Original article

High preoperative serum vascular endothelial growth factor levels predict poor clinical outcome after curative resection of gastric cancer

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Background: **Tumour vascular endothelial growth factor (VEGF) and tumour urokinase-type plasminogen activator (uPA) are prognostic factors in gastric cancer but surgical specimens are required for testing. The prognostic value of preoperative serum VEGF (s-VEGF) and serum uPA (s-uPA) levels was evaluated in patients undergoing potentially curative (R0) gastric cancer resection.**

Methods: **Concentrations of s-VEGF and s-uPA were measured 97 patients with gastric cancer and 20 controls. Angiogenesis was measured *in vitro* based on human endothelial cell tube formation.**

Results: **Levels of s-VEGF were higher in patients with gastric cancer than controls (median 288 *versus* 189 pg/ml respectively; $P = 0{\cdot}002$). They were associated with pathological tumour node metastasis (pTNM) stage, pT, pN, lymph node ratio and perineural invasion, and correlated with platelet counts. In multivariable analysis, s-VEGF over 320 pg/ml was the only preoperative predictor of both recurrence and disease-specific survival. Serum from patients with raised s-VEGF levels enhanced angiogenesis *in vitro* significantly more than serum from those with a s-VEGF level of 320 pg/ml or less.**

Conclusion: **High preoperative s-VEGF level is an independent prognostic factor for recurrence and survival after R0 resection of gastric cancer. This may provide a useful guide to decision making regarding neoadjuvant and adjuvant therapies.**

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Introduction

Vascular endothelial growth factor (VEGF) is a key angiogenic factor mediating neovascularization. VEGF binds exclusively to endothelial cells promoting proliferation. It is involved in the 'angiogenic switch' from the initial avascular phase of a microscopic tumour into a rapidly growing and metastasizing tumour by stimulating new vessel formation[1]. The urokinase-type plasminogen activator (uPA) system is strongly implicated in degrading extracellular matrix as well as stimulating angiogenesis, mitogenesis, cell migration and cell adhesion[2]. Several studies have shown that expression of VEGF in the tumour is an independent prognostic factor of survival in patients with gastric cancer[3–7]. The prognostic significance of immunohistochemically assessed uPA in patients with gastric cancer remains controversial[7–9]. Evaluation of tumour expression of angiogenic factors depends on the availability of resected surgical specimens or biopsy material, and there is considerable observer-related variability when using semiquantitative techniques such as

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immunohistochemical staining[10]. In addition, intratumoral heterogeneity may also be a confounding factor.

VEGF is a soluble and diffusible peptide secreted by tumours, and its serum levels can be quantified[1]. It has been suggested that measurement of serum VEGF (s-VEGF) concentrations could be a less observer-dependent method of quantifying angiogenesis and such levels could act as a surrogate marker of tumour angiogenesis[10]. In a variety of malignancies an increased s-VEGF level is associated with advanced tumour stage and can be used as a predictor of poor long-term survival[11,12]. There is little information on the prognostic value of plasma or s-VEGF levels in the field of gastric cancer. Three previous studies have evaluated the usefulness of this marker in heterogeneous clinical series of patients with gastric cancer undergoing palliative bypass surgery or potentially curative resection[13–15].

A prospective study was therefore conducted to assess the relationship between preoperative s-VEGF and serum uPA (s-uPA) levels and various clinicopathological parameters in patients undergoing potentially curative resection for gastric cancer, to investigate the value of s-VEGF and s-uPA levels as prognostic factors for long-term outcome. The effect of patients' sera on *in vitro* angiogenesis was also examined.

Methods

Between 1997 and 2003, patients who underwent a curative (R0) resection of primary gastric adenocarcinoma, at the Hospital Clínic, Barcelona, Spain, were recruited prospectively to the study. Patients who died in the immediate postoperative period were excluded. The study design was approved by the ethics committee of the hospital. None of the patients included in the study had evidence of distant metastases or received neoadjuvant therapy but some received adjuvant chemotherapy.

Clinicopathological data for all operated patients were collected in a database. Tumours were classified according to the tumour node metastasis (TNM) system of the American Joint Committee on Cancer[16]. The curability grade was defined according to the guidelines of the Japanese Gastric Cancer Association[17]. Patients in group A (no evidence of residual disease with a high probability of cure) had tumour stage T1 or T2; N0 treated with D1 or D2 lymphadenectomy or N1 treated with D2 lymphadenectomy; M0, no malignant cells in the abdominal washing fluid; and resection margins greater than 10 mm. Patients in group B also had no evidence of residual disease, but had D1 lymphadenectomy in the presence of N1; or had margins of resection smaller than 10 mm.

Blood samples were collected 1–2 h before surgery. They were allowed to coagulate at room temperature and centrifuged at 2060*g* for 10 min. Serum was separated, aliquoted into a minimum of 250 ml to allow duplicate VEGF and uPA measurements, and stored at −20°C until further processing. Serum samples were also obtained from 20 age- and sex-matched healthy volunteers.

Analysis of serum vascular endothelial growth factor and urokinase-type plasminogen activator

The concentration of s-VEGF was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) designed to measure $VEGF_{165}$ levels (Quantikine® human VEGF; R&D Systems, Minneapolis, Minnesota, USA). The assay employs a quantitative sandwich enzyme immunoassay technique using Sf21-expressed recombinant human $VEGF_{165}$ and antibodies raised against the recombinant protein. The assay shows no significant cross-reactivity with other angiogenesis factors and has a sensitivity of 9·0 pg/ml. Optical density was measured at 450 nm using a microtitre plate reader (MR 5000; Dynatech Laboratories, Chantilly, Virginia, USA). All samples were assayed in duplicate. To correct for variation in platelet counts, VEGF per platelet (pg per 10^6 platelets) was calculated by dividing s-VEGF concentration (pg/ml) by the platelet count ($\times 10^6$/ ml)[18–20]. A commercial manual ELISA kit (Dako, Glostrup, Denmark) was used to quantify uPA in serum samples. Measurements were made by single investigator blinded to the patients' clinicopathological data.

Tumour vascular endothelial growth factor and urokinase-type plasminogen activator expression

Immunostaining for VEGF and uPA was performed as described previously[4,7]. Expression of VEGF and uPA was based on staining intensity, and was assessed in malignant epithelial cells. Smooth muscle cells were used as positive internal controls for VEGF immunoreactivity[21]. The degree of expression of VEGF was classified into one of three categories as the percentage of immunoreactive cells in the total cells counted: score 0, carcinoma cells were stained less intensely than normal smooth muscle; score 1, 30 per cent or fewer carcinoma cells were stained, or the staining intensity of carcinoma cells was similar to that of normal smooth muscle; and score 2, more than 30 per cent of carcinoma cells were stained more intensely than normal smooth muscle[7]. Sections with a score of 1 or 2 were considered positive. The degree of expression of uPA

was graded as negative (no immunostaining in tumour cells or staining equivalent to or less intense than that in non-malignant epithelium) or positive (more intensely stained than control), regardless of the number of cells stained[7,9]. Immunohistochemical procedures were performed by two investigators without knowledge of the s-VEGF or s-uPA levels, or of clinicopathological data.

In vitro angiogenesis assay

Twenty-four-well plates were coated with Matrigel™ (Becton Dickinson, Franklin Lakes, New Jersey, USA) at 10 mg/ml (300 µl), following the manufacturer's instructions, and incubated at 37°C for 30 min. Human umbilical vein endothelial cells (HUVECs) were harvested with trypsin and seeded on coated plates at 85 000–100 000 cells per well and incubated for 24 h at 37°C in the presence of different sera. Tube formation was monitored by an inverted phase-contrast microscope (Leica Microsystems, DMIRB, Heidelberg, Germany). Time-lapse, phase-contrast recording of HUVECs was carried out for 24 h. Morphometric analysis of capillary-like networks on Matrigel™ was performed from phase-contrast images. Ten images on Matrigel™ were analysed from random fields. Capillaries were defined as multicellular cords between two cell aggregates. The length of capillary structures was measured using Quantimet® software (Leica Cambridge, Cambridge, UK). The angiogenic assay was evaluated in 30 samples at a serum concentration of 5 per cent. The results are expressed as a percentage of the control result. Tube formation by untreated HUVECs in endothelial cell basal medium was used as a negative control.

Follow-up

Chemotherapy (10 mg/m^2 mitomycin C intravenously on day 1 and 400 mg tegafur–uracil given orally every 12 h, over a 6-week cycle, until four cycles were completed) was administered after surgery in 39 patients (40 per cent). Outpatient follow-up was every 3 months for the first 2 years, and every 6 months thereafter. Histological confirmation of tumour recurrence was sought in all instances.

Statistical analysis

Continuous data are expressed as median (range). Categorical data were compared by the χ^2 test or Fisher's exact test. The Kruskal–Wallis and Mann–Whitney *U* tests were used to evaluate differences between observations. Correlations between continuous data were evaluated by means of the Spearman rank test.

Time to recurrence and disease-specific survival were the main endpoints. Time to recurrence was defined as the time from the date of surgery to the date of the first confirmed recurrence. Disease-specific survival was calculated from the date of surgery until death due to gastric cancer. Kaplan–Meier curves were plotted and compared using log rank statistics. A Cox proportional hazards regression model was used for multivariable analyses. $P < 0{\cdot}050$ was considered statistically significant. Analyses were performed using SPSS® software for Windows® version 12 (SPSS, Chicago, Illinois, USA).

Results

A total of 276 patients with gastric cancer had palliative or curative surgery during the study period. Ninety-seven patients undergoing curative resection of primary gastric cancer for whom serum samples and tumour tissue for VEGF and uPA testing were available formed the study population.

Complications occurred after surgery in 21 (22 per cent) of 97 patients. Twelve developed intestinal complications (eight had anastomotic leakage at the oesophagojejunal anastomosis and four had duodenal stump leakage). Four oesophagojejunal leaks were a diagnostic finding during barium swallow examination. Among the 12 patients with leaks, two required surgery, six were treated with percutaneous drainage and antibiotics, three with antibiotics only and one patient required no treatment. Other complications were wound infection in six patients and pneumonia in three.

The median follow-up time was 50 (range 1–126) months. Clinicopathological characteristics of the patients are shown in *Table 1*. Four patients were lost to follow-up. During the study, 33 patients (35 per cent) died from gastric cancer progression, four (4 per cent) died from other causes and 56 (60 per cent) remained alive, two with documented tumour recurrence. Overall, 40 per cent of patients (37 of 93) developed recurrence; 15 (16 per cent) presented with peritoneal or distant metastases, and 22 (24 per cent) with local and regional recurrences.

Associations between preoperative VEGF and uPA levels and clinicopathological characteristics

Preoperative s-VEGF levels among the 97 patients were significantly higher than those in the 20 healthy control subjects (median (range) 288 (28–1862) *versus* 189 (118–327) pg/ml; $P = 0{\cdot}002$).

Table 1 Clinicopathological characteristics of the 97 patients

	No. of patients
Age (years)*	70 (34–90)
Sex	
M	45 (46)
F	52 (54)
Laurén classification	
Intestinal	63 (65)
Diffuse	34 (35)
Grade of differentiation	
Good	8 (8)
Moderate	45 (46)
Poor	44 (45)
Lymphatic invasion	
No	65 (67)
Yes	32 (33)
Microvascular invasion	
No	80 (82)
Yes	17 (18)
Perineural invasion	
No	77 (79)
Yes	20 (21)
Extent of lymphadenectomy	
D1	12 (12)
D2	85 (88)
Grade of curability†	
A	50 (52)
B	47 (48)
pT category	
T1	18 (19)
T2	50 (52)
T3	29 (30)
pN category	
N0	43 (44)
N1	33 (34)
N2	11 (11)
N3	10 (10)
pTNM stage	
I	36 (37)
II	32 (33)
III	19 (20)
IV	10 (10)
Lymph node ratio (%)	
≤ 25	73 (75)
> 25	24 (25)
Adjuvant therapy	
No	58 (60)
Yes	39 (40)
t-VEGF staining	
Negative	49 (51)
Positive	48 (49)
t-uPA staining	
Negative	86 (89)
Positive	11 (11)

Values in parentheses are percentages unless indicated otherwise; *values are mean (range). †According to the Japanese Gastric Cancer Association[17]. pTNM, pathological tumour node metastasis; t-VEGF, tumour vascular endothelial growth factor; t-uPA, tumour urokinase-type plasminogen activator.

Relationships between s-VEGF levels and clinicopathological variables are shown in *Table 2*. Preoperative s-VEGF levels were significantly associated with perineural invasion ($P = 0{\cdot}021$), grade of curability ($P = 0{\cdot}002$), pathological (p) T category ($P = 0{\cdot}005$), pN category ($P = 0{\cdot}042$), pTNM stage ($P = 0{\cdot}032$) and lymph node ratio ($P = 0{\cdot}004$).

The median platelet count was 238 (range 110–491) $\times$ 10^6/ml. There was a significant correlation between s-VEGF level and platelet count ($r_s = 0{\cdot}47$, $P = 0{\cdot}001$). Median s-VEGF level was 1·3 (range 0·19–4·90) pg per 10^6 platelets. There was no significant association between positive tumour immunostaining of VEGF and higher serum VEGF level. However, positive tumour inmunostaining of VEGF was associated with a higher s-VEGF level per platelet than negative VEGF staining (median 2·09 *versus* 0·83 per 10^6 platelets respectively; $P = 0{\cdot}050$).

Preoperative s-uPA levels in patients with gastric cancer were similar to those in the control subjects (median (range) 754 (195–2434) *versus* 715 (101–1913) pg/ml; $P = 0{\cdot}435$). No significant correlation was observed between s-uPA levels and demographic, histological or therapeutic data. There was no significant association between s-uPA and tumour uPA expression. There was, however, a significant correlation between s-VEGF and s-uPA levels ($r_s = 0{\cdot}38$, $P = 0{\cdot}001$).

Prognostic factors for tumour recurrence

There was a skewed distribution of s-VEGF levels. High s-VEGF levels were defined as being greater than the 95th percentile value in the healthy control group, in accordance with previous recommendations[13,22]. This resulted in a cut-off value of 320 pg/ml as a definition for high s-VEGF concentrations. Using this value, high s-VEGF levels were found in 44 patients (45 per cent). High s-VEGF platelet levels were defined as being greater than the median value. This resulted in a cut-off value of 1·3 pg per 10^6 platelets.

At the end of follow-up, the estimated mean time to recurrence was 26 (range 4–60) months. During follow-up, 14 of 53 patients with s-VEGF levels of 320 pg/ml or less and 23 of 44 of those with s-VEGF levels of more than 320 pg/ml had tumour recurrence ($P = 0{\cdot}047$). Univariable analysis revealed that s-VEGF ($P = 0{\cdot}026$), platelet count ($P = 0{\cdot}002$), s-VEGF per platelet ($P = 0{\cdot}001$), tumour VEGF (t-VEGF) expression ($P = 0{\cdot}001$), extent of lymphadenectomy ($P = 0{\cdot}002$), lymph node ratio ($P = 0{\cdot}001$), pTNM stage ($P = 0{\cdot}001$) and pN category ($P = 0{\cdot}031$) were significant prognostic factors affecting tumour recurrence. The probability of tumour

Table 2 Relationship between serum vascular endothelial growth factor levels and clinicopathological variables

	No. of patients*	s-VEGF (pg/ml)†	*P*§
Age (years)			0·295
≤ 71	49 (51)	358 (28–1528)	
> 71	48 (49)	277 (53–1862)	
Sex			0·236
M	45 (46)	349 (28–1036)	
F	52 (54)	279 (53–1862)	
Laurén classification			0·287
Intestinal	63 (65)	317 (28–1862)	
Diffuse	34 (35)	265 (79–1538)	
Grade of differentiation			0·403
Good	8 (8)	325 (53–533)	
Moderate	45 (46)	334 (28–1862)	
Poor	44 (45)	243 (62–1538)	
Lymphatic invasion			0·387
No	65 (67)	287 (28–1862)	
Yes	32 (33)	311 (62–1012)	
Microvascular invasion			0·314
No	80 (82)	299 (28–1862)	
Yes	17 (18)	279 (131–1538)	
Perineural invasion			0·021
No	77 (79)	279 (28–1862)	
Yes	20 (21)	406 (192–1277)	
Grade of curability‡			0·002
A	50 (52)	234 (28–1862)	
B	47 (48)	497 (162–1009)	
pT category			0·005
T1	18 (19)	199 (28–449)	
T2	50 (52)	358 (62–1862)	
T3	29 (30)	299 (62–1809)	
pN category			0·042
N0	43 (44)	253 (53–1346)	
N1	33 (34)	300 (28–1862)	
N2	11 (11)	657 (205–1538)	
N3	10 (10)	272 (192–597)	
pTNM stage			0·032
Early (I–II)	68 (70)	272 (28–1862)	
Advanced (III–IV)	29 (30)	365 (81–1809)	
Lymph node ratio (%)			0·004
≤ 25	73 (75)	267 (28–1862)	
> 25	24 (25)	439 (192–1809)	
Adjuvant therapy			0·533
No	58 (60)	294 (53–1862)	
Yes	39 (40)	279 (28–1538)	
t-VEGF staining			0·541
Negative	49 (51)	288 (53–1862)	
Positive	48 (49)	292 (28–1809)	
t-uPA staining			0·891
Negative	86 (89)	279 (28–1862)	
Positive	11 (11)	371 (69–1036)	

*Values in parentheses are percentages; †values are median (range). ‡According to the Japanese Gastric Cancer Association[17]. s-VEGF, serum vascular endothelial growth factor; pTNM, pathological tumour node metastasis; t-VEGF, tumour vascular endothelial growth factor; t-uPA, tumour urokinase-type plasminogen activator. §Kruskal–Wallis or Mann–Whitney *U* test as appropriate.

recurrence in patients grouped according to preoperative s-VEGF is shown in *Fig. 1a*. The probability of being free from tumour recurrence at 5 years was 73 (95 per cent confidence interval (c.i.) 65 to 86) per cent in patients with s-VEGF levels of less than 320 pg/ml compared with 27 (12 to 88) per cent in those with levels of more than 320 pg/ml ($P = 0·028$).

In the multivariable analysis, high s-VEGF level ($P = 0·033$), extent of lymphadenectomy D1 ($P = 0·001$) and advanced pTNM stage ($P = 0·001$) remained as independent prognostic factors for time to recurrence (*Table 3*).

Prognostic factors for disease-specific survival

At a median follow-up of 50 (range 1–126) months, 33 patients had died as a consequence of cancer progression. Univariable analysis revealed that s-VEGF level ($P = 0·023$), platelet count ($P = 0·016$), s-VEGF per platelet ($P = 0·001$), t-VEGF expression ($P = 0·001$), extent of lymphadenectomy ($P = 0·002$), lymph node ratio ($P = 0·001$), pTNM stage ($P = 0·001$), pT stage ($P = 0·005$), pN stage ($P = 0·001$), perineural invasion ($P = 0·041$) and grade of curability ($P = 0·007$) were significant prognostic indicators for disease-specific survival. The cumulative disease-specific survival curves of patients grouped according to s-VEGF level are shown in *Fig. 1b*. The 5-year disease-specific survival rate was 75 (95 per cent c.i. 57 to 97) per cent in patients with s-VEGF levels of 320 pg/ml or less compared with 55 (13 to 86) per cent in those with levels of more than 320 pg/ml ($P = 0·010$).

When multivariable analysis was performed following the same criteria as for evaluation of tumour recurrence, high s-VEGF ($P = 0·004$), extent of lymphadenectomy D1 ($P = 0·001$), pT1 *versus* pT2 ($P = 0·010$), pT1 *versus* pT3 ($P = 0·018$), pN0 *versus* pN1 ($P = 0·022$), pN0 *versus* pN2 ($P = 0·002$) and pN0 *versus* pN3 ($P = 0·029$) were identified as independent prognostic factors for disease-specific survival (*Table 4*).

In vitro angiogenic effect of serum samples

Thirty serum samples (20 with high s-VEGF levels and ten with low s-VEGF levels) were available for this *in vitro* study. After 24 h of incubation, cultures containing serum samples at 5 per cent concentration from patients with high s-VEGF levels showed significantly greater angiogenesis than cultures containing serum samples at the same concentration from patients with low s-VEGF levels, as demonstrated by increased endothelial cell tube formation (*Fig. 2*).

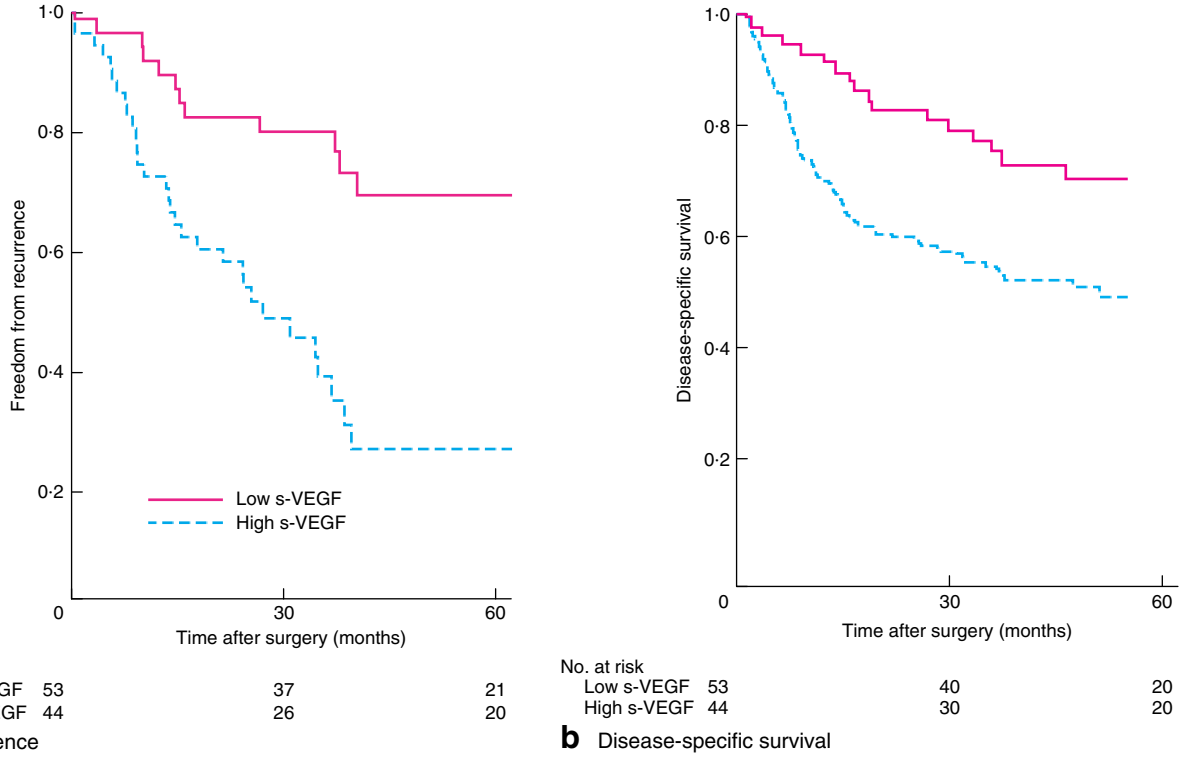

Fig. 1 Kaplan–Meier estimates of the probability of **a** tumour recurrence and **b** disease-specific survival according to serum vascular endothelial growth factor (s-VEGF) levels (low, 320 pg/ml or less; high, over 320 pg/ml) in 97 patients with gastric cancer who had potentially curative resection. **a** $P = 0{\cdot}028$, **b** $P = 0{\cdot}010$ (log rank test)

Table 3 Multivariable analysis of significant prognostic factors for tumour recurrence

	Hazard ratio	*P**
s-VEGF > 320 pg/ml	2·16 (1·12, 3·70)	0·033
D1 lymphadenectomy	9·03 (1·67, 14·91)	0·001
pTNM stage III–IV	5·00 (1·62, 21·06)	0·001

Values in parentheses are 95 per cent confidence intervals. s-VEGF, serum vascular endothelial growth factor; pTNM, pathological tumour node metastasis. *Cox proportional hazards regression model, adjusted for the effects of adjuvant chemotherapy; tumour vascular endothelial growth factor excluded.

Table 4 Significant prognostic factors for disease-specific survival

	Hazard ratio	*P**
s-VEGF > 320 pg/ml	4·00 (1·13, 8·48)	0·004
D1 lymphadenectomy	9·03 (1·87, 16·15)	0·001
pT category		
T1 *versus* T2	2·62 (1·00, 11·17)	0·010
T1 *versus* T3	5·91 (1·15, 26·01)	0·018
pN category		
N0 *versus* N1	2·94 (1·16, 7·31)	0·022
N0 *versus* N2	5·64 (1·83, 16·27)	0·002
N0 *versus* N3	8·22 (2·90, 23·09)	0·029

Values in parentheses are 95 per cent confidence intervals. s-VEGF, serum vascular endothelial growth factor; pT, pathological tumour; pN, pathological node. *Cox proportional hazards regression model, adjusted for the effects of adjuvant chemotherapy; tumour vascular endothelial growth factor excluded.

Discussion

Serum levels of VEGF in patients with gastric cancer were significantly higher than those in healthy controls. Higher preoperative s-VEGF levels were associated with advanced disease (advanced pTNM stage, perineural invasion and high lymph node ratio), a lower probability of being free from recurrence and shorter disease-specific survival in patients undergoing R0 resection for gastric cancer. Cultures containing serum from a subset of patients with high s-VEGF levels showed higher endothelial cell tube formation than those containing serum from patients with low s-VEGF levels. No significant findings were observed in relation to s-uPA as a prognostic factor.

One of the potential clinical implications of tumour angiogenesis is its prognostic value. Measurement of

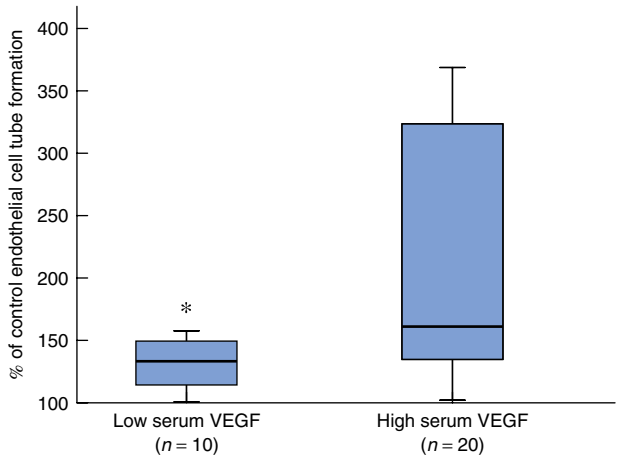

Fig. 2 Angiogenesis as measured *in vitro* by human umbilical vein endothelial cell tube formation after addition of serum from patients with low (320 pg/ml or less) or high (over 320 pg/ml) serum vascular endothelial growth factor (s-VEGF) levels. Horizontal lines, boxes and whiskers represent median, interquartile range and range respectively. $^*P = 0{\cdot}036$ *versus* high s-VEGF level (Mann–Whitney *U* test)

circulating levels of proangiogenic proteins has several advantages over direct assessment of angiogenesis within the tumour. It does not require a tumour specimen, is technically simpler, and is more objective and reproducible in its evaluation than semiquantitative inmunohistochemistry[10].

Within the bloodstream, VEGF is largely concentrated in platelets[23] and a significant correlation between platelet number and s-VEGF level has been documented in patients with cancer[24–26]. These findings, together with the correlation between the platelet load of VEGF in the circulation and the expression of VEGF in tumours, suggest that s-VEGF may be used as an indirect estimate of t-VEGF expression[26,27]. In agreement with previous studies[24,26], s-VEGF levels were found to correlate with platelet count. Positive tumour immunostaining of VEGF was also associated with higher s-VEGF corrected for variation in platelet counts (VEGF per platelet). A similar association has been described in patients with hepatocellular carcinoma[26].

Previous studies have shown that patients with advanced stage and metastatic cancer of different histological types have higher s-VEGF levels than with those with localized tumours[28,29]. From the limited number of studies available in gastric cancer[13,14,30,31], the present findings confirm previous data showing that preoperative s-VEGF levels were significantly higher in patients with advanced stage cancer (III–IV), higher lymph node ratio (more than 25 per cent) and perineural invasion.

s-VEGF may be useful as a prognostic biomarker in patients with gastric cancer. The present study group comprised a larger number of patients after potentially curative gastric cancer resection and with a longer follow-up (5 years) than three previous reports[13,15,30]. A significant association existed between preoperative s-VEGF level and both recurrence and disease-specific survival, with high s-VEGF level acting as an independent indicator for worse prognosis in multivariable analysis. Tumour expression of VEGF was not included in the multivariable analysis because the main goal of the study was to confirm whether s-VEGF could act as a prognostic marker of survival, overcoming the subjectivity associated with scoring tumour VEGF expression. Karayiannakis and colleagues[13] found a significant association between preoperative s-VEGF level and overall survival, with s-VEGF being an independent prognostic factor in multivariable analysis. That analysis, however, included 42 patients with radically resected gastric cancer and 16 with unresectable tumours undergoing palliative bypass surgery.

A limitation of the present study is that 40 per cent of patients received chemotherapy following surgery. The potential confounding effect of this adjuvant chemotherapy was taken into account in the Cox regression analysis, and s-VEGF maintained its status as an independent prognostic factor.

The biological significance of circulating VEGF remains unknown. The fact that the serum of patients with high s-VEGF enhanced *in vitro* capillary tube formation suggests that a biologically relevant level of angiogenic activity can be induced in this subset of patients with gastric cancer. Two previous studies tested the *in vitro* effect of sera from patients with breast cancer or gastrointestinal cancer on the proliferation of HUVECs. Both observed that high proliferative activity of HUVECs was more frequent in patients with a high serum level of VEGF[32,33]. It is tempting to speculate that the detection of high preoperative s-VEGF levels reflects a particularly aggressive biological behaviour that might facilitate recurrence. Two previous studies assessed the clinical relevance of serum angiogenic activity in patients with transitional cell cancer of the bladder and colorectal cancer. Results were contradictory in that patients with lower serum proliferative activity on HUVECs had a worse outcome[34,35]. Additional studies with large series of patients are needed to verify this paradoxical observation.

In addition to its prognostic value, another implication of the results is that serum VEGF might be a useful marker for selecting patients who require neoadjuvant therapy before surgery. Two recent trials have demonstrated a survival benefit with a perioperative systemic approach in operable gastric cancer[36,37]. Moreover, blocking the action of VEGF using presently available anti-VEGF agents appears to be a promising antiangiogenic therapeutic approach[38]. It would be reasonable to envisage that the circulating level of VEGF might be useful in predicting the response of a tumour to anti-VEGF therapy. Recent data, however, suggest that the efficacy of anti-VEGF agents does not relate directly to pretreatment serum VEGF levels in gastrointestinal cancer, although a correlation has been observed in patients with renal tumours[39,40].

High preoperative s-VEGF level is an independent factor indicating poor prognosis and might be helpful in guiding the selection of future therapeutic strategies in the neoadjuvant and adjuvant setting. Larger, prospective studies are required to validate s-VEGF as a marker of poor outcome in patients with gastric cancer.

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