

**Keywords:** interleukin-6; tumour necrosis; systemic inflammatory response; local inflammatory response; colorectal cancer

# Circulating IL-6 concentrations link tumour necrosis and systemic and local inflammatory responses in patients undergoing resection for colorectal cancer

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**Background:** Cancer-associated inflammation, in the form of local and systemic inflammatory responses, appear to be linked to tumour necrosis and have prognostic value in patients with colorectal cancer. However, their relationship with circulating biochemical mediators is unclear. The aim of the present study was to examine the interrelationships between circulating mediators, in particular interleukin-6 (IL-6) and tumour necrosis, and local and systemic inflammatory responses in patients undergoing resection for colorectal cancer.

**Methods:** Data were collected from preoperative blood tests for 118 patients who underwent resection for colorectal cancer. Analysis of circulating IL-6, IL-10, vascular endothelial growth factor (VEGF), differential white cell count, C-reactive protein, and albumin were carried out. Routine pathology specimens were examined for tumour characteristics including necrosis and the extent of the inflammatory cell infiltrate. Body composition was examined using body mass index (BMI), total body fat, subcutaneous body fat, visceral fat, and skeletal muscle mass.

**Results:** Circulating IL-6 concentrations were significantly associated with increased T stage ( $P < 0.05$ ), tumour necrosis ( $P < 0.001$ ), IL-10 ( $P < 0.001$ ), VEGF ( $P < 0.001$ ), modified Glasgow Prognostic Score (mGPS;  $P < 0.001$ ), white cell ( $P < 0.01$ ) and platelet ( $P < 0.01$ ) counts, and low skeletal muscle index ( $P < 0.01$ ). When normalised for T stage, tumour necrosis was associated with IL-6 ( $P < 0.001$ ), IL-10 ( $P < 0.01$ ), VEGF ( $P < 0.001$ ), mGPS ( $P < 0.001$ ), neutrophil-lymphocyte ratio (NLR;  $P < 0.05$ ), white cell ( $P < 0.001$ ), neutrophil ( $P < 0.05$ ), and platelet counts ( $P < 0.005$ ), and skeletal muscle index ( $P < 0.001$ ).

**Conclusion:** The present study provides, for the first time, supportive evidence for the hypothesis that tumour necrosis, independent of T stage, is associated with elevated circulating IL-6 concentrations, thereby modulating both local and systemic inflammatory responses including angiogenesis that, in turn, may promote tumour progression and metastases.

Colorectal cancer is the second most common cause of cancer death in both men and women in the United Kingdom, with 16 000 deaths per year (Cancerstats, 2005–2010). Despite improvements in treatment, outcomes remain poor, with approximately half of those undergoing curative resection dying from the disease (Oliphant *et al*, 2013).

In recent years, it has become increasingly apparent that cancer-associated inflammation, in the form of local and systemic

inflammatory responses, is a key determinant of disease progression and survival in colorectal cancer. Indeed, there is consistent evidence that the presence of a high-grade inflammatory or immune cell infiltrate in the tumour and in the immediate microenvironment predicts survival independent of tumour stage in colorectal cancer (Klintrup *et al*, 2005; Galon *et al*, 2006; Roxburgh and Mcmillan, 2012). Furthermore, many studies have reported that markers of the systemic inflammatory response, in

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particular C-reactive protein, albumin, and their combination in the modified Glasgow Prognostic Score (mGPS), and the neutrophil-lymphocyte ratio (NLR) predict survival independent of tumour stage (Guthrie *et al*, 2013a; Mcmillan, 2013).

Despite the strong evidence linking the local and systemic inflammatory responses to colorectal cancer survival and their apparent independent prognostic value, the mechanisms by which these two related inflammatory processes are activated, maintained, and interact are not clear. Recent work has highlighted tumour necrosis as a prognostic factor in colorectal cancer and its close associations with the local and systemic inflammatory responses (Pollheimer *et al*, 2010; Richards *et al*, 2012b). However, what remains unclear is the mechanism linking tumour necrosis and the local and systemic inflammatory responses.

One hypothesis is that tumour necrosis may link these two key inflammatory processes via immunological mediators, in particular interleukin-6 (IL-6), present in the tumour microenvironment and in the circulation (Tripathi *et al*, 2003; Guthrie *et al*, 2013b).

The molecular links between tumour necrosis and these key inflammatory processes are likely to be complex. The 'dirty', hypoxia-driven, unscheduled cell death occurring in tumour necrosis makes it likely that this process may stimulate a variety of inflammatory mediators that can influence both the systemic and local inflammatory responses. Other potential candidates for such mediators include circulating IL-10 and vascular endothelial growth factor (VEGF; Mocellin *et al*, 2005; Hsu and Chung, 2006).

Therefore, the aim of the present study was to examine whether circulating mediators, in particular IL-6, may be a link between tumour necrosis and local and systemic inflammatory responses in patients undergoing curative resection for colorectal cancer.

## PATIENTS AND METHODS

Patients with colorectal cancer who, on the basis of preoperative staging and laparotomy findings, were considered to have undergone an elective, potentially curative resection of colorectal cancer between April 2004 and July 2009 in a single surgical unit at Glasgow Royal Infirmary were included in the study. Tumours were staged using the conventional tumour, node, metastasis (TNM) staging system, 7th edition, 2010 (AJCC, 2010). Patients with conditions known to elicit an acute or chronic systemic inflammatory response were excluded. These were namely (1) preoperative chemoradiotherapy, (2) clinical evidence of active preoperative infection, or (3) chronic active inflammatory diseases such as rheumatoid arthritis.

Blood samples were collected before surgery for routine laboratory analysis of full blood count, white cell and lymphocyte counts, albumin, and C-reactive protein.

The mGPS was constructed based on routine preoperative blood tests (McMillan, 2013). Briefly, patients with both an elevated C-reactive protein ( $>10 \text{ mg l}^{-1}$ ) and low albumin ( $<35 \text{ g l}^{-1}$ ) were allocated a score of 2; patients in whom only C-reactive protein was elevated ( $>10 \text{ mg l}^{-1}$ ) were allocated a score of 1; and patients with a normal C-reactive protein were allocated a score of 0.

The NLR ratio was constructed using previously documented thresholds (Guthrie *et al*, 2013a). Briefly, NLR was determined by dividing the absolute neutrophil count by the absolute lymphocyte count, and the NLR data were then dichotomised and given a score of 0 ( $<5:1$ ) and 1 ( $>5:1$ ).

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

Blood samples were centrifuged and the serum stored at  $-80^\circ\text{C}$  before analysis of IL-6, IL-10, and VEGF. Circulating concentrations of IL-6, IL-10, and VEGF were measured using

commercially available human colorimetric enzyme-linked immunosorbent assays (Quantikine ELISA, R&D Systems, Europe Ltd, Abingdon, UK). The minimum detectable concentrations were  $2 \text{ pg ml}^{-1}$  for IL-6,  $4 \text{ pg ml}^{-1}$  for IL-10, and  $9 \text{ pg ml}^{-1}$  for VEGF.

Assessment of the local inflammatory cell infiltrate and tumour necrosis was performed on original haematoxylin and eosin-stained full sections of the tumour, felt to represent the maximum depth of tumour invasion. The local inflammatory response was evaluated previously in this cohort using the method described by Klintrup and Makinen (K-M; Klintrup *et al*, 2005). Briefly, the K-M criterion is a four-point scale with scores as follows: a score of 0 indicates no increase in inflammatory cells at the invasive margin, a score of 1 indicates presence of a mild/patchy increase in inflammatory cell reaction at the invasive margin but no destruction of invading cancer cell islets, a score of 2 indicates observation of a band-like inflammatory reaction at the invasive margin, and a score of 3 indicates observation of a florid inflammatory reaction with cup-like inflammatory infiltrate at the invasive margin. The scores were then dichotomised to 'high' and 'low' grade inflammation in line with the previously published literature (Klintrup *et al*, 2005).

Tumour necrosis was assessed using the method described by Pollheimer *et al* (2010). Briefly, at  $\times 40$  magnification, the full sections were examined for evidence of tumour necrosis. Tumour necrosis was graded as 'absent' (none), 'focal' ( $<10\%$  of tumour surface area), 'moderate' (10–30% tumour surface area), or 'extensive' ( $>30\%$  of tumour surface area) in each section before an assessment of overall extent of necrosis was made.

To test the reliability of the evaluation of necrosis, sections of 30 patients (average of 3 slides per patient) were examined independently by two observers (GJG and CSDR) blinded to clinical outcome and clinicopathological variables. The intraclass correlation coefficient (ICC) for the assessment of local inflammatory cell infiltrate was 0.81 and for tumour necrosis was 0.70.

To evaluate metabolic upset, the body composition parameters, body mass index (BMI), total body fat, subcutaneous body fat, visceral fat, and skeletal muscle mass, as previously described (Richards *et al*, 2012c), were used to assess the relationship between systemic and local inflammation, tumour necrosis and circulating immunological factors, and cancer cachexia.

**Statistics.** Data are presented as median and range. Grouping of variables was carried out using standard thresholds for laboratory parameters. As there are no widely accepted thresholds for IL-6, IL-10, and VEGF, the values were grouped as tertiles (Lee *et al*, 2012). The concentrations of IL-6, IL-10, and VEGF below the threshold of sensitivity of the respective assays were expressed as equal to this threshold. The relationships between the groups of patients was carried out using Mantel-Haenszel ( $\chi^2$ ) test for trend and the Kruskal-Wallis test and Spearman's rank correlation as appropriate. The relationship between IL-6 and cancer-specific survival was examined using the Kaplan-Meier method. Analysis was performed using SPSS software version 19 (SPSS Inc., Chicago, IL, USA).

## RESULTS

The majority of patients were  $\geq 65$  years old (66%), were male (51%), had TNM stage I/II disease (62%), and the majority had C-reactive protein (68%) and albumin (72%) concentrations in the normal range and a normal mGPS (68%). Of the 33 patients with hypoalbuminaemia, 16 (48%) had an elevated C-reactive protein. The majority of patients also had total white cell counts (71%), neutrophil counts (89%), lymphocyte counts (82%), and platelet counts (87%) in the normal range and a normal NLR (80%).

Serum IL-6 concentrations were divided into tertiles. The tertiles had approximately equal numbers of patients: tertile 1 (39 patients), tertile 2 (39 patients), and tertile 3 (40 patients). Tertile 1 (median concentration 2 pg ml<sup>-1</sup>, range 0.8–3.38) the lowest, tertile 2 moderately elevated concentration (median concentration 4.85 pg ml<sup>-1</sup>, range 3.43–6.01), and tertile 3 (median concentration 9.97 pg ml<sup>-1</sup>, range 6.1–252.46) the highest.

On assessment of tumour necrosis, 48% had no evidence of tumour necrosis, 29% had focal areas of tumour necrosis, 11% had moderate tumour necrosis, whereas 12% had extensive tumour necrosis. On assessment of the local tumour inflammatory cell response using the K-M criteria, the majority of patients were considered to have a high-grade inflammatory cell infiltrate (55%).

On Spearman's rank correlation of individual values, there were significant associations between circulating concentrations of IL-6 and IL-10 ( $r=0.56$ ,  $P<0.001$ ), C-reactive protein ( $r=0.45$ ,  $P<0.005$ ), albumin ( $r=-0.65$ ,  $P<0.001$ ), and the skeletal muscle index ( $r=-0.38$ ,  $P=0.056$ ). Circulating concentrations of IL-10 were significantly associated with albumin ( $r=0.40$ ,  $P<0.05$ ). Circulating concentrations of VEGF were significantly associated with C-reactive protein ( $r=0.32$ ,  $P<0.05$ ).

The relationship between circulating IL-6 tertiles, tumour characteristics, and systemic responses in all patients with

colorectal cancer is shown in Table 1. When all patients were considered, circulating IL-6 was associated with gender ( $P<0.05$ ), increased IL-10 ( $P<0.001$ ), VEGF ( $P<0.001$ ), tumour site

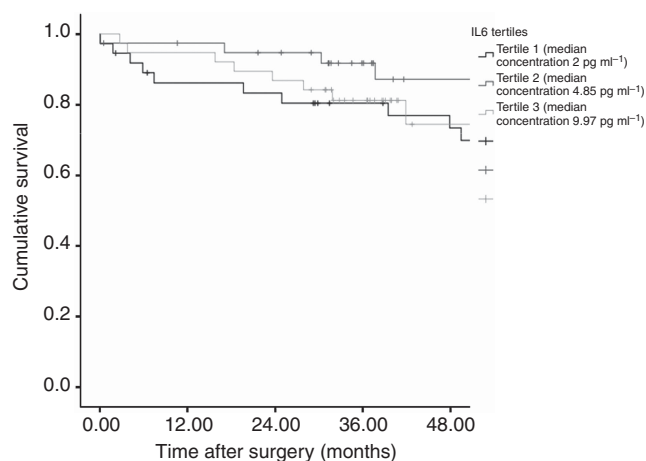


Figure 1. Kaplan–Meier analysis of cancer survival in relation to serum IL-6 concentration ( $P=0.292$ ).

Table 1. The relationships between circulating IL-6, tumour characteristics, and systemic responses in patients with colorectal cancer

Patient characteristics	Circulating IL-6 (pg ml <sup>-1</sup> )			P-value
	<3.4 pg ml <sup>-1</sup> (n = 39)	3.4–6.0 pg ml <sup>-1</sup> (n = 39)	>6.0 pg ml <sup>-1</sup> (n = 40)	
Age (<65/ 65–74/≥75 years)	18/12/10	13/16/18	8/11/12	0.080
Sex (male/female)	25/14	20/19	15/25	0.019
<b>Tumour characteristics</b>				
Presentation (elective/emergency)	36/1	37/0	32/1	0.948
Tumour site (colon/ rectum)	17/21	21/17	24/10	0.028
T stage (T <sub>1</sub> /T <sub>2</sub> /T <sub>3</sub> /T <sub>4</sub> )	4/7/23	2/5/25/7	1/2/25/12	0.007
N stage (N <sub>0</sub> / N <sub>1</sub> /N <sub>2</sub> )	29/4/6	23/12/4	21/14/5	0.237
Tumour necrosis (low/high)	24/13	24/12	6/34	<0.001
Vascular invasion (yes/no)	14/25	19/20	14/26	0.927
Inflammatory cell infiltrate (K-M low grade/high grade)	19/20	18/19	13/24	0.239
<b>Circulating mediators</b>				
IL-10 (tertiles)	19/12/7	11/12/7	5/9/20	<0.001
VEGF (tertiles pg ml <sup>-1</sup> )	25/0/0	10/29/0	0/5/35	<0.001
<b>Systemic responses</b>				
Systemic inflammatory response (mGPS 0/1/2)	34/4/1	31/8/0	15/10/15	<0.001
NLR (low <5/high >5)	34/5	30/9	30/10	0.182
Anaemia	12/27	13/26	11/29	0.751
> 13 g dl <sup>-1</sup> (men), > 11.5 g dl <sup>-1</sup> (women)				
< 13 g dl <sup>-1</sup> men, < 11.5 g dl <sup>-1</sup> (women)				
White cell count (<8.5/8.5–11.5/> 11.5 × 10 <sup>9</sup> per l)	30/6/3	32/6/1	22/11/7	0.030
Neutrophil count (<7.5/>7.5 × 10 <sup>9</sup> per l)	35/4	38/1	32/8	0.164
Lymphocyte count (≥1/<1 × 10 <sup>9</sup> per l)	6/33/0	9/28/2	4/36/0	0.545
Monocyte count (<1/>1 × 10 <sup>9</sup> per l)	38/1	34/5	34/6	0.069
Platelets (≤400/>400 × 10 <sup>6</sup> per l)	37/2	35/4	31/9	0.021
BMI (kg m <sup>-2</sup> ; normal weight, overweight, obese)	1/6/2	6/2/5	8/7/8	0.818
Total fat index (cm <sup>2</sup> m <sup>-2</sup> ; low/medium/high)	0/3/6	6/4/3	8/6/9	0.161
Subcutaneous fat index (cm <sup>2</sup> m <sup>-2</sup> ; low/medium/high)	0/4/5	6/3/4	11/2/10	0.163
Visceral fat index (cm <sup>2</sup> m <sup>-2</sup> ; low/medium/high)	1/5/3	6/6/1	8/7/8	0.795
Skeletal muscle index (cm <sup>2</sup> m <sup>-2</sup> ; low/medium/high)	0/1/8	2/5/6	7/10/6	0.002

Abbreviations: BMI = body mass index; IL-6 = interleukin-6; IL-10 = interleukin-10; K-M = Klintrup and Makinen criteria; mGPS = modified Glasgow Prognostic Score; NLR = neutrophil-lymphocyte ratio; VEGF = vascular endothelial growth factor.

**Table 2.** The interrelationships between different pathological and clinical parameters in patients with T3 stage disease undergoing potentially curative resection for colorectal cancer ( $n = 73$ )

	Tumour characteristics		Circulating mediators			Systemic responses			
	Tumour necrosis	K-M (low/high grade)	IL-6	IL-10	VEGF	Platelet count	mGPS	NLR	Skeletal muscle index
Site	0.111	0.787	0.023	0.644	0.008	0.173	0.042	0.868	0.427
Tumour necrosis		0.140	<0.001	0.008	<0.001	0.004	<0.001	0.044	0.009
K-M (low/high grade)			0.768	0.837	0.428	0.719	0.320	0.886	0.496
IL-6				<0.001	<0.001	0.038	<0.001	0.301	0.133
IL-10					0.004	0.222	0.017	0.904	0.297
VEGF						0.017	<0.001	0.161	0.195
Platelet count							0.001	0.619	0.537
mGPS								0.339	0.600

Abbreviations: IL-6 = interleukin-6; IL-10 = interleukin-10; K-M = Klintrup and Makinen criteria; mGPS = modified Glasgow Prognostic Score; NLR = neutrophil-lymphocyte ratio; VEGF = vascular endothelial growth factor.

( $P < 0.05$ ), increased T stage ( $P < 0.01$ ), tumour necrosis ( $P < 0.001$ ), increased mGPS ( $P < 0.001$ ), increased white cell ( $P < 0.05$ ) and platelet ( $P < 0.05$ ) counts, and low skeletal muscle index ( $P < 0.01$ ).

When all patients were considered together, tumour necrosis was associated with increased T stage ( $P < 0.005$ ), decreased local inflammatory cell infiltrate ( $P < 0.05$ ), increased IL-6 ( $P < 0.001$ ), increased IL-10 ( $P < 0.005$ ), increased VEGF ( $P < 0.001$ ), mGPS ( $P < 0.001$ ), anaemia ( $P < 0.05$ ), elevated white cell ( $P < 0.005$ ), neutrophil count ( $P < 0.05$ ), and platelet counts ( $P < 0.001$ ), and skeletal muscle index ( $P < 0.001$ ).

In the present study, the median follow-up for the survivors was 53 (range 25–91) months. During this period, 29 patients died from colorectal cancer and 11 from other causes. Neither IL-6 and IL-10 nor VEGF were significantly associated with cancer-specific survival (Figure 1).

In order to account for the effect of T stage, the relationship between tumour characteristics, circulating mediators, and systemic responses in patients with stage T3 colorectal cancer ( $n = 73$ ) was examined (Table 2). When T3 colon tumours were considered alone, the relationships between IL-6 and IL-10 ( $P < 0.001$ ), VEGF ( $P < 0.001$ ), mGPS ( $P < 0.001$ ), platelet count ( $P < 0.05$ ), and tumour necrosis ( $P < 0.001$ ) remained significant.

## DISCUSSION

The results of the present study show that tumour necrosis, independent of tumour size, was significantly associated with elevated concentrations of IL-6, IL-10, VEGF, mGPS, NLR, platelet count, and the skeletal muscle index. Furthermore, IL-6 concentrations were significantly associated with IL-10, VEGF, platelet counts, mGPS, and the skeletal muscle index. In addition, IL-10 and VEGF were significantly associated with each other and both were significantly associated with systemic inflammation, as evidenced by the mGPS. Taken together, the results of the present study indicate that IL-6 is indeed a plausible mediator of the relationship between tumour necrosis and systemic inflammatory responses in patients with operable colorectal cancer.

In the present study, circulating IL-6 concentrations were not significantly associated with the extent of the general local inflammatory cell infiltrate as evidenced by K-M criteria or

cancer-specific survival. This does not preclude IL-6 concentrations being associated with specific inflammatory cell types. Also, the relatively small number of cancer deaths limits the conclusions that can be made about the prognostic value of IL-6. Furthermore, although most inflammatory cell types increase with a high K-M grade, macrophage counts have recently been reported to be similar in both low- and high-grade K-M grades (Richards *et al*, 2012a). It was therefore of interest that circulating concentrations of IL-6 were directly associated with IL-10 as tumour IL-10 levels can be sustained by the production of IL-6 by tumour-infiltrating macrophages (Herbeuval *et al*, 2004; Mocellin *et al*, 2005). Although the exact mechanism by which IL-10 exerts its effect in the tumour microenvironment is unclear, it is accepted that this cytokine has multiple stimulatory and inhibitory effects on innate and adaptive immune responses respectively. Indeed, it has been proposed that IL-10 is a key anti-inflammatory cytokine involved in the switch from Th1 to Th2 immune responses (De Vita *et al*, 2000).

Therefore, it may be the balance between IL-6 and IL-10 in the tumour microenvironment that may be important in determining the nature of local and systemic inflammatory responses in colorectal cancer.

Recent work has highlighted a direct relationship between the systemic inflammatory responses (mGPS) and the loss of skeletal muscle in patients with colorectal cancer (Richards *et al*, 2012c). In the present study, although there was a relatively small number of observations, there was also an association between elevated concentrations of circulating IL-6 and a low skeletal muscle mass. These results are consistent with work in experimental models of cancer cachexia that have implicated proinflammatory cytokines and their downstream signalling cascades in cancer-associated muscle wasting (Strassmann *et al*, 1992; Argiles *et al*, 2005; Bonetto *et al*, 2012). Prominent among these is the IL-6/JAK/STAT3 cascade (Bonetto *et al*, 2012) and therefore confirms the plausibility of the present hypothesis in patients with colorectal cancer.

The mechanism whereby tumour necrosis results in an increase in circulating IL-6 concentrations is not clear. One plausible hypothesis is that hypoxic tumour cells activate hypoxia-inducible factors that result in the production of IL-6 and VEGF and that these spill into the circulation raising circulating concentrations (see Figure 2). However, it is of interest that IL-6 concentrations do not appear to normalise following potentially curative resection of

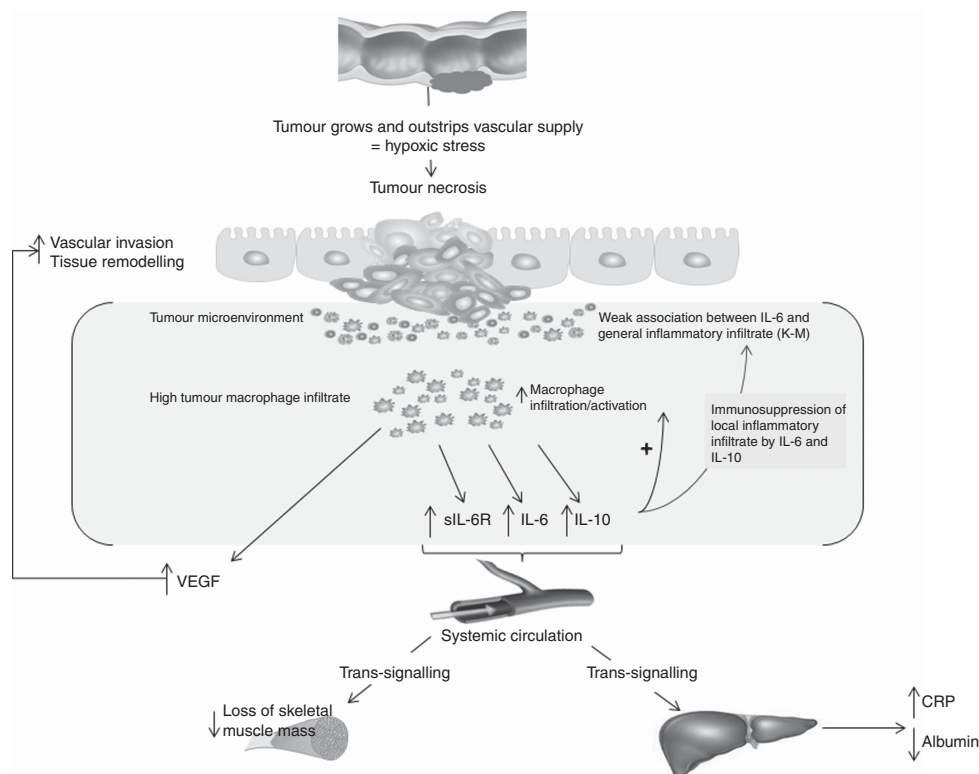


Figure 2. Proposed pathway linking tumour necrosis, IL-6, and systemic and local inflammatory responses in patients with colorectal cancer.

tumours, similar to C-reactive protein. Thus, it is also plausible that IL-6 concentrations may be determined by tissues other than that of the tumour (Guthrie *et al*, 2013b). Indeed, it has been reported that tumour-associated macrophages (TAMs) may play an important role, as they are known to produce significant amounts of IL-6 and are reported to be a major source of IL-6 in both the serum and tumour microenvironment of patients with colorectal cancer (Lieubeau *et al*, 1999; Piancatelli *et al*, 1999). Indeed, numerous studies have reported the presence of macrophages in the tumour microenvironment (Roxburgh and Mcmillan, 2012). Despite this, the prognostic value of tumour-infiltrating macrophages remains unclear. At least nine studies have previously reported a relationship between TAMs and survival. Of these, five studies reported a strong association between the density of macrophages at the invasive margin and survival, whereas the remainder reported no significant association with survival (Roxburgh and Mcmillan, 2012).

Although some studies have reported that high macrophage infiltration is associated with improved survival (Lackner *et al*, 2004; Forssell *et al*, 2007; Zhou *et al*, 2010; Algars *et al*, 2012; Edin *et al*, 2012; Kinouchi *et al*, 2012), recent work that has identified the two main subpopulations of macrophages present in the tumour microenvironment suggests a more complex relationship between macrophage infiltration and survival (Sica *et al*, 2006; Solinas *et al*, 2009). Indeed, it has been reported that the distribution of the M1 and M2 subpopulations of macrophages in the tumour microenvironment may influence survival and that these subpopulations may localise to different areas of the tumour depending on the prevailing conditions, including tissue hypoxia (Movahedi *et al*, 2010; Laoui *et al*, 2011; Richards *et al*, 2012d). Indeed, it has long been recognised that TAMs localise to hypoxic regions of the tumour microenvironment (Harris, 2002). Therefore, it is plausible that the relative density of macrophages (perhaps M2) are important in such cytokine alterations and the elaboration of the systemic inflammatory response in patients with colorectal cancer and this requires further investigation.

Interestingly, recent work by Kantola *et al* (2012) and Guthrie and Mcmillan (2013) has highlighted the potential relationship between the systemic inflammatory response, as evidenced by mGPS, and alterations in a variety of serum cytokine concentrations and this, along with the results of the present study, may provide new insight into the inflammatory cells associated with the upregulation of the systemic inflammatory response in patients with colorectal cancer.

Indeed, with the exception of macrophages, few inflammatory cells can produce such a spectrum of cytokines and growth factors, and this is consistent with recent reports that macrophages are abundant in tumour microenvironments even in the absence of other inflammatory cells (Mohammed *et al*, 2012; Richards *et al*, 2012a). Therefore, the above findings are also consistent with the hypothesis that tumour necrosis (increasing with T stage) and IL-6 play an important role in linking local and systemic inflammatory responses in patients with colorectal cancer (Richards *et al*, 2012b).

It has also previously been suggested that IL-6 may stimulate systemic inflammatory responses through the trans-signalling pathway involving the soluble IL-6 receptor (Scheller *et al*, 2006; Rose-John *et al*, 2007), and it is plausible that intratumoural and circulating macrophages may contribute to this trans-signalling pathway (Scheller *et al*, 2006; Rose-John *et al*, 2007), and therefore play a key role in the evolution of a chronic systemic inflammatory response. Indeed, Chua *et al* (2011) proposed a similar scheme that proinflammatory cytokines may modulate both the local tumour microenvironment and a chronic systemic inflammatory response that affects normal organs, including liver and muscle. Irrespective, the relationship between this 'IL-6 trans-signalling' pathway and both local and systemic inflammatory responses in patients with colorectal cancer also merits further evaluation.

In summary, the present study therefore provides, for the first time, supportive evidence for the hypothesis that tumour necrosis, independent of T stage, elevates circulating IL-6 concentrations, thereby modulating both local and systemic inflammatory responses including angiogenesis that, in turn, may promote

tumour progression and metastases. Further evaluation of the relationships between cells that produce IL-6 (e.g., macrophages) in the tumour microenvironment and in the circulation is of considerable interest.

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