undergoing potentially curative resection of oesophageal adenocarcinoma was examined. Given that, of the tumour inflammatory cell infiltrate, the macrophages were most closely associated with survival, it would be of interest to examine the phenotype (eg. M1 and M2) of the macrophages in detail, in patients with oesophageal adenocarcinoma.

In the context of the present results it is also important to acknowledge that, that there is some recent evidence that CD68+ can be expressed on non-myeloid cells including carcinomas (Gottfried et al. 2008). Indeed, Gottfried and coworkers (2008) concluded that CD68+ is not a selective macrophage marker but rather a lysosomal protein that is enriched in macrophages. In the present study the morphology of the cell type was clearly considered during the assessment of CD68+ expression and, although unlikely, it is conceivable that the results of the present study may be have been influenced by expression of CD68 by non-macrophages cells. Further work is required to examine the extent of possible confounding in the present study.

It was of interest therefore, in the present study, that CD68+ macrophages was strongly associated with CD8 + T lymphocytes and tumour proliferative index (Ki67) in oesophageal adenocarcinoma. Taken together with the other results above, this would suggest that high tumour lymphocytic infiltrate prevents tumour progression and a high tumour macrophage infiltration promotes tumour proliferation. Given that they are both

generally increased together it is likely that the balance of these inflammatory cells determines whether there is tumour progression. However, more work is required to be undertaken to better define these relationships in oesophageal adenocarcinoma.

In summary, of the tissue based factors examined in the present study, tumour macrophage infiltration appeared to play a central role in the proliferative activity and the coordination of the inflammatory cell infiltrate and was independently associated with poor outcome in patients with oesophageal adenocarcinoma.

Table 6-1: Clinico-pathological characteristic of patients undergoing potentially curative resection for oesophageal cancer (n=121)

	Adeno (n=98)	Squamous (n=23)	P value
Patient related factors			
Age (<65/65 –74/≥75 years)	57/35/6	15/6/2	0.755
Sex (Male/ Female)	83/15	10/13	<0.001
mGPS (0/1/2)	87/9/2	18/5/0	0.362
Klintrup-Makinen score (Low grade/ High grade)	77/21	17/6	0.591
CD8+ tertiles (1/2/3)	25/39/34	17/5/1	<0.001
CD68+ tertiles (1/2/3)	25/35/38	12/9/2	0.002
Tumour related factors			
TNM Stage (I/II/III)	15/33/50	7/11/5	0.011
Tumour differentiation (well-mod/poor)	60/38	11/12	0.251
Resection Margin (R0/R1)	75/23	20/3	0.399
LNR (0/≤ 0.2/>0.2)	35/35/28	11/9/3	0.132
Necrosis Score (Low grade/ High grade)	48/50	9/14	0.488
Ki67 tertiles (1/2/3)	34/29/35	5/8/10	0.284
CD34+ tertiles (1/2/3)	26/33/39	15/8/0	<0.001
Neo-adjuvant therapy (no/yes)	53/45	21/2	0.001
Adjuvant therapy (no/yes)	81/17	22/1	0.191
Alive	45	8	
Cancer specific death	49	11	
Non cancer death	4	4	0.893*

*Log rank test(Mantel- Cox)

Table 6-2: Relationships between clinic-pathological factors and cancer specific survival, in patients selected for potentially curative resection for oesophageal adeno-carcinoma (n=98)

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Patient related factors				
Age (<65/65 –74/≥75 years)	1.69 (1.11-2.59)	0.016	1.93 (1.23-3.04)	0.004
Sex (male/female)	0.47 (0.17-1.31)	0.148		
mGPS (0/1/2)	2.39 (1.36-4.18)	0.002	2.91 (1.51-5.62)	0.001
Tumour related factors				
TNM Stage (I/II/III)	2.27 (1.42-3.64)	0.001		0.345
Tumour differentiation (well-mod/poor)	2.52 (1.43-4.44)	0.001		0.202
Resection Margin (R0/R1)	1.80 (0.99-3.29)	0.053		0.805
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	2.47 (1.71-3.58)	<0.001	2.38 (1.60-3.52)	<0.001
Neo-adjuvant therapy (no/yes)	1.39 (0.74-2.61)	0.304		
Adjuvant therapy (no/yes)	1.74 (0.83-3.65)	0.141		
Klintrup-Makinen score (Low / High grade)	0.35 (0.15-0.82)	0.016		0.076
Necrosis score (Low / High grade)	1.12 (0.64-1.97)	0.695		
CD8 tertiles (1/2/3)	0.69 (0.48-0.99)	0.048		0.697
CD68 tertiles (1/2/3)	1.38 (0.99-1.94)	0.061	1.49 (1.02-2.18)	0.041
Ki67 tertiles (1/2/3)	1.46 (1.01-2.12)	0.048		0.479
CD34 tertiles (1/2/3)	0.94 (0.67-1.34)	0.736		

Table 6-3: The relationship between the mGPS and clinicopathological characteristics in patients undergoing potentially curative resection for oesophageal adenocarcinoma (n= 98).

	mGPS 0 (n= 87)	mGPS 1&2 (n= 11)	P value
Patient related factors			
Age (<65/65 –74/≥75 years)	53/28/6	4/7/0	0.368
Sex (male/female)	75/12	8/3	0.222
Tumour related factors			
TNM Stage (I/II/III)	14/29/44	1/4/6	0.812
Tumour differentiation (well-mod/poor)	55/32	5/6	0.207
Resection Margin (R0/R1)	68/19	7/4	0.236
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	30/32/25	5/3/3	0.628
Neo-adjuvant therapy (no/yes)	47/40	6/5	0.615
Adjuvant therapy (no/yes)	73/14	8/3	0.290
Klintrup-Makinen score (Low / High grade)	68/19	9/2	0.568
Necrosis score (Low / High grade)	44/43	4/7	0.286
CD8 tertiles (1/2/3)	20/34/33	5/5/1	0.038
CD68 tertiles (1/2/3)	22/31/34	3/4/4	0.853
Ki67 tertiles (1/2/3)	31/25/31	3/4/4	0.736
CD34 tertiles (1/2/3)	21/32/34	5/1/5	0.563

Table 6-4: Interrelationships between different pathological and clinical parameters in patients selected for potentially curative resection for oesophageal adenocarcinoma (n=98).

	Sex	mGPS	TNM Stage	Tumour differentiation	Resection Margin	LNR	Klintrup-Makinen score	Necrosis score	CD8 tertiles	CD68 tertiles	Ki67 tertiles	CD34 tertiles	Neo-adjuvant therapy
Age in years (<65/ 65-74/ ≥75 years)	0.199	0.248	0.688	0.939	0.991	0.586	0.977	0.505	0.510	0.749	0.110	0.923	0.639
Sex (male/ female)		0.155	0.605	0.008	1.00	0.170	0.302	0.091	0.609	0.203	0.813	0.693	0.400
mGPS			0.823	0.616	0.569	0.328	0.894	0.228	0.243	0.838	0.311	0.723	0.988
TNM Stage (I/ II/ III)				0.008	<0.001	<0.001	0.403	0.754	0.203	0.593	0.430	0.081	0.420
Tumour differentiation (well-mod/poor)					1.00	<0.001	0.322	0.012	0.422	0.024	0.655	0.922	0.838
Resection Margin (R0/R1)						0.004	0.144	0.344	0.004	0.362	0.307	0.719	0.242
LNR							0.009	0.388	0.093	0.332	0.293	0.168	0.417
Klintrup-Makinen score (Low / High grade)								1.00	0.002	0.078	0.105	0.813	1.00
Necrosis score (Low / High grade)									0.219	0.724	0.140	0.109	0.839
CD8 tertiles (1/2/3)										0.002	0.051	0.044	0.016
CD68 tertiles (1/2/3)											<0.001	0.994	0.004
Ki67 tertiles (1/2/3)												0.050	<0.001
CD34 tertiles (1/2/3)													0.261

Table 6-5: Interrelationships between different pathological and clinical parameters in patients selected for potentially curative resection for oesophageal adenocarcinoma without neo-adjuvant therapy (n=53).

	Sex	mGPS	TNM Stage	Tumour differentiation	Resection Margin	LNR	Klintrup-Makinen score	Necrosis score	CD8 tertiles	CD68 tertiles	Ki67 tertiles	CD34 tertiles
Age in years (<65/ 65-74/ ≥75 years)	0.131	0.262	0.706	0.978	0.674	0.412	0.991	0.266	0.951	0.753	0.375	0.952
Sex (male/ female)		0.545	0.654	0.070	0.706	0.259	0.416	0.302	0.615	0.580	0.433	0.726
mGPS			0.624	0.645	0.988	0.085	0.697	0.833	0.661	0.440	0.454	0.806
TNM Stage (I/ II/ III)				0.034	0.001	<0.001	0.325	0.380	0.169	0.229	0.503	0.183
Tumour differentiation (well-mod/poor)					1.00	0.001	0.503	0.053	0.906	0.028	0.740	0.849
Resection Margin (R0/R1)						0.011	0.149	0.074	0.032	0.347	0.789	0.726
LNR							0.008	0.921	0.042	0.076	0.198	0.318
Klintrup-Makinen score (Low / High grade)								0.509	0.011	0.722	0.160	0.412
Necrosis score (Low / High grade)									0.485	0.359	0.051	0.685
CD8 tertiles (1/2/3)										0.083	0.570	0.799
CD68 tertiles (1/2/3)											0.259	0.709
Ki67 tertiles (1/2/3)												0.507

Table 6-6: Relationships between clinico-pathological factors and survival, in patients selected for potentially curative resection for oesophageal adeno-carcinoma without neo-adjuvant therapy (n=53)

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Patient related factors				
Age (<65/65 –74/≥75 years)	2.07 (1.13-3.77)	0.018	2.62 (1.27-5.39)	0.009
Sex (male/female)	0.77 (0.27-2.21)	0.625		
mGPS (0/1/2)	4.14 (1.80-9.51)	0.001	12.71 (4.15-38.94)	<0.001
Tumour related factors				
TNM Stage (I/II/III)	2.46 (1.34-4.49)	0.004		0.246
Tumour differentiation (well-mod/poor)	2.35 (1.13-4.92)	0.023		0.552
Resection Margin (R0/R1)	2.13 (1.02-4.49)	0.046		0.607
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	2.45 (1.55-3.89)	<0.001	3.18 (1.77-5.72)	<0.001
Adjuvant therapy (no/yes)	2.45 (0.83-7.22)	0.105		
Klintrup-Makinen score (Low / High grade)	0.28 (0.08-0.92)	0.036		0.055
Necrosis score (Low / High grade)	1.29 (0.61-2.70)	0.505		
CD8 tertiles (1/2/3)	0.68 (0.43-1.10)	0.117		
CD68 tertiles (1/2/3)	1.49 (0.97-2.28)	0.066	1.88 (1.12-3.15)	0.017
Ki67 tertiles (1/2/3)	1.35 (0.78-2.35)	0.289		
CD34 tertiles (1/2/3)	0.73 (0.45-1.20)	0.214		

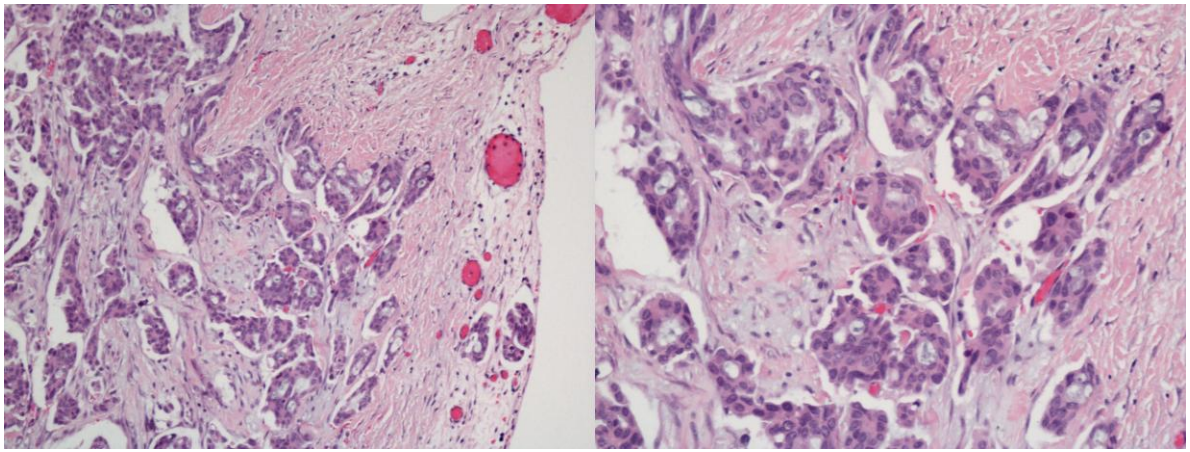

Figure 6-1: Example of "low grade" local tumor inflammatory infiltrate (low power and high power view).

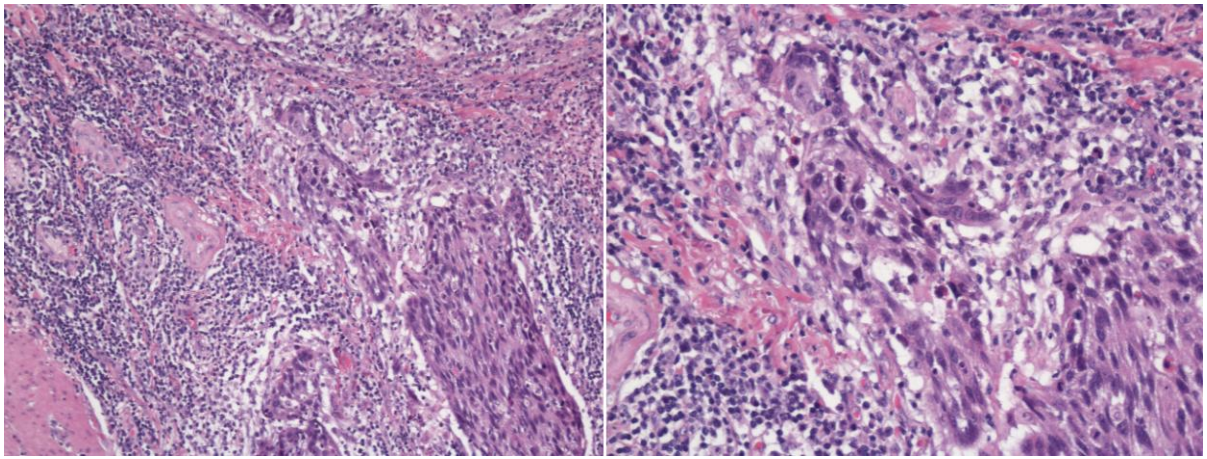

Figure 6-2: Example of "high grade" local tumor inflammatory infiltrate (low power and high power view).

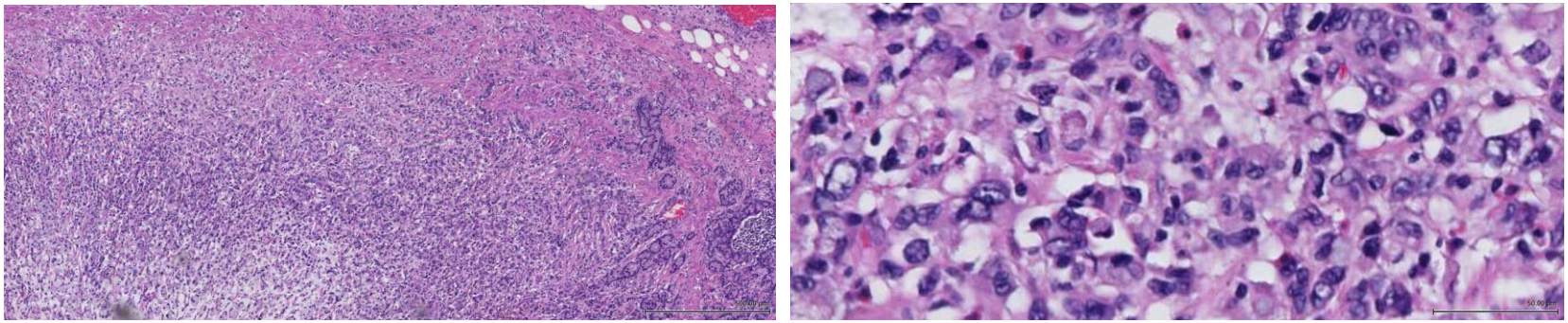

Figure 6-3: Example of absence of necrosis (low power and high power view).

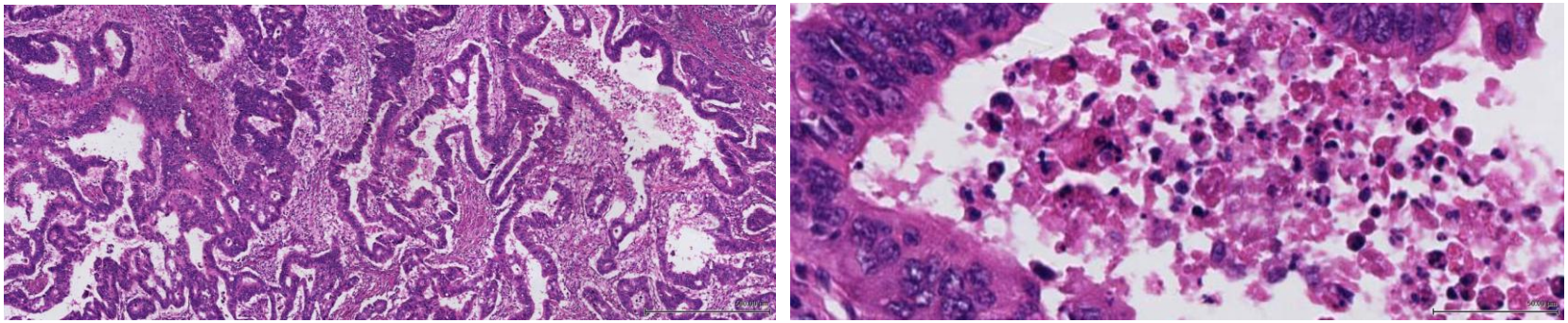

Figure 6-4: Example of extensive necrosis (low power and high power view).

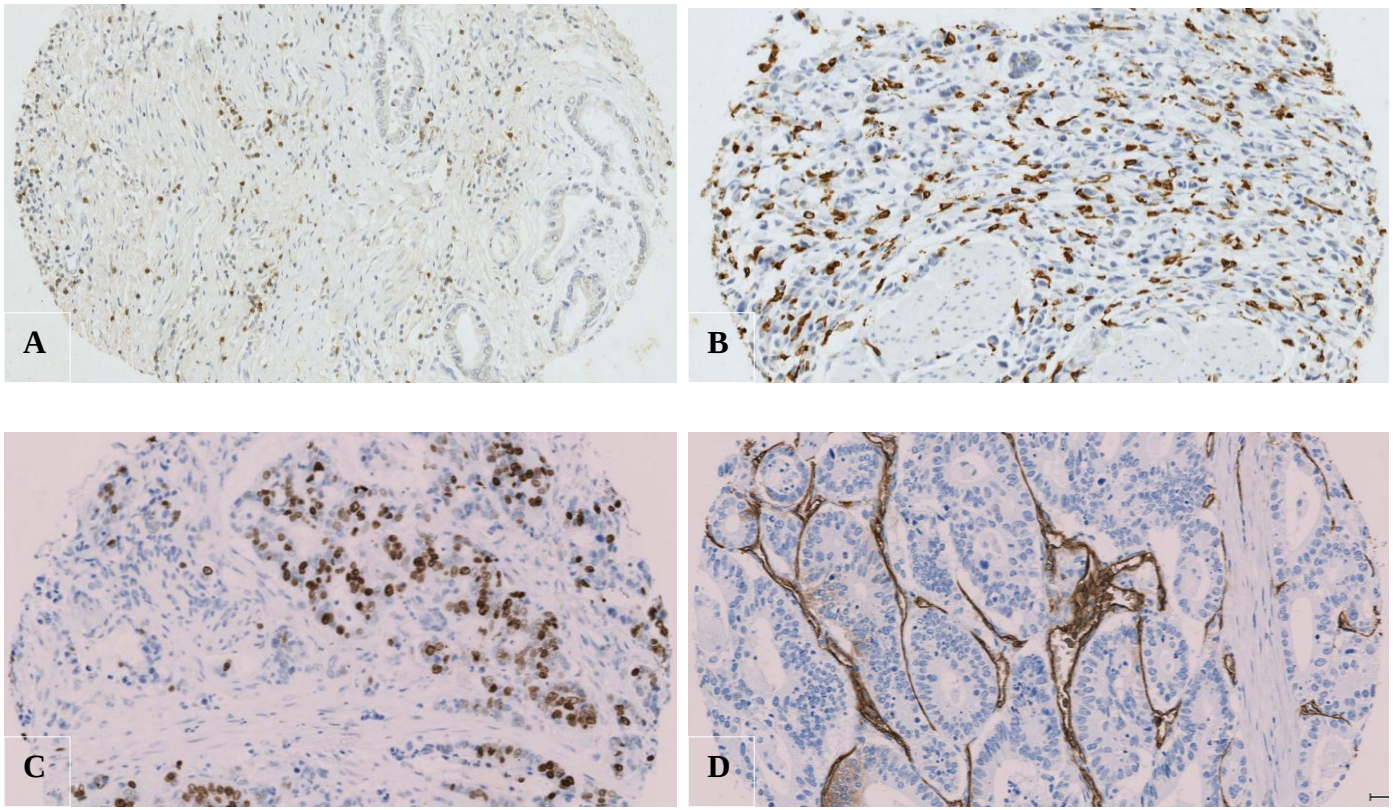

Figure 6-5: Immunohistochemisry of tissue microarray for CD8+ (A); CD68+ (B); Ki67 (C) and CD34+ (D). Positive cells are stained brown. All pictures are in 200x magnification.

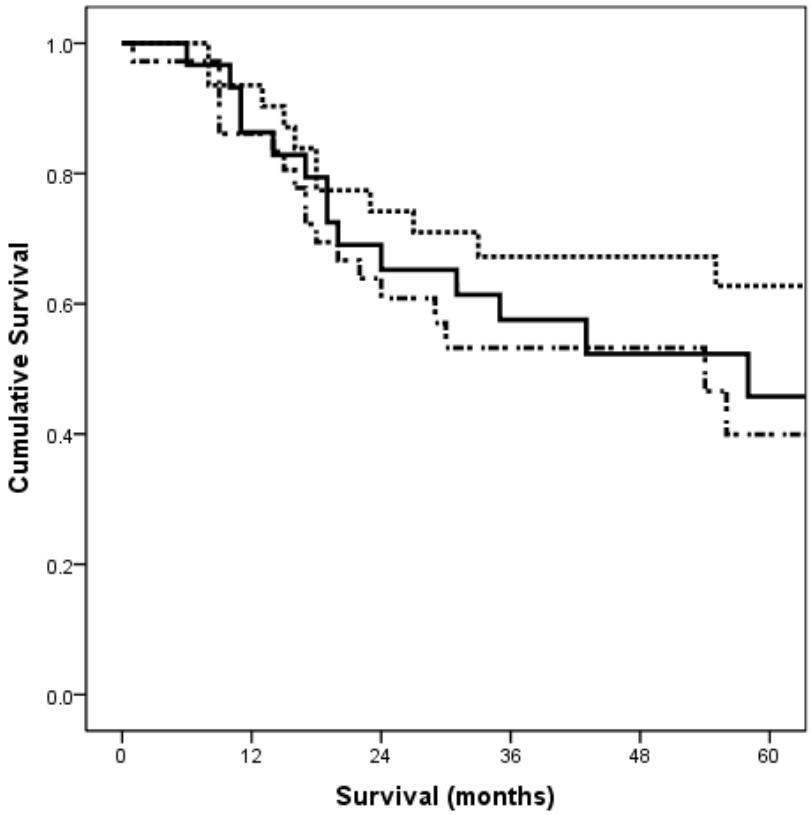

Figure 6-6: The relationship between tumour CD68+ infiltration (top to bottom, tertiles 1/2/3) and cancer specific survival in patients undergoing resection for oesophageal adenocarcinoma.

7 Chapter VII: The relationship between tumour necrosis, tumour proliferation, local and systemic inflammation and microvessel density and survival in patients undergoing potentially curative resection of gastric adenocarcinoma

7.1 Introduction

It is increasingly recognised that outcomes for patients with cancer are determined by host as well as tumour-related factors. Important host-related factors include local and systemic inflammatory responses (McMillan 2009, Roxburgh, McMillan 2010, Hanahan, Weinberg 2011, Colotta et al. 2009). Increased systemic inflammatory response i.e. elevated C-reactive protein, prior to surgery in gastric cancer has been reported to have an independent prognostic value (Wang, Sun 2009, Chang et al. 2010, Nozoe et al. 2011). For example, on a comprehensive examination of the prognostic value of both tumour and patient related factors, only mGPS and positive to total lymph node ratio (LNR) were independent predictors of cancer specific survival in gastric cancer (Chapter 5).

With reference to the local inflammatory response, a pronounced tumour inflammatory cell infiltrate has been reported to be associated with improved survival in gastric cancer (Davessar et al. 1990, Ma et al. 1994, Songun et al. 1996). It is of interest therefore, that Klintrup, Makinen and colleagues reported a simplified subjective assessment of the inflammatory infiltrate at the invasive margin of colorectal cancer, including all inflammatory cell types and classifying the infiltrate as low or high grade, had independent prognostic value (Klintrup et al. 2005). This method, in addition to being validated in an independent cohort of colorectal cancer patients (Roxburgh et al. 2009a, Roxburgh et al. 2009b), has also been validated in an unselected cohort of patients with gastro-oesophageal cancer (Crumley et al. 2011).

More specifically, CD8+ T-lymphocytes have been reported to provide prognostic information in gastric cancer (Lee et al. 2008, Milasiene, Stratilatovas & Norkiene 2007).

Also, tumour associated macrophages (CD68+) appear to have prognostic value in patients with gastric cancer (Ishigami et al. 2003, Kawahara et al. 2010, Ohno et al. 2003, Wang et al. 2011) . Therefore, it would appear that the type and location of tumour inflammatory cells are important in determining cancer outcome. However, to date the few reports e.g. Crumley and coworkers (2011) have been in heterogeneous cohorts of patients including esophageal, junctional, and gastric sites and squamous and adenocarcinomas (Crumley et al 2011). Therefore, the prognostic value of measures of the local inflammatory response in patients with gastric cancer remains to be clearly established.

The basis of the relationship between the tumour and local and systemic inflammatory responses and outcome is not clear. However, a plausible hypothesis is that rapidly proliferating tumours outgrow their blood supply becoming hypoxic and necrotic thereby stimulating both local and systemic inflammatory responses and angiogenesis that, in turn, promote tumour progression and metastases (Vakkila, Lotze 2004, Degenhardt et al. 2006). Indeed, it has been reported that high tumour proliferative activity is associated with poorer survival in gastric cancer (Tzanakis et al. 2009, Solcia et al. 2009). Also, it has been reported that CD34+ positive intra tumoral microvessel density was associated in gastric cancer survival (Chen et al. 2008, Osinsky et al. 2011). It has also been recognised the role of hypoxia in neoangiogenesis and tumour progression in gastric cancer (Osinsky et al. 2011, Cabuk et al. 2007).

This hypothesis has recently been examined in patients undergoing potentially curative resection for oesophageal cancer (Chapter 6). It was shown that the extent of the inflammatory infiltrate and angiogenesis was greater in oesophageal adenocarcinoma compared with squamous carcinoma. Within the adenocarcinoma cohort, although tumour necrosis was not associated with tumour proliferative activity, inflammatory cell infiltrate and angiogenesis, tumour proliferative activity was directly associated with the extent of

macrophage infiltration and intra-tumoral macrophage infiltration had independent predictive value (Chapter 6).

To our knowledge the prognostic of value of tumour necrosis in patients with gastric adenocarcinoma, has not been previously reported. However, histological evidence of tumour necrosis is recognised to be associated with decreased survival in other gastrointestinal malignancies such as gastrointestinal stromal tumour, pancreas and colorectal (Hiraoka et al. 2010, Pollheimer et al. 2010, Fujimoto et al. 2003).

The aim of the present study was to examine the relationship between tumour necrosis, tumour proliferation, local and systemic inflammation and micro vessel density and survival in patients undergoing potentially curative resection of gastric cancer.

7.2 Patients and Methods

One hundred and four patients undergoing potentially curative resection of gastric cancer (including type III tumours of the gastro-oesophageal junction (Siewert, Stein 1996)) in the upper GI surgical unit, Glasgow Royal Infirmary between January 1996 and May 2009 were included in the study.

The tumours were staged according to the tumour node metastasis (TNM) Criteria from the 6th edition of the International Union Against Cancer (UICC) Classification of the Malignant Tumours (Sobin, Wittekind & editors. April 2002). Tumours of the gastro-oesophageal junction were further subdivided according to site, using the Siewert classification; Type I and II lesions of the gastro-oesophageal junction were designated, as cancers of the oesophagus and were therefore excluded from the study. Type III tumours of the cardia were designated as gastric cancers (Siewert, Stein 1996). Furthermore, only patients with TNM stage I to III tumours were considered amenable to curative surgical resection and included in the study.

All patients underwent potentially curative en-bloc resection and the majority had D2 lymph-adenectomy and survived at least 30 days following surgery. Thirty six patients received neo-adjuvant chemotherapy, mainly in last two years of study period. Gastric cancer patients (including type III gastro-oesophageal junctional tumour patients) received 3 cycles of pre operative and post-operative epirubicin, cisplatin and 5-flurouracil (ECF) (the MRC Adjuvant Gastric Infusional Chemotherapy; MAGIC trial) (Cunningham et al. 2006).

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

7.2.1 Biochemical data

The coefficient of variation for laboratory measurements of albumin and C-reactive protein, over the range of measurement, was less than 10% as established by laboratory quality control procedures. The limit of detection of the assay was a C-reactive protein concentration lower than 6mg/l. A C-reactive protein concentration greater than 10mg/l was considered to indicate the presence of a systemic inflammatory response (McMillan et al. 2001)

The mGPS was constructed as previously described (McMillan 2008). An elevated C-reactive protein (>10 mg/l) were assigned a modified GPS score (mGPS) of 1 or 2 depending on the absence or presence of hypo-albuminaemia (<35g/l). Patients in whom neither abnormality was present were allocated an mGPS score 0.

7.2.2 Assessment of tumour necrosis

The same routine haematoxylin and eosin slides from the resected tumour specimens were used to evaluate tumour necrosis over the entire area of invasive carcinoma available. The scoring method for evaluating necrosis was adapted from a previously published protocol (Ikpatt, Ndoma-Egba & Collan 2002) where it was

subjectively graded into three categories using full haematoxylin and eosin-stained histological sections. Score 0, absent (no confluent necrosis at all, i.e. only single-cell death (apoptosis) identifiable. Score 1, mild: confluent areas of invasive carcinoma cell necrosis in fewer than 25% of x40 fields. Score 2, moderate: confluent areas of invasive carcinoma cell necrosis in 25-50% of x40 fields. Score 3, extensive: confluent areas of invasive carcinoma cell necrosis in >50% of x40 fields. These scores were aggregated as low grade (scores 0 and 1) or high grade (scores 2 and 3). Confluent necrosis was defined as areas of definite death of small or large foci of carcinoma cells with some or all of the following features: condensation, darker staining, fragmentation or total loss of tumour cell nuclei; increased cytoplasmic eosinophilia, loss of cytological detail, granular eosinophilic debris, occasionally with calcification. All cases were scored independently by two observers (SD, JG) who were blinded to clinical outcomes. The cases with different scores were reviewed by both of the observers together and an agreed score was determined.

7.2.3 Assessment of Tumour Inflammatory infiltrate

The routine haematoxylin and eosin slides from the resected tumour specimens were retrieved from the pathology archive and scored as described by Klintrup and colleagues (Klintrup et al. 2005). Tumours were scored based on the appearance at the deepest area of tumour invasion on a four point score. A score of 0 indicated that there was no increase in the inflammatory cells at the deepest point of the tumours invasive margin; score 1 denoted a mild and patchy increase in the inflammatory cells; score 2 denoted a prominent inflammatory reaction forming a continuous 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). All cases were scored independently by two observers (SD or AC and JG). Observers were blinded to the clinical outcome of the

patient. The inter-observer intraclass correlation coefficient for tumour inflammatory infiltrate was 0.86.

7.2.4 Tissue micro array (TMA) construction:

The routine haematoxylin and eosin slides of the resected tumour specimens along with corresponding paraffin blocks were retrieved from the pathology archive for all patients in our study group. A minimum of three representative areas of tumour were defined by the researcher (SD) and the pathologist (JG). Tissue micro arrays were then constructed in triplicate cores 0.6-mm in diameter from each tumour which were placed in separate TMA blocks (Beecher Scientific, Silver Spring, Maryland , USA) as previously described (Kononen et al. 1998). Sections 2.5 µm thick from each TMA block were mounted on silanised glass slides. These sections were used to perform immunohistochemistry for CD8+ T cells, CD68+ (tumour-associated macrophages), Ki-67 (tumour proliferative index) and CD34+ (for micro vessel density). This was performed in the Department of Pathology, Western Infirmary, Glasgow.

7.2.5 Immunohistochemistry

Immunohistochemistry of TMA slides was performed using the ChemMate Dako Envision method (Dako, Cambridgeshire, UK). The primary antibody for CD8+ was monoclonal mouse anti-human CD8+, Clone CD8+/144B (DAKO, Glostrup, Denmark) at a dilution of 1:100 (overnight incubation) and for CD68+ was monoclonal mouse anti-human CD68+, Clone PG-M1 (DAKO, Glostrup, Denmark) at a dilution of 1:200 (1 hour incubation). The primary antibody for Ki-67 was monoclonal mouse anti-human Ki-67, Clone MIB-1(DAKO, Glostrup, Denmark) at a dilution of 1:50 (overnight incubation) and for CD34+ was monoclonal mouse anti-human, CD34+ Class II, Clone QBEnd 10 (DAKO, Glostrup, Denmark) at a dilution of 1:150 (30 minutes incubation).

Cores were dewaxed and rehydrated. Antigen retrieval was performed by keeping the slides in Tris EDTA buffer (pH 8), in pressure cooker for 5 minutes. Endogenous peroxidise was blocked by incubation in 3% hydrogen peroxide for 10 minutes. The cores were then incubated with the normal horse serum at dilution 1:20 for 20 minutes at 25ºC to block non- specific binding sites. Respectively primary antibody was added in appropriate concentrations. Sites of binding were detected using the Envision technique (DAKO code K5007, Glostrup, Denmark) with DAB (3-3´ diaminobenzidine, Vector code SK 4001, USA), a chromogenic substrate, according to the manufacturer's instruction. Cores were counterstained with haematoxylin, dehydrated and mounted with DPX. Appropriate positive controls were included in each run and negative controls were run with the omission of the primary antibody.

7.2.6 Morphometry

For automated image analysis of digitised slides were accessed through the Slidepath Image Analysis system and evaluated with the program's nuclear (for Ki-67), cytoplasmic (for CD68+) and membranous (for CD8+) scoring algorithm.

Individual TMA cores were identified, annotated on the scanned image and associated with TMA map entries. Individual nuclei stained with haematoxylin and/or polymerised diaminobenzidine were identified by a thresholding and segmentation algorithm which outlines nuclei and separates touching nuclei. Nuclear size (area) limits can be specified to accept or reject individual nuclei to be quantified. Staining for Ki-67 in each nucleus is classified as positive or negative based on the threshold specified by the observer. Pseudo-colours (red / orange / yellow / blue) display these staining intensity measurements for individual nuclei, allowing thresholds to be chosen appropriately. These thresholds were chosen using a sample of TMA cores from the whole cohort and once chosen were used for analysis over the entire patient cohort without further adjustment.

For CD68+, intracellular and positive pixel detection algorithm was used and for the CD8+, algorithm for thin cell membrane was used. Each of the TMA cores were crosschecked by one of the authors (SD) to detect any obvious error. Moreover, at least 25% of the cases were manually scored to produce inter-observer reproducibility. The inter-observer intraclass correlation coefficient values for Ki67, CD68+ and CD8+ were 0.96, 0.86 and 0.75 respectively.

To assess intra-tumoral micro-vessel density, immuno-histochemical staining for CD34+ was performed. Quantitative assessment of CD34+ was performed by manual counting of CD34+ positive endothelial cells or cluster regardless of whether a vessel lumen was seen in each of the TMA cores. All cores were counted by one of us (SD) and at least 25% of the cases were counted by a second observer (ZM) and interclass correlation coefficient for CD34+ was 0.87.

7.2.7 Statistical analysis

Survival analysis of the group variables was performed using the Cox proportional hazard model including deaths up to the end of May 2011. Multivariate survival analysis, including all significant covariates ($P \leq 0.1$) 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. The relationships between the mGPS and other variables were analysed using the Mantel–Haenszel (X^2) test for trend as appropriate. Analysis was performed using SPSS software (SPSS Inc., Chicago IL, USA).

7.3 Results

One hundred and four patients with gastric adenocarcinoma were included in our study. Overall, the majority of patients were male (66%), less than 75 years of age (82%)

and had an mGPS of 0 (80%). Most of the patients had TNM stage I or II (65%), had well to moderately differentiated tumour (54%), clear resection margin (89%) and LNR ≤0.2 (80%). Only 27 patients had high grade peri-tumour inflammatory infiltrate according to Klintrup-Makinen criteria (26%) and 36 patients had high grade necrosis score (35%). The median values for CD8+, CD68+ and Ki-67 were 5.3%, 17.2% and 19.7% respectively and for CD34+ the median value was 43. The majority did not receive neo-adjuvant (65%) therapy.

The relationship between patients without neo-adjuvant therapy and with neo-adjuvant therapy and clinico-pathological characteristics are shown in Table 7-1. Patients with neo-adjuvant therapy had higher infiltration of CD8+ ($p<0.01$) and higher Ki67 proliferation index ($p<0.05$). Whereas patients without neo-adjuvant therapy had higher TNM stage ($p=0.001$) and higher positive to total lymph node ratio ($p<0.01$). Cancer specific survival was better in patients with neo-adjuvant therapy ($p<0.001$).

The relationship between clinic-pathological factors and cancer specific survival was examined also in a subgroup of gastric cancer patients who did not receive neo-adjuvant therapy (n=68, Tables 7-2). In patients with gastric cancer, the median follow up of survivors was 62 months with a minimum of 21 months. During this period 44 (42%) patients died of their cancer and 7 (7%) patients died of non-cancer causes. On univariate analysis, only mGPS ($p=0.001$), TNM stage ($p=0.001$), tumour differentiation ($p<0.01$), LNR ($p<0.001$) and tumour necrosis (<0.01) were significantly associated with cancer specific survival. On multivariate analysis only, mGPS (HR 2.22, 95% CI 1.16-4.27, $p=0.017$), tumour differentiation (HR 2.44, 95% CI 1.10-5.42, $p=0.028$), LNR (HR 1.82, 95% CI 1.06-3.14, $p=0.030$) and tumour necrosis (HR 2.43, 95% CI 1.10-5.40, $p=0.029$) retained independent significance (Tables 7-2 and Figure 7-1).

The relationship between the mGPS and clinicopathological characteristics in patients undergoing potentially curative resection for gastric adenocarcinoma without neo-adjuvant therapy is shown in Table 7-3. There was no association between pre operative mGPS and clinicopathological parameters in this cohort.

Interrelationships between clinical and pathological charecteristics of patents who did not receive neo-adjuvant chemotherapy are shown in Table 7-4 (n=68). TNM stage was directly associated with poor tumour differentiation ($p=0.001$) and LNR($p<0.001$). Poor differentiation was directly associated with a LNR ($p<0.05$). Positive resection margin (R1) was directly associated with necrosis ($p<0.05$) and CD68+ infiltration ($p<0.05$). The Klintrup-Makinen score was directly associated with CD8+ infiltration ($p<0.05$), CD68+ infiltration ($p=0.001$) and Ki67 proloferation index ($p<0.05$). The CD8 infiltration was positively associated with CD68 ($p<0.001$). CD68+ infiltration in gastric cancer was directly associated with Ki67 proliferation index ($p<0.05$).

Interrelationships between clinical and pathological charecteristics of patents who did receive neo-adjuvant chemotherapy are shown in Table 7-5 (n=36). Advanced age was associated with higher necrosis score ($p<0.01$). TNM stage was directly associated with positive resection margin ($p<0.05$) and LNR($p<0.001$). Poor differentiation was directly associated with a positive resection margin ($p<0.01$) and higher CD68+ infiltration ($p<0.01$). Positive resection margin (R1) was directly associated with LNR ($p<0.05$). The Klintrup-Makinen score was directly associated with CD68+ infiltration ($p=0.01$) and Ki67 proloferation index ($p<0.01$). CD68+ infiltration in gastric cancer was directly associated with Ki67 proliferation index ($p<0.05$).

7.4 Discussion

The results of the present study show that, infiltration of CD8+ lymphocytes and proliferation index is higher in patients who received neo-adjuvant chemotherapy for their gastric cancer. Moreover, tumour proliferative activity was directly associated with the extent of macrophage infiltration, although tumour necrosis was not associated with proliferative activity, inflammatory cell infiltrate and angiogenesis. When the prognostic value of such tissue factors were examined, only tumour necrosis retained significance independent of the lymph node ratio and the systemic inflammatory response. Taken together these results would suggest that the relationship tumour necrosis and the inflammatory responses is more complex than proposed but that tumour necrosis plays a role in cancer survival following resection of gastric cancer.

In the present study tumour necrosis was an independent predictor of survival in patients who did not receive neo-adjuvant therapy. These results are consistent with the recent review reporting the prognostic value of tumour necrosis in other solid organ malignancies such as breast, renal, lung and colorectal cancer (Richards et al. 2011). Therefore, the basis of the impact of tumour necrosis on cancer survival is worthy of further study.

Low grade peri-tumoral inflammatory infiltrate was reported to be associated with poorer cancer specific survival in gastro-oesophageal cancer (Crumley et al. 2011) and colorectal cancer (Klintrup et al. 2005, Roxburgh et al. 2009a, Roxburgh et al. 2009b). The results of the present study suggest that role of the Klintrup-Makinen score as a prognostic indicator is inferior to that of tumour necrosis in gastric cancer.

With reference to the other tissue factors examined, the results of the present study are consistent with previous literature. Indeed, some previously reported studies in gastric cancer did not find any relationship between CD34+ microvessel density and Ki-67 proliferaration index and survival (Mao et al. 2007, Kim et al. 2002, Tenderenda et al. 2001, Suzuki et al. 2010, Li, Wei & Xue 2009) (Muller et al. 1996, Setala et al. 1998, Victorzon et al. 1997). Similarly, CD8+ inflammatory cell infiltrate was not associated with survival (Haas et al. 2009, Chiaravalli et al. 2006, Shen et al. 2010). However, there have been conflicting reports regarding the predictive value of the macrophages in gastric cancer. Ishigami and colleagues reported that higher infiltration of macrophages was associated with poorer outcome in gastric cancer (Ishigami et al. 2003). In contrast, Ohno et al reported a better outcome of patients with high tumour infiltration of macrophages in a study with similar number of patients with gastric cancer (Ohno et al. 2003). In the present study, intra tumoral macrophages were not associated with cancer specific survival. Although, it was associated with peri-tumoral inflammatory infiltrate, intra tumoral CD8+ lymphocyte infiltration, tumour proliferation and tumour differentiation.

In summary, of the tissue factors examined in the present study, tumour necrosis appeared to play a dominant role in the poor outcome in patients with gastric cancer.

Table 7-1: Clinico-pathological characteristic of patients undergoing potentially curative resection for gastric cancer (n=104)

	No neo-adjuvant (n=68)	Neo-adjuvant (n=36)	P value
Patient related factors			
Age (<65/65 –74/≥75 years)	29/28/11	11/18/7	0.300
Sex (Male/ Female)	46/22	23/13	0.828
mGPS (0/1/2)	53/13/2	28/5/3	0.620
Klintrup-Makinen score (Low grade/ High grade)	52/16	25/11	0.485
CD8+ tertiles (1/2/3)	29/21/18	7/10/19	0.004
CD68+ tertiles (1/2/3)	22/26/20	13/8/15	0.617
CD34+ tertiles (1/2/3)	24/23/18	10/10/16	0.238
Tumour related factors			
TNM Stage (I/II/III)	22/14/32	20/12/4	0.001
Tumour differentiation (well-mod/poor)	38/30	18/18	0.680
Resection Margin (R0/R1)	63/5	29/7	0.104
LNR (0/≤ 0.2/>0.2)	27/24/17	27/5/4	0.003
Necrosis Score (Low grade/ High grade)	45/23	23/13	0.831
Ki67 tertiles (1/2/3)	26/24/17	8/11/17	0.023
Alive/ Cancer specific death/ Non cancer death	28/33/7	25/11/0	<0.001*

*Log rank test

Table 7-2: Relationships between clinico-pathological factors and survival, in patients selected for potentially curative resection for gastric without neo-adjuvant therapy (n=68)

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Patient related factors				
Age (<65/65 –74/≥75 years)	1.01 (0.98-1.05)	0.501		
Sex (male/female)	1.27 (0.61-2.62)	0.522		
mGPS (0/1/2)	2.60 (1.45-4.67)	0.001	2.22 (1.16-4.27)	0.017
Tumour related factors				
TNM Stage (I/II/III)	2.20 (1.37-3.53)	0.001		0.737
Tumour differentiation (well-mod/poor)	2.74 (1.34-5.63)	0.006	2.44 (1.10-5.42)	0.028
Resection Margin (R0/R1)	2.08 (0.62-6.96)	0.234		
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	2.35 (1.48-3.72)	<0.001	1.82 (1.06-3.14)	0.030
Klintrup-Makinen score (Low / High grade)	0.45 (0.17-1.17)	0.103		
Necrosis score (Low / High grade)	2.50 (1.26-4.97)	0.009	2.43 (1.10-5.40)	0.029
CD8 tertiles (1/2/3)	0.98(0.64-1.50)	0.925		
CD68 tertiles (1/2/3)	0.87 (0.56-1.35)	0.541		
Ki67 tertiles (1/2/3)	0.78 (0.51-1.24)	0.316		
CD34 tertiles (1/2/3)	0.99 (0.63-1.54)	0.950		

Table 7-3: The relationship between the mGPS and clinicopathological characteristics in patients undergoing potentially curative resection for gastric adenocarcinoma without neo-adjuvant therapy (n= 68).

	mGPS 0 (n= 53)	mGPS 1&2 (n= 15)	P value
Patient related factors			
Age (<65/65 –74/≥75 years)	24/20/9	5/8/2	0.695
Sex (male/female)	37/16	9/6	0.337
Tumour related factors			
TNM Stage (I/II/III)	17/12/24	5/2/8	0.793
Tumour differentiation (well-mod/poor)	32/21	6/9	0.134
Resection Margin (R0/R1)	50/3	13/2	0.303
Positive to total lymph node ratio (0/≤ 0.2/>0.2)	21/19/13	6/5/4	0.940
Klintrup-Makinen score (Low / High grade)	40/13	12/3	0.506
Necrosis score (Low / High grade)	36/17	9/6	0.390
CD8 tertiles (1/2/3)	23/16/14	6/5/4	0.879
CD68 tertiles (1/2/3)	18/21/14	4/5/6	0.367
Ki67 tertiles (1/2/3)	24/16/12	2/8/5	0.065
CD34 tertiles (1/2/3)	16/20/14	8/3/4	0.339

Table 7-4: Interrelationships between different pathological and clinical parameters in patients selected for potentially curative resection for gastric without neo-adjuvant therapy (n=68).

	Sex	mGPS	TNM Stage	Tumour differentiation	Resection Margin	LNR	Klintrup Makinen score	Necrosis score	CD8+ tertiles	CD68+ tertiles	Ki67 tertiles	CD34+ tertiles
Age in years (<65/ 65-74/ ≥75 years)	0.172	0.400	0.206	0.721	0.137	0.120	0.926	0.148	0.422	0.910	0.469	0.746
Sex (male/ female)		0.195	0.418	0.541	0.476	0.293	0.415	0.051	0.649	0.832	0.733	0.497
mGPS			0.890	0.222	0.486	0.878	0.567	0.522	0.941	0.440	0.104	0.458
TNM Stage (I/ II/ III)				0.001	0.235	<0.001	0.909	0.181	0.816	0.073	0.575	0.362
Tumour differentiation (well-mod/poor)					0.613	0.049	0.602	0.085	0.800	0.069	0.557	0.433
Resection Margin (R0/R1)						0.111	0.335	0.041	0.306	0.015	0.438	0.789
LNR							0.342	0.159	0.625	0.802	0.402	0.353
Klintrup-Makinen score (Low / High grade)								0.528	0.022	0.001	0.027	0.852
Necrosis score (Low / High grade)									0.690	0.688	0.988	0.992
CD8+ tertiles (1/2/3)										<0.001	0.902	0.131
CD68+ tertiles (1/2/3)											0.033	0.180
Ki67 tertiles (1/2/3)												0.645

Table 7-5: Interrelationships between different pathological and clinical parameters in patients selected for potentially curative resection for gastric with neo-adjuvant therapy (n=36).

	Sex	mGPS	TNM Stage	Tumour differentiation	Resection Margin	LNR	Klintrup Makinen score	Necrosis score	CD8+ tertiles	CD68+ tertiles	Ki67 tertiles	CD34+ tertiles
Age in years (<65/ 65-74/ ≥75 years)	0.231	0.766	0.789	0.638	0.644	0.876	0.156	0.008	0.482	0.635	0.374	0.707
Sex (male/ female)		0.260	0.267	1.00	1.00	0.724	1.00	0.292	0.884	0.066	0.067	0.632
mGPS			0.462	0.423	0.561	0.421	0.834	0.568	0.210	0.906	0.801	0.301
TNM Stage (I/ II/ III)				0.055	0.013	<0.001	0.563	0.542	0.474	0.183	0.763	0.443
Tumour differentiation (well-mod/poor)					0.008	0.222	0.471	0.489	1.00	0.009	0.836	0.237
Resection Margin (R0/R1)						0.032	0.076	1.00	0.479	0.447	0.361	0.561
LNR							0.606	0.241	0.917	0.527	0.701	0.406
Klintrup-Makinen score (Low / High grade)								0.708	0.287	0.010	0.005	0.354
Necrosis score (Low / High grade)									0.884	0.619	0.914	0.632
CD8+ tertiles (1/2/3)										0.301	0.428	0.801
CD68+ tertiles (1/2/3)											0.014	0.296
Ki67 tertiles (1/2/3)												0.385

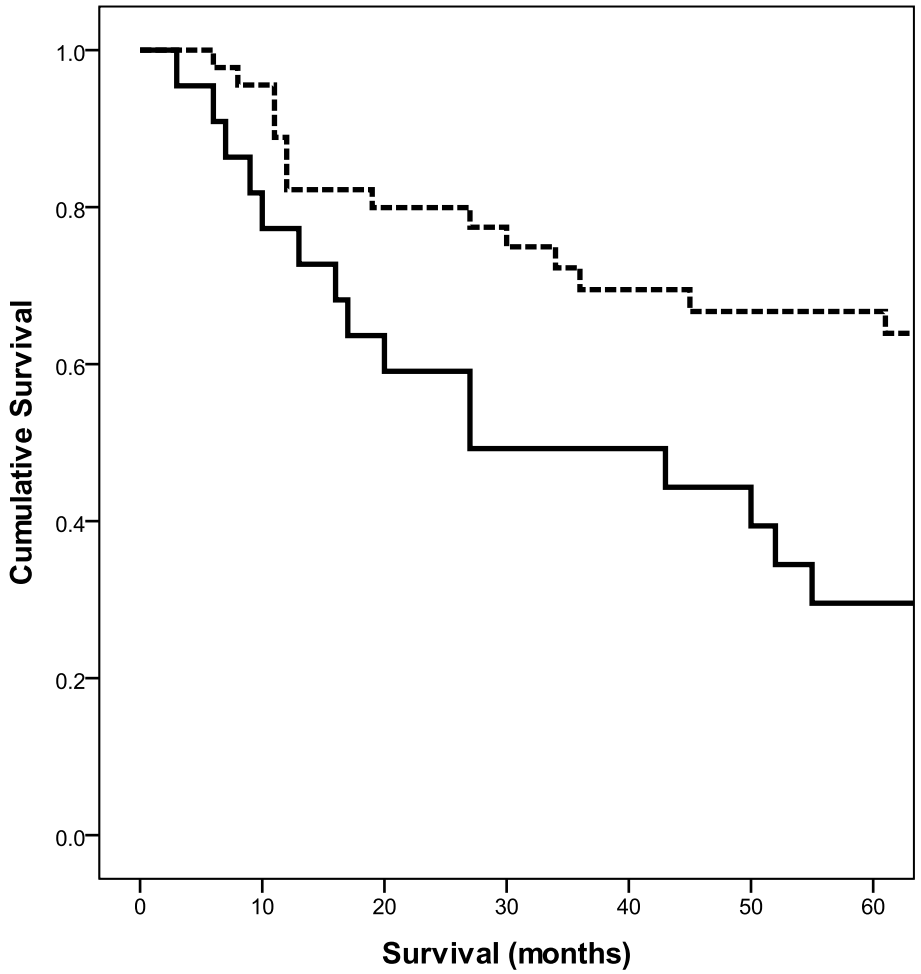

Figure 7-1: Relationship between tumour necrosis (top to bottom, low-grade and high grade) and cancer-specific survival in patients undergoing resection for gastric cancer without neo-adjuvant therapy.

8 Chapter VIII: Discussion

The overall aim of the thesis was to examine the relationships between tumour based factors, patient co-morbidity and the inflammatory responses (local and systemic) and outcome in patients undergoing resection for oesophageal and gastric cancer.

An underlying hypothesis in the present thesis work is that a sustained elevation of the systemic inflammatory response whether pre-, peri- or postoperative will compromise both short and long term survival through its relationship with the tumour microenvironment in patients undergoing surgery for oesophageal and gastric cancer.

The results of the present work have demonstrated that, in addition to tumour based factors, pre operative patient comorbidity and systemic inflammation have a role in predicting post operative outcome (short term and long term respectively) in patients with oesophageal and gastric cancer. Moreover, the perioperative systemic inflammatory response was useful in predicting the likelihood of developing a post-operative infective complication. These results parallel those recently reported in patients with colorectal cancer (Richards et al. 2011a).

In terms of short term outcome, the present studies did not confirm the pre operative elevated systemic inflammatory response predicts the post operative complications. This is in contrast to that reported in colorectal cancer (Richards et al. 2011a, Moyes et al. 2009) and oesophageal cancer (Vashist et al. 2011) in larger cohorts. It may be that the present studies were not large enough to detect a significant association. However, in large series of patients with curative resection of gastric cancer (n=1071) it was reported that preoperative elevated systemic inflammation was not associated with post operative complication but was a predictor of long term survival (Kubota et al. 2012). Therefore, further studies in larger patient's cohorts are required to define the relationship

between the presence of a pre-operative systemic inflammatory response and post-operative complications in patients with oesophageal and gastric cancer.

It is also of interest that although there have been reports of a relationship between post operative infective complications such as anastomotic leak are associated with poor long term outcome in colorectal cancer (McArdle, McMillan & Hole 2005, Walker et al. 2004) as well as in gastric cancer (Kubota et al. 2012, Nagasako et al. 2012, Sierzega et al. 2010). This was not confirmed in the present thesis work. Again, it may be that the present studies were not large enough to detect a significant association. Therefore, further studies in larger patients' cohorts are required to define the relationship between the presence of a post-operative systemic inflammatory response and complications and long term survival in patients with oesophageal and gastric cancer.

In terms of long term outcome, a pre-operative systemic inflammatory response was significantly associated with poor outcome in both oesophageal and gastric cancer. These results are consistent with a large number of studies in patients undergoing surgery for a variety of common solid tumours (Roxburgh and McMillan, 2010; Vashist et al. 2011; Jiang et al. 2012)).

Therefore, in long term survival, it would appear that the pre-operative systemic inflammatory response is of more importance compared with the peri-operative systemic inflammatory response. The results are consistent with recent work in patients with colorectal cancer (Richards et al. 2011a).

The mechanisms by which the systemic inflammatory response impacts on cancer specific survival is, however, still unclear. The present work confirmed the relationship between local inflammatory response and aggressive tumour behaviour. However, there was no significant relationship between the tumour inflammatory cell infiltrate and the

systemic inflammatory response, as evidenced by the mGPS, in patients with oesophageal and gastric cancer. One possible explanation of the enhanced malignant potential of tumour in presence of chronic elevated systemic inflammatory response is that the systemic inflammatory response is a surrogate for occult metastases. If this were the case it may be that more extensive pre-operative tumour staging will shed further light on this relationship. Alternatively, the use of neoadjuvant therapy would also be useful in teasing out this relationship.

However, this theory of occult metastasis is unlikely to fully explain the ability of the systemic inflammatory response to consistently and independently predict poor cancer survival in a variety of tumour types and at different stages of disease (Roxburgh, McMillan 2010; McMillan, 2012). From these reports and the results of the present study, it is more likely that the presence of a systemic inflammatory response enhances the malignant potential of the tumour. Indeed, it has been suggested that pre operative anti inflammatory intervention might reduce the recurrence rate and improve survival (Roxburgh, McMillan 2010; Clarke et al. 2011; McMillan, 2012). Clearly these issues of targeted anti-inflammatory therapies need to be evaluated by prospective controlled trials.

In the present thesis it was also hypothesised that the link between systemic inflammatory response and the local inflammatory response might be tumour hypoxia and necrosis. Whereby tumour necrosis activates tumour cells and macrophages to produce pro-inflammatory cytokines and growth factors such as vascular endothelial growth factor. As a result of that lympho-vascular invasion and tumour dissemination is increased. Indeed, tumoural necrotic cell death releases pro-inflammatory signals into the tumour microenvironment i.e. necrosis can recruit inflammatory cells which has tumouricidal activity. Also, such necrotic cells can release bio active regulatory factors like IL-1∞ that in turn stimulates tumour proliferation (Grivennikov, Greten & Karin 2010) and induces IL-6 production that stimulate hepatocytes to produce C-reactive protein. A recent review

by Guthrie and coworkers (2013) has developed such a scheme and have proposed IL-6 as a key mediator of the relationship between tumour necrosis, local and systemic inflammatory response and outcome in patients with colorectal cancer (Guthrie et al. 2013. The results from the thesis do not support such scheme in oesophageal and gastric cancer since a direct relationship between the tumour necrosis, inflammatory cell infiltrate and the systemic inflammatory response was not demonstrated. Therefore, it may be that the molecular link between tumour necrosis, local and systemic inflammatory response in oesophageal and gastric cancer is more complex. A detailed study of such relationships of IL-6 and pro-inflammatory cytokines may be useful.

Tumour associated macrophages have been reported to be one of the most important players in the tumour associated inflammatory responses. They may have anti-tumour as well as pro-tumour activity. However, generally a high tumour infiltrate of macrophages is associated with poor prognosis (Grivennikov, Greten & Karin 2010). Indeed, there was some support for the tumour promoting role of the macrophages in oesophageal adenocarcinoma in the present thesis.

Macrophages infiltrate the tumour at a very early stage of tumorigenesis and usually precede other leucocytes (e.g. lymphocytes; Clark et al. 2007). Detailed research in the last decade has enhanced our understanding of the promoting role of macrophages in tumour progression, metastasis and angiogenesis (Allavena, Mantovani 2012). Tumour associated macrophages can be of two subtypes i.e. M1 and M2. M1 macrophages are thought to have anti-tumour immune response whereas M2 subtype is thought to have a tumour progression role. Tumour associated macrophages are known to produce IL-6 in the tumour tissue by NF-kB signaling pathway. Moreover macrophages are known to accumulate in the hypoxic areas of the tumour and hypoxia induced factor (HIF) -1 alpha activates NF-kB pathways in such macrophages. In turn, IL-6 is known to activate the signal transducer and activator of transcription 3 (STAT3) pathways that in turn promotes

tumour proliferation and progression (Aggarwal, Vijayalekshmi & Sung 2009). Lastly, tumour associated macrophages are known to stimulate angiogenic switches in the tumour through vascular endothelial growth factor (VEGF) and other angiogenic molecules. In the present work there was not a significant relationship between intra tumoural microvessel density and intra tumoural macrophage counts. Therefore more detailed studies involving subtypes of tumour associated macrophages (M1 and M2), IL-6, VEGF expression and hypoxia-inducible factor 1 alpha (HIF1∞) and various signalling pathways (i.e. STAT3 and NF-kB) may provide a more detailed insight into the complex interplay between the tumour and host responses.

There is an accumulating body of evidence indicates that several anticancer chemotheraputic agents target tumour specific immune responses (Galluzzi et al. 2012). Furthermore, several large clinical studies are currently underway to examine the predictive value of various immune infiltrates in solid tumour (Senovilla et al. 2012). The potential pro-tumourigenic role of macrophages has made them an attractive target for anti cancer therapy and such therapy has been reported to have a better effect compared with conventional chemotherapy and anti-angiogenic therapy (Marigo et al. 2008, Bianchi et al. 2011, De Palma et al. 2007, Welford et al. 2011, Ferrara 2010). In the era of preoperative anti cancer treatment (neo-adjuvant) in oesophageal and gastric cancer, the role of tumoral and systemic inflammatory response biomarkers are likely to become more crucial to the determination of therapeutic response, risk stratification and long term outcome.

Therefore, as discussed above the work in the present thesis points to a number of avenues of future research. In particular, (a) the better stratification and allocation of treatment in patients with oesophago-gastric cancer. Although there are consistent reports in observational studies that markers of the local and systemic inflammatory responses have prognostic value independent of tumour characteristics. The next step is for such markers is that they will be shown to stratify patients in post-hoc analysis of existing

randomised controlled trials (RCTs). Once the post hoc RCTs analysis confirms the clinical utility of these factors they will then become stratification factors for prospective RCTs. There is evidence that markers of the systemic inflammatory response such as the GPS and the NLR are reaching this stage (McMillan 2013) (b) further examination of the complex interplay between local and systemic inflammatory response and survival. For example, the present thesis work does not distinguish whether an impaired local inflammatory response or an elevated systemic inflammatory response merely reflects the presence of occult metastases or whether these promote the development of micrometastases. In this case treatments that target the tumour (such as neoadjuvant therapy) or treatments that target the local and systemic inflammatory responses (such as NSAIDs administration) will provide further insight into the mechanisms underlying the observations of the present thesis.

From the above it is clear that the pre operative systemic inflammatory response, as evidenced by the mGPS, is of paramount importance (compared with cellular components of the local and systemic inflammatory responses) in determining long term outcome following oesophageal and gastric cancer resection. Furthermore, the mGPS is a simple and objective measure which is well standardised and easily reproducible and can be readily included in the clinical staging of all patients. Therefore, it would be reasonable to incorporate inflammatory biomarkers into pre operative stratification of patients with oesophageal and gastric cancer that it is possible to select those patients who will benefit from the extensive surgery and those who are unlikely to derive benefit. Also, pre-operative optimisation of patients is likely to become of increasing importance in achieving better survival especially in the modern era of neo-adjuvant therapy for oesophageal and gastric cancer. Further prospective studies are required to establish the complex interplay between patient's pre operative condition and survival in oesophageal and gastric cancer.

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