Prognostic impact of matched preoperative plasma and serum VEGF in patients with primary colorectal carcinoma

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In serum, the major part of vascular endothelial growth factor derives from in vitro degranulation of granulocytes and platelets. Therefore, plasma may be preferred for vascular endothelial growth factor measurements. However, which specimen is the best predictor of survival is still debated. The present study analyzed the prognostic value of matched preoperative serum and plasma vascular endothelial growth factor concentrations in patients with colorectal cancer. To establish the reference range among healthy people, vascular endothelial growth factor was analyzed in 50 matched EDTA-plasma and serum samples from healthy blood donors. Preoperatively, in 524 patients with colorectal cancer, matched plasma and serum vascular endothelial growth factor concentrations were analyzed. In the colorectal cancer patients, the median plasma vascular endothelial growth factor concentration (44 pg ml−1) was significantly (P=0.01) higher than the median plasma vascular endothelial growth factor concentration (30 pg ml−1) in the healthy blood donors. In serum, no significant (P=0.30) difference in the median vascular endothelial growth factor concentration was found between colorectal cancer patients (268 pg ml−1) and healthy blood donors (220 pg ml−1). The preoperative vascular endothelial growth factor concentration distributions were dichotomized by the 95th percentile of the healthy blood donors (plasma=112 pg ml−1, serum=533 pg ml−1). In univariate survival analyses, both high plasma vascular endothelial growth factor (>112 pg ml−1) and high serum vascular endothelial growth factor (>533 pg ml−1) independently predicted a reduced survival. In multivariate survival analyses, high serum vascular endothelial growth factor (>533 pg ml−1) independently predicted a reduced survival (HR=1.65, P=0.015), while high plasma vascular endothelial growth factor (>112 pg ml−1) did not (HR=1.27, P=0.23). This study indicates that preoperative serum vascular endothelial growth factor apparently is a better predictor of overall survival than the preoperative plasma vascular endothelial growth factor.

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Vascular endothelial growth factor (VEGF) is one of the strongest promoters of angiogenesis, and it has been indicated, that the preoperative serum VEGF concentration is a prognostic marker in a variety of solid tumours (Salven et al, 1999a; Chin et al, 2000). In a previous study including 614 patients, it was shown that preoperative serum VEGF concentration, independent of Dukes stage, was a strong predictor of overall survival of patients with colorectal cancer (CRC) (Werther et al, 2000). However, VEGF is stored in circulating white blood cells and platelets (Nielsen et al, 1999; Salven et al, 1999b) and several reports have indicated, that elevated VEGF concentrations in serum may be a reflection of degranulation of platelets and white blood cells during in vitro clotting, rather than a reflection of an ongoing angiogenic activity in the tumour (Webb et al, 1998). In plasma, white cell and platelet degranulation is minimized by adding anticoagulatives to the blood samples, and as a consequence, plasma VEGF concentrations are up to 20 times lower than the matched serum VEGF concentrations (Banks et al, 1998). Therefore, it was suggested that plasma should be preferred as specimen for VEGF measurements and that serum was unsuitable (Banks et al, 1998).

The aims of the present study were to compare the prognostic significance of matched preoperative plasma and serum VEGF concentrations in patients with CRC and to evaluate whether serum or plasma was the best predictor of overall survival.

MATERIALS AND METHODS

Healthy volunteers

To establish the reference range among healthy people, matched plasma and serum VEGF concentrations were measured in 50 healthy volunteer blood donors. Their median age was 59 (55–65) years and there were 30 men and 20 women.

Patients

The study included 524 consecutive patients scheduled to undergo elective resection of primary CRC. The median age of the patients at the time of operation was 69 (33–90) years, and 316 men and 208 women were included. All patients had their primary tumours resected and none were given chemotherapy or radiotherapy before or after the operation. All patients had histologically verified carcinoma localized in the colon or in the rectum and were staged...
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RESULTS

VEGF concentrations in plasma

In the 50 healthy blood donors, the median plasma VEGF concentration was 30 pg ml$^{-1}$ (range 0 – 369). There was no significant correlation ($r_s=0.009$, $P=0.95$) between age and plasma VEGF among the blood donors and no significant difference in plasma VEGF ($P=0.86$) between men and women.

In the 524 patients with CRC, the median plasma VEGF concentration was 44 pg ml$^{-1}$ (range 0 – 1185). There was no significant correlation ($r_s=0.05$, $P=0.21$) between age and plasma VEGF among the CRC patients, and no significant difference in plasma VEGF ($P=0.32$) between men and women. The percentile plot of the plasma VEGF measurements in the 524 CRC patients and in the 50 blood donors is shown in Figure 1A. The median preoperative concentration of plasma VEGF for patients with CRC was significantly ($P=0.01$) higher than the median value for the healthy blood donors.

Stratified by Dukes stage, the median plasma VEGF concentration among the CRC patients was as follows: Stage A: 27 pg ml$^{-1}$ (range 0 – 440); stage B: 44 pg ml$^{-1}$ (range 0 – 1185); stage C: 44 pg ml$^{-1}$ (range 0 – 706), and stage D: 74 pg ml$^{-1}$ (range 0 – 543). The median plasma VEGF concentration significantly ($P=0.001$) increased with advanced Dukes stage. Patients with the primary tumour localized in the colon, had significantly ($P=0.01$) higher median plasma VEGF concentrations than patients with the primary tumour localized in the rectum.

VEGF concentrations in serum

In the 50 healthy blood donors, the median serum VEGF concentration was 220 pg ml$^{-1}$ (range 46 – 983). There was no significant correlation ($r_s=0.07$, $P=0.61$) between age and serum VEGF among the healthy donors, and no significant difference in serum VEGF ($P=0.74$) between men and women was found.

In the 524 patients with CRC, the median concentration of serum VEGF was 268 pg ml$^{-1}$ (range 9 – 2500). There was no significant correlation ($r_s=0.05$, $P=0.21$) between age and serum VEGF among the patients, and no significant difference in serum VEGF ($P=0.22$) between men and women. The percentile plot of the serum VEGF measurements in the 524 CRC patients and in the 50 blood donors is shown in Figure 1B. Thus, the median preoperative serum VEGF concentration in the CRC patients (268 pg ml$^{-1}$) was higher than the median serum VEGF concentration in the healthy controls (220 pg ml$^{-1}$). However, in contrast to the median plasma VEGF concentration between CRC patients and healthy blood donors, the difference in the median serum VEGF concentration was not statistically significant ($P=0.30$).

Stratified by Dukes stage, the median serum VEGF concentration among the colorectal cancer patients was as follows: Stage A: 261 pg ml$^{-1}$ (range 9 – 1500); stage B: 266 pg ml$^{-1}$ (range 15 – 1975); stage C: 263 pg ml$^{-1}$ (range 15 – 2500), and stage D: 304 pg ml$^{-1}$ (range 19 – 1475). Patients with Dukes stage D disease had significantly ($P=0.001$) higher serum VEGF concentrations compared to patients with Dukes stage A, B and C disease, while the latter three groups had comparable concentrations. Patients with the primary tumour localized in the colon, had significantly ($P=0.01$) higher serum VEGF levels than patients with the primary tumour localized in the rectum.

Correlation between matched preoperative serum and plasma VEGF concentrations

The correlation ($r_s=0.64$) between the 524 matched serum and plasma VEGF measurements is shown in Figure 2. Dichotomizing VEGF in serum and plasma by the 95th percentile in the relevant control showed that the plasma measurements had significantly more positives than the serum measurements ($P=0.01$, McNemars test). This
analysis indicated that, although serum and plasma concentrations are correlated, grouping by the 95th percentile of normal donors, significantly more positives are identified by plasma VEGF.

**Prognostic significance of the preoperative VEGF level**

In univariate analyses, by classifying the CRC patients in two groups, based on the upper VEGF limit of the 95th percentile of healthy controls, it was shown that patients with plasma VEGF concentrations above 112 pg ml$^{-1}$ (n=105) had a reduced overall survival (although not significant, $P=0.06$) compared to the patients (n=419) with plasma VEGF concentrations equal to or below this level (Figure 3A). In the group of patients with serum VEGF concentrations above the 95th percentile of healthy persons (533 pg ml$^{-1}$), (n=81), overall survival was significantly ($P=0.006$) reduced, compared to patients with VEGF concentrations below or equal to this level (n=443) (Figure 3B). In the subgroup of patients with colon cancer, the patients with plasma VEGF above 112 pg ml$^{-1}$ (n=71) had significantly ($P=0.01$) reduced overall survival, compared to colon cancer patients (n=223) with lower VEGF concentrations (Figure 4A). This difference was not shown in patients with rectum cancer ($P=0.93$), (Figure 4B). Dichotomizing the serum VEGF, using the cut point 533 pg ml$^{-1}$, the subgroup of patients with colon cancer and high VEGF concentration (n=57) had significantly ($P=0.003$) reduced survival compared to the patients (n=237) with low VEGF concentration (Figure 5A). This difference was not observed in the subgroup of patients with rectal cancer ($P=0.3$) (Figure 5B).

In the above-mentioned calculations, the cut-off point was set to the upper limit of the 95th percentile of healthy controls. However, this level may not be the relevant scoring. In order to study if a trend could be detected, strata were defined by the 10th and 90th percentiles of the plasma and serum VEGF concentrations. This division as seen in Figure 6 indicates that CRC patients with preoperative serum VEGF concentrations higher than 670 pg ml$^{-1}$ had a significantly ($P<0.0001$) reduced survival compared to the patients with lower concentrations. Additionally, CRC patients with preoperative serum VEGF concentrations lower than 76 pg ml$^{-1}$ had a significantly better prognosis than CRC patients with VEGF concentrations above this level. Using the 25th and the 75th percentiles for stratification shows a less pronounced effect although statistically significant ($P=0.02$). This effect was not seen with plasma VEGF.
Figure 3  Survival curves of the 524 colorectal cancer patients dichotomized by the upper limit of the 95th percentile of healthy volunteer blood donors. The end-point for survival analysis was death of all causes. Differences between the two survival curves were assessed by the log rank test, the hazard rate with 95% confidence interval was calculated by the Cox regression model. The number of events in each group and the number of patients at risk after each 24-month interval up to 72 months is indicated below the curve. (A) The two curves represent patients with plasma VEGF values below or equal to 112 pg ml\(^{-1}\) (n=419, upper curve), and patients with plasma VEGF above this level (n=105, lower curve). (B) The two curves represent patients with serum VEGF values below or equal to 533 pg ml\(^{-1}\) (n=443, upper curve), and patients with serum VEGF above this level (n=81, lower curve).

Figure 4  Survival curves of 294 colon cancer (A) and the 230 rectal cancer (B) patients dichotomized by the upper limit of the 95th percentile of healthy volunteer blood donors. The end-point for survival analysis was death of all causes. Differences between the two survival curves were assessed by the log rank test, the hazard rate with 95% confidence interval was calculated by the Cox regression model. The number of events in each group and the number of patients at risk after each 24-month interval up to 72 months is indicated below the curve. (A) The two curves represent colon cancer patients with plasma VEGF values below or equal to 112 pg ml\(^{-1}\) (n=223, upper curve), and colon cancer patients with plasma VEGF above this level (n=71, lower curve). (B) The two curves represent rectal cancer patients with plasma VEGF values below or equal to 112 pg ml\(^{-1}\) (n=196, upper curve), and rectal cancer patients with plasma VEGF above this level (n=34, lower curve).
Multivariate analysis was performed including Dukes stage, gender, age, topographical tumour localization, and VEGF level (Table 2). As expected, advanced Dukes stage was an independent predictor of overall survival. In patients with colon cancer, high

![Survival curve](image)

**Figure 5** Survival curves of 294 colon cancer (A) and the 230 rectal cancer (B) patients dichotomized by the upper limit of the 95th percentile of healthy volunteer blood donors. The end-point for survival analysis was death of all causes. Differences between the two survival curves were assessed by the log rank test, the hazard rate with 95% confidence interval was calculated by the Cox regression model. The number of events in each group and the number of patients at risk after each 24-month interval up to 72 months is indicated below the curve. (A) The two curves represent colon cancer patients with serum VEGF values below or equal to 533 pg ml$^{-1}$ ($n=237$, upper curve), and colon cancer patients with plasma VEGF above this level ($n=57$, lower curve). (B) The two curves represent rectal cancer patients with serum VEGF values below or equal to 533 pg ml$^{-1}$ ($n=206$, upper curve), and rectal cancer patients with plasma VEGF above this level ($n=24$, lower curve).

![Survival curve](image)

**Figure 6** Survival curves of the 524 colorectal cancer patients grouped into three strata by the 10th and 90th percentiles of the preoperative serum VEGF concentrations. The end-point for survival analysis was death of all causes. Differences between the survival curves were assessed by the log rank test, the hazard rate with 95% confidence interval was calculated by the Cox regression model. The number of events in each group and the number of patients at risk after each 24-month interval up to 72 months is indicated below the curve. The three curves represent colorectal cancer patients with the following preoperative serum VEGF concentrations: I: $<76$ pg ml$^{-1}$; II: 76$–$670 pg ml$^{-1}$; III: $>670$ pg ml$^{-1}$.

**Table 2** Multivariate survival analysis of the 524 colorectal cancer patients, using the cut-off levels 112 and 533 pg ml$^{-1}$ for the preoperative plasma and serum VEGF concentrations respectively. The analysis includes Dukes stage, gender, age, topographical tumour localization and preoperative serum and plasma VEGF concentrations.

| Dukes stage | HR  | 95% CI   | P-value |
|-------------|-----|----------|---------|
| A           | 1   |          |         |
| B           | 1.84| 1.13--3.00| 0.01    |
| C           | 4.06| 2.51--6.56| <0.0001 |
| D           | 29.74| 17.6--51.6| <0.0001 |
| P-VEGF > 112|     |          |         |
| Colon       | 1.27| 0.86--1.86| 0.23    |
| Rectum      | 0.81| 0.50--1.33| 0.41    |
| s-VEGF > 533|     |          |         |
| Colon       | 1.65| 1.10--2.47| 0.015   |
| Rectum vs colon | 1.44| 0.84--2.48| 0.19    |
| Age (in years) | 1.03| 1.02--1.04| <0.0001 |
| Gender (m/w) | 1.35| 1.08--1.70| 0.009   |

P-VEGF=plasma VEGF; S-VEGF=serum VEGF; HR=hazard ratio; 95% CI=95% confidence interval.

Multivariate analysis

Multivariate survival analysis was performed including Dukes stage, gender, age, topographical tumour localization, and VEGF level (Table 2). As expected, advanced Dukes stage was an independent predictor of overall survival. In patients with colon cancer, high
preoperative serum VEGF (>533 pg ml\(^{-1}\)) significantly (P=0.013) predicted a reduced overall survival, while high pre-operative plasma VEGF level (>112 pg ml\(^{-1}\)) did not (P=0.23). In patients with rectal cancer, neither high serum VEGF concentration nor high plasma VEGF concentration independently predicted a reduced overall survival (P=0.19 and 0.41 respectively). Additionally, patients with the tumour located in the rectum had a significantly (P<0.0001) worse prognosis than patients with the primary tumour located in the colon. In Table 3, multivariate survival analyses using the cut-off points 76 and 670 pg ml\(^{-1}\) are shown. This survival analysis indicates that the 95th percentile may not be the relevant clinical cut-off point for serum VEGF. Furthermore, the analysis showed that the patients with the highest 10% of the serum VEGF concentrations had a significantly (P=0.0005) reduced survival compared to the CRC patients with lower VEGF concentrations, while the patients with the lowest 10% of the serum VEGF concentrations had a significantly (P=0.04) better prognosis than the patients with higher preoperative serum VEGF concentrations.

**DISCUSSION**

The present study showed that preoperative concentrations of plasma and serum VEGF in patients with colorectal cancer were higher than in healthy controls, although the difference in the serum concentrations between the two groups was not significant. Additionally, the study indicated that high plasma and serum VEGF levels might be predictors of reduced overall survival in patients with CRC. In many aspects, the information obtained from the matched preoperative serum and plasma VEGF measurements were similar. The preoperative VEGF levels in plasma and serum were both higher than in healthy controls, and no correlation was found between gender and age in the two types of specimen. Additionally, in univariate survival analyses, high VEGF values predicted a reduced overall survival in both cases, although the preoperative serum VEGF concentration is affected to capillary walls at distant sites (Hejna et al, 1999). Therefore, although the preoperative serum VEGF concentration is affected by in vitro degradation of platelets, the increased platelet-derived VEGF may influence the biology of a present tumour in vivo, and may presumably reflect tumour burden at the time of surgery.

Previously, most clinical studies have addressed the prognostic impact of preoperative serum VEGF concentrations. Recently, it was demonstrated that plasma VEGF was increased in patients with colorectal cancer compared with controls (George et al, 2000) and that high plasma VEGF concentrations tended to occur with more advanced disease (Nakayama et al, 2000). The present study supports these observations and indicates furthermore, in a univariate analysis, that high concentrations may predict a reduced overall survival. However, in the multivariate analysis, in the subgroup of patients with colon cancer, high preoperative plasma VEGF concentration was not an independent predictor of reduced overall survival while a high preoperative serum VEGF concentration was. These findings may indicate that the preoperative serum VEGF concentration is a better prognostic parameter than the preoperative plasma VEGF concentration. However, since platelets and white cells in peripheral blood samples contribute to the concentration of VEGF in serum, a large prospective clinical study should investigate the prognostic significance of these parameters and their correlation to the VEGF concentration in serum and plasma.

**Table 3** Multivariate survival analysis of the 524 colorectal cancer patients, using the cut-off levels 76 and 760 pg ml\(^{-1}\) for the preoperative serum VEGF concentrations. The analysis includes Dukes stage, gender, age, topographical tumour localization, and preoperative serum VEGF.

|                          | HR 95% CI | P-value |
|--------------------------|-----------|---------|
| Dukes stage A            | 1         |         |
| Dukes stage B            | 1.84      | 1.13 – 2.99 | 0.01 |
| Dukes stage C            | 4.02      | 2.49 – 6.50 | <0.0001 |
| Dukes stage D            | 28.16     | 16.7 – 47.6 | <0.0001 |
| S-VEGF=76 – 670          | 1         |         |
| S-VEGF > 670             | 1.85      | 1.31 – 2.61 | 0.0005 |
| S-VEGF < 76              | 0.62      | 0.39 – 0.97 | 0.04 |
| Rectum vs colon          | 1.57      | 1.25 – 1.97 | <0.0001 |
| Age (in years)           | 1.03      | 1.02 – 1.04 | 0.0003 |
| Gender (m/w)             | 1.38      | 1.10 – 1.73 | 0.04 |

S-VEGF=serum VEGF; HR=Hazard ratio; 95% CI=95% Confidence interval.

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APPENDIX

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