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von Willebrand factor antigen levels in plasma of patients with malignant breast disease

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Abstract

von Willebrand factor (vWF) is a protein that mediates platelet adherence to the subendothelium during primary hemostasis. High plasma vWF concentrations have been reported in patients with various types of cancer, such as head and neck, laryngeal and prostatic cancer, probably representing an acute phase reactant. In the present study we determined the plasma levels of vWF antigen (vWF:Ag) by quantitative immunoelectrophoresis in 128 female patients with breast cancer as well as in 47 women with benign breast disease and in 27 healthy female controls. The levels of vWF:Ag were 170.7 ± 78 U/dl in patients with cancer, 148.4 ± 59 U/dl in patients with benign disease and 130.6 ± 45 U/dl in controls ($P<0.005$). We also detected a significant increase in the levels of vWF:Ag ($P<0.0001$) in patients with advanced stages of the disease (stage IV = 263.3 ± 113 U/dl, stage IIIB = 194.0 ± 44 U/dl) as compared to those with earlier stages of the disease (stage I = 155.3 ± 65 U/dl, stage IIA = 146.9 ± 75 U/dl). In conclusion, vWF levels were increased in plasma of patients with malignant breast disease, and these levels correlated with tumor progression.

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• Breast cancer
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Introduction

von Willebrand factor (vWF) is a plasma glycoprotein synthesized mainly in endothelial cells (1,2). It plays an essential role in initial hemostasis, mediating adhesion of platelets to subendothelial surfaces at sites of vascular injury and acting as a carrier protein for factor VIII (3). Increased plasma levels of von Willebrand factor antigen (vWF:Ag) have been reported in several clinical conditions such as liver diseases, diabetes mellitus, myocardial infarction, connective tissue diseases and acute infections, probably as a result of increased endothelial cell damage (4-8). Plasma concentrations are also influenced by ABO blood group, with individuals with group O having lower mean vWF:Ag levels than individuals with other ABO blood groups (9).

High plasma levels of vWF:Ag have been also observed in patients with several types of malignant diseases, such as head and neck, laryngeal and prostatic cancer, and this phenomenon has been associated with tumor-induced endothelial growth during the an-

giogenic process (10-12). This increase may reflect endothelial proliferation and/or may be part of the acute phase reaction in response to vascular abnormalities (5,7).

In experimental models, vWF was shown to be involved in pathogenesis of metastasis by promoting the binding of tumor cells to platelets. This effect leads to the formation of heterotypic cellular emboli, which increase the probability of tumor cell attachment to endothelial surfaces (13-17).

Recent studies have suggested that the levels of vWF:Ag in plasma of cancer patients increase with clinical staging and may be of prognostic significance (18-22). In the present study we determined the levels of vWF in plasma of a series of patients with breast cancer at different stages, in patients with benign breast diseases, and in normal controls.

Patients and Methods

Eligibility criteria

Newly diagnosed female patients aged 18 to 70 years with histologically proven breast cancer at TNM (tumor, node, metastasis) stages I, II, III and IV were included in this study. Other eligibility criteria included no prior radiation, hormonal or cytotoxic therapy, no breast surgery during the previous 12 months, and absence of any concomitant diseases such as diabetes mellitus, coronary artery disease, hypertension, liver, rheumatic or connective tissue disorders, or active infectious or inflammatory disease. No concomitant medications known to interfere with vWF levels or measurements were allowed. Female patients with benign breast disease and normal controls were also included in the study. Informed consent was obtained from all participants.

Laboratory measurements

Patients had to undergo a routine staging procedure which included a complete medical history and physical examination, full blood counts, biochemistry, liver and renal tests, as well as imaging tests (chest X-ray, abdominal ultrasound, bone scintigraphy, and other tests when indicated). A complete coagulation assessment was performed in all patients and included a total platelet count, prothrombin time (PT) and partial thromboplastin time (PTT) and the measurements of coagulation factors. For the measurement of vWF levels in plasma, 5 ml of blood was drawn by venipuncture and collected into a 1:10 volume of 3.8% trisodium citrate. After centrifugation at 3500 *g* for 10 min, the platelet-poor plasma was stored at -80°C until testing. vWF:Ag levels were measured by quantitative immunoelectrophoresis using a rabbit polyclonal antibody against human vWF (23,24).

Statistical analysis

The results obtained for patients with breast cancer at each stage of the disease, patients with benign breast disease and normal controls were analyzed using the nonparametric Kruskal-Wallis test. Mean values (± SD) were obtained for each study group and the results were used for the calculations.

Results

Patient characteristics

A total of 128 patients with malignant breast disease, 47 patients with benign breast disease and 27 control healthy women were included in the study. The main characteristics of the patients are summarized in Table 1. The main histopathological types of disease in patients with breast cancer were infiltrating ductal carcinoma in 85.2% of cancer patients (N = 109), and lobular carcinoma in 6.25% (N = 8). The most common histopathological types of benign breast disease were fibroadenoma in 57.4% of cases

(N = 27) and fibrocystic changes in 23.4% (N = 11).

Laboratory measurements

The results obtained from the laboratory tests performed on patients with breast cancer, patients with benign breast disease and normal controls are summarized in Table 2. All results for patients with benign breast disease and controls were within normal values. There were no differences in biochemistry or liver and renal function tests, or in common routine coagulation parameters such as mean platelet counts, PT and PTT levels between the study groups.

In patients with breast cancer, the values of routine laboratory tests were all normal for patients with stages I, II and III, while alkaline phosphatase, gamma-glutamyl transferase and liver transaminases were elevated in some patients with stage IV disease due to liver and/or bone metastatic involvement.

The mean level of vWF (reported as U/dl) was 130.6 ± 45 for the control group, 148.4 ± 59 for the group with benign breast disease and 170.7 ± 78 for the group with malignant breast disease. There was a statistically significant difference in vWF levels between patients with malignancy and patients with benign disease or normal controls ($P<0.005$). vWF levels were also analyzed according to disease stage in patients with breast cancer. vWF:Ag levels were higher in women with advanced stages of breast cancer, with a statistically significant difference being observed between stages I/II and stages III/IV, with mean values of 154.9 and 199.1, respectively ($P<0.0001$). Mean vWF values are presented in Table 2.

Discussion

High plasma vWF levels have been reported in different types of cancers (10-13). The mechanisms involved in this process are not completely understood, but there is evidence that this phenomenon may be related to accelerated endothelial synthesis associated with tumor-dependent angiogenesis (10-12). In addition, tumor thrombin release was shown to induce vWF production in endothelial cells and to influence tumor cell adhesion (25-27). More recently, a decrease in the cleavage of vWF by its protease control system has been reported in malignancy (28).

Experimental studies have demonstrated a direct interaction between vWF and neoplastic cells (29). The expression of the surface complexes GpIIb-IIIa and GpIb, the adhesive ligands for vWF, has been reported to occur in tumor cells (26,28,30). In plasma, vWF seems to contribute to the metastatic process by promoting the binding of malignant cells to platelets. Such interaction results in heterotypic cell aggregates which are more capable of producing adherence to en-

Table 1. Characteristics of the women with breast disease and normal controls.

Characteristic	Controls	Benign disease	Malignant disease
Number	27	47	128
Mean age (± SD)	44.7 ± 13	40.4 ± 14	53.8 ± 14
ABO blood type	33%	34%	42%

Table 2. von Willebrand factor antigen (vWF:Ag) levels in patients with breast cancer according to stage of the disease determined by the TNM system (U/dl).

TNM stage	N	vWF:Ag
Normal controls	27	130.6 ± 45
Benign breast disease	47	148.4 ± 59
Malignant breast disease		
I	23	155.3 ± 65
IIA	37	146.9 ± 75
IIB	22	163.3 ± 52
IIIA	13	134.2 ± 38
IIIB	18	194.1 ± 44
IV	15	263.3 ± 113

Data are reported as means ± SD. $P<0.0001$ for the differences in vWF:Ag values between patients with stages I and II versus patients with stages III and IV (Kruskal-Wallis test). TNM: tumor, node, metastasis.

dothelial surfaces compared to single tumor cells (16-18). Notably, animal studies have shown that anti-platelet and anti-vWF antibodies can reduce substantially the occurrence and number of metastases. The same occurs after the inhibition of the GpIIb-IIIa and GpIb receptor sites in platelets and tumor cells (16,17,26-28,30).

vWF:Ag is an acute phase reactant which may vary substantially under several physiological conditions, such as menstrual cycle, age or ABO blood group, as well as in several benign and malignant diseases (3,4, 10,31). Although the data reported in the present study did not have enough statistical power to allow a detailed subgroup analysis, they showed a significant increase in plasma vWF levels in patients with malignant breast disease compared to patients with benign breast disease and normal controls. In addition, patients with metastatic disease were those with the highest plasma vWF levels.

It should be pointed out that no firm conclusion can be drawn from our data concerning the role of vWF for the diagnosis of breast cancer or its prognosis. Further studies including a larger patient population are warranted to clarify the role of this factor in the above processes. Although differences in vWF activity were observed between cancer patients and the other groups, our measurements did not include the determination of integrity of vWF multimers. In healthy individuals, a 1:1 relationship between the two parameters should be observed. In addition, the influence of vWF-specific proteases, which can be present in some patients with cancer, should also be important in the interpretation of the results.

In conclusion, our study gives support to previous observations of other tumor types which demonstrated that vWF levels are increased in plasma of cancer patients compared to patients with benign disease and normal controls (10-12). Thus, breast cancer should be added to the list of malignancies, such as head and neck, prostate, laryngeal cancer and others, in which a correlation between plasma vWF levels and malignancy has been observed (18-22). Further studies are warranted to evaluate vWF activity in patients in terms of angiogenic and endothelial cell response to malignancy.

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