Cancer patients often show an imbalance between coagulation and fibrinolysis systems, which results in their prothrombotic condition. Several blood molecular factors, such as von Willebrand factor (vWF), presenting higher plasma concentrations in these patients, play a key role in this process. In the angiogenesis that takes place during tumour growth, vascular endothelial growth factor (VEGF) contributes to proliferation and differentiation of endothelium, main vWF producer, resulting in increased plasma levels of vWF in cancer patients. In addition, platelets are considered to be VEGF carriers in blood, being indirectly responsible for its concentration in serum. A role for blood clotting and platelet aggregation induced by tumour cells adhering to platelets has been demonstrated by the discovery of platelet, endothelial and tumour cells membrane integrins and their functions. Moreover, several clinical trials have shown high rates of plasma vWF in cancer patients, with elevated levels being associated with more advanced stages. In addition, higher pre-surgical vWF values appear to be determinants of decreased survival rates after surgery.

To date, there has been a lack of prospective clinical trials, correlating analytical findings with response to treatment and long-term survival, which makes the role of vWF and VEGF as tumour markers in cancer patients still controversial. The promising results of several clinical trials raise the question of whether these markers could be used as prognostic or predictive factors in cancer patients.

**Introduction.** von Willebrand factor (vWF) is thought to mediate binding of tumour cells to platelets and to favour their systemic spreading capacity. Platelets involved in tumour angiogenesis are capable of releasing vascular endothelial growth factor (VEGF). Hence, levels of vWF and VEGF may correlate with cancer stage. The objectives are to determine the impact of surgery and chemotherapy on vWF and VEGF in colorectal cancer (CRC) patients.

**Material and methods.** Twenty healthy volunteers (group 1), 14 patients with locally advanced CRC (group 2) and 12 patients with metastatic CRC (group 3) were enrolled. Blood samples were taken at recruitment in group 1, and before and after surgery and chemotherapy in groups 2 and 3, respectively. Blood levels of vWF, VEGF, platelet count, C-reactive protein (CRP), ceruloplasmin and carcinoembrionary antigen (CEA) were measured.

**Results.** At baseline, group 3 showed higher concentrations of vWF than the other groups (p<0.05). In group 2, vWF became elevated 40% post-surgery (p=0.016), independently of changes in CRP or ceruloplasmin. In group 3, chemotherapy caused a 42% reduction in VEGF (p=0.015).

**Conclusions.** There was a strong correlation between higher vWF levels and more advanced CRC stage at diagnosis. These levels were elevated post-surgery in patients with locally advanced CRC. Chemotherapy significantly decreased VEGF in metastatic CRC patients before CEA showed any significant change.

**Key words:** angiogenesis, colorectal cancer, VEGF, vWF.

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**Background**

Cancer patients often show an imbalance between coagulation and fibrinolysis systems, which results in their prothrombotic condition. Several blood molecular factors, such as von Willebrand factor (vWF), presenting higher plasma concentrations in these patients, play a key role in this process. In the angiogenesis that takes place during tumour growth, vascular endothelial growth factor (VEGF) contributes to proliferation and differentiation of endothelium, main vWF producer, resulting in increased plasma levels of vWF in cancer patients. In addition, platelets are considered to be VEGF carriers in blood, being indirectly responsible for its concentration in serum. A role for blood clotting and platelet aggregation induced by tumour cells adhering to platelets has been demonstrated by the discovery of platelet, endothelial and tumour cells membrane integrins and their functions. Moreover, several clinical trials have shown high rates of plasma vWF in cancer patients, with elevated levels being associated with more advanced stages. In addition, higher pre-surgical vWF values appear to be determinants of decreased survival rates after surgery.

To date, there has been a lack of prospective clinical trials, correlating analytical findings with response to treatment and long-term survival, which makes the role of vWF and VEGF as tumour markers in cancer patients still controversial. The promising results of several clinical trials raise the question of whether these markers could be used as prognostic or predictive factors in cancer patients.

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**ORIGINAL ARTICLES**

Impact of surgery and chemotherapy on von Willebrand factor and vascular endothelial growth factor levels in colorectal cancer

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Key words: angiogenesis, colorectal cancer, VEGF, vWF.
anti-aggregation/anti-coagulation therapies in combination with new anti-angiogenic agents in these patients have paved the way for the development of new therapeutic targets in cancer treatment.

The aim of this study was to determine the impact of surgery and chemotherapy on vWF plasma and VEGF serum levels in both localized and metastatic colorectal cancer (CRC) patients who had not been treated previously.

PATIENTS AND METHODS

Inclusion criteria

From January 2001 to February 2003, untreated CRC patients aged 18 to 85 attending the Departments of General Surgery and Oncology of the University Clinic, University of Navarra, were invited to join the study. Patients and controls received detailed information on the purposes and design of the study and gave informed consent according to the guidelines of the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of the University Clinic, University of Navarra.

Exclusion criteria

None of the patients or healthy volunteers had received anti-coagulation or anti-aggregation drugs in the 6 months prior to the study, nor received them during the study. Subjects with diabetes mellitus, chronic arterial hypertension, ischaemic heart disease, acute inflammation or infectious active process, connective tissue disease, rheumatoid arthritis, systemic lupus erythematosus, renal and hepatic failure, or major surgery performed in the three months preceding the trial enrolment, were excluded from the study (vWF is a known acute phase reactant and endothelial damage marker in plasma). Recent venous thrombosis and/or pulmonary embolism, radiotherapy within the last year and less than 6 months of life expectancy were also exclusion criteria.

Patient groups and blood samples

A total of 20 healthy volunteers and 26 colorectal cancer patients at different stages of disease were enrolled. Fourteen of the patients were diagnosed as having local or locally advanced cancer (stages I to III) and underwent rectum resection, sigmoidectomy, hemicolectomy or colectomy as initial therapy (group 2). The 12 patients who had distant metastases (group 3), underwent 3 cycles of 5-fluorouracil-based chemotherapy. Twenty healthy controls who were unrelated to the patients and who were of comparable age and gender served as the control group (group 1). At enrolment time (group 1) and before and after treatment (groups 2 and 3), blood samples were obtained by cubital venipuncture and stored until further processing. A total of 92 blood samples were obtained and processed.

Study variables and techniques

Blood samples were immediately centrifuged at 2500 g for 20 minutes (plasma) or 5500 g for 10 minutes (serum) at 4 °C. The plasma and serum samples were aliquoted and, for long-term storage, kept at -20 and -80 °C respectively, until further analysis.

Serum VEGF determination

For the measurement of serum VEGF, a commercially available ELISA kit was used (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN) according to the recommendations of the manufacturer. The assay has been shown to be reproducible. It showed no significant cross-reactivity with other angiogenic factors and had a sensitivity of 9 pg/ml.

Results were calculated from a standard curve (generated with recombinant human VEGF 165; range 31.2 pg/ml to 2000 pg/ml) and expressed in picograms per millilitre (pg/ml). All samples were assayed in duplicate.

Serum C-reactive protein determination

Levels of C-reactive protein (CRP) in serum samples were measured by an immunoturbidimetric assay following the Tina-quant CRP protocol (Roche) in the Hitachi Modular equipment (Roche).

Serum ceruloplasmin concentration

Serum ceruloplasmin levels were determined through an immunonephelometric procedure using the IMMAGE equipment (Beckman).

Serum carcinoembryonic antigen levels

Determination of serum carcinoembryonic antigen (CEA) levels was carried out employing the COBAS Core II machine (Roche) following the directions of the manufacturer.

Plasma vWF determination

vWF was measured in stored plasma samples by the immunological Asserachrom vWF method (Stago), using the STA Compact analyser (Stago). Values are expressed as percentage with reference to a standard curve using a pool of normal plasma (100% activity).