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AN INVESTIGATION OF THE RELATIONSHIP BETWEEN THE SYSTEMIC INFLAMMATORY RESPONSE, CYTOKINE PROFILE AND OUTCOME IN PATIENTS WITH RENAL CANCER

Sara Ramsey MB ChB MRCS

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Dr DC McMillan	University Dept of Surgery, Glasgow Royal Infirmary
Mr M Aitchison	Dept of Urology, Gartnavel General Hospital
Dr JD Graham	Beatson Oncology Centre,
Dr A Gracie	Dept of Immunology, Glasgow Royal Infirmary
Mrs M Cunningham	Beatson Oncology Centre
Mr GWA Lamb	Gartnavel General Hospital, Glasgow

Declaration

The work presented in this thesis was carried out in the Department of Urology, Gartnavel General Hospital, Glasgow, and the University Departments of Surgery, Royal Infirmary, Glasgow.

I declare that the work carried out in this thesis was carried out by myself, with technical support during the Luminex experiments provided by Dr Gracie, (Senior Lecturer) Dept of Immunology, Glasgow Royal Infirmary, and statistical analysis under the supervision of Dr DC McMillan (Senior Lecturer), University Departments of Surgery, Glasgow Royal Infirmary.

Publications arising from this thesis

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CRP	C-reactive protein
CT	Computerised tomography
ECOG-ps	Eastern Cooperative Oncology Group Performance Status
ELISA	Enzyme-linked immunosorbent assay
GPS	Glasgow Prognostic Score
HPRC	Hereditary papillary renal cancer
IFN	Interferon
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-10	Interleukin-10
LDH	Lactate dehydrogenase
MRCPS	Metastatic Renal Cancer Comprehensive Prognostic System
MSKCC	Memorial Sloan Kettring Cancer Centre
RCC	Renal cell carcinoma
TNM	Tumour Nodes Metastases
SSIGN	Stage Size Grade Necrosis
UISS	UCLA Integrated Staging System

Summary

Renal cancer is the 14th most common cancer in the UK, but is the most lethal of urological cancers. 40% of patients present with distant metastases, and 30% of patients undergoing a curative nephrectomy will subsequently develop metastases. For localized disease, the mainstay of treatment is radical nephrectomy. For patients with metastatic disease immunotherapy is the current standard of care, though the median survival is only nine months. In Chapter one, the epidemiology, pathology, clinical presentation and treatment of renal cancer are discussed.

In the second chapter we have reported the prognostic value of the cumulative Glasgow Prognostic Score (based on the combination of an elevated C-reactive protein and hypoalbuminaemia) in patients with metastatic renal cancer commencing immunotherapy. The Glasgow Prognostic Score was independently associated with cancer specific survival, in addition to the Memorial-Sloan Kettring Cancer Centre Score, with median survivals of 28, 11 and 3 months for patients with GPS of 0, 1 and 2 respectively. The GPS was also superior in predicting outcomes to another commonly used prognostic score, the Metastatic Renal Cancer Comprehensive Prognostic System.

In Chapter three we reported the prognostic significance of C-reactive protein, but not hypoalbuminaemia, in addition to the Leibovich score in UISS low and intermediate risk patients undergoing potentially curative nephrectomy. The presence of an elevated C-reactive protein was independently associated with cancer specific survival in addition to the Leibovich score. Both the Leibovich score and C-reactive protein were superior to the SSIGN score in predicting cancer specific survival.

In Chapter four we examined the role of circulating cytokines associated with T-lymphocyte subpopulations in patients with renal cancer. In the presence of a systemic inflammatory response, there was an association with increased cytokine concentrations from both T-helper 1 and T-helper 2 responses. However, cytokine concentrations measured using the Luminex technology were variable, and appeared less reliable than those measured using conventional ELISA technology.

In Chapter five, a longitudinal study of cytokine concentrations and circulating T-lymphocytes was performed in patients undergoing immunotherapy for metastatic renal cancer. Analysis of C-reactive protein, circulating T-lymphocytes, circulating cytokines was performed prior to commencement of immunotherapy, and after two to four weeks of treatment. Concentrations of C-reactive protein and interleukin-6 did not alter significantly following instigation of immunotherapy, nor did the numbers of circulating T-lymphocyte subsets. However, there was a significant increase in Interleukin-10 concentrations following commencement of immunotherapy.

In Chapter 6, we examined the longitudinal relationship between the systemic inflammatory response, and circulating cytokines in patients undergoing nephrectomy. Both interleukin-6 and interleukin-10 concentrations were elevated in patients with evidence of a systemic inflammatory response. However, on multiple regression analysis only interleukin-6 was significantly correlated with C-reactive protein concentrations. Following nephrectomy, the proportion of patients with an elevated C-reactive protein did not change significantly, nor did concentrations of interleukin-6 normalise. In contrast there was a trend towards significance in the elevation of Interleukin-10 concentrations following nephrectomy.

It has been previously suggested that the presence of the systemic inflammatory response in patients with renal cancer is due to secretion of pro-inflammatory cytokines by the tumour itself. It appears clear from the investigations carried out during the course of this thesis that the presence of systemic inflammatory response appears unlikely to be solely be determined by the tumour, but may be as a result of a disordered immune response from the host.

1.0 Introduction

1. 1 Incidence

Renal cell cancer (RCC) accounts for 3% of all cancers worldwide and was ranked as the 14th most common cancer in the UK in 2004, with over 6,000 new cases per year(Cancer Research UK). It is approximately twice as common in men as women and incidence rates increase with age with over 45% of cases occurring in patients aged over 65 (Landis *et al,* 1999, Office for National Statistics 2001). The incidence of renal cancer has been rising steadily over the last 50 years world-wide (Mathew *et al,* 2002) with the most significant increases occurring in black population groups (Chow *et al,* 1999). The greatest proportional increase in diagnosis has been for small tumours. It has been proposed this is due to serendipitous detection resulting from the increased use of non-invasive imaging for a wide variety of urological and non-urological conditions (Jayson & Saunders 1998, Konnak & Grossman 1985). However, there has also been an increase in detection of advanced and metastatic tumours which is not explained by earlier detection and subsequent lead time bias (Chow et *al,* 1999, Hock *et al,* 2002). Similarly, the mortality rate has also increased by a small but significant amount suggesting the increased incidence is not wholly due to earlier detection (Ries *et al,* 2001). In general, all stages of renal cell carcinoma are increasing in incidence and it is postulated this may be due to an unidentified aetiological factor.

1.2 Aetiology

The majority of RCC is sporadic, though 10% of cases are related to inherited predispositions or syndromes. Sporadic RCC is a disease of the 5th and 6th decades of life though familial syndromes such as VHL may occur in younger patients.

1.2.1 Smoking

In keeping with other common cancers, smoking is associated with an increased risk of developing renal cancer. In contrast to other cancers, the increased relative risk of cigarette smoking is only around 1.4 – 2.46, with a slightly greater risk for tobacco chewers and pipe smokers though interestingly women appear to exhibit a less strong association between smoking and the development of RCC (Kantor 1977, Goodman *et al,* 1986).

1.2.2 Hypertension

Hypertension has been linked to renal cancer, particularly uncontrolled hypertension and it is theorised that hypertensive damage to the nephron may initiate tumorigenesis. A patients with a diastolic blood pressure of 90 mmHg or greater has approximately twice the risk of developing RCC than a patient with a diastolic BP of 70mmHg or less (Chow *et al,* 2000). Previous studies have suggested users of diuretics have an small increased risk of RCC (McLaughlin *et al,* 1995) but on correction for hypertension there appears to be no excess relative risk (Mellemgaard *et al,* 1994). However, users of non-diuretic antihypertensives appear to have an increased risk even with correction for hypertension (McLaughlin *et al,* 1995). Other renal-specific associations include end stage renal failure and acquired cystic disease of the kidney

which both lead to an increased incidence of papillary variant renal cancer (Ishikawa *et al,* 1993).

1.2.3 Obesity

Obesity is associated with development of a number of cancers such as breast and colon, but also RCC. A patient with a Body Mass Index of 30 or greater may have between 3 and 6 fold increased risk of developing RCC, with the effect most strongly reported in women. (Yu *et al,* 1986, Chow *et al,* 2000) It is postulated that this is due to higher levels of circulating oestrogens, or the secreted Insulin-like Growth factor -1 from adipose tissue (Longcope *et al,* 1986).

Hormone-related factors have been implicated in the aetiology of renal cancer due to the lesser number of women affected, the association with obesity in women, and also the phenomenon of renal carcinogenesis in hamsters treated with diethylstilbestrol. However, in human case-controlled studies there is no association between RCC and hormone replacement therapy and a negative association with oral contraceptive use. There is an increased risk of developing RCC in highly parous women, but the effect is not linear, and other factors such as socioeconomics may be implicated (Linblad *et al,* 1995).

1.2.4 Environmental and occupational factors

Older studies have suggested RCC is an urban disease (Kantor, 1977) but the rural area of Bas-Rhin in France had the world's highest incidence of RCC between 1972 and 1992 (Mathew *et al,* 2002). Low socioeconomic status is associated with increased risk of developing renal cancer but no specific causative factor has been found (Kantor, 1977).

Occupational exposure to chemical agents has been extensively investigated with multiple agents postulated, but no single factor has been demonstrated to consistently and significantly increase risk. Occupational contacts with dry-cleaning solvents, cadmium and asbestos have demonstrated an increased relative risk of 1.4, 2.0 and 1.4 respectively though the risk is not consistent for duration of exposure and dose considerations (Mandel *et al,* 1995). There has been no association demonstrated between radiation exposure and RCC. Animal studies have suggested a link between hormones such as diethylstilboestrol, lead compounds and aflatoxins but no correlation has been shown in humans (Kantor, 1977).

1.2.5 Genetics

RCC describes several distinct histological subsets, and hence there are several distinct genetic syndromes associated with RCC development.

1.2.5(i) Von Hippel Lindau Syndrome

The most common mutation associated with the development of renal cell cancer is loss of the von Hippel-Lindau (vHL) tumour suppressor gene. This gene is located on Chromosome 3p25 and conforms to the Two-Hit Hypothesis (Kaelin *et al,* 1998). proposed by Knudson and Strong in 1972. VHL disease occurs in approximately one per 36,000 head of population and is inherited in an autosomal dominant fashion with over 80% penetrance. Patients with VHL disease may develop multiple tumours including retinal angiomata, CNS haemangioblastoma and phaeochromocytomas as well as clear cell RCC, which may often be multi-centric or bilateral in young adults. The VHL gene codes for a protein which indirectly regulates the cellular response to hypoxia. It forms a complex with 2 other proteins (ElonginB +C) which targets a protein known as Hypoxia Inducible Factor 1α (HIF1-α). HIF1-α controls the transcription of a number of growth factors including vascular endothelial growth factor(VEGF), platelet derived growth factor(PDGF), transforming growth factor-α (TGF-α) and erythropoietin. Under normal VHL control and oxygen tension, the VHL complex is responsible for degrading HIF1-α and limiting the production of growth factors. Under hypoxic conditions, or if both copies of the VHL gene are mutated, HIF1-α is not degraded. The subsequent increase in production of vascular and autocrine growth factors are thought to be crucial to the developing neoplastic cells, and the hypervascular nature of RCC. (Linehan *et al,* 2003).

Careful screening of affected families is required to identify renal and other tumours at an early stage.

1.2.5(ii) Hereditary Papillary Renal Cancer

A further form of hereditary RCC was described in 1994 following pedigree studies of affected families. This is known as Hereditary Papillary Renal Carcinoma (HPRC) (Zbar *et al,* 1994). It is transmitted in an autosomal dominant fashion and has high and consistent penetrance. Tumours are often of later onset, in the 5^{th} and 6^{th} decade, but may be multi-focal or bilateral. The gene for HPRC is located on the long arm of Chromosome 7 (7q31) (Linehan *et al,* 2003) and is a proto-oncogene. This region codes for the MET gene, which belongs to a family of tyrosine kinase transmembrane receptors responsible for sending signals from the cell surface to the cell nucleus. The MET protein is bound by Hepatocyte Growth Factor (HGF.) Normally when HGF binds, a growth stimulating cascade is stimulated. If the MET gene region becomes mutated, the receptor is activated without binding, leading to uncontrolled cell growth and proliferation.

1.2.5(iii) Birt-Hogg-Dube Syndrome

Birt-Hogg-Dube Syndrome was first described as a dermatological condition in 1977 with a triad of benign skin tumours; fibrofolliculomas, trichodiscomas and acrochordons appearing in the third or fourth decade of life (Birt *et al,* 1977). A case report of a patient with Birt-Hogg-Dube (BHD) syndrome and bilateral renal cancers was published in 1993 (Roth *et al,* 1993) and a population screening study identified an association with familial renal tumours (Toro *et al,* 1999). The syndrome is inherited in an autosomal dominant fashion with the genetic abnormality mapped to chromosome 17 which codes for a protein called folliculin (Schmidt *et al,* 2001). Approximately 15-30%

of patients with BHD will develop renal tumours, though unlike the other familial syndromes, the pathology is variable. The most common tumour subtype is chromophobe, though oncocytic renal cell carcinomas and pure oncocytomas have also been reported.

1.2.5(iv) Genetic abnormalities and sporadic tumours

In cases of sporadic clear cell RCC there is loss of heterozygosity of the VHL gene in nearly 100% of cases with an accompanying mutation on the remaining allele in 35-85% of cases (Gnarra *et al,* 1994, Shuin *et al,* 1994). Other genetic abnormalities associated with clear cell RCC are the loss of genetic material from the p arm of chromosome 3 and gain on the q arm of chromosome 5 forming an unbalanced translocation (Pavlovich *et al,* 2003).

Unlike the VHL mutation, sporadic cases of papillary RCC express the MET oncogene in less than 15% of cases (Schmidt *et al,* 1999). Papillary RCC is also associated with a translocation between the Y chromosome and Chromosome 17 and also trisomy in Ch7 and Ch17 (Lager *et al,* 1995).

1.3 Presentation

1.3.1 Classical presentation

The classical presentation of RCC is a triad of symptoms and signs; haematuria, loin pain, and a palpable flank mass. This triad is now often referred to as the "too late triad" and is rarely seen, comprising less than 2% and 10% of symptomatic presentations in 1998 and 1971 respectively (Jayson & Saunders 1998; Skinner 1971). The overall presentation of RCC is changing, with increasing numbers of patients diagnosed with asymptomatic, incidentally detected tumours. The figures are now historical, but it was estimated that over 60% of renal tumours were diagnosed incidentally in the 1990s, compared with around 10% in the early 1970s (Jayson & Saunders 1998; Skinner 1971). This change has been linked to a significant increase in the use of non-invasive imaging such as ultrasound and CT throughout the population, and a population that is ageing as a whole. However, no figures regarding modes of presentation in the 21st century have been published to confirm this trend.

1.3.2 Local effects

Local effects from the primary tumour may include loin pain and haematuria. Local invasion may cause pain, but also disruption of the invaded structure. Right sided tumours may invade or compress the duodenum or biliary tree leading to gastrointestinal symptoms and occasionally jaundice.

RCC is unique in its propensity to propagate along the venous system into the inferior vena cava and then cranially towards the right atrium. This involvement whether by tumour thrombus or direct invasion of the vessel wall may cause a number of presenting symptoms. These include a left-sided varicocoele, or varicocoele that persists

despite recumbency, due to obstruction of the left renal vein. Rarely, a right-sided varicocoele may result from extensive IVC thrombosis obstructing the right gonadal vein. Bilateral lower limb oedema or the presence of bilateral deep vein thrombosis may also result from IVC involvement. Tumour thrombus may be identified during routine echocardiography it if has propagated to the right atrium, or due to symptomatic pulmonary emboli.

1.3.3 Distant

The pattern of metastases in renal cancer is diverse, with a propensity for unusual metastatic sites. Virtually every organ in the body has been recorded as a site of metastasis from RCC. Subsequently, symptomatic presentation of metastatic disease is heterogeneous, and in addition, patients may be completely asymptomatic despite disseminated RCC. Lung, bone and brain are among the most frequently encountered metastatic sites and common presenting symptoms related to these are dyspnoea, bone pain and neurological disturbances respectively. Cutaneous deposits are also relatively common though the underlying diagnosis may only be made after excision biopsy. Table 1.1 illustrates the frequency of various metastatic sites recorded in one study of patients with metastatic RCC (Maldayz & DeKernion, 1986).

1.3.4 Paraneoplastic

RCC is probably associated with the widest range of paraneoplastic phenomena of any tumour. Paraneoplastic syndromes are constitutional symptoms or derangement of normal biochemistry or haematology which are associated with the presence of a neoplasm, though not caused directly by the tumour. They are estimated to occur in 10-40% of patients with RCC (Gold *et al,* 1996). Paraneoplastic disorders are more usually

associated with metastatic disease, but may be present in patients with localised primary tumours, and tend to resolve after curative resection. They can be subdivided into endocrine and non-endocrine syndromes, depending on the nature of the proteins secreted by the tumour.

Endocrine paraneoplastic effects may be due to hypersecretion of a substance normally associated with the kidney such as renin; or due to secretion of an abnormal hormone, or hormone-like protein. Syndromes associated with normal kidney secretion include hypertensions due to excess renin secretion, and polycythaemia secondary to increased erythropoietin release. Abnormal endocrine secretions include Parathormone-related peptide which mimics parathyroid hormone (PTH) leading to hypercalcaemia without bony metastatic involvement (Gold *et al,* 1996).

Non-endocrine paraneoplastic phenomena include weight loss, fever, night sweats and fatigue, and may be related to the development of a systemic inflammatory response. It has been suggested that these systemic symptoms are associated with IL-6 secretion (Blay *et al,* 1997). Other non-endocrine syndromes include non-metastatic hepatic dysfunction, known as Stauffer syndrome (Stauffer, 1961). This was first described in 1961 where 5 cases were recorded with hepatosplenomegaly, and grossly abnormal liver function tests in the absence of metastatic hepatic involvement. This dysfunction is reversible, and biochemical parameters may return to normal after nephrectomy. Anaemia is common despite the endocrine paraneoplastic secretion of erythropoietin. It is estimated that 25-33% of patients with RCC will have a normochromic normocytic anaemia whilst only 3% of patients will have polycythaemia (Chisholm & Roy, 1971, Marshall & Walsh 1977).

1.4 Diagnosis

Bosniak devised a classification of renal cysts based on their radiological appearance to stratify the likelihood of malignancy, see Table 1.2 (Bosniak 1986). The grading is based in the complexity of the cysts, and includes features such as multi-septated walls, calcification and enhancement after contrast administration. Solid renal lesions that enhance after contrast administration are said to have a 95% rate of malignancy, and the majority of renal tumours are currently diagnosed on CT imaging alone.

Fine needle aspiration biopsy (FNAB) of renal masses has a low sensitivity and specificity, with a false negative rate of around 10% (Rybicki et al, 2003). When compared with the high specificity of CT scanning, FNAB is not used routinely for diagnosis of RCC, though there are certain specific circumstances where renal biopsy is indicated. Patients with a complex renal mass and pre-existing cancer diagnosis require FNA to differentiate between renal metastasis and a second primary. Follow-up imaging after nephrectomy may identify a further mass in the solitary kidney or renal remnant and confirmation of RCC would be advisable before radical surgery which may render the patient anephric. Tissue diagnosis via FNAB to confirm RCC is also essential in patients who will receive immunotherapy as first line or neoadjuvant therapy; or as part of a clinical trial. Complications are relatively rare and those that occur most commonly are related to damage to adjacent structures such as bleeding and pneumothorax. Tumour seeding is a greatly feared complication but evidence from the literature suggests only five cases have been reported worldwide arising from biopsy of an RCC (Herts & Baker, 1995).

1.5 Pathology

Renal cell carcinomas are often 5 -8 cm in gross dimensions though can vary extensively. Most are round or ovoid and often have a pseudocapsule of compressed renal tissue around them. The appearance of the tumour can be extremely heterogeneous and may feature yellow or tan tumour along with cysts, necrosis, haemorrhagic areas and calcification. The pathological classification of renal cancers was revised and simplified by international consensus in 1997 (Storkel *et al,* 1997) following advances in molecular genetics and greater understanding of the behaviours of the different subsets of renal cancer. The current classification in use was proposed by Kovacs and colleagues in 1993 and is also referred to as the Heidelberg Classification (Kovacs *et al,* 1997). The classification defines 5 main subsets of renal cancer based on differences in genetics and molecular biology.

1.5.1 Conventional renal carcinoma

This is the most common form of renal cancer, and represents 70-80% of all cases. These tumours arise from the proximal convoluted tubule and are associated with loss of heterozygozity of the VHL gene on chromosome 3 in around 80% of spontaneous cases (Clifford *et al,* 1998). No other subset of RCC is associated with VHL mutation. These tumours are also referred to as "clear cell" due to their characteristic microscopic appearance. The tumour cells have an abundant cytoplasm containing glycogen and cholesterol which washes out during routine staining to produce the classic "clear cell." The majority of studies examining treatment in metastatic RCC describe clear cell tumour types only.

1.5.2 Papillary renal carcinoma

This is the second most common renal cancer, estimated to occur in 10-15% of cases and occasionally referred to as "chromophil renal cancer." It is thought this tumour arises from more distal in the nephron than conventional renal cancer and may develop in an adenoma-carcinoma sequence. Papillary renal cancer occurs more frequently in specific populations such as those with end stage renal failure, and cystic disease of the kidney (Ishikawa & Kovacs, 1993). Papillary renal cancer is also more likely to be multicentric and tends to be hypovascular as it lacks the VHL mutation leading to production of VEGF and subsequent angiogenesis (Renshaw & Corlees, 1995). As well as the papillary architecture of malignant epithelial cells around a central fibrovascular core, classical microscopic features include psammoma bodies and foamy macrophages. Previous subdivision into basophilic and eosinophilic variants was made on the degree of cytoplasmic staining. This convention has now been abandoned as it is believed a spectrum of staining exists within the subtype rather than two distinct entities.
The genetic abnormalities most commonly encountered with cases of papillary RCC are trisomy of chromosome 7, 16 or 17 and a loss of the Y chromosome.

Controversy exists over outcome in papillary RCC. Blath and coworkers reported that 80% of papillary cancers in his series were confined to the kidney (T1 or T2) and often of low grade, whilst Lager's series suggested increased numbers of higher stage and grade, associated with aggressive disease (Blath *et al,* 1976; Lager *et al,* 1995). It has been suggested that papillary renal carcinoma is subdivided into Type 1 and 2 to stratify the various behaviour. Type 2 tumours are larger, tend to occur in younger patients, and

are frequently of higher stage than Type 1 tumours, explaining the apparent contradictions in behaviour (Delahunt & Eble, 1997).

1.5.3 Chromophobe renal cancer

Chromophobe renal carcinoma occurs in 4-5% of RCC cases and is derived from the cortical portion of the collecting duct. Unlike the more common subsets of renal cancer, chromophobe renal carcinoma is not associated with specific genetic mutations. Its appearance has been described as "plantlike" on microscopy as it has a transparent cytoplasm, and fine reticular pattern. Electron microscopy reveals characteristic microvesicles. In a similar fashion to papillary renal cancer, there is dispute over the natural history of this tumour. Thoenes and colleagues reported the behaviour of chromophobe tumours as low grade and often localised to the kidney, whilst Renshaw and co-workers depicted a high grade tumour frequently associated with metastatic disease (Thoenes *et al,* 1988; Renshaw *et al,* 1996).

1.5.4 Collecting Duct Renal Cell Carcinoma

This also an extremely rare form of RCC and is thought to arise from the distal collecting duct cells as the name suggests. Only 100 cases exist in the literature (Chao *et al,* 2002) and whilst such small numbers make inferences difficult, it is thought to affect younger patients, presents with advanced disease and has an aggressive course.

1.5.5 Unclassified Renal Cell Carcinoma

It is estimated that 3-5% of renal tumours will not be classifiable by the 1997 consensus (Kovacs *et al,* 1997). These tumours may have unidentifiable cell types, or have complete sarcomatoid features without residual renal epithelial cells. Unclassified tumours are often excluded from studies but have been shown to be considerably more aggressive, with 90% of patients having nodal disease or distant metastasis at presentation. (Zisman *et al,* 2002). Numbers are small but survival is significantly reduced when compared with clear cell RCC, and there is also a propensity for the primary tumour to be inoperable due to local invasion (Zisman *et al,* 2002).

1.5.6 Other tumour types

1.5.6(i) Sarcomatoid variation

Sarcomatoid renal cell carcinoma was removed as a distinct histological subset during the revision of pathological subsets in 1997(Störkel *et al,* 1997). It is now thought to represent areas of poorly differentiated RCC with spindle-shaped morphology. It occurs in 1-5% of RCCs, particularly in conventional RCC. Sarcomatoid variation is associated with aggressive tumour behaviour and a poor prognosis. In two studies, over three-quarters of patients with sarcomatoid tumours had metastases at presentation. Survival after cytoreductive nephrectomy and immunotherapy was a median 8.5 months, and only 5 months for patients with metastatic disease receiving immunotherapy only (Mian *et al,* 2002, Cangiano *et al,* 1999). If the underlying RCC tissue is not identifiable, the tumour is ascribed to the unclassified subset.

1.5.6(ii) Medullary renal carcinoma

This is an exceptionally rare form of RCC first decribed in 1995 by Davis. It is virtually always found in patients with sickle cell trait, and is believed to develop from the calyceal epithelium near the renal papilla. Less than 50 cases exist in the literature (Figenshau *et al,* 1998) and it is highly aggressive with a mean survival of 15 weeks post-nephrectomy (Davis *et al,* 1995).

1.5.7 Nuclear grade

In keeping with the gross appearance of the kidney, the nuclear appearance of RCCs is also variable. The most commonly adopted system of classification is Fuhrman's grading (Fuhrman *et al,* 1982), which is stratified from grade 1 through to 4 (Table 1.3). There can be high levels of inter-observer variability, (Al-Aynati *et al,* 2003) particularly in determining intermediate grades, and some experts have suggested simplifying the system into only three levels. Nuclear grade has prognostic significance independent of tumour stage, but is inferior to Stage in determining outcome (Fuhrman *et al,* 1982).

1.6 Disease Staging

Staging of any cancer requires an understanding of the natural history of the disease to allow accurate risk stratification and outcome prediction. Accurate staging is essential for decisions regarding definitive treatment, adjuvant therapy and predictions of life expectancy. Use of a consistent world-wide staging system allows for comparison between centres and meaningful multi-centre clinical trials.

1.6.1 Current and historical staging systems

Tumour stage defines the anatomical extent of the disease. The first staging system used in RCC was created by Flocks and Kadesky in 1958 as a simple 4 stage system. It was further revised by Robson in 1969 to include vascular invasion but was maintained as a four stage system. (Robson 1969, Table 1.4) The Tumour-Nodes-Metastases system was devised by Denoix in 1948 and was used to develop the TNM classification for RCC by the Union Internationale Contre le Cancer in 1978. The current TNM classification (Table 1.5) is the 5th major revision, with further modifications adopted in 2001 to reflect the introduction of partial nephrectomy (Guinan *et al,* 1997). The TNM classification is now the most widely used system worldwide to stratify risk in patients with RCC, though some American groups still favour the Robson system. The strengths of the TNM classification lie in the adaptability of the classification to accurately represent the patient's tumour, with 72 possible variations, combined to produce a 4 stage system. TNM stage classification has been shown to accurately predict 5 year survival for patients with RCC (Bassil *et al,* 1985; Tsui *et al*, 2000a; Hermanek &

Schrott, 1990; Table 1.6) whilst the Robson system correlates poorly with prognosis (Pantuck *et al,* 2001).

1.6.2 Areas of controversy and potential modifications

It could be argued that some of the current controversies regarding the current staging system are related to areas of tumour biology still not fully understood in RCC. Discrimination between T1 and T2 tumours and subsequently TNM Stage I and Stage II is based purely on tumour size. The cut-off point between the two stages has undergone numerous revisions, being lowest in 1987 at 2.5 cm, and raised to 7cm in the 1997 revision. This most recent breakpoint was chosen arbitrarily as it represented the mean size of tumours in the SEER database, and it had been argued that the lower 2.5cm point did not lead to a significant survival difference between stages. However, similar criticisms have been applied to the current breakpoint. A number of authors have examined prognosis with different size subdivisions, and the general consensus is that the current 7cm cut-off is too high. However, there is no clear consensus as to where the reduced breakpoint should be placed. Various authors have proposed new cut-offs from 3.5 to 5.5cm (Wunderlich *et al,* 2002; Ficarra *et al,* 2004) though the most common breakpoint suggested is 5cm, which may be accepted in future revisions (Zucchi *et al,* 2003; Elmore *et al,* 2003).

Adrenal involvement is more common in upper pole tumours and is estimated to occur in less than 5% of cases (Sagalowsky *et al,* 1994). Ipsilateral adrenal involvement is currently classified as involvement via direct spread or as a metastatic deposit, T3a and M1 respectively. However, patients with direct invasion of the adrenal have been shown

to have poorer prognosis than other patients with capsular penetration and perinephric fat invasion, other T3a tumours (Han *et al*, 2003). It has been proposed that adrenal involvement by direct spread is reclassified as T4 disease.

Nodal disease is known to convey a poorer prognosis even with a favourable T stage and no distant disease. The 5 year survival for patients with nodal invasion is estimated to be between 7 and 17% even after apparently curative nephrectomy (Bassil *et al,* 1985). In view of this, it has been suggested that N1 nodal disease should be upstaged to Stage IV as the outcome is clearly poorer than patients with perinephric fat or venous involvement in Stage III.

1.7 Prognosis

Stage, as categorized by the TNM system, is the single most important determinant of outcome and prognosis in patients with RCC. Patients with Stage IV metastatic disease have a median survival of 9 months after diagnosis, and a 5 year survival of around 10%, compared to 90% survival at 5 years for patients with Stage I disease (Maldazys & Dekernion, 1986; Tsui *et al,* 2000a, Table 1.5.) Other prognostic factors include further tumour factors, and clinical factors, though they differ between metastatic RCC and localised RCC so will be dealt with separately.

1.7.1 Metastatic renal cancer

Prognostic scoring systems for metastatic renal cancer tend to include a number of variables encompassing tumour factors, clinical factors and haematological and biochemical parameters, probably reflecting the heterogeneous nature of the disease. The Atzpodien predictive score uses 6 biochemical and clinical variables with various weightings to stratify patients into three prognostic categories (Atzpodien *et al,* 2003). The most highly weighted variable in the system is a raised neutrophil count; with raised CRP, elevated lactate dehydrogenase (LDH,) time from diagnosis to metastatic disease, number of metastases and the presence of bony metastases included on univariate analysis but given lesser weighting. Another commonly used prognostic score is the Memorial-Sloan Kettering Cancer Centre system which uses low Karnofsky performace score, high LDH, low haemoglobin, high corrected serum calcium and absence of prior nephrectomy to stratify patients into three groups (Motzer *et al,* 1999). Further different variables are included in the Leibovich score for prognosis following cytoreductive

nephrectomy, including sarcomatoid differentiation, constitutional symptoms,and Thyroid Stimulating Hormone levels (Leibovich *et al,* 2003b).

The Karnofsky performance score is used in the Memorial-Sloan Kettering prognostic scoring system. Performance scores reflect the impact of the disease on the patient and their capability to carry out normal activities, and the eponymous Karnofsky score was developed in 1949 as the first quantative measure of the general condition of a patient. The Karnofsky score has now largely been superseded by the by the Eastern Cooperative Oncology Group (ECOG) performance scale (Oken *et al,* 1982) due to its relative simplicity and excellent reproducibility (Blagden *et al,* 2003). The ECOG performance scale is shown in Table 1.7. It has a major role as a single independent prognostic factor in determining patient eligibility for entry into clinical trials. A poor ECOG performance status is associated with increased risk of progressive disease, reduced response to treatment and reduced overall survival in RCC as well as other tumours.

An International Kidney Cancer Working Group has recently been established with the aim of creating a unified prognostic model for patients with metastatic RCC (Bukowski *et al,* 2004).

1.7.2 Localised renal cancer

In patients with localised tumours the important prognostic factors are closely linked to the discriminating factors between tumour stages in the TNM classification. Poorer prognosis is conferred by increasing size of the tumour, the presence of capsular invasion, and lymphatic or vascular invasion. The presence of nodal involvement is a

poor prognostic factor independent of T stage. Nuclear grade, as measured by the Fuhrman system, is also a significant prognostic feature (Fuhrman *et al,* 1982).

Histological subtype may have prognostic significance with improved 5 year survival for patients with papillary and chromophobe shown in small studies, though in a recent larger study, prognostic significance was lost on multivariate analysis (Amin *et al,* 2002, Patard *et al,* 2005). The two extremely rare subsets of RCC, medullary RCC and collecting duct RCC both confer an extremely poor prognosis due to the aggression of the tumour, along with the unclassified RCC. The presence of sarcomatoid features, or necrosis within the primary tumour is associated with shorter time to recurrence and reduced overall survival, independent of RCC tumour type (Sevinc *et al,* 2003). Microvascular invasion is also associated with poorer outcome, though this is thought to be due to its association with higher grade tumours (Sevinc *et al,* 2003).

Two major models have been developed to utilise the recognised prognostic tumour factors in addition to the TNM stage, both featuring the presence of intratumoral histological necrosis. These are the SSIGN score, which encompasses TNM stage, size, grade and histological necrosis (Frank *et al,* 2002) and the Leibovich score which includes T stage, nodal status, tumour size, grade and tumour necrosis (Leibovich *et al,* 2003a). No consensus exists as to the superiority of one model over another.

Clinical factors are thought to be of less importance in the prognosis of localised tumours than in metastatic disease. Mode of presentation may have prognostic significance simply because increasing numbers of RCC are diagnosed incidentally, (Gelb, 1997), and incidental tumours tend to be of lower stage and grade, conveying a better prognosis (Leslie *et al,* 2003, Tsui *et al,* 2000a). The presences of specific

paraneoplastic phenomena such as weight loss, anorexia, malaise, and hypoalbuminaemia have also been associated with poorer outcome independent of stage (Kim *et al,* 2003). The Memorial Sloan Kettring postoperative prognostic nomogram includes presenting symptoms but this variable is given significantly less weighting than tumour factors (Kattan *et al,* 2001). The only combined scoring system validated in localised renal cancer as well as metastatic disease is the UCLA Integrated Staging System (Zisman *et al,* 2001) which combines tumour factors of TNM Stage and Fuhrman grade with ECOG performance status to stratify patients into 5 groups corresponding to low, medium and high risk.

1.8 Management of localised (T1-T3a) RCC

Localised RCC describes a tumour contained within Gerota's fascia and without evidence of distant metastases. Complete surgical removal of localised disease provides the best outlook for disease free and overall 5 year survival. The operative management of localised renal cell carcinoma will be discussed in this section, including the role of nephron-sparing surgery, radiofrequency ablation and observation, as well as the most commonly performed radical nephrectomy.

1.8.1 Radical Nephrectomy

The first planned nephrectomy was performed in 1869 by Simmon, for a ureteral fistula. Simple nephrectomy is performed by urologists for benign and malignant conditions alike and entails removal of the kidney from within the perinephric fascia, leaving perinephric fat, adrenal and regional lymph nodes in situ. The first description of a radical nephrectomy with cancer principles in mind was by Robson in 1963 though Berg reporting from Mount Sinai hospital in 1913 suggested primary control of the renal vessels to prevent disseminating tumour. Robson's operative principles were removal of the primary with a wide margin, early control of vessels to prevent tumour embolisation, and removal of potential sites of lymphatic drainage.

Robson's radical nephrectomy involved a thoraco-abdominal approach with early ligation of the renal artery. The kidney and tumour would be removed in continuity with perinephric fat within Gerota's fascia, including the ipsilateral adrenal. Ideally nodal dissection would be carried out as part of the main dissection of tumour, but often due to the size of the tumour, lymphadenectomy would need to be performed following removal

of the kidney. His study reported a 5 year survival rate of 66% following radical nephrectomy for Stage 1 tumours and 64% for Stage 2 tumours, a significant improvement in comparison with simple nephrectomy. His results in 1969 demonstrated a statistically significant improvement in 5 year survival following radical nephrectomy, with an overall 5 year survival of 66% for patients with localised disease. It has been suggested his excellent results were partly due to precise exclusion of patients with early metastatic disease with use of novel imaging techniques such as CT and mediastinoscopy.

There have been subsequent modifications to Robson's classical radical nephrectomy. The rate of adrenal involvement is around 10% overall and may be as low as 0.6% in Stage I lower pole tumours (Tsui *et al,* 2000b). It is believed the majority of adrenal involvement develops from direct extension in upper pole tumours, rather than haematogenous spread. It has been proposed that the indications for ipsilateral adrenalectomy include an abnormal adrenal on CT, upper pole tumour or involvement of the left renal vein, otherwise the adrenal can be left in situ (Sagalowsky *et al,* 1994; Tsui *et al* 2000b).

Robson's initial paper reported involvement of regional nodes in 22.7% of cases. Lymph node involvement confers poor prognosis even with a favourable T stage and absence of metastases, though controversy still exists regarding the role of routine lymphadenectomy as part of radical nephrectomy. The role of lymphadenectomy is to further stratify patients, allowing consideration for adjuvant therapy for those with node positive disease; whilst removing those with poorer prognosis from the localised disease group, improving this group's survival figures. The morbidity associated with lymphadenectomy is relatively low, though it adds time and complexity to the operation,

and it has been suggested that the scientific principles behind it are erroneous. The lymphatic drainage varies between left and right kidney. The left drains via an anterior and posterior group to involve the left lateral lumbar, left diaphragmatic, pre- and post-aortic nodes; whilst the right kidney has three main regions of lymphatic, posterior, anterior, and middle channels which drain to the lateral caval, pre- and post- caval and inter-aortocaval nodes. However, only the regional groups of nodes drain predictably, and there are also multiple small interconnecting lymphatic channels between regions (Marshall & Powell, 1982). Metastatic disease commonly occurs without enlargement of the regional lymph nodes, as the disease does not progress in a stepwise fashion (Pantuck *et al,* 2003). Lymph node enlargement on pre-operative CT staging is commonly due to reactive inflammatory nodes rather than micrometastases (Studer *et al,* 1990). Primary results only are available from the EORTC (European Organisation for Research and Treatment of Cancer) prospective randomised controlled trial No. 30881 comparing radical nephrectomy alone with radical nephrectomy and lymph node dissection. Early results suggest no difference between surgical outcomes (Blom *et al,* 1999), but no long-term results have been published to compare oncological outcomes. It has been shown that very few patients, 2% on one study, will have positive nodes without clinical suspicion either at laparotomy or pre-operative screening, and it is proposed that nodal dissection is not performed routinely unless otherwise indicated (Minervini *et al,* 2001).

Developments in laparoscopic surgery have led to description of the laparoscopic radical nephrectomy as the gold standard for surgical management of RCC. The first laparoscopic nephrectomy was performed in 1990 for an oncocytoma by Clayman and co-workers (Clayman *et al,* 1991). Laparoscopic nephrectomy is performed most

commonly via the transperitoneal approach, though increasingly the retroperitoneal approach is used (Allan *et al*, 2001). Laparoscopy has shown favourable surgical outcomes when compared with open surgery, with similar operative complication rates, but significantly shorter post-operative stay and convalescence (Kavoussi *et al,* 1993). Many of the initial studies on laparoscopic radical nephrectomy were based on short-term outcomes, and did not have the long-term follow-up to fully compare oncological efficacy (McDougall *et al,* 1996; Ono *et al,* 1997). Longitudinal studies comparing the oncological efficacy of open radical nephrectomy with laparoscopic nephrectomy over 10 years are currently awaited to confirm laparoscopic nephrectomy is indeed the new gold standard.

1.8.2 Nephron-sparing surgery (NSS)

Nephron-sparing surgery has been pioneered due to the increasing numbers of patients with bilateral tumours, both synchronous and metachronous, the VHL population, and those with an anatomical or functional single kidney with unilateral tumours. The aim is to give surgical clearance whilst preserving as much healthy kidney as possible, and reconstructing the collecting system as necessary. Vermooten was the first to describe the feasibility of nephron-sparing surgery in the 1950s but was oblivious to the possibility of multi-centric tumours, or the presence of vascular and lymphatic invasion. Following Robson's work on radical nephrectomy, techniques of partial nephrectomy were developed in the mid sixties (Morgan & Zincke, 1990) and included simple enucleation, partial nephrectomy, and extracorporeal excision with autotransplantation. The latter has been shown to have significantly higher complications

and has been essentially abandoned. The current practice is to perform an extraperitoneal approach to the kidney and mobilise the vessels. Small peripheral tumours may not require temporary occlusion of the renal vein and artery, but larger and more centrally placed tumours usually do. The tumour and overlying perinephric fat is resected with a cuff of at least 0.5 cm of normal kidney. In order to reduce ischaemic damage to the remainder of the kidney, mannitol is given before vessel occlusion, and the kidney remnant is cooled with ice (Uzzo & Novick, 2001; Herr, 1999.) Some centres use intra-operative ultrasound to confirm intra-renal extent of tumours, and it is also recommended that frozen section is used to confirm the excision margins are clear before repairing the kidney (Uzzo & Novick, 2001).

Partial nephrectomy gained popularity to obviate the need for long-term dialysis in certain patients, but in its infancy, surgeons experienced technical problems with 45% of patients developing a urinary fistula, and one third of patients developing acute renal failure in the post-operative period (Campbell *et al,* 1994). Fortunately, the discovery that urinary fistulas respond well to expectant management and endoscopic stenting improved outcomes and reduced the requirements for further open surgery.

The major concern regarding nephron-sparing surgery is the potentially increased risk of local recurrence. It has been suggested that patients with small peripherally located tumours should be offered partial nephrectomy despite a normal contralateral kidney (Herr *et al,* 1999) but this can only be done if partial nephrectomy is of equal oncological effectiveness. Cancer free survival after nephron-sparing surgery has been shown to be significantly better in patients with tumours less than 4 cm (Hafez *et al,* 1999) and has led to the subclassification of the T1 tumour into T1a (<4cm) and T1b

(4cm – 7cm)in the 2001 revision of the TNM staging system. Fergany and co-workers have also reported that results from nephron-sparing surgery are comparable to results from radical nephrectomy over 10 year follow-up (Fergany *et al,* 2000).

1.8.3 Minimally invasive therapy

1.8.3(i) Radiofrequency ablation

Radiofrequency ablation of tumours using a needle electrode to cause local heating and tissue destruction was initially developed for small metastatic liver lesions. It was noted that the heating and tissue destruction were remarkably constant in ex vivo use, (Zlotta *et al,* 1997) and the first case report of successful radiofrequency ablation of a human renal cancer was described in 1998 by McGovern. Early follow-up suggests promising results and as the procedure appears well tolerated under conscious sedation (Pavlovich *et al,* 2002) this treatment may well be used increasingly in the elderly population who are unfit for a general anaesthetic as well as those with hereditary or multiple tumours, though it is still an considered an experimental technique.

1.8.3(ii) Cryotherapy

Similar to RFA, cryotherapy was pioneered on hepatic lesions, with the first use on a human RCC in vivo reported in 1996 (Delworth *et al,* 1996). Cryotherapy is usually delivered using an argon gas probe, and has two phases of tissue destruction. Initial freezing produces an iceball with total destruction of all tissue through rupture of cell membranes up to the few millimetres in the periphery. Subsequent thawing allows circulation to return with development of an acute inflammatory response in the residual tissue, thought to be due to damage to endothelial cells (Anderson & Havranek, 2004).

Again, long-term follow-up is limited, but promising results have been reported following cryotherapy for small tumours in patients unfit for nephrectomy (Gill *et al,* 2005).

1.8.4 Observation.

Despite advances in surgical technique and anaesthesia, nephrectomy remains a major operation, and in patients with significant co-morbidity complication rates are high. Due to this, and the increasing number of asymptomatic tumours discovered, a policy of watchful waiting has been suggested in RCC. The first advocate of the watchful waiting policy was Berg in 1913 though his stance was derived from the high mortality rate of early operative intervention for RCC. Bosniak has suggested that small tumours have a slow growth rate and lesser propensity to metastasis (Bosniak, 1995). These findings are supported in a larger study by Lamb and colleagues who reported growth rate as independent of tumour size, with metastasis a rare event in the cohort of elderly patients who were actively observed (Lamb *et al,* 2004).

1.9 Management of locally advanced renal cancer

1.9.1 T3b and T3c tumours

Renal cancer is unique in its propensity for tumour thrombus propagation into the renal vein and the inferior vena cava (IVC) and right atrium. IVC involvement is estimated to occur in 4-10% of cases of RCC (Marshall *et al,* 1970). Accurate pre-operative imaging of the tumour is required, not only regarding the level of caval tumour extension, but also regarding the presence of distant metastases as there is currently no evidence to demonstrate any survival benefit for patients undergoing nephrectomy and caval exploration with concurrent distant metastases (Bissada *et al,* 2003). Suprahepatic extension of tumour thrombus may necessitate the planned involvement of hepatic or cardiothoracic surgeon to perform hepatic mobilisation, cardiac bypass, and rarely cardioplegia to allow for vessel control and thrombectomy (Jibiki *et al,* 2004; Langenburg *et al,* 1994).

The mainstay of treatment in T3b or T3c disease is radical surgery, aiming to remove all tumour with negative surgical margins. The first published description of caval exploration with nephrectomy is by Berg in 1913 who described gaining initial control of the renal vessels, followed by caval clamping and milking out of tumour thrombus in 6 patients with only 1 death. It took until 1972 for RCC with caval extension to be thought of as a potentially curable lesion, with successful resection in 75% of patients (Skinner *et al,* 1972). An improvement in surgical techniques, including hepatic mobilisation, cardiopulmonary bypass, and the use of prosthetic grafts to replace resected portions of IVC have all lead to increasing numbers of patients with T3b tumours having successful resections (Bissada *et al,* 2003).

There has been controversy over the revision of the TNM classification in 1997 to classify renal vein and infra-diaphragmatic IVC involvement as T3b, based on the evidence that no statistically significant difference in prognosis has been reported between patients with renal vein involvement alone, and renal vein and inferior vena cava involvement below the diaphragm (Kim *et al,* 2004). However, tumour extension above the diaphragm has a significantly worse prognosis and has been classified as T3c to reflect the poorer outcome (Sosa *et al,* 1984). As with localised disease, the presence of regional lymph node metastases is an independent prognostic sign of poor outcome (Kuczyk *et al,* 1997). It is also important to differentiate IVC involvement as either tumour thombus propagation or direct vessel wall invasion, as these have different prognostic values. Tumour thrombus present in the IVC can be milked out relatively easily and 5-year survival rates are around 68%. In contrast, invasion of the caval wall with positive surgical margins has a 5 –year survival rate of 26%, rising to 57% if the involved caval wall is fully resected (Hatcher *et al,* 1991).

1.9.2 T4 tumours

T4 tumours in the TNM classification are tumours that have extended outwith Gerotas fascia and may have invaded into adjacent structures. Patients may present with pain from invasion of local nerve roots, or paraspinous muscles, but many still have incidentally detected tumours despite the advanced tumour stage.

The primary consideration of the surgeon treating a patient with a T4 tumour is whether the disease is resectable. The results from simply debulking tumours have been very poor; with 1 year survival rates of around 12% (DeKernion 1978). Radical surgery

may require excision of neighbouring structures such as colon, pancreas and diaphragm to provide clear excision margins. In patients with apparently unresectable disease and good performance status, neo-adjuvant immuno-therapy may be advocated in the hope even a partial response may render the tumour operable. However, there is a striking lack of literature regarding neo-adjuvant immunotherapy and the management of patients with T4 disease in general.

1.10 Adjuvant therapy following nephrectomy for patients at high risk of recurrence

Despite improvements in diagnosis and also improvements in surgical technique, many patients are at high risk of recurrence following an apparently curative nephrectomy and relapse rates may be as high as 30% (Sandock *et al,* 1995). High-risk groups include patients with nodal disease, patients with macroscopic venous or microvascular invasion and also patients with positive surgical margins. A variety of adjuvant therapies have been tried to reduce the recurrence rates and hence improve overall survival in this group.

1.10.1 Radiotherapy

A number of studies have examined the role of post-operative radiotherapy to the renal bed following nephrectomy following the success of the BAUS series in the fifties (Riches, 1966) which suggested an increased survival benefit and reduced recurrence rate. However, this series was published before uniform staging systems were widespread. Repeat studies based on standard pathological classification demonstrated no survival benefit or increased time to recurrence with post-operative radiotherapy using a number of regimes (Finney, 1973; Kjaer *et al,* 1986.) The effects of radiotherapy on neighbouring organs, in particular the liver, duodenum, and small bowel, have been shown to increase morbidity and mortality and adjuvant radiotherapy for RCC has become essentially obsolete.

1.10.2 Immunotherapy

A number of clinical trials have used immunotherapy in an adjuvant role to prevent recurrence by stimulating immune surveillance. Treatment with interferon-alpha has not been associated with any delay in time to recurrence or reduced incidence of disease recurrence in several trials (Porzsolt *et al,* 2001; Pizzocaro, 1992).

At present there is no standard adjuvant treatment for patients at high risk of recurrence and they require intensive follow-up to allow for early detection and treatment of metastases. A Europe-wide Phase III trial "HYDRA" is currently running to establish the efficacy of combination immunochemotherapy in the form of IL-2, 5-FU and IFN-α in comparison with observation alone as an adjuvant therapy for high risk patients with recruitment closing in July 2006.

1.11 Management of metastatic disease

Several modalities of treatment have been used in metastatic disease and these will be addressed in turn. The overall outlook for patients with distant metastases is poor, with a 5 year survival rate of around 10% (DeKernion, 1978). The phenomenon of spontaneous regression of RCC is estimated to occur in less than 1% of cases (Young 1998) but it has been suggested spontaneous resolution may explain the similarity of responses in patients treated in both placebo and active arms of clinical trials (Oliver *et al,* 1989).

1.11.1 Chemotherapy

Metastatic renal cell ca is relatively chemotherapy insensitive. A meta-review of 33 chemotherapy agents in 51 Phase II trials was carried out, which involved 1,347 patients (Motzer & Russo, 2000). No chemotherapy drug produced significant response rates to support single agent chemotherapy as a treatment option for patients with metastatic RCC. Combination chemotherapy regimes and chemotherapy plus hormonal agents also produced no increase in rates with additional toxicity

Chemoresistance in RCC is due to underlying cell biology. RCC cells produce P-glycoprotein, the protein product of the MDR-1 gene (multi drug resistant) gene. This plasma membrane protein provides an ATP driven mechanism for transporting hydrophobic drugs from intracellular to extracellular. It actively effluxes many chemotherapeutic agents, including vinka alkaloids, promoting broad resistance (Mickish *et al,* 1990). However, agents used to block the MDR protein such as verapamil have not improved responses when used in combination with chemotherapy (Yagoda *et al,* 1995)

and other features of tumour biology may also be important in the chemoresistance of RCC.

1.11.2 Radiotherapy

Renal cell carcinoma is also relatively radio-resistant. The main role of radiotherapy in metastatic renal cancer is for symptom control in a palliative setting. This may be appropriate for symptoms from the primary tumour such as pain, or continuing haemorrhage, though is more commonly used for symptoms from metastatic disease. Indications for radiotherapy in metastatic disease include haemorrhage, bone pain, tumour mass effects, and neurological symptoms from cord involvement or brain metastases. Relatively small doses are highly effective in reducing symptoms from bony metastases. However, radiotherapy is less effective in the treatment of neurological metastases than neurosurgery in patients with an isolated recurrence and a good performance status (Halperrin & Harisiadis, 1983).

1.11.3 Hormonal therapy

In animal studies oestrogen has been used to produce renal cancers in rodents, particularly hamsters and subsequent use of antioestrogens blocked tumour induction (Li & Li, 1984). Despite this, no role has been identified for endocrine blockade such as tamoxifen in treating metastatic RCC (Atzpodien *et al,* 2001) and immunohistochemisty suggests minimal androgen receptor expression within renal tumours (Langner *et al,* 2004).

Both medroxyprogesterone acetate (Provera) and Megestrol acetate (Megace) have been used in metastatic RCC on a limited basis. Neither drug has shown an increased response rate to suggest anti-tumour activity but both have been used in patients in advanced disease for palliative purposes (Schacter *et al,* 1989; Harris, 1983.) Both increase appetite and may improve fatigue, particularly in patients with cachexia and it is reported that minimal adrenal suppression may reduce the systemic inflammatory response driving the cachexia (Naing *et al,* 1999).

1.11.4 Immunotherapy

Immunotherapy is treatment using cytokine preparations to stimulate or modify the body's immune response. The two main types of immunotherapy used are the interferons and interleukins.

1.11.4(i) Interferons

Interferons are a family of proteins containing around 166 amino acids and were first discovered in the 1950s as antiviral agents, named due to their "interference" with viral replication. They are coded for by a number of genes on chromosome 9 in leucoyctes and monocytes (Dorr, 1993). In vivo the amino acids in the molecule are bound to an essentially inert sugar molecule to produce a molecular weight of around 37,000D. There are three main subgroups, interferon-α, β and γ. The exact mechanism of action of IFN is as yet unclear. Cell surfaces carry a specific IFN receptor which allows for internalisation of the IFN. Within the cell, IFN activates a specific protein kinase which binds to double stranded RNA, blocking RNA synthesis and protein production. IFN also inhibits a number of oncogenes including c-myc and c-fos and the gene coding

for an enxyme ornithine decarboxylase (Dorr, 1993). This is believed to be the underlying mechanism of IFN's activity in slowing cell division and increasing the time cells spend in the resting (G0) phase.A number of tumours including non-small cell lung cancer, glioma and acute lymphoblastic leukaemia show a deletion of the area on chromosome 9 responsible for interferons, (Olopade *et al,* 1993; Diaz *et al,* 1990) leading to suggestions that IFNα is a natural tumour inhibitor.

Interferons have a significant number of immunoregulatory actions. They can activate monocytes and macrophages, induce tumour antigen expression, increase natural killer and cytotoxic T-cell acitivity. Interferons have also been shown to modify the T helper differentiation in murine studies. IFNγ is released by TH-1 cells, and in turn inhibits TH-2 cells (Dorr *et al,* 1993). Patients with RCC have a predominantly TH-2 response and IFN may help to downregulate this in favour for T-cell maturation. IFNs have a weak antiangiogenic action, but even when used in high dose and in combination with other antiangiogenic agents to treat RCC results have been disappointing (Hernberg *et al,* 2003).

Interferon-γ has appeared to be a more active immune modulator in vivo than other subgroups but Phase III trials administering IFN-γ have suggested its response rate in patients with metastatic RCC is no better than placebo (Venner *et al,* 1997; Gleave *et al,* 1998). Its use has now been discontinued in favour of IFN-α in clinical trials. Artificial or therapeutic IFNα is produced by recombinant DNA technology and administered by subcutaneous injection. The optimum dosage and administration regime varies between country and trial group but it is generally established that low-to medium doses of around 10 megaunits intermittently are as effective as continuous high doses

(Krown, 1987). The dose and duration of treatment usually is limited by patient tolerance of side effects, though one study has shown patients with stable disease may tolerate more than12 months of treatment (Kankuri *et al,* 2001). The Medical Research Council trial of IFNα versus medroxyprogesterone acetate reported a 12% improvement in survival in the IFN group, an improvement in median survival of 2.5 months (MRCC, 1999).

Responses to IFN vary, and have been reported as between 12 and 30 % (DeKernion 1983; Neidhart 1986). However, some trials have considered stable disease to be evidence of a response to IFN, whilst others have defined a response to be at least a 50% reduction in the disease. The average time to an objective response is between 2 and 4 months (Wirth, 1993) though responses after more than 1 year's treatment have been reported (Kankuri *et al,* 2001).The average duration of response has been shown to be between 8 and 10 months though on rare occasions may be sustained for 24 months (Wirth, 1993). Highest response rates reported tend to come from specialist centres and are probably due to careful patient selection. Improved outcomes are reported in asymptomatic patients with high performance status, previous nephrectomy, and low volume pulmonary metastases (Minasian *et al,* 1993). Other factors predictive of favourable response are the absence of a systemic inflammatory response (Bromwich *et al,* 2004) and normal white cell count (Royston *et al,* 2004).

Side-effects can be considerable and lead to a significant deterioration in ECOG-PS. They include fatigue, nausea, flu-like symptoms, diarrhoea and itch and whilst modulated to some degree with paracetamol may require dose reduction or even treatment cessation in severe cases.

1.11.4(ii) Interleukins

Interleukin-2 was discovered as part of the search for T-cell growth factors in 1976. Interleukin-2 is primarily a cytokine of the TH-1 response and is a 15,000 dalton glycoprotein secreted by activated CD4+ lymphocytes. Its effects are T-cell proliferation and differentiation, and it also enhances natural killer cell function, and causes proliferation of granular lymphocytes which develop as lymphokine-activated killer cells (LAK) Its antitumour activity is primarily through immunological actions as it has no direct antiproliferative actions (Gitlitz & Figlin, 2003). High dose intravenous IL-2 was approved as a treatment for metastatic RCC in 1992 by the American FDA following overall response rates of 15% in Phase III trials with 255 patients (Fyfe *et al,* 1995). There was a significant increase in the duration of the response with a median response of 23 months, and also in complete responses, though responses only occurred in patients with an ECOG PS of 0 or 1.

Toxicity is the major concern in patients treated with high-dose IL-2, with side-effects including hypotension, and cardiac problems which have been fatal, with a 4% incidence of treatment-related death reported (Fyfe *et al,* 1995). The underlying mechanism of toxicity appears to be increased capillary permeability leading to hypotension and pulmonary oedema (Haas *et al,* 1993). Side effects are dose-dependent, and considerable adjustment has been made to minimise side effects whilst preserving response rates. Attempts to reduce side-effects with regimens of subcutaneous low-dose IL-2 has produced better tolerance, with similar response rates, though it is suspected responses may be of a shorter duration (Law *et al,* 1995.) It has also been suggested that a

rapid deterioration in a patients' quality of life whilst receiving IL-2 is associated with a better outcome, which may motivate patients to persevere with treatment (Atzpodien *et al,* 2003b).

Relatively new mechanisms of administration have included local use of IL-2 to reduce systemic side-effects such as inhaled IL-2 for pulmonary metastases. This has been trialled in high risk patients and has been associated with increased stable disease rather than any objective response (Huland & Heinzer, 2004).

1.11.5 Combination chemo/immunotherapy

Early studies of both IFNα and IL-2 suggested that the different mechanisms of action could lead to synergy from combined therapy. However in a multi-centre trial, response rates from dual therapy were similar to those of IL-2 alone, but with additional toxicities (Vogelzang *et al,* 1993). One large study has demonstrated a significant increase in response rates to 18.6% in patients treated with dual therapy, when compared with monotherapy interleukin-2 or interferon alfa with responses of 7.5% and 6.5% respectively (Negrier *et al,* 1998). However, the overall survival rates showed no difference between those receiving single therapy and dual therapy.

Experimental and Phase II studies have also suggested a synergistic effect between fluorouracil and interferon-α. A biochemotherapy triple regime of interleukin-2, interferon-alpha and fluorouracil over an eight week cycle, pioneered by Atzpodien has reported response rates of over 40 % and with similar toxicities to dual immunotherapy (Atzpodien *et al,* 1993). Whilst some centres running Phase II trials have achieved similar response rates of 35%, (Hofmockel *et al,* 1996) other clinical trial centres have shown no

additional benefit from the addition of 5-FU to the dual therapy of interleukin-2 and interferon-α (Negrier *et al,* 2000). It has been argued that the lack of reproducibility with the triple regime represents a less highly selected group of patients with poorer prognostic factors. Currently a European multicentre randomised trial is in progress to compare "triple therapy" with IFN alone in patients with metastatic RCC (EORTC 79).

1.11.6 Multi-modality therapy

Increased response rates to Interferon and Interleukin-2 have been recorded in patients with metastatic RCC who have undergone nephrectomy, so known as cytoreductive nephrectomy. However, nephrectomy alone has no survival benefit in patients with metastatic renal cancer (Middleton, 1967) and in patients unfit for subsequent immunotherapy nephrectomy should only be performed for palliative reasons such as haemorrhage. It is believed that removal of the large primary tumour may at least partially reverse any immunosuppressive effect prior to stimulatory immunotherapy. Similarly, even multiple pulmonary metastases are of relatively low volume when compared with an in situ renal primary, hence the "cytoreductive" role of nephrectomy. Surgery followed by immunotherapy with interferon led to a survival advantage of 5.8 months respectively when compared with interferon alone (Flanigan *et al,* 2004).

Controversy exists over the timing of immunotherapy and cytoreductive nephrectomy. A survival advantage exists for those patients who have undergone primary nephrectomy but performing major surgery on patients with a relatively poor survival may be inappropriate. Patients may have a delayed recovery following their nephrectomy, deterioration in performance status, or operative complications such as myocardial

infarction which would render them unsuitable for IL-2 therapy. Patients with rapidly progressive disease may also not be deemed suitable for a trial of immunotherapy after surgery. A number of studies have reported that approximately 40% of patients who undergo cytoreductive nephrectomy subsequently do not receive post-operative immunotherapy, usually due to operative morbidity or disease progression (Walther *et al,* 1993). The only significant factor predicting failure to undergo immunotherapy in these studies was a pre-operative ECOG PS of 2 or greater which emphasises the importance of careful patient selection for multi-modality therapy. Despite a policy of active consideration for surgery, the numbers of patients suitable for primary cytoreductive nephrectomy are small and represent seven percent of all patients with renal cancer (Bromwich *et al,* 2002).

It has also been proposed that immunotherapy should be given initially as a neoadjuvant treatment (Sella *et al,* 1993). Patients who respond to immunotherapy would then undergo cytoreductive nephrectomy and radical excision of any remaining metastases. Advantages of this approach include better selection of patients undergoing the major procedure of a nephrectomy, and potential shrinkage of the primary leading to enhanced operability. However, numbers undergoing nephrectomy would be even smaller than the current rates for cytoreductive nephrectomy. One study has suggested slight survival benefit of 14 months compared with 12 months for patients treated with initial immunotherapy but numbers were small (Rackley *et al,* 1994). At present the Southwest Oncology Group (SWOG) is running a multicentre trial to compare these two treatment regimes though no results have been published to date.

1.11.7 Surgery for metastatic disease

Whilst the mainstay of treatment in patients with metastatic RCC is immunotherapy, either alone or as part of a clinical trial, there are specific indications where surgery can play a useful role in the treatment of metastatic disease.

The first role is in the treatment of the isolated local recurrence. This is a relatively rare occurrence and is estimated to occur in less than five percent of patients within five years of the potentially curative nephrectomy (Itano *et al,* 2000). Controversy exists as to whether renal bed recurrence represents microscopic residual disease from the time of nephrectomy, or a specific form of metastatic disease. In these patients, complete surgical excision of residual tumour including any adjacent involved organs is essential to maximise disease-free survival. The five year survival rate for patients undergoing successful en-bloc resection of recurrence is approximately 50% whilst conservative treatment or immunotherapy carries a five year survival of around 15% (Itano *et al,* 2000; Sandhu *et al,* 2005).

The second indication for surgery in metastatic disease is in patients with discrete single metastases or low volume disease. In these patients, careful evaluation needs to be made to ensure there are no other concomitant metastases as it is estimated that the incidence of a solitary metastasis is approximately two and a half percent (O'Dea *et al,* 1978). The first description of successful surgery for solitary pulmonary metastases was in 1939, with the patient living 23 years after surgery (Middleton, 1967). Metastasectomy is most commonly carried out for pulmonary metastases in the form of wedge resection or lobectomy, but can also include partial liver resection, adrenalectomy and soft tissue resection. There is a significant survival benefit for those presenting with a solitary

metastasis some time after their primary tumour when compared to patients with concomitant metastasis and primary tumour, with 50% survival at five years, and 22% at two years respectively (O'Dea *et al,* 1978). The site of metastasis may also have prognostic significance as work by O'Dea suggested patients with solitary bony metastases had a relatively poorer outcome, though more recent work from a specialised Orthopaedic Oncology unit reports a five year survival of approximately 50% for patients with favourable characteristics treated aggressively with surgical resection (Althausen *et al,* 1997). In some circumstances, such as recurrent solitary pulmonary metastases, repeat metastasectomy may be appropriate but should not be performed if there is evidence of disseminated disease (Fourquier *et al,* 1997).

1. 12 RESEARCH PROPOSAL

Examining the prognostic indicators in metastatic and localised RCC, various aspects of the systemic inflammatory response, namely neutrophil count and CRP, are clearly associated with poorer outcomes. Recently a cumulative prognostic score based on elevated CRP and hypoalbuminaemia, markers of the systemic inflammatory response, has been developed. This has been shown to have prognostic value in patients with non-small-cell lung cancer, independent of stage and performance status (Forrest *et al*, 2003.) The value of this prognostic score has yet to be examined in metastatic or localised RCC.

1.12.1 CRP and Renal Cancer

C-reactive protein (CRP) was first discovered in 1930 and is the prototypical acute phase protein. In response to infection or injury, hepatic synthesis can rapidly increase its circulating concentration up to one thousand fold (Gabay *et al*, 1999, see Fig 1.1). Its secretion is thought to be primarily regulated by Interleukin-6 (IL-6,) and corticosteroids, though the mechanism controlling secretion is not fully understood (Black *et al,* 2004) CRP has both pro-inflammatory and anti-inflammatory activities. It activates complement and enhances phagocytosis, stimulates IL-8 release, a strong chemotactant factor for neutrophils, and increases the release of IL-1, IL-6 and IL-18. However, it also induces IL-1Ra expression, and may increase secretion of IL-10 whilst decreasing secretion of interferon-γ. CRP has a protective role against bacterial infection though requires synergism with the complement system. CRP is the most widely used determinant of the presence of a systemic inflammatory response in vivo as CRP

concentrations have no clinical variations with age or gender, and are readily measured by standardised reliable assays (Gabay *et al,* 1999).

The underlying stimulus driving CRP secretion in RCC has yet to be established. It has also to be determined whether abnormalities in the host immune system lead to the development of an inflammatory response, or whether this is in response to secretions from the tumour itself.

1. 12. 2 Interleukin-6 and Renal Cancer

IL-6 was first described in 1985 as a "B-cell differentiation factor (BSF-2)." It is a 26 kDa cytokine synthesised by macrophages and vascular endothelial cells in response to IL-1 and TNF. It is a pro-inflammatory cytokine, and also has importance in regulating the Th1/Th2 balance (Fig 1.2). It acts on hepatocytes in association with IL-1 and TNF to trigger synthesis of acute phase proteins, particularly CRP secretion.

1. 12. 2(i) Interleukin-6 and advanced cancer

Serum IL-6 has been shown to be a prognostic indicator similar to CRP in patients with metastatic RCC undergoing immunotherapy with IL-2. Patients with detectable IL-6 or a CRP >50mg/l were found to have a poorer response rate to immunotherapy, and a shorter overall survival (Blay *et al,* 1992). The presence of paraneoplastic syndromes in association with RCC is well-documented. IL-6 concentrations have been found to be significantly higher in patients with paraneoplastic phenomena such as weight loss, pyrexia, hypoalbuminaemia and Stauffer syndrome (Blay *et al,* 1997; McLellan *et al,* 1998). Levels of serum IL-6 are higher in patients with metastatic RCC when compared

with localised disease, (Ljungberg *et al,* 1997) with one study suggesting over 70% of patients with metastatic RCC had elevated serum IL-6 concentrations (Negrier *et al,* 2004).

1.12.2(ii) Interleukin-6 and localised disease

An increased expression of IL-6 has been associated with higher grade tumours (Paule *et al,* 2000) and also in higher tumour stage (Ljungberg *et al,* 1997). Yoshida and co-workers have also reported similar findings of increased concentrations of serum IL-6 in patients with higher grade tumours, and an association between IL-6 and tumour size in RCC (Yoshida *et al,* 2002). This group also confirmed the association between serum IL-6 levels and CRP without examining the underlying mechanism. In 1989, Miki and co-workers demonstrated the expression of IL-6 mRNA in freshly isolated RCC cells, and proposed that IL-6 behaved as an autocrine growth factor tissue for RCC in vitro. Further work by Takenawa and colleagues confirmed the secretion of IL-6 by RCC cell lines, and the presence of IL-6 receptor within cell lines and fresh RCC tissue. An association between raised IL-6 levels and elevated CRP was also noted by this group (Takenawa *et al,* 1991).

Blay and co-workers proposed that IL-6 acts as an autocrine growth factor in vivo in RCC, and correlates well with serum CRP levels (Blay *et al,* 1994).

Both CRP and IL-6 have been shown to have prognostic significance in RCC, but whether one is superior as a prognostic indicator has yet to be examined, nor is their inter-relationship fully understood. The source of IL-6 secretion has also to be verified as it is often ascribed to the tumour itself, though circulating levels after nephrectomy have yet to be examined.

1.12.3 Interleukin-10 and Renal Cancer

IL-10 was first described in 1989 as "cytokine synthesis inhibitory factor" (Fiorentino *et al,* 1991) and is produced by macrophages and T-helper 2 cells. IL-10 is a homodimer with a molecular mass of 37 kDa (Asadullah *et al,* 2003). The IL-10 gene is located on Chromosome 1 and codes for 5 exons. IL-10 is an inhibitory cytokine that suppresses the production of pro-inflammatory cytokines, Th1 cytokine release and antigen presenting cells (see Fig 1.2).

1.12.3(i) IL-10 and advanced cancer

Elevated IL-10 serum concentrations in patients with metastatic RCC have been shown to be predictive of a poorer survival, median 11 months, in comparison to patients with low or undetectable IL-10, median survival 27 months (Wittke *et al,* 1999).
In contrast, Uwatoko and colleagues have demonstrated an association between absence of IL-10 expression and distant metastases in RCC, suggesting a tumour inhibitory role for IL-10. However, circulating IL-10 concentrations were not quantified, and patients with bilateral, metastatic and primary tumours were included in their analysis (Uwatoko *et al* 2002).

1.12.3(ii) Interleukin-10 and localised disease

In renal cancer, IL-10 mRNA is detectable in vivo, and RCC cell lines have been shown to induce IL-10 production by monocytes in vitro (Menetrier-Caux *et al,* 1999). Tumour infiltrating lymphocytes isolated from RCC have also been shown to express IL-10 mRNA (Maeurer *et al,* 1995). The role of IL-10 secretion in RCC, and its relationship with other cytokines such as IL-6 has not been fully explored. Elevated serum IL-10

concentrations were associated with higher tumour stage, and elevated IL-6 in patients with colorectal cancer (Ordemann *et al,* 2002). In a small study elevated pre-operative IL-10 and IL-6 concentrations used in combination were also predictive for non-curative resection in colorectal cancer (Galizia *et al,* 2002). The relationship between IL-6 and IL-10 and CRP concentrations in patients with RCC has not been examined.

1.12.4 Immune Dysfunction

Historical clinical observations created early interest in the role of immune dysfunction in RCC. The rare event of regression of metastases after nephrectomy has been recorded since 1928 (Kavoussi *et al,* 1986) and has prompted speculation over the role of immune mediation in metastatic regression. Similarly, the development of distant metastases many years after nephrectomy has also led to suggestions that delayed failure of immune surveillance may be responsible.

To mount an active host anti-tumour immune response requires activation of T-lymphocytes in association with presentation of tumour antigen peptides on various antigen-presenting cells. T-lymphocytes produce two differing responses when stimulated. Type I (Th-1) responses generally act to stimulate cell-mediated immunity, characterised by the production of cytokines such as TNF, IL-2 and IFN-γ; whilst Type II (Th-2) stimulate humoral immunity and the production of antibodies by B cells, characterised by cytokines IL-2, IL-4, IL-5, IL-6, and IL-10. There is cross-regulation between the two responses with IL-10 known to down-regulate the Type I response, whilst IFN-γ and IL-2 reduce humoral immunity by down–regulating Th-2 cells and their cytokine production (Fiorentino *et al,* 1989).

It is has been shown there is a shift from the usual balance of responses to a Type II bias, with a reduced level of circulating Th1 cytokines in patients with solid tumours (Goto *et al,* 1999). It has been suggested that increasing tumour stage in RCC is associated with this shift to Th2 predominance, whilst mounting an effective cell-mediated antitumour response requires a shift back to Th1 cytokines (Derweesh *et al,* 2003).

Examination of resected primary tumours has revealed the presence of a significant tumour infiltration by mononuclear cells, in particular T-lymphocytes and macrophages suggesting another important role for immune dysfunction in the pathogenesis of RCC. These tumour infiltrating lymphocytes (TILs) have been shown to be dysfunctional, with reduced cellular signalling and responsiveness, along with reduced antigen presenting capacity. A high TIL count is associated with higher stage tumours, increased necrosis and a higher pathological grade (Kolbeck *et al,* 1992).

The subsets of TILs have also demonstrated prognostic significance though results from different groups are conflicting. Bromwich and colleagues have demonstrated that a high TIL CD4+ count is associated with a poorer disease-free survival in patients undergoing curative nephrectomy (Bromwich *et al,* 2003). In contrast, Nakano and co-workers have reported that a high intratumoral CD8+ in RCC is prognostic for poorer outcome, and is associated with increasing tumour grade and proliferation (Nakano *et al,* 2001). These findings are in direct opposition to their findings in colorectal cancer where a high CD8+ count predicts better prognosis (Naito *et al,* 1998). Igarashi and co-workers also reported an association between elevated TIL levels of CD8+ and progressive disease in patients treated with interferon (Igarashi *et al,*

2002). Interestingly, his research has also suggested an association between a raised CD8+ and raised CRP. It has been proposed that tumour secretion of IL-10 may play a role as IL-10 is chemo-attractant for CD8+ cells but suppresses IL-8 related migration in CD4+ cells (Jinquan *et al,* 1993).

Examining circulating peripheral lymphocytes, it has been reported that a high proportion of CD8+ cells is predictive of increased recurrence after nephrectomy, and associated with progressive disease in patients with metastatic RCC treated with IFN (Arima *et al,* 1996). However, the role of CD4 lymphocytes as a predictive tool in patients with metastatic RCC treated with immunotherapy has yet to be examined. Similarly, there are no studies investigating the relationship between the systemic inflammatory response and immune dysfunction in metastatic RCC

1.13 Hypothesis

A complex balance of pro-inflammatory and anti-inflammatory cytokines controls the systemic inflammatory response. It is hypothesized that the presence of a systemic inflammatory response, as evidenced by an elevated C-reactive protein concentration, is due to an imbalance between increased synthesis of pro-inflammatory cytokines such as IL-6, and subsequent increase of anti-inflammatory cytokines such as IL-10, leading to immune dysfunction and impaired Th1 antitumour response.

1.14 Aims

- To evaluate the prognostic value of an inflammation based prognostic score in patients with metastatic renal cancer.
- To evaluate the prognostic role of an inflammation based prognostic score in patients with primary operable renal cancer.
- To examine the relationship between the systemic inflammatory response and a Th1/Th2 cytokine profile in patients with undergoing nephrectomy for renal cancer.
- To examine the longitudinal relationship between the systemic inflammatory response, circulating T-lymphocytes, interleukin-6 and interleukin-10 in patients with metastatic renal cancer treated with immunotherapy.
- To examine the longitudinal relationship between the systemic inflammatory response, interleukin-6 and interleukin-10 in patients undergoing nephrectomy for primary operable renal cancer.

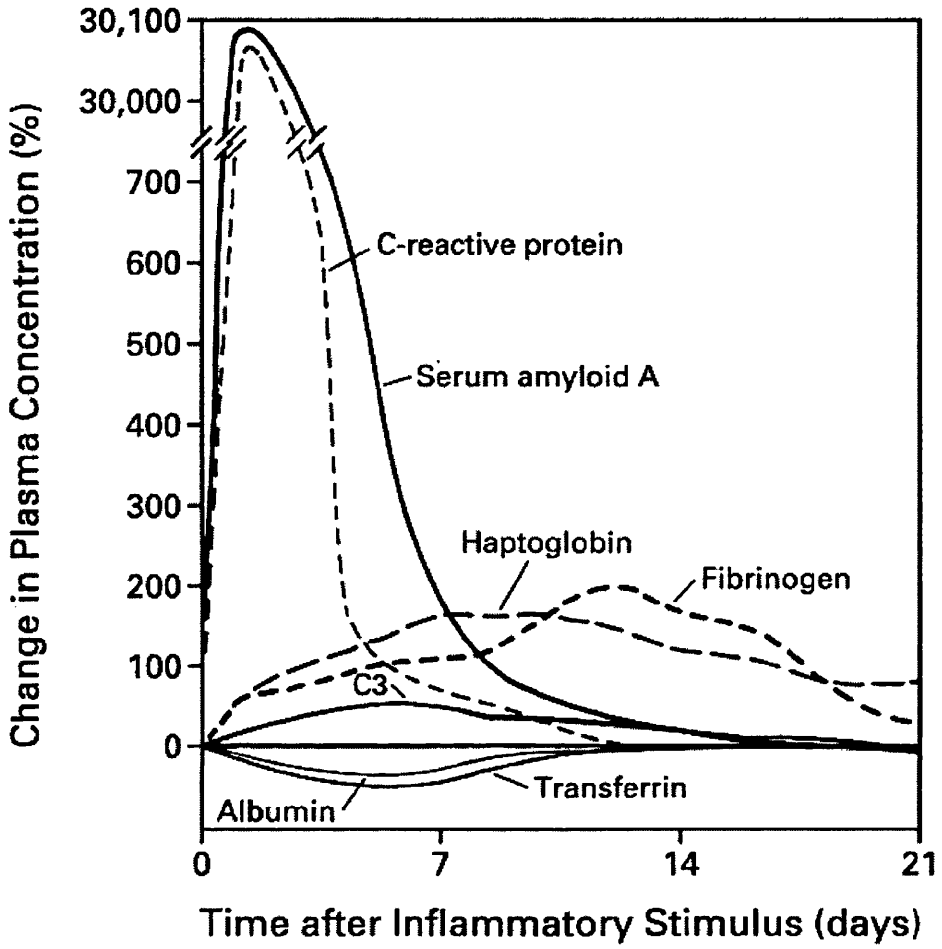

Figure 1.1 Changing pattern of acute phase proteins after a moderate inflammatory stimulus (Adapted from Gabay and Kushner, 1999).

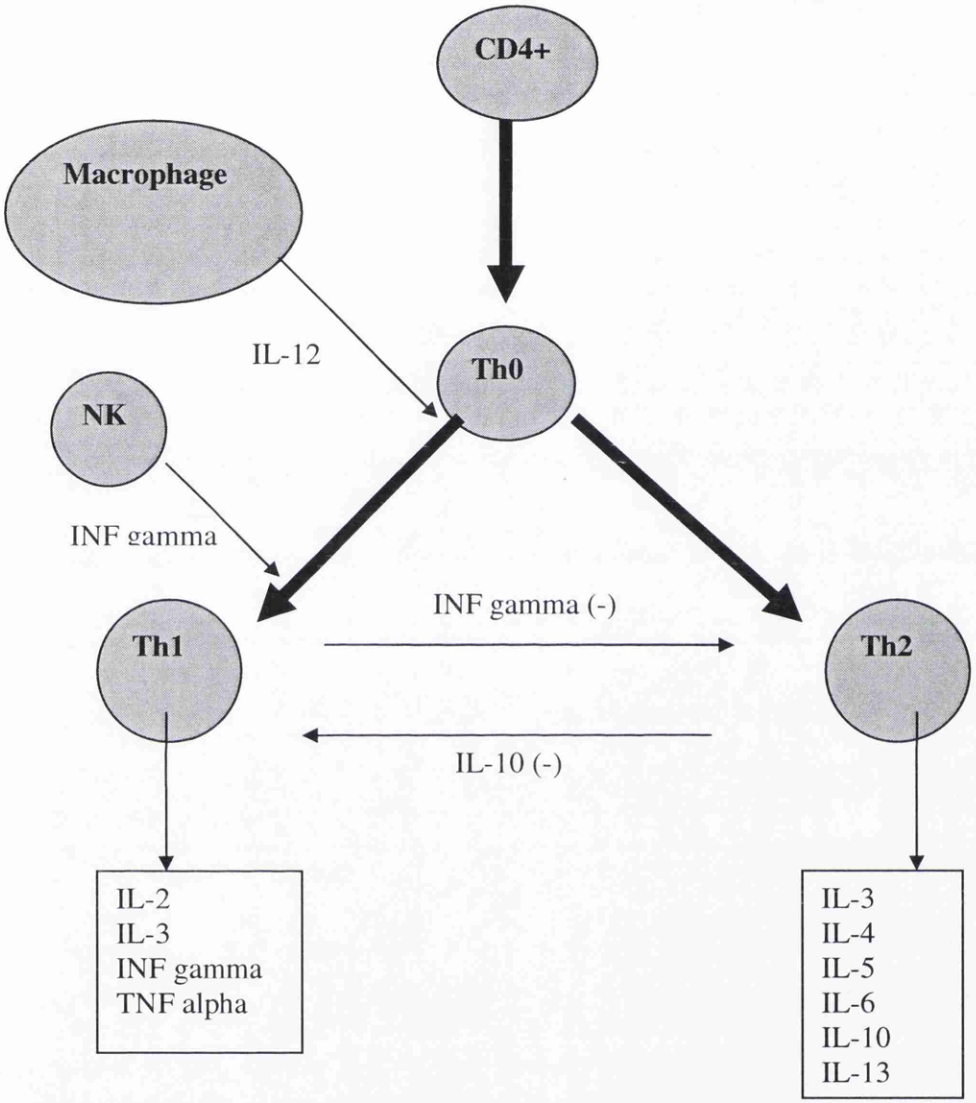

Figure 1.2 The production of cytokines.

Table 1.1 Location of metastases from primary renal cancer

Site of metastases	Single Site (%)	Multiple Sites (%)
Lung	72	77
Soft Tissue	10	54
Bone	11	26
Liver	5.5	26
Central Nervous System	1.4	12
Skin	0	13
Other	0	7

Table 1.2 Bosniak Classification of Renal Cysts

Class	Description	Nature
I	Uncomplicated, simple	Benign
II	Minimally complicated, some findings to cause concern eg septae, hyper dense cysts	Benign
III	More complicated – irregular margins, thickened septae, thick irregular calcification	Uncertain
IV	Large, cystic, irregular shaggy margins, areas of solid enhancing tissue	Malignant

Table 1.3 Fuhrman System of Nuclear Grading

Grade	Nuclear Size	Nuclear Outline	Nucleoli
1	10 mm	Round, uniform	Absent / inconspicuous
2	15 mm	Irregular	Small
3	20 mm	Irregular	Prominent
4	>20 mm	Bizarre, multi-lobed	Prominent, heavy chromatin

Table 1.4 Robson Staging System

Stage	Substage	Description
I		Confined to the kidney
II		Perirenal fat involvement, but within Gerota's fascia
III	A	Gross renal vein or inferior vena cava involvement
	B	Lymphatic involvement
	C	Vascular and lymphatic involvement
IV	A	Adjacent organs other than adrenal involved
	B	Distant metastases

Table 1.5 TNM Staging System 2001

TUMOUR		
	Tx	Primary tumour cannot be assessed
	T0	No evidence of primary tumour
	T1a	Tumour less than 4 cm, limited to the kidney
	T1b	Tumour greater than 4 cm, no greater than 7 cm, limited to the kidney
	T2	Tumour greater than 7 in greatest dimension, limited to the kidney
	T3	Tumour extends into major veins or perinephric fat but not beyond Gerota's fascia. **T3a**: Tumour invades perinephric tissues but not beyond Gerota's fascia **T3b**: Tumour grossly extends into renal vein(s) or vena cava below diaphragm **T3c**: Tumour grossly extends into vena cava above diaphragm
	T4	Tumour invades beyond Gerota's fascia
NODES		
	Nx	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastasis
	N1	Metastasis in a single regional lymph node
	N2	Metastasis in more than one regional lymph node
METASTASES		
	Mx	Distant metastases cannot be assessed
	M0	No distant metastases
	M1	Distant metastases

Table 1.5b: TNM Stage classification

Stage	TNM		
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1	N1	M0
	T2	N1	M0
	T3	N0,1	M0
Stage IV	T4	N0,1	M0
	Any T	N2	M0
	Any T	Any N	M1

Table 1.6 Five year survival according to TNM stage

Stage	Robson	Tsui	Hermanek	Storkel
I	66%	91%	91%	98
II	64%	74%	92%	93
III	42%	67%	64%	58
IV	11%	32%	15%	33

NB – Robson cohort uses Robson staging system

Table 1.7 ECOG Performance Status

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature eg light house work, office work
2	Ambulatory and capable of self-care but unable to carry out any work activites. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self care. Totally confined to bed or chair
5	Dead

2.0 Patient Study: Evaluation of an inflammation based prognostic score in patients with metastatic renal cancer.

2.1 Introduction

Renal cell cancer, although the 12th most common cause of cancer death is one of the most lethal urological cancers. Each year in the UK, there are approximately 3,500 new cases and approximately 30% of patients present with metastases, with a further 40% developing metastases after a potentially curative resection (Cancer stats, Cancer Research UK).

As previously discussed in Chapter one, the mainstay of treatment in metastatic renal cancer is immunotherapy though in specific circumstances there is a role for surgery such as cytoreductive nephrectomy or metastasectomy. Whilst interferon-based immunotherapy has been shown to provide a consistent survival advantage in 4 randomised placebo controlled trials, this is measured in months and is a toxic treatment with considerable side-effects (Steineck *et al*, 1990; Kriegmair *et al*, 1995; Pyrhonen *et al*, 1999; MRC RCC *et al*, 1999).

There is increasing interest in improving selection of patients with good prognosis for more active treatment, and patients with poor prognosis for less active and less toxic treatment since this will improve overall patient outcomes. At present, performance status (Eastern Cooperative Oncology Group, ECOG-ps) is widely used as a prognostic tool in patients with metastatic disease. However, the assessment of performance status is subjective variable with significant inter-observer variability (Ando *et al*, 2001). For these factors to be clinically useful they should be routinely available, well standardized and validated in different patient cohorts.

There is increasing evidence that the presence of a systemic inflammatory response, as evidenced by an elevated circulating C-reactive protein concentration or hypoalbuminaemia, is an independent prognostic factor in patients with advanced cancer (McMillan *et al*, 2001; Maltoni *et al*, 2005). With reference to renal cancer an elevated C-reactive protein has been shown to be associated with poorer cancer specific survival in patients with advanced disease (Atzpodien *et al*, 2003; Bromwich *et al*, 2004; Casamassima *et al*, 2005).

It is therefore of interest that the combination of hypoalbuminaemia and an elevated C-reactive protein, as in the Glasgow Prognostic Score (GPS), has been shown to provide additional prognostic information in patients with advanced cancer (Forrest *et al*, 2003; Forrest *et al*, 2004; Al-Murri *et al*, 2006; Crumley *et al*, 2006; Glen *et al*, 2006).

A number of prognostic scoring systems are in use to stratify patients with metastatic renal cancer into risk groups (Motzer *et al*, 1999; Atzpodien *et al*, 2003). However, these systems incorporate a variety of different biochemical, histological and radiological features and it is not known how these scoring systems compare.

Therefore, the aim of the present study was to examine the value of the GPS, in comparison with established scoring systems, in predicting cancer specific survival in patients with metastatic renal cancer

2.2 Patients and Methods

Patients with metastatic renal cancer, who, on the basis of clinical findings and computed tomography of chest and abdomen were diagnosed with metastatic disease between 2001 and 2005 in the West of Scotland, and referred to the specialist renal cancer unit, were included in the study. The out-patient treatment regimes were either subcutaneous alpha-interferon given at 10 mega-units three times weekly on a 12-weekly basis, or a modified Atzpodien regime of subcutaneous alpha-interferon, subcutaneous interleukin-2 for five weeks, with an additional 3 weeks of 5-flourauracil given intravenously.

Patients who underwent surgery were staged pathologically according to the 1997 UICC TNM classification of renal tumours (Sobin & Wittekind, 1997). Tumours were graded according to criteria set out by Fuhrman *et al* (1982) if biopsies or surgery had been performed. Clinical stage and performance status (Eastern Cooperative Oncology Group, ECOG-ps) were recorded prior to treatment initiation. Prior to commencement of treatment for metastases routine laboratory measurements of lactate dehydrogenase, haemoglobin, calcium, differential white cell count, albumin and C-reactive protein were carried out.

The Research Ethics Committee of North Glasgow NHS Trust approved the study.

The Memorial Sloan Kettering Cancer Centre (MSKCC) score was derived as previously described (Motzer *et al*, 1999). In this system an abnormal haemoglobin, calcium, lactate dehydrogenase three times greater than the upper limit of normal, Karnofsky performance status <80% and the absence of prior nephrectomy are

considered as risk factors. Patients with no risk factors are classified as favourable, one to two as intermediate, and three or greater as poor risk.

The Metastatic Renal Carcinoma Comprehensive Prognostic System (MRCCPS) was scored as previously described (Atzpodien *et al*, 2003). Briefly, six pre-treatment variables are used to categorise patients into low, intermediate and high risk groups. Neutrophil count is given the highest weighting, the remainder of the factors are C-reactive protein greater than 10 mg/l, lactate dehydrogenase greater than 220 U/l, the presence of bone metastases, and onset of metastatic disease of less than three years from diagnosis all assigned one point. Patients with zero or one point are low risk, two or three intermediate risk, and four or greater are classified as high risk.

The GPS was constructed as previously described (Forrest *et al*, 2003, 2004). In essence, patients with both an elevated C-reactive protein (>10 mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of two. Patients in whom only one of these biochemical abnormalities was present were allocated a score of one. Patients in whom neither of these abnormalities was present were allocated a score of zero.

2.2.1: Statistics

Comparisons between groups of patients were carried out using contingency table analysis (X^2) as appropriate.Grouping of the laboratory variables haemoglobin, white cell, lymphocyte counts, albumin and C-reactive protein was carried out using standard thresholds (O'Gorman *et al*, 2000; McMillan *et al*, 2001; Maltoni *et al*, 2005). Survival (cancer-specific) analysis of the group variables was performed using the Cox proportional hazard model. Deaths up to the end of July 2006 have been included in the

analysis. Multivariate survival analysis, including all covariates that were significant on univariate analysis, 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 0.10. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

2.3 Results

The characteristics of patients with metastatic renal cancer (n=119) are shown in Table 2.1. The majority were male and over the age of 60 years and had prior surgical treatment. The majority of patients had an ECOG performance status of 0 and had lactate dehydrogenase, calcium, white cell count, neutrophil count, percentage lymphocytes, and albumin measurements in the normal range. In contrast, the majority of patients had an elevated C-reactive protein concentration and of the 18 patients with hypoalbuminaemia 15 (83%) had an elevated C-reactive protein concentration.

The minimum follow-up of patients was 5 months, the median follow-up of the survivors was 15 months. During this period 78 patients died of their cancer, and the median survival was eight months. On univariate survival analysis of individual variables, lactate dehydrogenase ($p<0.10$), haemoglobin ($p<0.01$), calcium ($p<0.001$), white cell count ($p<0.01$), neutrophil count ($p<0.05$), albumin ($p<0.01$) and C-reactive protein were significantly associated with cancer specific survival. On multivariate analysis of these significant factors only calcium (HR 3.21, 95%CI 1.51-6.83, p=0.002), white cell count (HR 1.66, 95%CI 1.17-2.35, p=0.004), albumin (HR2.63, 95%CI 1.38-5.03) and C-reactive protein (HR 2.85, 95%CI 1.49-5.45, p=0.002) were independently associated with cancer specific survival.

On univariate survival analysis of the scoring systems, the MSKCC ($p<0.001$, Figure 2.1), the MRCCPS ($p<0.01$, Figure 2.2), and the GPS ($p<0.001$, Figure 2.3) were significantly associated with cancer specific survival. On multivariate analysis of these scoring systems, the MSKCC (HR 1.88, 95%CI 1.22-2.88, p=0.004), the MRCCPS (HR

1.42, 95%CI 0.97-2.09, p=0.071), and the GPS (HR 2.35, 95%CI 1.51-3.67, p<0.001) were independently associated with cancer specific survival.

The relationship between clinicopathological characteristics and an inflammation-based prognostic score (GPS) in patients with metastatic renal cancer is shown in Table 2.2. An increasing GPS was associated with a lower haemoglobin (p<0.001), higher calcium (p<0.001), lower percentage lymphocytes (p<0.01) and greater risk of poor survival as estimated by the MSKCC and the MRCCPS scoring systems. The median survival in these patients was 28 months, 11 months and 3 months for a GPS of 0, 1 and 2, respectively.

2.4: Discussion

In the present study we have shown that the presence of a systemic inflammatory response, as evidenced by an elevated C-reactive protein concentration and hypoalbuminaemia, predicts cancer-specific survival, independent of other established prognostic factors such as performance status, calcium, lactate dehydrogenase and neutrophil counts in patients with metastatic renal cancer. Moreover, we have shown that the GPS compares favourably with MSKCC and is superior to the MRCCPS scoring systems.

At the time of diagnosis, there are well-established prognostic factors on which to base the prediction of likely survival in cancer patients. As a result, clinicians often overestimate survival (Glare, 2005). The results of the present study suggest that the GPS may be useful in the assessment of survival in patients with metastatic renal cancer. It is simple to use and based on routinely available, well standardized measurements. Therefore it may be that this simply derived inflammation-based score will be a useful tool in the prediction of survival and possible stratification, at diagnosis, of patients with metastatic renal cancer.

The mechanisms by which a systemic inflammatory response might influence cancer survival in these patients are not clear. However, it may be that the presence of a systemic inflammatory response and the associated nutritional decline (McMillan *et al*, 1998; Scott *et al*, 2002) influences tolerance and compliance with active treatment (Bromwich *et al*, 2004; Forrest *et al*, 2004). It may also reflect the proinflammatory cytokine activity, in particular interleukin-6 (McKeown *et al*, 2004), which not only stimulates renal tumour growth (Trikha *et al*., 2003), but also produce profound catabolic

effects on host metabolism (McMillan *et al*, 1998; Kotler, 2000). In this way, the presence and magnitude of a chronic systemic inflammatory response, as reflected by the GPS, may produce progressive nutritional and functional decline, ultimately resulting in reduced survival. Indeed, this concept is consistent with the observation in the present study that almost all patients with hypoalbuminemia had an elevated C-reactive protein concentration.

Due to the high toxicities and low response rates of immunotherapy, there has been considerable interest in the use of new targeted agents such as sorafenib and sunitinib in patients with metastatic renal cancer (Motzer *et al*, 2006). Early reports of increased progression-free survival, and more recently of increased overall survival may lead to these drugs being adopted as the first-line treatment. It remains to be established whether the GPS predicts which patients will benefit from these regimens.

In summary, the prognosis for patients diagnosed with metastatic renal cancer, even with active treatment, remains poor. The presence of a systemic inflammatory response (an elevated GPS) appears to be a useful indicator of outcome among these patients, independent of established scoring systems. Moreover, the GPS has the advantage of being simple to measure, routinely available and well standardised.

Table 2.1 Clinicopathological characteristics and cancer specific survival in metastatic patients undergoing treatment for renal cancer. Univariate analysis

	Patients (n= 119)	Hazard ratio (95%CI)	p-value
Age group (≤60/>60 yrs)	56/ 64	1.46 (0.93–2.30)	0.102
Sex (male/ female)	85/ 35	1.34 (0.84–2.15)	0.216
ECOG-PS (0/ ≥1)	93/ 26	1.07 (0.62-1.86)	0.812
Lactate dehydrogenase (<300/ ≥300 U/l)	108/ 9	1.89 (0.90–3.96)	0.094
Haemoglobin (≥13/ <13 g/dl M, ≥11.5/ <11.5 g/dl F)	56/ 63	2.04 (1.28–3.23)	0.003
Calcium (≤2.5/ >2.5mmol/l)	100/ 19	3.67 (2.01-6.69)	<0.001
Prior surgical treatment (curative/ cytoreductive/ none)	48/ 22/ 50	0.85 (0.62–1.17)	0.328
Disease free interval (>1/ ≤1 year)	35/ 35	0.96 (0.52–1.78)	0.897
Immunotherapy (Interferon/ Interferon, Interleukin-2, 5-FU)	100/ 19	0.96 (0.52-1.79)	0.910
Number of metastatic sites (1/ ≥2)	61/ 58	0.83 (0.53-1.29)	0.402
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	86/ 23/ 11	1.56 (1.13–2.15)	0.007
Neutrophil count (<6.5/ ≥6.5 10^9/l)	92/ 27	1.87 (1.13-3.11)	0.015
Lymphocyte percentage (20 –40/ 12– 19.9/ 0 – 11.9 %)	58/ 47/ 15	1.30 (0.93–1.81)	0.122
Platelets (≤400/ >400 10^9/l)	73/ 23	3.71 (2.17–6.32)	<0.001
Albumin (≥35/ <35g/l)	103/ 16	2.52 (1.38-4.61)	0.003
C-reactive protein (≤10/ >10mg/l)	35/ 84	3.75 (2.02-6.97)	<0.001
MSKCC (favourable/ intermediate/ poor)	31/ 72/ 13	2.44 (1.60-3.70)	<0.001
MRCCPS (low/ intermediate/ high risk)	27/ 61/ 29	1.70 (1.21-2.39)	0.002
GPS (0/ 1/ 2)	28/ 70/ 14	3.03 (2.01–4.56)	<0.001

* Median (range)

Table 2.2 The relationship between clinicopathological characteristics and an inflammation-based prognostic score (GPS) in patients with metastatic renal cancer.

	GPS 0 (n= 33)	GPS 1 (n=72)	GPS 2 (n= 14)	p-value
Age group (≤60/>60 yrs)	10/ 23	39/ 33	7/ 7	0.074
Sex (male/ female)	23/ 10	51/ 21	10/ 4	0.892
ECOG-PS (0/ ≥1)	23/ 9	60/ 12	9/ 5	0.990
Lactate dehydrogenase (<300/ ≥300 U/l)	31/ 2	66/ 4	11/ 3	0.165
Haemoglobin (≥13/ <13 g/dl M, ≥11.5/ <11.5 g/dl F)	25/ 8	28/ 44	3/ 11	<0.001
Calcium (≤2.5/ >2.5mmol/l)	30/ 0	15/ 14	9/ 5	0.001
Prior surgical treatment (curative/ cytoreductive/ none)	12/ 14/ 7	31/ 32/ 9	7/ 2/ 5	0.738
Disease free interval (>1/ ≤1 year)	24/ 9	49/ 23	11/ 3	0.892
Immunotherapy (Interferon/ Interferon, Interleukin-2, 5-FU)	26/ 7	64/ 8	10/ 4	0.989
Number of metastatic sites (1/ ≥2)	18/ 15	35/ 37	8/ 6	0.938
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	27/ 4/ 2	50/ 15/ 7	9/ 3/ 2	0.162
Neutrophil count (<6.5/ ≥6.5 10^9/l)	30/ 3	52/ 20	10/ 4	0.057
Lymphocyte percentage (20 –40/ 12– 19.9/ 0 – 11.9 %)	20/ 12/ 0	31/ 30/ 11	6/ 4/ 4	0.009
MSKCC (favourable/ intermediate/ poor)	15/ 16/ 1	15/ 49/ 6	1/ 7/ 6	<0.001
MRCCPS (low/ intermediate/ high risk)	16/ 14/ 3	10/ 38/ 22	1/ 9/ 4	<0.001
Survival (months)*	28.3 (18.8-37.8)	10.7 (9.1-12.4)	3.4 (2.2-4.6)	<0.001

*Median (range)

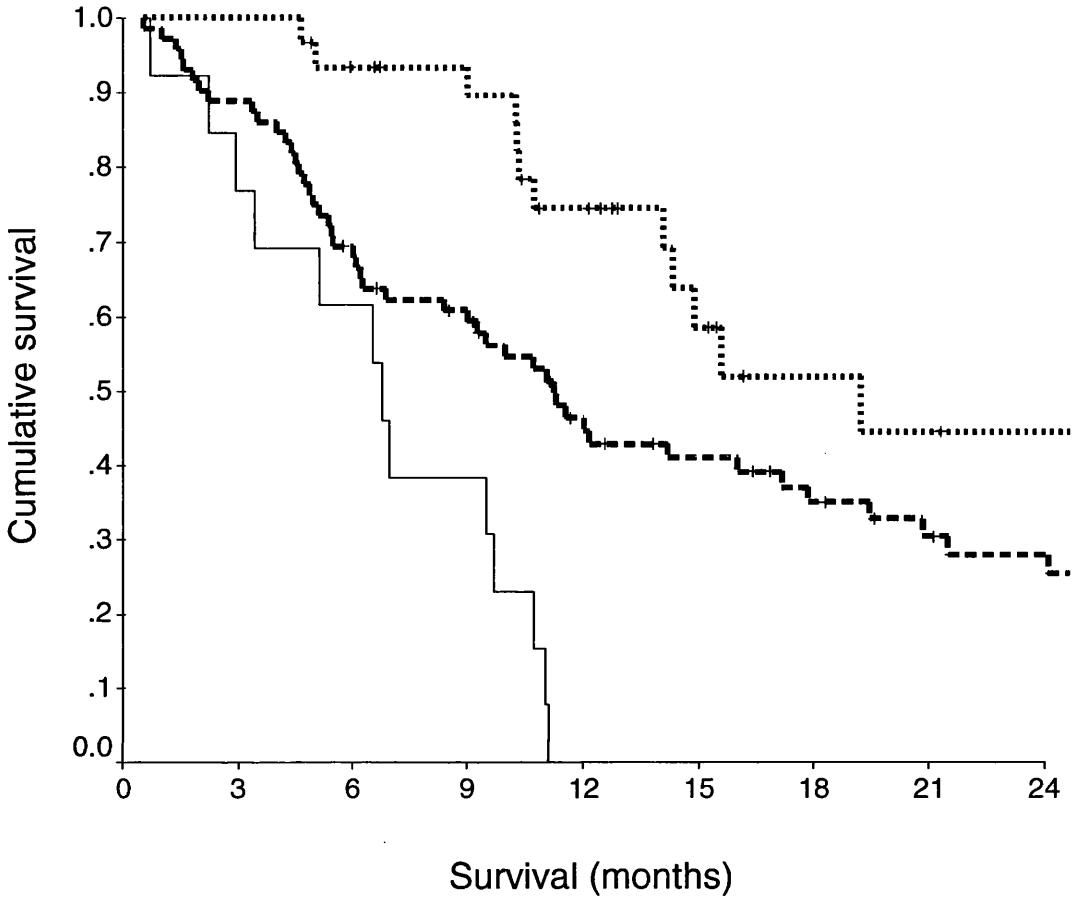

Figure 2.1 Relationship between the MSKCC (favourable/ intermediate/ poor risk from top to bottom) and cancer-specific survival in patients with metastatic renal cancer.

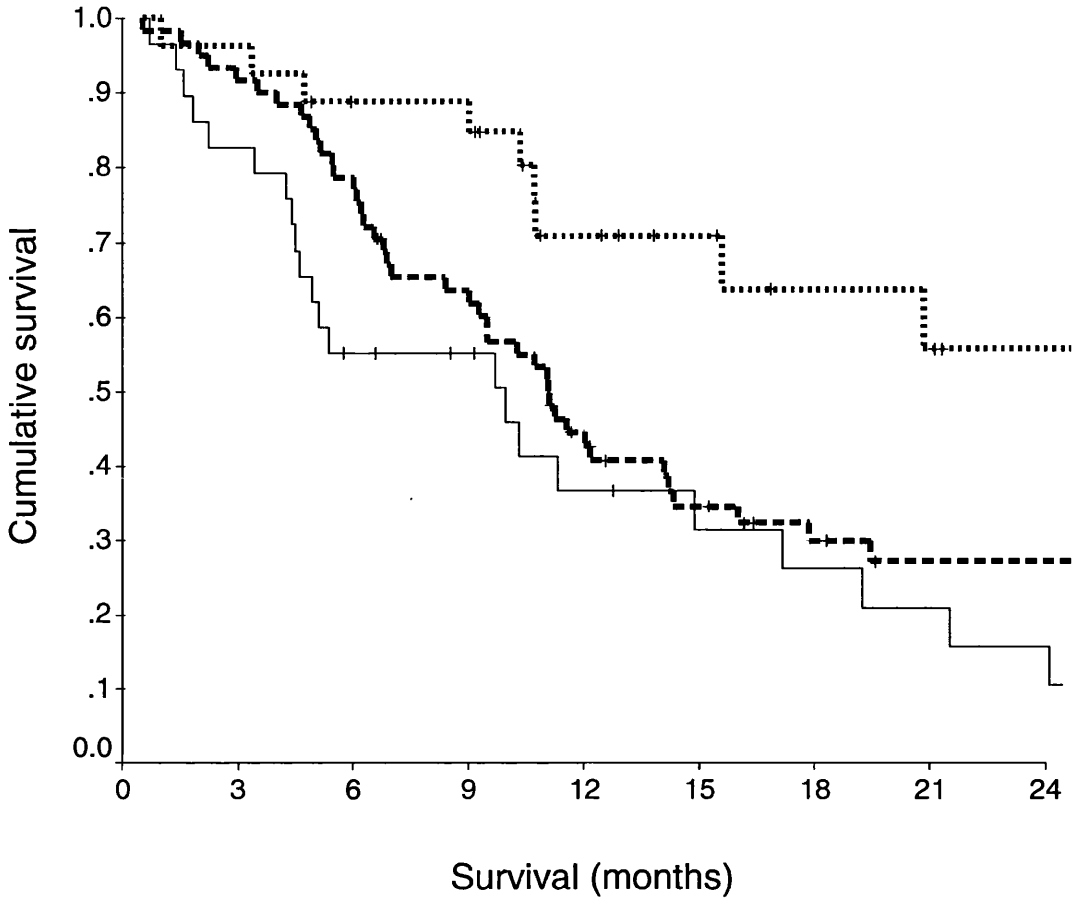

Figure 2.2 Relationship between the MRCCPS (low/ intermediate/ high risk from top to bottom) and cancer-specific survival in patients with metastatic renal cancer.

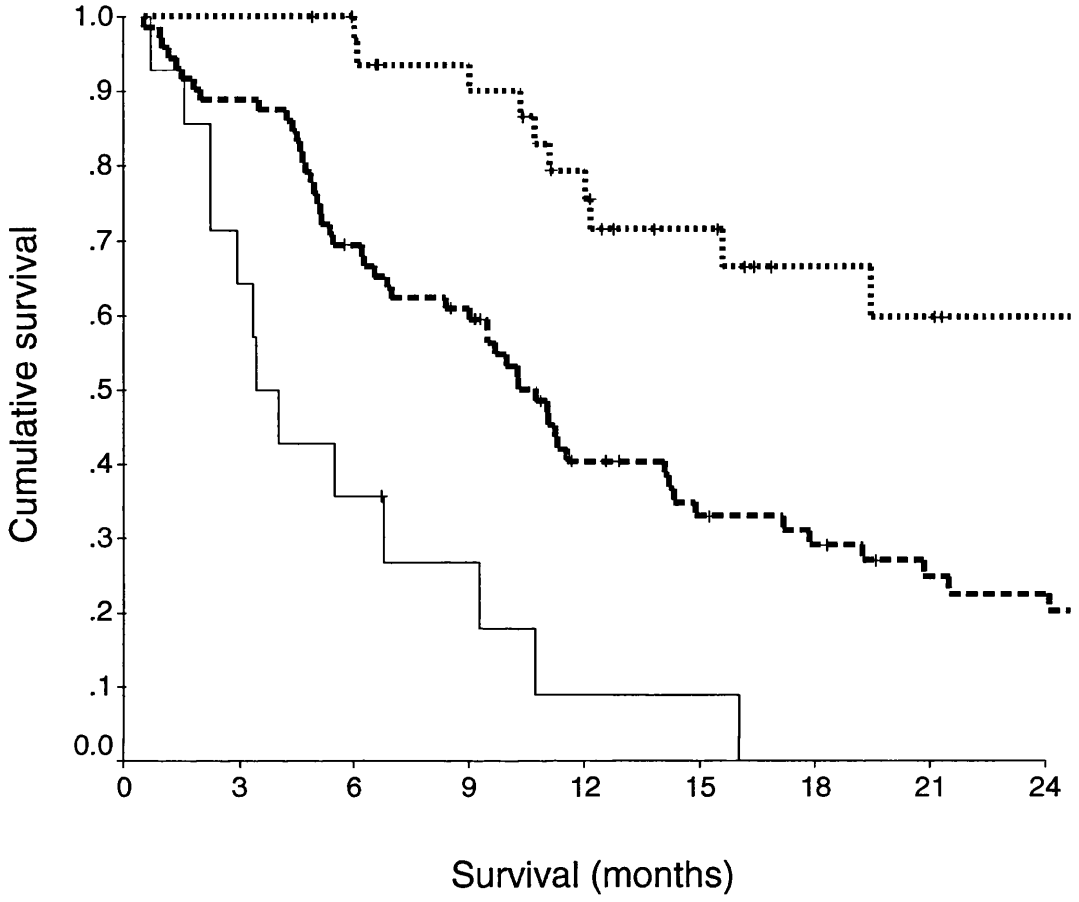

Figure 2.3 Relationship between the GPS (0/ 1/ 2 from top to bottom) and cancer-specific survival in patients with metastatic renal cancer.

3.0 Patient Study: The relationship between the systemic inflammatory response, prognostic scoring systems and cancer specific survival in patients undergoing potentially curative resection for renal cancer.

3.1 Introduction

As approximately 30% of patients who have undergone a curative nephrectomy will subsequently develop metastatic disease, there has been long-standing interest in identifying those patients most likely to die of renal cancer. Ideally, a factor or combination of factors would clearly stratify patients who will remain disease free and are "cured" from those who will ultimately die from their cancer. Currently TNM stage is the most widely used tool to predict likely outcome. However, despite revisions there is considerable "overlap" in survival between the stages. A number of cumulative prognostic scores, which include TNM stage and other host factors, have been developed to improve the prediction of survival in primary operable renal cancer. These include the combination of TNM stage, Fuhrman grade, and ECOG performance status (Eastern Cooperative Oncology Group) to form the UCLA Integrated Staging System (UISS, Zisman *et al*, 2002).

Recently, we have shown that the presence of a systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, provided additional prognostic value in patients with low and intermediate risk UISS scores undergoing potentially curative nephrectomy for renal cancer (Lamb *et al*, 2006). However, there are additional validated prognostic algorithms such as the SSIGN (Frank *et al*, 2003) and the Leibovich (Leibovich *et al*, 2002) scores.

The aim of the present study was to examine the relationship between the systemic inflammatory response, prognostic scoring systems and cancer specific survival in patients undergoing potentially curative resection for renal cancer.

3.2 Patients and Methods

Patients with renal cell carcinoma, who, on the basis of surgical findings and pre-operative computerized tomography of the chest and abdomen underwent potentially curative resection between August 1996 and July 2005 in the North Glasgow NHS Trust were included in the study. No patient had T4 or metastatic disease, and all macroscopic tumour was removed at nephrectomy. The UISS score was derived as previously described (Zisman *et al*, 2002). Briefly, tumour stage, Fuhrman grade and ECOG performance status are combined to stratify patients into low, intermediate or high risk. Patients with high risk UISS scores were excluded.

Patients were staged pathologically according to the 1997 UICC TNM classification of renal tumours (Sobin & Wittekind 1997). Tumours were graded according to criteria set out by Fuhrman and co-workers (1982). Clinical stage and performance status (Eastern Cooperative Oncology Group, ECOG-ps) were recorded prior to surgery. Routine laboratory measurements including haemoglobin, white cell, neutrophil, lymphocyte, albumin and C-reactive protein were performed pre-operatively.

Data for 1996–2000 (n= 48) were collected retrospectively, and that for 2001–2005 (n= 63) prospectively. The Research Ethics Committee of North Glasgow NHS Trust approved the study.

3.2.1 Experimental design

The coefficient of variation for the routine laboratory measurements of haemoglobin, white cell, neutrophil and lymphocyte 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 systemic inflammatory response (O'Gorman *et al*, 2000; McMillan *et al*, 2001).

The SSIGN score was derived as previously described (Frank *et al*, 2002; Table 3.1). Patients are awarded scores based on T stage, nodal disease, tumour size, nuclear grade, presence or absence of tumour necrosis and the presence or absence of metastases (Table 3.1). The Leibovich score was derived as previously described (Leibovich et al, 2002, Table 3.1). Patients are awarded scores based on T stage, nodal stage, tumour size, nuclear grade, presence or absence of tumour necrosis (Table 3.1). The Leibovich score is similar to the SSIGN score, though weighting of individual variables differs (Table 3.1). Patients with scores of zero to two were classified as low risk, three to five as intermediate, and six or greater as high risk.

3.2.2 Statistics

Comparisons between groups of patients were carried out using contingency table analysis (X^2) as appropriate. Survival analysis was performed using the Cox's proportional-hazards model. Deaths up to the end of August 2006 were included in the analysis. Multivariate survival analysis 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 0.10. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

3.3 Results

The characteristics of patients with renal cancer who underwent potentially curative resection (n= 111) are shown in Table 3.2. The majority were male, over the age of 60 years, had good performance status, had T stage I/ II disease and absent tumour necrosis. The majority had haemoglobin, white cell, neutrophil and lymphocyte counts, albumin and C-reactive protein concentrations in the normal range. Of these parameters C-reactive protein was most commonly abnormal (37%).

The minimum follow-up was 14 months; the median follow-up of the survivors was 47 months. During this period 28 patients died; 20 patients of their cancer and eight of intercurrent disease. On univariate survival analysis, tumour stage ($p \leq 0.001$), Fuhrman grade ($p<0.01$), tumour necrosis ($p<0.001$), haemoglobin ($p<0.01$), C-reactive protein ($p<0.01$), SSIGN ($p<0.001$) and Leibovich ($p<0.001$) scores were significant predictors of cancer specific survival. On multivariate analysis with haemoglobin, C-reactive protein, SIGN and Leibovich entered as covariates, only C-reactive protein (HR 2.69, 95% CI 1.00–7.23, p=0.050) and Leibovich (HR 4.44, 95% CI 1.91–10.28, $p<0.001$) were significant independent predictors of cancer-specific survival.

The preoperative values of C-reactive protein at the thresholds of greater than five, and greater than ten mg/l were compared in multivariate survival analysis. In this analysis, the prognostic significance of the ten mg/l (p=0.003) was greater than the five mg/l (p=0.110).

The relationship between the presence of a systemic inflammatory response and the clinicopathological characteristics are shown in Table 3.3. There was no significant

difference in age or sex between the inflammatory and non-inflammatory groups. An elevated C-reactive protein was associated with a greater number of patients with advanced tumour stage ($p<0.001$), lower haemoglobin ($p\leq0.001$) and poor UISS ($p<0.01$), SIGN ($p<0.001$) and Leibovich scores ($p\leq0.001$).

3.4 Discussion

The present study evaluated the prognostic value of the systemic inflammatory response and compared, for the first time, a number of prognostic scoring systems in patients undergoing potentially curative resection for colorectal cancer. From this comparison it is clear that, in the present cohort, that the Leibovich algorithm is superior to the others and that an elevated C-reactive protein provides additional prognostic value.

In the present study the patients studied were either low or intermediate risk as defined by the UISS criteria (tumour stage, Fuhrman grade and performance status) and patient survival was not significantly different between these risk groups. In contrast, increasing SSIGN and Leibovich and scores derived from additional criteria (tumour size and tumour necrosis) were significantly associated with poor cancer specific survival. These results would suggest that such additional criteria, in particular tumour necrosis, are of considerable prognostic value in patients undergoing potentially curative resection for renal cancer.

In the present study although SSIGN and Leibovich scores were derived using similar criteria, on multivariate analysis the Leibovich score appeared to be superior in predicting cancer specific survival. This may have been due to the fact that tumour stage was more highly scored compared with that of the SSIGN score. This may be related to the SSIGN score having been developed in a group of patients which included patients who had proven metastatic disease, whereas the Leibovich score was developed from a cohort of patients without evidence of metastatic disease.

We have previously reported that an elevated C-reactive protein had additional prognostic value to that of the UISS score (Lamb *et al*, 2006). In the present study we

were able to confirm this finding and also establish that an elevated C-reactive protein had additional prognostic value to the SSIGN and Leibovich scores. An elevated C-reactive protein concentration has recently been incorporated into a prognostic score for patients with metastatic renal cancer (Atzpodien *et al*, 2003; Royston *et al*, 2006), and the previous chapter has reported the prognostic value of a cumulative inflammation based score (GPS). Given this, the results of the present and previous studies (Masuda *et al*, 1998; Lamb *et al*, 2006) would support the inclusion of C-reactive protein concentrations into prognostic scoring systems for patients with primary operable renal cancer. Of interest, hypoalbuminaemia appears to be a rare event in patients with operable renal cancer, and as such the GPS could not be applied to this cohort.

It was of interest that the threshold for C-reactive protein (>10 mg/l) which was originally established in studies of patients with gastrointestinal cancer (O'Gorman et al, 2000) and subsequently verified in patients with renal cancer (Bromwich et al, 2004) was superior to that (>5 mg/l) used recently in a similar study of patients with operable renal cancer (Komai et al, 2006).

This is a relatively small study in a single centre and requires verification in large cohorts in other centres. If an elevated C-reactive protein concentration is confirmed to predict a poorer prognosis, decision making regarding radical nephrectomy in patients with extensive co-morbidity, or those with high-risk locally advanced tumours, may be influenced by the presence of a high inflammatory profile pre-operatively. Alternatively, modulation of the systemic inflammatory response may be a useful approach in these patients in the pre-operative period. In summary, the results of the present study indicate that in patients selected to undergo potentially curative resection for renal cancer, the

presence of an elevated C-reactive protein concentration pre-operatively (>10mg/l) is an independent predictor of poor cancer-specific survival.

Table 3.1 SSIGN and Leibovich scoring algorithms

Feature	SSIGN score	Feature	Leibovich Score
T Stage: pT1a	0	T Stage: pT1a	0
pT1b	0	pT1b	2
pT2	1	pT2	3
pT3a	2	pT3a	4
pT3b	2	pT3b	4
pT3c	2	pT3c	4
pT4	0	pT4	4
Nuclear grade:		Nuclear grade:	
1	0	1	0
2	0	2	0
3	1	3	1
4	3	4	3
N Stage: pNx/	0	N Stage: pNx/	0
pN0	0	pN0	0
pN1/2	2	pN1/2	2
Tumour size <5 cm	0	<10 cm	0
≥5cm	2	≥10 cm	1
Histologic tumour necrosis:			
Absent	0	Absent	0
Present	2	Present	1
M Stage: pM0	0		
pM1	4		

Table 3.2 Baseline characteristics and cancer specific survival in patients undergoing potentially curative nephrectomy. Univariate analysis

	Patients (n= 111)	Hazard ratio (95%CI)	p-value
UISS (low/ intermediate risk)	29/ 82	3.06 (0.71-13.31)	0.135
Age group (≤60/>60 yrs)	47/ 64	0.93 (0.38-2.26)	0.868
Sex (male/ female)	67/ 44	1.26 (0.52-3.03)	0.613
ECOG PS (0/ ≥1)	93/ 18	1.03 (0.34-3.08)	0.965
T Stage (I/ II/ III)	51/ 22/ 38	2.99 (1.52-5.85)	0.001
Fuhrman Grade (1/ 2/ 3/ 4)	20/ 46/ 34/ 11	2.06 (1.22-3.48)	0.007
Tumour necrosis (Absent/ present)	73/ 38	4.76 (1.89-11.98)	<0.001
Haemoglobin (M≥13/ <13 g/dl, F≥11.5/ <11.5 g/dl)	80/ 24	4.19 (1.52-11.56)	0.006
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	85/ 12/ 7	0.99 (0.45-2.16)	0.971
Neutrophil count (<6.5/ ≥6.5 10^9/l)	92/ 9	0.76 (0.10-5.84)	0.794
Percentage total lymphocytes (20-40/ 12.0-19.9/ 0-11.9 %)	76/ 20/ 5	1.60 (0.68-3.77)	0.278
Albumin (≥35/ <35 g/l)	108/ 1		0.841
C-reactive protein (≤10/ >10 mg/l)	70/ 41	4.39 (1.69-11.44)	0.003
SIGN (0-3/ 4-6/ ≥7)	56/ 40/ 15	3.73 (2.03-6.85)	<0.001
Leibovich score (low/ intermediate/ high)	39/ 50/ 22	4.94 (2.26-10.79)	<0.001

Table 3.3 The relationship between the presence of a systemic inflammatory response and clinicopathological characteristics in patients with operable renal cancer.

	C-reactive protein (≤10 mg/l, n= 70)	C-reactive protein (>10 mg/l, n= 41)	p-value
UISS (low/ intermediate risk)	25/ 45	4/ 37	0.003
Age group (≤60/>60 yrs)	33/ 37	14/ 27	0.183
Sex (male/ female)	44/ 26	23/ 18	0.484
ECOG-PS (0/ ≥1)	61/ 9	32/ 9	0.212
T Stage (I/ II/ III)	41/ 12/ 17	10/ 10/ 21	<0.001
Fuhrman Grade (1/ 2/ 3/ 4)	13/ 32/ 21/ 4	7/ 14/ 13/ 7	0.137
Tumour necrosis (Absent/ present)	50/ 20	23/ 18	0.102
Haemoglobin (≥13/ <13 g/dl M, ≥11.5/ <11.5 g/dl F)	58/ 8	22/ 16	0.001
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	56/ 6/ 4	29/ 6/ 3	0.372
Neutrophil count (<6.5/ ≥6.5 10^9/l)	60/ 4	32/ 5	0.219
Percentage total lymphocytes (20-40/ 12.0-19.9/ 0-11.9 %)	50/ 10/ 4	26/ 10/ 1	0.708
Albumin (≥35/ <35 g/l)	68/ 1	40/ 0	0.446
SIGN (0-3/ 4-6/ ≥7)	44/ 21/ 5	12/ 19/ 10	<0.001
Leibovich score (low/ intermediate/ high)	32/ 29/ 9	7/ 21/ 13	0.001
Survival (months) Mean (95% CI)	97.2 (89.3-105.1)	81.5 (58.6-104.4)	<0.001

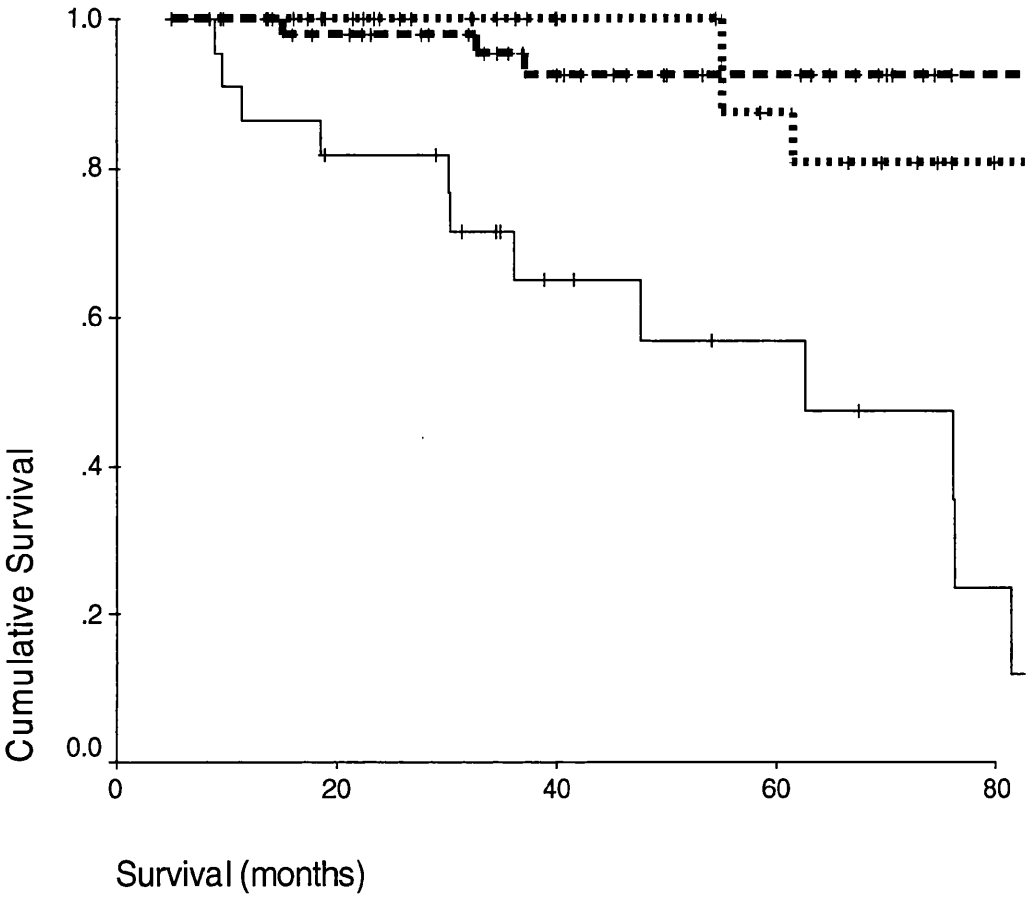

Fig 3.1 Relationship between the Leibovich score (favourable/ intermediate/ poor risk from top to bottom) and cancer-specific survival in patients with operable renal cancer

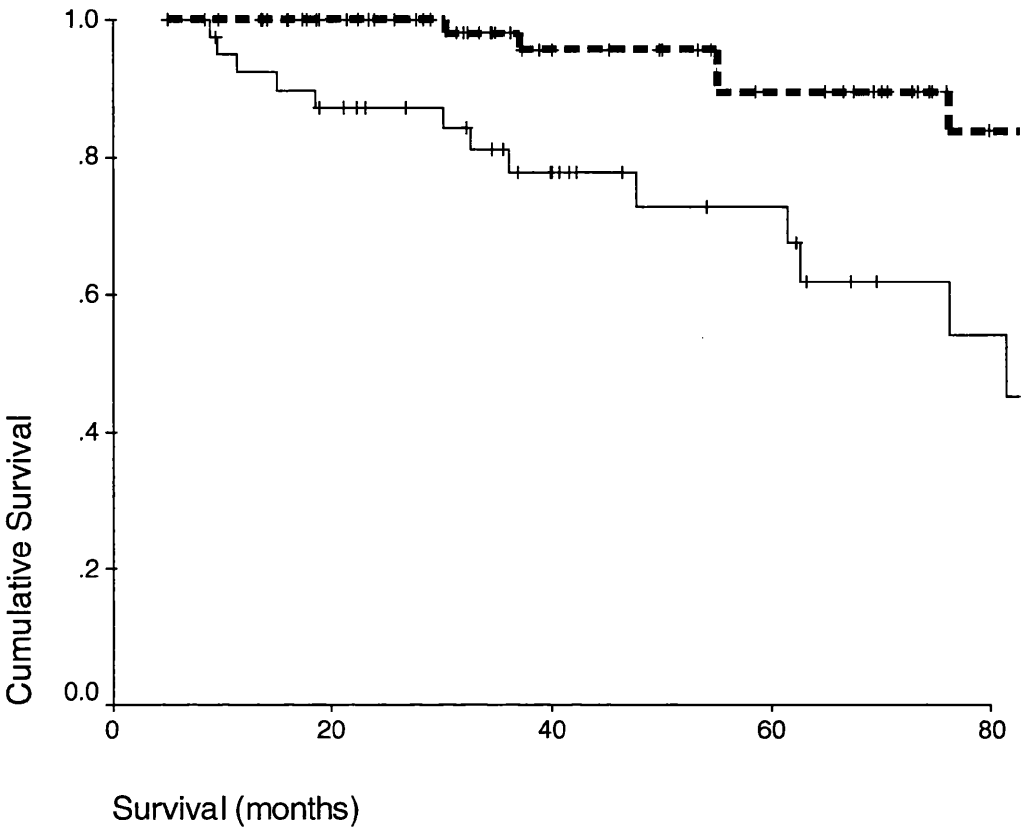

Fig 3.2 Relationship between C-reactive protein concentrations (≤10 mg/l / >10 mg/l from top to bottom) and cancer-specific survival in patients with operable renal cancer

4.0 Patient study: The relationship between the systemic inflammatory response and Th1/Th2 cytokines in patients with primary operable renal cancer.

4.1 Introduction

It is increasingly recognised that, in addition to recognised risk factors such as tumour stage and grade, disease recurrence and subsequent outcome depends on a complex interaction of the tumour and host inflammatory response (Balkwill & Mantovani, 2001; Coussens & Werb, 2002; Vakkila & Lotze, 2004)). In Chapter two we have shown that the systemic inflammatory response, as evidenced by circulating concentrations of C-reactive protein and hypoalbuminaemia, the GPS, had prognostic value independent of the MSKCC score (Motzer *et al*, 1999) in patients with metastatic renal cancer. In Chapter three, C-reactive protein, in addition to the Leibovich score (Leibovich *et al*, 2003) was associated with poorer cancer specific survival in patients with primary operable renal cancer.

It is now recognised that elevated circulating concentrations of C-reactive protein reflect the net effect of hormones and cytokines (Gabay & Kushner, 1999). To date, few studies have examined the cytokine profile associated with an elevated C-reactive protein in patients with cancer (McKeown *et al*, 2004) and to our knowledge none in renal cancer. Such profiles may be useful in identifying the underlying immune cell activation that may result in the elaboration of CRP.

It is of interest that tumour infiltration by T-lymphocytes has been reported to have prognostic significance in patients with renal cancer. Nakano and co-workers (2001) reported an association between increased intra-tumoral CD8+ T-lymphocytes

and reduced cancer specific survival. In contrast, Bromwich and colleagues (2003) found no association between CD8+ T-lymphocytes and poor prognosis; though there was an association between increased CD4+ T-lymphocytes and reduced cancer specific survival.

Activation and suppression of Tl-lymphcyte function is tightly regulated by circulating cytokines. Type I (Th-1) responses generally act to stimulate cell-mediated immunity, characterised by the production of cytokines such as Tumour Necrosis Factor (TNF), IL-2 and IFN-γ: Type II (Th-2) stimulate humoral immunity and the production of antibodies by B cells, characterised by cytokines IL-4, IL-5, IL-6, IL-10. There is a cross regulation between the two responses with IL-10 known to down-regulate the Type I response, whilst IFN-γ and IL-2 down-regulate Th-2 cells and their cytokine production (Fiorentino *et al*, 1989). A Th-2 bias, with a reduced level of circulating Th-1 cytokines has been reported in a number of solid tumours including renal cancer (Rayman *et al*, 2004).

The aim of the present study was to examine the relationship between circulating concentrations of CRP and cytokines produced by the T-lymphocyte subset populations in patients with renal cancer.

4.2 Patients and Methods

Patients with primary operable renal cancer, who underwent nephrectomy between March 2003 and October 2005 in the North Glasgow NHS Trust were included in the study. Patients were staged pathologically according to the 1997 UICC TNM classification of renal tumours (Sobin & Wittekind,1997).Tumours were graded according to criteria set out by Fuhrman and coworkers (1982). Clinical stage and performance status (Eastern Cooperative Oncology Group, ECOG-ps) were recorded prior to surgery.

The Research Ethics Committee of North Glasgow NHS Trust approved the study.

4.2.1 Experimental design

A blood sample was collected prior to resection for routine laboratory analysis including C-reactive protein quantification. A further pre-operative sample of blood was drawn, centrifuged at 5,000 rpm for 8 minutes before aliquots of serum were snap frozen and stored at -80°C.

The limit of detection of the assay for C-reactive protein was 6mg/l. The coefficient of variation, over the range of measurement was less than 5% as established by routine quality control procedures. A C-reactive protein measurement of greater than 10mg/dl was considered to indicate the presence of a systemic inflammatory response (O'Gorman *et al,* 2000; Maltoni *et al*, 2005).

From a single serum sample (50μl) IFN-γ, IL-2, IL-4, IL-5 and IL-10 were measured using Luminex multianalyte profiling technology. Luminex technology utilises patented pre-dyed fluorescent microspheres occupying different regions of the optical spectrum,

allowing up to one hundred analytes to be measured simultaneously in a single microtitre well. Briefly, an individual bead set is coated with a capture antibody for the analyte (in this case a cytokine) of choice. Combinations of these bead sets are then incubated with the sample, and after washing, further incubated with a cocktail of detection antibodies. Further washing is performed to remove any unbound antibodies. Finally, incubation with a fluorochrome is performed, to produce a sample-antibody-indicator sandwich, in a similar fashion to a conventional sandwich ELISA. Detection of beads and quantification of analytes was performed using a Multiplex system and Bio-plex software with five-parametric curve fitting (Bio-Rad, California, USA). In the present study, the analysis was carried out as per the manufacturer's instruction using a preconfigured multiplex cytokine kit. (Biosource, California, USA). According to the product data sheet, the minimum detectable concentration for IFN-γ was less than five pg/ml, IL-2 less than six pg/ml, IL-4 less than five pg/ml, IL-5 less than three pg/ml, and IL-10 less than five pg/ml.

From a single serum sample (200μl) IL-10 was measured using ELISA technology (RnD systems, Abingdon, UK). In summary, a monoclonal antibody specific for IL-10 is pre-coated onto a microplate. Any IL-10 in the sample added to each microwell is bound, and unbound substances removed by washing. Subsequently, an enzyme-linked polyclonal antibody specific for IL-10 is added to the wells. Following further washing, a substrate solution is added, and colour develops in proportion to the IL-10 bound initially. The colour changes are visible to the naked eye, but are read by an optical platemeter.

4. 2. 2 Statistical Analysis

Data are presented as median and range. Cytokine concentrations below the threshold of sensitivity were expressed as equal to this threshold. Where appropriate, data were tested for statistical significance using the Mann-Whitney U test. Analysis was performed using SPSS software (SPSS Inc, Chicago, IL, USA).

4.3 Results

The clinicopathological characteristics and cytokine concentrations of patients undergoing nephrectomy (n=53) grouped according to the presence or absence of a systemic inflammatory response are shown in Table 4.1. Age, gender and Furhman grade were similar between both groups. However, T-stage was significantly higher in the inflammatory group ($p<0.05$) and there were significantly more cytoreductive nephrectomies in the inflammatory compared with the non-inflammatory group ($p<0.005$). In those cytokines measured using the Luminex technology the circulating concentrations of IFN-γ ($p\leq0.01$), IL-2 ($p<0.005$), IL-4 ($p<0.005$) and IL-5 ($p<0.005$) were significantly higher in the inflammatory group.

The inter-relationships between C-reactive protein, IFN-γ, IL-2, IL-4, IL-5 and IL-10 are shown in Table 4.2. C-reactive protein was correlated with IFN-γ ($p<0.001$), IL-2 ($p<0.01$), IL-4 ($p<0.001$), IL-5 ($p<0.001$) and IL-10 ($p\leq0.001$). IFN-γ, IL-2, IL-4, IL-5 and IL-10 were all highly correlated with each other ($r_s>0.75$, $p<0.001$).

Circulating concentrations of IL-10 measured using the ELISA technique were significantly higher in the inflammatory group ($p<0.001$). When the IL-concnetrations measured using the ELISA technique were compared with those measured with the Luminex technology, IL-10 concentrations were significantly higher in the later ($p<0.001$) The Luminex IL-10 and ELISA IL-10 concentrations were weakly, but significantly correlated ($r_s=0.37$, $p=0.003$) and ELISA Il-10 concentrations were more significantly correlated with C-reactive protein ($r_s=0.75$, $p<0.001$).

4.4 Discussion

It is well recognised cytokines are secreted by and are regulators of immune system and the systemic inflammatory response. Furthermore, different cytokine functions are complex and overlapping and therefore the analysis of multiple related cytokines may be more informative with regard to their source than the measurement of a single cytokine. In the present study we examined the circulating concentrations of those cytokines recognised to have a role in T-lymphocyte function.

In the present study circulating concentrations of IFN-γ, IL-2, IL-4, IL-5 and IL-10 measured by the Luminex technology were increased in those patients with evidence of a systemic inflammatory response. Also, IL-10 concentrations measured by the ELISA technique were increased in patients with evidence of a systemic inflammatory response. However, the ELISA values for IL-10 were lower, showed a lesser degree of variation, and were more significantly correlated with C-reactive protein when compared with IL-10 concentrations measured by the Luminex technology. The Luminex values for the other cytokines analysed showed similar variation to that of IL-10 measured by this approach.

Taken together the results of the present study would suggest firstly that the Luminex values are more variable and potentially less accurate compared with the more established ELISA technique. Secondly, that the systemic inflammatory response is associated with increased circulating concentrations of a variety of cytokines associated with both Th-1 and Th-2 responses.

The reasons for the wide range of values obtained using the Luminex technology are not clear. The method relies on the use of a filter mesh to suspend the beads and allow

effective washing to remove residual unbound compounds. If the filter mesh had become clogged with particulate matter prior to wash cycles unbound cytokines may have remained in the wells prior to analysis. However the filter wells were not macroscopically clogged during the experiment. To date, multiplex analysis of cytokines has been almost exclusively limited to cell culture supernatants. In terms of the number of peptides and proteins such supernatants are much less complex than that of a plasma or serum sample. Indeed, the problems of analysing plasma or serum samples have been suggested by De Jager and colleagues (2003) who note that when sera are used as the analytical matrix the matrix should be carefully monitored for blocking substances such as heterophilic antibodies. Taken together the above observations would suggest that there is a need to pre-treat plasma or serum samples prior to multiplex analysis of cytokines.

In summary, there remain a number of technical challenges to be overcome before multiple cytokine analysis can be applied to identify the nature of the immune response and relationship to the systemic inflammatory response in patients with renal cell carcinoma. However, IL-10 concentrations measured with the ELISA technique were strongly correlated with C-reactive protein and worthy of further investigation.

Table 4.1 Clinicopathological characteristics and cytokine profile according to the presence of absence of a systemic inflammatory response in patients with undergoing nephrectomy for renal cancer

	C-reactive protein ≤10mg/l (n=26)	C-reactive protein >10mg/l (n=27)	p-value
Age (≤60/ >60 years)	14/ 12	17/ 10	0.505
Gender (male/ female)	17/ 9	17/ 10	0.856
Curative/ Cytoreductive	25/ 1	20/ 7	0.026
Stage I/ II/ III/ IV	16/ 1/ 8/ 1	7/ 2/ 9/ 9	0.004
Grade 1/ 2/ 3/ 4	4/ 8/ 7/ 3	2/ 5/ 11/ 6	0.176
Luminex measurement			
IFN gamma (pg/ml)	474 (15-14514)	770 (115-1803)	0.010
IL-2 (pg/ml)	56 (6-655)	273 (8-1107)	0.003
IL-4 (pg/ml)	56 (5-356)	195 (12-551)	0.003
IL-5 (pg/ml)	43 (3-249)	141 (3-382)	0.002
IL-10 (pg/ml)	25 (5-2954)	62 (5-256)	0.058
ELISA measurement			
IL-10 (pg/ml)	3.80 (3.80-9.66)	7.55 (3.80-23.62)	<0.001

Table 4.2 The inter-relationships between C-reactive protein and T-lymphocyte cytokine profile in patients with renal cancer.

	IFN γ (pg/ml) r_s (p-value)	IL-2 (pg/ml) r_s (p-value)	IL-4 (pg/ml) r_s (p-value)	IL-5 (pg/ml) r_s (p-value)	IL-10 (pg/ml) r_s (p-value)
C-reactive protein (mg/l)	0.54 (<0.001)	0.56 (<0.001)	0.53 (<0.001)	0.57 (<0.001)	0.43 (0.001)
IFN γ (pg/ml)		0.84 (<0.001)	0.85 (<0.001)	0.87 (<0.001)	0.80 (<0.001)
IL-2 (pg/ml)	0.84 (<0.001)		0.97 (<0.001)	0.97 (<0.001)	0.75 (<0.001)
IL-4 (pg/ml)	0.85 (<0.001)	0.97 (<0.001)		0.99 (<0.001)	0.71 (<0.001)
IL-5 (pg/ml)	0.89 (<0.001)	0.97 (<0.001)	0.99 (<0.001)		0.73 (<0.001
IL-10 (pg/ml)	0.80 (<0.001)	0.75 (<0.001)	0.71 (<0.001)	0.73 (<0.001)	

5.0 Patient Study: The longitudinal relationship between the systemic inflammatory response, interleukin-6, interleukin-10 and circulating T-lymphocytes in patients undergoing immunotherapy for metastatic renal cancer.

5.1 Introduction

In the second chapter, we reported the prognostic significance of the systemic inflammatory response, as evidenced by the GPS, in patients with metastatic renal cancer treated with immunotherapy. However, the basis of the independent relationship between elevated C-reactive protein concentrations, poor response rates and poor survival in patients with metastatic renal cancer is not clear. One explanation is that an elevated C-reactive protein identifies an impaired T-lymphocytic response (Berczi *et al*, 2000) to the tumour (Maccio *et al*, 1998; Canna *et al*, 2005). If this were the case then it might be expected that circulating concentrations of interleukin-6 and interleukin-10, key mediators of T-lymphocyte stimulation and suppression respectively (Gabay & Kushner, 1999; Jee *et al*, 2001; Trikha *et al*, 2003; Mocellin *et al*, 2005), would be abnormal. Indeed, in the previous Chapter we reported increased concentrations of various cytokines, but in particular a correlation between IL-10 and C-reactive protein. Interleukin-6 has been previously used as an indicator of the systemic inflammatory response and prognostic factor in patients with metastatic renal cancer (Blay *et al*, 1993), and is thought to be responsible for around 50% of C-reactive protein secretion in vivo (Gabay & Kushner, 1999). To our knowledge the longitudinal relationships between these inflammatory markers and T-lymphocytes have not been previously examined in patients undergoing immunotherapy for renal cancer.

The aim of the present study was to examine the longitudinal relationship between C-reactive protein, interleukin-6, interleukin-10, and circulating T-lymphocyte sub-populations in patients undergoing alpha-interferon-based immunotherapy for metastatic renal cancer.

5.2 Patients and Methods

Patients with metastatic renal cancer who were immunotherapy naive and due to commence out-patient immunotherapy between May 2005 and February 2006 were included in the study. The out-patient regimes were either subcutaneous alpha-interferon given at 10 mega-units three times weekly on a 12-weekly basis, or a modified Atzpodien regime of subcutaneous alpha-interferon, subcutaneous interleukin-2 for five weeks, with an additional 3 weeks of 5-flourauracil given intravenously, both defined as one cycle. Patients were staged pathologically according to the 1997 UICC TNM classification of renal tumours (Sobin & Wittekind, 1997). Tumours were graded according to criteria set out by Fuhrman and coworkers (1982). Clinical stage and performance status (Eastern Cooperative Oncology Group, ECOG-ps) were recorded prior to treatment initiation

The Research Ethics Committee of North Glasgow NHS Trust approved the study.

5.2.1 Experimental design

A blood sample was collected, prior to initiation of immunotherapy, for routine laboratory analysis of haemoglobin, white cell count, lactate dehydrogenase, lymphocyte count, albumin and C-reactive protein. The limit of detection of the assay was a C-reactive protein concentration lower than 6mg/l. The coefficients of variation of these methods, over the range of measurements, was less than 10% as established by routine quality control procedures.

A further blood sample was taken, centrifuged, and the serum stored at –80°C prior to analysis of interleukin-6 and interleukin-10. Circulating concentrations of these

cytokines were measured using an enzyme linked immunosorbent assay (ELISA) technique. The minimum detectable concentrations were 2 pg/ml for interleukin-6 and 4pg/ml for interleukin-10 (Quantikine ELISA, R&D Systems Europe Ltd, Abingdon, UK). Inter- and intra-assay variability was less than 10% for both assays. Cytokine concentrations below the threshold of sensitivity of the respective assays were expressed as equal to this threshold.

The blood sampling and analyses were repeated two to four weeks after commencing treatment at the first outpatient review. Performance status was recorded, along with any dose reductions or treatment breaks. CT scanning was performed after approximately eight to twelve weeks of immunotherapy to assess for disease response or progression.

The GPS was constructed as previously described (Forrest *et al*, 2003, 2004). Briefly, patients with both an elevated C-reactive protein (>10 mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of two. Patients in whom only one of these biochemical abnormalities was present were allocated a score of one. Patients in whom neither of these abnormalities was present were allocated a score of zero.

The MSKCC scoring system (Motzer *et al*, 1999) was used to stratify patients into favourable, intermediate and poor risk groups. In this system an abnormal haemoglobin, calcium, LDH three times greater than the upper limit of normal, Karnofsky performance stautus <80% and the absence of prior nephrectomy are considered as risk factors. Patients with no risk factors are classified as favourable, one to two as intermediate, and three or greater as poor risk.

5.2.2 Statistics

Data are presented as median and range. Comparisons between groups of patients were carried out using contingency table analysis (X^2) as appropriate. Grouping of the laboratory variables haemoglobin, white cell and lymphocyte counts and lactate dehydrogenase was carried out using standard thresholds (Maltoni *et al*, 2005). Where appropriate, data were tested for statistical significance using Mann–Whitney U test and the Wilcoxon signed rank test. As the distribution of C-reactive protein and the cytokines were skewed, they were logarithmically transformed prior to stepwise multiple regression analysis for the examination of independent associations with C-reactive protein. Survival (cancer-specific) analysis of the group variables was performed using the Cox proportional hazard model. Deaths up to the end of August 2006 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

5.3 Results

The characteristics of patients with advanced renal cancer receiving alpha-interferon immunotherapy (n=23) are shown in Table 5.1. The majority were male and over the age of 60 years and had prior curative surgery. All patients had an ECOG performance status of less than or equal to one, haemoglobin and adjusted calcium in the normal range and a favourable or intermediate MSKCC risk. The majority of patients had lactate dehydrogenase, albumin, C-reactive protein, white cell and lymphocyte counts in the normal range. In contrast, the majority of patients had an elevated GPS, interleukin-6 and interleukin-10. Of the seven patients with hypoalbuminaemia six (86%) had an elevated C-reactive protein concentration.

The patients were grouped according to whether or not the patients completed one cycle of treatment with no dose reduction of their immunotherapy regimen (Table 5.2). The patients who completed one cycle of immunotherapy were more likely to have a normal haemoglobin ($p<0.01$) and a favourable MSKCC score ($p<0.05$). In contrast, patients who did not complete one cycle of immunotherapy were more likely to have hypoalbumaemia ($p<0.05$), an elevated GPS ($p<0.05$) and a low percentage lymphocytes ($p<0.05$). There was a trend towards a higher interleukin-6 concentration ($p<0.10$).

The relationship between baseline clinicopathological criteria and cancer specific survival of patients with advanced renal cancer receiving immunotherapy (n=23) are shown in Table 5.2. The minimum follow-up was four months and nine (39%) patients died during the follow-up period. On univariate survival analysis, MSKCC ($p<0.01$), the GPS ($p\leq0.01$), interleukin-6 ($p\leq0.05$) and interleukin-10 ($p<0.05$) were significantly associated with poorer survival. On multivariate analysis of these significant factors only

the MSKCC (HR 3.31, 95%CI 0.87-12.68, p=0.080) and the GPS (HR 3.15, 95%CI 0.88-11.24, p=0.077) were independently associated with cancer specific survival.

The before and after values of those patients completing one cycle of treatment are shown in Table 5.3. ECOG-ps, haemoglobin, adjusted calcium, MSKCC, lactate dehydrogenase, albumin, C-reactive protein, the GPS, white cell and lymphocyte counts and iterleukin-6 did not change significantly. In contrast, concentrations of interleukin-10 increased from pre-treatment values when measured at two weeks($p<0.01$).

5.4 Discussion

In the present prospective study of patients undergoing immunotherapy for metastatic renal cancer the failure to complete immunotherapy and poor cancer specific survival was significantly associated with the MSKCC risk classification and the systemic inflammatory response, as evidenced by the GPS. Both these prognostic scores were also associated with cancer specific survival and did not alter following one cycle of immunotherapy. These results confirm the importance of the MSKCC classification and the systemic inflammatory response in predicting the outcomes for patients with metastatic renal cancer treated with immunotherapy (Motzer *et al*, 2004; Bromwich *et al*, 2004).

In contrast, the proportions of CD4+ and CD8+ T-lymphocytes were not significantly associated with the failure to complete immunotherapy, cancer specific survival and were not significantly altered during immunotherapy. These results would suggest that an impaired T-lymphocytic response is not a major factor in the failure of immunotherapy in these patients. Few studies have examined the role of circulating T-lymphocytes in immunotherapy treatment. One study of 25 patients treated with interferon suggested an elevated CD8+ T lymphocyte count was associated with reduced survival (Arima *et al*, 1996). However, this study has used arbitrary criteria to subdivide the patients, and multivariate analysis was not performed on the results derived from the patients treated with interferon.

In the present study there was a trend towards a higher interleukin-6 being associated with the failure to complete immunotherapy, poorer cancer specific survival and interleukin-6 concentrations were not altered following immunotherapy. However,

compared with the MSKCC and the GPS classifications the value of interleukin-6 in predicting the response to immunotherapy appears limited. Therefore, these results do not confirm a major role for interleukin-6, proposed by Negrier and coworkers (2004), in determining treatment for patients with metastatic renal cancer.

It was of interest that, following immunotherapy, circulating concentrations of interleukin-10 were significantly increased. It has previously been reported that concentrations of interleukin-10 are progressively increased during treatment with high-dose interleukin-2 (Engelhardt *et al*, 1997). The basis of the increase in interleukin-10 concentrations is not clear. However, as the proportions of T-lymphocytes before and during immunotherapy did not change significantly, this would suggest a source of interleukin-10 secretion other than the T-lymphocytes. Since C-reactive protein is known to activate macrophages (Du Clos & Mold, 2004) it may be that macrophages are a major source of interleukin-10 (Moore *et al*, 2001). Nevertheless, the results of the present study do not confirm a predictive role for interleukin-10 as proposed by Wittke and coworkers (1999). Therefore, it may be that the increase in interleukin-10 concentrations is part of anti-inflammatory response to immunotherapy.

In summary, longitudinal measurements of circulating interleukin-6, interleukin-10 and T-lymphocytes do not appear to offer information in addition to the MSKCC and GPS risk classifications in patients receiving immunotherapy for metastatic renal cancer.

Table 5.1 Baseline characteristics in patients undergoing alpha-interferon based immunotherapy for metastatic renal cancer according completion of immunotherapy.

	Immunotherapy not completed (n= 12)	Immunotherapy completed (n = 11)	p-value
Age group (≤60/>60 yrs)	5/ 7	4/ 7	0.791
Sex (male/ female)	9/ 3	9/ 2	0.699
Prior surgical treatment (curative/ cytoreductive/ none)	5/ 2/ 5	8/ 1/ 2	0.153
ECOG-PS (≤1/ >1)	12/ 0	11/ 0	1.000
Haemoglobin (≥13/ <13 g/dl M, ≥11.5/ <11.5 g/dl F)	4/ 8	10/ 1	0.009
Adjusted Calcium (<2.5/ >2.5)	7/ 5	9/ 2	0.232
MSKCC (favourable/ intermediate/ poor)	2/ 8/ 2	7/ 4/ 0	0.016
Lactate dehydrogenase (<300/ ≥300 U/l)	11/ 1	11/ 0	1.000
Albumin (≥35/ <35 g/l)	6/ 6	10/ 1	0.037
C-reactive protein (≤10/ >10 mg/l)	3/ 9	6 / 5	0.156
GPS (0, 1, 2)	2/ 5/ 5	6/ 4/ 1	0.033
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	8/ 2/ 2	9/ 2/ 0	0.239
Percentage total lymphocytes (20-40/ 12.0-19.9/ 0-11.9 %)	4/ 4/ 4	7/ 4/ 0	0.046
CD4+ T-lymphocytes (%)	43 (30-62)*	41 (14-58)*	0.341
CD8+ T-lymphocytes (%)	27 (14-40)*	31 (12-50)*	0.844
Interleukin-6 (pg/ml)	39 (12-133)*	15 (12-41)*	0.074
Interleukin-10 (pg/ml)	28 (11-272)*	27 (12-238)*	0.424
Chemotherapy (alpha-interferon/ alpha interferon plus)	11/ 1	8/ 3	0.242

* Median (range)

Table 5.2 Baseline characteristics and cancer specific survival in patients undergoing alpha-interferon based immunotherapy for metastatic renal cancer. Univariate analysis

	Patients (n= 23)	Hazard ratio (95%CI)	p-value
Age group (≤60/>60 yrs)	9/ 14	1.35 (0.34-5.41)	0.673
Sex (male/ female)	18/ 5	1.01 (0.21-4.92)	0.989
Prior surgical treatment			
(curative/ cytoreductive/ none)	13/ 3/ 7	0.39 (0.12-1.21)	0.103
MSKCC (Favourable/ intermediate/ poor)	9/ 12/ 2	6.36 (1.70-23.73)	0.006
Lactate dehydrogenase (<300/ ≥300 U/l)	22/ 1	0.57 (0.12-2.78)	0.150
GPS (0/ 1/ 2)	8/ 9/ 6	4.27 (1.43-12.75)	0.010
White cell count			
(<8.5/ 8.5-11.0/ >11.0 10^9/l)	17/ 4/ 2	1.60 (0.61-4.16)	0.340
Percentage total lymphocytes			
(20-40/ 12.0-19.9/ 0-11.9 %)	11/ 8/ 4	1.57 (0.66-3.72)	0.310
CD4+ T-lymphocytes (%)	42 (14-62)*	1.00 (0.94 - 1.07)	0.950
CD8+ T-lymphocytes (%)	29 (12-50)*	1.01 (0.94 - 1.08)	0.772
Interleukin-6 (pg/ml)	17.7 (11.8-132.9)*	1.02 (1.00 - 1.03)	0.055
Interleukin-10 (pg/ml)	27.2 (11.0-272.4)*	1.01 (1.00 – 1.01)	0.038
Chemotherapy			
(alpha-interferon/ alpha interferon plus)	19/ 4	0.58 (0.07-4.69)	0.614
Chemotherapy completed (no/ yes)	12/ 11		0.101

* Median (range)

Table 5.3 Clinicopathological characteristics before and during chemotherapy in patients completing one cycle of treatment

	Prior to Chemotherapy (n = 11)	During chemotherapy (n=11)	p-value
ECOG-PS (≤1/ >1)	11/ 0	10/ 1	0.317
Haemoglobin (≥13/ <13 g/dl M, ≥11.5/ <11.5 g/dl F	10/ 1	7/ 4	0.083
Adjusted Calcium (<2.50/ >2.50)	9/ 2	11/ 0	0.157
MSKCC (favourable/ intermediate/ poor)	7/ 4/ 0	6/ 5/ 0	0.564
Lactate dehydrogenase (<300/ ≥300 U/l)	11/ 0	11/ 0	1.000
Albumin (≥35/ <35 g/l)	10/ 1	10/ 1	1.000
C-reactive protein (≤10/ >10 mg/l)	6/ 5	4/ 7	0.317
GPS (0, 1, 2)	6/ 4/ 1	4/ 6/ 1	0.317
White cell count (<8.5/ 8.5-11.0/>11.0 10^9/l)	9/ 2/ 0	8/ 2/ 1	0.317
Percentage total lymphocytes (20-40/ 12.0-19.9/ 0-11.9 %)	7/ 4/ 0	9/ 2/ 0	0.414
CD4+ T-lymphocytes (%)	423 (29-58)*	47 (23–65)*	0.160
CD8+ T-lymphocytes (%)	27 (12–35)*	22 (14–32)*	0.725
Interleukin-6 (pg/ml)	16 (13-41)*	19 (12–82)*	0.859
Interleukin-10 (pg/ml)	27 (12–238)*	81 (17–240)*	0.008
Chemotherapy (alpha-interferon/ alpha interferon plus)	8/ 3		

* Median (range)

6.0 Patient Study: The longitudinal relationship between circulating concentrations of C-reactive protein, interleukin-6 and interleukin-10 in patients undergoing resection for renal cancer

6.1 Introduction

In the third chapter, we reported the prognostic value of the systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, in patients with primary operable renal cancer. This has been reported as a prognostic factor in patients undergoing potentially curative surgery for a number of solid tumours including colorectal (Nielsen *et al*, 2000; McMillan *et al*, 2003); gastro-oesophageal (Ikeda *et al*, 2003; Crumley *et al*, 2006), pancreatic (Jamieson *et al*, 2004) and urinary bladder (Hilmy *et al*, 2005) cancers.

However, the basis of the independent relationship between an elevated C-reactive protein concentration and poor survival in renal cancer is not clear. Specifically, it is not clear whether the systemic inflammatory response arises from the tumour per se or as a result of an impaired immune cytokine response. Interleukin-6 and interleukin-10 are likely to be key cytokines in such a response since they appear to have stimulant and suppressive actions respectively on immune cells, in particular T-lymphocytes (Gabay & Kushner, 1999; Jee *et al*, 2001; Trika *et al*, 2003). Interleukin-6 is recognised as an autocrine growth factor for tumours, but also has a tumour suppressive role in promoting anti-tumour activity of macrophages (Trika *et al*, 2003). More recently, interleukin-10 has been recognised to be an important immunosuppressive cytokine for the Th1 anti-tumour response and may be important in determining tumour growth and metastases (Mocellin *et al*, 2005).

The aim of the present study was to examine C-reactive protein, interleukin-6 and interleukin-10 concentrations before and following curative resection of renal cancer.

6.2 Patients and Methods

Patients with benign and malignant renal disease, who underwent resection between March 2003 and October 2005 in the North Glasgow NHS Trust were included in the study. Patients were staged pathologically according to the 1997 UICC TNM classification of renal tumours (Sobin & Wittekind, 1997). Tumours were graded according to criteria set out by Fuhrman and coworkers (1982). Clinical stage and performance status (Eastern Cooperative Oncology Group, ECOG-ps) were recorded prior to surgery.

The Research Ethics Committee of North Glasgow NHS Trust approved the study.

6.2.1 Experimental design

A blood sample was collected prior to resection for routine laboratory analysis of haemoglobin, white cell count, percentage lymphocyte count, albumin and C-reactive protein. The limit of detection of the assay was a C-reactive protein concentration lower than 6mg/l. The inter- and intra-assay variability of haemoglobin, white cell count, albumin and C-reactive, protein were less than 10%. A C-reactive protein concentration of greater than 10mg/l was considered to indicate the presence of systemic inflammatory response (O'Gorman *et al*, 2000; McMillan *et al*, 2001). A further blood sample taken prior to surgery was centrifuged and the serum stored at –80°C prior to analysis of interleukin-6 and interleukin-10. Circulating concentrations of these cytokines were measured using an enzyme linked immunosorbent assay (ELISA) technique. The minimum detectable concentrations were two pg/ml for interleukin-6 and four pg/ml for

interleukin-10 (Quantikine ELISA, R&D Systems Europe Ltd, Abingdon, UK). Inter- and intra-assay variability were less than 10% for both assays.

A second blood sample was obtained approximately three months following nephrectomy for routine laboratory analysis and cytokine quantification using the methods above.

6.2.2 Statistics

Data are presented as median and range. Comparisons between groups of patients were carried out using contingency table analysis (X^2) as appropriate. Cytokine concentrations below the threshold of sensitivity of the respective assays were expressed as equal to this threshold. Where appropriate, data were tested for statistical significance using Mann–Whitney U test and the Wilcoxon signed rank test. As the distribution of C-reactive protein and the cytokines were skewed, they were logarithmically transformed prior to stepwise multiple regression analysis for the examination of independent associations with C-reactive protein. Univariate survival analysis was performed using the Kaplan–Meier method with the logrank test. Deaths up to the end of July 2006 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

6.3 Results

The clinicopathological characteristics of patients who underwent resection for benign (n=12) and malignant (n=64) renal disease are shown in Table 6.1. Age, sex, ECOG-ps, haemoglobin, white cell count, percentage lymphocyte count, albumin and C-reactive protein were similar in the two groups. Circulating concentrations of both interleukin-6 and interleukin-10 ($p \leq 0.01$) were higher and a greater proportion were elevated ($p<0.05$) in malignant compared with benign disease.

The renal cancer patients were grouped according to whether they had evidence of a systemic inflammatory response prior to nephrectomy (C-reactive protein >10 mg/ l, Table 6.2). The groups were similar in terms of age, sex, tumour volume, Fuhrman grade, white cell count and percentage lymphocytes. In the inflammatory group T stage was higher ($p<0.01$), number of cytoreductive operations greater ($p<0.05$), both interleukin-6 and interleukin-10 concentrations were higher ($p<0.001$) and elevated ($p<0.10$) compared with the non-inflammatory group. In contrast, haemoglobin ($p<0.01$) and albumin ($p<0.10$) concentrations and ECOG-ps ($p<0.05$) were lower in the inflammatory group. Tumour volume was weakly correlated with C-reactive protein ($r^2= 0.20$, $p=0.002$), interleukin-6 ($r^2= 0.20$, $p=0.002$) and interleukin-10 ($r^2= 0.24$, $p=0.001$)

The minimum follow-up was seven months or until date of death; the median follow-up of the survivors was 25 months. During this period, 15 (20%) patients died: 11 patients of their cancer and four of intercurrent disease. On univariate analysis, an elevated C-reactive protein concentration prior to resection was associated with reduction in cancer specific survival ($p=0.014$).

In the cancer patients who had detectable circulating pre-operative C-reactive protein concentrations (n= 41), log transformed concentrations of C-reactive protein were significantly correlated with interleukin-6 (r^2= 0.62, p<0.001, Figure 1a) and interleukin-10 (r^2= 0.33, p<0.001, Figure 1b). On multiple regression analysis of both interleukin-6 and interleukin-10 on C-reactive protein, only interleukin-6 (r^2= 0.63, p<0.001) retained independent significance. Interleukin-6 was significantly correlated with interleukin-10 (r^2= 0.49, p<0.001).

The clinicopathological characteristics of the patients who had undergone a potentially curative operation, prior to and approximately three months following nephrectomy, are shown in Table 6.3. The proportion of patients with a low percentage lymphocyte count, albumin and an elevated C-reactive protein concentration did not change significantly over this period. In contrast, there was a fall in performance status (p<0.01), haemoglobin (p<0.01) and an increase in white cell count during this period. Changes in interleukin-6 and interleukin-10 concentrations did not reach statistical significance.

In the cancer patients who had detectable circulating post-operative C-reactive protein concentrations (n= 35), log transformed concentrations of C-reactive protein were significantly correlated with those of interleukin-6 (r^2= 0.66, p<0.001) and interleukin-10 (r^2= 0.33, p<0.001). On multiple regression analysis of both interleukin-6 and interleukin-10 on C-reactive protein, only interleukin-6 (r^2= 0.66, p<0.001) retained independent significance. Interleukin-6 was significantly correlated with interleukin-10 (r^2= 0.51, p<0.001).

6.4 Discussion

In the present study both interleukin-6 and interleukin-10 concentrations were greater in malignant compared with benign renal disease. Furthermore, both were directly associated with C-reactive protein and did not appear to normalise on resection of the primary renal tumour.

These results appear to contradict the report of Ljundberg and coworkers (1995) who, in a similar study design of 56 patients with stage I renal cancer reported that a number of acute phase proteins including C-reactive protein fell significantly approximately six months after resection. However, in the present study, when the analysis was confined to those patients with stage I disease, neither C-reactive protein, interleukin-6 or interleukin-10 appeared to normalise on resection of the primary tumour.

Galizia and coworkers (2002) in a similar study design in 50 patients with colon cancer reported that that both interleukin-6 and interleukin-10 concentrations fell by day 16 following resection. However, it was of interest that, in their study, the median concentrations of interleukin-6 and interleukin-10, prior to surgery, were higher (8 and 15 pg/ml respectively) compared with the results (3 and 5pg/ml respectively) in the present study. Nevertheless, consistent with the present study Galizia and coworkers (2002) observed that the majority of patients did not normalise their cytokine concentrations following radical resection.

The basis of the discrepancies between the present and previous studies is not clear. Nevertheless, the results of the present study are consistent with previous pre-/ post-operative C-reactive protein findings in colorectal, pancreatic and bladder cancer (McMillan *et al*, 2003; Jamieson *et al*, 2005; Hilmy *et al*, 2005). Furthermore, if there

were to be a significant conversion rate from a systemic inflammatory state (C-reactive protein >10mg/l) to a non-inflammatory state (C-reactive protein ≤10mg/l) following resection then the prognostic value of markers of the systemic inflammatory response would be significantly degraded.

The elevated circulating concentrations of interleukin-6 and interleukin-10 following resection of renal cancer may reflect a continuing Th-2 cytokine response since increased intra-tumoural CD4+ T-lymphocyte infiltrate has been shown to be associated with poor outcome, independent of grade, in patients with renal clear-cell cancer (Bromwich *et al*, 2003). This would be consistent with the observations in the present study that circulating interleukin-6 and interleukin-10 concentrations were not strongly correlated with tumour volume but were similarly correlated with each other before and after resection of the renal tumour. Moderation of this cytokine response may be important in the regulation of the systemic inflammatory response and warrants further clinical investigation.

Given the considerable variability of the effect of resection on C-reactive protein, interleukin-6 and interleukin-10 seen in the present study it would require a much larger study to absolutely preclude the possibility that surgical resection of renal cancer does not alter C-reactive protein, interleukin-6 and interleukin-10 concentrations. Nevertheless, the results of the present study would suggest that the presence of systemic inflammatory response is not solely determined by the elaboration of cytokines from the tumour.

In summary, an elevated pre-operative C-reactive protein was associated with increased tumour stage, interleukin-6 and interleukin-10 concentrations. However, resection of the primary tumour did not appear to be associated with significant

normalisation of circulating concentrations of C-reactive protein, interleukin-6 or interleukin-10. Therefore, the presence of systemic inflammatory response is unlikely to be solely be determined by the tumour itself, but may be as a result of an impaired immune cytokine response in patients with renal cancer.

Table 6.1 Clinicopathological characteristics in patients with benign and malignant renal disease prior to nephrectomy

	Benign disease (n = 12)	Renal cancer (n= 64)	p-value
Age group (≤60/>60 yrs)	9/ 3	35/ 29	0.194
Sex (male/ female)	4/ 8	42/ 22	0.037
T stage (1/ 2/ 3/ 4)		27/ 4/ 21/ 12	
Tumour volume (cm^3)		112 (1-4864)	
Fuhrman grade (1/ 2/ 3/ 4)		8/ 16/ 21/ 13	
Operation (curative/ cytoreductive)		54/ 10	
ECOG-PS (0/ 1)	12/ 0	53/ 11	0.123
Haemoglobin (≥12/ <12g/ dl)	9/ 3	50/ 14	0.813
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	7/ 5/ 0	48/ 13/ 3	0.486
Lymphocyte percentage (20-40/ 12.0-19.9/ 0-11.9 %)	10/ 2/ 0	43/ 15/ 6	0.199
Albumin (≥35/ <35g/l)	11/ 0	59/ 3	0.459
C-reactive protein (≤10/ >10mg/l)	8/ 4	34/ 30	0.390
Interleukin-6 (pg/ml)	<2 (<2–19)	4 (<2–142)	0.007
Interleukin-6 (≤4/ >4pg/ml)	10/ 1	33/ 31	0.015
Interleukin-10 (pg/ml)	<4 (<4–7)	6 (<4–66)	0.012
Interleukin-10 (≤10/ >10pg/ml)	12/ 0	45/ 19	0.030

Median (range)

Table 6.2 Clinicopathological characteristics of inflammatory and non-inflammatory patients with renal cancer prior to nephrectomy.

	C-reactive protein ≤10mg/l (n= 34)	C-reactive protein >10mg/l (n= 30)	p-value
	Median <6mg/l	Median 35mg/l	
Age group (≤60/>60 yrs)	17/ 17	18/ 12	0.426
Sex (male/ female)	23/ 11	19/ 11	0.719
T stage (1/ 2/ 3/ 4)	19/ 2/ 11/ 2	8/ 2/ 10/ 10	0.005
Tumour volume (cm^3)	64 (1-1331)	179 (9-4864)	0.123
Fuhrman grade (1/ 2/ 3/ 4)	5/ 11/ 9/ 6	3/ 5/ 12/ 7	0.193
Operation (curative/ cytoreductive)	32/ 2	22/ 8	0.023
ECOG-PS (0/ 1)	32/ 2	21/ 9	0.011
Haemoglobin (≥12/ <12g/ dl)	31/ 3	19/ 11	0.008
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	26/ 8/ 0	22/ 5/ 3	0.344
Lymphocyte percentage (20-40/ 12.0-19.9/ 0-11.9 %)	26/ 6/ 2	17/ 9/ 4	0.100
Albumin (≥35/ <35g/l)	33/ 0	26/ 3	0.060
Interleukin-6 (pg/ml)	<2 (<2–20)	16 (<2–142)	0.001
Interleukin-6 (≤4/ >4pg/ml)	22/ 12	11/ 19	0.026
Interleukin-10 (pg/ml)	<4 (<4–66)	8 (<4–35)	0.013
Interleukin-10 (≤10/ >10pg/ml)	27/ 7	18/ 12	0.092
Cancer specific survival (months)*	34.8 (32.6-37.1)	28.1 (23.1-33.0)	0.014

Median (range), *mean (95%CI)

Table 6.3 Clinicopathological characteristics of patients with renal cancer prior to and approximately 3 months following potentially curative nephrectomy for localised renal cancer.

	Pre-nephrectomy (n = 54)	Post-nephrectomy (n= 54)	p-value
Age group (≤60/>60 yrs)	26/ 28		
Sex (male/ female)	34/ 20		
T stage (1/ 2/ 3/ 4)	27/ 4/ 21/ 2		
Tumour volume (cm^3)	112 (1-4864)		
Fuhrman grade (1/ 2/ 3/ 4)	8/ 14/ 16/ 10		
ECOG-PS (0/ 1)	48/ 6	39/ 15	0.007
Haemoglobin (≥12/ <12g/ dl)	45/ 9	33/ 21	0.007
White cell count (<8.5/ 8.5-11.0/ >11.0 10^9/l)	41/ 11/ 2	31/ 20/ 3	0.029
Lymphocyte percentage (20-40/ 12.0-19.9/ 0-11.9 %)	39/ 11/ 4	37/ 12/ 4	0.874
Albumin (≥35/ <35g/l) [a]	51/ 2	45/ 1	0.317
C-reactive protein (<10/ >10mg/l)	32/ 22	35/ 19	0.439
Interleukin-6 (pg/ml) [a]	3 (<2–99)	<2 (<2-44)	0.227
	10 (18)[b]	6 (9)[b]	
Interleukin-6 (≤4/ >4pg/ml) [a]	31/ 23	28/ 9	0.059
Interleukin-10 (pg/ml) [a]	5 (<4–66)	6 (<4-112)	0.056
	12 (17)[b]	18 (26)[b]	
Interleukin-10 (≤10/ >10pg/ml) [a]	42/ 12	24/ 13	0.257

[a] post-nephrectomy n= 37, [b] mean (standard deviation), median (range)

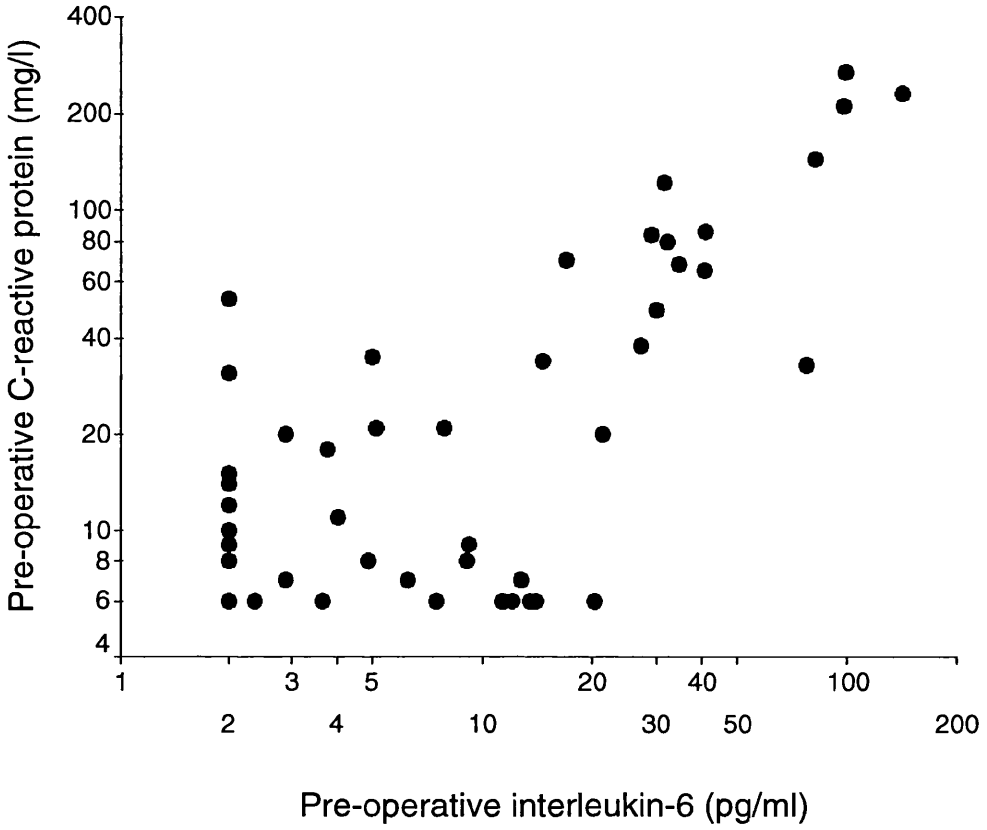

Figure 6.1 Relationship between circulating concentrations of interleukin-6 and C-reactive protein in patients with renal cancer.

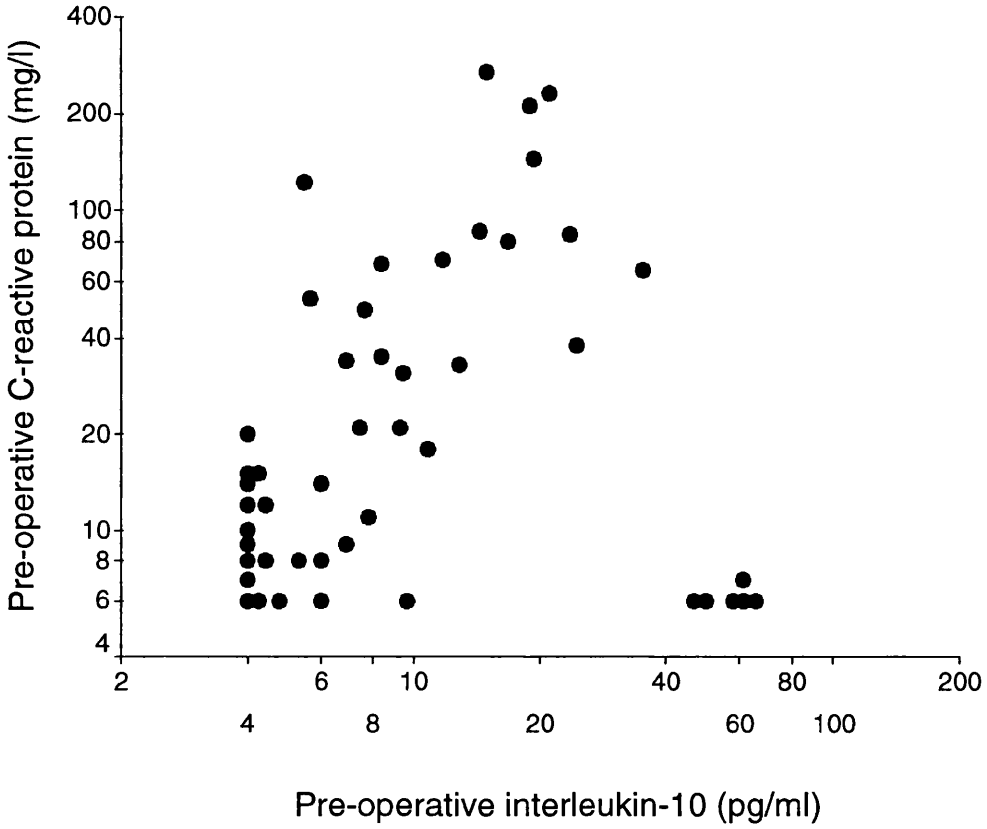

Figure 6.2 Relationship between circulating concentrations of interleukin-10 and C-reactive protein in patients with renal cancer.

7.0 Conclusions

It has long been recognised that disease progression in cancer patients is not solely determined by the characteristics of the tumour, but also by aspects of the host response (Balwill & Mantovani, 2001). Indeed, there is increasing evidence that both the local and systemic inflammatory responses play an important role in the progression of a variety of common solid tumours (Coussens & Webb, 2002; Vakkila & Lotze, 2004). The use of immunotherapy in treating patients with metastatic renal cancer, and the rare phenomenon of spontaneous tumour regression, emphasise the immunogenic nature of renal cancer and the importance of host responses. The main aim of this thesis was to investigate the prognostic value of the systemic inflammatory response and its underlying mechanisms in patients with renal cancer. As the mechanisms underlying systemic inflammation may be different in patients with metastatic renal cancer compared to those with localised disease, these will be considered separately. Patients with metastatic renal cancer were studied in Chapters two and five, whilst Chapters three, four and six are based on studies of patients with primary operable renal cancer.

7.1 Metastatic renal cancer

In the second chapter it was shown that a cumulative inflammation-based Glasgow Prognostic Score had independent prognostic value in a study of 119 patients. Patients with GPS of 0, 1 and 2 had median survivals of 28, 11 and 3 months respectively. These results are consistent with the concept that the systemic inflammatory response is associated with profound catabolic effects on host protein metabolism (McMillan et al, 1998; Kotler, 2000) including albumin (Fearon et al., 1998). Indeed, in the thesis study, only 15% of the patients with hypoalbuminaemia had normal C-reactive

protein concentrations. As such, the GPS quantifies the chronic systemic inflammatory response, which may lead to progressive nutritional and functional decline, subsequent cancer cachexia, and ultimately resulting in reduced survival.

A number of cytokine factors are thought to mediate increased concentrations of C-reactive protein in advanced cancer. In particular, interleukin-6 has been reported to account for 50-60% of the variation in circulating concentrations of C-reactive protein (Gabay & Kushner, 1999; McKeown *et al*, 2004). In Chapter five, it was shown that an elevated C-reactive protein concentration was associated with increases in both the "pro-inflammatory" cytokine interleukin-6 and the "immunosuppressive" cytokine interleukin-10 in 23 patients with metastatic renal cancer. In the same study following immunotherapy T-lymphocyte subsets, including the CD4 population, did not change despite a subsequent significant increase in interleukin-10 concentrations. These results would suggest that there may another source of interleukin-10. A recognised source of interleukin-10 secretion in vivo is the macrophage; and it may be that macrophage activation plays an important role in the activation systemic inflammatory response in patients with metastatic renal cancer. This hypothesis is consistent with the recent observation that increased tumour macrophage infiltration is associated with poor outcome, independent of subtype, in patients with renal cancer (Webster *et al*, 2006). Clearly, in the thesis study, macrophage activation may have occurred as a response to immunotherapy, with subsequent anti-inflammatory interleukin-10 secretion. It has yet to be determined if concentrations of interleukin-10 are independently associated with cancer specific survival in patients with metastatic renal cancer.

Currently, the International Kidney Cancer Working Group is forming a collaborative effort to produce a unified prognostic scoring system for effective stratification of patients with metastatic renal cancer. It remains to be seen if this will include features of the systemic inflammatory response, but evidence from this thesis indicates the GPS may be a useful inclusion in the unified prognostic scoring system. Moreover, it is cheap, simple to assess and based on reliable analyses.

Whilst the mechanism of inflammation remains unclear, moderation of the systemic inflammatory response in patients with metastatic renal cancer could potentially reduce nutritional and functional decline and improve survival substantially. Based on our data, this survival benefit may as much as 17 months. Studies in patients with gastro-intestinal cancer have reported that treatment with Non-Steroidal-Anti-Inflammatory Drugs may halt this functional decline (McMillan *et al*, 1999; Lundholm *et al*, 2004). However, a previous nephrectomy is a relative contra-indication to NSAID therapy as these can reduce renal blood flow, leading to renal impairment. As such, no studies have evaluated the role of NSAIDs in the management or palliation of patients with advanced renal cancer.

Alternatively, steroids could be used to suppress the systemic inflammatory response. Short courses of steroids are currently used in the management of the systemic inflammatory response in sepsis, guided by an abnormal short-synacthen test, a dynamic test of the hypothalamic-pituitary-adrenal axis. To date, few studies have examined the possibility of abnormal cortisol secretion in patients with advanced cancer or the role of steroids such as dexamethasone in metastatic renal cancer. However, they have been used empirically in certain circumstances such as cerebral metastases. Future research could be

tailored towards investigating the role of endogenous cortisol secretion in the moderation of the systemic inflammatory response in patients with metastatic renal cancer. The short-synacthen test could be used as a screening test prior to commencement of immunotherapy, or the new tyrosine kinase inhibitor drugs. Patients with abnormal cortisol secretion could then be treated with a short course of steroids and the systemic inflammatory response monitored.

7.2 Operable renal cancer

In Chapter three of this thesis the prognostic significance of C-reactive protein in 111 patients undergoing potentially curative nephrectomy was reported. This significance was in addition to the Leibovich score. Both an elevated C-reactive protein and the Leibovich score were superior to the SSIGN score.

It was of interest that all but one patient had albumin concentrations within the normal range, and as such, the GPS could not be investigated for additional prognostic value in this cohort of patients. It may be that, unlike gastro-intestinal tumours where hypoalbuminaemia is a more common event, patients with renal cancer are relatively less nutritionally compromised at the time of their potentially curative surgery.

In Chapter six, the longitudinal relationship between the systemic inflammatory response, and circulating cytokines in patients with primary operable renal cancer was inverstigated. Interleukin-6 and interleukin-10 concentrations were both elevated in the presence of a systemic inflammatory response and were directly correlated. However, on multiple regression analysis only interleukin-6 was significantly correlated with C-reactive protein concentrations. Consequently, it may be that concentrations of

interleukin-10 are increased in response to raised concentrations of interleukin-6 in a bid by the host to limit the systemic inflammatory response.

Following nephrectomy, the proportion of patients with an elevated C-reactive protein did not change significantly, nor did concentrations of interleukin-6 normalise. In contrast, there was a trend towards significance in the elevation of interleukin-10 concentrations following nephrectomy. Therefore, the presence of systemic inflammatory response appears unlikely to be solely determined by the tumour itself, but may be a result of a disordered immune response. Again, the source of interleukin-10 secretion in response to nephrectomy is unclear, but may be a macrophage-derived response, similar to the mechanism postulated during immunotherapy treatment. However, it remains to be seen if either interleukin-6 or interleukin-10 are superior to C-reactive protein in predicting cancer-specific survival these patients. Further follow-up of this cohort of patients will allow this important question to be addressed.

This longitudinal study, and the results from Chapter five suggest there may be a role for the macrophage in the development and persistence of the systemic inflammatory response. Whilst macrophages were not specifically examined during the studies, it would be of interest to repeat the analyses of circulating cytokines specifically associated with macrophages and the relationship with the systemic inflammatory response. In addition, macrophages have been implicated in angiogenesis via growth factors such as VEGF. If activation of macrophages leads to angiogenesis, and also secretion of Interleukin-10 which can suppress effective anti-tumour response, this potentially could be a mechanism for tumour progression and ultimately development of metastases.

There are a number of prognostic algorithms in use for patients with operable or localized renal cancer, but the majority of these are based on pathological factors. C-reactive protein concentrations are easily measured as part of a pre-operative work up, and add prognostic information to that provided by cross-sectional imaging prior to nephrectomy. Ultimately, the majority of patients fit for nephrectomy with localized disease will undergo resection. However, decision making regarding surgery in elderly patients, those with extensive co-morbidity, or high-risk locally advanced tumours may be influenced by the presence of a high inflammatory profile pre-operatively.

As with metastatic disease, moderation of the systemic inflammatory response could theoretically lead to improved time to progression, and cancer specific survival in patients with operable renal cancer. However, investigating this could require several years of essentially adjuvant therapy before effectiveness could be assessed. If steroids were used to suppress the systemic inflammatory response, complications relating to long-term use such as gastro-intestinal bleeding, osteopenia, and muscle wasting would be of concern. As such, it is likely any interventional study would need to investigate the effects of inflammation-modification in patients with metastatic renal cancer first, and an adjuvant study would only be possible if a significant survival benefit had been established for patients with metastatic disease.

7.3 Conclusions

In Chapter two the prognostic significance of the GPS in patients with metastatic renal cancer was reported. In Chapter three, an elevated C-reactive protein was shown to have prognostic value in addition to the Leibovich score in patients with primary operable renal cancer. In Chapter four, concentrations of circulating cytokines from both the Th-1

and Th-2 lymphocytes were increased in the presence of a systemic inflammatory response. In Chapter five, a longitudinal study of patients commencing immunotherapy for metastatic renal cancer, immunotherapy did not alter circulating concentrations of T-lymphocytes though there was an increase in circulating interleukin-10. In the final study, a longitudinal study of patients undergoing potentially curative nephrectomy was performed. Elevated concentrations of C-reactive protein were associated with elevated interleukin-6 concentrations. At three months following nephrectomy, the proportion of patients with an evidence of a systemic inflammatory response had not altered significantly

In summary, the results of the present thesis are consistent with the hypothesis that the presence of a systemic inflammatory response is a significant factor in the functional decline and survival in patients with metastatic renal cancer, and the progression of disease in patients with primary operable renal cancer.

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9.0 Appendix 1

9.1 Chapter 2 data

Pt No	Male1 Female2	IFN1TRI2	NEPH01	LIVER01	BONE01	NOMETS	TIMRECUR	AGEYRS	ECOGPS	ALBUMIN	CRP	GPS
1	1	2	.00	.00	.00	1.00	.00	70	0	43	6	0
2	1	1	.00	.00	.00	1.00	.00	61	0	45	21	1
3	2	1	.00	.00	.00	1.00	.00	59	0	37	11	1
4	1	2	.00	.00	.00	1.00	.00	59	0	34	22	2
5	2	1	.00	.00	.00	1.00	.00	61	0	45	19	1
6	2	1	.00	.00	.00	1.00	.00	72	0	36	94	1
7	1	1	.00	.00	.00	1.00	.00	71	1	35	6	0
8	1	1	.00	.00	.00	1.00	.00	51	1	45	51	1
9	1	2	.00	.00	.00	1.00	.00	68	0	40	226	1
10	2	1	.00	.00	.00	1.00	.00	74	0	46	40	1
11	1	1	.00	.00	.00	1.00	.00	50	1	37	191	1
12	1	1	.00	.00	.00	1.00	.00	67	0	43	62	1
13	2	1	.00	.00	.00	1.00	.00	65	1	42	20	1
14	2	1	.00	.00	.00	1.00	.00	74	0	37	10	0
15	1	1	.00	.00	.00	1.00	.00	49	0	39	70	1
16	2	1	.00	.00	.00	1.00	.00	61	0	42	115	1
17	1	1	.00	.00	.00	1.00	.00	36	1	46	37	1
18	1	1	2.00	.00	.00	1.00	1.58	57	0	43	73	1
19	1	1	2.00	.00	1.00	1.00	1.61	50	0	44	7	0
20	1	1	2.00	.00	.00	1.00	1.61	53	0	38	206	1
21	1	1	2.00	.00	.00	1.00	1.65	49	0	49	6	0
22	1	1	2.00	.00	.00	1.00	1.71	54	0	32	79	2
23	1	2	2.00	.00	.00	1.00	2.03	83	0	39	7	0
24	1	1	2.00	.00	.00	1.00	2.10	63	1	31	46	2
25	1	1	2.00	.00	.00	1.00	2.39	75	1	43	6	0
26	1	1	2.00	.00	.00	1.00	2.61	71	1	45	6	0
27	1	1	2.00	.00	.00	1.00	2.74	66	0	47	23	1
28	2	1	2.00	.00	.00	1.00	4.13	68	0	30	136	2
29	1	1	1.00	.00	.00	1.00	4.23	44	0	40	290	1
30	1	1	1.00	.00	.00	1.00	6.58	67	0	46	102	1

31	1	2	1.00	.00	.00	1.00	7.81	64	0	43	10	0
32	1	1	2.00	.00	.00	1.00	8.35	73	0	44	155	1
33	1	2	1.00	.00	.00	1.00	9.90	59	0	43	53	1
34	2	2	1.00	.00	.00	1.00	10.87	43	0	37	11	1
35	2	1	1.00	.00	.00	1.00	11.58	67	0	35	29	1
36	2	1	1.00	.00	.00	1.00	11.58	49	0	46	6	0
37	1	1	.00	.00	.00	2.00	.00	53	0	34	6	1
38	2	1	.00	.00	1.00	2.00	.00	67	0	45	6	0
39	1	1	.00	.00	.00	3.00	.00	71	1	44	28	1
40	1	1	.00	.00	.00	2.00	.00	75	0	40	6	0
41	2	1	.00	.00	.00	2.00	.00	66	3	46	6	0
42	1	1	.00	.00	1.00	2.00	.00	72	1	42	6	0
43	1	1	.00	.00	.00	2.00	.00	58	0	45	29	1
44	1	2	.00	.00	.00	2.00	.00	57	0	39	28	1
45	2	1	.00	.00	.00	2.00	.00	42	0	37	73	1
46	1	1	.00	.00	.00	2.00	.00	43	0	43	50	1
47	2	1	.00	1.00	.00	1.00	.00	70	1	40	9	0
48	1	1	.00	.00	.00	2.00	.00	73	3	32	18	2
49	2	1	.00	.00	.00	2.00	.00	66	0	41	24	1
50	1	1	.00	.00	.00	3.00	.00	56	0	42	17	1
51	1	1	.00	.00	1.00	2.00	.00	52	1	43	11	1
52	1	1	.00	.00	1.00	2.00	.00	74	0	38	48	1
53	2	1	.00	.00	1.00	2.00	.00	61	0	46	23	1
54	2	1	.00	.00	.00	2.00	.00	73	0	41	194	1
55	1	1	.00	.00	.00	3.00	.00	71	1	31	170	2
56	1	1	.00	.00	1.00	2.00	.00	74	1	42	6	0
57	1	1	.00	.00	.00	2.00	.00	58	0	43	1	0
58	1	2	.00	.00	.00	1.00	.00	54	0	31	29	2
59	1	1	.00	.00	1.00	1.00	.00	51	0	42	168	1
60	1	1	.00	.00	.00	1.00	.00	75	0	40	20	1
61	2	1	.00	.00	.00	1.00	.00	69	0	40	69	1
62	1	1	.00	.00	1.00	1.00	.00	47	0	43	9	0
63	1	1	.00	.00	.00	1.00	.00	58	1	28	141	2
64	1	1	1.00	.00	.00	2.00	1.23	59	0	44	16	1
65	1	1	2.00	.00	.00	2.00	1.32	58	0	48	44	1
66	1	1	2.00	.00	.00	2.00	1.81	59	0	42	31	1

67	1	1	1.00	.00	.00	2.00	2.19	69	0	49	6	0
68	1	2	2.00	1.00	.00	1.00	2.32	55	0	45	45	1
69	1	1	2.00	.00	.00	2.00	2.58	58	0	32	33	2
70	1	2	2.00	1.00	.00	1.00	3.13	55	0	43	7	0
71	2	2	1.00	.00	1.00	2.00	3.61	52	0	38	98	1
72	1	1	1.00	.00	.00	2.00	4.32	77	0	42	6	0
73	1	1	1.00	.00	.00	3.00	4.48	57	1	39	177	1
74	1	1	1.00	.00	.00	3.00	4.84	68	0	45	6	0
75	2	1	2.00	.00	.00	1.00	8.10	67	1	42	6	0
76	1	1	1.00	.00	.00	3.00	11.81	50	0	36	151	1
77	1	1	1.00	.00	.00	1.00	13.19	67	0	40	91	1
78	1	1	2.00	.00	1.00	1.00	19.23	49	0	33	30	2
79	1	2	1.00	.00	.00	1.00	20.52	48	0	42	144	1
80	2	1	1.00	.00	.00	1.00	32.42	64	0	47	8	0
81	2	1	1.00	.00	1.00	1.00	47.03	52	0	38	26	1
82	2	1	1.00	.00	.00	1.00	51.32	53	0	47	89	1
83	1	1	1.00	.00	.00	1.00	56.68	73	0	43	15	1
84	1	2	1.00	.00	.00	1.00	60.32	60	0	45	6	0
85	1	1	1.00	.00	.00	1.00	67.26	76	0	39	13	1
86	2	2	.00	1.00	.00	2.00	.00	47	0	34	98	2
87	1	1	.00	1.00	.00	3.00	.00	70	0	45	7	0
88	1	1	.00	1.00	.00	2.00	.00	70	1	42	51	1
89	2	1	.00	.00	.00	2.00	.00	67	1	35	152	1
90	1	1	.00	.00	.00	2.00	.00	76	1	38	34	1
91	2	2	.00	1.00	.00	1.00	.00	65	0	31	51	2
92	1	1	2.00	.00	.00	2.00	2.10	52	1	37	87	1
93	1	1	1.00	1.00	.00	3.00	9.68	75	0	36	220	1
94	1	1	1.00	.00	1.00	3.00	12.39	49	0	38	17	1
95	2	2	1.00	1.00	.00	1.00	14.71	56	0	46	6	0
96	1	1	1.00	.00	1.00	3.00	19.23	59	0	38	308	1
97	2	1	1.00	1.00	.00	1.00	22.94	54	1	36	12	1
98	1	2	1.00	.00	.00	2.00	26.55	48	0	40	6	0
99	2	1	1.00	.00	.00	2.00	27.00	44	0	47	6	0
100	2	1	1.00	1.00	.00	1.00	27.77	61	0	44	6	0
101	1	1	1.00	.00	.00	2.00	35.45	57	0	35	295	1
102	1	1	1.00	.00	.00	2.00	36.52	50	0	28	6	1

103	1	1	1.00	.00	1.00	2.00	50.45	67	1	35	6	0
104	1	1	1.00	.00	1.00	2.00	58.45	51		40	6	0
105	1	1	1.00	1.00	.00	1.00	59.90	50	0	43	16	1
106	2	1	1.00	.00	.00	2.00	61.39	72	0	32	12	2
107	1	1	1.00	.00	.00	2.00	61.84	62	1	28	18	2
108	1	1	1.00	.00	.00	2.00	77.81	66	0	41	6	0
109	2	1	1.00	.00	.00	2.00	83.84	55	0	45	28	1
110	1	1	1.00	.00	.00	2.00	89.23	76	0	38	23	1
111	1	1	1.00	.00	.00	2.00	96.61	62	0	36	42	1
112	2	1	1.00	.00	.00	2.00	107.35	71	0	40	11	1
113	1	1	1.00	.00	.00	2.00	121.26	70	0	37	14	1
114	1	1	1.00	1.00	.00	1.00	154.26	76	0	39	18	1
115	1	1	1.00	1.00	.00	2.00	13.77	59	0	42	11	1
116	1	1	1.00	.00	.00	2.00	15.35	39	0	41	71	1
117	1	1	2.00	1.00	.00	2.00	32.13	73	0	44	13	1
118	2	2	1.00	.00	.00	2.00	43.77	71	0	48	12	1
119	1	1	1.00	1.00	.00	2.00	83.87	55	0	45	17	1

Pt No	CCAL	HB	WCC	NEUT	%LYM	PLATELET	LDH	MOTSCORE	AZSCORE	Alive0/ Cancer Death1	Survival (months)
1	2.25	14.50	5.11	2.67	20.94	266	177	1	0	.00	21
2	2.23	13.40	5.96	3.64	27.85	225	208	1	1	1.00	9
3	2.16	9.40	4.55	3.70	10.33	284	188	1	1	.00	30
4	2.13	10.40	6.18	3.75	37.54	292	203	1	1	1.00	9
5	2.46	10.30	5.79	3.86	21.76	226	191	1	1	1.00	7
6	2.58	9.30	7.56	4.54	26.85	211	194	2	1	1.00	7
7	2.43	13.20	8.13	4.68	29.64		243	1	1	.00	7
8	2.32	15.60	7.40	4.88	19.59	245	196	1	1	.00	20
9	2.52	10.10	6.64	4.94	13.25	416	166	2	1	1.00	5
10	2.63	13.70	9.52	5.10	29.41	431	211	1	1	1.00	1
11	2.38	11.50	6.99	5.13	18.31	244	153	1	1	.00	12
12	2.39	9.00	7.77	5.49	14.93	669	194	1	1	1.00	6
13	2.40	12.80	7.57	5.59	15.06	395	198	1	1	.00	28
14	2.30	10.30	7.39	5.83	15.56	178	245	1	1	1.00	6
15	2.49	11.90	8.75	6.37	12.46	270	135	1	1	1.00	5
16	2.42	10.40	9.34	6.84	15.85	471	246	1	2	1.00	5
17	2.60	15.10	9.17	7.25	13.09		186	1	2	.00	9
18	2.22	13.20	6.98	4.68	22.35	208	188	0	1	1.00	10
19	2.31	12.90	6.49	4.74	15.41	196	132	1	1	1.00	12
20	3.15	8.60	8.57	7.32	9.57	224	161	1	2	1.00	1
21	2.30	13.20	4.30	2.15	32.56	217	227	0	1	.00	30
22	2.32	10.80	8.06	5.29	19.98	582	175	1	1	1.00	2
23	2.25	14.50	9.80	6.10	22.76	319	225	0	1	.00	16
24	3.29	10.80	8.05	5.82	13.79		139	1	1	1.00	4
25	2.30	12.90	8.10	5.17	28.64	186	167	1	0	.00	14
26	2.39	13.20	9.06	5.67	25.06	254	153	0	0	1.00	9
27	2.34	14.60	8.21	5.89	15.47	328	160	0	1	1.00	14
28	2.43	11.30	10.21	5.95	28.11	517	201	1	1	1.00	5
29	2.55	9.60	10.67	7.23	19.59	555	212	1	2	1.00	5
30	2.38	11.60	6.13	3.91	20.88	286	139	1	1	1.00	6
31	2.35	14.40	7.26	5.12	16.12	227	189	0	0	1.00	10
32	2.40	10.50	10.52	7.50	20.91	476	153	1	2	1.00	17
33	2.43	12.40	6.51	5.69	7.07	110	140	1	1	.00	18

34	2.20	12.20	7.72	4.62	30.18	309	135	0	1	.00	43
35	2.51	9.80	6.29	3.93	27.66	433	315	2	1	1.00	11
36	2.27	12.80	9.53	6.40	21.83	355	329	1	1	1.00	12
37	2.11	10.70	3.20	2.53	12.19	193	181	1	0	.00	31
38	2.34	11.50	4.12	2.79	19.17	238	167	1	1	1.00	28
39	2.11	8.10	4.65	3.58	11.61	277	219	1	1	1.00	11
40	2.20	13.00	5.05	3.63	16.63	216	253	1	1	.00	16
41	2.50	12.90	6.43	3.66	31.26	357	328	2	1	1.00	11
42	2.21	14.00	5.50	3.82	18.73		192	1	1	1.00	6
43	2.17	11.40	5.84	3.83	22.95	169	203	1	1	.00	30
44	2.67	10.10	7.11	4.09	28.55	262	150	2	1	1.00	7
45	2.30	12.00	5.46	4.18	8.24	381				1.00	1
46	2.84	11.70	6.81	4.39	19.24	329	150	2	1	1.00	9
47	2.45	10.10	8.00	5.00	28.25	295	191	1	0	1.00	11
48	2.33	11.00	6.80	5.15	7.06	310	157	2	1	1.00	11
49	2.20	10.00	8.32	5.23	20.67	404	286	1	1	1.00	9
50	2.37	12.90	7.62	5.34	20.08	204	263	1	2	1.00	5
51	2.31	14.10	7.67	5.49	20.47	243	206	1	1	1.00	26
52	2.50	9.30	8.86	5.62	24.60	313	205	1	1	1.00	5
53	3.31	13.30	7.63	5.69	13.24	532	203	1	1	1.00	2
54	2.33	9.50	8.27	5.70	15.60	552	211	1	1	1.00	11
55	2.90	11.50	7.66	5.98	13.19		212	2	1	1.00	3
56	2.35	10.90	6.06	6.06	28.38	316	271	1	1	.00	11
57	2.18	14.70	8.34	6.34	12.83	313	213	1	0	.00	17
58	2.41	12.10	9.27	7.56	10.03		742	2	2	1.00	2
59	2.28	13.40	11.33	7.96	16.86	372	208	1	2	1.00	21
60	2.39	11.70	12.31	10.03	14.13	467	370	2	2	1.00	10
61	2.35	11.80	12.34	10.35	10.37	411	253	1	2	1.00	10
62	2.37	13.50	15.23	12.35	13.20	168	299	1	2	.00	24
63	2.62	9.90	38.47	35.01	3.64		181	2	2	1.00	1
64	2.15	14.50	9.79	7.36	15.63	261	182	0	2	.00	24
65	2.45	11.20	9.55	7.25	17.07	312	186	1	2	1.00	4
66	2.29	11.20	6.59	3.04	37.63	231	155	1	1	.00	27
67	2.36	15.30	8.02	5.05	27.56	246	224	0	1	.00	40
68	2.35	12.30	5.43	2.85	23.57	277	163	1	1	1.00	12
69	2.14	8.90	5.09	3.07	22.20	280	118	1	1	1.00	16

70	2.44	14.10	5.78	3.43	29.24		247	0	1	.00	12
71	2.12	10.90	8.33	4.68	31.33	786	145	1	1	1.00	1
72	2.43	14.10	6.44	3.70	33.39		201	0	0	.00	6
73	2.41	10.90	5.71	3.89	24.87		639	1	2	.00	9
74	2.42	13.10	10.92	7.26	23.26		168	0	2	.00	13
75	2.45	12.20	13.62	7.69	32.38		297	0	2	.00	7
76	2.24	8.10	6.35	4.94	11.97	593	99	1	1	1.00	8
77	2.30	10.40	10.19	7.30	14.13	469	248	1	2	1.00	11
78	2.56	12.60	10.21	7.15	15.38	506	355	2	2	1.00	3
79	2.19	10.00	9.86	7.46	16.53	662	135	1	2	1.00	24
80	2.36	13.30	7.51	4.91	26.76	288	179	0	0	.00	10
81	2.28	9.80	5.51	3.12	30.67	224	342	1	1	1.00	18
82	2.34	12.10	5.91	4.22	22.67	180	157	0	0	1.00	25
83	2.26	12.30	5.20	3.25	18.85	198	227	1	1	1.00	3
84	2.25	15.30	5.36	2.56	41.60	169	209	0	0	.00	15
85	2.55	15.80	6.73	4.58	22.88		210	1	0	.00	9
86	2.25	10.30	5.97	3.47	33.84	212	561	2	1	1.00	7
87	2.21	14.60	7.49	4.55	26.17	219	109	1	1	1.00	19
88	2.24	13.80	8.23	5.86	19.20	275	187	1	1	1.00	14
89	2.33	8.90	10.49	7.86	17.16		162	1	2	1.00	2
90	2.22	13.10	13.04	10.58	7.90	268				1.00	1
91	2.39	12.40	38.52	30.02	4.00	401	238	1	2	1.00	2
92	2.70	12.00	12.84	9.16	16.90	753	158	1	2	.00	6
93	2.93	9.00	8.46	6.55	11.35	605	160	1	2	1.00	4
94	2.26	15.10	7.91	4.70	23.64	329	154	0	2	1.00	15
95	2.38	13.70	5.97	3.61	25.46	231	143	0	0	1.00	16
96	2.17	8.10	5.00	3.86	12.80	642	135	1	2	1.00	5
97	2.45	11.90	4.65	2.66	27.53		243	0	1	1.00	5
98	2.38	13.70	7.35	4.31	29.93		181	0	0	.00	5
99	2.16	11.40	7.74	5.28	20.16	340	144	1	0	.00	31
100	2.34	13.80	7.55	5.68	17.75		204	0	0	.00	12
101	2.75	9.10	7.18	6.02	10.31		179	1	1	.00	13
102	2.40	11.30	3.44	2.17	23.26		262	1	0	.00	9
103	2.37	11.80	6.72	4.40	19.05	279	179	1	0	1.00	33
104	2.35	12.40	7.02	4.56	19.80	307	162		0	.00	30
105	2.37	13.70	6.54	3.79	33.79	106	160	0	0	1.00	11

106	2.45	13.40	5.99	3.52	18.70		254	0	1	.00	7
107	2.58	14.00	8.23	5.90	20.41		155	1	0	1.00	3
108	2.20	13.90	6.67	4.54	16.04	205	209	0	0	.00	21
109	2.41	15.00	10.19	7.47	16.29	287	250	0	2	1.00	10
110	2.08	13.30	6.21	4.36	11.59	161	190	0	0	.00	11
111	2.38	16.60	7.05	5.16	15.04		145	0	0	.00	13
112	2.19	11.10	2.34	.49	38.89	157	140	1	0	1.00	21
113	2.18	10.20	6.17	3.45	27.39		206	1	0	1.00	1
114	2.48	11.60	6.13	3.95	25.29	377	193	1	0	1.00	5
115	2.32	14.40	5.37	3.28	29.42	71	149	0	1	1.00	14
116	2.21	8.70	11.61	10.04	8.10	386	213	1	2	1.00	4
117	2.31	14.70	9.53	6.61	19.73	170	189	0	2	1.00	19
118	2.32	13.60	11.76	8.79	17.52	320	192	0	1	1.00	5
119	2.44	19.50	5.76	3.76	20.49	236	286	0	1	.00	15

9.2 Chapter 3 data

Pt No	M1F2	TUMSIZE	NECR01	LVI01	AGEYRS	TNMSTAGE	GRADE	ECOGPS	UISS	ALBUMIN	CRP
1	1.00	2.80	.00	.00	27	1	1	0	0	47	0
2	1.00	4.00	.00	.00	54	1	1	0	0	43	0
3	2.00	5.00	1.00	.00	70	1	1	0	0	43	0
4	1.00	4.00	.00	.00	44	1	2	0	0	47	0
5	1.00	3.00	1.00	.00	53	1	2	0	0	42	0
6	1.00	3.20	.00	.00	69	1	2	0	0	40	0
7	1.00	3.50	1.00	.00	75	1	2	0	0	42	0
8	2.00	5.00	.00	.00	79	1	2	0	0	42	0
9	2.00	2.00	.00	.00	78	1	2	0	0	44	0
10	1.00	7.00	1.00	.00	62	1	2	0	0	45	0
11	1.00	3.50	1.00	.00	79	1	1	2	1	38	0
12	1.00	6.50	.00	.00	42	1	3	0	1	46	0
13	1.00	7.00	.00	.00	60	1	3	1	1	35	0
14	2.00	6.00	1.00	.00	65	1	4	0	1	46	0
15	2.00	8.00	.00	.00	77	2	1	0	1	42	0
16	2.00	8.00	.00	.00	56	2	2	0	1	41	0
17	1.00	16.00	.00	.00	58	2	2	2	1	46	0
18	1.00	10.00	.00	.00	55	2	2	0	1	47	0
19	1.00	6.50	1.00	.00	71	2	2	0	1	40	0
20	1.00	13.00	1.00	.00	55	2	3	0	1	44	0
21	1.00	11.00	1.00	.00	54	3	1	0	1	43	0
22	2.00	5.50	.00	.00	68	3	1	0	1	45	0
23	2.00	10.00	1.00	1.00	54	3	2	0	1	42	0
24	1.00	7.00	.00	.00	63	3	3	0	1	46	0
25	2.00	6.00	.00	.00	65	3	3	0	1	41	0
26	1.00	7.50	.00	1.00	53	3	3	0	1	39	0
27	1.00	4.00	1.00	.00	47	3	4	0	1	46	0
28	2.00	7.00	.00	.00	48	3	4	0	1	46	0
29	2.00	3.00	.00	.00	78	1	1	0	0	36	5
30	1.00	5.00	1.00	.00	58	1	1	0	0	45	6
31	1.00	6.00	.00	.00	56	1	2	0	0	48	6
32	1.00	3.50	.00	.00	61	1	2	0	0	46	6
33	1.00	7.00	.00	.00	65	1	2	0	0	45	6

34	1.00	6.00	1.00	.00	65	1	2	0	0	46	6
35	1.00	2.50	.00	.00	37	1	2	0	0	31	6
36	2.00	2.50	.00	.00	66	1	2	0	0	47	6
37	1.00	3.00	.00	.00	45	1	2	0	0	44	6
38	1.00	2.50	.00	.00	57	1	2	0	0	39	6
39	2.00	2.00	.00	.00	64	1	2	0	0	49	6
40	2.00	3.70	.00	.00	67	1	2	1	1	45	6
41	2.00	3.80	.00	.00	66	1	2	1	1	48	6
42	1.00	3.50	.00	1.00	59	1	3	0	1	49	6
43	2.00	3.00	.00	.00	64	1	3	0	1	46	6
44	1.00	4.00	.00	.00	62	1	3	0	1	45	6
45	1.00	4.00	.00	.00	39	1	3	0	1	43	6
46	1.00	3.00	.00	1.00	67	1	3	0	1	42	6
47	2.00	2.00	1.00	.00	64	1	3	0	1	42	6
48	1.00	2.00	.00	.00	47	1	4	0	1	46	6
49	1.00	7.00	.00	.00	45	2	2	0	1	44	6
50	2.00	8.00	.00	.00	65	2	3	0	1	47	6
51	1.00	10.00	1.00	.00	65	2	3	0	1		6
52	1.00	8.00	.00	1.00	78	3	3	0	1	46	6
53	2.00	6.00	.00	1.00	57	3	3	0	1	49	6
54	1.00	15.00	1.00	.00	52	3	3	0	1	42	6
55	2.00	6.00	1.00	.00	78	3	3	0	1	44	6
56	1.00	8.50	.00	.00	49	2	3	0	1	44	8
57	2.00	5.00	.00	.00	41	1	1	0	0	45	10
58	2.00	4.00	.00	.00	60	1	1	0	0	42	10
59	2.00	4.00	1.00	.00	45	1	2	0	0	47	10
60	2.00	4.00	.00	.00	70	1	2	0	0	42	10
61	1.00	3.50	.00	.00	66	1	2	2	1	45	10
62	2.00	7.00	.00	.00	63	1	3	2	1	41	10
63	1.00	3.00	1.00	.00	56	1	3	0	1	44	10
64	1.00	5.00	1.00	.00	69	2	1	2	1	43	10
65	1.00	17.00	.00	.00	48	2	2	0	1	48	10
66	2.00	4.00	.00	.00	81	3	1	1	1	45	10
67	1.00	7.00	.00	1.00	67	3	2	0	1	46	10
68	1.00	4.00	.00	.00	67	3	2	0	1	41	10
69	1.00	9.00	.00	1.00	51	3	2	0	1	53	10

70	1.00	9.00	.00	.00	75	3	2	0	1	45	10
71	1.00	4.00	.00	.00	49	1	2	0	0	46	11
72	1.00	4.00	.00	.00	60	3	2	0	1	46	11
73	1.00	7.50	.00	.00	68	2	3	0	1	42	12
74	2.00	2.00	.00	1.00	74	3	2	0	1	48	12
75	2.00	11.00	.00	.00	63	3	1	0	1	42	13
76	1.00	5.00	1.00	.00	59	3	3	0	1	42	13
77	1.00	4.50	1.00	.00	51	1	3	1	1	45	14
78	2.00	2.50	.00	.00	49	1	1	0	0	45	15
79	1.00	13.50	1.00	.00	50	2	2	0	1	42	15
80	2.00	8.50	.00	.00	67	2	2	0	1	40	17
81	1.00	7.50	1.00	.00	78	2	4	0	1	42	20
82	2.00	4.00	.00	.00	76	1	2	0	0	43	21
83	2.00	9.00	.00	.00	69	2	1	1	1	45	21
84	1.00	13.00	.00	.00	39	2	3	0	1	42	21
85	1.00	16.00	1.00	1.00	37	3	3	0	1	46	21
86	2.00	7.00	1.00	1.00	82	3	4	0	1	42	22
87	2.00	7.60	.00	.00	73	3	2	0	1	43	24
88	2.00	3.00	.00	.00	72	1	1	0	0	40	26
89	2.00	9.00	.00	.00	59	3	4	0	1	47	26
90	1.00	11.00	.00	1.00	72	3	1	0	1	46	33
91	2.00	9.70	.00	.00	56	2	3	1	1	47	34
92	1.00	7.00	1.00	.00	74	3	1	0	1	47	34
93	1.00	5.70	.00	.00	74	3	3	0	1	44	35
94	1.00	6.00	.00	.00	67	3	2	0	1	44	36
95	2.00	5.00	.00	.00	75	1	2	1	1	40	48
96	2.00	5.00	.00	.00	67	1	1	1	1	45	49
97	1.00	7.50	.00	.00	43	3	3	0	1	41	49
98	1.00	8.00	1.00	1.00	78	3	3	0	1	40	50
99	1.00	3.00	.00	.00	68	1	3	2	1	40	57
100	2.00	6.00	.00	1.00	60	1	4	0	1	39	68
101	1.00	10.50	1.00	1.00	42	3	2	0	1	41	70
102	1.00	13.00	1.00	.00	62	3	2	0	1		77
103	1.00	10.00	.00	.00	59	3	3	0	1	38	78
104	2.00	5.00	1.00	.00	76	1	2	1	1	42	114
105	2.00	10.00	1.00	1.00	65	2	4	1	1	42	114

106	1.00	7.00	1.00	1.00	70	2	3	1	1	39	116
107	1.00	13.00	1.00	1.00	63	3	3	0	1	38	116
108	2.00	5.50	1.00	.00	61	3	2	0	1	44	117
109	1.00	15.00	1.00	1.00	57	2	2	0	1	46	139
110	2.00	12.00	1.00	1.00	82	3	4	0	1	39	159
111	1.00	8.50	1.00	.00	50	3	4	0	1	43	234

9.3 Chapter 4 data

Pt No	M1F2	AGE	NEPHRECT	STAGE	GRADE	CRP	EIL10	IFNG	IL2	IL4	IL5	LIL10
1	1	57	1	1	2	6	3.80	167.20	11.46	9.18	3.00	5.00
2	2	54	1	3	2	11	7.82	115.22	7.93	11.99	3.00	5.00
3	1	58	1	1	3	6	3.80	226.82	17.57	11.99	3.00	5.00
4	1	47	1	3	4	6	3.80	126.94	6.79	14.12	3.00	5.00
5	2	70	1	1	1	6	4.25	209.97	15.09	24.92	5.82	5.00
6	2	60	1	1	4	68	8.37	232.01	18.82	13.41	9.60	5.00
7	1	48	1	1		14	6.02	197.01	30.41	17.69	14.87	5.00
8	2	68	1	3	1	9	6.92	221.64	11.46	10.58	15.36	5.00
9	2	74	1	3	2	12	3.80	321.68	21.34	22.74	17.28	5.00
10	1	43	1	3	2	6	3.80	294.33	30.41	31.51	29.18	5.00
11	2	64	1	1	3	6	9.66	451.54	54.89	100.55	73.55	5.00
12	2	55	1	4	0	53	5.66	243.68	21.34	22.74	22.74	5.37
13	1	77	1	2	4	20	3.90	515.20	27.79	53.10	49.76	5.94
14	1	77	1	3	3	7	3.80	330.80	49.33	31.51	26.22	7.09
15	1	63	1	1	3	20	3.80	322.98	29.10	30.04	20.06	8.24
16	1	61	1	1	2	6	3.80	372.63	64.75	65.20	43.43	13.47
17	1	62	1	1	3	6	6.02	455.50	77.66	49.34	42.63	16.98
18	2	58	1	1	0	9	3.80	528.53	54.89	88.17	67.33	18.75
19	1	51	1	1	3	14	3.80	584.77	98.15	96.67	57.99	21.12
20	1	61	1	3	4	6	3.90	493.92	42.47	38.16	37.01	23.50
21	1	58	1	1		6	4.77	728.71	53.50	71.29	61.50	23.50
22	1	71	1	3	1	33	12.83	547.23	114.57	107.55	104.04	23.50
23	1	56	1	1	3	10	3.80	509.88	101.12	45.60	47.79	25.89
24	1	66	1	1	0	6	3.80	496.58	56.29	40.38	37.01	28.89
25	2	64	1	1	3	6	3.80	742.45	191.88	178.02	110.76	34.33
26	1	37	1	1	2	6	3.80	308.65	108.57	62.16	39.03	34.93
27	1	52	1	3	3	6	3.80	709.52	207.77	138.17	98.92	39.81
28	2	73	1	1	2	8	6.02	691.76	195.05	113.80	91.87	42.87
29	2	69	1	1	2	15	4.25	849.19	243.17	215.22	157.61	44.72
30	2	75	1	1	2	21	9.29	724.59	269.24	154.83	123.89	48.42
31	1	69	1	2	3	12	4.42	684.94	215.77	179.63	116.31	54.64
32	2	65	1	1	4	6	3.80	786.60	249.66	174.81	144.90	60.90
33	2	34	1	3	0	269	14.92	770.01	272.52	233.18	154.31	61.53

34	2	54	1	3	1	8	4.42	1132.88	463.22	355.79	248.53	76.76
35	1	71	1	2	2	6	3.80	280.03	166.72	75.88	52.51	78.04
36	1	43	1	3	3	49	7.64	1565.81	536.40	317.79	242.70	79.32
37	1	36	1	3	3	21	7.46	712.26	287.32	280.19	140.01	85.11
38	1	74	1	3	3	35	8.37	768.63	270.88	194.94	138.80	87.05
39	2	61	1	4	4	84	23.62	991.94	313.81	174.81	140.83	110.61
40	1	54	1	1	1	10	3.80	1266.61	655.20	248.77	217.92	154.43
41	1	74	1	3	1	34	6.92	1497.97	401.41	415.70	348.31	195.57
42	1	57	1	1	3	18	10.77	1706.17	1087.87	544.60	362.66	233.58
43	1	42	1	3	2	70	11.71	1205.67	750.77	377.93	270.82	237.22
44	1	51	1	1		6	3.80	15.46	6.00	5.00	3.00	1390.16
45	2	54	1	3	2	8	5.30	14513.75	6.00	5.00	3.00	2953.69
46	2	59	2	4	3	80	16.83	908.17	274.16	190.90	171.74	57.76
47	1	50	2	4	2	7	3.80	1089.90	251.29	233.99	165.06	62.79
48	2	54	2	4	3	212	18.95	1378.16	490.97	269.41	248.08	64.68
49	1	54	2	4	4	86	14.35	1168.80	352.29	213.59	185.21	100.07
50	1	53	2	4	4	231	21.08	1341.76	559.26	285.18	237.34	123.92
51	1	59	2	4	3	31	9.44	1497.97	571.61	313.59	273.58	146.22
52	1	57	2	4	3	122	5.48	1803.18	1106.74	550.85	381.71	220.56
53	1	58	2	4	4	145	19.33	1544.67	1037.07	503.82	368.14	255.52

9.4 Chapter 5 data

Pt No	AGE	Gender	NEPHCODE	IFN0/Tri1	ECOG_PS	PREHB	PREWCC	PRELYPER	ALBUMIN	CRP	LDH
1	54	Female	1.00	.00	1	11.90	4.65	27.53	36	12	243
2	67	Female	.00	.00	1	8.90	10.49	17.16	35	152	162
3	71	Male	.00	.00	1	11.50	7.66	13.19	31	170	212
4	62	Male	1.00	.00	0	16.60	7.05	15.04	36	42	145
5	68	Male	1.00	1.00	0	13.10	10.92	23.26	45	6	168
6	57	Male	1.00	.00	0	9.10	7.18	10.31	35	295	179
7	61	Female	1.00	.00	0	13.80	7.55	17.75	44	6	204
8	72	Male	.00	.00	1	14.00	5.50	18.73	42	6	192
9	55	Male	2.00	1.00	0	14.10	5.78	29.24	43	7	247
10	76	Male	1.00	.00	0	15.80	6.73	22.88	39	13	210
11	67	Female	2.00	.00	1	12.20	13.62	4.41	42	6	297
12	50	Male	1.00	.00	0	11.30	3.44	23.26	28	6	262
13	57	Male	1.00	.00	1	10.90	5.71	24.87	39	177	172
14	36	Male	.00	.00	1	15.10	9.17	13.09	46	37	186
15	70	Male	1.00	.00	0	10.20	6.17	27.39	37	14	206
16	58	Male	.00	.00	1	9.90	38.47	3.61	28	141	181
17	72	Female	1.00	.00	0	13.40	5.99	18.70	32	12	254
18	62	Male	1.00	.00	1	14.00	8.23	20.41	28	18	155
19	63	Male	2.00	.00	1	10.80	8.05	13.79	31	46	139
20	71	Male	.00	.00	1	13.20	8.13	29.64	35	6	243
21	54	Male	.00	1.00	0	12.10	9.27	10.03	31	29	742
22	77	Male	1.00	.00	0	14.10	6.44	33.39	42	6	201
23	48	Male	1.00	1.00	0	13.70	7.35	29.93	40	6	181

Pt No	PREIL6	PREIL10	CD4PC	CD8PC	POSTPS	POST CRP	POSTALB	POSTHB	POSTWCC	PSTLYMPE	POSTIL6	POSTIL10
1	28.94	16.88			2	6	31	10.40	3.74	10.96	55.17	32.20
2	132.94	22.67	43	40	2	110	32	9.70	4.53	27.81		
3	65.38	23.45	37	19	2	303	29	10.50	7.35	6.80		
4	20.80	14.50	38	12	0	33	38	16.10	5.23	21.99	21.97	16.50
5	12.29	11.51	43	17	1	70	40	13.20	17.70	15.65		
6	110.35	31.70	61	26	2	250	34	8.40	7.53	10.62	110.64	79.43
7	13.07	11.56	45	19	1	6	43	13.50	5.22	28.93	11.91	21.75
8	11.79	10.95	31	39	1	6	41	13.30	3.99	15.29	15.13	17.57
9	13.06	16.68	29	35	2	137	43	11.60	7.99	28.41	81.85	80.91
10	14.57	27.21	44	23	1	26	39	15.40	4.93	37.12	20.65	26.09
11	12.43	17.22	53	32	2	6	36	11.10	9.10	21.32	10.99	20.31
12	14.70	21.28	49	25	0	6	23	10.00	3.56	20.22		
13	40.59	21.18	41	32	1	76	40	8.30	5.43	61.51	19.03	240.14
14	19.19	238.31	58	31	1	6	40	12.80	2.51	35.86	13.55	166.19
15	13.31	205.88	30	31	1	12	37	10.20	3.68	27.17		
16	65.68	157.28	62	27								
17	17.69	144.52	55	15	1	18	33	13.10	3.94	27.16	19.25	194.53
18	51.04	272.44	59	14								
19	49.88	184.33	39	39	1	13	38				40.56	153.40
20	14.78	91.19	14	50	1	6	37	13.20	9.65	14.92	12.48	176.19
21	18.08	104.28	38	25	2	22	28	12.60	5.60	31.25	14.48	139.00
22	16.34	65.18	35	33	1	6	43	12.40	3.33	45.65	17.07	48.46
23	12.39	54.62	33	41	1	11	36	12.60	8.62	38.28		

Pt No	CD4_PC1	CD8PC1	LDH2	COMPLETE	Alive0/Cancer Death1	SURVIVAL
1	42.00	38.00	253.00	.00	1.00	5
2				.00	1.00	2
3				.00	1.00	3
4	45.00	14.00	161.00	1.00	.00	12
5			298.00	1.00	.00	12
6	55.00	28.00	78.00	.00	.00	12
7	49.00	23.00	223.00	1.00	.00	12
8	41.00	36.00	187.00	.00	1.00	6
9	31.00	30.00	220.00	1.00	.00	12
10	52.00	21.00	188.00	1.00	.00	9
11				.00	.00	6
12				.00	.00	8
13	23.00	17.00	147.00	1.00	.00	8
14	65.00	32.00	186.00	1.00	.00	8
15	45.00	31.00		.00	1.00	1
16				.00	1.00	1
17	64.00	17.00	271.00	1.00	.00	6
18				.00	1.00	1
19	33.00	42.00	139.00	.00	1.00	4
20			271.00	1.00	.00	6
21	48.00	19.00	863.00	.00	1.00	2
22	43.00	32.00	242.00	1.00	.00	5
23			225.00	1.00	.00	4

9.5 Chapter 6 data

Pt No	M1F2	Age	ECOG-ps	UISS	NEPHRE	STAGE	GRADE	TUMVOL	CRP	ALBUMIN	WCC	LYMPH%	HB	PRE IL6	PRE IL10
1	1.00	74	0	1	1	3	3	133.95	35	44	6.58	21.88	11.60	5.02	8.37
2	1.00	30	0		0	0	0		9		4.60	33.48	15.60	2.00	4.00
3	2.00	58	0	0	1	1	1		9	50	9.68	23.35	14.10	2.00	4.00
4	1.00	54	0	0	1	1	1	360.00	10	44	8.69	23.71	16.90	2.00	4.00
5	1.00	68	0	1	1	2	3	225.00	12	42	6.43	27.37	12.80	2.00	4.42
6	2.00	49	0	0	1	1	1	9.38	15	45	9.97	21.87	12.80	2.00	4.00
7	2.00	54	0	1	1	3	1	1331.00	8	44	5.46	34.62	12.40	4.90	4.42
8	1.00	58	1	2	2	4	4		145	36	6.79	13.84	9.70	82.09	19.33
9	1.00	74	0	1	1	3	1	243.75	34	47	8.46	19.86	13.10	14.60	6.92
10	2.00	34	1		1	3		1430.00	269	31	11.82	11.76	11.40	99.01	14.92
11	2.00	28	0		0	0	0		6	49	6.44	20.65	13.30	2.00	4.77
12	2.00	78	0	0	1	1	2	11.50	8	44	5.72	27.97	14.20	2.00	4.00
13	2.00	54	0	1	1	3	2	1000.00	8	42	8.50	26.24	13.10	9.08	5.30
14	1.00	37	0	0	1	1	2	4.00	6	46	6.31	24.56	15.00	2.00	4.00
15	2.00	60	0	0	1	1	4	111.60	68	39	4.49	20.27	9.80	34.68	8.37
16	2.00	61	1	2	1	4	4	4864.00	84	34	17.32	9.58	17.32	29.18	23.62
17	2.00	55	0	2	1	4			53	43	5.85	20.68	12.00	2.00	5.66
18	1.00	47	0	1	1	3	4	72.00	6	46	7.64	20.29	16.30	3.64	4.00
19	2.00	48	0		0	0	0		89	39	9.59	12.10	10.60	18.61	4.60
20	2.00	68	0	1	1	3	1	125.00	9	45	7.80	19.49	13.40	9.20	6.92
21	2.00	49	0		0	0	0		6	47	7.33	40.25	13.50	2.00	4.00
22	1.00	72	0	1	1	3	1	357.50	33	45	7.72	19.95	11.50	77.70	12.83
23	1.00	50	0	2	2	4	2		7		5.40	21.67	13.70	2.88	4.00
24	1.00	37	0	1	1	3	3	960.00	21	46	7.19	25.17	14.50	7.86	7.46
25	1.00	62	0	0	1	1	2	231.00	6	45	8.28	26.69	15.50	2.00	4.00
26	1.00	54	1	2	2	4	4		86	38	5.13	24.95	9.60	40.82	14.35
27	1.00	42	0	1	1	3	2	866.25	70	41	6.21	30.76	12.90	16.96	11.71
28	2.00	70	0	0	1	1	2	68.80	15	42	5.50	29.45	13.90	2.00	4.25
29	2.00	76	0	0	1	1	2	72.00	21	43	7.09	21.44	12.90	5.15	9.29
30	2.00	70	0	0	1	1	1	135.00	6	43	6.48	30.25	13.00	2.00	4.25
31	2.00	67	0		0	0	0		9	39	6.44	25.16	13.30	2.00	4.00
32	2.00	65	0	0	1	1	4	115.01	6	46	5.04	25.40	12.10	2.00	4.00
33	1.00	59	0	2	2	4	3		31	45	8.32	21.27	12.20	2.00	9.44

34	1.00	57	0	2	2	4	3		122	43	6.02	18.60	15.60	31.48	5.48
35	1.00	63	0	0	1	1	3		20	41	5.85	19.32	14.30	2.88	4.00
36	1.00	56	0	0	1	1	3	12.00	18	42	8.36	25.00	15.10	3.77	10.77
37	1.00	66	0		1	1		1.20	6	48	4.84	28.51	16.10	2.00	4.00
38	1.00	48	0		1	1		48.00	14		5.94	20.03	13.10	2.00	6.02
39	2.00	54	1	2	2	4	3		212	35	11.57	6.48	11.20	98.36	18.95
40	2.00	52	0		0	0	0		6	45	5.56	53.06	13.50	2.00	4.00
41	1.00	52	0	1	1	3	3	960.00	6	42	9.68	18.70	13.60	2.00	4.00
42	2.00	59	1	2	2	4	3		80	39	6.50	14.62	10.40	32.16	16.83
43	2.00	73	0	0	1	1	2	42.88	8	44	6.24	21.79	9.40	2.00	6.02
44	1.00	71	0	1	1	2	2	178.75	6	40	6.77	21.12	16.50	2.00	4.00
45	1.00	51	0		1	1		54.00	6	44	4.52	12.83	12.30	2.00	4.00
46	1.00	53	0	2	2	4	4		231	40	8.50	18.35	9.70	142.23	21.08
47	1.00	58	0		1	1		52.50	6	45	5.84	38.53	15.90	2.00	4.77
48	2.00	56	0		0	0	0		26	44	8.68	28.69	10.60	2.00	4.00
49	1.00	43	0		0	0	0		16	48	9.82	14.15	10.90	2.00	4.00
50	2.00	67	0		0	0	0		25	45	7.86	30.15	14.00	2.00	4.00
51	1.00	59	0	0	1	1	3	42.88	6	49	6.42	30.69	12.80	2.00	4.00
52	2.00	64	0	0	1	1	3	6.75	6	42	5.11	32.88	14.50	2.00	9.66
53	1.00	43	0	1	1	3	2	15.00	6	48	8.47	27.63	15.30	2.00	4.00
54	2.00	64	0	0	1	1	3	15.00	6	46	5.96	25.17	11.90	2.00	4.00
55	1.00	51	0	0	1	1	3	54.00	14	45	7.79	32.22	15.80	2.00	4.00
56	1.00	56	0	0	1	1	3	27.00	10	39	6.97	19.66	13.80	2.00	4.00
57	2.00	74	1	2	1	3	2	30.00	12	48	6.12	21.57	13.30	2.00	4.00
58	2.00	54	0	1	1	3	2	288.00	11	45	7.13	21.04	10.80	4.02	7.82
59	1.00	62	0	0	1	1	3	64.00	6	45	10.08	9.33	15.40	2.36	6.02
60	1.00	43	0	1	1	3	3	316.88	49	41	11.47	16.13	11.30	29.98	7.64
61	1.00	61	0	1	1	3	4	336.00	6	47	8.64	23.96	14.70	7.49	4.00
62	1.00	63	0		0	0	0		6	42	8.88	27.48	13.60	2.00	4.00
63	1.00	77	0	1	1	3	3	343.00	7	38	5.63	11.72	10.20	6.26	4.00
64	1.00	78	0	1	1	2	4	341.25	20	42	7.95	21.26	12.10	21.30	4.00
65	1.00	57	1	1	1	1	2	15.63	6	39	6.32	19.46	15.10	2.00	4.00
66	2.00	44	0		0	0	0		8	45	10.24	21.78	14.40		4.25
67	1.00	60	1	2	1	3	4	112.00	38	44	11.11	16.29	15.50	27.19	24.51
68	1.00	53	0		0	0	0		6	47	7.33	20.05	14.20	2.00	7.21
69	2.00	65	0	1	1	2	3		6	47	10.07	31.78	14.70	12.06	49.83

70	1.00	55	0	2	2	4	2	6	43	6.74	21.81	13.90	14.05	57.95
71	1.00	67	0	0	1	1	2	6	42	4.91	20.37	14.80	11.42	65.52
72	1.00	47	0	0	1	1	4	6	46	6.48	27.62	14.70	11.32	61.41
73	1.00	71	1	2	1	3	3	6	46	7.87	21.35	13.90	20.31	46.75
74	1.00	54	0	1	1	3	4	6	46	6.22	15.92	12.50	13.47	61.24
75	1.00	64	0	1	1	3	4	7	45	9.48	21.20	14.00	12.80	61.13
76	1.00	63	1	2	2	4	3	65	31	11.49	9.31	13.40	40.64	35.37