Preoperative Serum Vascular Endothelial Growth Factor Can Predict Stage in Colorectal Cancer¹

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ABSTRACT

Neovascularization has been shown to be essential for the growth of solid tumors. Vascular endothelial growth factor (VEGF) is one of the most important mediators of angiogenesis. This study was conducted to determine the significance of this cytokine as a tumor marker for staging colorectal cancer. Preoperative serum VEGF was measured in 108 colorectal cancer patients and in 136 normal healthy controls. The results of this study showed a significant difference between the four T classes, Union International Contre Cancer (UICC) stages, and Dukes’ stages. In comparison to serum levels in controls (median, 173.8 pg/ml), VEGF levels were significantly elevated in T2 (P = 0.003), T3, and T4 (P < 0.0005); UICC I (P = 0.001), UICC II, UICC III, and UICC IV (P < 0.0005); and Dukes’ A (P = 0.001), Dukes’ B, and Dukes’ C (P < 0.0005). Serum VEGF showed a significant elevation over control in node-negative (P < 0.0005) and in node-positive colorectal cancer (P < 0.0005) patients. Node-positive cancer had a significant elevation of serum VEGF compared to node-negative cancer (P = 0.008). This study reveals that preoperative serum VEGF can detect all but very early colorectal cancer i.e., T1 (P = 0.06).

INTRODUCTION

VEGF³ is a dimeric heparin-binding glycoprotein with a molecular weight of about M, 45,000 (1). VEGF is one of the most potent mitogens of vascular endothelial cells (2). In addition to its mitogenic effect, the increased permeability caused by VEGF (3) results in extravasation of macromolecules such as fibrinogen from the circulation, which provides a fibrin gel meshwork or substratum for the migration and organization of endothelial cells as well as tumor cells (4). VEGF is expressed by a wide variety of tumor cells in vitro and in vivo (5–9).

Serum VEGF levels have previously been shown to be raised in patients with various tumors, including brain, renal, melanoma, breast, and gastrointestinal malignancies (5–10). VEGF is essential for angiogenesis and for the growth of colorectal cancer, and its expression in a tumor may be a good prognostic indicator (11). Other studies have highlighted the significance of angiogenesis in colorectal cancer. In one study, node-negative colon cancer patients living 5 years had significantly lower angiogenesis scores than did nonsurvivors (12). The same study showed that angiogenesis score correlated with local recurrence as well (12). Angiogenesis score correlated with tumor size in rectal cancer, and higher scores were seen in patients who died within 3 and 5 years when compared with survivors at these same time intervals (13). VEGF may have a role to play in the metastatic potential of the tumor, as seen in melanoma (7) and colorectal cancer cell lines (11). We set out to study whether preoperative serum VEGF could be used as a tumor marker for colorectal cancer and also to determine its relationship with disease stage at presentation.

PATIENTS AND METHODS

Study Population. One hundred eight patients with primary colorectal cancer were recruited. No patient had received any blood transfusion, radiotherapy, or chemotherapy before the study. One hundred thirty-six healthy sex- and age-matched subjects were also recruited as control subjects.

Storage of the Sera. Blood (7 ml) was drawn preoperatively in a plain tube. The serum was separated after 20–30 min of coagulation at room temperature and was stored at −80°C until the assay. Repeated thawing and freezing of samples was avoided.

VEGF Assay. Serum was assayed for VEGF by quantitative solid-phase ELISA (R&D Systems, Oxford, United Kingdom). The assay uses the quantitative sandwich enzyme immunnoassay technique using Sf-21-expressed recombinant human VEGF 165 and antibodies raised against the recombinant protein. This ELISA will detect both VEGF 165 and VEGF 121. The absorbance of each well was measured by dual wavelength (450 nm and a wavelength correction set to 540 nm), and the VEGF levels were expressed as pg/ml. All of the serum samples and standards were assayed in duplicate. The minimum detectable level of VEGF was 9 pg/ml.

Tumor Staging. A single pathologist reported on the resected tumor specimens and staged them according to TNM, UICC, and Dukes’ classifications (Table 1). Staging was done based on radiological reports and pathological records.

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³ The abbreviations used are: VEGF, vascular endothelial growth factor; IQR, interquartile range.
Table 1  Cancer stages

<table>
<thead>
<tr>
<th>Dukes' stages*</th>
<th>Lesion limited to mucosa</th>
<th>Lesion extending into muscularis propria but not through it; negative lymph nodes</th>
<th>Lesion penetrating muscularis and extending into the serosa; negative lymph nodes</th>
<th>Lesion involves any layer of bowel wall except serosa; positive lymph nodes</th>
<th>Lesion involves all layers of the bowel wall including serosa; positive lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage A</td>
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<tr>
<td>Stage B1</td>
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<td>Stage B2</td>
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<td>Stage C1</td>
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<td>Stage C2</td>
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</table>

UICC stages*  

<table>
<thead>
<tr>
<th>Stage I</th>
<th>T_0^c</th>
<th>N_0^d</th>
<th>M_0^c</th>
<th>T_1</th>
<th>N_0</th>
<th>M_0</th>
<th>Dukes' A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage II</td>
<td>T_1</td>
<td>N_0</td>
<td>M_0</td>
<td>T_2</td>
<td>N_0</td>
<td>M_0</td>
<td>Dukes' A</td>
</tr>
<tr>
<td>Stage III</td>
<td>Any T</td>
<td>N_1</td>
<td>M_0</td>
<td>Any T</td>
<td>N_2N_3</td>
<td>M_0</td>
<td>Dukes' C</td>
</tr>
</tbody>
</table>

* From Ref. 28.
* From Ref. 29.

T_0^c, carcinoma in situ; T_1, tumor invades submucosa; T_2, tumor invades muscularis propria; T_3, tumor invades the subserosa or nonperitonealized pericolic or perirectal tissue; T_4, tumor perforates the visceral peritoneum or directly invades other organs or structures.

N_0, no regional lymph nodes; N_1, one to three pericolic or perirectal nodes; N_2, four or more pericolic or perirectal nodes; N_3, any positive node along the course of a named vascular trunk.

M_0, no distant metastasis; M_1, distant metastasis.

Table 2  Normal distribution of VEGF

<table>
<thead>
<tr>
<th>n</th>
<th>Age, years</th>
<th>VEGF, pg/ml</th>
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<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
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<tr>
<td>Controls</td>
<td>37.5 (30.0–51.7)</td>
<td>173.8 (92.5–282.3)</td>
</tr>
<tr>
<td>Male controls</td>
<td>32.0 (28.0–58.3)</td>
<td>171.4 (95.3–289.9)</td>
</tr>
<tr>
<td>Female controls</td>
<td>38.5 (32.0–44.3)</td>
<td>173.8 (91.9–252.4)</td>
</tr>
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</table>

Statistical Analysis. Statistical analysis was performed with the SPSS software package for Windows [SPSS (UK) Ltd., Surrey, United Kingdom]. ANOVA was used to determine the difference between the means of the groups. Further analysis was carried out using a nonparametric test for two independent samples (Mann-Whitney U test).

RESULTS

Controls. The details of the control subjects are summarized in Table 2. Mean serum VEGF levels in the control subjects was 216.9 pg/ml (median, 173.4; IQR, 92.5–282.3; Table 2). There was no statistical difference in VEGF levels between sexes (P = 0.969; Fig. 1a). We found that age had no influence on the expression of serum VEGF when controls were grouped according to the decade of their age (P = 0.676; Fig. 1b).

Colorectal Cancer Group. ANOVA showed a significant difference between the different Dukes’ stages (P = 0.007), T classes (P = 0.001), and UICC stages (P = 0.001). There was a trend toward increasing VEGF levels with increasing UICC and Dukes’ stages and increasing T classes. There was a significant correlation between UICC stages (p = 0.64; P < 0.005), Dukes’ stages (p = 0.633; P < 0.0005), T classes (p = 0.624; P < 0.0005), and serum VEGF (Spearman correlation with significance at 0.01 level). Sensitivity of serum VEGF in detecting any colorectal cancer, using mean VEGF of controls (216.9 pg/ml), was found to be 90.7% with 61.0% specificity. The positive predictive value of this level of serum VEGF in

Fig. 1  a, scatter plot of VEGF in controls. There was no significant difference in VEGF levels between sexes (P = 0.969). b, scatter plot of VEGF in controls based on decade of their age. There was no influence of age on VEGF levels (P = 0.676). a and b, reference line, mean VEGF (216.9 pg/ml).
detecting any colorectal cancer was 64.9%, and negative predictive value was 89.2% (Table 3; Fig. 2).

**T Classes.** T1 tumors with a median VEGF of 291.3 pg/ml (IQR, 230.5–510.3) were not significantly elevated compared to controls \( (P = 0.06). \) However, T2 (median VEGF, 313.1 pg/ml), T3 (median VEGF, 554.6 pg/ml), and T4 (median VEGF, 772.3 pg/ml) had significantly elevated VEGF levels over controls \( (P = 0.003, P < 0.0005, \) and \( P < 0.0005, \) respectively; Table 4; Fig. 3, a and b). VEGF levels in T1 and T4 classes of colorectal cancer (median, 628.5 pg/ml) were significantly higher compared to serum VEGF levels in T1 and T2 classes (median, 307.4 pg/ml; \( P < 0.0005; \) Table 4). Using mean levels of T1 and T2 tumors (370 pg/ml), serum VEGF could predict T3 and T4 classes of colorectal cancer with 73.3% sensitivity and 82.4% specificity. The positive predictive value was 72.4%, and these levels of VEGF could exclude T1 and T2 tumor classes at a negative predictive value of 83.0% (Table 3; Fig. 4).

**UICC Stages.** Patients at all UICC stages had elevated serum VEGF compared to controls. The significance of difference from controls increased with the increasing stage. There were 19 patients with UICC stage I, 42 with stage II, 34 with stage III, and 13 with stage IV colorectal cancer. UICC I (median VEGF, 291.1 pg/ml), UICC II (median VEGF, 593.5 pg/ml), UICC III (median VEGF, 625.0 pg/ml), and UICC IV (median VEGF, 701.3 pg/ml) had significantly elevated level of serum VEGF compared to controls \( (P < 0.0005, <0.0005, <0.0005, \) and \( <0.0005, \) respectively; Table 4; Fig. 5, a and b). In addition, UICC stage III and IV VEGF levels (median, 676.0 pg/ml) were significantly elevated compared to UICC I and II stages (median, 418.0 pg/ml; \( P = 0.006; \) Table 4).

**Dukes’ Stages.** Serum VEGF levels in Dukes’ A (median VEGF, 307.4 pg/ml), Dukes’ B (median VEGF, 593.5 pg/ml), and Dukes’ C (median VEGF, 662.5 pg/ml) were significantly higher than the control group \( (P = 0.001, <0.0005, \) and \( <0.0005, \) respectively; Table 4; Fig. 6, a and b). Serum VEGF levels of 370 pg/ml (similar to the cutoff used for T classes) predicted higher Dukes’ stages (B and C) with 72.2% sensitivity and 82.4% specificity and had a positive predictive value of 73.0% and a negative predictive value of 81.8% (Table 3; Fig. 7).

**Metastatic Status.** Colorectal cancer patients with evidence of liver metastases had significantly elevated serum VEGF levels (median VEGF, 701.0 pg/ml) over controls \( (P < 0.0005, \) and also over colorectal cancers without liver metastases (median VEGF, 540.3 pg/ml; \( P = 0.03; \) Table 5; Fig. 8). Using mean levels of the metastasis-free colorectal cancer group (600 pg/ml), serum VEGF could exclude liver metastasis with a negative predictive value of 97.0%, sensitivity of 69.2%, and specificity of 96.3% (Table 3; Fig. 9).

**Nodal Status.** Serum VEGF levels in node-positive cancers (median VEGF, 680.3 pg/ml) were significantly elevated compared to controls \( (P < 0.0005, \) and also compared to node-negative disease (median VEGF, 403.4 pg/ml; \( P = 0.008; \) Table 5; Fig. 10). Using mean levels of node-negative colorectal cancer (500 pg/ml), serum VEGF was 68.3% sensitive and 91.2% specific in detecting node-positive disease. The chances of node-positive disease at or below these levels were 9.5% (negative predictive value, 90.5%; Table 3; Fig. 11).
Fig. 4 VEGF at 370 pg/ml cutoff is 73.3% sensitive and 82.4% specific in predicting T3 and T4 classes of colorectal cancer (also see Table 3).

Fig. 3 a, except for T1, all T classes showed a significant elevation in VEGF compared to controls (Ps: for T1, 0.06; for T2, 0.003; and for T3 and T4, <0.0005; see Table 4). Box, IQR; whiskers range. b, VEGF levels in T2 (P = 0.003), T3 (P < 0.0005), and T4 (P < 0.0005) were significantly higher than controls except for T1 (P = 0.06). Reference line, mean VEGF (216.9 pg/ml).

DISCUSSION

VEGF is produced by a wide variety of tumor cells, helping the growth and dissemination of the tumor by making it more vascular (5–8). The four isoforms of VEGF (121, 165, 189, and 206) are produced by alternate splicing of mRNA (14, 15). VEGF 165 is the most abundant form of VEGF (15). VEGF 121 and 165 are the secreted forms, whereas the bigger forms are insoluble (15). VEGF acts in a paracrine fashion via two high-affinity tyrosine kinase receptors called flt-1 (or VEGF R1) and KDR (or VEGF R2) expressed mainly on the endothelial cells (16). These receptors, apart from being associated with tumor angiogenesis, are also involved in normal embryonic development (16, 17). The target cells for VEGF were identified by in vitro ligand autoradiography with iodinated recombinant human VEGF in adult rat tissues. The binding sites identified were brain, spinal cord, adrenal cortex, glandular stomach, lung.

Fig. 5 a, all UICC stages had VEGF levels significantly elevated over controls (Ps: stage I, 0.001; stages II, III, and IV, <0.0005). Box, IQR; whiskers range. b, scatter plot showing distribution of VEGF in controls and UICC stages. VEGF levels in all stages were significantly higher than controls. Reference line, mean VEGF (216.9 pg/ml).
spleen, and pancreas (18). VEGF has also been shown to be
expressed by fibroblasts, and protein kinase C is involved in the
signal transduction (19). The receptors binding VEGF have been
demonstrated on mononuclear phagocytes (20).

The prognostic value of VEGF has been shown in breast
gastric cancer based on VEGF expression in tumor tissue
detected by immunohistochemistry (8, 21), with VEGF concen-
trations being higher in vascularly rich breast tumors (8). The
VEGF positivity in gastric cancer correlated with vessel in-
volvement, lymph node metastasis, and liver metastasis and was
associated with an overall poorer prognosis (21). Warren et al.
(22) showed that various immortal colorectal cancer cell lines
express VEGF, which was also expressed by human colorectal
cancer liver metastases. In experimental studies, neutralizing
monoclonal antibodies against VEGF not only restricted the
growth of colorectal cancer cells (HM7 and LS LiM6) at the
primary site (flank of nude mice) of inoculation but resulted in
fewer and smaller liver metastases after animals were inoculated
with colorectal cancer cells (HM7) by splenic-portal injection
(22). In an experimental model, similar tumor loads in anti-
VEGF-treated animals resulted in fewer and smaller liver me-
tastases (from the human epidermoid carcinoma cell line) com-
pared to untreated animals, suggesting that VEGF seems to have
a mechanism discernibly different in promoting liver metastasis
from the one aiding the primary growth of tumors (23).

Of the four isoforms, VEGF 165 is considered to be the
most abundant form of VEGF (14, 24, 25); however, the ELISA
kits we used detect VEGF 121 as well. The range of age in our
control group is 22-79 and is representative of the study group,
as the incidence of colorectal cancer increases markedly after 50
years of age. Given that it was shown in our study that age has
little bearing on the degree of serum VEGF expression, an exact
age match is not relevant. The range of serum VEGF among
controls in our study was from undetectable to 830.8 pg/ml
(mean, 216.9 pg/ml; 2SD, 347.0 pg/ml). The relevance of nor-
mal levels of VEGF at present is not clear. However, the high
density of receptors in the endothelial lining of the heart valves,
which are constantly subjected to the shear forces of blood flow,
emphasizes that VEGF may be important for continual repair
and maintenance in regions that are susceptible to endothelial
erosions (18).

<table>
<thead>
<tr>
<th>Table 5</th>
<th>VEGF comparison according to node and metastatic status</th>
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</table>
| Node-negative disease | 67 | 403.4 (275.6-713.0) | <0.0005*
| Node-positive disease | 41 | 680.3 (400.3-880.1) | <0.0005*
| Metastasis-free disease | 95 | 547.6 (296.0-767.7) | <0.0005*
| Metastatic disease present | 13 | 858.4 (574.6-948.6) | <0.0005*
| N<sub>0</sub> versus N<sub>+</sub> | 0.008 |
| M<sub>0</sub> versus M<sub>+</sub> | 0.03 |

* Mann-Whitney U test.
* N<sub>0</sub>, node negative; N<sub>+</sub>, node positive.
* M<sub>0</sub>, metastasis free; M<sub>+</sub>, metastatic disease.

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**Fig. 6** a, all Dukes’ stages showed significant elevation in VEGF levels over controls (Ps: Dukes’ A, 0.001; Dukes’ B and C, <0.0005). Box, IQR: whiskers, range. b, scatter plot of VEGF distribution in Dukes’ stages and controls. All stages had significantly higher VEGF levels than controls (see Table 4). Reference line, mean VEGF (216.9 pg/ml).

**Fig. 7** VEGF at 370 pg/ml cutoff is 72.2% sensitive and 82.4% specific in predicting Dukes’ B and C (also see Table 3).
In the present study, the colorectal cancer group as a whole showed rising levels of serum VEGF levels with increasing tumor load. This is the first documentation of the role of serum VEGF in staging colorectal cancer. In this present study, all but T1 tumors showed significantly elevated levels of serum VEGF compared to controls. However, small numbers of T1 (n = 4) in our study preclude any significant conclusion regarding this class of colorectal cancer. T2, T3, and T4 classes of tumor and UICC stages I, II, III, and IV had significantly elevated levels compared to control subjects. A similar pattern was seen in all three Dukes’ stages. This is a reiteration of the hypothesis that as a tumor grows, it needs to recruit more vessels and therefore results in increased expression of VEGF, which is reflected in the serum levels as well. A recent in vitro study has shown that human colon carcinoma cell density can regulate VEGF expression (26).

We found that the sensitivity of serum VEGF to detect colorectal cancer was 90.7% with a negative predictive value of 89.2% at a cutoff value of 217 pg/ml. Serum VEGF at 500 pg/ml can detect node-positive disease with 68.3% sensitivity and 91.2% specificity and has an excluding power of 90.5% (negative predic-

Fig. 8 VEGF was elevated in metastatic disease over control (P < 0.0005) and also compared to metastasis-free disease (P = 0.03; see Table 5). Box, IQR; whiskers, range.

Fig. 9 VEGF at 600 pg/ml cutoff is 69.2% sensitive and 96.3% specific in predicting metastatic disease (also see Table 3).

Fig. 10 VEGF in node-positive disease was elevated over controls and node-free disease (P < 0.0005 and 0.008, respectively; see Table 5). Box, IQR; whiskers, range.

Fig. 11 VEGF at 500 pg/ml cutoff predicts node-positive disease with 68.3% sensitivity and 91.2% specificity (also see Table 3).

4 H. Kumar, K. Heer, and J. R. T. Monson, unpublished data.
direction, the role of VEGF will need to be elucidated thoroughly vis-à-vis tumorigenesis and its kinetics in normal individuals and treated patients.

In the changing scenario of the treatment of colorectal cancer there is a need for an easy, economical, and fairly accurate staging tool to select patients with tumor load suitable for preoperative chemo- and/or radiotherapy. Serum VEGF shows a promise in that direction. Furthermore, the study of Shen et al. that shows that mononuclear phagocytes have the ability to bind VEGF, which induces the migration of these cells, has opened up an important issue of interaction between VEGF and the host response (20). In conclusion, this study demonstrates that serum VEGF can be used as a diagnostic marker for all but very early colorectal cancer and may be used to predict high tumor load, nodal involvement, and liver metastases and thus has a potentially important role to play in planning patient management.

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REFERENCES


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