Original Article

Prognosis of Colorectal Cancer Patients with Elevated Endothelin-1 Concentrations

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AIM: Prognostic indicators from clinical, laboratory and pathological data of patients with colorectal cancer are essential to identify high-risk groups in whom adjuvant therapy could be beneficial. Endothelin-1 (ET-1), a growth factor, has been associated with the development and spread of solid tumours. This prospective study was performed to determine whether preoperative plasma big ET-1 concentrations might be useful as a prognostic indicator in patients with colorectal carcinoma.

METHODS: Overall, 65 consecutive patients with colorectal cancer confirmed by biopsy were included prospectively in this study from 1998 to 2001. Plasma samples from a peripheral vein were obtained prior to surgery. Univariate analysis of survival used age (less than or more than 70 years), gender, Dukes' stage (A/B vs C), tumour size (less than or more than 50 mm), vascular invasion, and plasma big ET-1 concentrations, and significant factors were then analysed using a Cox regression model.

RESULTS: Three variables, age, Dukes' tumour stage and plasma big ET-1 concentration, had prognostic significance ($p < 0.05$). Factors associated with a poorer prognosis were age more than 70 years ($p = 0.02$), Dukes' C ($p = 0.04$) and plasma big ET-1 concentration more than 4.2 pg/mL ($p = 0.02$). The Cox regression model identified the same three variables as having independent prognostic value for overall survival.

CONCLUSION: Preoperative plasma big ET-1 concentrations may be useful in predicting overall survival in patients with colorectal cancer. Plasma big ET-1 concentrations may be useful in the selection of high-risk, lymph node-negative patients with colorectal cancer for adjuvant therapy. [*Asian J Surg* 2004;27(1):4–9]

Introduction

Colorectal carcinoma is the second most common cancer in the UK after lung cancer and accounts for approximately 20,000 patient deaths per year.[1] Approximately 50% of patients who undergo supposedly curative resection of colorectal cancer die from metastatic disease within 5 years. The prognosis for these patients mainly depends on tumour stage. Staging of colorectal cancer has remained unchanged since Dukes' classification of rectal cancer. Lymph node metastasis in colorectal cancer remains the most important prognostic factor in non-metastatic cancer, with approximately 60% to 70% of lymph node-positive patients dying within 5 years of surgery. Other factors such as age, gender and histopathological variables can influence survival. Almost 30% of node-negative patients relapse and die of disseminated disease. Recently, abnormalities in hepatic haemodynamics have been shown to occur in patients and animals with hepatic metastases, notably decreased blood flow through the portal vein caused by splanchnic vasoconstriction.[2] Furthermore, such abnormal hepatic haemodynamics may be useful in predicting poor outcome in patients with colorectal cancer.[3]

The blood vessels supplying colorectal hepatic metastases are quite different from the normal hepatic vessels and lack

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both smooth muscle and neuropeptide innervation, suggesting the presence of vasoactive substances causing these changes in hepatic blood flow.[4] To date, three isoforms of endothelin (ET) have been identified (ET-1, ET-2 and ET-3), each composed of 21 amino acids.[5] ET-1, a potent vasoconstrictor peptide, is the principal isoform and was originally isolated from the cultured media of aortic endothelial cells.[6] Due to a low circulating concentration and a short plasma half-life (about 1.5 minutes), measurement of plasma ET-1 concentrations has proven to be difficult. Big ET-1, the precursor, is a stable peptide with a plasma half-life of 30 minutes, making the measurement of plasma big ET-1 concentrations a sensitive indicator of endothelin system activation.[7] We speculate that ET could be the vasoactive factor involved in the haemodynamic changes noted in colorectal hepatic metastases.

This prospective study was performed to determine whether preoperative plasma big ET-1 concentrations might be useful as a prognostic factor in patients with colorectal carcinoma.

Patients and methods

To determine the normal range of plasma big ET-1 concentrations, peripheral plasma samples were obtained from an age–sex-matched group with no history of cancer.

A total of 65 consecutive patients with colorectal cancer confirmed by biopsy who underwent surgery were included prospectively in this study from 1998 to 2001. Patients with co-morbid conditions that are associated with elevated big ET-1 concentrations, such as hypertension, cardiac failure, myocardial infarction and rheumatic diseases, were excluded.

Local ethical approval was obtained prior to the start of this study. Patients were given an information leaflet about the study and informed consent was then obtained.

Blood collection

Plasma samples from a peripheral vein were obtained prior to surgery. Samples were taken in a seated position. All blood samples were collected in EDTA specimen tubes, placed immediately into an ice bag and centrifuged at 2,000 rpm (Sigma centrifuge model no. 4k15, Sigma Laboratories, Dorset, UK) for 10 minutes at 4°C within 15 minutes of blood collection. The plasma supernatant was drawn off, snap frozen in liquid nitrogen and stored at –80°C.

An individual who was blinded to the plasma big ET-1 concentrations collected data from pathology reports on the colorectal cancer specimen and patients' details independently for all patients. Previous studies have analysed clinicopathological variables such as age (> 70 years), gender, tumour size, vascular invasion and tumour stage.[8,9] However, in this study, we also assessed plasma big ET-1 concentrations as a clinical variable prior to surgery. A plasma concentration of more than 4.2 pg/mL was chosen because this was the upper limit of big ET-1 concentrations in age–sex-matched controls. Only variables predicting survival in the univariate analysis were used in the multivariate analysis.

Assessment of plasma big ET-1 concentrations

Plasma big ET-1 concentrations were measured within 2 months of sample collection using a one-step sandwich enzyme immunoassay kit (Biomedica, Vienna, Austria) in accordance with the manufacturer's protocol. All standards and patient samples were analysed in duplicate and the mean value taken.

The kit consisted of purified polyclonal antibody and monoclonal detection antibody highly specific for big ET-1 (1-38). Big ET-1 binds to the pre-coated antibody and forms a sandwich with the detection antibody. Big ET-1 was quantified using an enzyme-catalyzed colour change detectable on a standard enzyme-linked immunosorbent assay (ELISA) reader (Denley scan, R&D Systems, Abingdon, UK). The detection range for this assay was 0.125–39 pg/mL, and the cross reactivity with human big ET-1 (22-38), ET-1, ET-2 and ET-3 was less than 1%. The intra- and inter-assay coefficient variations for this assay kit were 5% and 8%, respectively. Plasma big ET-1 concentrations were calculated by extrapolation from a standard curve. A separate standard curve was constructed for each ELISA batch.

Statistical analysis

Kaplan-Meier survival curves and the log rank test were used to analyse survival differences. Univariate and multivariate analyses (Cox's proportional hazard) of all clinicopathological variables were performed using SPSS version 10.1 (SPSS Inc, Chicago, IL, USA). A p value of less than 0.05 was considered significant. Only variables shown by univariate analysis to be associated with survival were entered into the multivariate analysis.

Results

A total of 69 patients with primary colorectal cancer without evidence of distant metastasis were included in this study (41

males and 28 females). The median age was 69 years (range, 42–92 years). Although all patients had apparently curative resections for their primary tumour, two patients with locally advanced tumours underwent palliative resection only and they were excluded from the study. Overall survival at a mean of 14 months (range, 2–36 months) from surgery was 72.3%. Patients who died within 30 days of surgery (n = 2) were also excluded from the study. Sixty-five patients were therefore available for analysis.

Plasma big ET-1 concentration and disease status

Of the 65 patients, 26 (40%) had elevated plasma big ET-1 concentrations (> 4.2 pg/mL; Table 1). Of the 31 patients with Dukes' A/B colorectal cancer, 14 (45%) had elevated plasma big ET-1 concentrations. Similarly, of 34 patients with Dukes' C cancer, 12 (35%) had elevated plasma big ET-1 concentrations (Table 2). To assess whether elevated plasma big ET-1 concentrations were related to tumour load and vascular invasion, these variables were compared to big ET-1 concentrations. Of 36 patients with smaller primary tumours (< 5 cm), 22 (61%) had plasma big ET-1 concentrations of less than 4.2 pg/mL. Furthermore, only 16 of 40 patients (40%) with evidence of vascular invasion had elevated plasma big ET-1 concentrations. Interestingly, 29% of patients with high concentrations of plasma big ET-1 in the Dukes' A/B group died (4/14), but only 6% of patients with low concentrations of big ET-1 in this group died (1/17).

Univariate analysis of survival

Three variables (age, Dukes' tumour stage and plasma big ET-1 concentration) had prognostic significance ($p < 0.05$). Factors associated with a poorer prognosis were a tumour of Dukes' stage C, a plasma big ET-1 concentration of more than 4.2 pg/mL, and age more than 70 years (Figures 1–4). There was no significant survival difference with respect to gender, tumour size and presence of vascular invasion on tumour sections (Table 3).

Multivariate analysis of survival

The Cox regression model identified the same three variables as having independent prognostic value for overall survival (Table 4). There was a significant difference in survival among those more than 70 years old compared to those less than 70 years old (p = 0.02). Survival was significantly better in patients with Dukes' A/B cancer than those with Dukes' C cancer (p = 0.01). Similarly, preoperative plasma big ET-1 concentrations predicated survival (< vs > 4.2 pg/mL; p = 0.01).

Table 1. Characteristics of clinical and pathological variables

	Number of patients
Gender	
Male	39
Female	26
Age	
< 70 yr	29
> 70 yr	36
Dukes' staging	
A/B	31
C	34
Vascular invasion	
Present	40
Absent	25
Tumour size (largest diameter)	
< 50 mm	36
> 50 mm	29
Plasma big ET-1	
< 4.2 pg/mL	39
> 4.2 pg/mL	26

ET-1 = endothelin-1.

Table 2. Relationship between plasma big endothelin-1 (ET-1) concentrations and Dukes' staging

	Duke's stage	
	A/B, *n* (%)	C, *n* (%)
Plasma big ET-1		
< 4.2 pg/mL	17 (55)	22 (65)
> 4.2 pg/mL	14 (45)	12 (35)
Total	31	34

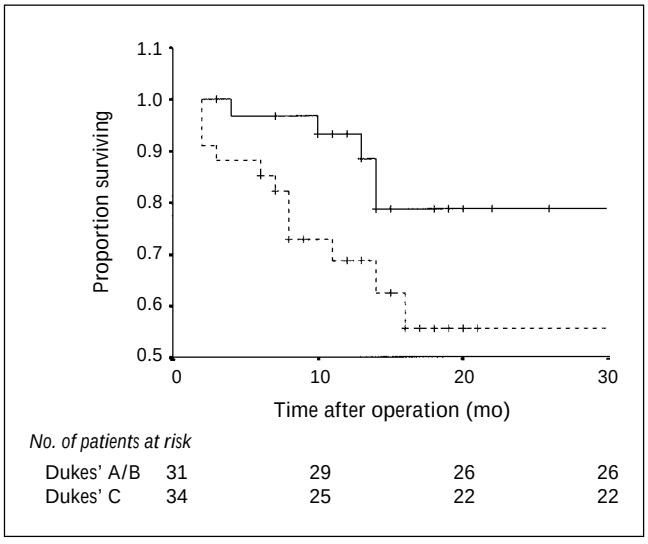

Figure 1. Kaplan-Meier survival curves for patients with Dukes' A/B cancer (solid line) and Dukes' C cancer (dotted line).

Discussion

In this study, we demonstrated that age, Dukes' stage and plasma big ET-1 concentration were significant prognostic factors both by univariate and multivariate analyses in patients with colorectal cancer. Overall survival decreased in patients older than 70 years in this study; other groups have reported similar results.[8,10,11] The significance of age is frequently reviewed and yet remains controversial. Some groups have shown similar cancer-related death outcomes in both the

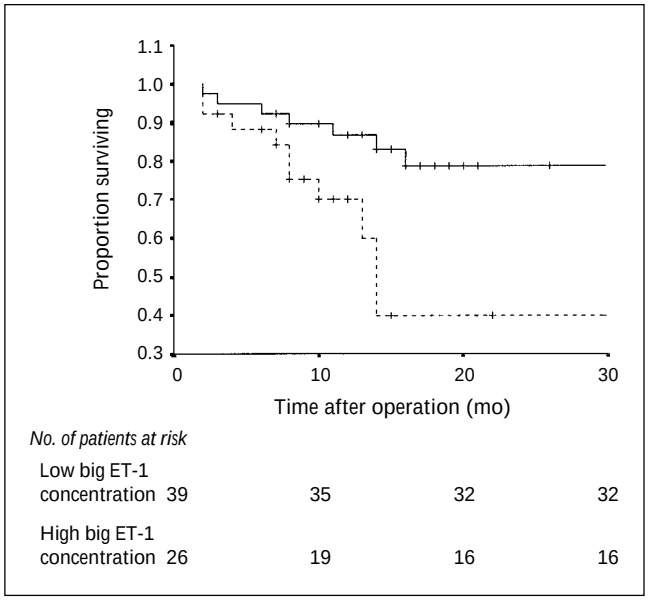

Figure 2. Kaplan-Meier survival curves for patients with plasma big endothelin-1 (ET-1) concentrations below 4.2 pg/mL (solid line) and above 4.2 pg/mL (dotted line).

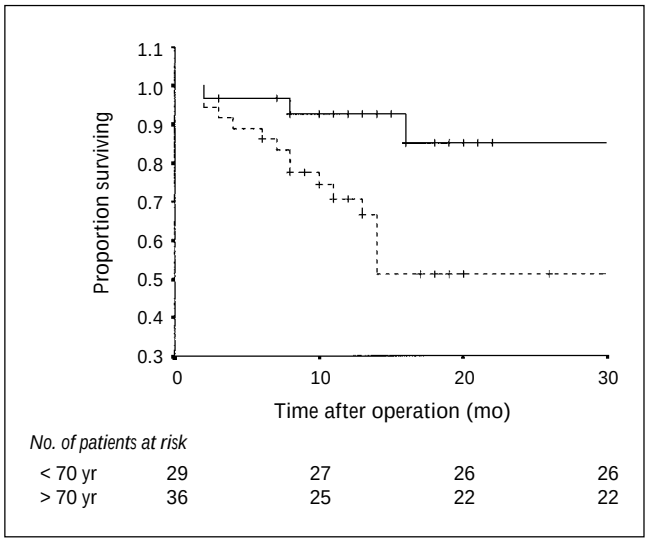

Figure 3. Kaplan-Meier survival curves for patients less than 70 years old (solid line) and more than 70 years old (dotted line).

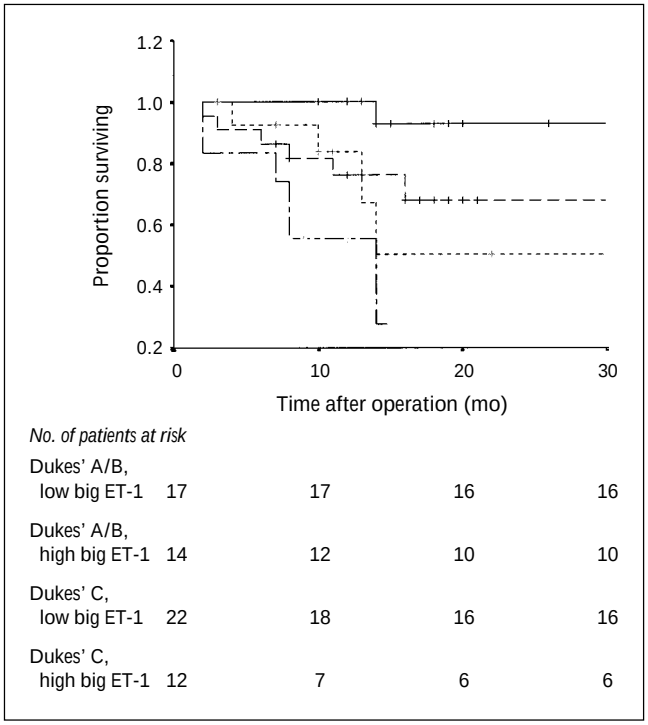

Figure 4. Kaplan-Meier survival curves for patients with Dukes' A/B and plasma big endothelin-1 (ET-1) concentrations less than 4.2 pg/mL (solid line), Dukes' A/B and plasma big ET-1 concentrations more than 4.2 pg/mL (dashed line), Dukes' C and plasma big ET-1 concentrations less than 4.2 pg/mL (dotted line), and Dukes' C and plasma big ET-1 concentrations more than 4.2 pg/mL (dashed line with centre dots).

Table 3. Results of the univariate analysis

	Hazard ratio	Confidence interval	Log-rank test *p*
Gender			
Male	1.00		
Female	1.51	0.56–4.03	0.41
Age			
< 70 yr	1.00		
> 70 yr	4.43	1.27–15.48	0.02
Dukes' staging			
A/B	1.00		
C	2.86	1.00–8.67	0.04
Vascular invasion			
Absent	1.00		
Present	2.43	0.16–1.08	
Tumour size (largest diameter)			
< 50 mm	1.00		
> 50 mm	2.44	0.91–6.53	0.08
Plasma big ET-1			
< 4.2 pg/mL	1.00		
> 4.2 pg/mL	3.34	1.25–8.98	0.02

ET-1 = endothelin-1.

Table 4. Results of the multivariate analysis (Cox proportional hazard)

	Hazard ratio	Confidence interval	*p*
Age			
< 70 yr	1.00		
> 70 yr	4.42	1.25–15.57	0.02
Dukes' staging			
A/B	1.00		
C	3.79	1.30–11.03	0.01
Plasma big ET-1			
< 4.2 pg/mL	1.00		
> 4.2 pg/mL	3.57	1.29–9.88	0.01

ET-1 = endothelin-1.

elderly and younger age groups.[12] Many have suggested that the poor prognosis in elderly patients is due to co-existing disease and poor physiological reserve.[11,13] Although patients with serious illness were excluded from the study to avoid the confounding effect of raised plasma big ET-1 concentrations in these conditions, we found that the elderly group did poorly in terms of survival following surgery for colorectal cancer.

This study reinforces the importance of Dukes' classification in predicting overall outcome after colorectal cancer surgery. However, this information is known only in the postoperative period. Dukes reported a crude 5-year survival rate of 82% for patients with Dukes' A cancer, instead of the 100% expected for this type of cancer when there is no evidence of spread beyond the bowel. The reporting of cancer sections could vary between centres because careful dissection to identify tumour spread beyond the muscularis mucosa is different due to technical reasons, and small lymph node metastases may be missed. In spite of these disadvantages, Dukes' staging is still used as a standard method. Moreover, attempts to create a new staging system have resulted in more confusion than refinement.

Plasma big ET-1 concentrations are elevated among colorectal cancer patients with liver metastases in comparison to colorectal cancer patients without metastases.[14] Plasma ET-1 concentrations increase with increasing size of liver metastases,[15] with no significant difference in concentrations between patients with primary colorectal cancer without metastases and a control group. These findings suggest that ET-1 could be involved in the spread of colorectal cancer to the liver. Ferrari-Bravo et al investigated 14 consecutive patients who underwent surgery for gastric cancer.[16] Mean plasma concentrations of ET-1 reduced significantly 50 days after surgery compared to preoperative concentrations. Interestingly, the reduction in postoperative ET concentration was significantly smaller among patients with less advanced disease than advanced disease. These results indicate that ET-1 may be secreted by the cancer and, when the tumour is removed, the ET-1 concentration will fall in less advanced cancer. Advanced gastric cancer may still have micrometastases that continue to produce ET-1.

In this study, preoperative plasma big ET-1 concentrations were an important independent prognostic factor in patients with colorectal cancer. We speculate that increased plasma ET concentrations may indicate unidentified micrometastatic disease, predicting a poor outcome in a proportion of patients with favourable staging. Of node-negative colorectal cancer patients, 30% develop recurrent disease, yet these patients do not routinely receive adjuvant chemotherapy to prevent recurrence. Our study identified 29% of patients with elevated ET concentrations and Dukes' A/B staging who subsequently died. Measurement of plasma big ET-1 concentrations could be used to identify patients with node-negative disease who may benefit from adjuvant therapy. Gender, tumour size and vascular invasion were not associated with any survival difference among the cancer patients. Follow-up of these patients for another 3 years may show the significance of other variables that were not significant in this study.

In conclusion, preoperative plasma big ET-1 concentrations may be useful in predicting survival in colorectal cancer patients. Plasma big ET-1 concentrations may be useful in the selection of high-risk lymph node-negative patients with colorectal cancer for adjuvant therapy.

Acknowledgement

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