AN INVESTIGATION OF THE ROLE OF THE SYSTEMIC AND LOCAL INFLAMMATORY RESPONSE IN PATIENTS UNDERGOING RESECTION FOR RENAL CELL CARCINOMA

By

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From research conducted in

The University Department of Surgery Glasgow Royal Infirmary University NHS Trust and The Department of Urology Gartnavel General Hospital North Glasgow NHS Trust.

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IV ACKNOWLEDGEMENTS

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V DECLARATION

I declare that the work presented in this thesis has been solely carried out by myself except where indicated below

Measurements of C-reactive protein were performed in the Institute of Biochemistry Glasgow Royal Infirmary and the Department of Biochemistry Western Infirmary North Glasgow University NHS Trust.

Assistance with immunostaining for CD4 and CD8 was carried out by the University Department of Pathology, Glasgow Royal Infirmary.

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

GWA Lamb, DC McMillan, S Ramsay, M Aitchison (2006). The relationship between the preoperative systemic inflammatory response and cancer-specific survival in patients undergoing potentially curative resection for renal clear cell cancer. *Br J Cancer*

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This work has also been presented at the Urological Research Society Meeting London (2004), West of Scotland Urology Meeting Ayr 2005, and is due to be presented at the British Association of Urological Surgeons Annual Conference Manchester 2006.

VI DEDICATION

I wish to dedicate this work to my wonderful partner Kirsty and my three fantastic children Christiaan, Morgan and Poppy.

VII SUMMARY

Although renal cell carcinoma accounts for only 2% of all cancers it is responsible for 12% of all cancer deaths making it a particularly lethal malignancy. Over the last 50 years there has been an increased incidence in all stages of disease with the greatest rise observed in localised lesions found incidentally due to increased used of radiological imaging.

The mainstay of treatment for the 60% of patients presenting with only localised disease is surgery however, despite aggressive surgical management 30% of patients with no evidence of metastases at the time of surgery will subsequently develop metastases. The median survival of patients either presenting or developing metastatic disease is 8.5 months even in those who are suitable for immunotherapy.

Currently staging and consequently the prognosis of patients with renal cancer is based on macroscopic tumour characteristics and the presence or absence of lymph node and distant metastases as defined by the American Joint Committee on Cancer TNM staging system. Stage alone has been shown to be inadequate in predicting outcome and combination with grade and histological subtype has been shown to improve prognostication in patients with renal cancer.

Additionally patient factors are thought to contribute to patients overall cancer specific survival including the presence of cachexia and poor performance status. The reasons for these factors influencing survival are not clear but their potential relationship with the systemic inflammatory response, as evidenced by raised circulating concentrations of C-reactive protein is thought to be significant.

Previous work has shown that elevated C-reactive protein signifying a systemic inflammatory response is of independent prognostic value in patients undergoing potentially curative resection for a variety of common solid tumours.

In chapter 2 we examined patients undergoing potentially curative nephrectomy for renal cancer, the presence of a systemic inflammatory response has been tested for its prognostic significance against the UISS (UCLA Integrated Staging System) an internationally validated prognostic algorithm incorporating stage, grade and performance status.

In this study of 100 patients a raised C-reactive protein was found to be an independent prognostic factor for cancer specific survival on both univariate and multivariate analysis. Furthermore inclusion into the UISS allowed better stratification of patients defined by this algorithm as low or intermediate risk of progression.

Previous studies have suggested that the local inflammatory response as defined by the presence of a pronounced lymphocytic infiltration, in particular tumour CD4+ and CD8+ T-lymphocytes, are associated with poorer cancer specific survival in renal cancer. How this local response relates to the systemic response in renal cancer has not been previously examined.

It was of interest how these effectors of immune function related to the mediators of inflammation within the tumour micro-environment. Cyclo-oxygenase-2 is the enzyme responsible for the production of prostaglandins the prime mediators of inflammation and where over-expressed has been implicated with poor outcome in a number of common solid tumours. Cyclo-oxygenase-2 has been reported to stimulate production of interleukin-6 a potent pro-inflammatory cytokine which acting through its receptor has been linked with tumour genesis and progression in renal cancer. Few studies have examined the expression of cyclo-oxygenase-2 in renal tumours.

In chapter 3 patients undergoing nephrectomy for renal cancer were examined for CD4+ and CD8+ tumour lymphocytic infiltrate, interleukin-6 receptor expression, cyclo-oxygenase-2 expression as well as concentrations of circulating C-reactive protein

with relation to cancer specific survival. Both elevated C-reactive protein and increased numbers of CD4+ lymphocytic infiltrate were associated with poorer cancer specific survival. A positive relationship was also demonstrated between T-lymphocytic infiltrate and C-reactive protein and were both significantly associated with increased grade of tumour suggesting an association with more aggressive tumour biology. Cyclo-oxygenase-2 and interleukin-6 receptor expression were found to be independent of tumour stage, grade, lymphocytic infiltrate and not related to cancer specific survival.

In conclusion the work presented in this thesis suggests that both the presence of a systemic or a local inflammatory response are negative prognostic factors in patients undergoing nephrectomy for renal cancer. Systemic C-reactive protein and tumour lymphocytic infiltrate are significantly associated with each other. Tumour expression of cyclo-oxygenase-2 and interleukin-6 receptor are independent of circulating C-reactive protein and local lymphocytic infiltrate and are therefore the basis for the link between the local and systemic inflammation observed in renal cancer is unclear and warrants further study.

Chapter 1

1.0 INTRODUCTION

1.1 Incidence and Mortality of Renal Cancer

Malignant tumours of the kidney account for around 2% of all new cancer cases and deaths in North America (Landis *et al.*, 1998). Internationally the overall incidence of RCC has been seen to be increasing at a greater rate relative to other cancers (Reis *et al.*, 1998). Renal cancer has demonstrated increased incidence in all age groups over the past 50 years, with the greatest rise observed in localised tumours (Chow *et al.*, 1999). All stages including localised, regional and distant have shown an increase as well as an increase in renal carcinoma mortality rates (Hock *et al.*, 2002). The reason for this increased incidence is unclear. For example in North America the increase in incidence of renal cancer has been noted to be more rapid in the black population relative to whites. Black men have shown an increase rate of 3.9% and black women by 4.3% per year, compared with 2.3% for white men and 3.1% for white women (Chow *et al.*, 1999). As a result the black population now have a higher incidence rate than the white population (Kosary *et al.*, 1993).

In Scotland cancer of the kidney accounts for 2.7% of all cancers in males (324 new cases/ year) and 1.8% of all cancers in females (217 new cases/ year) (Scottish Health Statistics, 2000).

Renal cell carcinoma is predominantly a disease of the elderly with a median age of diagnosis of 65 (Vogelzang *et al.*, 1998). Men are approximately twice as likely to develop RCC as women, with in Scotland, a median age of 67 in men and 69 in women. The Scottish figures show a male to female ratio of 3:2.

At the time of presentation approximately one third of patients will have metastatic disease. Of patients who undergo radical surgery with curative intent 30-

50% will ultimately develop distant metastases (Flannigan and Yonover, 1996). Therefore over the course of their disease, approximately 60% of all patients with renal cancer will progress to metastatic disease, which carries a very poor prognosis with median survival of approximately 8 months (DeKiernon *et al.*, 1978).

The pattern of presentation has altered over the past two decades with the amount of lesions diagnosed incidentally increasing due to the rise in use of ultrasound scanning, computerised tomography (CT) and magnetic resonance imaging (MRI). However this increased detection of asymptomatic lesions due to higher levels of imaging does not fully explain the rise in incidence of renal cancer.

In their study of 9 cancer registries Hock and co-workers (2002) concluded that there was no difference in stage at presentation of renal carcinoma diagnosed 1973-85 compared to 1986-98. Increased incidence in all 3 stages; localised, regional and distant were noted over this time period. Significant reduction in distant disease has not been observed despite the increased detection of localised and regional tumours through incidental findings on imaging. Indeed a more contemporary study has confirmed 39% patients continue to present with metastatic disease (Leslie et al. 2003)

This would suggest that many such incidentally diagnosed masses are slow growing, and of uncertain malignant behaviour. This is consistent with the fact that the rise of incidentally diagnosed renal carcinoma treated by nephrectomy has not significantly altered the overall mortality rate for RCC (Chow *et al.*, 1999; Rendon *et al.*, 2000). Leslie and co-workers observed a significant difference in 5 year disease specific survival between incidental lesions and symptomatic lesions at diagnosis of 94 and 35% respectively treated by nephrectomy. This survival difference may represent tumours detected before they would ultimately become symptomatic and follow a more aggressive course or alternatively are inherently less likely to progress.

In summary, over the past two decades the method of presentation of the majority or renal cancers has seen a shift toward incidentally detected lesion, without any evidence to suggest an overall improvement in survival. This would indicate that a number of tumours continue to behave in an inherently aggressive manner, whilst others follow a more indolent course. Identifying those tumours which will behave in a more aggressive manner remains of utmost priority if we are to improve outcome.

1.2 Aetiology of Renal Cell Cancer

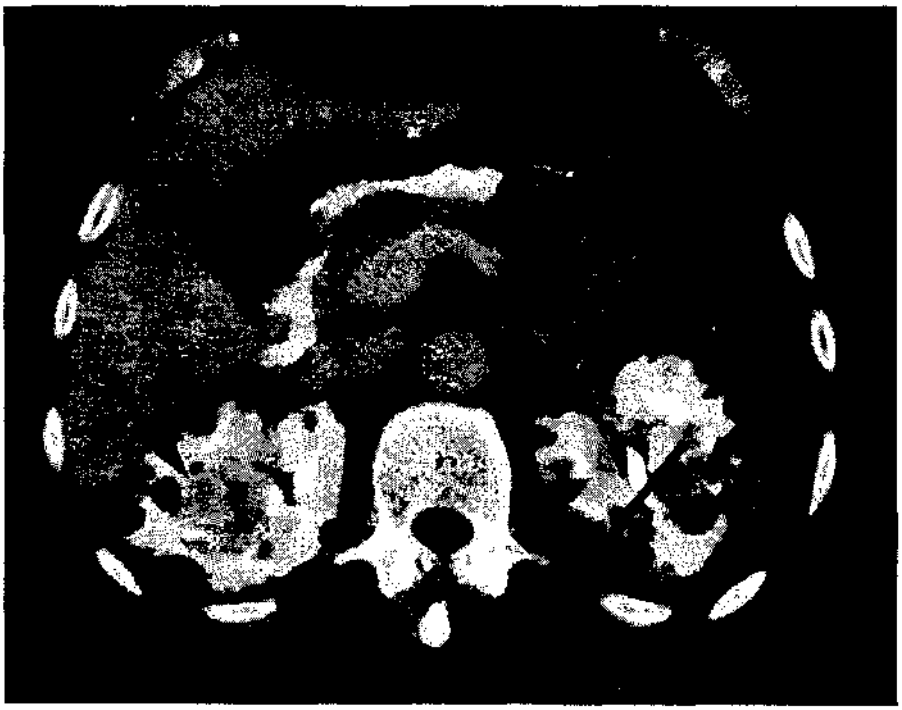
The precise aetiology of renal cancer is unknown, however sporadic RCC accounts for approximately 90% of all cases. A number of risk factors for the development of renal cancer have been identified.

1.2.1 Genetics of Renal Cancer

The most well characterised familial syndrome associated with renal cancer is the von Hippel-Lindau syndrome (VHL). This syndrome is associated with highly vascular tumours of multiple organs including the central nervous system, kidney, adrenal and pancreas (Lamiell *et al.*, 1989). VHL syndrome inherited in an autosomal dominant manner, occurs in 1: 40,000 live births and is associated with a 70% probability of developing RCC by the seventh decade (Maher and Kaelin, 2001). The syndrome was first described by Cushing and Bailey (Cushing and Bailey 1926), with the VHL defect ultimately identified and isolated to the VHL tumour suppressor gene 3p 25-26 in 1993 (Latif *et al.*, 1993).

In these patients renal cancer presents earlier in life than in sporadic tumours with a mean age of 35 years. The histology is of clear cell carcinoma and is present as multiple microscopic tumour foci which can grow and develop. For example a 37 year old patient with VHL may have more than 600 independent solid tumours and 1100 cysts (Walther *et al.*, 1995) Figure1.1.

Figure 1.1 A computerised tomography image of patient with Von-Hippel Lindau disease demonstrating bilateral multiple renal cysts with three solid tumours (arrowed) (Tao L., www.cortlandtforum.com).



For tumours to develop both alleles for the VHL suppressor gene must be lost. Patients with VHL are born with a germline mutation at one VHL locus. As only a single further mutation is required of the opposite allele the chances of tumour formation in these patients is substantially increased (Maranchie and Linehan, 2003). Such a mutation may arise by loss of heterozygosity, mutation of the second allele itself or through silencing of the VHL promoter by DNA hypermethylation (Prowse *et al.*, 1997).

There is also a high frequency of VHL alteration identified in sporadic RCC; 50-65% of sporadic clear cell carcinomas are found to have VHL mutations of which 85% are through loss of heterozygosity at the VHL locus (Gnarra *et al.*, 1996). Such mutations do not arise in the other histological types of RCC, namely papillary or chromophobe tumours.

Genetic factors observed in other histological variant familial cases are not commonly observed in respective sporadic cases. Hereditary papillary renal carcinoma demonstrates MET gene mutations on chromosome 7 which are not found in the majority of sporadic papillary tumours suggesting an alternate pathway in the development of these more common lesions (Linehan *et al.*, 2003). Familial chromophobe renal cancer is associated with Birt-Hogg-Dube. The Birt-Hogg-Dube gene normally functions as a tumour suppressor gene (Nickerson *et al.*, 2002). It is yet to be determined whether such mutations occur in sporadic forms of chromophobe renal cell carcinoma.

1.2.2 Hypoxia Inducible Factor role in sporadic RCC

VHL is thought to act predominantly to regulate cell cycle arrest and the transition to quiescence under conditions in which cells transit to G0 (Pause *et al.*, 1997). It does this by changing the stability of p27. It has also been noted that loss of VHL function correlates with an increase in expression of vascular endothelial growth factor which is over expressed in well vascularised tumours such as renal cell cancer (Siemeister *et al.*, 1996).

Glucose transporter-1 and transforming growth factor α along with vascular endothelial growth factor are hypoxia induced genes (Gnarra *et al.*, 1996; Iliopoulos *et al.*, 1996). The expression of these genes is controlled by hypoxia-inducible

transcription factors. These factors are activated in response to reduced cellular oxygen tension and mediate their effects through hypoxia response elements to increase transcription of hypoxia induced genes. Hypoxia inducible factors are heterodimers comprised of a hypoxia inducible factor α subunit and the aryl hydrocarbon nuclear translator (Semenza *et al.*, 2000). Hypoxia inducible factor α subunits are continually synthesised but rapidly ubiquitinylated and broken down by proteosomal degradation in normoxic conditions. VHL protein functions as the substrate recognition subunit of a multiprotein E3 ubiquitin ligase (Patton *et al.*, 1998). Where VHL is mutated or absent hypoxia inducible factor α subunits are not able to be ubiquitinylated resulting in increased expression of hypoxia inducible genes which are thought to be responsible both for the development and propagation of renal tumours.

1.2.3 Risk factors for renal cancer

Hypertension

There is an extensive literature on the association between hypertension and renal cancer. However, difficulty arises in separating hypertension from other confounding variables. Two studies adjusted for age, tobacco smoking and Body Mass Index but not antihypertensives have demonstrated an association between hypertension and renal cancer (Mellemgaard *et al.*, 1994; Schlehofer *et al.*, 1996). In one of the largest studies, carried out in Sweden, a direct and significant relationship was observed between elevated systolic and diastolic pressure (Chow *et al.*, 2000). Diastolic pressure of 90 mm Hg or more had twice the incidence of renal cancer than those men with diastolic less than 70 mm Hg. A systolic blood pressure greater than 150 mm Hg was associated with a 60 – 70% higher risk than those with a pressure below 120 mm Hg.

There was also a decrease in renal cancer risk with a decrease in blood pressure over time.

The proposed mechanism for hypertension increasing the risk of renal cell carcinoma is related to the changes observed in renal parenchyma secondary to development of hypertension. Hypertensive nephropathy may result in the kidney becoming more sensitive to carcinogens. Alternatively the increase in angiogenic and other growth factors seen with hypertension may be responsible for this association with the development of renal cancer (Schena *et al.*, 1999).

Obesity

Obesity has been consistently shown to be associated with renal cell carcinoma, particularly among women (McLaughlin *et al.*, 1984; Mellemgaard *et al.*, 1995). The association is observed only in the severely obese who have a Body Mass Index above the fourth quartile. Mellemgaard and co-workers (1995) reported that weight cycling or large swings in weight appeared to play a significant role in the development of renal cancer. However, the age at which obesity develops does not influence this association. The mechanism for the effect of obesity on renal cancer is unclear. Although studies are often not adjusted for hypertension, which in many instances accompanies obesity, there is broad agreement among a large number of studies that classify obesity as a risk factor for renal cancer. Scotland has rising levels of obesity.

Tobacco

A dose-response association has been demonstrated in relation to cigarette consumption and development of renal cell carcinoma (Chow *et al.*, 2000). Heavy smoking increases the risk of renal cancer by 2 – 2.5 times that of non smokers (Yuan *et*

al., 1998). Smoking in men appears to result in a higher incidence of renal cancer (20-30%) relative to the increase in smoking women (10-20%) (Yuan *et al.*, 1998).

In summary while genetic defects are common, particular at the VHL locus in approximately 75% of sporadic tumours, the causes of these genetic mutations which precipitate this chain of events are largely unknown but a number of risk factors have been described. It should be noted that the principle risk factors for renal cancer are also those for cardiovascular disease. In addressing these risk factors consideration must be taken into the relative rare incidence of renal cell carcinoma. These risk factors should be tackled on the basis of their cardio protective basis rather than any relative weak association with renal cancer. The presence of these risk factors may in time allow a rationale for targeted screening in the future along with abdominal aortic aneurysm, a condition with similar associations.

1.3 **Pathology of Renal Cancer**

1.3.1 Morphology

Renal tumours may arise from any portion of the kidney, but more commonly arises from one of the poles (Robbins et al., 1989). Lesions are usually solitary and unilateral and are spherical in shape. The cut surface is often haemorrhagic and may appear bright yellow due to the high lipid content of these tumours. Areas of ischaemic, opaque, gray/ white necrosis are commonly observed.

Renal tumours are expansile in nature often compressing adjacent renal parenchyma into a pseudocapsule (McGee *et al.*, 1992). As they enlarge they may protrude into the calyces, eventually fungating through the wall of the collecting system and even into the ureter. Often where the collecting system is involved patients will experience frank haematuria.

1.3.2 Histogenesis

Renal cell carcinoma is thought to arise from renal tubules. The presence of a readily demonstrable brush border found on ultrastructural analysis suggests the origin to be from the proximal tubular epithelium (McGee *et al.*, 1992). The cellular origins of renal cancer have been further studied using immuno-histochemistry techniques utilising monoclonal antibodies specific to antigens of different tubular cells. Most renal cell carcinomas express antigens from the proximal tubular epithelium, but many express antigens of the distal tubule and both antigens may coexist in the same tumour. This may be explained by aberrant differentiation of renal tumour cells along with the possible origins of tumours from various parts of the nephron.

1.3.3 Histology

The classification of renal cell neoplasms derived from renal epithelial cells follows a standardised nomenclature summarised below Table 1.1 (Storkel *et al.*, 1997).

Table 1.1 Classification of renal tumours.

Classification of renal tumours		Proportion
Malignant	Clear cell/ conventional	70-80%
	Papillary	7-15%
	Chromophobe	~5%
	Collecting Duct	~1%
	Unclassified	~1%
Benign	Adenoma	<1%
	Oncocytoma	~5%
	Metanephric Adenoma	<1%

Clear Cell/ Conventional Renal Cell Carcinoma

Clear cell carcinoma represents the commonest cell type found in neoplasms of the kidney. These account for 70-80% of renal cancers. The name clear cell is derived from the histological appearance. These cells have an abundant apparently empty cytoplasm due to the high lipid content, which is removed during processing for embedding in paraffin wax. The cytoplasm contains vacuoles which are usually peripheral with the rest of the cytoplasm centrally placed. They also contain glycogen Figure 1.2. Some cells are found to have a more granular cytoplasm, hence the move to rename these as conventional rather than clear cell type.

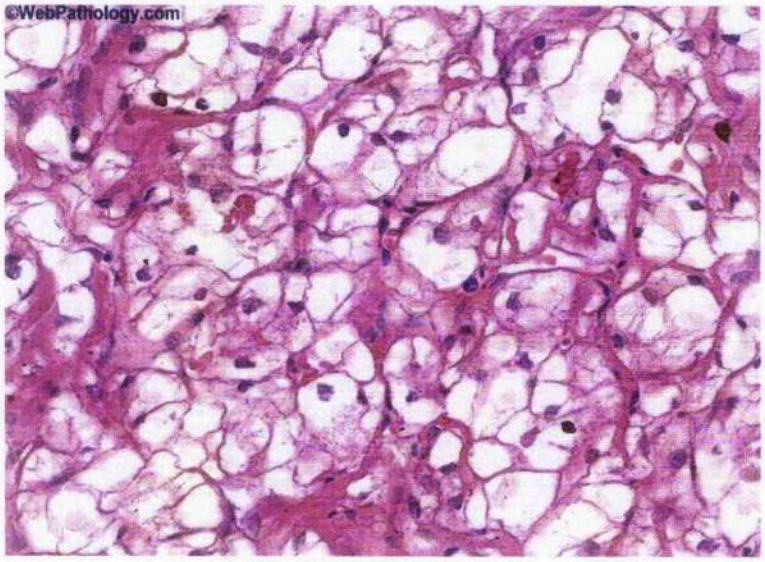


Figure 1.2 A H&E histological slide of a clear cell carcinoma of kidney (webpathology.com).

Clear cells may be round or polygonal in shape and tend to be arranged in a tubular or acinar pattern. Some may display papillary features particularly when within cysts (McGee *et al.*, 1992). The cells are usually supported by a rich sinusoidal vascular architecture. High grade variants show loss of this cytology and architectural pattern. The nucleus has a typical prominent nucleolus and may be eccentric to the cell, and may even appear partially extruded (Renshaw *et al.*, 2000). Higher grade tumours have more isolated cells and less cytoplasmic vacuolisation. Tumours tend to have a fibrous pseudocapsule but the presence of a less well defined infiltrative margin is an ominous feature and suggests sarcomatoid transformation (Fleming and O'Donnell, 2000). Approximately 10-15% of these tumours will be cystic or pseudocystic as a consequence of tumour necrosis and haemorrhage (Eble and Bosnib, 1998).

The most useful immunohistochemical markers to define clear cell carcinoma are cytokeratin, vimentin and EMA (Fleming and Symes, 1987; Rahilly *et al.*, 1992; Fleming *et al.*, 1985). In most cases diagnosis is straightforward but metastatic tumours can be more difficult to diagnose due to cells becoming very "bland". Immunohistochemistry has particular use in such cases.

Papillary Renal Carcinoma

For tumours to be defined as papillary at least 50% true papillae must be observed (Renshaw and Corless, 1995). Papillary tumours represent 7-15% of all renal cell carcinomas. The gross appearance has characteristic flecks of gold in a background of haemorrhage (Renshaw and Corless, 1997). The cells lining the papillae are either cuboidal or columnar type Figure 1.3. Studies have suggested that the cellular subtype defines prognosis with cuboidal (Type 1) having a better outcome than papillary (Type 2) (Delahunt and Eble, 1997). Cells are either basophilic with scant cytoplasm or eosinophilic with abundant granular cytoplasm. Papillae may be recognisable or may appear as spherules or tubules. Often fibrovascular cores distended with macrophages are seen (Weir and Pitman, 1996).

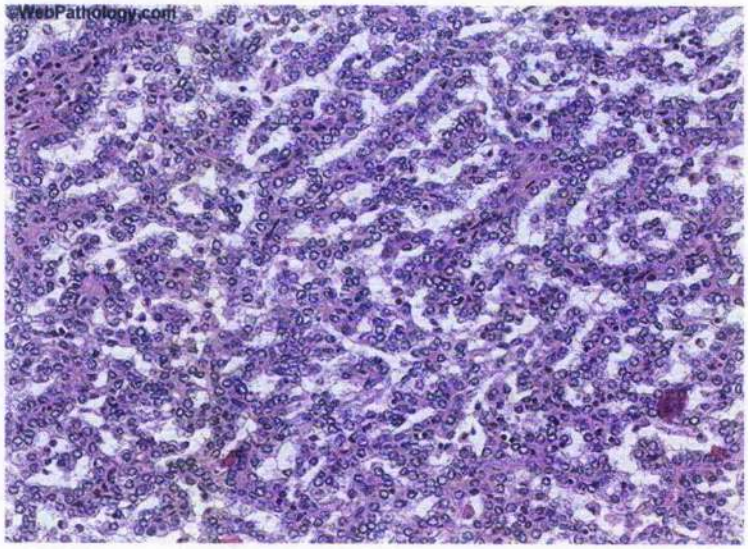


Figure 1.3 A H&E histological slide of a papillary carcinoma of kidney (webpathology.com).

Papillary carcinoma is usually accompanied by the presence of papillary adenomas in the cortex. It is the type of renal carcinoma which complicates renal cystic disease most frequently in the dialysis population (Ishikawa and Kovacs, 1993).

Papillary tumours like clear cell show cytokeratin and EMA reactivity but are less likely to be vimentin positive. They are also positive for cytokeratin 7 which may prove to be a useful diagnostic marker (Renshaw and Corless, 1995).

Chromophobe Carcinoma

Five percent of all renal cell carcinomas are chromophobe tumours which carry a better prognosis unless large or multifocal (Renshaw *et al.*, 1996). On gross inspection these tumours are uniformly soft and grey, and can occasionally have central stellate scarring to mimic oncocytoma. Tumour cells show weak uptake of eosin staining hence their name, with pale staining membrane bound vesicles and perinuclear halo formation (Thoenes *et al.*, 1985) Figure 1.4.

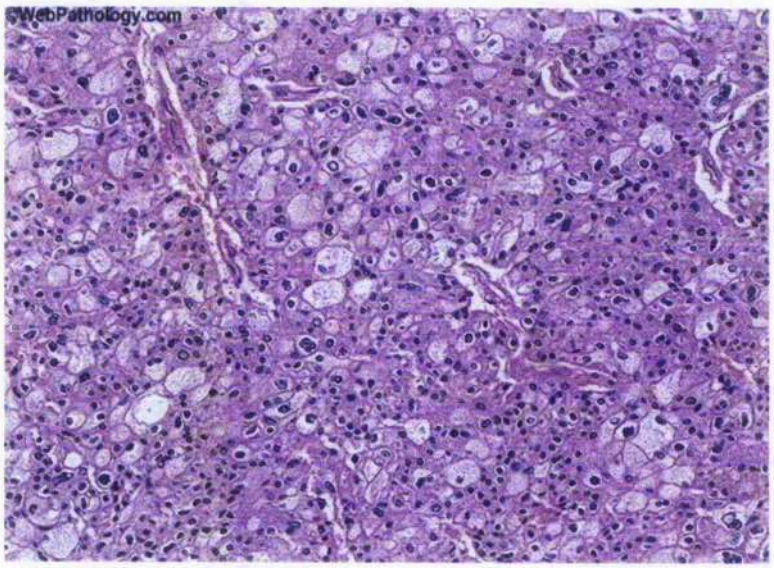


Figure 1.4 A H&E histological slide of a chromophobe carcinoma of kidney (webpathology.com).

Collecting Duct Carcinoma

Only 1% or renal cancers are collecting duct tumours, but these behave in a highly aggressive manner, affecting a younger age group with 90% of those diagnosed succumbing to their disease (Fleming and Lewi, 1986). It demonstrates tubulopapillary histology with high-grade cytology and prominent desmoplasia (Baer *et al.*, 1993) Figure 1.5.

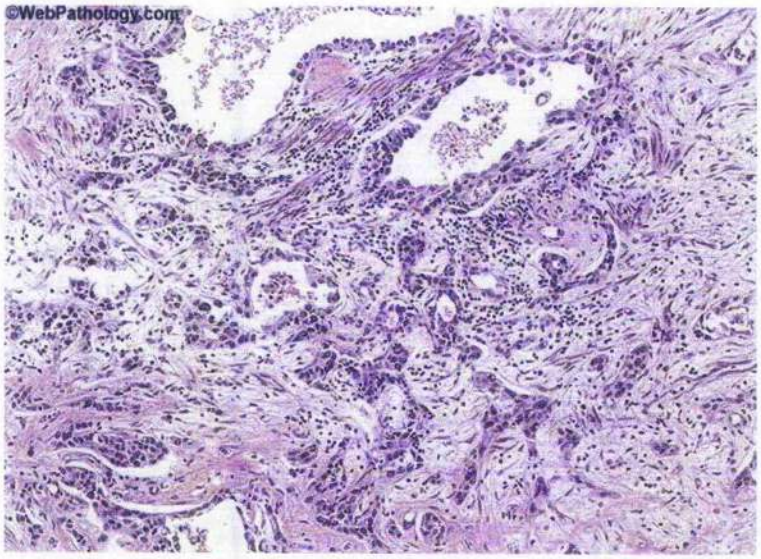


Figure 1.5 A H&E histological slide of a collecting duct carcinoma of kidney (webpathology.com).

Unclassifiable Renal Malignancy

As the name suggests there are a number of tumours in which the original epitheliod element cannot be identified and classified. These tumours tend to have mixtures of components, unrecognizable architecture or sarcomatoid pattern (Fleming and O'Donnell, 2000).

Oncocytoma

This represents the commonest benign lesion of the kidney and accounts for 5% of all surgically resected renal masses. They are well circumscribed, tan coloured tumours with a central stellate scar (Fleming and O'Donnell, 2000). Vascular channels are present at the margins of the lesion which gives a classic cartwheel appearance on angiography (Lieber *et al.*, 1981). Histologically it appears as nests and sheets of intensely granular eosinophillic cells. The nests are found in a mucopolysaccharide rich extracellular matrix (Amen *et al.*, 1997). They show minimal nuclear pleomorphism except in ischaemic areas and mitotic activity is rare.

Renal Cortical Adenoma

These are benign lesions that are histologically, immunohistochemically and cytogenetically indistinguishable from low grade papillary renal cell carcinomas except by size (Grignon and Eble, 1998). They are defined by size and are always less than 5mm and usually less than 2mm and are identified incidentally in 4-37% of kidneys at autopsy (Xipell, 1971).

Metanephric Adenoma

These are benign lesions most commonly arising in women in the 5th decade (Strong and Ro, 1996). They are characterised by a well circumscribed margin, white appearance and are usually firm although larger lesions are softer. Tumours are composed of tubules and papillae forming "glomeruloid bodies" lined by uniformly bland cells, with occasional psammoma bodies (Renshaw *et al.*, 2000).

1.3.4 Grade

Histological grade has been identified as an independent factor of prognosis in many studies (Medeiros *et al.*, 1988; Hofmockel *et al.*, 1995; Bretheau *et al.*, 1995). The most commonly used system for grading renal tumours is that proposed by Fuhrman (Fuhrman *et al.*, 1982). This study examined 103 patients undergoing nephrectomy between 1961 and 1974. Four nuclear grades were defined in order of increasing nuclear size, irregularity and nucleolar prominence shown below (Table 1.2).

Table 1.2 Fuhrman grading system for renal cell carcinoma.

Fuhrman Grade	Histological features	Malignant Potential*
Grade 1	Small (approx. 10μ) round uniform nuclei with inconspicuous or absent nucleoli.	9%
Grade 2	Larger (approx. 15μ) nuclei which exhibited irregularities in outline and nucleoli under high x400 power.	61%
Grade 3	Large (approx. 20μ) nuclei with an obviously irregular outline and prominent nucleoli even at low x100 power.	79%
Grade 4	Similar features to G3 with additional bizarre, often multilobed nuclei and heavy chromatin clumps.	67%

*Denotes the percentage of patients developing metastases independent of stage according to Fuhrman's original series over 5 year follow up.

Sarcomatoid Differentiation

Sarcomatoid change is recognised as a progression factor with loss of epithelial phenotype rather than a distinct tumour type. It is characterised by a spindle cell histologic appearance with ultrastructural or immunohistochemical evidence of epithelial and mesenchymal differentiation. Sarcomatoid change has been reported in all distinct histological subtypes of renal cancers (de Peralta-Venturina *et al.*, 2001).

It occurs in approximately 8% of renal tumours and can be associated with any histological subtype, grade or stage of tumour. Sarcomatoid change confers an adverse prognosis. This is more pronounced when the percentage of sarcomatoid component exceeds 50% (de Peralta-Venturina *et al.*, 2001).

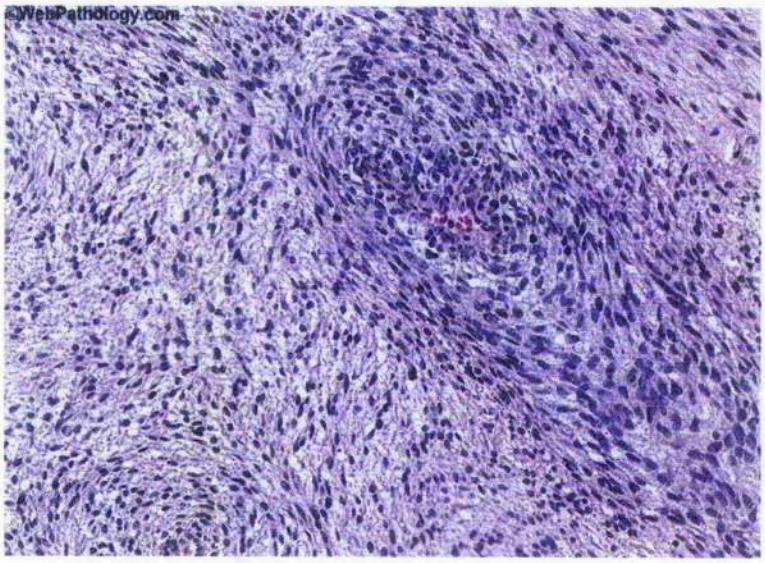


Figure 1.6 A H&E histological slide of a sarcomatoid carcinoma of kidney (webpathology.com).

1.4 Clinical Features of Renal Cell Carcinoma

The classical triad of symptoms of haematuria, loin pain and an abdominal mass are rarely all present in patients with newly diagnosed renal cancer. More commonly patients present with one or no symptoms to suggest a diagnosis of renal cell carcinoma. Kim and co-workers (2003) examined 1046 patients with localised and metastatic renal cancer and found that haematuria was present in 35.2% of cases, loin pain 19.5% and an abdominal mass 4.4%of patients. Localised to metastatic ratios for haematuria, loin pain and abdominal masses are respectively 0.8, 0.6 and 0.4 demonstrating patients with metastatic disease are more likely to present symptomatically. Patients with metastatic disease and have a higher incidence of all paraneoplastic features particularly weight loss, anorexia and other cachexia related features (Kim *et al.*, 2003).

Approximately 20% of patients diagnosed with RCC present with paraneoplastic symptoms, with a further 10-40% developing these features during their disease course (McDougal and Garnick, 1995). Paraneoplastic symptoms can include weight loss, hepatic dysfunction, anaemia, malaise, hypercalcaemia, anorexia, thrombocytosis, fever, night sweats, hypertension and erythrocytosis. Anaemia (52.1%), hepatic dysfunction (31.5%) and weight loss 22.9% represent the commonest paraneoplastic symptoms (Kim *et al.*, 2003). Such presenting symptoms are also found in localised as well as metastatic RCC cases, suggesting that these findings reflect tumour biology rather than simply the local or distant extent of the tumour.

These paraneoplastic symptoms may be the result of release of tumour associated proteins. The proteins responsible for the paraneoplastic effect may be shed directly from the tumour or generated by the immune system in response to the tumour. It has been suggested that cachexia and malaise may be due to the secretion of proinflammatory cytokines by the tumour infiltrating cells (Laski and Vugrin, 1987).

The general condition of a patient combining symptoms and co-morbidity has been shown to be an independent predictor of survival. An Eastern Cooperative Oncology Group (ECOG) performance status of 2 or greater is an independent prognostic factor associated with shorter survival and poorer response to immunotherapy (Lissoni *et al.*, 1994; Zisman *et al.*, 2002).

The increased use of abdominal imaging has lead to a greater number of tumours being identified in the absence of symptoms and is thought to account for a degree of stage migration towards localised disease. In the 1970s only 10% of patients were diagnosed incidentally compared with 61% in the 1990s (Zisman *et al.*, 2001). Compared to symptomatic tumours it has been shown that tumours found fortuitously are of a lower histological grade and stage with a lower risk of metastases and patients noted to survive longer (Bretheau *et al.* 1995; Tsui *et al.*, 2000).

1.5 Assessment of renal cancer prior to treatment

Previously known as the "internists tumour" due to the presentation as various paraneoplastic phenomenon; it is now known as the "radiologists tumour" as increasing numbers of patients are diagnosed on incidental radiology. Therefore radiology has a role in diagnosis, staging, planning surgery and obtaining histological biopsy of renal lesions. Renal masses can be identified on plain radiograph, intravenous urography, ultrasound, magnetic resonance imaging (MRI) and computed tomography.

The greater access to ultrasound for most departments means that this modality commonly identifies incidental lesions and is highly sensitive in distinguishing between cystic and solid lesions (Helenon *et al.*, 2001) Figure 1.6. To accurately characterise and plan treatment magnetic resonance imaging and computerised tomography are used to image renal masses.

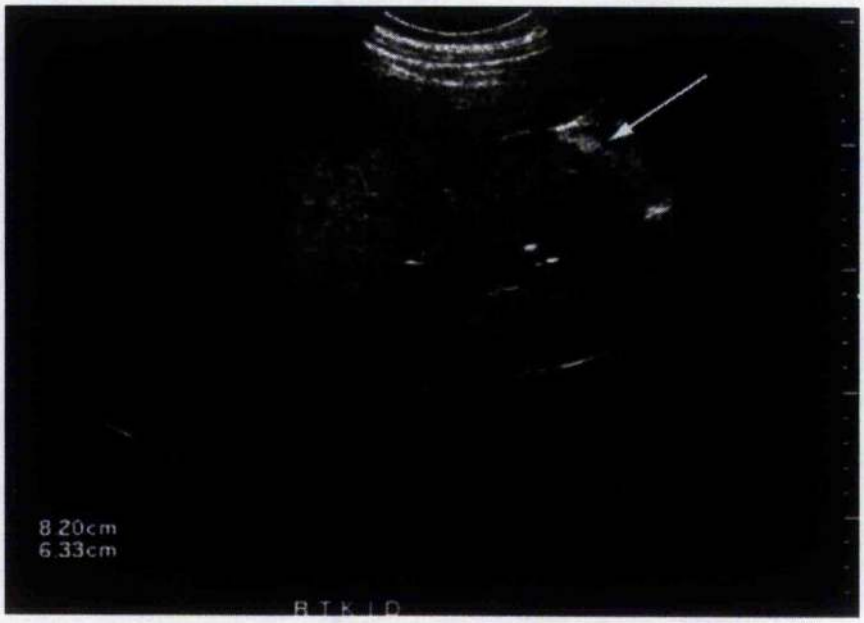


Figure 1.7 An Ultrasound image of solid lesion arising from the right kidney (Patterson B et al., www.cortlandtforum.com).

Computerised tomography scanning is the gold standard for diagnosis, characterisation and staging of renal tumours (Herts, 2003). Thin section helical computerised tomography reduces volume averaging, eliminates misregistration from respiratory or patient motion, and allows rapid scanning in different phases of contrast enhancement (Herts, 2003) Figure 1.7.

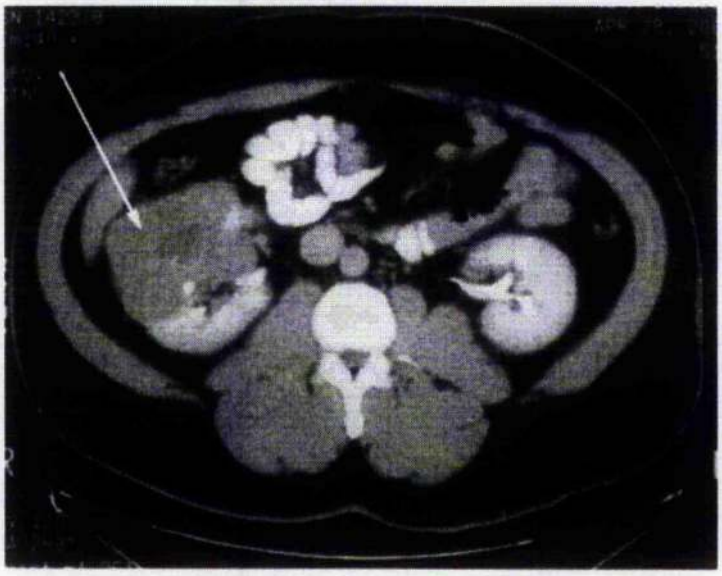


Figure1.8 A computerised tomography image of an enhancing solid renal mass arising from right kidney (arrowed) (Patterson B et al., www.cortlandtforum.com).

Traditional two phase contrast computerised tomography scanning includes a pre-contrast and a post-contrast scan. New triphasic helical computerised tomography includes pre-contrast unenhanced phase (to assess for calculi and baseline attenuation), corticomedullary phase (timed to visualise vascular structures) and nephrographic phase (most sensitive in detecting and determining enhancement) (Yuh and Cohan, 1999). It is difficult to distinguish on computerised tomography between renal cell carcinoma and oncocytoma. Papillary lesions are suggested to enhance less than clear cell lesions (Jinzaki *et al.*, 2000). The effective radiation dose for an abdominal computerised

tomography scan is 2200 mrad which is over 7 times the annual natural background radiation of 300 mrad (Prasad *et al.*, 2002).

Computerised tomography assessment not only has a role in diagnosis, but also in accurate staging of renal tumours. This staging accuracy approaches 91% (Catalano *et al.*, 2003). Limitations exist, particularly when defining perinephric fat invasion, in distinguishing inflammatory changes from tumour infiltration (Johnson *et al.*, 1987). This means that accurately differentiating T1/2 tumours from T3a is difficult and accounts for more than half of the staging errors using computerised tomography in assessment of renal tumours (Bechtold and Zagoria, 1997). Difficulties also exist in defining accurately renal vein and caval involvement where flow artefact may occur.

The prognosis of patients with nodal disease is significantly poorer when compared to those without lymph node metastases. Computerised tomography has a reported sensitivity of 83% and specificity of 88% in identifying lymphadenopathy in patients with renal cell carcinoma (Johnson *et al.*, 1987). Direct invasion of adjacent organs in stage 4 disease can be difficult as 15% of patients can demonstrate obliteration of the fat plane and the adjacent organ without actual involvement (Catalano *et al.*, 2003).

Distant spread is accurately assessed using computerised tomography scanning. The most common sites of metastases are the lungs, lymph-nodes, liver and bone but can occur anywhere throughout the body. Using lung settings pulmonary metastases can be readily seen as solid pulmonary nodules, but to accurately define liver lesions arterial phase scanning is required to increase the sensitivity (Yoon and Herts, 2003).

Surgical Planning

Helical computerised tomography with three dimensional image post processing has been shown to be accurate and reliable in illustrating the relative position within a kidney of a mass being considered for surgery (Coll *et al.*, 2000). This has particular use when planning nephron sparing surgery. 3-Dimensional reconstruction demonstrates the renal vasculature accurately removing the requirement for pre-operative angiography (Roy *et al.*, 1999). Magnetic resonance imaging can be used to characterise renal lesions in patients with renal insufficiency or contrast allergy as it can be performed both with and without gadolinium contrast agents that are not nephrotoxic. It is more sensitive than computerised tomography where contrast enhancement is equivocal and is better in assessment of renal vein and inferior vena cava tumour extension (Remer *et al.*, 2000).

In addition to planning nephron-sparing surgery imaging can be used to assist in gaining satisfactory clearance and preserve optimal renal function. The use of intraoperative ultrasound is advised to allow precise determination of the extent of renal lesions and polycentricity (Marshall *et al.*, 1992; Assimos *et al.*, 1991). It is also reported to assist in accurate assessment of the feasibility of partial nephrectomy intraoperatively. Ultrasound scanning provides a guide to more accurate nephrotomy, facilitating the attainment of negative resection margins during partial nephrectomy (Assimos *et al.*, 1991).

Positron Emission Tomography

This is an experimental modality of renal cancer imaging and operates on the principle of an increased rate of glycolysis in cancer cell. A radioactive positron-emitting analogue of glucose (fluorine-2-D-deoxyglucose) is taken up and phosphorylated in cells to remain trapped and detectable using a detector ring. The role in renal cancer is emerging. It is good at initially identifying disease and better than conventional imaging at assessing local disease recurrence and metastatic disease (Hain and Maisey, 2003).

Radiological Guided Biopsy

Despite the advances in radiological imaging a number of solid renal lesions will turn out to be benign tumours of the kidney. The standard treatment for such solid lesions is either partial or radical nephrectomy with formal histology only obtained after surgery. Studies to examine the role of fine needle biopsy have demonstrated an excellent positive predictive value for carcinoma on biopsy of greater than 94% (Dechet *et al.*, 1999). Unfortunately overall accuracy is only 75% with the greatest degree of error in defining benign lesions, therefore the standard of care remains to remove these lesions without requirement of pre-operative biopsy. The principle role remains in excluding other rare causes of a renal mass, or in gaining positive histology in the absence of nephrectomy prior to commencing systemic treatment.

1.6 Management of Renal Cell Carcinoma

1.6.1 Localised Disease

Surgery remains the only proven cure for localised renal cancer. Robson demonstrated that the removal of perinephric tissue, including the ipsilateral adrenal gland and lymphatic drainage including paracaval or para-aortic lymph nodes, was associated with better survival than simple nephrectomy (Robson *et al.*, 1969).

Robson's original operation has been modified since it was first described, to reflect the observed lack of survival benefit achieved in routine removal of the ipsilateral adrenal gland. Sagalowski and coworkers (1994) demonstrated that the routine removal of the ipsilateral adrenal gland could contribute to cure in only 0.43% of all patients, and suggested that adrenalectomy be reserved for patients with large upper pole tumours.

With emerging technology and advances in surgery there has been a drive to provide an alternative surgical procedure to minimise the relative morbidity of formal open radical nephrectomy. The first described laparoscopic nephrectomy was carried out in 1990 at Washington University by Clayman et al (Clayman *et al.*, 1991). A total nephrectomy was performed removing the kidney, most of Gerota's fascia and peri-renal fat, with the adrenal left behind. Subsequent groups have extended the procedure, to more closely define laparoscopically the radical nephrectomy described by Robson, by including the adrenal, perinephric fat and Gerota's fascia in the dissection (Dunn *et al.*, 2000; Ono *et al.*, 2001; Barrett *et al.*, 1998; Abbou *et al.*, 1999). Various adaptations have been described using retroperitoneal or transperitoneal approaches, as well as hand assisted procedures. This closer approximation in gross resection has led to the comparison of long term cancer free survival with open radical nephrectomy.

Portis and colleagues (2002), compared patients undergoing laparoscopic radical nephrectomy with a representative group of patients undergoing open radical nephrectomy within the same institutions, with a mean follow up of 54 months. The result was no significant difference in 5-year disease free survival or overall survival, but this study was carried out retrospectively with relatively small numbers. Other centres have shown similar results for short term data, but to date no formal randomised controlled study has been performed to compare cancer specific survival following laparoscopic versus open radical nephrectomy.

Nephron-sparing surgery, or partial nephrectomy has evolved primarily for the treatment of patients who would otherwise be rendered anephric by traditional radical nephrectomy. Such patients include those with renal cell carcinoma arising in an anatomically or functionally solitary kidney, or alternatively in patients with bilateral synchronous renal lesions. The indications have extended to patients with solid renal lesions in whom the contra lateral kidney is threatened by local, systemic or genetic conditions that may affect its future function.

Data are accumulating to suggest that preservation of renal function can be achieved following partial nephrectomy without sacrificing cancer control (Uzzo *et al.*, 2001). Current evidence suggests that adequate surgical clearance of RCC can be achieved with a minimal margin of 5 millimetres, as opposed to the previous recommended margins of 10-20 millimeters (Sutherland *et al.*, 2002). Having confirmed intraoperative clear margins local recurrence is unlikely, which essentially assures local cancer control. Sutherland and colleagues (2002) describe no cases of local recurrence with negative margins.

The excellent results reported following nephron sparing surgery in patients with a normal contra lateral kidney has led to the suggestion that in selected patients this treatment can be extended to such patients on an elective indication (Butler *et al.*, 1995; Lerner *et al.*, 1996). Concerns exist regarding the impact of tumour size on patient survival and localised recurrence after partial nephrectomy. Following a large study at the Cleveland Clinic examining 485 patients it was observed that a significantly lower rate of recurrence and significantly improved survival after nephron sparing surgery for tumours 4cm or less compared with those greater than 4cm (Hafez *et al.*, 1999). Accordingly this guideline is increasingly accepted that elective partial nephrectomy should be offered only to patient with tumours less than 4cm.

Further to the development of open partial nephrectomy there are a number of current experimental techniques under investigation to provide yet more nephron sparing minimally invasive options in the management of small, localised tumours. These include laparoscopic partial nephrectomy (Rassweler *et al.*, 2000), and a variety of energy based tissue ablative procedures like cryoablation (Gill *et al.*, 2000), radiofrequency ablation (Gill et al 2000), high intensity focussed ultrasound (Chapelon *et al.*, 1992), microwave thermotherapy (Kigure *et al.*, 1996), and interstitial photon irradiation (Chan *et al.*, 1999). None of these treatments are of proven efficacy, much of the studies having been carried out in animal models and further work will have to be performed before any conclusions can be drawn with regard to their place in treatment of renal cancer.

1.6.2 Management of Metastatic Disease

Immunotherapy

Metastatic renal cancer remains highly resistant to systemic therapy with no proven cure in advanced disease (Motzer and Russo, 2000). Immunotherapy with interleukin 2 and/ or interferon-α achieves response in 10-20% of patients, only a very small number of which are durable (Motzer *et al.*, 1997; Fisher *et al.*, 1997). Most studies define response to treatment as either static disease of tumour regression, with complete response accounting for only a very small number of patients.

The median survival of patients on interferon therapy is 8.5 months compared with 6 months for patients given medroxyprogesterone acetate (Bower *et al.*, 1998). In those patients who respond (12%) longer survival is associated with high performance status, prior nephrectomy and predominantly lung metastases (Minasian *et al.*, 1993; Fossa *et al.*, 1991). Duration of response rarely exceeds 2 years (Motzer and Russo, 2000). Treatment dosage and duration vary between trials. A dose of 5 to 20 million units of recombinant interferon daily is thought to have maximal efficacy without the toxicity of the higher doses (Krown, 1987). Any marginal benefit in overall survival must be weighed against the toxic side effects of interferon which can be severe including general malaise, fevers, rigors, anorexia and nausea.

Interleukin-2 has been shown to achieve complete and partial responses in 19% of patients with a median duration of response of 22 months (Bower *et al.*, 1998). Bolus Interleukin-2 is notably more toxic than interferon and has an associated treatment-related mortality of 4%.

Combination regimens have been suggested to have greater response rates compared to single agent interferon or interleukin-2 (Negrier *et al.*, 1998). Negrier and co-workers (1998) demonstrated objective response in 19% of patients on combination treatment compared with 7% and 8% for interleukin-2 and interferon respectively. Overall survival was not significantly different in this study with the combination arm experiencing greater toxicities. Chemotherapy in isolation has no proven benefit in the treatment of renal cell carcinoma. Evidence is emerging that 5-fluorouracil in combination with immunotherapy does improve response rates (Samland *et al.*, 1999). Current investigations into the response rates for combination treatment including interferon, 5-fluorouracil and interleukin-2 versus single agent interferon are ongoing in the EORTC REO4 trial.

Immunotherapy has been considered in an adjuvant role in treating patients at high risk of relapse after surgery. To date studies have failed to demonstrate significant survival benefit using adjuvant immunotherapy (Blasting *et al.*, 1999). The lack of prospective data prompted the EORTC adjuvant renal study to be carried out, but this has had to be halted due to poor patient recruitment.

Radiotherapy

Renal cell carcinoma is historically regarded as a radio-resistant tumour, but evidence continues to indicate a role for radiotherapy in the palliation of symptoms. Radiotherapy has most commonly been used in the treatment of symptomatic metastases to bone and brain, but may also be used in lung, spinal cord and soft tissue (Onufrey and Mohiuddin, 1985). Radiotherapy can achieve a palliative response in 86% of patients and a complete palliative response in 49% (DiBiase *et al.*, 1997).

Response rates are noted to be higher in those patients with better performance status and those receiving a higher biologically active effective dose (greater than 50Gy).

Cytoreductive Nephrectomy

With the increase in understanding of the biology of renal tumours has come a move towards cytoreductive nephrectomy. In the late 1970s before the advent of immunotherapy the natural history of metastatic renal carcinoma was not affected by adjunctive nephrectomy (de Kernion *et al.*, 1978). At this time nephrectomy was considered as debulking surgery and principally management of significant symptoms such as haematuria and loin pain. Currently there is increasing evidence to suggest that de-bulking of renal cell carcinoma may actually remove tumour growth associated factors (eg transforming growth factor beta) which are potent immunosuppressive factors and which, when, removed, make the host more capable of responding to systemic immunological treatment (Filgin, 1999).

Studies have compared immunotherapy regimens along with cytoreductive nephrectomy and have demonstrated an improved survival benefit. The University of California-Los Angeles group compared their experience with interleukin-2 immunotherapy after cytoreductive nephrectomy with matched interferon plus nephrectomy cases (Pantuck *et al.*, 2002). This study demonstrated a 30% improvement in both groups with 5 year survival a 19.6% in the Il-2 + nephrectomy group compared with 10% in the IFN + nephrectomy group. This study also demonstrated a doubling of survival compared with the interferon only group.

Concerns exist regarding the timing of nephrectomy due to the associated morbidity and perioperative mortality along with the potential for patients to not recover sufficiently to undergo systemic immunotherapy. Due to these concerns it has been

suggested that nephrectomy be reserved only for those patients who demonstrate a response to immunotherapy prior to nephrectomy. Levy and coworkers (1998) report 3% peri-operative mortality with 82% of patients proceeding to immunotherapy in carefully selected patients. In an attempt to reduce the prolonged recovery period and morbidity after open nephrectomy the role of laparoscopic procedures has been proposed in the cytoreductive setting.

The current recommendations from South West Oncology Group state that in suitable patients (especially those with a good performance status), nephrectomy followed by immunotherapy using interferon [alpha]-2b with or without other cytokine therapy should be the control arm for future phase III trials (Yonover and Flannigan, 2000).

Metastasectomy

The resistance of renal cancer to traditional systemic chemotherapy, combined with the poor sustained response rates to immunotherapy, has lead to the exploration of treatment, where metastases appear to be isolated, of surgical metastasectomy. In this special, small subgroup of patients several studies have demonstrated prolonged survival following removal of solitary metastases.

Post mortem data demonstrates single organ confined metastases to occur with a frequency of 8 – 11% (Saitoh *et al.*, 1982). The lung is the commonest site of metastases, and where identified in isolation, confers the best prognosis following metastasectomy increasing median survival to 43 months (Maldazys and deKiernon 1986; Van Der Poel *et al.*, 1999; Piltz *et al.*, 2002). Bone is the second commonest resectable site for renal carcinoma to occur, with between 15 and 30% of skeletal

metastases thought to be solitary, but confers a poorer prognosis (Maldazys *et al.*, 1986).

Solitary brain metastases account for 8.1% of solitary metastases seen in relation to renal cancer. Those younger patients, with good performance status, demonstrate the most benefit from surgery with a median survival of 15.3 months versus 10.3 months treated with radiotherapy (Pomer *et al.*, 1997).

1.7 Prognosis of Renal Cell Carcinoma

1.7.1 Pathological criteria

The extent of anatomical extension of disease is one of the principle prognostic factors in renal cancer, as with many other tumours. Stage was originally defined according to Robson's criteria, but today a more refined TNM system is preferred (Table 1.3, 1.4) (Robson *et al.*, 1969; American Joint Committee on Cancer 2002). There have been a number of changes in the system between 1987 and 2003, which principally involve the stratification between T1 and T2 lesions. T1 lesions prior to 1997 were 2.5 cm or less with greater confined lesions all being T2. In 1997 the size of T1 lesions was increased to 7cm or less but concerns arose relating to significant variability in clinical outcome within this group (Zisman *et al.*, 2001). Accordingly the American Joint Committee on Cancer 6th edition of TNM staging has subdivided T1 lesions into T1a <4cm and T1b >4cm < 7cm.

The presence of venous invasion confers a poorer prognosis than those patients with lesions confined to the kidney (Giberti *et al.*, 1997). Studies have suggested that the presence of renal vein or caval involvement associated with invasion of the vessel wall carries an even higher rate of recurrence. Van-poppell and coworkers (1997) demonstrated a 45% recurrence rate at one year post nephrectomy where microvascular invasion was present.

Microvascular invasion has been shown to be correlated with disease progression but is not an independent prognostic factor (Lang *et al.*, 2000). It is also been reported that disease invasion into the veins of the renal sinus may identify patients at risk of metastatic progression even when the tumour is confined to the kidney (Bosnib *et al.*, 2000). This is reflected in the new TNM guidelines (Table 1.3).

Lymphadenectomy is not routinely performed in the absence of enlarged nodes detected prior to surgery. Several studies have demonstrated that such regional lymph node dissection in patients with clinically negative nodes is unnecessary and offers limited staging information and no benefit in terms of decreasing disease recurrence or improving survival (Minervini *et al.*, 2001; Pantuck *et al.*, 2003). In patients with positive lymph nodes on preoperative staging, dissection is however associated with improved survival in carefully selected patients undergoing cytoreductive nephrectomy and post operative immunotherapy (Pantuck *et al.*, 2003).

Stage specific survival as reported by the US National Cancer Database is shown in Table 1.5. These are overall survival rates and do not account for other prognostic factors such as tumour grade, histological subtype, patient age or treatment modality (Marshall *et al.*, 1997). For those patients managed with nephrectomy the survival is significantly higher than overall data would suggest, particularly for localised tumours confined to the kidney. Five year cancer specific survival rates for T1 (T1a + T1b) and T2 lesions treated by open radical nephrectomy are reported at 95% and 87% respectively (Portis *et al.*, 2002). In further subdividing pT1 lesions 3 and 10 year survival for pT1a lesions is 98.6% and 86.5% respectively. For pT1b the survival rates are 93.7% and 87.9% (Masuda *et al.*, 2003).

Table 1.3 TNM classification of renal tumours according to the American Joint Committee on Cancer 6th edition of TNM staging.

T	Tx	Tumour not assessable
	T0	Absence of tumour
	T1a	Tumour less than or equal to 4cm limited to the kidney
	T1b	Tumour greater than 4cm up to 7cm limited to the kidney
	T2	Tumour greater than 7cm limited to the kidney
	T3a	Invasion of the perirenal fat, renal sinus invasion or adrenal gland
	T3b	Invasion of the renal vein or subdiaphragmatic inferior vena cava
	T3c	Invasion of the supradiphragmatic vena cava
	T4	Tumour invasion of Gerota's fascia
N	Nx	Lymph nodes not assessable
	N0	Absence of lymph node metastases
	N1	Only 1 lymph node metastasis
	N2	Several lymph node metastases
M	M0	Absence of distant metastasis
	M1	Distant metastases

Table 1.4 Stage grouping according to American Joint Committee on Cancer 6th Edition TNM Staging.

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 1.5 Five-year cumulative relative survival rates by AJCC/ UICC stage at diagnosis.

Years After Diagnosis		1	2	3	4	5
% Survival	Stage I	95	91	90	87	85
	Stage II	94	90	87	85	83
	Stage III	83	73	68	63	60
	Stage IV	37	23	17	13	11

1.7.2 Performance Status

Currently the most commonly used measure for performance status is the Eastern Cooperative Oncology Group ECOG-ps, which defines the ability to perform the daily activities of life, such as house-work and self care (Table 1.6). Several studies have demonstrated that a poorer performance status has a negative impact on survival (Elson *et al.*, 1988; Giberti *et al.*, 1997). This has been observed in both localised tumours predicting recurrence and in metastatic disease in predicting response to immunotherapy (Zisman *et al.*, 2002).

It is unclear in localised disease whether it is because the performance status is low that the response to surgery is less effective or whether the performance status is low because the tumour is more aggressive. In metastatic disease it has been suggested that the reason for poorer outcome with reduced performance status is principally related to the fact that these patients receive significantly less chemotherapy and develop more toxicities rather than a specific reduced response to treatment (Andreyev *et al.*,. 1998). The effect of performance status on overall survival has been utilised in stratifying patients according to overall risk in the University of California-Los Angeles group Integrated Staging System (UISS) (Zisman *et al.*, 2002 Table 1.7). This prognostic algorithm combines stage, grade and performance status to predict overall outcome in both metastatic and localised disease.

Performance status does not fully take into account other symptoms related to the tumour such as cachexia. As cachexia like symptoms have been demonstrated to be independent prognostic factors it has been suggested that ECOG-ps should be expanded to include these parameters (Kim *et al.*, 2003).

Table 1.6 Eastern Cooperative Oncology Group Performance Status (ECOG-ps) scale.

Performance Status	Criteria
0	Fully active, able to carry on all pre-diseases performance without restriction. (Karnofsky 90-100)
1	Restricted physically in strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (eg light housework, office work). (Karnofsky 70-80)
2	Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours. (Karnofsky 50-60)
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours. (Karnofsky 30-40)
4	Completely disabled. Cannot carry out any self care. Totally confined to bed or chair. (Karnofsky 10-20)

Table 1.7 The UISS Prognostic algorithm (Zisman et al. 2002).

1.7.1 N0, M0 nephrectomised patients

T Stage	1				2	3				4
Grade	1-2		3-4		Any	1		>1		Any
ECOG	0	≥1	0	≥1	Any	0	≥1	0	≥1	Any
Risk	Low	Intermediate (Int)							High	

1.7.2 N1, N2 or M1 nephrectomised patients.

Stage	N1M0	N2M0/ M1							
Grade	Any	1		2		3		4	
ECOG	Any	0	≥1	0	≥1	0	≥1	0	≥1
Risk	Low		Int	Low	Int				High

1.8 The Inflammatory Response

As previously discussed the principle management strategy in metastatic disease is recruitment of host immunity using interferon or interleukin-2 immunotherapy. Immunotherapy has been shown to have a direct inhibitory effect on tumour cell growth/ functions including down-regulation of oncogene expression and induction of tumour suppressor genes (Pfeffer et al., 1998). Additionally immunotherapy can increase major histocompatibilty complex 1 expression which combined with the effect of increased natural killer cell and T cell activity results in enhanced tumour cell recognition (Takahashi et al., 1994; Pfeffer et al., 1998). The resistance of renal cancer to respond to treatments other than immunotherapy would suggest that the inflammatory immune response is of particular relevance in development and progression of renal cell carcinoma.

It is now recognised that both the local and systemic inflammatory response play a crucial role in neoplastic development and the progression of many tumours (Coussens and Werb, 2002). Inflammation involves the activation of many promoters involved in cell proliferation, production of reactive oxidative species leading to oxidative DNA damage as well as recruiting many chemokines involved in cell migration and adhesion. In normal inflammation this process is self-limiting, but where tumour formation occurs it appears to persist. The reason for this continued response is not known, however there is evidence that a variety of cytokines and chemokines are produced by the host and the tumour cells. The resultant inflammatory component of a developing neoplasm includes a diverse leukocyte population of lymphocytes, neutrophils, dendritic cells, macrophages, eosinophils and mast cells.

1.8.1 The Systemic Inflammatory Response

1.8.1.1 Serum C-Reactive Protein

C-reactive protein is a 130kDa acute phase protein synthesised in the liver and constructed of five polypeptide subunits. It is present in minute quantities in normal serum (<1mg/L) and is thought to mediate binding of foreign polysaccharides, phospholipids, complex polyions and also activating complement via the classical pathway (Baynes and Dominiczak, 1999).

Interleukin-6 is the cytokine most likely to be responsible for elevated C-reactive protein levels in patients with renal cancer (Blay *et al.*, 1992). Elevated interleukin-6 and consequently elevated C-reactive protein are markers of a poor response to immunotherapy (Blay *et al.*, 1992).

C-reactive protein levels have been examined in patients undergoing cytoreductive nephrectomy. Patients with an elevated C-reactive protein treated with cytoreductive nephrectomy in addition to immunotherapy have been suggested to do better than patients with an elevated C-reactive protein treated with immunotherapy alone (Fujikawa *et al.*, 1999). Furthermore patients in whom postoperative serum C-reactive protein decreases to within normal limits are observed too have a better prognosis than those in whom it remained elevated (Fujikawa *et al.*, 1999).

Cytoreductive nephrectomy may decrease the total amount of interleukin-6 secretion by the tumour and improve the response to immunotherapy. Patients with an elevated postoperative nadir C-reactive protein may signify interleukin-6 secretion at a higher rate from metastatic lesions. These patients therefore have biologically more aggressive lesions and are less responsive to immunotherapy compared with those who show a decline to normal C-reactive protein postoperatively. The same study also noted

that patients with a normal C-reactive protein undergoing cytoreductive nephrectomy did no better than patients with a normal C-reactive protein treated by immunotherapy alone, suggesting surgery is of greatest benefit in patients with evidence of an inflammatory response (Fujikawa *et al.*, 1999).

1.8.2 The Local Inflammatory Response

1.8.2.1 Tumour Lymphocytic Infiltrate

The presence of a significant tumour mononuclear cellular infiltrate is thought to be evidence of the host attempting to mount a local cell mediated immune response (Schendel *et al.*, 1993). This response, in the later stages at least, is insufficient to prevent tumour growth and fails to stop progression.

The characteristics of the lymphocytic tumour infiltrate associated with better outcome and prognosis have been defined in a number of solid tumours. Increased tumour T-lymphocyte CD8 subsets have been shown to be associated with better survival in colorectal cancer (Naito *et al.*, 1998). Conversely, greater numbers of CD8+ and CD4+ T lymphocytes are associated with increased tumour recurrence and poorer survival in patients with renal cell carcinoma (Kolbeck *et al.*, 1992; Nakano *et al.* 2001, Bromwich *et al.*, 2003). This relationship, opposite to that observed in other solid tumours, suggests that the tumour infiltrate in renal cancer is more likely to be ineffective in mounting host anti-tumour immunity.

There are two distinct subsets of CD4+ T helper (Th) cells differentiated from one another according to their cytokine profile. Only Th1 cells produce interleukin - 2, interferon-[gamma] and tumour necrosis factor -[beta] which are principally associated with cytotoxic lymphocyte activation (Cher and Mosmann, 1987). Th2 cells produce

interleukin-4, interleukin-5, interleukin-6 and interleukin-10 and are associated with humoral immunity and cytotoxic lymphocyte suppression (Stevens *et al.*, 1988; Steiner *et al.*, 1999).

The major goal of immunotherapy in the treatment of renal cancer is the activation of T helper cells and cytotoxic CD8+ lymphocytes. Studies examining the intratumoral immunological environment in patients with renal cancer have shown a tendency to produce Th-2 related cytokines in accordance with stage and grade of tumour (Onishi *et al.*, 1999). These results are consistent with the work suggesting that higher numbers of infiltrating T-lymphocytes confers a poorer prognosis as the infiltrate is not effective in recruiting an appropriate cell mediated response.

The precise nature by which the immune response, innate or acquired, is rendered inadequate is unclear. In renal cancer it may be due to immune suppressor agent shedding, induction of T-cell apoptosis or aberrant or inadequate expression of major histocompatibility antigens on the tumour cell surface (Uzzo *et al.*, 1999; Akdis and Blaser, 2001).

1.8.2.2 Interleukin-6

Interleukin-6 is a cytokine of approximately 26kD, which is synthesised by mononuclear phagocytes, vascular endothelial cells, fibroblasts and other cells in response to Interleukin-1 and tumour necrosis factor. It functions as a pro-inflammatory cytokine and induces hepatocytes to synthesise acute phase proteins, which compliment the proteins induced by interleukin-1 and tumour necrosis factor. It also acts as a cellular growth factor, initially described in relation to plasma B cells but has also been shown to be a co-stimulator in the growth of bone marrow and stem cells.

The presence of interleukin-6 mRNA and active secretion has been demonstrated in freshly isolated renal cancer tissues (Miki *et al.*, 1989). Further studies have revealed renal cancer cell lines to express mRNA for both interleukin-6 and its receptor (Takenawa *et al.*, 1991). Patients with higher mRNA levels are noted to have a greater incidence of both lymph node and distant metastases. Blay and co workers (1992) have proposed that interleukin-6 acts as an autocrine growth factor, and correlates well with serum C-reactive protein concentrations. This suggests that interleukin-6 is the pro-inflammatory cytokine most closely associated with increased C-reactive protein production. This work has identified patients with a poorer prognosis as having detectable serum interleukin-6 and C-reactive protein greater than 50. Yoshida and co workers (2002) as well as confirming the close correlation with C-reactive protein, also noted significantly higher interleukin-6 levels with stage IV tumours, G3 tumours and a positive correlation with tumour size.

The source of interleukin-6 production in renal cell carcinoma has not been satisfactorily identified in vivo. Miki and co workers (1989) using immunostaining techniques in cell lines identified 2-9% of cultured cells to positively stain for anti-interleukin-6 antibody. Direct staining of renal cancer tissues for interleukin-6 to differentiate between the vascular endothelial cells and renal carcinoma cells, has shown that 70% of tumour cells themselves stain positively for interleukin-6 expression (Takenawa *et al.*, 1991; Paule *et al.*, 2000). These studies did not relate direct tissue positive staining to serum interleukin-6 concentrations.

Interleukin-6 is therefore implicated in the inflammatory response, which confers a poorer prognosis, and would seem to be stage dependent. C-reactive protein would appear to be produced in response to interleukin-6 hepatocyte stimulation as a

principal acute phase protein. It has been suggested that interleukin-6 acts as an autocrine growth factor secreted by renal cancer cells, however its mechanism of action has not been fully identified.

1.8.2.3 Interleukin-6 Receptor

Interleukin-6 function is mediated by two membrane proteins, a 80kDa binding receptor and the gp130 protein that transduces the signal (Kishimoto, 1989). Soluble gp80 binds to interleukin-6 and the interleukin-6/ soluble interleukin-6 receptor complex binds and activates the gp130 transducer chain (Yasukawa *et al.*, 1990). Previous work has shown interleukin-6 receptor expression in 26% of renal cancers (Takenawa *et al.*, 1991).

Further work to define interleukin-6 receptor expression using immunohistochemistry demonstrated again 26% of renal cancer patients to express interleukin-6 receptor. Also, 83% of patients with metastatic disease were positive for the receptor (Costes *et al.*, 1997). In this study interleukin-6 receptor expression was associated with increasing tumour stage, higher grade, greater proliferative index and detectable serum interleukin-6. Interleukin-6 receptor is a marker of patients with more aggressive disease and is the mediator of interleukin-6 function which is known to confer a poorer prognosis.

1.8.2.4 Cyclo-oxygenase 2

Arachidonic acid metabolism is of fundamental importance in inflammation. Primary inflammatory cytokines such as tumour necrosis factor α, interleukin-1α, interleukin-1β and consequently interleukin-6 induce the cyco-oxygenase-2 gene to produce cyclo-oxygenase-2 enzyme. This enzyme synthesises pro-inflammatory prostaglandins (Dinarello., 1995).

Cyclo-oxygenase-2 is one of two cyclo-oxygenase isoforms, cyclo-oxygenase 1 & 2, which posses many similar enzymatic propertics, however differ in many ways. Cyclo-oxygenase 1 is constitutively expressed, however in the normal adult cyclo-oxygenase-2 is principally confined to the macula densa of the kidney and the brain. Cyclo-oxygenase-2 is up regulated in response to growth factors, tumour promoters and cytokines, and appears to bc the isoform most intrinsically related to acute inflammatory responses (Herschman *et al.*, 1996). Oncogenes havc also been noted to result in cyclo-oxygenase-2 expression including v-src, HER2/neu, Wntl and v-Ha-ras (Fosslien., 2000).

Expression of cyclo-oxygenase-2 can be induced through binding at the promoter region of the gene located on chromosome 1q25.2-3. These binding sites include those for nuclear factor-kappa B, cyclic AMP response element motifs, transforming growth factor-B response elements and nuclear factor interleukin-6 motifs (Chen *et al.*, 1999). Several elements are thought to act through this induction region including epidermal growth factor, transform growth factor-B, intracellular nitric oxide synthase, benzo[a]pyrene, interleukin-6 and androgens. Tumour necrosis factor α and interleukin-1 act to directly induce nuclear factor-kappa B which promotes cyclo-oxygenase-2 transcription.

Cyclo-oxygenase-2 and Neoplasia

Cyclo-oxygenase-2 over expression was first identified in colorectal carcinoma when compared to undetectable levels found in normal mucosa (Eberhart *et al.*, 1994). Subsequently aberrant cyclo-oxygenase-2 expression has now been identified in cancers affecting numerous organs, including lung, pancreas, stomach, oesophagus, prostate, bladder, cervix, skin and head and neck (Dannenberg *et al.*, 2001). Cyclo-oxygenase-2 is also implicated early in tumour genesis, as demonstrated in rodent models of intestinal tumour genesis, where premalignant adenomas are noted to express cyclo-oxygenase-2 (Boolbol *et al.*, 1996).

This implication of cyclo-oxygenase-2 mediated inflammation and tumour genesis has been highlighted in epidemiological studies examining the incidence of carcinoma in people regularly using non steroidal anti-inflammatory drugs which inhibit cyclo-oxygenase. Long term follow up over 35 years of patients treated with non-aspirin non steroidal anti-inflammatory drugs for at least 48 months had half the expected level of colorectal carcinoma development than the normal population (Cibere *et al.*, 1997).

Cyclo-oxygenase-2 is thought to mediate its effect on cancer formation via the formation of prostaglandins, in particular prostaglandin E2. These prostaglandins as well as having direct effects on cell proliferation are also thought to interfere with apoptosis, hence adding to tumour growth (Bandyopadhyay *et al.*, 1987). This impairment of apoptosis is thought to derive from increased levels of Bcl-2 secondary to prostaglandin synthesis (Sheng *et al.*, 1998). Bcl-2 expression facilitates closure of mitochondrial pores which prevents the escape of cytochrome c hence prevents normal apoptosis.

As well as preventing apoptosis cyclo-oxygenase-2 can have an inhibitory effect on E-cadherin to facilitate cell migration and invasion. It also induces hepatocyte growth factor production by surrounding stromal cells which enhances invasion, metastases and angiogenesis as well as functioning in a positive feedback mechanism in cyclo-oxygenase-2 induction (Hori *et al.*, 1993). Prostaglandins also increase the production of interleukin-6 forming a positive feedback loop supporting the potential autocrine growth function of this cytokine in renal cancer.

Cyclo-oxygenase-2 and Renal Cancer

Cyclo-oxygenase-2 is expressed in the normal kidney and is an important mediator of renal function. This makes renal cancer unique among the other solid tumours in which cyclo-oxygenase-2 has been examined. It is expressed in the macula densa, cortical thick ascending limb, inner medullary collecting duct cells and intercalated cells in the renal cortex (Fergusson *et al.*, 1999; Harris, 2003).

Normal renal physiology requires the active production and metabolism of prostaglandins (Norian *et al.*, 2002). Prostaglandin E2 (PGE2) is the most abundant form found in the renal tubules and regulates sodium and chloride transport in the loop of Henle as well as modulating water transport and renal medullary blood flow. Prostaglandin I2 is the most abundant prostaglandin in the renal cortex and regulates renal vascular tone, glomerular filtration rate and renin release (Dunn, 1984). Cyclo-oxygenase-2 is implicated in all of these processes.

The inflammatory response is associated with poor prognosis in renal cancer. The induction of cyclo-oxygenase-2 through inflammatory mediators, such as interleukin-6, would suggest likely enhanced cyclo-oxygenase-2 expression would be present in renal cancer tissue. Cyclo-oxygenase-2 is also induced by benzo[a]pyrene, a

product of cigarette smoking, and androgens, via described nuclear factor kappa B activation pathways, both known risk factors in the development of renal cancer. These known carcinogens may result in the constitutive expression of nuclear factor kappa B, and accordingly promote the interplay of interleukin-6 and cyclo-oxygenase-2 as the final common pathway. But cyclo-oxygenase-2 is constitutively expressed by renal tubules in health.

To date there are little data on the relationship between cyclo-oxygenase-2 expression and influence in renal cancer. The first study was reported in canine models, where increased expression was noted, and thought to represent cancer cells reverting to a less differentiated form (Khan *et al.*, 2001). In the only published series on human tumours Miyata and co workers (2003) found that 53.4% of renal tumours expressed cyclo-oxygenase-2. This is in contrast with other tumours such as colorectal where higher levels of expression are observed in 80-90% of cancers (Tomozawa *et al.*, 2000). With relation to prognosis cyclo-oxygenase-2 expression was not shown to be significantly associated with prognosis (Miyata *et al.*, 2003).

The implications of cyclo-oxygenase-2 expression in renal cancer are difficult to interpret due to its role in physiology. Should higher concentrations of interleukin-6 along with aberrant cyclo-oxygenase-2 expression be demonstrated this may indicate a potential point of intervention with cyclo-oxygenase-2 inhibitors. Cyclo-oxygenase-2 inhibitors have been shown to decrease endothelial tubule formation in vitro, and it is from these cells that tumours are thought to arise in the kidney (Jones *et al.*, 1999).

1.9 Summary and Aims

In summary the inflammatory mechanisms of tumour development are as yet poorly understood in relation to renal cancer. The systemic inflammatory response, evidenced by elevated C-reactive protein concentration, would appear to be of considerable importance in the relationship between tumour, host and prognosis. Preliminary work suggests that the lymphocytic infiltrate behaves in a different manner in renal cancer to other solid tumours. The link between this impaired T-lymphocytic response and the biologic nature of renal cancer cells may be mediated through the cytokine profile, in particular interleukin-6.

Precise localisation of pro-inflammatory cytokines to tumour cells or inflammatory cells within the tumour, particularly in paraffin embedded tissues, remains problematical. However, interleukin-6 activity has been assessed by staining for IL-6 receptor expression in a variety of solid tumours, including renal cancer (Costes et al., 1997). By staining the tumour specimen for interleukin-6 receptor and correlating this with systemic C-reactive protein measurements our aim would be to define if such association existed and hence the tumour responsible for the systemic inflammatory response.

Infiltration of tumours with T- lymphocytes has been reliably demonstrated to have prognostic significance in a variety of solid tumours, including renal cancer (Nakano et al., 2001; Bromwich et al., 2003). No study to date has examined or demonstrated interleukin-6 to be correlated with an impaired T lymphocytic response.

Central to the local inflammatory response is cyclooxygenase-2 and increased expression has been shown to be associated with poor survival in a number of common solid tumours (Dannenberg et al., 2001; Subbaramaiah and Dannenberg 2003).Whether the effect of interleukin-6 is dictated by its activation of cyclo-oxygenase-2 is not

known in renal cancer. Examination of cyclo-oxygenase-2 in renal cancer is of particular relevance when considering the intrinsic relationship between cyclo-oxygenase-2 and normal renal function.

Aims

1. To examine the relationship between the pre-operative systemic inflammatory response and cancer specific survival in patients undergoing potentially curative resection for renal clear cell cancer.

2. To examine the relationship between the systemic inflammatory response, tumour T-lymphocyte infiltration, interleukin-6 receptor and COX-2 expression and survival in patients undergoing resection for renal cancer

Chapter 2

2 THE RELATIONSHIP BETWEEN THE PRE-OPERATIVE SYSTEMIC INFLAMMATORY RESPONSE AND CANCER SPECIFIC SURVIVAL IN PATIENTS UNDERGOING POTENTIALLY CURATIVE RESECTION FOR RENAL CLEAR CELL CANCER.

2.1 Introduction

The ideal prognostic score for patients undergoing potentially curative resection of a primary renal cancer should clearly distinguish those who will eventually succumb to the disease from those who are cured. While TNM stage has been widely used, it fails to provide clear separation between these groups. This has lead to the development of a number of cumulative prognostic scores including TNM stage. TNM stage has been combined with tumour grade and performance status to form the UCLA Integrated Staging System (UISS, Zisman et al., 2002).

It is recognised that, in addition to tumour stage and proliferative activity, disease progression is dependent on a complex interaction of the tumour and host inflammatory response (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Vakkila and Lotze, 2004). Recently, the systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, has been shown to be a stage independent prognostic factor in patients undergoing potentially curative resection for colorectal cancer (McMillan et al., 2003), pancreatic cancer (Jamieson et al., 2005) and urinary bladder cancer (Hilmy et al., 2005).

The aim of the present study was to examine the prognostic value of the systemic inflammatory response in patients undergoing potentially curative resection for renal cancer.

2.2 Patients and Methods

Patients with renal clear cell cancer, who, on the basis of surgical findings and pre-operative computed tomography of chest abdomen and pelvis underwent potentially curative resection between August 1996 and November 2004 in the West of Scotland, were included in the study. To be defined potentially curative post operative histology had to determine surgically clear margins and no pathological evidence of nodal involvement and no CT evidence of metastases.

Data for 1996–2000 (n= 57) were collected retrospectively from the West of Scotland Renal Cancer Database hence are not truly sequential and that for 2001–2004 (n= 43) consequential and prospectively. Clinical stage and performance status (Eastern Cooperative Oncology Group, ECOG-ps) were recorded prior to surgery. Also, routine laboratory measurement of C-reactive protein was carried out.

Patients were staged pathologically according to the 1997 UICC TNM classification of renal tumours (Sobin et al., 1997). Tumours were graded according to criteria set out by Fuhrman and co-workers (1982). Tumour stage, grade and ECOG-ps were collated into the UCLA Integrated Staging System (UISS, Zisman et al., 2002, Table 1.7.1).

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

2.2.1 C-Reactive Protein Assay

Routine laboratory measurement of patient's serum for C-reactive protein concentration was performed. The limit of detection of the assay was a C-reactive protein concentration lower than 6mg/l. The coefficient of variation, over the range of measurement, was less than 5% as established by routine quality control procedures. C-reactive protein measurement of greater than 10mg/l was considered to indicate the presence of systemic inflammatory response (O'Gorman et al., 2000).

2.2.2 Statistical Analysis

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 October 2005 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).

2.3 Results

The characteristics of patients with renal cancer who underwent potentially curative resection (n= 100) are shown in Table 2.1 and Appendix I. The majority were male, over the age of 60 years, had good performance status and were defined as UISS intermediate risk. Approximately 40% had an elevated C-reactive protein (>10mg/l).

The minimum follow-up was 12 months; the median follow-up of the survivors was 59 months. During this period 25 patients died; 18 patients of their cancer and 7 of intercurrent disease. On univariate survival analysis, sex (p=0.050), tumour stage ($p \leq 0.001$), Fuhrman grade (p<0.001), UISS (p<0.001) and C-reactive protein (p<0.01) were significant predictors of cancer specific survival.

On multivariate analysis with sex, tumour stage, Fuhrman grade, performance status and C-reactive protein entered as covariates, only sex (HR 0.25, 95% CI 0.06–0.99, p=0.048), Fuhrman grade (HR 2.91, 95% CI 1.29–6.56, p=0.010) and C-reactive protein (HR 7.67, 95% CI 1.64–35.84, p=0.010) were significant independent predictors of cancer specific survival.

On multivariate analysis with sex, UISS and C-reactive protein entered as covariates, only UISS (HR 2.70, 95% CI 1.00–7.30, p=0.050) and C-reactive protein (HR 4.00, 95% CI 1.21–13.31, p=0.024) were significant independent predictors of cancer specific survival. A greater proportion of females compared with males had Fuhrman grade I tumours (10% vs 29%, p=0.030, Fisher's exact test).

The relationship between the systemic inflammatory response and the clinicopathological characteristics are shown in Table 2.2. 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 ($p<0.01$), increased grade ($p<0.05$), poorer performance status ($p<0.01$) and consequently a high UISS ($p<0.01$). Those patients with an elevated pre-operative C-reactive protein concentration (>10mg/l) had a mean cancer specific survival of 71 months compared with 96 months ($p<0.001$) in those patients with a C-reactive protein concentration in the normal range (≤10mg/l).

In those patients with a UISS risk classified as "low" or "intermediate" (n= 91) an elevated C-reactive protein concentration was associated with a decrease in cancer specific survival ($p= 0.008$, Figure 2.1).

2.4 Discussion

Surgical resection remains the only prospect for long term survival in patients with renal clear cell cancer. Currently, in patients undergoing surgery prognosis is based on tumour stage and grade and performances status (UISS, Zisman et al., 2002). In the present study the pre-operative measurement of C-reactive protein provided additional prognostic information.

To our knowledge this is only the second study to examine the role C-reactive in predicting survival following potentially curative resection for renal cancer. Masuda and colleagues (1998) in a retrospective study of 111 patients reported that C-reactive protein was a prognostic factor independent of tumour stage and grade. However, performance status was not considered and thresholds for C-reactive protein were not defined in the survival analysis.

Few studies have identified factors which give prognostic information in addition to the UISS criteria, namely 'T' stage, Fuhrman grade and ECOG-ps, in patients undergoing potentially curative resection for renal cancer. Recently, Shvarts and coworkers (2005) reported that tumour p53 expression was related to Fuhrman grade and displaced it in multivariate analysis. Similarly, Lam and colleagues (2005) reported that tumour Ki-67, unlike carbonic anhydrase, was related to the degree of necrosis and had independent prognostic value. Further studies are required to determine whether an elevated C-reactive protein has prognostic value independent of these other biological factors, in particular Ki-67.

It has been previously shown that, in patients undergoing potentially curative surgery for colorectal and pancreatic cancer, approximately one third and one half respectively, of patients had elevated circulating concentration of C-reactive protein

pre-operatively and that these patients had a significantly lower cancer specific survival (McMillan et al., 2003; Jamieson et al., 2005). It was of interest that, in the present study, the proportion of patients with an elevated pre-operative C-reactive protein concentration was similar and that these patients also had a poorer outcome.

It may be that because C-reactive protein concentration has prognostic value independent of UISS criteria, it might be added to these criteria to improve the prediction outcome, in particular the large group of "low or intermediate risk" patients with renal cancer. Indeed, this approach has recently been used to improve the prediction of outcome in patients who underwent potentially curative resection for colorectal cancer (Canna et al, 2005).

An elevated circulating C-reactive protein concentration is also an established poor prognostic factor in patients with metastatic renal cancer (Atzpodien et al., 2003; Bromwich et al., 2004; Casamassima et al., 2005). In our previous study of patients with metastatic renal cancer (Bromwich et al., 2004) approximately 70% had an elevated C-reactive protein (>10mg/l) compared with approximately 40% in the present study of primary operable disease. This is consistent with previous observations that the systemic inflammatory response increases with advancing disease (Mahmoud and Rivera, 2002). However, the basis of the independent relationship between an elevated C-reactive protein concentration and poor survival in renal cancer is not clear. There are a number of possible explanations. Firstly, that an elevated C-reactive protein identifies tumours capable of producing significant amounts of pro-inflammatory cytokines, in particular interleukin-6 (Kinoshita et al., 1999; McKeown et al., 2004) and therefore with the potential for more rapid growth of tumour cells (Jee et al., 2001; Trika et al., 2003). Also, that an elevated C-reactive protein identifies those patients with T-lymphocyte impairment (Maccio et al., 1998; Canna et al., 2005) or patients with

a pro-angiogenic environment (Kofler et al., 2005; Xavier et al., 2006) allowing unrestrained tumour growth and dissemination. Clearly, both these tumour and host mechanisms may be related and required for the greater malignant potential associated with an elevated C-reactive protein concentration.

This is a relatively small, partially retrospective study and requires verification in larger prospective cohorts. However, if an elevated C-reactive protein concentration is shown to offer prognostic value in addition to the current UISS criteria it would improve our staging of these patients. Moreover, C-reactive protein may offer a useful pre-operative therapeutic target in patients undergoing potentially curative surgery for renal clear cell cancer.

In summary, the presence of a pre-operative systemic inflammatory response predicts poor cancer specific survival in patients who have undergone potentially curative resection for renal clear cell cancer.

Table 2.1 Clinicopathological characteristics in patients undergoing potentially curative resection for renal cancer.

	Patients (n= 100)
Age group (≤60/ >60 yrs)	43/ 57
Sex (male/ female)	59/ 41
T stage (1/ 2/ 3/ 4)	36/ 19/ 42/ 3
Fuhrman grade (I/ II/ III/ IV)	18/ 39/ 26/ 13
ECOG-ps (0/ ≥1)	86/ 14
UISS (low/ intermediate/ high)	22/ 69/ 9
C-reactive protein (≤10/ >10mg/l)	58/ 42
Alive/ dead	75/ 25
Cancer specific/ intercurrent disease	18/ 7

Table 2.2 The relationship between the presence of a pre-operative systemic inflammatory response and clinicopathological characteristics of renal clear cell cancer.

	C-reactive protein ≤ 10mg/l (n= 58)	C-reactive protein > 10mg/l (n = 42)	p-value
Age group (≤60/ >60 yrs)	26/ 32	17/ 25	0.664
Sex (male/ female)	36/ 22	23/ 19	0.463
Tumour stage (1/ 2/ 3/ 4)	28/ 12/ 18/ 0	8/ 7/ 24/ 3	0.003
Fuhrman grade (I/ II/ III/ IV)	10/ 28/ 14/ 3	8/ 11/ 12/ 10	0.021
ECOG-ps (0/ ≥1)	54/ 4	32/ 10	0.016
UISS (low/ intermediate/ high)	17/ 40/ 1	5/ 29/ 8	0.003
Cancer specific Survival (months)*	95.9 (89.8-102.0)	70.8 (57.8-83.8)	<0.001

*Mean (95%CI)

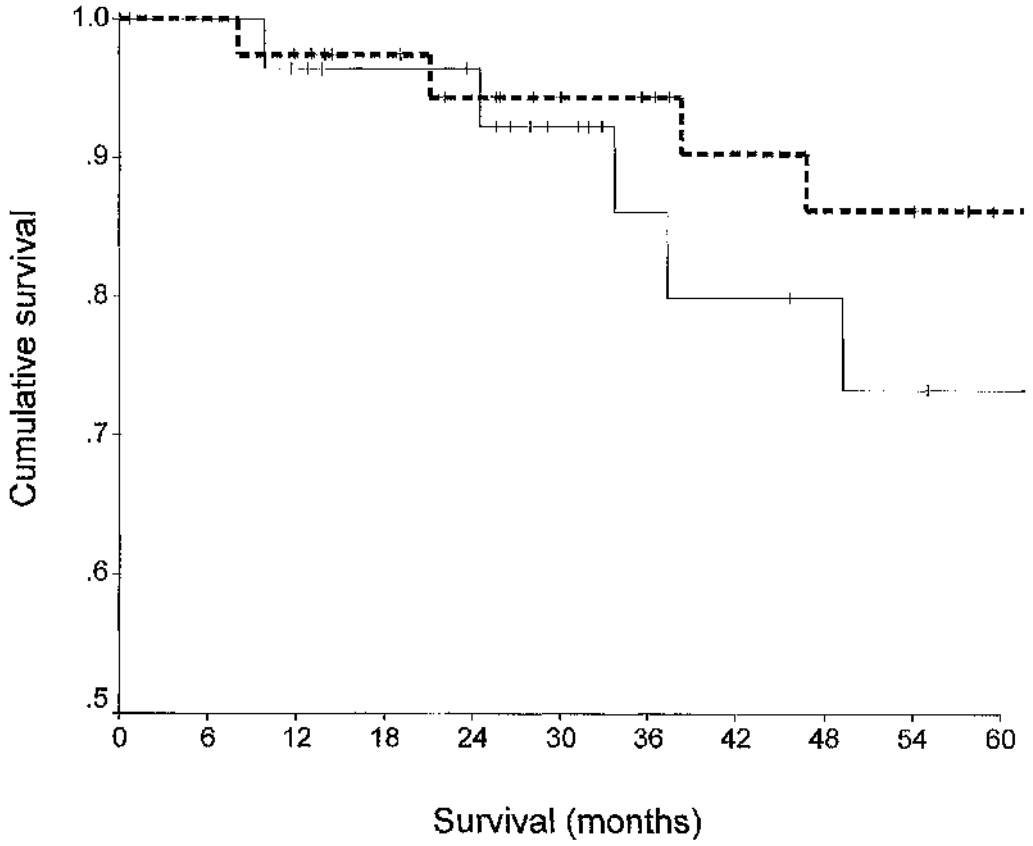


Figure 2.1. Relationship between preoperative C-reactive protein (≤10/ >10mg/l from top to bottom) and cancer-specific survival in "low or intermediate risk" patients (n= 91) undergoing potentially curative resection for renal cancer.

Chapter 3

3 THE RELATIONSHIP BETWEEN THE SYSTEMIC INFLAMMATORY RESPONSE, TUMOUR T-LYMPHOCYTE INFILTRATION, INTERLEUKIN-6 RECEPTOR AND COX-2 EXPRESSION AND SURVIVAL IN PATIENTS UNDERGOING RESECTION FOR RENAL CANCER

3.1 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. Overall survival is poor; even in those who undergo potentially curative resection, only approximately half survive 5 years (Cancerstats, www.cancerresearchuk.org).

It is increasingly recognised that cancer progression is dependent on a complex interaction of the tumour and host inflammatory response (O'Byrne and Dalgleish, 2001; Coussens and Werb, 2002; Vakkila and Lotze, 2004). Recently, the systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, has been shown to be independently associated with poorer survival in patients with other advanced malignancies (O'Gorman et al., 2000; McMillan et al., 2001; Maltoni et al., 2005). There is also evidence that C-reactive protein has independent prognostic value in a variety of primary operable cancers (McMillan et al., 2003; Ikeda et al., 2003; Hilmy et al., 2005; Jamieson et al., 2005). It would appear that the systemic inflammatory response is of considerable importance in the relationship between the tumour, the host and outcome in patients with cancer. Indeed, it has been reported that an elevated C-reactive protein is associated with poor cancer specific survival in patients with operable renal cancer independent of tumour stage and grade (Masuda et al., 1998; Chapter 2).

The basis of the independent relationship between an elevated C-reactive protein concentration and poor survival in cancer is not clear. There are a number of possible explanations. Firstly, that an elevated C-reactive protein identifies tumours capable of producing significant amounts of pro-inflammatory cytokines, in particular interleukin-6 (Kinoshita et al., 1999; McKeown et al., 2004) and therefore with the potential for more rapid growth of tumour cells (Jee et al., 2001; Trikha et al., 2003) or to impair T-lymphocytic function (Maccio et al., 1998; Canna et al., 2005) allowing unrestrained tumour growth and dissemination.

Precise localisation of pro-inflammatory cytokines to tumour cells or inflammatory cells within the tumour, particularly in paraffin embedded tissues, remains problematical (Canna et al., 2005) and the molecular basis of the increased production of interleukin-6 by the renal tumour remains to be defined. However, interleukin-6 activity has been assessed by staining for interleukin-6 receptor expression in a variety of solid tumours, including renal cancer (Costes et al., 1997) and infiltration of tumours with T- lymphocytes has been reliably demonstrated in a variety of solid tumours, including renal cancer (Nakano et al., 2001; Bromwich et al., 2003).

Central to the local inflammatory response is cyclooxygenase-2 and increased expression has been shown to be associated with poor survival in a number of common solid tumours (Dannenberg et al., 2001; Subbaramaiah and Dannenberg 2003). Cyclo-oxygenase-2 is the rate limiting enzyme in the synthesis of prostaglandin E2 which is known to stimulate proliferation and inhibit apoptosis. Prostaglandin E2 is also recognised to induce interleukin-6 (Fosslien, 2000) and non-steroidal anti-inflammatory drugs have been shown to reduce interleukin-6 concentrations in cancer patients (McMillan et al., 1995; Shimizu et al., 2001) and animal cancer models (Cahlin et al., 2000).

In normal tissues cyclo-oxygenase-2 is usually absent, however, the kidney is unusual in that there is expression of cyclo-oxygenase-2 in health (Harris, 2003). Few studies have examined the role of cyclo-oxygenase-2 in renal cancer. There is conflicting evidence as to whether increased cyclo-oxygenasse-2 expression is associated with poor survival in renal cancer (Miyata et al., 2003; Tuna et al., 2004).

The aim of the present study was therefore to examine the relationship between the systemic inflammatory response (C-reactive protein), interleukin-6 receptor expression, T-lymphocyte (CD4+, CD8+) infiltration and cyclo-oxygenase-2 expression and cancer specific survival in patients with renal cell carcinoma.

3.2 Patients and Methods

Patients diagnosed with renal clear cell cancer, who underwent resection between November 1997 and April 2004 in the West of Scotland, were included in the study.

Patients were staged clinically on the basis of surgical findings and pre-operative computed tomography of chest abdomen and pelvis and pathologically according to the 1997 UICC TNM classification of renal tumours (Sobin et al., 1997). Tumours were graded by experienced pathologists according to criteria set out by Fuhrman and coworkers (1982). Also, routine laboratory measurement of C-reactive protein was carried out.

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

3.2.1 C-Reactive Protein

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

3.2.2 Immunohistochemistry

Blocks from the primary tumour were fixed in 10% buffered formalin and embedded in paraffin wax. One representative block of tumour was selected for each patient. Sections (4 mm) were cut and mounted on slides coated with aminopropyltriethoxysilane. Slides were dewaxed in xylene and rehydrated using graded alcohols 100, 90 and 70% and water. Slides were then immersed in 0.3% hydrogen peroxide for 10 minutes (20 minutes for cyclo-oxygenase-2 and interleukin-6 receptor) to block endogenous peroxidases.

The following monoclonal antibodies were used; CD4 mouse monoclonal (Vector, Peterborough, UK) antibody 1:50 dilution. CD8 mouse monoclonal (Dako, Cambridgeshire, UK) antibody 1:100. Cyclo-oxygenase-2 IgG (Cayman Chemical Co., MI, USA) antibody 1:800 dilution. Interleukin-6 receptor α rabbit polyclonal (Santa Cruz Biotechnology Inc, USA) 1:500.

Antigen retrieval for CD4+ and interleukin-6 receptor was carried out by incubating in 10mM citrate buffer (Epitope retrieval solution x 10, Dako) in a calibrated water bath at 96°C for 75 minutes and 20 minutes respectively. Antigen retrieval for CD8+ and cyclo-oxygenase-2 was carried out by microwaving in 1 mM Tris EDTA buffer (5mM Tris base pH 8.0 and 1mM Sodium EDTA) for 5 minutes under full pressure in a plastic pressure cooker in a 850W microwave on full power.

Non specific background staining was blocked using 1.5% v/v normal horse serum in tri-phosphate buffered saline (TBS) and incubated for 15 minutes at room temperature. Only cyclo-oxygenase-2 underwent further block with avidin-biotin horseradish peroxidase complex solution (Vector Labs Inc., CA, USA) as per manufacturer's instructions. Sections were then blotted and incubated with CD4+ or

CD8+ or cyclo-oxygenase-2 or interleukin-6 receptor primary antibody at the above dilutions for 30 minutes at 27°C in a temperature controlled moisture chamber (chamber and same temperature used for all antibody incubations). CD4+ and CD8+ sections were then immunostained using the peroxidase-based Envision (Dako, Cambridgeshire, UK) technique Figures 3.1 and 3.2. Cyclo-oxygenase-2 and interleukin-6 receptor sections were immunostained using avidin-biotin based Lsab Plus kit (Dako, Cambridgeshire, UK) Figures 3.3 and 3.4. Sections were counterstained with haematoxylin, dehydrated, cleared and mounted with Pertex.

3.2.3 Morphometry

Quantitative analysis of the lymphoid infiltrate was performed using point counting with a random sampling technique as previously described (Bromwich et al, 2003). With this method, the volume occupied by any given component (volume density) is expressed as a percentage of the total volume of the tissue. In the present study, the volumes of CD4+ and CD8+ immunopositive cells were calculated as percentage of the total tumour volume. A 100 point ocular grid was used at x 400 magnification and 30 fields were counted per case for each antibody. Fields were scored according to the number of positively stained cells lying in contact with the vertical and horizontal cross hatch of the hundred point grid. Only fields within the tumour (including cancer cell nests and surrounding tissue stroma) were counted. Any normal tissue on the slide was excluded from the analysis. This final method was designed on the basis of a pilot study, which demonstrated that the volume density of CD4+ and CD8+ of two observers reached a plateau after 25-30 fields. This pilot study also demonstrated that CD4+ and CD8+ counts were equivalent to the CD3+ counts (unpublished data). The observers were blinded to the clinical outcome of the patient.

Cyclo-oxygenase-2 and interleukin-6 receptor expression were assessed as previously described (Wendrum et al., 2003). Percentage tumour cells stained were scored as 0-20% = 1, 21-50% = 2 and 51-100% = 3. This score is then multiplied by the predominant intensity of staining; 0 = no stain, 1 = weak stain, 2 = moderate stain and 3 = strong staining gaining a final score ranging from 0 – 9. For analysis, scores were grouped in tertiles.

Slides were examined by independent observers (GWAL, SR and PAM) blind to the clinical outcome of each patient.

Figure 3.1 Immunohistochemical staining of CD4+ T-lymphocytes (brown) within clear cell carcinoma of kidney (blue).

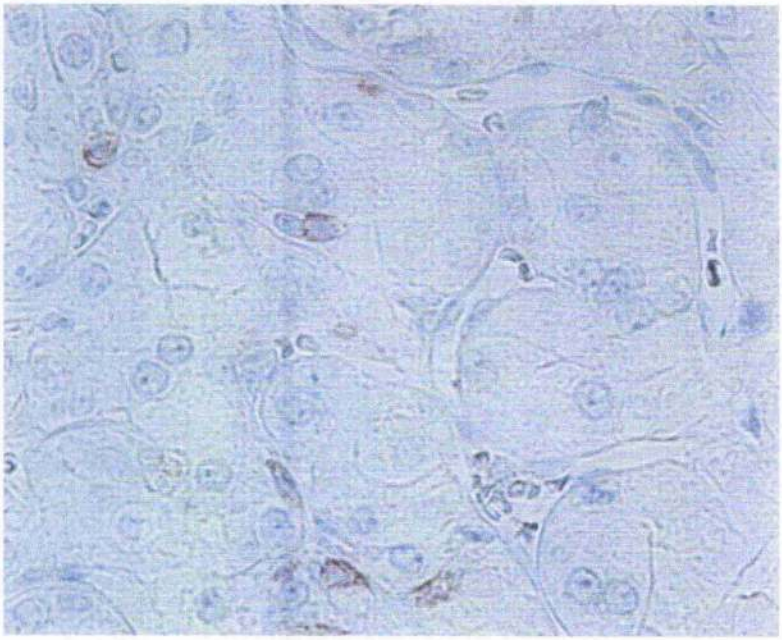


Figure 3.2 Immunohistochemical staining of CD8+ T-lymphocytes (brown) within clear cell carcinoma of kidney (blue)

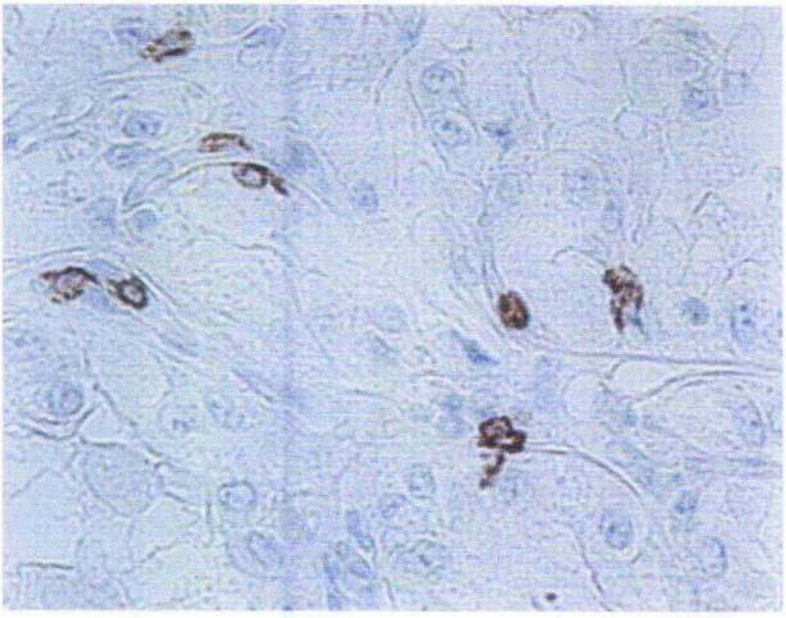


Figure 3.3 Immunohistochemical staining of cyclo-oxygenase-2 (brown) within renal clear cell cancer (blue).

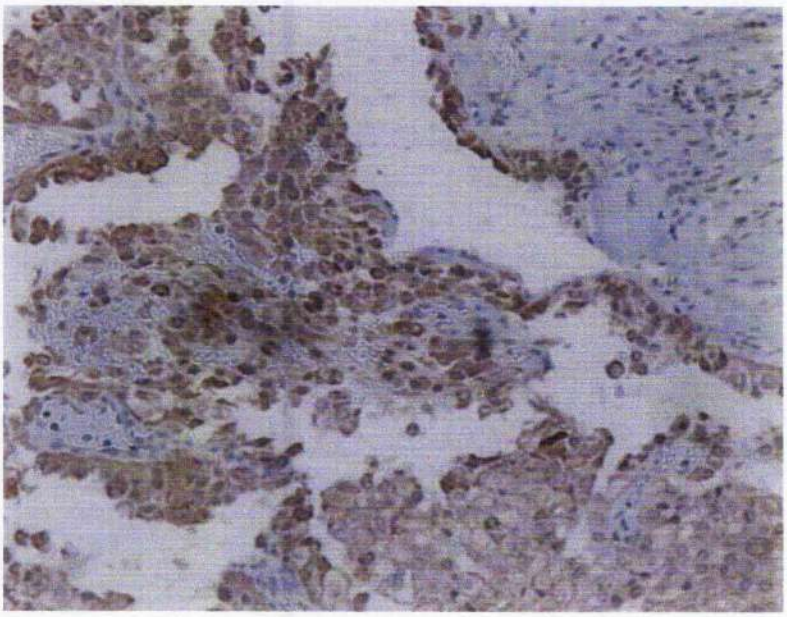
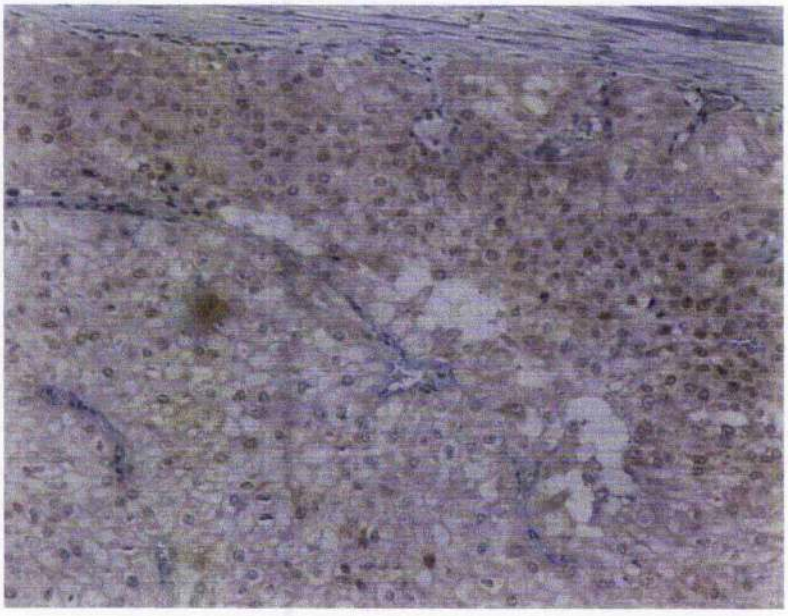


Figure 3.4 Immunohistochemical staining of interleukin-6 receptor (brown) within renal clear cell carcinoma (blue).



3.2.4 Statistics

Comparisons between groups of patients were carried out using contingency table analysis (X^2) for trend as appropriate. Survival analysis was performed using the Cox's proportional-hazards model. Deaths up to the end of February 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 and cancer specific survival of patients with renal cancer who underwent resection (n= 72) are shown in Table 3.1 and Appendix II. The majority of patients were male and similar numbers had localised or advanced disease. Immunohistochemical scores were grouped in tertiles for analysis. Approximately 40% had an elevated C-reactive protein concentration (>10mg/l).

The minimum follow-up was 23 months; the median follow-up of the survivors was 62 months. During this period 22 patients died; 16 patients of their cancer and 6 of intercurrent disease. On univariate survival analysis, age ($p<0.05$), tumour stage ($p<0.01$), Fuhrman grade ($p<0.001$), tumour CD4+ T-lymphocytic infiltration ($p<0.05$), tumour cyclo-oxygenase-2 expression ($p<0.10$) and C-reactive protein ($p<0.01$) were significant predictors of cancer specific survival. On multivariate analysis with age, tumour stage, grade, tumour CD4+ T-lymphocytic infiltration, cyclo-oxygenase-2 expression and C-reactive protein entered as covariates, tumour stage (HR 7.73, 95%CI 1.63-36.62, $p=0.010$), grade (HR 3.08, 95%CI 1.60-5.94, $p<0.001$) and C-reactive

protein (HR 2.78, 95% CI 1.00–8.84, p=0.046) were independent predictors of cancer specific survival.

Patients grouped according to stage are shown in Table 3.2. The groups were similar in terms of age, sex, tumour T-lymphocytic infiltration, cyclo-oxygenase-2 expression and interleukin-6 receptor expression. An increased tumour stage was associated with an increase in grade ($p<0.05$), and C-reactive protein ($p<0.05$).

The inter-relationships between the clinicopathological characteristics in patients undergoing resection for renal cancer are shown in Table 3.3. An increase in tumour CD4+ T-lymphocytic infiltration was associated with increased Fuhrman grade ($p<0.01$) and CD8+ T-lymphocytic infiltration ($p<0.001$). An increase in tumour CD8+ T-lymphocytic infiltration was associated with an increase in cyclo-oxygenase-2 expression ($p<0.05$). An increase in interleukin-6 receptor expression was associated with an elevated C-reactive protein ($p<0.10$). An elevated C-reactive protein was associated with increased tumour grade ($p<0.01$).

There was a significant direct relationship between C-reactive protein and percentage tumour CD4+ T-lymphocytes ($r_s=0.34$, $p=0.003$) and CD8+ T-lymphocytes ($r_s=0.29$, $p=0.014$).

3.4 Discussion

In the present study the presence of both local (tumour CD4+ lymphocytic infiltrate, cyclo-oxygenase-2 expression) and systemic (C-reactive protein) inflammatory responses were associated with poor cancer specific survival in patients undergoing resection for renal cancer. However, only C-reactive protein had independent prognostic value.

The relationship between increased tumour CD4+ lymphocytic infiltrate and poor cancer-specific survival is the opposite of that previously reported in patients with primary operable colorectal cancer (Canna et al., 2005). Furthermore, Canna and coworkers (2005) reported that increased tumour CD4+ lymphocytic infiltrate was inversely, rather than directly, associated with C-reactive protein. Nevertheless, consistent with our data, C-reactive protein had independent prognostic value whereas tumour CD4+ lymphocytic infiltrate did not (Canna et al., 2005).

The reasons for the contrasting relationships between tumour CD4+ lymphocytic infiltrate and C-reactive protein in these different tumours is not clear. However, it is well recognised that, in contrast to colorectal cancer, renal cancer is strongly immunogenic (Zou, 2005). In the present study, tumour CD4+ lymphocytic infiltrate was strongly associated with tumour grade and would be consistent with the tumour CD4+ lymphocytic infiltrate being directly linked to the malignant potential of the tumour.

There was a direct correlation between CD8+ lymphocytic infiltrate and cyclo-oxygense-2 expression in renal cancer patients. This is in contrast with observations in endometrial cancer where an inverse relationship has been observed (Ohno et al., 2005). Ohno and co workers suggest that in endometrial cancer cyclo-oxygenase-2 produced

by tumour cells reduces CD8+ infiltration and subsequently confers a poorer prognosis. Neither CD8+ lymphocytic infiltrate or cyclo-oxygenase-2 expression were independently prognostic in renal cancer and although significantly associated this would not appear to be an important step in tumour progression.

Elevated circulating C-reactive protein concentrations are primarily determined by circulating interleukin-6 (Gabay and Kushner, 1999). Interleukin-6 is expressed in the majority of renal cancers and has been proposed as an autocrine growth factor (Takenawa *et al.*, 1991; Blay et al 1992; Paule *et al.*, 2000). Furthermore, circulating interleukin-6 is associated with increased tumour grade, volume and metastasis in patients with renal cancer (Yoshida et al., 2002). In our laboratory, previous groups have attempted to assess interleukin-6 within the paraffin embedded tumour samples using different methods of antigen retrieval and staining and the use of negative and positive controls. Co-workers in our laboratory have been unable to reliably identify regions of interleukin-6 expression due to deep background staining, which precludes accurate scoring of interleukin-6-positive cells in the tumour tissue (Canna et al., 2005). Interleukin-6 receptor expression is readily stained for and has been shown to correlate strongly with tumour interleukin-6 concentrations in a variety of solid tumours (Miki et al., 1989; Kinoshita et al., 1999). However, our results show tumour expression of interleukin-6 receptor not to be strongly associated with C-reactive protein. Further investigation of the relationship between tumour interleukin-6 immunohistochemical staining and circulating concentrations of C-reactive protein is warranted.

To our knowledge only one previous study has examined the prognostic value of tumour expression of interleukin-6 receptor (Costes et al., 1997). Costes and coworkers (1997) reported a significant association between the presence of the interleukin-6

receptor in renal tumours and stage and grade. Furthermore, they reported that those patients with positive staining of interleukin-6 receptor had poorer survival. This is in contrast to our data where interleukin-6 receptor demonstrated no association with stage, grade or survival. This previous study examined 38 patients with mean follow-up 14 months compared with 72 patients and a mean follow-up of approximately 5 years in the our cohort.

Few studies have examined the expression of cyclo-oxygenase-2 in renal cancer. Some studies have suggested that it is increased with tumour stage and grade (Miyata et al., 2003, Hashimoto et al., 2003; Tuna et al., 2004). In contrast, other studies have shown no relationship (Cho et al., 2005). To date no studies have shown that tumour cyclo-oxygenase-2 expression has prognostic value (Miyata et al., 2003; Tuna et al., 2004; Cho et al., 2005). The basis of such differences in the relationship between tumour cyclo-oxygenase-2 expression and tumour stage and grade in different studies is not clear. However, the lack of a relationship between tumour cyclo-oxygenase-2 expression and tumour stage and grade in the present study and that of Cho and coworkers (2005) is consistent with tumour cyclo-oxygenase-2 expression having limited prognostic value. Additionally cyclo-oxygenase-2 expression was not associated with circulating C-reactive protein suggesting that in renal cancer the stimulating factors influencing the systemic inflammatory response are independent of local prostaglandin synthesis. This may be a consequence of the fact that cyclo-oxygenase-2 is expressed in normal renal endothelial tubular cells from which tumour cells are thought to arise (Jones et al., 1999).

In summary, the results of the present study show that up-regulation of both local and systemic inflammatory responses are associated with poor cancer specific survival in patients undergoing resection for renal cancer. However, only the systemic inflammatory response (C-reactive protein) appears to have independent prognostic value.

Table 3.1 Clinicopathological characteristics and survival in patients undergoing resection for renal cancer: univariate analysis.

	Patients (n= 72)	Hazard ratio (95%CI)	p-value
Age group (≤60/ >60 yrs)	35/ 37	0.20 (0.06-0.71)	0.013
Sex (male/ female)	43/ 29	0.67 (0.23-1.94)	0.463
TNM stage (≤II/ >II)	37/ 35	10.27 (2.33-45.35)	0.002
Fuhrman grade (I/ II/ III/ IV)	16/ 29/ 14/ 12	3.48 (1.94-6.24)	<0.001
T-lymphocytes (% tumour volume)			
CD4+ (tertiles 1, 2, 3)	0.45 (0.01-6.30)*	2.32 (1.14-4.71)	0.020
CD8+ (tertiles 1, 2, 3)	0.90 (0.01-10.80)*	1.39 (0.75-2.58)	0.296
Cyclo-oxygenase-2 (tertiles 1, 2, 3)	100 (0-200)*	1.73 (0.92-3.23)	0.088
Interleukin-6 receptor (tertiles 1, 2, 3)	140 (0-300)*	0.95 (0.51-1.77)	0.878
C-reactive protein (≤10/ >10 mg/l)	40/ 32	5.20 (1.67-16.20)	0.005

*median range

Table 3.2 The relationship between tumour stage and clinicopathological characteristics in patients undergoing resection for renal cancer.

	TNM stage ≤II (n= 37)	TNM stage >II (n= 35)	P-value
Age group (≤60/ >60 yrs)	15/ 22	20/ 15	0.162
Sex (male/ female)	21/ 16	22/ 13	0.600
Fuhrman grade (I/ II/ III/ IV)	10/ 18/ 5/ 3	6/ 11/ 9/ 9	0.017
T-lymphocytes (% tumour volume)			
CD4+ (tertiles 1, 2, 3)	12/ 16/ 9	12/ 8/ 15	0.390
CD8+ (tertiles 1, 2, 3)	11/ 14/ 11	12/ 10/ 13	0.774
Cyclo-oxygenase-2 (tertiles 1, 2, 3)	14/ 14/ 9	10/ 10/ 15	0.152
Interleukin-6 receptor (tertiles 1, 2, 3)	10/ 12/ 15	14/ 12/ 9	0.348
C-reactive protein (≤10/ >10 mg/l)	25/ 12	15/ 20	0.036

Table 3.3 The inter-relationships between the clinicopathological characteristics in patients undergoing resection for renal cancer.

	CD4+	CD8+	Cyclo-oxygenase-2	Interleukin-6 receptor	C-reactive protein
	(tertiles 1, 2, 3)	(tertiles 1, 2, 3)	(tertiles 1, 2, 3)	(tertiles 1, 2, 3)	(≤10/ >10 mg/l)
Fuhrman grade	p-value	p-value	p-value	p-value	p-value
(I/ II/ III/ IV)	0.003	0.209	0.161	0.497	0.003
T-lymphocytes					
(% tumour volume)		p-value	p-value	p-value	p-value
CD4+ (tertiles 1, 2, 3)		<0.001	0.726	0.861	0.249
			p-value	p-value	p-value
CD8+ (tertiles 1, 2, 3)			0.035	0.160	0.149
Cyclo-oxygenase-2				p-value	p-value
(tertiles 1, 2, 3)				0.114	0.773
Interleukin-6 receptor					p-value
(tertiles 1, 2, 3)					0.084

Chapter 4

4 DISCUSSION

4.1 Introduction

In Chapter 1 the aims of this thesis were defined as follows:

1. To examine the relationship between the pre-operative systemic inflammatory response and cancer specific survival in patients undergoing potentially curative resection for renal clear cell cancer.

2. To examine the relationship between the systemic inflammatory response, tumour T-lymphocyte infiltration, interleukin-6 receptor and COX-2 expression and survival in patients undergoing resection for renal cancer

4.2 General Discussion

Other than stage and grade there are few universally accepted criteria for predicting relapse in patients undergoing potentially curative resection for renal cancer. Patient factors are thought to be of importance and accordingly, in designing the UCLA Integrated Staging System (UISS), performance status was combined with stage and grade to give an overall risk of relapse (Zisman et al., 2002).

The UISS has been developed from data accrued from American surgical practice where traditionally a more aggressive approach is observed in the management of patients with potentially respectable disease. By definition, according to the criteria described in the original paper, patients defined as high risk of relapse have either T4 disease indicating invasion of gerotas fascia and surrounding structures or have locally advanced disease including renal vein involvement with a poor performance status.

Considering patients with T4 disease which equates to TNM stage 4 disease only 37% of patients will be alive after 1 year (Table 1.5). With regard to the locally advanced patients with poor performance status there is good evidence to suggest that

more advance tumours and poor performance status result in a significant increased risk of peri-operative mortality in a mainly elderly population of patients (Stephenson et al., 2004; Gupta et al., 2004). These UISS high risk of relapse patients are less likely to be offered surgery on this basis in the U.K. This is reflected in our distribution of patients taken from a specialist centre for renal cancer where 22 were low, 69 intermediate and only 9 high risk patients. This is compared to the original paper which describes equal distribution of patients among the three categories (Zisman et al., 2002). This makes the UISS potentially limited in application to a UK population as there is little stratification of UK patients undergoing nephrectomy.

Previous work has suggested that the presence of a systemic inflammatory response is a stage independent negative prognostic factor in colorectal cancer (McMillan et al., 2003), pancreatic cancer (Jamieson et al., 2005) and urinary bladder cancer (Hilmy et al., 2005). The study described in Chapter 2 shows that elevated circulating C-reactive protein is also an independent prognostic factor in patients undergoing potentially curative resection for renal cancer.

Elevated C-reactive protein as compared to ECOG-performance status is an objective variable with defined threshold and reproducible values (O'Gorman et al., 2000). Indeed studies incorporating both of these variables together have shown C-reactive protein to be of greater prognostic value on multivariate analysis (O'Gorman et al., 2000; Bromwich et al., 2003). This would suggest that compared to the somewhat more subjective performance status assessment versus the ease of measurement of C-reactive protein with greater numbers and longer follow up a more clinically useful prognostic score for a UK renal cancer population could be developed. This is further substantiated by the fact that within the large low and intermediate risk group described

in chapter 2 we were able to demonstrate prognostic significance of elevated C-reactive protein in this sub-group analysis.

Further to this body of work myself and co-workers have pursued application and validation of the systemic inflammatory response into defining a Glasgow Prognostic Score (GPS) for renal cancer. The GPS was constructed as previously described by Forrest and co-workers (2003) in small cell lung cancer. Briefly, patients with both an elevated C-reactive protein (>10 mg/l) and hypoalbuminaemia (<35g/l) are allocated a score of 2. Patients in whom only one of these biochemical abnormalities is present were allocated a score of 1. Patients in which neither of these abnormalities are present are allocated a score of 0.

This was first published examining patients with metastatic renal cancer comparing the established Memorial Sloan Kettering Cancer Centre (MSKCC) and The Metastatic Renal Carcinoma Comprehensive Prognostic System (MRCCPS) scores with the GPS. The GPS predicts survival, independent of established scoring systems, in patients with metastatic renal cancer (Ramsey et al., 2007).

In a subsequent study C-reactive protein has been compared with other circulating systemic inflammatory markers namely neutrophil, lymphocyte, platelet and albumin concentration in conjunction with the validated Kattan, UCLA and Ssign score (Ramsey et al., 2007). C-reactive protein demonstrated superiority over all other systemic inflammatory markers in predicting outcome and stratification in patients undergoing curative nephrectomy for localized renal cancer. Indeed C-reactive protein alone proved superior to the Kattan score in predicting disease specific survival after nephrectomy. Within this study due to the independent superiority of C-reactive protein and tumour necrosis over albumin a refinement to the Glasgow Renal Cancer Prognostic Score should include both of these parameters. This represents the fulfillment of the

pilot work carried out and described in chapter 2 of this thesis. Larger cohort studies with longer follow up as well as other institutions are required to internationally validate such a predictive score however the ease of application will potentially allow for greater uptake of its use in clinical practice.

Having examined the influence of the systemic inflammatory response on outcome in Chapter 3 we examined the relationship of the systemic and the local inflammatory response. To do this we looked at the local cellular mediators of the local inflammatory response defined by tumour lymphocytic infiltrate and the potential effector mediators of inflammation in the form of cyclo-oxygenase-2 and interleukin-6 receptor.

The relationship between systemic and local inflammatory response has previously been reported in colorectal cancer where there was an inverse correlation between tumour CD4+ T-lymphocytic infiltration and circulating concentrations of C-reactive protein in patients undergoing resection (Canna et al., 2005).

By contrast we found a direct relationship between C-reactive protein and CD4+ lymphocytic infiltrate in patients with renal cancer. Renal cancer is a highly immunogenic tumour with the only proven treatment of advanced disease being immunotherapy (Bower et al., 1998).

It is of interest that despite a more pronounced CD4+ tumour lymphocytic infiltrate these patients have a worse outcome after surgery. This would suggest that despite being present in high numbers the T-lymphocytes are ineffective in mounting an appropriate response to either control development or prevent progression of the disease.

It is perhaps less surprising that with such greater numbers of tumour inflammatory cells there is a direct correlation with C-reactive protein and the systemic inflammatory response. How these two factors are linked is not clear, but interleukin-6 may be implicated as this cytokine has been previously suggested by Blay and co-workers to act as an autocrine growth factor in renal cancer, and correlated well with serum C-reactive protein levels (1992). The site and source of production of interleukin-6 is controversial. Either the tumour cells or the inflammatory infiltrate may excrete interleukin-6.

Accordingly we examined the local mediators of inflammation within renal cancer. Cyclo-oxygenase 2 is an important enzyme responsible for prostaglandin production and inflammation and has been implicated in the tumour genesis and progression of a number of solid tumours (Subbaramaiah and Dannenberg, 2003). Cyclo-oxygenase 2 produced prostaglandins are recognised to induce interleukin-6 production (Fosslien, 2000)

As discussed in Chapter 3 direct staining of tissues for interleukin-6 itself has proven difficult by co-workers in our laboratory and accordingly staining for its receptor was used as a surrogate marker. Our study did not show any association between cyclo-oxygenase-2 expression and interleukin-6 receptor expression. Additionally, neither factor was an independent predictor of cancer specific survival. The lack of association would refute the hypothesis that tumour cyclo-oxygenase-2 expression and consequently local prostaglandin synthesis is responsible for local interleukin-6 production in renal cancer. Both increased cyclo-oxygenase-2 expression and an increase in local interleukin-6 production in renal cancer were independent of the systemic inflammatory response. The limited prognostic value of cyclo-oxygenase-2

and independence from the systemic inflammatory response may be related to renal tissue being unique in its constitutive expression of cyclo-oxygenase-2 in health.

Local prostaglandin cyclo-oxygenase-2 mediated inflammatory response would appear to be independent of the tumour CD4 lymphocytic infiltrate. Prostaglandins in renal cancer would therefore not seem to be important factors involved in recruitment of CD4+ lymphocytes previously shown to have independent prognostic significance.

Tumour cell expression of interleukin-6 receptor as a surrogate marker for interleukin-6 was independent of circulating C-reactive protein indicating that the interleukin-6 responsible for the systemic inflammatory response is produced by inflammatory supporting cells rather than the tumour itself. Indeed there was a positive relationship between CD4+ tumour lymphocytic infiltrate and C-reactive protein suggesting that these cells may be a potential source of interleukin-6.

This may offer an explanation as to why this infiltrate is ineffective in preventing tumour progression in renal cancer. Th2 cells which are a subset of CD4+ T-lymphocytes, produce interleukin-6 as part of their cytokine profile promoting predominantly humoral immunity and a suppression of cytotoxic cellular response such as natural killer cells and CD8+ lymphocytes (Cher et al 1987; Stevens et al., 1988; Steiner et al., 1999). In comparison the Th1 subset of CD4+ lymphocytes which produce both interleuikin-2 and interferons which have been shown to improve survival in advanced cancer and promote a cytotoxic cell mediated immunity (Bower et al., 1998). Therefore, it may be that a predominance of the Th2 subset in those patients with greater T-lymphocytic infiltrate would explain the poorer cancer specific survival.

Further work has demonstrated that patients with an elevated systemic inflammatory response pre-operatively treated with nephrectomy show little in the way

of normalisation of C-reactive protein, serum interleukin-6 and serum interleukin-10 at 3 months post procedure (Ramsey et al. 2006). Fujikawa and co-workers (1999) examining C-reactive protein in patients undergoing cytoreductive nephrectomy propose that patients with an elevated postoperative nadir C-reactive protein may signify interleukin-6 secretion at a higher rate from metastatic lesions. The more probable explanation based on the work published in this thesis and subsequent study by Ramsey and co-workers (2006) would suggest that this persistently elevated systemic inflammatory response is unlikely to be determined by the tumour or the metastatic lesions but rather an impaired immune cytokine response in patients with renal cancer.

The assertion that the source of interleukin-6 and interleuikin-10 is out with the tumour itself is supported by this thesis. Interleukin-6 receptor expression was not significantly associated with systemic C-reactive protein. Preliminary unpublished work by myself and co-workers has failed to show any direct link between tissue interleukin-6 receptor expression and elevated serum interleukin-6 concentrations in a small number of patients studied. A biological model of a patient with impaired cytokine mediated immune response facilitating tumour development and progression could be explained on the basis of these results. To expand this theory the tissue micro-environment is based on a systemic decline rather than anything local to the tumour itself and as such a systemic therapy is required to address the poor prognosis of patients with evidenced elevated systemic inflammatory response.

As mentioned earlier in this discussion the possibility of the local infiltrate CD4+ lymphocytic helper subset determining the extent and the effect of the CD8+ lymphocytic infiltrate may be suggested by the correlation between CD4+ lymphocytic infiltrate and systemic C-reactive protein. In light of the study by Ramsey and co-

workers (2006) which suggests the source of the inflammatory cytokines to be separate from the tumour this would appear an inadequate explanation.

One hypothesis based on the work in this thesis would be that the tumour lymphocytic infiltrate is a local representation of the immune system as a whole. Furthermore a generic alteration in the cytokine profile of active CD4+ lymphocytes throughout the body is skewed towards the Th2 subset hence fitting the biological model of a patient with impaired cytokine mediated immune response facilitating tumour development and progression. Ramsey and co-workers (2006) demonstrated a positive significant association between the hallmark cytokines of Th2 CD4+ lymphocytes, systemic interleuikin-6 and interleukin-10 in patient with renal cancer. Further work is warranted to study these serological markers of cytokine expression along with the tissue based lymphocytic infiltrate.

The growing body of evidence including the work presented in this thesis suggest that both the local and systemic inflammatory response are of significant importance in determining survival in patients with renal cancer. Interleukin-6 remains the most probable inflammatory mediator to link the inflammatory processes and although our data may speculatively propose that the inflammatory cells and an impaired immune system are responsible, the site and source of production remain unclear and further study is warranted.

Chapter 5

5 CONCLUSIONS

The outcome of this work would suggest that the presence of a systemic inflammatory response is a negative prognostic factor. Measurement of C-reactive protein and the association with outcome in renal cancer would appear to be of significant importance. Currently the West Of Scotland Renal Cancer Database continues to collect prospective data including C-reactive protein on patients undergoing nephrectomy for renal cancer. With increasing patient numbers and follow up of these additional patients and those reported in this thesis the primary objective of this data would be the further development of the Glasgow Prognostic Score algorithm.

The systemic response was directly related to an increased T-lymphocytic infiltrate. Why this lymphocytic infiltrate is ineffective in preventing tumour progression is unclear, but on the basis of this research it would appear to be independent of cyclo-oxygenase-2 mediated local interleukin-6 production.

Further work is required to define the possible mechanisms by which the local inflammatory infiltrate has a negative effect on outcome in renal cancer whereas it has survival benefit in other tumours. Interleukin-6 is a cytokine associated with a CD4+ type 2 humoral immunity and suppresses the cytotoxic immune response. Another important inhibitory cytokine is interleukin-10 known to attenuate immune function and produced by the same CD4+ type 2 lymphocytic subset. It would be of interest to examine how serum and tissue levels of interleukin-10 relate to circulating C-reactive protein and tumour lymphocytic infiltrate in renal cancer.

In conclusion the present studies would suggest the systemic inflammatory response may be a useful therapeutic target in patients with primary renal clear cell carcinoma.

REFERENCES

Abbou CC, Cicco A, Gasman D, Hoznek A, Antiphon P, Chopin DK, Solomon L (1999). Retroperitoneal laparoscopic versus open radical nephrectomy. *J Urol*, **161**, 1776-1780.

Akdis CA, Blaser K (2001). Mechanisms of interleukin-10 mediated immune suppression. *Immunology*, **103**, 131-136.

Amen MA, Crotty TB, Tickoo SK, Farrow GM (1997). Renal oncocytoma: a reappraisal of morphological features with clinicopathological findings in 80 cases. *Am J Surg Pathol*, **21**, 1-12.

American Joint Committee on Cancer (AJCC). AJCC Cancer Staging Manual, Sixth Edition (2002). pub Springer-Verlag NY.

Andreyev HJ, Norman AR, Oates J, Cunningham D (1998). Why do patients with weight loss have a worse outcome when undergoing chemotherapy for gastrointestinal malignancies? *Eur J Cancer*, **34**, 503-509.

Assimos DG, Boyce WH, Woodruff RD, Harrison LH, McCullough DL, Kroovand RL (1991). Intraoperative renal ultrasonography: a useful adjunct to partial nephrectomy. *J Urol*, **146**, 1218-1220.

Atzpodien J, Royston P, Wandert T, Reitz M; DGCIN -- German Cooperative Renal Carcinoma Chemo-Immunotherapy Trials Group. (2003) Metastatic renal carcinoma comprehensive prognostic system. *Br J Cancer*. **88**:348-53.

Baer SC, Ro JY, Ordonez NG (1993). Sarcomatoid collecting duct carcinoma: a clinicopathologic and immunohistochemical study of five cases. *Hum Pathol*, **24**, 1017-1022.

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

Bandyopadhyay GK, Imagawa W, Wallace D, Nandi S (1987). Lieolate metabolites enhance the in vitro proliferative response of mouse mammary epithelial cells to epidermal growth factor. *J Biol Chem*, **262**, 2750-2756.

Barrett PH, Fentie DD, Taranger LA (1998). Laparoscopic radical nephrectomy with morcellation for renal cell carcinoma: the Saskatoon experience. *Urology*, **52**, 23-28.

Baynes J, Dominiczak MH. Medical Biochemistry. (1999) 1st Ed Harcourt Brace and Co Ltd. p.29

Bechtold RE, Zagoria RJ (1997). Imaging approach to staging of renal cell carcinoma. *Urol Clin North Am*, **24**, 507-522.

Blasting R, Corvin S, Handel D, Hinke A, Schmidt D (1999). Adjuvant immunotherapy in renal cell carcinoma: comparison of interferona alpha treatment with an untreated control. *Anticancer Res*, **19**, 1545-1548.

Blay JY, Negrier S, Combarlet V, Attali S, Goillot E, Merrouche Y, Maercatello A, ravault A, Tourani JM, Moskovtenko JF (1992). Serum level of Il-6 as a prognostic factor in metastatic renal cell carcinoma. *Cancer Res*, **52**, 3317-3322.

Boolbol SK, Dannenberg AJ, Chadburn A, Martucci C, Guo XJ, Ramonetti JT, Abrou-Goris M, Newmark HC, Lipkin ML, Bertagnoli MM (1996). Cyclooxygenase-2 overexpression and tumour formation are blocked by sulindac in murine model of familial polyposis. *Cancer Res*, **56**, 2556-2560.

Bosnib SM, Gibson D, Mhoon M, Greene GF (2000). Renal sinus involvement in renal cell carcinomas. *Am J Surg Pathol*, **24**, 451-458.

Bower M, Roylance R, Waxman J (1998). Immunotherapy for renal cell cancer. *Q J Med*, **91**, 597-602.

Bretheau D, Lechevallier E, de Fromont M, Sault MC, Rampal M, Coulange C (1995). Prognostic value of nuclear grade of renal cell carcinoma. *Cancer*, **76**, 2543-2549.

Bretheau D, Lechevallier E, Eghazarian C, Grisoni V, Coulange C (1995). Prognostic significance of incidental renal cell carcinoma. *Eur Urol*, **27**, 319-323.

Bromwich EJ, McArdle PA, Canna K, McMillan DC, McNicol A-M, Brown M Aitchison M. (2003) The relationship between T-lymphocyte infiltration, stage, tumour grade and survival in patients undergoing curative surgery for renal cell carcinoma. *Br J Cancer*, **89**, 1906-1908.

Bromwich EJ, McMillan DC, Lamb GWA, Vasey PA, Aitchison M (2004). The systemic inflammatory response, performance status and survival in patients undergoing alpha interferon treatment for advanced renal cancer. *Br J Cancer*, 91, 1236-1238

Butler BP, Novick AC, Miller DP Campbell SA, Licht MR (1995). Management of small unilateral renal cell carcinomas: radical versus nephron sparing surgery. *Urology*, **45**, 34-40.

Cahlin C. Korner A. Axelsson H. Wang W. Lundholm K. Svanberg E (2000). Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon-gamma, and tumor necrosis factor alpha evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia. *Cancer Research*, **60**, 5488-5493

Canna K, McArdle PA, McMillan DC, McNicol A-M, Smith GW, McKee RF, McArdle CS (2005) The relationship between tumour T-lymphocyte infiltration, the systemic inflammatory response and survival in patients undergoing curative resection for colorectal cancer. *Br J Cancer*, **92**, 651-654.

Canna K, McMillan DC, McKee RF, McNicol A-M, Horgan PG, McArdle CS (2004). Evaluation of a cumulative prognostic score based on the systemic inflammatory response in patients undergoing potentially curative surgery for colorectal cancer. *Br J Cancer,* **90,** 1707-1709.

Casamassima A, Picciariello M, Quaranta M, Berardino R, Ranieri C, Paradiso A, Lorusso V, Guida M. (2005) C-reactive protein: a biomarker of survival in patients with metastatic renal cell carcinoma treated with subcutaneous interleukin-2 based immunotherapy. *J Urol.* **173**:52-5.

Catalano C, Fraioli F, Laghi A (2003). High resolution multidetector CT in the preoperative evaluation of patients with renal cell carcinoma. *Am J Radiol*, **180**, 1271-1277.

Chan DY, Koniaris L, Magee C, Ferrell M, Solomon S, Lee BR, Anderson JH, Smith DO, Czapski J, Kavoussi LR (1999). Feasibility of ablating normal renal parenchyma by interstitial photon radiation energy: study in a canine model. *J Endourol*, **14**, 111-116.

Chapelon JY, Margonari J, Theillere Y, Gorry F, Vernier F, Blanc E, Gelet A (1992). Effects of high intensity focused ultrasound on kidney tissue in the rat and dog. *Eur Urol*, **22**, 147-152.

Chen G, Wood EG, Wang SH, Warner TD (1999). Expression of cyclooxygenase-2 in rat vascular smooth muscle cells is related to NF-kB activation. *Life Sci*, **64**, 1231-1242.

Cher DJ, Mosmann TR (1987). Two types of murine helper T cell clone. II delayed-type hypersensitivity is mediated by Th1 clones. *J Immunol*, **138**, 3688-3694.

Cho DS. Joo HJ. Oh DK. Kang JH. Kim YS. Lee KB. Kim SJ (2005). Cyclooxygenase-2 and p53 expression as prognostic indicators in conventional renal cell carcinoma. *Yonsei Med J,*. **46**, 133-40.

Chow WH, Devesa SS, Warren JL, Fraumeni JF jr (1999). Rising incidence of renal cell cancer in the United States. *JAMA,* **281**, 1628-1631.

Chow WH, Gridley G, Fraumeni JF Jr, Jarvholm B (2000). Obesity and hypertension, and the risk of renal cancer in men. *N Engl J Med*, **343**, 1305-1311.

Cibere J, Sibley J, Haga M (1997). Rheumatoid arthritis and the risk of malignancy. *Arthritis Rheum*, **40**, 1580-1586.

Clayman RV, Kavoussi LR, Soper NJ, Dierks SM, Meretyk S, Darcy MD, Roemer FD, Pingleton ED, Thomson PG, Long SR (1991). Laparoscopic nephrectomy: initial case report. *J Urol*, **146**, 278-282.

Coll DM, Hertz BR, Davros WJ (2000). Preoperative use of 3-Dimensional volume rendering to demonstrate renal tumours and renal anatomy. *Radiographics*, **20**, 431-438.

Costes V, Liautard J, Picot MC, Robert M, Lequeux N, Brochier J, Baldet P, Rossi JF (1997). Expression of the interleukin 6 receptor in primary renal cell carcinoma. *J Clin Pathol*, **50**, 835-840.

Coussens LM, Werb Z (2002). Inflammation and cancer. *Nature*, **420**, 860-867.

Cushing H, Bailey P (1926). Haemangiomas of cerebellum and retina (Lindau's disease), with a report of a case. *Arch Ophthalomol,* **57**, 447-463.

Dannenberg AJ, Altorki NK, Boyle JO, Lin DT, Subaramaiah K (2001). Cyclooxygenase-2: A pharmacological target for the prevention of cancer. *Lancet Oncol*, **2**, 544-551.

De Paralta-Venturina M, Moch H, Amin M, Tamboli P, Hailemariam S, Mihatsch M, Javidan J, Stricker H, Roe JY, Amin MB (2001). Sarcomatoid differentiation in renal carcinoma: a study of 101 cases. *Am J Surg Pathol*, **25**, 275-284.

Dechet CB, Sebo T, Farrow G, Blute ML, Engen DE, Zinke H (1999). Prospective analysis of intraoperative frozen needle biopsy of solid renal masses in adults. *J Urol*, **162**, 1282-1284.

DeKernion JB, Ramming KP, Smith RB (1978). The natural history of metastatic renal cell carcinoma: a computer analysis. *J Urol.*, **120**, 148-52.

Delahunt B, Eble JN (1997). Papillary renal cell carcinoma: a clinicopathological and immunohistochemical study of 105 tumors. *Mod Pathol*, **10**, 537-544.

DiBiase SJ, Valicenti RK, Schultz D, Xie Y, Gomella LG, Corn BW (1997). Palliative irradiation for focally symptomatic metastatic renal cell carcinoma: Support for dose escalation based on a biological model. *J Urol*, **158**, 746-749.

Dinarello CA (1995). Controlling the production of interleukin 1 and TNF in disease. *Nutrition*, **1**, 695-697.

Dunn MD, Portis AJ, Shalav AS, Elbahnasy AM, Heidorn C, McDougal EM, Clayman RV (2000). Laparoscopic versus open radical nephrectomy: a 9 year experience. *J Urol*, **164**, 1153-1159.

Dunn MJ (1984). Role of eicosanoids in control of renal function in severe hepatic disease. *Gastroenterology*, **87**, 1392-1395.

Eberhart CE, Coffey RJ, Radhika A, Giardello FM, Ferrenbach S, Dubois RN (1994). Up regulation of COX-2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, **107**, 1183-1188.

Eble JN, Bonsib SM (1998). Extensively cystic renal neoplasms. *Sem Diagn. Pathol*, **15**, 2-20.

Elson PJ, Witte RS, Trump DL (1988). Prognostic factors for survival in patients with recurrent or metastatic renal cell carcinoma. *Cancer Res*, **48**, 7310-7313.

Fegrusson S, Herbert RL, Laneuville O (1999). NS-398 upregulates constitutive cyclooxygenase-2 expression in the M-1 cortical collecting duct cell line. *J Am Soc Nephrol*, **10**, 2261-2271.

Filgin RA (1999). Renal cell carcinoma: Management of advanced disease. *J Urol*, **161**, 381-387.

Fisher RI, Rosenberg SA, Sznol M (1997). High-dose aldesleukin in renal cell carcinoma: long term survival update. *Cancer J Sci Am, suppl.* **3**, S70.

Flanigan RC, Yonover PM (1996). The role of surgery in patients with metastatic renal cell carcinoma. *Semin Urol Oncol*, **14**, 227-229.

Fleming S, Lewi HJE (1986). Collecting duct carcinoma of the kidney. *Histopathology*, **10**, 1131-1141.

Fleming S, Lindop GBM, Gibson AAM (1985). The distribution of epithelial membrane antigen in the kidney and its tumours. *Histopathology*, **9**, 729-739.

Fleming S, O'Donnell M (2000). Surgical pathology of renal epithelial neoplasms: recent advances and current status. *Histopathology*, **36**, 195-202.

Fleming S, Symes CE (1987). Cytokeratin antigens in the kidney and its tumours. *Histopathology*, **11**, 157-170.

Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ (2003). Evaluation of cumulative prognostic scores based on the systemic inflammatory response in patients with inoperable non-small-cell lung cancer. *Br J Cancer*; **89**, 1028-1030.

Fossa SD, Martinelli G, Otto U, Schneider G, Wander H, Oberling F, Bauer HW, Achtnicht U, Holdener EE (1992). Recombinant interferon alfa-2a with or without vinbalstine in metastatic renal cell carcinoma: Results of a European multicentre phase III study. *Ann Oncol*, **9**, 301-305.

Fosslien E (2000). Molecular pathology of cyclo-oxygenase-2 in neoplasia. *Ann Clin Lab Sci*, **30**, 3-21.

Fuhrman SA, Lasky LC, Limas C (1982). Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol*, **6**, 655-663.

Fujikawa K, Matsui Y, Oka H, Fukuzawa S, Takeuchi H (1999). Serum C-reactive protein level and the impact of cytoreductive surgery in patients with metastatic renal cell carcinoma. *J Urol*, **162**, 1934-1937.

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

Giberti C, Oneto F, Martorana G, Rovida S, Carmignani G (1997). Radical nephrectomy for renal cell carcinoma: long term results and prognostic factors on a series of 328 cases. *Eur Urol*, **31**, 40-48.

Gill IS, Hsu THS, Fox RL, Matamoros A, Miller CD, Leveen RF, Grunc MT, Song GT, Fidler ME (2000). Laparoscopic and percutaneous radiofrequency ablation of the kidney: acute and chronic porcine study. *Urology*, **56**, 197-200.

Gill IS, Novick AC, Meraney AM, Chen RN, Hobart MG, Song GT, Hale J, schweizer DK, Remer EM (2000). Laparoscopic renal cryoablation in 32 patients. *Urology*, **56**, 748-753.

Gnarra JR, Zhou S, Merrill MJ, Wagner JR, Krumm A, Papavassiliou E, Oldfield EH, Klausner RD, Linchan WM (1996). Post transcriptional regulation of vascular endothelial growth factor mRNA by the product of the VHL suppressor gene. *Proc. Natl. Acad. Sci. USA*, **95**, 589-594.

Grignon DJ, Eble JN (1998). Papillary and metanephric adenomas of the kidney. *Sem Diagn Pathol*, **15**, 41-53.

Gupta R. Kawashima T. Ryu M. Okada T. Cho A. Takayama W. (2004). Role of curative resection in octogenarians with malignancy. *Am J Surg*, **188**, 282-287.

Hafez KS, Fergany AF, Novick AC (1999). Nephron sparing surgery for localised renal cell carcinoma: impact of tumour size on patient survival, tumour recurrence and TNM staging. *J Urol*, **162**, 1930-1933.

Hain SF, Maisey MN (2003). Positron emission tomography for urological tumours. *BJU Int*, **92**, 159-164.

Harris RC. (2003) Interactions between COX-2 and the renin-angiotensin system in the kidney. *Acta Physiol Scand*, **177**, 423-427.

Hashimoto Y, Kondo Y, Kimura G, Matsuzawa I, Sato S, Ishizaki M, Imura N, Akimoto M, Hara S (2004) Cyclooxygenase-2 expression and relationship to tumour progression in human renal cell carcinoma. *Histopathology*, **44**, 353–359

Helenon O, Correas JM, Balleyguier C (2001). Ultrasound for renal tumours. *Eur Radiol*, **11**, 1890-1901.

Herschman HR (1996). Prostaglandin synthase 2. *Biochim Biophys Acta*, **1299**, 125-140.

Herts BR (2003). Imaging for renal tumours. *Curr Opin Urol*, **13**, 181-186.

Hilmy M, Bartlett JM, Underwood MA, McMillan DC. (2005) The relationship between the systemic inflammatory response and survival in patients with transitional cell carcinoma of the urinary bladder. *Br J Cancer*, **92**, 625-627.

Hirano T, Ishihara K, Hibi M. (2000) Roles of Stat-3 in mediating the cell growth, differentiation and survival signals relayed through the Il-6 family of cytokine receptors. *Oncogene*, **19**, 2548-2556.

Hock LM, Lynch J, Balaji KC (2002). Increasing incidence of all stages of kidney cancer in the last 2 decades in the United States: An analysis of surveillance, epidemiology and end results program data. *J Urol*, **167**, 57-60.

Hofmockel G, Tsatalapas P, Muller H, Dammrich J, Poot M, Maurer-Schultz B, Muller-Hermelink HK, Frohmuller HG, Bassukas ID. Significance of conventional and new prognostic factors for locally confined renal cell carcinoma. *Cancer*, **76**, 296-306.

Hori T, Shibamoto S, Hayakawa M, Takeuchi K, Oku N, Miyazawa K, Kitamura N, Ito F (1993). Stimulation of prostaglandin production by hepatocyte growth factor in human gastric carcinoma cells. *FEBS Lett*, **334**, 331-334.

Ikeda M. Natsugoe S. Ueno S. Baba M. Aikou T (2003). Significant host- and tumor-related factors for predicting prognosis in patients with esophageal carcinoma. *Annals of Surg*, **238**, 197-202.

Iliopolous O, Levy AP, Jiang C, Kaelin WG, Goldberg MA (1996). Negative regulation of hypoxia inducible genes by the VHL protein. *Proc. Natl. Acad. Sci. USA,* **93**, 10595-10599.

Ishikawa I, Kovacs G (1993). High incidence of papillary renal cell tumours inpatients on chronic haemodialysis. *Histopathology*, **22**, 135-139.

Jamieson NB, Glen P, McMillan DC, McKay CJ, Foulis AK, Carter R, Imrie CW. (2005) Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma head of pancreas. *Br J Cancer*, **92,** 21-23.

Jayson M, Sanders H. (1998) Increased incidence of serendipitously discovered renal cell carcinoma. *Urology,* **51**, 203-205

Jee SH, Shen SC, Chiu HC, Tsai WL, Kuo ML. (2001) Overexpression of interleukin-6 in human basal cell carcinoma cell lines increases anti-apoptotic activity and tumorigenic potency. *Oncogene*, **20**, 198-208.

Jinzaki M, Tanimoto A, Mukai M, Ikeda E, Kobyashi S, Yuasa Y, Narimatsu Y, Murai M (2000). Double phase helical CT of small renal parenchymal neoplasms: correlation with pathologic findings and tumour angiogenesis. *J Comput Assist Tomogr*, **24**, 835-842.

Johnson CD, Dunnick NR, Cohan RH (1987). Renal adenocarcinoma: CT staging of 100 tumours. *Am J Radiol*, **148**, 59-63.

Jones MK, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh IJ, Tarnawski AS (1999). Inhibition of angiogenesis by non-steroidal anti-inflammatory drugs: Insight into mechanisms and implications for cancer growth and ulcer healing. *Nat Med*, **5**, 1418-1423.

Khan KNM, Stanfield KM, Trajikovic C, Knapp DW (2001). Expression of Cyclooxygenase-2 in canine renal cell carcinoma. *Vet Pathol*, **38**, 116-119.

Kigure T, Harada T, Yuri Y, Satoh Y, Yoshida K (1996). Laparoscopic microwave thermotherapy on small renal tumours: experimental using implanted VX-2 tumours in rabbits. *Eur Urol*, **30**, 377-382.

Kim HL, Belldebrun AS, Freitas DG, Bui MHT, Han K, Dorey FJ, Figlin RA (2003). Papraneoplastic signs and symptoms of renal cell carcinoma: implications for prognosis. *J Urol*, **170**, 1742-1746.

Kinoshita T, Ito H, Miki C. (1999) Serum interleukin-6 level reflects the tumour proliferative activity in patients with colorectal carcinoma. *Cancer*, **85**, 2526-2531

Kishimoto T (1989). The biology of interleukin-6. *Blood*, **74**, 1-10.

Kofler S, Nickel T, Weis M. (2005) Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. *Clin Sci (Lond)*. **108**:205-13.

Kolebeck PC, Kaveggia FF, Johansson SL, Grune MT, Taylor RJ (1992). The relationships among tumour infiltrating lymphocytes, histopathalogic findings, and long term clinical follow up in renal cell carcinoma. *Mod Pathol*, **5**, 420-425.

Kosary CL, McLaughlin JK (1993). Kidney and renal pelvis, in Miller BA, Reis LAG, Hankey BF et al (eds): Cancer Statistics Review 1973-1990. NIH Publication No. 93-2789. Bethesda, MD, National Cancer Institute, 1993, pp X1-X22.

Krown SE (1987). Interferon treatment of renal cell carcinoma: current status and future prospects. *Cancer*, **59**, 647-651.

Lam JS, Shvarts O, Said JW, Pantuck AJ, Seligson DB, Aldridge ME, Bui MH, Liu X, Horvath S, Figlin RA, Belldegrun AS. (2005) Clinicopathologic and molecular

correlations of necrosis in the primary tumor of patients with renal cell carcinoma. *Cancer*. **103**:2517-25.

Lamiell JM, Salazar FG, Hsia YE (1989). Von Hippel-Lindau disease affecting 43 members of a single kindred. *Medicine,* **68,** 1-29.

Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1998. (1998) *CA Cancer J Clin,* **48,** 6-29.

Lang H, Lindner V, Saussine C, Havel D, Faure F, Jacqmin D (2000). Microscopic venous invasion: a prognostic factor in renal cell carcinoma. *Eur Urol*, **38**, 600-605.

Laski ME, Vugrin D (1987). Paraneoplastic syndromes in hypernephroma. *Semin Nephrol*, 7, 123-130.

Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I (1993). Identification of the von Hippel-Lindau disease tumour suppressor gene. *Science*, **260**, 1317-1320.

Lerner SE, Hawkins CA, Blute ML, Grabner A, Wollan PC, Eickholt JT, Zinke H (1996). Disease outcome in patients with low stage renal cell carcinoma treated with nephron sparing or radical surgery. *J Urol*, **155**, 1868-1873.

Leslie, JA, Prihoda T, Thompson IM (2003). Serendipitous renal cell carcinoma in the post CT era: continued evidence in improved outcomes. *Uro Oncol,* **21,** 39-44.

Levy DA, Swanson DA, Slaton JW, Ellenhost J, Dinney CP (1998). Timely delivery of biological therapy after cytoreductive nephrectomy in carefully selected patients with metastatic renal cell carcinoma. *J Urol,* **159**, 1168-1173.

Lieber MM, Tomera KM, Farrow GM (1981). Renal oncocytoma. *J Urol*, **125**, 481-485.

Linehan WM, Walther MM, Zbar B (2003). The genetic basis of cancer of the kidney. *J Urol*, **170**, 2163-2172.

Lissoni P, Barni S, Ardizzoia A, Crispino S, Paolorossi F, Andres M Scardino E, Tancini G (1994). Prognostic factors of clinical response to subcutaneous immunotherapy with interleukin alone in patients with metastatic renal cell carcinoma. *Oncology*, **51**, 59-62.

Liu XH, Kirschenbaum A, Lu M, Yao S, Klausner A, Preston C, Holland JF, Levine AC. (2002) Prostaglandin E2 stimulates prostatic intraepithelial neoplasia cell growth through activation of the interleukin-6/gp130/stat-3 signalling pathway. *Biochem Biophys Res Commnications,* **290**, 249-255

Maccio A, Lai P, Santona MC, Pagliara I., Melis GB, Mantovani G. (1998) High serum levels of soluble IL-2 receptor, cytokines, and C reactive protein correlate with impairment of T cell response in patients with advanced epithelial ovarian cancer. *Gynecol Oncol*, **69**, 248-252.

Maher ER, Kaelin Jr WG (2001). Von Hippel-Lindau disease. *Surv Ophthalmol*, **46**, 117-142.

Mahmoud FA, Rivera NI. (2002) The role of C-reactive protein as a prognostic indicator in advanced cancer. *Curr Oncol Rep.* 4:250-5.

Maldazys JD, deKiernon JB (1986). Prognostic factors in metastatic renal carcinoma. *J Urol*, **136**, 376-379.

Maltoni M, Caraceni A, Brunelli C, Broeckaert B, Christakis N, Eychmueller S, Glare P, Nabal M, Vigano A, Larkin P, De Conno F, Hanks G, Kaasa S (2005) Steering Committee of the European Association for Palliative Care.Prognostic factors in advanced cancer patients: evidence-based clinical recommendations--a study by the

Steering Committee of the European Association for Palliative Care. *J Clin Oncol.* **23**:6240-8.

Maranchie JK, Linehan WM (2003). Genetic disorders and renal cell carcinoma. *Urol Clin North Am*, **30**, 133-141.

Marshall FF, Holdford SS, Hamper UM (1992). Intraoperative sonography of renal tumours. *J Urol*, **148**, 1393-1396.

Marshall FF, Stewart AK, Menck HR (1997). The national cancer database: Report on kidney cancers. *Cancer*, **80**, 2167-2174.

Masuda A, Aoki M, Masatoshi T, Usui Y, Abe T, Miyakita H, Kinoshita H, Kawamura N, Terachi T (2003). Clinicopathological factors predicting recurrence of N0M0 renal cell carcinoma: A case series analysis of one facility. *Int J Urol*, **10**, 511-517.

Masuda H, Kurita Y, Fukuta K, Mugiya S, Suzuki K, Fujita K. (1998) Significant prognostic factors for 5-year survival after curative resection of renal cell carcinoma. *Int J Urol*. **5**, 418-422.

McDougal WS, Garnick MB (1995). Clinical signs and symptoms of renal cell carcinoma. Comprehensive Textbook of Genitourinary Oncology. Baltimore: Williams & Wilkins Co 154-159 + 111-115.

McGee JO, Isaacson PG, Wright NA (1992). *Oxford textbook of pathology. Oxford University Press*, **Vol 2a,** 1501-1503.

McKeown DJ, Brown DJ, Kelly A, Wallace AM, McMillan DC. (2004) The relationship between circulating concentrations of C-reactive protein, inflammatory cytokines and cytokine receptors in patients with non-small-cell lung cancer. *Br J Cancer*, **91**, 1993-1995.

McLaughlin JK, Mandel JS, Blot WJ, Schuman LM, Mehl ES, Fraumeni JF jr (1984). Population based case-controlled study of renal cell carcinoma. *J Natl Cancer Inst*, **72**, 275-284.

McMillan DC, Canna K, McArdle CS (2003). Systemic inflammatory response predicts survival following curative resection of colorectal cancer. *Br J Surgery*, **90**, 215-219.

McMillan DC, Elahi MM, Sattar N, Angerson WJ, Johnstone J, McArdle CS (2001). Measurement of the systemic inflammatory response predicts cancer-specific and non-cancer survival in patients with cancer. *Nutrition & Cancer*, **41**, 64-69.

McMillan DC, Leen E, Smith J, Sturgeon C, Preston T, Cooke TG, McArdle CS (1995). Effect of extended ibuprofen administration on the acute phase protein response in colorectal cancer patients. *Eur J Surg Oncology*, **21**, 531-534

Mediros LJ, Gelb AB, Weiss LM (1988). Renal cell carcinoma. Prognostic significance of morphologic parameters in 121 cases. *Cancer*, **61**, 1639-1651.

Mellemgaard A, Lindbald P, Schlehofer B, Bergstorm R, Mandel JS, McCreadie M, McLaughlin JK, Niwa S, Okda N, Pommer W (1995). International renal-cell cancer study III. Role of weight, height, physical activity, and use of amphetamines. *Int J Cancer*, **60**, 345-349.

Mellemgaard A, Niwa S, Mehl ES, Engholm G, McLaughlin JK, Olsen JH (1994). Risk factors for renal cell carcinoma in Denmark: Role of medication and medical history. *Int J Epidemiol*, **23**, 923-930.

Miki S, Iwano M, Miki Y, Yamamoto M, Tang B, Yokokawa K, Sosonda T (1989). Interleukin-6 functions as an in vitro autocrine growth factor in renal cell carcinomas. *FEBS Lett*, **250**, 607-610.

Minasian LM, Motzer RJ, Gluck L Mazumadar M, Vlamis V, Krown SE (1993). Interferon alfa-2a in advanced renal cell carcinoma: treatment results and survival in 159 patients with long term follow up. *J Clin Oncol*, **11**, 1368-1375.

Minervini A, Lilas L, Morelli G, Traversi C, Battaglia S, Cristofani R, Minervini R (2001). Regional lymph node dissection in the treatment of renal cell carcinoma: is it useful in patients with no suspected adenopathy before or during surgery? *BJU Int*, **88**, 169-172.

Miyata Y, Koga S, Kanda S, Nishikido M, Hayashi T, Kanetake H (2003). Expression of cyclooxygenase-2 in renal cell carcinoma: correlation with tumor cell proliferation, apoptosis, angiogenesis, expression of matrix metalloproteinase-2, and survival. *Clin Cancer Res*, **9**, 1741-1749.

Motzer RJ, Russo P (2000). Systemic therapy for renal cell carcinoma. *J Urol*, **163**, 408-417.

Motzer RJ, Russo P, Nanus DM, Berg WJ (1997). Renal cell carcinoma. *Curr Problems in Cancer*, **21**, 189-232.

Naito Y, Saito M, Shiiba K, Ohuchi A, Saiqenji K, Naqura H, Ohtani H (1998). CD8+ T cells infiltrated within cancer cell nests as prognostic factor in human colorectal cancer. *Cancer Res*, **58**, 3491-3494.

Nakano 0, Sato M, Naito Y, Suzuki K, Orikasa S, Aizawa M, Suzuki Y, Shintaku I, Nagura H, Ohtani H (2001). Proliferation activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathological demonstration of antitumour immunity. *Cancer Res*, **61**, 5132-5136.

Negrier S, Escudier B, Lasset C, Douillard JY, Savary J, Chevreau C (1998). Recombinant human interleukin-2 recombinant human interferon alfa-2a, or both in metastatic renal cell carcinoma. *N Engl J Med*, **338**, 1272-1278.

Nickerson ML, Warren MB, Toro JR, Mastrova V, Glenn G, Turner ML (2002). Mutations in a novel gene lead to kidney tumours, lung wall defects, and benign tumours of the hair follicle in patients with Birt Hogg-Dube syndrome. *Cancer Cell*, **2**, 157-164.

Norian G, Clive D (2002). Cyclo-oxygenase-2 inhibitors and the kidney: A case for caution. *Drug Safety*, **25**, 165-172.

O'Byrne KJ, Dalgleish AG (2001) Chronic immune activation and inflammation as the cause of malignancy. *Br J Cancer*, **85**, 473-483.

O'Gorman P, McMillan DC, McArdle CS (2000) Prognostic factors in advanced gastrointestinal cancer patients with weight loss. *Nutr Cancer*, **37**, 36–40.

Ohno Y. Ohno S. Suzuki N. Kamei T. Inagawa H. Soma G. Inoue M (2005). Role of cyclooxygenase-2 in immunomodulation and prognosis of endometrial carcinoma. *Int J Cancer*, **114**, 696-701

Onishi T, Ohishi Y, Imagawa K, Ohmoto Y, Murata K (1999). An assessment of the immunological environment based on intratumoral cytokine production in renal cell carcinoma. *BJU Int*, **83**, 488-492.

Ono Y, Kinukawa T, Hattori R, Gotoh M, Kamihira O, Ohshima S (2001). The long term outcome for laparoscopic radical nephrectomy for small renal cell carcinoma. *J. Urol*, **165**, 1867-1870.

Onufrey V, Mohiuddin M (1985). Radiation therapy in the treatment of metastatic renal cell carcinoma. *Int J Rad Oncol. Biol. Phys*, **11**, 2007-2009.

Pantuck AJ, Zisman A, Chao D, Dorey F, Flannigan R, DeKernion JB (2002). Survival advantage of interleukin-2 over interferon immunotherapy when compared with cytoreductive nephrectomy based on SWOG 8949-matched populations. *J Urol suppl*, **167**; 166.

Pantuck AJ, Zisman A, Dorey F, Chao DH, Han K, Said J, Gitlitz BJ, Filgin RA, Belldegrun AS (2003). Renal cell carcinoma with retroperitoneal lymph nodes: role of lymph node dissection. *J Urol*, **169**, 2076-2083.

Patton EE, Willems AR, Tyres M (1998). Combinatorial control in ubiquitin dependent proteolysis: Don't skip the F-box hypothesis. *Trends Genet*, **14**, 236-243.

Paule B, Belot J, Rudant C, Coulombel C, Abbou CC (2000). The importance of Il-6 protein expression in primary human renal cell carcinoma: an immunohistochemical study. *J Clin Pathol*, **53**, 388-390.

Pause A, Lee S, Worrell RA, Chen DY, Burgess WH, Linehan WM, Klausner RD (1997). The von Hippel-Lindau tumour suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. *Proc Natl Acad Sci USA*, **94**, 2156-2161.

Pfeffer LM, Dinarello CA, Herberman RB et al. (1998) Biological properties of recombinant alpha interferons: 40th anniversary of the discovery of interferons. *Cancer Res*, **58**, 2489-2499.

Piltz S, Meimarakis G, Wichmann MW, Hatz R, Schildberg FW, Fuerts H (2002). Long term results after pulmonary resection of renal cell carcinoma metastases. *Ann Thorac Surg*, **73**, 1082-1087.

Pomer S, Klopp M, Steiner HH, Brkovic D, Staeher G, Cabillin-Engenhart R (1997). Brain metastases in renal cell carcinoma. *Urologe*, **36**, 117-125.

Portis AJ, Yan Y, Landman J, Chen C, Barrett P, Fentie D, Ono Y, McDougall E, Clayman R (2002). Long-term follow up after laparoscopic radical nephrectomy. *J Urol*, **167**, 1257-1262.

Prasad SR, Saini S, Stewart S, Hahn PF, Halpern EF (2002). CT characterisation of "Indeterminate" renal masses: targeting or comprehensive scanning? *J Comput Assist Tomogr*, **26**, 725-727.

Prowse AH, Webster AR, Richards FM (1997). Somatic innactivation of the VHL gene in von Hippel-Lindau disease tumours. *Am J Hum Genet*, **60**, 765-771.

Pruthi RS, Derksen E, Gaston K. (2003) Cyclo-oxygenase as a potential target in the prevention and treatment of genitourinary tumors: A review. *J Urol*, **169**, 2352-2359.

Rahilly MA, Salter DM, Fleming S (1991). Composition and organisation of cell substratum contacts in normal and neoplastic renal epithelium. *J Pathol*, **165**, 163-172.

Ramsey S, Aitchison M, McMillan DC. (2005) Re: C-reactive protein: a biomarker of survival in patients with metastatic renal cell carcinoma treated with subcutaneous interleukin-2 based immunotherapy. *J Urol*, **174**, 2422-2423.

Ramsey S, Lamb GW, Aitchison M, McMillan DC (2006). The longitudinal relationship between circulating concentrations of C-reactive protein, interleukin-6 and interleukin-10 in patients undergoing resection for renal cancer. *Br J Cancer*, **95**, 1076-1080.

Ramsey S, Lamb GW, Aitchison M, Graham J, McMillan DC (2007). Evaluation of an inflammation based prognostic score in patients with metastatic renal cancer. *Cancer*, **109**, 205-212.

Ramsey S, Lamb GW, Aitchison M, McMillan DC (2007) Prospective study of the relationship between the systemic inflammatory response, prognostic scoring systems and relapse free and cancer specific survival in patients undergoing potentially curative resection for renal cancer. Accepted for publication BJU Int Sept 5th 2007

Rassweiler JJ, Abbou C, Janetschek G, Jeschke K (2000). Laparoscopic partial nephrectomy. The European experience. *Urol Clin North Am*, **27**, 721-736.

Reis LAG, Kosary CL, Hankey BF (1998). SEER Cancer Statistics Review, 1973-1995. Bethesda MD, National Cancer Institute.

Remer EM, Weinberg EJ, Oto A (2000). MR imaging of the kidneys after laparoscopic cryoablation. *Am J Roentgenol*, **174**, 635-640.

Rendon RA, Stanietzky N, Panzarella T, Robinette M, Klotz LH, Thurston W, Jewett MAS (2000). The natural history of small renal masses. *J Urol*, **164**, 1143-1147.

Renshaw AA (2002). Subclassification of renal cell neoplasms: an update for the practising pathologist. *Histopathology*, **41**, 283-300.

Renshaw AA, Corless CL (1995). Papillary renal cell carcinoma: histology and immunohistochemistry. *Am J Surg Pathol*, **19**, 842-849.

Renshaw AA, Corless CL (1997). Papillary renal cell carcinoma: gross features and histologic correlates. *J Urol Pathol*, 7, 9-19.

Renshaw AA, Freyer DR, Hammers YA (2000). Metastatic metanephric adenoma in a child. *Am J Surg Pathol*, **24**, 570-574.

Renshaw AA, Henske EP, Loughlin KR, Shapiro C, Weinberg DS (1996). Aggressive variants of chromophobe renal cell carcinoma. *Cancer*, **78**, 1756-1761.

Robbins SL, Cotran RS, Kumar V (1989). *Pathologic basis of disease.* **5th Ed** WB Saunders Co 986-987.

Robson CB, Churchill BM, Anderson W (1969). The results of radical nephrectomy for renal cell carcinoma. *J Urol*, **101**, 297-301.

Roy C, Tuchman C, Morel M, Saussine C, Jacqmin D, Tongio J (1999). Is there still a place for angiography in the management of renal mass lesions? *Eur Radiol*, **9**, 329-335.

Sagalowski AI, Kadesky KT, Ewalt DM, Kennedy TJ (1994). Factors influencing adrenal metastases in renal cell carcinoma. *J Urol*, **151**, 1181-1184.

Saitoh H, Hida M, Nakamura K, Shiramizu T, Shimbo T, Satoh T (1982). Metastatic processes and a potential indication for treatment from metastatic lesions of renal adenocarcinoma. *J Urol*, **128**, 916-918.

Samland D, Steinbach F, Reiher F, Schmidt U, Gruss A, Allhoff EP (1999). Results of immunochemotherapy with interleukin-2, interferon alpha and 5-fuorouracil in the treatment of metastatic renal cell carcinoma. *Eur Urol*, **35**, 204-209.

Schena FP, Strippoli GF, Wankelmuth P (1999). Renal growth factors: past, present and future. *Am J Nephrol*, **19**, 308-312.

Schendel BJ, Gansbacher B, Oberneder R, Kriegmair M, Hofstetter A, Reithmuller G, Segurado OG (1993). Tumour specific lysis of human renal cell carcinomas by tumour infiltrating lymphocytes. I. HLA-A2-restricted recognition of autologous and allogeneic tumour lines. *J Immunol*, **151**, 4209-4213.

Schlehofer B, Pommer W, Mellemgaard A, Stewart JH, McCreadie M, Niwa S, Lindblad P, Mandel JS, McLaughlin JK, Wahrendorf J (1996). International renal cell cancer study – VI role of medical and family history. *Int J Cancer*, **66**, 723-726.

Semenza GL (2000). HIF-1 and human disease: One highly involved factor. *Genes Dev*, **14**, 1983-1991

Sheng H, Shao J, Morrow JD, Beuchamp RD, Dubois RN (1998). Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res*, **58**, 362-366.

Shimizu C. Kubo M. Takano K. Takano A. Kijima H. Saji H. Katsuyama I. Sasano H. Koike T (2001). Interleukin-6 (IL-6) producing phaeochromocytoma: direct IL-6 suppression by non-steroidal anti-inflammatory drugs. *Clinical Endocrinology,* **54**, 405-410

Shvarts O, Seligson D, Lam J, Shi T, Horvath S, Figlin R, Belldegrun A, Pantuck AJ. (2005) p53 is an independent predictor of tumor recurrence and progression after nephrectomy in patients with localized renal cell carcinoma. *J Urol.* **173**:725-8.

Siemeister G, Weindel K, Mohrs K, Barelon B, Martiny-Baron G, Marme D (1996). Reversion of deregulated expression of vascular endothelial growth factor in human renal cell carcinoma cells by von Hippel-Lindau suppressor protein. *Cancer Res*, **56**, 2299-2301.

Sobin L, Wittekind C (1997) International Union Against Cancer (UICC): TNM Classification of Malignant Tumors, 5th Edition. pp180-182, Wiley-Liss, New York.

Steiner T, Junker U, Wunderlich H, Schubert J (1999). Are renal cell carcinoma cells able to modulate the cytotoxic effect of tumour infiltrating lyphocytes by secretion of interleukin-6? *Anticancer Res*, **19**, 1533-1536.

Stephenson AJ. Hakimi AA. Snyder ME. Russo P. (2004) Complications of radical and partial nephrectomy in a large contemporary cohort. *J Urol,*. **171**, 130-134.

Stevens TL, Bossie A, Sanders VM, Fernandes-Botran R, Coffman RC, Mossman TR, Vietta ES (1988). Regulation of antibody isotype secretion by subsets of antigen specific helper T cells. *Nature*, **334**, 255-258.

Storkel S, Eble JN, Adlakha K (1997). Classification of renal cell carcinoma: Workgroup No.1 Union Internationale Contre Le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). *Cancer*, **80**, 987-989.

Strong JW, Ro JY (1996). Metanephric adenoma of the kidney a newly characterised entity. *Adv Anat Pathol*, **3**, 172-178.

Subbaramaiah K, Dannenberg AJ. (2003) Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends Pharmacol Sci*, **24**, 96-102.

Sutherland SE, Resnick MI, Maclennan GT, Goldman HB (2002). Does the size of the surgical margin in partial nephrectomy for renal cell cancer really matter? *J Urol*, **167**, 61-64.

Takahashi S, Tanigawa T, Imagawa M, Mimata H, Nomura Y, Ogata J. (1994) Interferon as adjunctive treatment for non-metastatic renal cell carcinoma. *BJU*, **74**, 11-14.

Takenawa J, Kawenko Y, Fukumoto M, Fukatsu A, Hirano T, Fukuyama H, Nakayama H, Fujita J, Yoshida O. (1991) Enhanced expression of interleukin-6 in primary human renal cell carcinoma. *J Natl Cancer Inst*, **83**, 1668-1672.

Thoenes W, Storkel S, Rumplet H-J (1985). Human chromophobe cell renal carcinoma. *Virchows Arch*, **48**, 207-217.

Tomozawa S, Tsuno NH, Sunami E, Hatano K, Kitayama J, Osada T (2000). Cyclooxygenase-2 overexpression correlates with tumour recurrence, especially haematogenous metastasis of colorectal cancer. *Br J Cancer*, **83**, 324-328.

Trikha M, Corringham R, Klein B, Rossi JF. (2003) Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. *Clin Cancer Res*, **9**, 4653-4665.

Tsui KH, Shvarts O, Smith RB, Figlin R, deKiernon JB, Belldegrun A (2000). Renal cell carcinoma, prognostic significance of incidentally detected tumours. *J Urol*, **163**, 426-430.

Tuna B, Yorukoglu K, Gurel D, Mungan U, Kirkali Z (2004). Significance of COX-2 expression in human renal cell carcinoma. *Urology*, **64**, 1116-1120.

Uzzo RG, Novick AC (2001). Nephron sparing surgery for renal tumours: Indications, techniques and outcomes. *J Urol*, **166**, 6-18.

Uzzo RG, Rayman P, Kolenko V, Clark PE, Bloom T, Ward AM, Molto L, Tannenbaum C, Worford LJ, bukowski R, Tobbs R, His ED, Bander NH, Novick AC, Finke JH (1999). Mechanisms of apoptosis in T cells from patients with renal cell carcinoma. *Clin Cancer Res*, **5**, 1219-1229.

Vakkila J, Lotze MT. (2004) Inflammation and necrosis promote tumour growth. *Nat Rev Immunol*, **4**, 641-648.

Van Der Poel H, Roukema JA, Horenblas S, van Geel AN, Debruyne H (1999). Metastasectomy in renal cell carcinoma: A multicentre retrospective analysis. *Eur Urol*, **35**, 197-203.

Van Poppel H, Vandendriessche H, Boel K, Mertens V, Goethuys H, Haustermans K, Van Damme B, Baert L (1997). Microscopic vascular invasion is the most relevant prognosticator after radical nephrectomy for clinically non-metastatic renal cell carcinoma. *J Urol*, **158**, 45-49.

Vogelzang NJ, Stadler WM (1998). Kidney cancer. *Lancet*, **352**, 1691-1696.

Walther MM, Lubensky IA, Venzon D (1995). Prevalence of microscopic lesions in grossly normal renal parenchyma from patients with von Hippel-Lindau disease, sporadic renal cell carcinoma and no renal disease: clinical implications. *J Urol*, **154**, 2010-2015.

Weir M, Pitman MB (1996). The vascular pattern of renal cell carcinomas on fine needle aspiration biopsy: an aid in the distinction from hepatocellular carcinoma (Abstract). *Acta Cytol*, **40**, 1081.

Wendrum D, Svrcek M, Rigau V, Boelle P, Sebbagh N, Parc R et al. (2003) COX-2, Inlammatory secreted PLA2, and Cytoplasmic PLA2 protein expression in small bowel adenocarcinomas compared with colorectal adenocarcinomas. *Modern Pathology,* **16,** 130-136.

World Health Organisation (2000). Incidence and Mortality Data. Geneva WHO.

Xavier P, Belo L, Beires J, Rebelo I, Martinez-de-Oliveira J, Lunet N, Barros H. (2006) Serum levels of VEGF and TNF-alpha and their association with C-reactive protein in patients with endometriosis. *Arch Gynecol Obstet.* **273**:227-31.

Xipell JM (1971). The incidence of benign renal nodules (a clinicopathological study). *J Urol*, **106**, 503-506.

Yasukawa K, Saito T, Fukunaga T, Sekimori Y, Koishihara Y, Fukui H (1990). Purification and characterisation of soluble human Il-6 receptor expressed in CHO cells. *J Biochem*, **108**, 673-676.

Yonover PM, Flanigan RC (2000). Should radical nephrectomy be performed in the face of surgically incurable disease? *Curr Opin Urol*, **10**, 429-434.

Yoon J, Herts BR (2003). Staging renal cell carcinoma with helical CT: the revised 1997 AJCC and UICC TNM criteria. *Crit Rev in Computed Tomography*, **44**, 229-249.

Yoshida N, Ikemoto S, Narita K, Sugimura K, Wada S, Yasumoto R, Kishimoto T, Nakatani T (2002). Interleukin-6, tumour necrosis factor α and interleukin-1β in patients with renal cell carcinoma. *Br J Cancer*, **86,** 1396-1400.

Yuan JM, Castelao JE, Gago-Dominguez M, Yu MC, Ross RK (1998). Tobacco use in relation to renal cell carcinoma. *Cancer Epidemiol Biomarkers Prev*, 7, 429-433.

Yuh BL, Cohan RC (1999). Different phases of renal enhancement: role in detecting and characterising renal masses during helical CT. *Am J Roentgenol*, **173**, 747-755.

Zambrano, NR, Lubensky IA, Merino, MJ, Linehan, W, Marston W, McClellan M. (1999). Histopathology and molecular genetics of renal tumours; toward unification of a classification system. *J Urol,* **162,** 1246-1258.

Zisman A, Pantuck AJ, Belldegrun AS, Schulam PG (2001). Laparoscopic radical nephrectomy. *Semi Uro Oncol*, **19**, 114-122.

Zisman A, Pantuck AJ, Chao D, Dorey F, Said JW, Gitlitz BJ, deKernion JB, Filgin RA, Beldegrun AS (2001). Reevaluation of the 1997 TNM classification for renal cell carcinoma: T1 and T2 cutoff point a 4.5 rather than 7cm better correlates with clinical outcome. *J Urol*, **166**, 54-58.

Zisman A, Pantuck AJ, Wieder J, Chao DH, Dorey F, Said JW, deKernion JB, Figlin RA, Belldegrun AS (2002). Risk group assessment and clinical outcome algorithm to predict the natural history of patients with surgically resected renal cell carcinoma. *J Clin. Oncol*, **20**, 4559-4566.

Zou W. (2005). Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nature Rev.* **5,** 263-272

APPENDIX I: Raw Data Chapter 2

Pat No	Sex	DOB	DODiag	T Stage	Grade	Side	CRP	ECOG	UCLA	Alive/ Dead	Cause of death	Date FU
1	M	12-Sep-40	17-Feb-00	3	3	L	78	0	2	Alive		31-Oct-05
2	M	28-Sep-20	06-Dec-99	1	1	L	0	2	2	Dead	Non-metastatic	05-Jan-03
3	F	30-Jan-34	05-Oct-00	1	1	L	49	1	2	alive		31-Oct-05
4	F	29-Nov-43	17-Feb-03	3	4	L	26	0	2	alive		31-Oct-05
5	M	17-Mar-28	20-Nov-97	2	3	1	116	1	2	DEAD	Metastatic Disease	29-Aug-00
6	M	27-May-41	01-Nov-99	2	2	R	0	0	2	alive		31-Oct-05
7	F	30-Sep-28	10-Nov-97	2	1	R	21	0	2	alive		31-Oct-05
8	F	17-Jun-55	20-Mar-00	1	2	R	6	0	1	alive		31-Oct-05
9	M	25-Nov-43	07-Jan-02	3	4	L	83	1	3	DEAD	Metastatic Disease	19-May-04
10	F	12-Jul-27	09-Aug-99	1	1	R	26	0	1	alive		31-Oct-05
11	M	02-Apr-49	08-Apr-03	1	1	R	0	0	1	alive		31-Oct-05
12	M	07-Feb-36	25-Nov-96	3	3	R	50	1	3	DEAD	Metastatic Disease	01-Jun-03
13	F	30-May-34	01-Dec-03	1	2	R	15	0	1	ALIVE		31-Oct-05
14	M	26-Oct-55	05-Aug-99	1	2	L	0	0	1	alive		31-Oct-05
15	F	04-Mar-36	23-Nov-98	3	1	R	13	0	2	alive		31-Oct-05
16	F	26-Apr-40	06-Sep-04	1	3	L	6	0	2	Alive		31-Oct-05
17	M	03-Mar-40	08-Jan-01	1	2	R	6	0	1	alive		31-Oct-05
18	M	09-Sep-30	15-Jul-98	3	.	R	6	0	2	DEAD	Metastatic Disease	16-Mar-99
19	M	03-Feb-67	23-Sep-03	3	3	L	21	0	2	DEAD	Metastatic Disease	16-Jul-04
20	M	07-Nov-53	30-Oct-02	2	3	L	0	0	2	alive		31-Oct-05
21	M	16-Sep-24	23-Nov-99	1	2	L	0	0	1	alive		31-Oct-05
22	M	27-Apr-52	29-Oct-01	3	4	L	234	0	2	DEAD	Non-metastatic	14-Oct-02
23	M	09-Feb-46	22-Aug-04	1	3	R	6	0	2	alive		31-Oct-05
24	M	09-Sep-42	21-Oct-98	1	2	L	0	0	1	ALIVE		31-Oct-05
25	M	02-Jul-56	15-Sep-03	3	4	R	0	0	2	alive		31-Oct-05
26	M	01-Oct-61	24-Nov-03	3	3	R	70	0	2	alive		31-Oct-05
27	F	06-Sep-25	30-Jun-03	1	2	R	0	0	1	alive		31-Oct-05
28	M	03-Jun-42	08-Nov-04	1	3	R	6	0	2	Alive		31-Oct-05
29	F	14-Sep-35	23-Sep-03	3	1	R	0	0	2	alive		31-Oct-05

Pat No	Sex	DOB	DODiag	T Stage	Grade	Side	CRP	ECOG	UCLA	Alive/ Dead	Cause of death	Date FU
30	F	10-Oct-22	15-Nov-04	3	4	L	159	1	3	Alive		31-Oct-05
31	F	04-Nov-21	28-Jun-00	1	2	R	0	0	1	alive		31-Oct-05
32	F	11-Dec-30	11-Oct-04	3	2	R	12	1	3	Alive		31-Oct-05
33	M	25-Mar-41	21-May-01	3	2	L	6	0	2	alive		31-Oct-05
34	M	01-Oct-29	17-Mar-03	3	3	R	35	0	2	ALIVE		31-Oct-05
35	M	19-Jan-42	03-Jun-99	2	2	R	139	0	2	alive		31-Oct-05
36	M	02-Jul-53	10-Jun-02	1	2	L	6	0	1	alive		31-Oct-05
37	F	13-Feb-51	29-Jul-99	3	4	L	0	0	2	Alive		31-Oct-05
38	M	11-Mar-28	17-May-99	3	2	L	20	0	2	DEAD	Metastatic Disease	20-May-01
39	F	23-Oct-41	02-Jun-97	2	2	R	0	0	2	alive		31-Oct-05
40	M	24-Feb-31	15-Nov-99	1	2	L	0	0	1	alive		31-Oct-05
41	M	10-May-33	12-Jun-00	3	2	L	6	0	2	alive		31-Oct-05
42	F	02-Jun-23	04-Nov-99	1	2	L	114	0	1	alive		31-Oct-05
43	M	16-Feb-34	27-Jul-98	4	4	L	179	0	3	DEAD	Metastatic Disease	27-Sep-98
44	F	10-Oct-19	18-Sep-00	3	1	R	0	0	2	alive		31-Oct-05
45	F	09-Jul-43	25-Aug-03	1	4	R	68	0	2	alive		31-Oct-05
46	M	25-Apr-56	01-Dec-97	1	3		0	0	2	alive		31-Oct-05
47	M	21-Sep-33	05-Apr-04	2	2	L	0	0	2	alive		31-Oct-05
48	F	03-Apr-54	14-Apr-03	1	1	R	15	0	1	alive		31-Oct-05
49	F	22-May-36	29-Jan-01	3	2	R	0	0	2	alive		31-Oct-05
50	M	14-Apr-54	11-Mar-97	3	3	R	272	0	2	alive		31-Oct-05
51	F	06-Jun-35	04-Sep-00	3	3	R	0	0	2	alive		31-Oct-05
52	M	11-Jul-48	04-Oct-04	1	3	R	10	0	2	Alive		31-Oct-05
53	F	04-Apr-35	08-Jun-98	1	3	L	0	1	2	alive		31-Oct-05
54	M	08-Nov-36	13-Sep-99	3	3	R	0	0	2	alive		31-Oct-05
55	F	08-Jul-49	13-May-03	3	1	L	0	0	2	alive		31-Oct-05
56	M	27-Nov-38	16-Jun-98	1	3	1	0	0	2	DEAD	Metastatic Disease	21-Apr-02
57	F	10-Feb-21	25-Jan-99	3	2	L	21	0	2	DEAD	Non-metastatic	28-Jan-99
58	M	27-Feb-32	23-Sep-03	3	1	R	33	0	2	alive		31-Oct-05
59	F	30-Oct-40	22-Aug-04	1	3	L	6	0	2	Alive		31-Oct-05
60	M	14-Oct-40	14-Dec-99	3	3	L	13	0	2	DEAD	Metastatic Disease	09-Apr-05
61	M	11-Nov-33	18-Dec-00	3	2	L	36	1	3	alive		31-Oct-05

Pat No	Sex	DOB	DODiag	T Stage	Grade	Side	CRP	ECOG	UCLA	Alive/ Dead	Cause of death	Date FU
62	F	12-Oct-33	15-Dec-03	1	1	L	0	0	1	ALIVE		31-Oct-05
63	F	13-Mar-58	23-Aug-99	1	1	L	13	0	1	DEAD	Metastatic Disease	04-Jun-01
64	F	09-Aug-31	29-Mar-04	1	2	R	8	1	2	DEAD	Non-metastatic	18-Apr-04
65	M	15-Jun-46	10-Dec-00	2	3	R	0	0	2	alive		31-Oct-05
66	M	18-Feb-40	02-Apr-01	3	2	R	0	1	3	DEAD	Non-metastatic	15-Aug-02
67	M	27-Sep-49	06-Sep-99	3	3	R	0	0	2	DEAD	Metastatic Disease	29-Oct-02
68	M	25-Apr-32	01-Mar-99	3	2	R	0	0	2	alive		31-Oct-05
69	M	07-Feb-34	22-May-00	1	2	L	0	0	1	alive		31-Oct-05
70	F	15-Mar-33	08-Dec-97	2	4		114	1	2	DEAD	Metastatic Disease	07-Nov-04
71	M	02-Sep-46	18-Oct-99	1	2	L	0	0	1	alive		31-Oct-05
72	F	17-Jul-52	16-Dec-97	2	2	L	6	0	2	alive		31-Oct-05
73	F	01-Oct-20	09-Nov-98	3	2	L	6	0	2	alive		31-Oct-05
74	M	19-Dec-33	13-Jul-98	2	3	R	6	0	2	DEAD	Non-metastatic	16-Jun-01
75	M	04-Nov-33	16-Aug-99	4	4	R	13	0	3	DEAD	Metastatic Disease	30-Oct-00
76	M	31-Aug-47	10-Nov-97	2	2	L	15	0	2	alive		31-Oct-05
77	F	02-May-42	10-Sep-03	4	4	L	89	1	3	DEAD	Metastatic Disease	12-Jan-04
78	M	15-Jul-54	08-Dec-98	3	.	L	17	0	2	DEAD	Metastatic Disease	22-May-05
79	F	19-Apr-28	13-Oct-97	2	.	L	6	0	2	DEAD	Non-metastatic	14-Oct-03
80	M	30-Oct-34	07-Apr-03	2	3	R	12	0	2	alive		31-Oct-05
81	F	15-Jan-45	23-Apr-01	2	3	L	34	1	2	alive		31-Oct-05
82	F	10-Mar-49	09-Jul-03	3	2	L	0	0	2	alive		31-Oct-05
83	M	16-Dec-72	25-Oct-99	1	1	L	0	0	1	alive		31-Oct-05
84	M	11-Aug-36	12-Sep-01	1	2	R	0	0	1	alive		31-Oct-05
85	F	12-Feb-23	08-May-00	2	1	R	0	0	2	alive		31-Oct-05
86	M	28-Jun-50	02-Nov-98	2	2	R	0	0	2	alive		31-Oct-05
87	M	27-Jun-29	09-Jun-03	3	1	R	34	0	2	alive		31-Oct-05
88	M	21-May-40	12-Oct-98	3	2	L	0	0	2	alive		31-Oct-05
89	F	04-Mar-39	05-Jan-04	1	4	R	0	0	2	ALIVE		31-Oct-05
90	F	21-Jan-31	14-Jul-03	3	2	R	24	0	2	alive		31-Oct-05
91	M	18-Feb-42	02-Nov-03	1	2	L	0	0	1	alive		31-Oct-05
92	F	18-Jul-50	11-Oct-04	3	2	R	11	0	2	Alive		31-Oct-05
93	M	22-Jul-31	12-Oct-00	2	1		0	0	2	alive		31-Oct-05

Pat No	Sex	DOB	DODiag	T Stage	Grade	Side	CRP	ECOG	UCLA	Alive/ Dead	Cause of death	Date FU
94	M	21-Oct-34	02-Aug-96	3	2	R	77	0	2	DEAD	Metastatic Disease	29-Aug-99
95	M	29-Feb-36	02-Sep-99	3	.	R	6	0	2	DEAD	Metastatic Disease	27-May-01
96	F	24-Feb-41	02-Oct-00	1	1	L	0	0	1	alive		31-Oct-05
97	F	16-Feb-20	28-Jan-02	3	4	R	18	0	2	alive		31-Oct-05
98	M	07-Feb-45	20-Mar-00	2	2	R	0	0	2	alive		31-Oct-05
99	M	15-Jul-53	13-Sep-04	1	3	L	14	0	2	Alive		31-Oct-05
100	M	24-Dec-20	21-Dec-98	3	3	L	50	0	2	DEAD	Metastatic Disease	08-Jan-03

APPENDIX II; Raw Data Chapter 3

Pat No	Sex	DOB	DODiag	TNM	Grade	CRP	CD4	CD4%	CD8	CD8%	CD4/8	CD4/8%	Tumour Vol	COX-2	IL-6 R	Alive/Dead	Cause of Death	Date FU
1	F	06-Sep-25	30-Jun-03	1	2	0	9	0.3	38	1.27	47	1.57	12.2	200	300	alive		01-Apr-06
2	M	02-Sep-46	18-Oct-99	1	2	0	19	0.63	24	0.8	43	1.43	15	60	120	alive		01-Apr-06
3	F	03-Apr-54	14-Apr-03	1	1	15	54	1.8	48	1.6	102	3.4	15.6	100	180	alive		01-Apr-06
4	F	17-Jun-55	20-Mar-00	1	2	6	13	0.43	87	2.9	100	3.33	16	50	0	alive		01-Apr-06
5	M	08-Nov-36	13-Sep-99	3	3	0	4	0.13	75	2.5	79	2.63	21.2	50	200	alive		01-Apr-06
6	M	16-Dec-72	25-Oct-99	1	1	0	18	0.6	26	0.87	44	1.47	23.5	20	180	alive		01-Apr-06
7	M	26-Oct-55	05-Aug-99	1	2	0	12	0.4	212	7.07	224	7.47	24	30	130	alive		01-Apr-06
8	M	03-Mar-40	08-Jan-01	1	2	6	18	0.6	5	0.17	23	0.77	27	100	200	alive		01-Apr-06
9	F	12-Jul-27	09-Aug-99	1	1	26	0	0	50	1.67	50	1.67	27	70	0	alive		01-Apr-06
10	M	24-Feb-31	15-Nov-99	1	2	0	16	0.53	50	1.67	66	2.2	30.07	30	200	alive		01-Apr-06
11	M	07-Feb-34	22-May-00	1	2	0	18	0.6	46	1.53	64	2.13	31.5	50	100	alive		01-Apr-06
12	M	15-Jan-56	23-Feb-04	1	.	14	4	0.13	12	0.4	16	0.53	48	0	0	Alive		01-Apr-06
13	F	10-Oct-19	18-Sep-00	3	1	0	24	0.8	17	0.57	41	1.37	48	90	120	alive		01-Apr-06
14	F	02-Jun-23	04-Nov-99	1	2	114	26	0.87	49	1.63	75	2.5	61.3	110	80	alive		01-Apr-06
15	M	02-Apr-49	08-Apr-03	1	1	0	0	0	2	0.07	2	0.07	64	100	200	alive		01-Apr-06
16	M	02-Jul-56	15-Sep-03	3	4	0	47	1.57	39	1.3	86	2.87	72	100	30	alive		01-Apr-06
17	M	10-May-33	12-Jun-00	3	2	6	19	0.63	62	2.07	81	2.7	72	60	60	alive		01-Apr-06
18	M	16-Sep-24	23-Nov-99	1	2	0	12	0.4	22	0.73	34	1.13	73.5	80	200	alive		01-Apr-06
19	F	30-May-34	01-Dec-03	1	2	15	17	0.57	27	0.9	44	1.47	79.5	30	20	ALIVE		01-Apr-06
20	F	30-Jan-34	05-Oct-00	1	1	49	10	0.33	9	0.3	19	0.63	84.7	60	200	alive		01-Apr-06
21	F	04-Nov-21	28-Jun-00	1	2	0	0	0	1	0.03	1	0.03	87.5	200	300	alive		01-Apr-06
22	M	09-Sep-42	21-Oct-98	1	2	0	7	0.23	28	0.93	35	1.17	96	4	200	ALIVE		01-Apr-06
23	F	09-Jul-43	25-Aug-03	1	4	68	18	0.6	8	0.27	26	0.87	111.6	100	200	alive		01-Apr-06
24	F	14-Sep-35	23-Sep-03	3	1	0	10	0.33	49	1.63	59	1.97	125	200	280	alive		01-Apr-06
25	M	22-Jul-31	12-Oct-00	2	1	0	36	1.2	80	2.67	116	3.87	129.6	80	110	alive		01-Apr-06
26	M	01-Oct-29	17-Mar-03	3	3	35	18	0.6	28	0.93	46	1.53	134	200	200	ALIVE		01-Apr-06
27	F	12-Oct-33	15-Dec-03	1	1	0	0	0	3	0.1	3	0.1	135	100	100	ALIVE		01-Apr-06
28	M	11-Nov-33	18-Dec-00	3	2	36	8	0.27	14	0.47	22	0.73	150	100	50	alive		01-Apr-06
29	F	04-Mar-39	05-Jan-04	1	4	6	43	1.43	35	1.17	78	2.6	174.2	100	300	alive		01-Apr-06
30	F	22-May-36	29-Jan-01	3	2	0	8	0.27	7	0.23	15	0.5	180	100	0	alive		01-Apr-06
31	M	21-Sep-33	05-Apr-04	2	2	0	16	0.53	14	0.47	30	1	196.7	100	200	alive		01-Apr-06

Pat No	Sex	DOB	DODiag	TNM	Grade	CRP	CD4	CD4%	CD8	CD8%	CD4/8	CD4/8%	Tumour Vol	COX-2	IL-6 R	Alive/Dead	Cause of Death	Date FU
32	M	30-Oct-34	07-Apr-03	2	3	12	2	0.07	28	0.93	30	1	225	100	0	alive		01-Apr-06
33	M	18-Feb-42	02-Nov-03	1	2	0	1	0.03	15	0.5	16	0.53	231	100	40	alive		01-Apr-06
34	M	25-Apr-56	01-Dec-97	1	3	0	2	0.07	7	0.23	9	0.3	274.6	190	300	alive		01-Apr-06
35	F	04-Mar-36	23-Nov-98	3	1	13	0	0	36	1.2	36	1.2	275	0	100	alive		01-Apr-06
36	F	12-Feb-23	08-May-00	2	1	0	0	0	8	0.27	8	0.27	330	90	100	alive		01-Apr-06
37	M	11-Aug-36	12-Sep-01	1	2	0	2	0.07	197	6.57	199	6.63	343	100	150	alive		01-Apr-06
38	M	27-Feb-32	23-Sep-03	3	1	33	3	0.1	15	0.5	18	0.6	357.5	200	0	alive		01-Apr-06
39	M	25-Apr-32	01-Mar-99	3	2	0	0	0	11	0.37	11	0.37	450	80	100	alive		01-Apr-06
40	F	01-Oct-47	16-Mar-98	4	2	32	2	0.07	4	0.13	6	0.2	520	200	0	Alive		01-Apr-06
41	F	15-Jan-45	23-Apr-01	2	3	34	46	1.53	75	2.5	121	4.03	611.1	100	150	alive		01-Apr-06
42	M	15-Jun-46	10-Dec-00	2	3	0	21	0.7	42	1.4	63	2.1	1092	90	0	alive		01-Apr-06
43	F	08-Jul-49	13-May-03	3	1	0	1	0.03	15	0.5	16	0.53	1331	100	70	alive		01-Apr-06
44	F	16-May-45	15-Mar-04	4	3	80	22	0.73	8	0.27	30	1	1331.7	200	200	Alive		01-Apr-06
45	M	27-Jun-29	09-Jun-03	3	1	34	1	0.03	6	0.2	7	0.23	1483	0	90	alive		01-Apr-06
46	M	28-Jun-50	02-Nov-98	2	2	0	1	0.03	4	0.13	5	0.17	2023	190	200	alive		01-Apr-06
47	F	24-Feb-41	02-Oct-00	1	1	0	22	0.73	71	2.37	93	3.1	3040	100	140	alive		01-Apr-06
48	M	27-May-41	01-Nov-99	2	2	0	13	0.43	6	0.2	19	0.63	3040	140	300	alive		01-Apr-06
49	F	10-Feb-21	25-Jan-99	3	2	21	25	0.83	23	0.77	48	1.6	150	130	80	DEAD	Non-metastatic	28-Jan-99
50	F	08-Dec-46	16-Nov-98	4	3	190	5	0.17	14	0.47	19	0.63	224	100	260	Dead	Metastatic Disease	06-Feb-99
51	M	06-Jan-55	01-Feb-99	4	4	242	64	2.13	324	10.8	388	12.93	472.5	80	180	Dead	Metastatic Disease	07-Dec-99
52	F	29-May-46	12-Jul-99	4	4	173	31	1.03	106	3.53	137	4.57	288	100	110	Dead	Metastatic Disease	29-Feb-00
53	M	17-Mar-28	20-Nov-97	2	3	116	32	1.07	38	1.27	70	2.33	157.5	40	260	DEAD	Metastatic Disease	29-Aug-00
54	M	17-Jul-25	13-Jul-00	3	2	0	20	0.67	18	0.6	38	1.27	229	80	150	Dead	Non-metastatic	12-Dec-00
55	F	05-Oct-25	08-Sep-00	1	2	48	32	1.07	73	2.43	105	3.5	40	80	90	Dead	Non-metastatic	26-May-01
56	M	18-Feb-40	02-Apr-01	3	2	0	22	0.73	29	0.97	51	1.7	541.5	40	0	DEAD	Non-metastatic	15-Aug-02
57	M	27-Apr-52	29-Oct-01	3	4	234	3	0.1	54	1.8	57	1.9	614.1	60	260	DEAD	Non-metastatic	14-Oct-02
58	M	27-Sep-49	06-Sep-99	3	3	0	9	0.3	12	0.4	21	0.7	168.8	120	200	DEAD	Metastatic Disease	29-Oct-02
59	M	28-Sep-20	06-Dec-99	1	1	0	0	0	1	0.03	1	0.03	42.9	190	200	Dead	Non-metastatic	05-Jan-03
60	M	24-Dec-20	21-Dec-98	3	3	50	4	0.13	6	0.2	10	0.33	408	120	140	DEAD	Metastatic Disease	08-Jan-03
61	F	20-Oct-42	30-Jul-00	4	2	0	4	0.13	2	0.07	6	0.2	562	180	80	Dead	Metastatic Disease	02-Dec-03
62	M	11-Dec-49	17-Nov-03	4	4	86	189	6.3	142	4.73	331	11.03	363	0	0	Dead	Metastatic Disease	18-Mar-04

Pat No	Sex	DOB	DODiag	TNM	Grade	CRP	CD4	CD4%	CD8	CD8%	CD4/8	CD4/8%	Tumour Vol	COX-2	IL-6 R	Alive/Dead	Cause of Death	Date FU
63	M	03-Feb-67	23-Sep-03	3	3	21	53	1.77	102	3.4	155	5.17	960	200	300	DEAD	Metastatic Disease	16-Jul-04
64	M	28-Jan-51	26-Apr-04	4	4	231	124	4.13	54	1.8	178	5.93	252	200	40	Dead	Metastatic Disease	27-Jul-04
65	F	15-Mar-33	08-Dec-97	2	4	114	9	0.3	27	0.9	36	1.2	700	140	40	DEAD	Metastatic Disease	07-Nov-04
66	M	05-May-47	19-Jan-04	4	4	122	112	3.73	152	5.07	264	8.8	1157.6	60	160	Dead	Metastatic Disease	24-Nov-04
67	M	13-Oct-45	19-May-03	4	4	173	186	6.2	183	6.1	369	12.3	274	200	180	Dead	Metastatic Disease	15-Dec-04
68	M	27-Sep-53	06-Oct-03	4	2	0	23	0.77	60	2	83	2.77	115.7	100	240	Dead	Metastatic Disease	24-Dec-04
69	M	14-Oct-40	14-Dec-99	3	3	13	19	0.63	8	0.27	27	0.9	61.3	10	110	DEAD	Metastatic Disease	09-Apr-05
70	F	13-Feb-51	29-Jul-99	3	4	0	28	0.93	9	0.3	37	1.23	252	80	300	Dead	Metastatic Disease	12-Jan-06
71	F	10-Mar-49	09-Jul-03	3	2	0	3	0.1	0	0	3	0.1	27	200	40	Alive		01-Apr-06
72	M	12-Sep-40	17-Feb-00	3	3	78	14	0.47	57	1.9	71	2.37	719	160	180	Alive		01-Apr-06