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# Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor

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**Abstract**

The activation of coagulation, angiogenesis and inflammatory cytokines are considered to be related with tumour growth and metastasis. We investigated the plasma levels of platelet microparticles (PMP), vascular endothelial growth factor (VEGF), IL-6, and the chemokine RANTES in patients with gastric cancer ($n=109$) and in healthy controls ($n=29$). The plasma levels of PMP, IL-6 and RANTES were significantly higher in the patients than in the healthy controls, and plasma levels of PMP, VEGF, IL-6 and RANTES were significantly higher in patients with stage IV disease than those in patients with stage I or stage II/III. In terms of predicting distant metastasis, the sensitivities of PMP, VEGF, IL-6 and RANTES were 93.3%, 56.7%, 70.0% and 81.8%, respectively, and the corresponding specificities were 91.1%, 64.6%, 79.7% and 50.0%. Among these parameters, PMP had the highest diagnostic accuracy. Significant correlations were found between PMP, VEGF, IL-6 and RANTES. This study demonstrates that the plasma levels of PMP, VEGF, IL-6 and RANTES were markedly increased in patients with stage IV disease, and that these increased plasma levels of IL-6, RANTES, and especially PMP, might be useful for identifying metastatic gastric patients.


*Keywords:* Platelet microparticles; VEGF; Interleukin-6; RANTES; Platelet activation; Angiogenesis; Metastasis; Gastric cancer

## 1. Introduction

Tumour angiogenesis is an important factor in tumour progression because without the development of new vessels a tumour cannot grow beyond 2–3 mm [1]. Vascular endothelial growth factor (VEGF), which is also known as vascular permeability factor, is one of the most pivotal angiogenic factors responsible for inducing tumour angiogenesis [2]. Circulating VEGF levels have been shown to be associated with a poor prognosis and to be a surrogate marker of angiogenic activity in various cancers [3]. Both plasma and serum VEGF levels are elevated in cancer patients [4]. Plasma VEGF levels are significantly lower than serum VEGF levels because the serum includes the VEGF released from platelets during blood clotting [5].

Platelets have been reported to contribute to metastasis formation and tumour growth [6,7]. Indeed, platelet activation has been shown to result in the release of numerous angiogenic factors including VEGF [8]. Moreover, cancer procoagulant and tissue factor generated by tumour cells induce platelet activation and thrombin generation. Recent results have shown that soft tissue sarcoma specimens express very high concentrations of VEGF and the presence of activated platelets in the intratumoral space [9]. Platelet activation results in the shedding of platelet microparticles (PMP), which are submicroscopic vesicles of the platelet membrane [10]. Elevated levels of microparticles have

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been detected in patients with disseminated intravascular coagulation, coronary artery diseases, transient ischaemic attacks and diabetes mellitus [11]. We also previously reported increased PMP in solid cancers [12].

lnterleukin-6 (IL-6) is a multifunctional cytokine that can regulate immune and inflammatory responses, hepatic acute-phase protein synthesis and bone metabolism [13]. IL-6 also increases platelet production and promotes coagulation and platelet activation [14]. IL-6 is produced in malignant tumours and in inflammatory tissues [15], and has a stimulatory effect on tumour growth [16] and direct angiogenic activity [17].

Regulated upon activation, normal T-cell expressed and secreted (RANTES) is one of the CC chemokines that serves as a chemoattractant for a variety of cells, and which is released by activated T lymphocytes, monocytes, epithelial cells and dermal fibroblasts [18]. It has been reported that platelets are also an important source of RANTES [19], and that RANTES is produced by several tumour cells [20]. Recently, it has been demonstrated that high plasma RANTES levels are correlated with advanced breast cancer [21] and that breast tumour cell-derived RANTES may promote breast cancer progression and metastasis [20].

The activation of coagulation, angiogenesis and inflammatory cytokines are considered to be associated with tumour growth and metastasis [1,8,22]. In this study, the plasma levels of PMP (as a platelet activation marker), VEGF (as an angiogenic factor), and IL-6 and RANTES (as inflammatory cytokines) were assessed in patients with gastric cancer to investigate the association between these markers and the tumour extent. These markers were also examined as possible predictors for metastasis in gastric cancer.

## 2. Patients and methods

### 2.1. Study population

We investigated 109 patients with gastric cancer (male: 75, female: 34, median age: 56 years) who were admitted to the Center for Gastric Cancer in National Cancer Center from September 2001 to April 2002. We excluded patients with diabetes mellitus, arteriosclerosis and acute illness, and those receiving anticoagulant medication. The characteristics of patients are presented in Table 1. No patients had received any form of previous anti-cancer treatment. Tumour pathology was diagnosed microscopically by examining haematoxylin-eosin stained sections of resected specimens. Staging was performed according to the 1997 American Joint Committee on Cancer [23]. The staging procedure included the following: clinical examination, oesophagogastro-duodenoscopy, standard chest radiography, abdominal computed tomography scans, and/or bone scintigraphy. Eighty-six patients underwent surgical resection of their gastric cancer.

The control group consisted of 29 healthy controls (male: 18, female: 11, median age: 55 years). ''Healthy'' was defined as being free from diabetes mellitus, arteriosclerosis and acute illness and without hospitalisation for any illness during the previous 3 years. This study was approved by the institutional review board and informed consent was received from all patients and controls.

### 2.2. Blood samples and assays

Peripheral blood was drawn from both healthy controls and patients into commercially available citrate-theophyllineadenosine-dipyridamole (CTAD) vacutainer tubes (Becton Dickinson, San Jose, CA, USA). The patients who underwent surgical resection were sampled before operation. To minimise platelet activation during the blood drawing, only a light tourniquet and 20-gauge needle were used and the first 2 ml of blood was discarded. The whole blood sample was centrifugated for 15 minutes at 1550 *g* at room temperature within 30 minutes of collection. Platelet-poor plasma was aliquoted and kept frozen at −70 °C.

PMP were measured according to the method reported by Combes and colleagues with some modification [24]. Twenty μl of plasma was incubated at room temperature with 3 μl platelet-specific antibody (CD41a-FITC) or isotype control (Becton Dickinson). After 10 min incubation, the sample was diluted in 0.5% paraformaldehyde/phosphate-buffered saline and 20 μl of Flow-Count Fluorospheres (Beckman Coulter) was added. The sample was analysed immediate using a Coulter Epics XL cytometer. PMP gate was defined using 1 μm diameter latex beads (Sigma) on a Log forward (FS) and Log side (SS) scatter cytogram. Among the events within this gate, PMP were discriminated from the background noise according to their positive fluorescence intensity on a Log fluorescence–Log SS dotplot. The intra-assay coefficient of variation (CV) was 6.3% and the interassay CV 11.1% [25].

Table 1
The characteristics of study populations

| | No. of subjects | Median age (interquartile range) | Sex (M/F) |
|---|---|---|---|
| Healthy control | 29 | 55 (44–61) | 18/11 |
| Gastric cancer | 109 | 56 (46–63) | 75/34 |
| Stage I | 28 | 55 (45–63) | 21/7 |
| Stage II | 12 | 53 (42–63) | 10/2 |
| Stage III | 28 | 61 (49–67) | 14/14 |
| Stage IV* | 41 | 57 (42–63) | 30/11 |

M, male; F, female.

* Distant metastatic sites: peritoneal (13); hepatic (12); multiple sites (4); bone (1).

Plasma levels of VEGF, IL-6 and RANTES were determined using three different commercial enzyme-linked immunosorbent assay (ELISA) kits (Quantikine human VEGF/Quantikine human IL-6/Quantikine human RANTES, R&D systems, Minneapolis, MN, USA) according to the manufacturer's guidelines. The intrassay CVs of VEGF, IL-6 and RANTES were 4.5%, 1.6% and 1.7%, respectively. The corresponding inter-assay CVs were 7.0%, 3.3%, and 6.4%, and the detection limits were 9 pg/ml, 0.7 pg/ml, and 8 pg/ml.

The upper limit of normal for these parameters was defined by the value of the 95 percentile of the values of healthy volunteers. These were $1.60\times10^9$/ml for PMP, 33.7 pg/ml for VEGF, 5.75 pg/ml for IL-6, and 551 pg/ml for RANTES.

### 2.3. Statistical analysis

Analysis was carried out using the Statistical Package for the Social Sciences (SPSS). Data were tested for normality and found to be non-normally distributed. Accordingly, all data are presented as medians (interquartile range). We placed patients with stage II and with stage III together, because patients with stage II and with stage III showed similar plasma levels of PMP, VEGF, IL-6 and RANTES. Comparison of continuous variables in different subgroups were performed using the Mann–Whitney *U* test or the Kruskal–Wallis test. Relationships between categorical variables were compared using the chi-square test. Coefficients of correlation (*r*) were calculated using the Spearmen's rank test. The sensitivities and specificities of PMP, VEGF, IL-6 and RANTES for detecting distant metastasis of gastric cancer were calculated using cut-off values which produced the best diagnostic accuracies. Odds ratios, as measures of the relative risk of distant metastasis, were estimated using multivariate logistic regression analysis, and 95% confidence intervals (CI) were computed. The plasma levels of PMP, VEGF, IL-6 and RANTES were categorised into two groups according to the cut-off values, which produced the best diagnostic accuracies. As covariates, we included age, sex and platelet count. Two sided *P* values $<0.05$ were considered significant.

## 3. Results

### 3.1. Correlation between PMP, VEGF, IL-6 and RANTES levels and gastric cancer stage

The plasma levels of PMP, IL-6 and RANTES in the patients with gastric cancer were significantly higher than in the healthy controls (Table 2). Plasma VEGF levels in patients with gastric cancer were slightly higher than in healthy controls, although this was statistically non-significant. The plasma levels of PMP, VEGF, IL-6 and RANTES in patients with stage IV were significantly higher than those in patients with stage I and stage II/III (Table 3, Fig. 1). No significant differences in the four marker levels were observed between patients with stage I and patients with stage II/III disease. The number of platelets was slightly higher in patients with advanced stage disease.

### 3.2. Plasma levels of PMP, VEGF, IL-6 and RANTES as a marker for detecting distant metastasis

The diagnostic accuracy for predicting distant metastasis were highest at the following cut-off values: $2.70\times10^9$/ml for PMP, 28.6 pg/ml for VEGF, 5.7 pg/ml for IL-6 and 488 pg/ml for RANTES (Table 4). When PMP was examined as a dichotomous variable (positive, $>2.70\times10^9$/ml; negative, $\leqslant2.70\times10^9$/ml), positive PMP correlated strongly with the presence of distant metastasis ($\chi^2$ test, $P<0.001$). Positive IL-6 (cut-off value, 5.7 pg/ml) and positive RANTES (cut-off value, 488 pg/ml) also correlated strongly with the presence of metastasis. A correlation was also found between positive VEGF (cut-off value, 28.6 pg/ml) and the presence of metastasis. Plasma levels of PMP, VEGF, IL-6 and RANTES were significantly different in patients with metastasis and in patients without metastasis. In terms of predicting distant metastasis, the sensitivities of PMP, VEGF, IL-6 and RANTES were 93.3%, 56.7%,

Table 2
Plasma levels of PMP, VEGF, IL-6 and RANTES in healthy controls and patients with gastric cancer

| | Healthy controls ($n=29$) | Gastric cancer ($n=109$) | *P* value[a] |
|---|---|---|---|
| PMP ($\times10^9$/mL) | | | |
| Median | 0.42 | 1.35 | $<0.001$ |
| Interquartile range | 0.28–0.70 | 0.67–4.80 | |
| Positivity[b] (%) | 1 (3) | 49 (45) | |
| VEGF (pg/ml) | | | |
| Median | 24.6 | 26.3 | 0.250 |
| Interquartile range | 22.4–28.5 | 21.9–34.4 | |
| Positivity[b] (%) | 1 (3) | 30 (28) | |
| IL-6 (pg/ml) | | | |
| Median | 2.95 | 4.40 | $<0.001$ |
| Interquartile range | 2.63–3.83 | 3.39–6.11 | |
| Positivity[b] (%) | 1 (3) | 37 (34) | |
| RANTES (pg/ml)[c] | | | |
| Median | 165 | 641 | $<0.001$ |
| Interquartile range | 141–330 | 368–1261 | |
| Positivity[b] (%) | 1 (3) | 50 (53) | |

[a] Mann–Whitney test was used.

[b] Positivity indicates that the plasma levels of PMP, VEGF, IL-6 and RANTES were $>$the upper normal limit value of $1.60\times10^9$/ml, 33.7 pg/ml, 5.75 pg/ml and 551 pg/ml, respectively.

[c] Data on RANTES were missing in 15 gastric cancers.

Table 3
Correlation of plasma levels of PMP, VEGF, IL-6 and RANTES with gastric cancer stage

| | Stage I ($n=28$) | Stage II/III ($n=40$) | Stage IV ($n=41$) | $P$ value[a] |
|---|---|---|---|---|
| Platelet ($\times 10^9$/ml) | | | | |
| Median | 234 | 227 | 274 | 0.044 |
| Interquartile range | 192–263 | 188–293 | 209–371 | |
| PMP ($\times 10^9$/ml) | | | | |
| Median | 0.76 | 0.87 | 7.16 | <0.001 |
| Interquartile range | 0.54–1.47 | 0.54–1.31 | 2.65–17.59 | |
| Positivity[b] (%) | 5 (18) | 7 (18) | 37 (90) | <0.001 |
| VEGF (pg/mL) | | | | |
| Median | 23.5 | 24.4 | 30.2 | 0.002 |
| (Interquartile range | 20.8–29.7 | 21.2–32.7 | 24.9–39.0 | |
| Positivity[b](%) | 4 (14) | 9 (23) | 17 (41) | 0.031 |
| IL-6 (pg/ml) | | | | |
| Median | 3.40 | 3.99 | 6.00 | <0.001 |
| Interquartile range | 3.10–4.55 | 3.24–5.43 | 4.52–12.00 | |
| Positivity[b] (%) | 4 (14) | 7 (18) | 26 (63) | <0.001 |
| RANTES (pg/ml)[c] | | | | |
| Median | 450 | 539 | 1130 | <0.001 |
| Interquartile range | 264–754 | 330–1022 | 505–1809 | |
| Positivity[b] (%) | 11 (41) | 18 (47) | 21 (72) | 0.017 |

[a] Chi-square and Kruskal–Wallis tests were used for categorical and continuous variables, respectively.

[b] Positivity indicates that the plasma levels of PMP, VEGF, IL-6 and RANTES were > the upper normal limit value of $1.60\times10^9$/ml, 33.7 pg/ml, 5.75 pg/ml and 551 pg/ml, respectively.

[c] Data on RANTES were missing in 1 stage I, 2 stage II/III, and 12 stage IV patients.

70.0% and 81.8%, respectively, and the corresponding specificities were 91.1%, 64.6%, 79.7% and 50.0%. Plasma PMP levels had a positive predictive value of 80.0% and a negative predictive value of 97.3% for predicting metastasis. Of these parameters, the diagnostic accuracy of PMP was highest.

The odds ratios of metastasis in each of the categorised levels are shown in Table 5, the low value group is used as a reference. Plasma PMP, IL-6 and RANTES levels were statistically significant predictors of distant metastasis. The sex, age and platelet count adjusted odds ratios for metastasis in the high value group versus the low value group of PMP was 208 (95% CI, 33 to 999). The adjusted odds ratios were 1.97 for VEGF, 9.79 for IL-6, and 3.87 for RANTES, respectively.

### 3.3. Relationship among the various variables in patients with gastric cancer

The strongest correlations were present between VEGF and IL-6 ($r=0.576$, $P<0.001$) and between PMP and RANTES ($r=0.538$, $P<0.001$) (Table 6). A highly significant correlation was also found between PMP and IL-6 ($r=0.407$, $P<0.001$), between platelet count and RANTES ($r=0.452$, $P<0.001$), and between IL-6 and RANTES ($r=0.396$, $P<0.001$). Platelet count was correlated with PMP ($r=0.285$, $P<0.05$) and IL-6 ($r=0.283$, $P<0.05$). In addition, PMP was related to VEGF ($r=0.210$, $P<0.05$) and VEGF to RANTES ($r=0.296$, $P<0.05$).

## 4. Discussion

The effect of platelets on tumour growth and wound healing has been studied extensively. Thrombocytosis has been shown to be an independent prognostic indicator in patients with colorectal cancer [26]. Moreover, antiplatelet agents, thrombocytopenia, and anticoagulants are recognised to have anti-metastatic effects [6], and there is evidence that platelets protect tumour cells by shielding them from the host's immune system [27]. In fact, platelet activation has been shown to result in the release of numerous angiogenic factors, including VEGF, basic fibroblast growth factor, hepatocyte growth factor, angiopoietin-1, insulin-like growth factor-1, epidermal growth factor, platelet-derived growth factor and sphingosine 1-phosphate, and anti-angiogenic factors, including platelet factor 4, thrombospondin-1, TGF-beta 1, plasminogen activator inhibitor type-1, alpha$_2$-antiplasmin and alpha$_2$-macroglobulin [8]. It has been postulated that platelets contribute to tumour angiogenesis through angiogenic factors released upon activation, and that activated platelet is critical determinator of the angiogenic effect in the tumour endothelium [28]. Markers of platelet activation such as beta-thromboglobulin, platelet factor 4 or thrombospondin have been shown to be elevated in patients with cancer [29,30]. Systemic abnormalities of haemostasis in cancer patients have increasingly been recognised, but whether abnormal haemostasis bears a certain significance remains unclear. In our study, plasma levels of PMP, as a platelet activation marker, were measured in patients with gastric cancer. The plasma levels of PMP in patients with stage IV disease were found to be significantly higher than in those with stage I and stage II/III. Moreover, a plasma level of PMP $>2.70\times10^9$/ml indicated metastasis in patients with gastric cancer with a very high sensitivity (93.3%) and specificity (91.1%). Because the platelet count was increased in advanced gastric cancer patients and showed a positive correlation with plasma PMP levels in our results, it therefore can not be excluded that PMP levels were influenced by platelet count. So, we performed a multivariate logistic analysis and showed that after adjustment for the effect of platelet count, plasma PMP levels were still significant predictors of metastasis. Gastrectomy is generally not indicated for disseminated

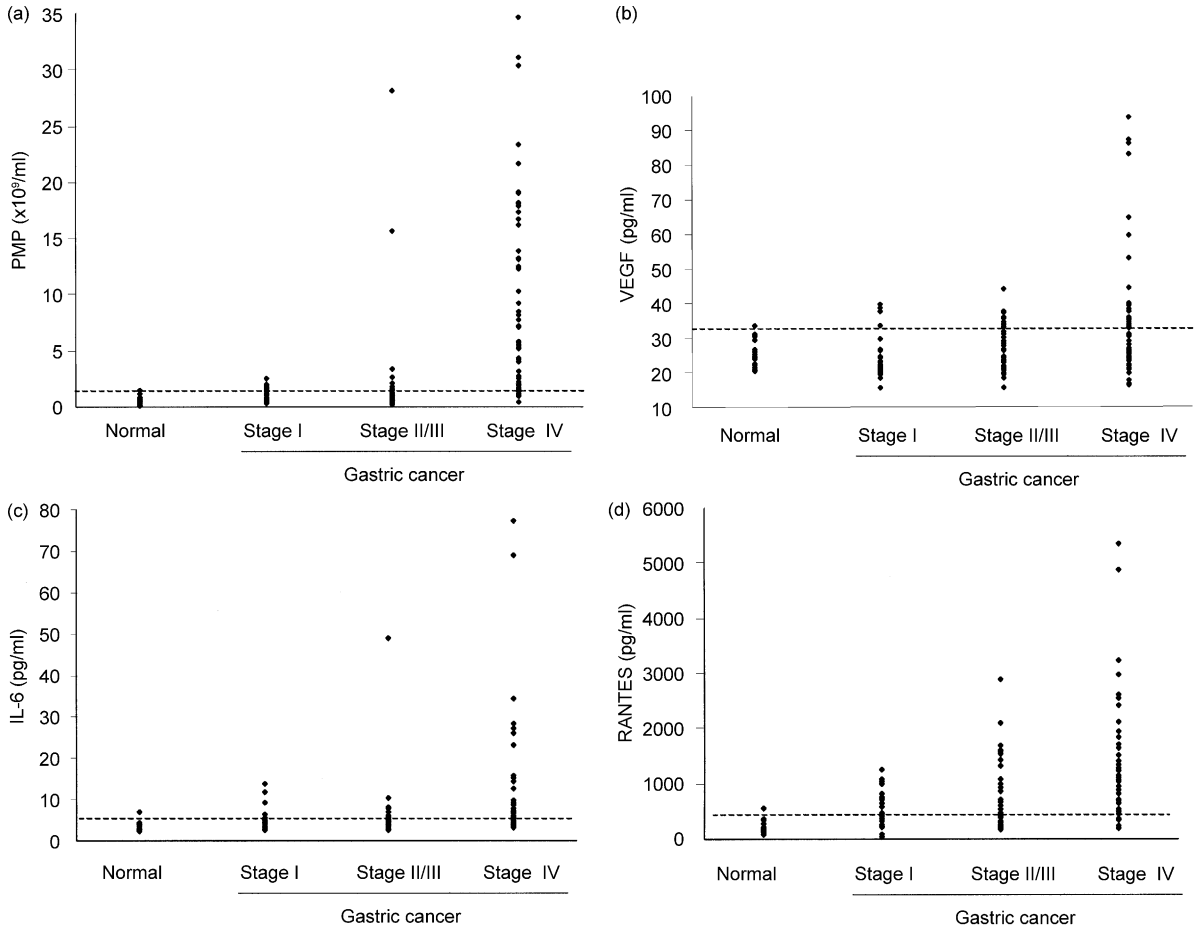


Fig. 1. Distributions of plasma levels of PMP (a), VEGF (b), IL-6 (c) and RANTES (d) in the differently staged patients. The dotted lines indicate the upper normal limits of each parameter.

cases because it does not improve prognosis and can impair the patient's quality of life. Therefore, the determination of PMP levels might be useful for identifying patients with metastasis, and for allowing more effective treatment strategies to be implemented.

Although the precise cause of platelet activation in cancer remains uncertain, suggested mechanisms have included the direct activation of coagulation by tumour secreting procoagulant molecules, such as tissue factor and cancer procoagulant and the indirect activation of host inflammatory cells, responding to tumour products such as tumour necrosis factor-α and interleukin-1β [31]. Nomura and colleagues demonstrated that leukaemic cell lines can generate PMP from intact platelets *in vitro* [32].

The functional importance of PMP *in vivo* has not yet been well defined. PMP have been reported to induce the expression of cyclooxygenase-2 and prostacyclin production in monocytes and endothelial cells via a protein kinase C/mitogen-activated protein kinase-dependent pathway [33]. PMP have also been reported to stimulate smooth muscle cell mitogenesis [34]. Recently, it was reported that PMP may transfer many platelet antigens to the surfaces of haematopoietic stem cells and may play a role in the homing of haematopoietic stem cells [35]. Although the role of PMP in cancer patients is unknown, PMP are biologically active molecules and may play a role in tumour growth and angiogenesis, as do platelets [28,29,30].

Platelets are known to be readily activated *in vitro*. Therefore, when platelet activation marker is measured, a standardised procedure for drawing blood is necessary to avoid even minor *in vitro* activation while collecting and processing samples [36]. In this study, we used a CTAD vacutainer as a vacuum blood collection tube. This contained sodium citrate and citric acid as anticoagulants, and theophylline, adenosine and dipyridamole as inhibitors of platelet activation. We also processed CTAD samples within 30 minutes of blood collection, because immediate measurement is important to avoid artifactual activation [25].

Because platelets are a major source of soluble VEGF in peripheral blood [37], a more accurate basal circulating VEGF level, which should reflect normal physiological angiogenesis, can be determined using CTAD or citrate plasma. Most studies have used serum for

Table 4
The comparison of four parameters in patients with metastasis versus patients without metastasis

| | | Distant metastasis | | | Sensitivity (%) | Specificity (%) | Diagnostic accuracy (%) |
|---|---|---|---|---|---|---|---|
| | | Presence ($n=30$) | Absence ($n=79$) | $P$ value[a] | | | |
| PMP | Positive[b] (n) | 28 (25.7%) | 7 (6.4%) | <0.001 | 93.3 | 91.1 | 91.7 |
| | Negative (n) | 2 (1.8%) | 72 (66.1%) | | | | |
| ($\times 10^9$/ml) | Median | 12.77 | 0.91 | <0.001 | | | |
| | Interquartile range | 5.48–18.39 | 0.56–1.56 | | | | |
| VEGF | Positive[b] (n) | 17 (15.6%) | 28 (25.7%) | 0.037 | 56.7 | 64.6 | 62.4 |
| | Negative (n) | 13 (11.9%) | 51 (46.8%) | | | | |
| (pg/ml) | Median | 30.2 | 24.6 | 0.003 | | | |
| | Interquartile range | 25.8–41.0 | 21.1–33.4 | | | | |
| IL-6 | Positive[b] (n) | 21 (19.3%) | 16 (14.7%) | <0.001 | 70.0 | 79.7 | 77.1 |
| | Negative (n) | 9 (8.3%) | 63 (57.8%) | | | | |
| (pg/ml) | Median | 6.35 | 3.98 | <0.001 | | | |
| | Interquartile range | 4.60–17.55 | 3.22–5.34 | | | | |
| RANTES[c] | Positive[b] (n) | 18 (19.1%) | 36 (38.3%) | 0.007 | 81.8 | 50.0 | 57.4 |
| | Negative (n) | 4 (4.3%) | 36 (38.3%) | | | | |
| (pg/ml) | Median | 1577 | 509 | <0.001 | | | |
| | Interquartile range | 527–2455 | 335–985 | | | | |

[a] Chi-square and Mann–Whitney tests were used for categorical and continuous variables, respectively.
[b] Positive indicates that the plasma levels of PMP, VEGF, IL-6 and RANTES were > the cut-off value of $2.70\times10^9$/ml, 28.6 pg/ml, 5.70 pg/ml, and 488 pg/ml, respectively. The cut-off values were determined as the values that produced the best diagnostic accuracy.
[c] Data on RANTES were missing in 15 gastric cancers.

Table 5
Odds ratios of four parameters for detecting distant metastasis

| | Cut-off value[a] | Odds ratio (95% confidence interval) | | | |
|---|---|---|---|---|---|
| | | Univariate | $P$ value | Multivariate[c] | $P$ value |
| PMP | $<2.70\times10^9$/ml | 1[b] | <0.001 | 1[b] | <0.001 |
| | $\geqslant2.70\times10^9$/ml | 144 (28–736) | | 208 (33–999) | |
| VEGF | <28.6 pg/ml | 1[b] | 0.047 | 1[b] | 0.139 |
| | ⩾28.6 pg/ml | 2.38 (1.01–5.61) | | 1.97 (0.80–4.84) | |
| IL-6 | <5.70 pg/ml | 1[b] | <0.001 | 1[b] | <0.001 |
| | ⩾5.70 pg/ml | 9.19 (3.54–23.86) | | 9.79 (3.36–28.54) | |
| RANTES | <488 pg/ml | 1[b] | 0.012 | 1[b] | 0.032 |
| | ⩾488 pg/ml | 4.50 (1.39–14.61) | | 3.87 (1.13–19.29) | |

[a] The cut-off values were determined as the values that produced the best diagnostic accuracy.
[b] Reference category.
[c] Adjusted for age, sex and platelet count.

Table 6
Correlation coefficients among various parameters in the patients with gastric cancer ($n=109$)

| | PMP | VEGF | IL-6 | RANTES[a] |
|---|---|---|---|---|
| Platelet | 0.285* | 0.112 | 0.283* | 0.452** |
| PMP | 1 | 0.210* | 0.407** | 0.538** |
| VEGF | | 1 | 0.576** | 0.296** |
| IL-6 | | | 1 | 0.396** |
| RANTES[a] | | | | 1 |

[a] Data on RANTES were missing in 15 gastric cancers.
* $P<0.05$.
** $P<0.001$ (Spearman's correlation).

measuring VEGF levels, which is now known to largely reflect the platelet-derived VEGF level. Adams and colleagues [38] have reported that plasma VEGF is a more sensitive measurement than serum VEGF in terms of it being significantly higher in patients with localised breast cancer than in controls or patients with benign disease, unlike serum VEGF. In the present study, we chose to use plasma VEGF to determine the amount of tumour-derived VEGF in the systemic circulation. The plasma levels of VEGF were increased in advanced cancer patients, but were much less dramatically elevated than PMP, IL-6 and RANTES levels. The amount of VEGF in

the peripheral blood of patients with cancer is likely to have been derived from various sources such as tumour cells, platelets and/or macrophages [39]. The elevated plasma VEGF levels observed in this study can be considered to emanate from *in vivo* activated platelets, tumour cells and/or macrophages.

It has been suggested that IL-6 may play a role in tumour-related angiogenesis by inducing tumour cell proliferation and VEGF expression in tumour cells [22,40,41]. In the present study, plasma levels of IL-6 were significantly elevated in the advanced gastric cancer patients. This result can be explained by IL-6 production in tumour cells and by the inflammatory cytokines produced by stromal cells, which stimulate IL-6 production in various cells [14,22,40]. Serum IL-6 has been reported to be an independent prognostic factor by multivariate analysis in patients with metastatic disease [42], and this result was confirmed by the present study, which found that plasma IL-6 is a predictor of metastasis in patients with gastric cancer.

It has been suggested that RANTES expression by breast tumour cells results not only in monocyte migration to the tumour site, but also in protumourigenic activity and in the expressions of proinflammatory cytokines that may facilitate metastasis formation and contribute to disease progression [22]. In the present study, we observed that RANTES levels were elevated in the plasma of two-thirds of the patients with stage IV gastric cancer. Moreover, patients with a RANTES plasma level $>488$ pg/ml appeared to have a more than three times risk of metastases compared with those with a RANTES plasma level of $\leqslant 488$ pg/ml.

The *in vivo* activation of platelets causes the release of angiogenic and inflammatory mediators such as VEGF and RANTES [4,5,19]. If one assumes that active coagulation and platelet activation occur in tumour tissues [43], it seems likely that the angiogenic and inflammatory mediators released in tumour tissues would be involved in tumour progression and metastasis. Thus, the observed correlations between the plasma levels of PMP, VEGF, IL-6 and RANTES in the present study may be explained by local release from tumour cells, from platelets activated at the tumour endothelium, and/or from host stromal cells such as macrophages, monocytes or endothelium [8,22].

In conclusion, this study demonstrates that the plasma levels of PMP, VEGF, IL-6 and RANTES in patients with stage IV disease are significantly higher than in patients with stages I and II/III diseases, and that increased plasma levels of IL-6, RANTES, and especially PMP, might be useful for identifying metastatic gastric patients, and allowing more effective treatment strategies to be implemented. Moreover, our results support the existence of an interaction between the coagulation, angiogenesis and inflammatory systems, which contribute to tumour metastasis and progression.

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