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Lamb, G.W. and McArdle, P.A. and Ramsey, S. and McNichol, A.M. and Edwards, J. and Aitchison, M. and McMillan, D.C. (2008) *The relationship between the local and systemic inflammatory responses and survival in patients undergoing resection for localized renal cancer.* BJU International, 102 (6). pp. 756-761. ISSN 1464-4096

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Deposited on: 27 March 2009

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The relationship between the local and systemic inflammatory responses and survival in patients undergoing resection for localized renal cancer

Gavin WA Lamb[1], Peter A McArdle[2], Sara Ramsey[1], Anne-Marie McNichol[3], Joanne Edwards[2], Michael Aitchison[1], Donald C McMillan[2]

1. Department of Urology, Gartnavel General Hospital, Glasgow G12 0YN.
2. University Department of Surgery, Royal Infirmary, Glasgow G31 2ER.
3. University Department of Pathology, Royal Infirmary, Glasgow G31 2ER

Keywords: Renal cancer, nephrectomy, tumour stage, grade, performance status, systemic inflammatory response, cancer specific survival.

Correspondence to:

Dr Donald C McMillan,

University Department of Surgery,

Royal Infirmary,

Glasgow G31 2ER, United Kingdom.

Tel No. 0141 211 5435

Fax No. 0141 552 3229

E-mail d.c.mcmillan@clinmed.gla.ac.uk

Summary

Objective: Both the local and systemic inflammatory responses appear to predict outcome in patients undergoing nephrectomy for renal cell carcinoma. To date almost all studies have examined such factors in isolation. The aim of the present study was therefore to examine the relationship between the systemic inflammatory response (C-reactive protein), tumour interleukin-6 receptor and COX-2 expression, tumour T-lymphocytic (CD4+, CD8+) infiltration and cancer survival in patients undergoing resection for renal cancer.

Patients and Methods: Sixty patients undergoing nephrectomy for localised renal cancer were studied. Pre-operative circulating C-reactive protein concentrations were measured and tumour interleukin-6 receptor and cyclo-oxygenase-2 expression, tumour CD4+ and CD8+ T-lymphocytes were assessed using immunohistochemical analysis.

Results: Median follow up was 78 months with 14 patients relapsing from their disease and 9 cancer specific deaths. On univariate and multivariate survival analysis tumour stage and grade and C-reactive protein were identified as significant factors associated with relapse-free and cancer specific survival. There was a significant direct relationship between Fuhrman grade and CD4+ T-lymphocytic infiltrate ($p<0.05$). An increase in tumour expression of interleukin-6 receptor was weakly associated with an increase in tumour CD8+ T-lymphocytic infiltration ($p=0.057$). An increase in tumour CD4+ T-lymphocytic infiltration was associated with an increase in CD8+ T-lymphocytic infiltration ($p<0.01$).

Conclusions: In summary, the results of the present study would suggest that tumour-based factors such as interleukin-6 receptor and COX-2 expression or T-lymphocytic subset infiltration are subordinate to systemic factors such as C-reactive protein in determining survival in patients with localised renal cancer.

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 these 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 [1,2,3]. Indeed, 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 advanced malignancies [4,5,6] including renal cancer [7,8,9,10,11].

There is also evidence that C-reactive protein has independent prognostic value in a variety of primary operable cancers [12,13,14,15]. Recently, it has been reported that an elevated pre-operative C-reactive protein is associated with poor cancer specific survival in patients with operable renal cancer independent of tumour stage and grade [16,17].

The basis of the independent relationship between an elevated pre-operative C-reactive protein concentration and poor cancer specific 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 [18,19], and therefore with the potential for more rapid growth of tumour cells [20,21]. Alternatively, C-reactive protein could directly impair immune function [22,23,24] allowing unrestrained tumour growth and dissemination.

Precise localisation of pro-inflammatory cytokines such as interleukin-6 to tumour cells or inflammatory cells within the tumour, particularly in paraffin embedded tissues, remains problematic [24]. However, interleukin-6 activity has been assessed by staining for

interleukin-6 receptor expression in a variety of solid tumours, including renal cancer [25] and infiltration of tumours with T- lymphocytes has been reliably demonstrated in a variety of solid tumours, including renal cancer [26,27].

The molecular basis of the increased production of interleukin-6 and other pro-inflammatory cytokines by the renal tumour remains to be defined. However, central to the local inflammatory response is cyclooxygenase-2 (COX-2) and increased expression has been shown to be associated with poor survival in a number of common solid tumours [28,29]. COX-2 is the rate limiting enzyme in the synthesis of prostaglandin E2 (PGE2) which is known to stimulate proliferation and inhibit apoptosis. PGE2 is also recognised to induce interleukin-6 [30] and NSAIDs have been shown to reduce interleukin-6 concentrations in cancer patients [31,32] or animal cancer models [33].

In normal tissues COX-2 is usually absent, however, the kidney is unusual in that there is expression of COX-2 in physiological health [34]. Few studies have examined the role of COX-2 in renal cancer. There is conflicting evidence as to whether increased COX-2 expression is associated with poor survival in renal cancer [35,36].

Recently, there have been comparisons between the local and systemic inflammatory responses and survival in colorectal [24] and bladder [14] cancer. However, to our knowledge such comparison has not been carried out in renal cancer. The aim of the present study was therefore to examine the relationship between the systemic inflammatory response (C-reactive protein), tumour interleukin-6 receptor and COX-2 expression, tumour T-lymphocytic (CD4+, CD8+) infiltration and cancer survival in patients undergoing resection for localised renal cell cancer.

Patients and Methods

Patients diagnosed with renal clear cell cancer, who underwent potentially curative resection for localized disease 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 [37]. At this time no patient showed clinical evidence of infection or other inflammatory conditions.

Tumours were graded by experienced pathologists according to criteria set out by Fuhrman and coworkers [38]. Also, routine laboratory measurement of patient's serum for C-reactive protein concentration prior to surgery 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 a systemic inflammatory response [4].

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

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. Serial individual sections (4 μm) were cut and mounted on slides coated with aminopropyltriethoxysilane for the immunohistochemistry of interleukin-6 receptor, CD4+ and CD8+ T-lymphocytes and COX-2 expression.

Interleukin-6 receptor

Slides were dewaxed in xylene and rehydrated using graded alcohols. Antigen retrieval for IL-6 receptor was carried out by incubating in 10mM citrate buffer (Epitope retrieval solution x 10, Dako, Cambridgeshire, UK) in a calibrated water bath at 96^{o}C for 20 minutes. Slides were then immersed in 0.3% hydrogen peroxide for 20 minutes to block endogenous peroxidases. The primary antibody for interleukin-6 receptor was rabbit polyclonal (Santa Cruz Biotechnology Inc, USA). Sections were immunostained using a streptavidin biotin technique (Dako, Cambridgeshire, UK). Sections were counterstained with haematoxylin, dehydrated, cleared and mounted.

CD4+ and CD8+ T-lymphocytes

Sections were immunostained using the peroxidase-based Envision technique (Dako, Cambridgeshire, UK) as described previously [27]. The primary antibody for CD4 was mouse monoclonal (Vector, Peterborough, UK) and that for CD8 was mouse monoclonal (Dako, Cambridgeshire, UK).

COX-2

Sections were immunostained using the biotinylated/streptavidin peroxidase complex technique (Dako, Cambridgeshire, UK) as previously described (Edwards et al, 2004). The primary antibody was human monoclonal antibody (Cayman Chemical Co., Annbor, Michigan, USA).

Morphometry

Quantitative analysis of the lymphoid infiltrate was performed using point counting [27] with a random sampling technique. With this method, the volume occupied by any given

component (volume density) is expressed as a percentage of the total volume of the tissue. A 100-point ocular grid was used at x400 magnification and 30 fields were counted per case for CD4+ and CD8+ immunopositive cells. 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.

COX-2 and IL-6 receptor expression were assessed as previously described [39]. 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, PAM and SR) blind to the clinical outcome of the patient.

Statistics

For the purpose of analysis, the tumour interleukin-6 receptor, CD4+ and CD8+ T-lymphocyte counts and COX-2 expression were grouped by tertiles. The relationships between these and other variables were analyzed using the Mantel–Haenszel (X^2) test for trend and Spearman's rank correlation as appropriate.

Survival analysis was performed using the Cox proportional hazard model. Multivariate survival analysis was performed using 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. Deaths up to the end of April 2007 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

The characteristics and cancer specific survival of patients who underwent a potentially curative resection for renal cancer (n= 60) are shown in Table 1. The majority of patients were over 60 years of age, were male, were TNM stage I/ II and had Fuhrman grade I-II disease. In the 23 patients who had TNM stage III disease this was based on T stage, no patients had involved lymph nodes. Approximately one third of patients had an elevated C-reactive protein concentration (>10mg/l).

The minimum follow-up was 37 months; the median follow-up of the survivors was 78 months. During this period, 14 patients relapsed and 9 died of their cancer; a further 8 patients died of intercurrent disease. On univariate survival analysis, age ($p<0.10$), TNM stage ($p<0.05$), Fuhrman grade ($p<0.01$) and C-reactive protein ($p<0.05$) were significant predictors of relapse-free survival. On multivariate analysis with age, TNM stage, Fuhrman grade, and C-reactive protein entered as covariates, age (HR 0.26, 95%CI 0.09-0.75, $p=0.0175$, TNM stage (HR 2.11, 95%CI 1.05-4.22, $p=0.0351$) and C-reactive protein (HR 2.66, 95% CI 0.88–8.03, $p=0.0838$) were independent predictors of relapse-free survival.

On univariate survival analysis, TNM stage ($p<0.01$), Fuhrman grade ($p<0.01$), COX-2 expression ($p<0.05$) and C-reactive protein ($p<0.05$) were significant predictors of cancer specific survival. On multivariate analysis with TNM stage, Fuhrman grade, COX-2 expression and C-reactive protein entered as covariates, TNM stage (HR 5.98, 95%CI 1.25-28.59, $p=0.0250$) and C-reactive protein (HR 3.40, 95% CI 0.81–14.38, $p=0.0950$) were independent predictors of cancer specific survival.

The inter-relationships between the clinicopathological characteristics in patients undergoing resection for renal cancer are shown in Table 2. An increase in Fuhrman grade was associated with increased tumour CD4+ T-lymphocytic infiltration ($p<0.05$). An increase in tumour expression of interleukin-6 receptor was weakly associated with an increase in

tumour CD8+ T-lymphocytic infiltration ($p=0.057$). An increase in tumour CD4+ T-lymphocytic infiltration was associated with an increase in CD8+ T-lymphocytic infiltration ($p<0.01$). The Spearman rank correlation between tumour CD4+ and CD8+ T-lymphocytic infiltration was 0.47 ($p<0.001$).

Discussion

In the present study, in addition to tumour stage and grade, the presence of a systemic (C-reactive protein) but not a local (tumour IL-6 receptor, COX-2 expression and CD4+/ CD8+ T lymphocytic infiltrate) inflammatory response was associated with poor relapse free and cancer specific survival in patients undergoing potentially curative resection for renal cancer. These results confirm the importance of circulating concentrations of C-reactive protein in the staging and prognosis of patients with localised renal cancer [16,17,40,41] and suggest that that tumour-based inflammation factors such as IL-6 receptor, COX-2 expression or T-lymphocytic infiltration are subordinate to systemic factors such as C-reactive protein in determining cancer outcome in these patients.

C-reactive protein concentrations above the threshold used in the present study (>10 mg/ l) are rare (<5%) in the general elderly population in the West of Scotland [42] and elevated circulating C-reactive protein concentrations are primarily determined by circulating IL-6 [43]. IL-6 is expressed in the majority of renal cancers and has been proposed as an autocrine growth factor [7,44,45]. Furthermore, circulating IL-6 has been shown to be associated with increased tumour grade, volume and metastasis in patients with renal cancer [46]. However, in the present study tumour cell expression of interleukin-6 receptor, a surrogate marker for interleukin-6, was not significantly associated with circulating concentrations of C-reactive protein. This would suggest that the main source of interleukin-6 responsible for an elevated C-reactive protein, in patients with localised renal cancer, was unlikely to be the tumour itself. However, a wide range of cell types are known to produce interleukin-6 and therefore the main source remains to be established [47].

In the present study, in contrast to previous studies [27,48] tumour CD4+ lymphocytic infiltrate was not associated with cancer specific survival. However, previous studies included patients, in terms of tumour stage and grade, with more advanced disease and

therefore the present results reflect early changes in the tumour inflammatory infiltrate. Nevertheless, the observation that, in the present study, tumour CD4+ lymphocytic infiltrate was directly associated with tumour grade and would be consistent with an increase in CD4+ T-lymphocytic infiltration being an early host inflammatory response in patients with renal cancer.

In the only other reported study, Costes and coworkers [25] reported a significant association between the presence of the IL-6 receptor in renal tumours and stage and grade. Furthermore, they reported that those patients with positive staining of IL-6R had poorer survival. In the present study tumour IL-6 receptor was not significantly associated with stage, grade or survival. Costes and coworkers [25] in their study examined 38 patients with mean follow-up 14 months compared with 60 patients and a mean follow-up of approximately 6 years in the present study. However, since both studies used a semi-quantitative assessment of IL-6 receptor further work is required to establish whether or not tumour expression of IL-6 receptor has prognostic value in patients with renal cancer.

Few studies have examined the expression of COX-2 in renal cancer. Some studies have suggested that it is increased with tumour stage and grade [35,36,49]. In contrast, consistent with other workers [50] we observed no such relationship. Also, consistent with previous studies [35,36,50] the present study showed little prognostic value for tumour COX-2 expression.

The results of the present study are consistent with the superior prognostic value of C-reactive protein compared with tumour T-lymphocytic infiltration and COX-2 expression in primary operable colorectal and bladder cancer [24,14]. One possible explanation is that C-reactive protein can be measured with greater accuracy and precision than tumour-based factors. Alternatively, it may be that C-reactive protein plays a more pivotal role in the tumour–host relationship. C-reactive protein is recognised to be not only an activator of

innate immunity but also a modulator of adaptive immunity [23] and its elevation appears to be a precursor to tumour dissemination and progressive involuntary loss of weight and lean tissue [51] which are key factors in determining cancer survival. Also, it is of interest that Jabs and co-workers [52] reported that tumour expression of C-reactive protein was directly associated with circulating concentration. It would therefore appear that tumour derived C-reactive protein concentration may make a contribution to circulating systemic levels. Therefore, the use of C-reactive protein, rather than tumour tissue based factors, in addition to tumour stage and grade, to more accurately stratify patients with localised renal cancer has much to commend it, particularly since it is well standardised worldwide.

In summary, the results of the present study would suggest that tumour-based factors such as interleukin-6 receptor and COX-2 expression or T-lymphocytic subset infiltration are subordinate to systemic factors such as C-reactive protein in determining survival in patients with localised renal cancer.

Acknowledgements

The authors gratefully acknowledge the funding provided by the “Fischer Foundation”, a charitable organisation dedicated to research in renal cancer. We also acknowledge the assistance of Mohamed Hadada, Emma Bromwich and David Murray.

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Table 1. Clinicopathological characteristics and survival in patients undergoing potentially curative resection for renal cancer: univariate analysis.

		Relapse free survival		Cancer specific survival	
	Patients	Hazard ratio	p-value	Hazard ratio	p-value
	n= 60	(95%CI)		(95%CI)	
Age group (≤60/ >60 yrs)	24/ 36	0.34 (0.11-1.01)	0.0530	0.36 (0.09-1.44)	0.1498
Sex (male/ female)	37/ 23	0.67 (0.21-2.13)	0.4943	0.80 (0.20-3.20)	0.7511
TNM stage (I/ II/ III)	27/10/ 23	2.12 (1.11-4.03)	0.0227	5.36 (1.55-18.47)	0.0079
Fuhrman grade (I/ II/ III/ IV)	16/ 25/ 12/ 6	2.46 (1.39-4.32)	0.0019	3.43 (1.64-7.17)	0.0010
IL-6 receptor (tertiles 1, 2, 3)	140 (0-300)*	1.03 (0.54-1.99)	0.9207	1.24 (0.52-2.98)	0.6148
COX-2 (tertiles 1, 2, 3)	100 (0-200)*	1.42 (0.71-2.82)	0.3229	2.55 (1.02-6.37)	0.0455
T-lymphocytes					
(% tumour volume)					
CD4+ (tertiles 1, 2, 3)	0.40 (0.01-1.80)*	1.70 (0.84-3.45)	0.1397	2.11 (0.80-5.56)	0.1294
CD8+ (tertiles 1, 2, 3)	0.88 (0.01-7.07)*	0.73 (0.39-1.39)	0.3143	0.57 (0.25-1.30)	0.1798
C-reactive protein					
(≤10/ >10 mg/l)	38/ 22	2.89 (1.00-8.39)	0.0416	4.83 (1.20-19.48)	0.0269

*median (range)

Table 2. The inter-relationships between the clinicopathological characteristics in patients undergoing potentially curative resection for renal cancer.

	Fuhrman grade (I/ II/ III/ IV) p-value	IL-6 receptor (tertiles 1, 2, 3) p-value	COX-2 (tertiles 1, 2, 3) p-value	CD4+ (tertiles 1, 2, 3) p-value	CD8+ (tertiles 1, 2, 3) p-value	C-reactive protein (≤10/ >10 mg/l) p-value
TNM stage (I/ II/ III)	0.112	0.184	0.210	0.586	0.731	0.312
Fuhrman grade (I/ II/ III/ IV)		0.274	0.334	0.033	0.750	0.135
IL-6 receptor (tertiles 1, 2, 3)			0.097	0.841	0.057	0.101
COX-2 (tertiles 1, 2, 3)				0.278	0.100	0.754
T-lymphocytes (% tumour volume)						
CD4+ (tertiles 1, 2, 3)					0.002	0.732
CD8+ (tertiles 1, 2, 3)						0.515