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Original Article

The clinical value of von Willebrand factor in colorectal carcinomas

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Abstract: Background: To identify the value of von Willebrand factor (vWF) as a clinical marker in colorectal carcinoma (CRC). Methods: Plasma levels of vWF were measured in 79 patients with UICC Stage I-IV CRC at time of operation and correlated with TNM categories, levels of the carcinoembryonic antigen (CEA), blood groups (BG) and 19 controls (CO). CO included cancer-free patients without bacterial or viral infections. For tissue analysis paraffin embedded tumour and mucosa sections of operation specimens were stained immunohistochemically for vWF and compared to vWF plasma levels as well as to TNM categories. Results: VWF plasma levels in CRC patients were significantly dependent on blood groups (p=0.012) and elevated compared to the normal ranges as well as to controls (BG 0: p=0.668, BG A/AB/B: p=0.020). CRC-Patients over 60 years of age presented with significantly higher vWF levels than patients below 60 years (BG 0: p=0.005; BG A/AB/B: p=0.035). There was no correlation of vWF plasma levels and UICC stages in CRC. Patients with elevated vWF plasma levels also presented with elevated CEA levels, but significance was missing (p=0.080). VWF concentration within the tumour tissue was independent of concentration within normal mucosa, blood groups, histopathological characteristics and did not correlate with plasma vWF levels. Conclusion: VWF plasma levels are elevated in CRC patients, but not in a stage dependent manner. Besides the tumour at least blood groups and age mainly influence plasma vWF levels. In our opinion vWF as a routinely used clinical marker in CRC cannot be recommended.

Keywords: von Willebrand factor, colorectal cancer, blood group

Introduction

Colorectal cancer (CRC) is one of the leading cancers in western countries and responsible for about 500 000 deaths per year worldwide [1]. Carcinoembryonic Antigen (CEA) is the most commonly used prognostic marker in CRC which is routinely investigated during follow up under clinical conditions. At the time of diagnosis only 39-50% of CRC patients present with elevated CEA levels but just in these patients CEA is useful for further postoperative monitoring [2-4]. Therefore more sensitive markers are needed in patients with CRC.

The glycoprotein von Willebrand factor (vWF) mediates the adhesion of platelets to subendothelial surfaces in primary haemostasis in case of vascular injury [5]. Plasma vWF levels vary over a very broad range extending from 40-240% of the mean value of 10µg/ml [6]. A major determinant of plasma vWF levels is the AB0 blood group but several clinical conditions, like myocardial infarction, diabetes mellitus, liver disease and acute infections induce increased plasma vWF concentrations [7-12]. There are some studies describing a correlation of vWF with different cancers like prostate cancer, cervical and ovarian cancer, head and neck cancer, larynx cancer and CRC [13-19]. Experimental models favour the hypothesis that vWF connects tumour cells to platelets and so assists during the pathogenesis of metastases. It is supposed, that these vWF-tumour-cell-embolies

Table 1. Characteristics of patients and controls

		BG 0 n (%)	BG A/AB/B n (%)
Patients: n=79		23 (29.1 %)	56 (70.9%)
Mean age in years		63; range: 37-78	68; range: 37-88
Gender			
	Females (n=29)	9 (39.1%)	20 (35.7%)
	Males (n=50)	14 (60.9%)	36 (64.3%)
UICC-Stages			
	UICC I (n=20)	6 (26.1%)	14 (25.0%)
	UICC II (n=27)	7 (30.4%)	20 (35.7%)
	UICC III (n=13)	4 (17.4%)	9 (16.1%)
	UICC IV (n=19)	6 (26.1%)	13 (23.2%)
vWF levels			
	within normal ranges	12 (52.2%)	33 (58.9%)
	elevated	11 (47.8%)	23 (41.1%)
Controls: n=19		8 (42.1%)	11 (57.9%)
Mean age in years		52; range: 40-61	59; range: 35-79
Gender			
	Females (n=10)	4 (50.0%)	6 (54.5%)
	Males (n=9)	4 (50.0%)	5 (45.5%)
vWF levels			
	within normal ranges	6 (75.0%)	9 (81.8%)
	elevated	2 (25.0%)	2 (18.2%)

are hardly detected by the immune system [20-22]. As vWF levels increase with progressive disease [18, 19] it is considered as a potential clinical marker in CRC.

The aim of the present study was to investigate the clinical value of vWF as a stage-dependent tumour marker in blood plasma and tissue of patients with CRC.

Patients and methods

Characteristics of patients and controls

79 patients with newly diagnosed histologically confirmed CRC receiving an elective surgery between 7/07 and 7/09 at our Department of surgery were included. None of the patients was pre-treated (by e.g. polypectomy, chemotherapy, radiation) or suffered from infections. The study group consisted of 29 females (36.7%) and 50 males (63.3%) at a mean age of 66.5 years (range: 37 to 88 years). 21 patients (26.6%) presented with rectal and 58 patients (73.4%) with colon cancer. In 42 patients (53.2%) blood group (BG) A, in 8 patients (10.1%) BG B, in 6 patients (7.6%) BG AB and in 23 patients (29.1%) BG 0 was confirmed.

VWF levels of patients were compared to 19 controls (ward patients without any evidence of malignancies or obvious infections) including 10 females (52.9%) and 9 males (47.1%) with the following arrangement of BG: 8 patients (42.1%) with BG 0, 8 (42.1%) with BG A, 1 (5.3%) with BG B und 2 (10.5%) with BG AB. Characteristics of patients and controls are presented in **Table 1**.

For this study institutional research ethical approval was received (Number 3402; 24.08.2005). All participants were informed personally and provided written informed consent for this study.

Immunohistochemical staining of tissue from CRC patients

VWF staining of formalin-fixed paraffin embedded sections from tumour and mucosal tissues of the operation specimens were performed as described before [23-25]. Tumour sections were confirmed by a pathologist, healthy mucosal tissues were gained from the tumour-free cranial or caudal resection margins. Antigen retrieval was carried out using target retrieval solution pH 6.1. For detection a mouse monoclonal APAAP system was used (DAKO Cytomation; Germany). The primary antibody vWF was diluted (1:75) and incubated for 1 hr. Staining

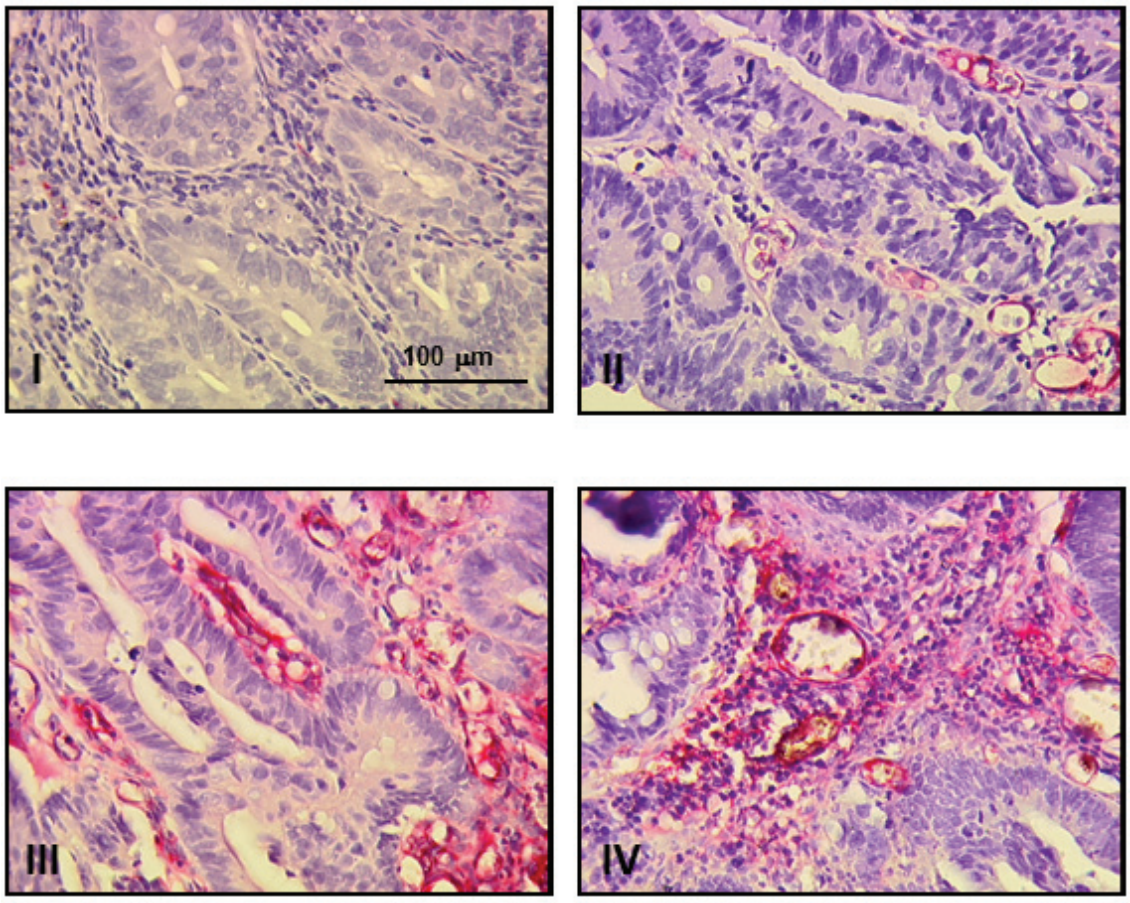

Figure 1. Examples for vWF concentrations within tissues. Displayed are examples for category I (0-25%), II (26-50%), III (51-75%) and IV (76-100% of the maximum of detected vWF).

was developed using chromogen red. All reagents were purchased from DAKOCytomation (Germany).

Stained sections were quantitatively evaluated by two persons. Tumour and mucosa sections were ordered by progressive amount of vWF and then separated in 4 categories: category I (0-25%), II (26-50%), III (51-75%) and IV (76-100% of the maximum recognised amount of vWF within the tissue). Samples for all categories are displayed in **Figure 1**.

Estimation of vWF in plasma

Blood draw was done at time of operation with a sodium citrate blood draw set (Sarstedt AG & Co., Nuembrecht, Germany) by routine venous punction. For estimation of human vWF an immunoturbidimetric assay according to manufacturer's instruction (STA LIATEST vWF, Diagnostica Stago, Asnieres, France) was used. In brief, latex covered microparticle coated by vWF specific antibodies coagulate in presence of vWF within the probe causing an increased turbidity. This turbidity was photometrically measured and is directly proportional to the vWF amount within the probe.

The normal levels of vWF are dependent on different blood groups, for BG 0 vWF of 40-145% and for BG A/AB/B vWF of 60-180% are within normal ranges. Therefore patients were analysed separately for blood group 0 and A/AB/B.

AB0- Blood group system

Blood was typed for common erythrocyte alloantigens: anti-A, anti-B, anti-0 and anti-AB by hemagglutination techniques according to the

Table 2. VWF concentration within the tumour tissues and histopathological tumour characteristics of 79 CRC patients

	vWF amount in tumour tissue				
	Category I n (%)	Category II n (%)	Category III n (%)	Category IV n (%)	P
Total	40 (51%)	22 (28%)	8 (10%)	9 (11%)	
T1/2	12 (52.2)	7 (30.4)	0	4 (17.4)	0.212
T3/4	28 (50.0)	15 (26.8)	8 (14.3)	5 (8.9)	
N0	25 (50.0)	13 (26.0)	5 (10.0)	7 (14.0)	0.828
N+	15 (51.7)	9 (31.0)	3 (10.3)	2 (7.0)	
M0	28 (46.7)	18 (30.0)	6 (10.0)	8 (13.3)	0.609
M+	12 (63.2)	4 (21.0)	2 (10.5)	1 (5.3)	
V0	38 (51.4)	20 (27.0)	8 (10.8)	8 (10.8)	0.646
V+	2 (40.0)	2 (40.0)	0	1 (40.0)	
L0	29 (50.9)	16 (28.1)	6 (10.5)	6 (10.5)	0.979
L+	11 (50.0)	6 (27.3)	2 (9.1)	3 (13.6)	
G1	1 (50.0)	1 (50.0)	0	0	0.817
G2	28 (52.8)	14 (26.4)	4 (7.6)	7 (13.2)	
G3	11 (45.8)	7 (29.2)	4 (16.7)	2 (8.3)	
UICC I	10 (50.0)	6 (30.0)	0	4 (20.0)	
UICC II	14 (51.9)	6 (22.2)	4 (14.8)	3 (11.1)	0.431
UICC III	4 (30.8)	6 (46.1)	2 (15.4)	1 (7.7)	
UICC IV	12 (63.2)	4 (21.0)	2 (10.5)	1 (5.3)	

German haemotherapy guidelines [26].

CEA

In patients with CRC five millilitres of venous blood were obtained at time of operation. For the quantitative measurement of CEA in human serum a microparticle enzyme immunoassay (AxSYM CEA; Abbott, Wiesbaden) was used according to the instructions. The cut-off value of serum CEA recommended by the manufacturer is 5ng/ml. Patients were classified in normal CEA levels (< 5ng/ml) and elevated CEA levels ($\geq$ 5ng/ml).

Statistical analysis

All statistical analyses were evaluated using the statistical software Statistical Package for Social Sciences (SPSS) for windows (version 18.0, SPSS Inc., Chicago, USA). After testing for normal distribution (Kolmogorov-Smirnov-Test) variables were compared by the Student´s t-test, chi^2 test or, in cases of smaller numbers than 5, by the exact Fisher-test and are expressed as mean $\pm$ standard deviation (SD). For comparing more than two medians the analysis of variance was used. A p-value $\leq$ 0.05 was determined significant.

Results

vWF concentration within tumour and mucosal tissues of CRC patients

The concentration of vWF within tissues was not influenced by blood groups (tumour p=0.199; mucosa p=0.202). Also the amount of vWF within tumour and corresponding mucosal tissues did not correlate (p=0.612; data not shown).

VWF concentrations within tumor tissues were not significantly influenced by TNM-categories, UICC-Stages or grading. Values are displayed in **Table 2**.

vWF concentration in tissue and plasma

In **Figure 2** vWF concentrations within the tumour (**Figure 2A**) and healthy mucosa (**Figure 2B**) were correlated with plasma vWF levels. Patients of BG 0 presented with increasing vWF plasma levels in case of higher vWF tissue

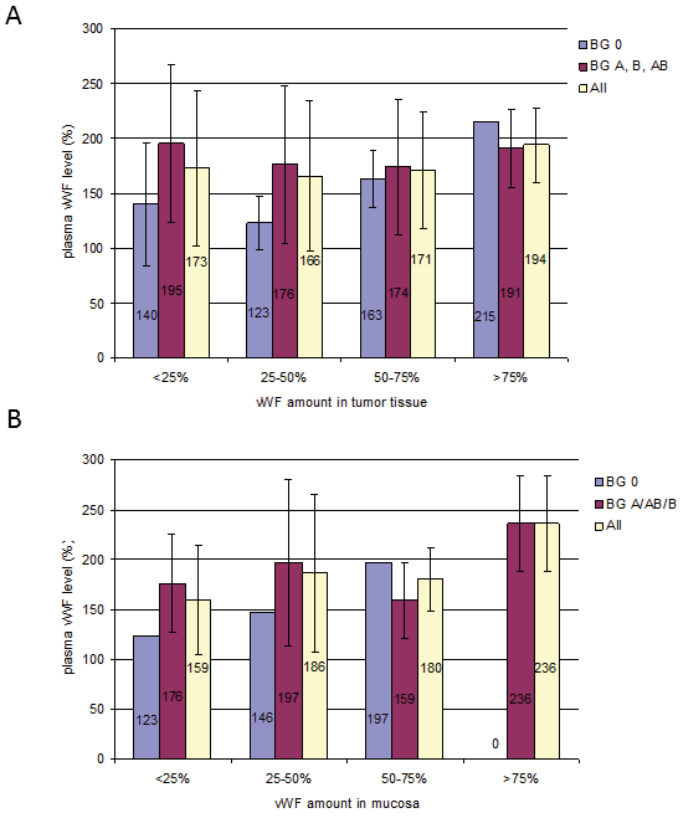

Figure 2. VWF amounts in tissues and corresponding mean plasma levels of vWF. In BG 0 plasma vWF levels were significantly influenced by vWF concentration within mucosal tissue (B) but not tumour (A) tissue. For BG A/AB/B an influence of plasma levels neither by tumour nor by mucosal tissue was detectable.

Table 3. Mean plasma levels of patients and controls separated for blood groups: VWF levels of CRC patients were elevated compared to controls and significantly (p=0.003) different for BG 0 and BG A/AB/B. Normal ranges for vWF of BG 0 are 40-145% and of BG A/AB/B 60-180%

	BG 0	BG A/AB/B	p-value
CRC Patients			
n= 79	23	56	
Mean vWF (SD) in %	142.5 (51.5)	186.3 (65.9)	0.003
Controls			
n= 19	8	11	
Mean vWF (SD) in %	133.1 (52.2)	139.9 (52.1)	0.783
p-value	0.668	0.020	

amounts reaching statistical significance for mucosal tissue (p=0.035) but not tumour tissue (p=0.431). For blood group A/AB/B neither for tumour nor mucosal tissues a correlation with plasma vWF was detectable (tumour tissue p=0.790; mucosal tissue p=0.407).

Plasma levels of vWF in CRC patients vs. controls

Patients over 60 years with CRC presented with significantly higher vWF levels than patients below 60 years (BG 0: p=0.005; BG A/AB/B: p=0.035). The mean plasma level of patients with CRC and blood group 0 (142.5%) was significantly lower (p=0.003) than the plasma vWF level of CRC patients with BG A/AB/B (186.3%) (**Table 3**).

Controls for BG 0 and BG A/AB/B presented with nearly equal plasma vWF levels (BG 0: 133.1% vs. BG: A/AB/B: 139.9%; p=0.783). Within the same blood group CRC patients presented with elevated mean vWF levels compared to controls (BG 0: 142.5% vs. 133.1%, p=0.668; BG A/AB/B 186.3% vs. 139.9%, p=0.020). See **Table 3**.

VWF plasma levels separated for blood groups

Analysing separated blood groups significant differences in plasma vWF levels were detectable (BG 0 < A < B < AB) in CRC patients (n=79). VWF was significantly higher in BG AB (225.2% (SD: 53.5); p=0.010) and A (178.4% (SD: 63.3); p=0.017) compared to BG 0 (142.5% (SD: 51.5%)). Regarding all 4 blood groups differences were significant (p=0.012).

In controls (n=19) there was no significant differences between several blood groups detectable (BG 0: 133.1% (SD: 52.2); BG A: 132.0% (SD: 54.6); BG B: 139.0% (SD 0); BG AB 172.0% (SD 60.8); p=0.812). Boxplots of patients and controls are dis-

played in **Figure 3**.

Plasma levels of vWF and correlation with histopathological tumour features

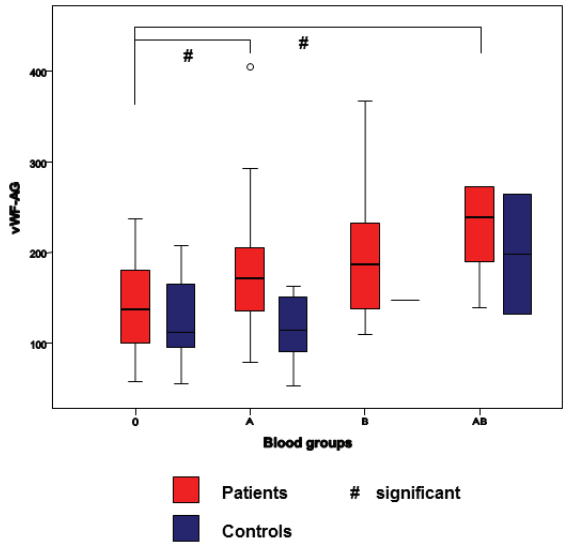

Figure 3. VWF plasma levels of CRC patients (red boxplots) displayed significant differences between several blood groups (BG 0 (n=23) vs. BG A (n=42) vs. BG B (n=8) vs. BG AB (n=6); p=0.012; #). In controls (blue boxplots) no significant differences between blood groups were detectable (BG 0 (n=8) vs. BG A (n=8) vs. BG B (n=1) vs. BG AB (n=2); p=0.812). Normal ranges for vWF of BG 0 are 40-145% and of BG A/AB/B 60-180%.

Separated for BG 0 and BG A/AB/B vWF plasma concentrations were compared with different histopathological tumour characteristics. There were no correlations with progressive tumour characteristics and elevated vWF plasma levels detectable. Mean levels and standard deviations are displayed in **Table 4**.

vWF plasma levels and UICC Stages

There was no correlation of UICC stages and plasma levels of vWF detectable (BG 0: 0.548; BG A/AB/B: 0.601). The mean plasma levels of vWF and corresponding UICC-stages are pictured in **Figure 4**.

vWF plasma levels and CEA

In 60 out of 79 patients preoperatively CEA was determined. The cut off of normal ranges is 5ng/ml. Patients with a CEA < 5ng/ml (n=39) showed lower mean levels of vWF (164.1%) than patients with a CEA ≥ 5ng/ml (n=21; 196.7%; p=0.080). The number of patients with blood group 0 was equal in both groups (p=0.620). See **Table 5**.

Discussion

The aim of this study was to evaluate the clinical relevance vWF in CRC. VWF is a multimeric glycoprotein synthesized in endothelial cells and megakaryocytes, stored within the Weibel-

Table 4. Different tumour characteristics and corresponding mean vWF plasma levels

	BG 0 (n=23)		BG A/AB/B (n=56)	
	Mean vWF in % (SD)	p-value	Mean vWF in % (SD)	p-value
T1/2	118.1 (33.6)	0.077	181.6 (62.5)	0.731
T3/4	153.2 (55.1)		188.2 (68.0)	
N0	145.8 (54.8)	0.739	186.7 (76.9)	0.953
N+	138.3 (49.4)		185.5 (38.1)	
M0	145.2 (49.4)	0.724	179.8 (66.7)	0.171
M+	135.0 (61.4)		207.7 (60.8)	
V0	140.9 (52.1)	0.494	187.5 (67.5)	0.630
V1	178.0 (0)		170.8 (43.8)	
L0	146.1 (52.0)	0.629	189.3 (69.4)	0.569
L1	134.4 (53.4)		177.9 (56.7)	
G1			139.0 (15.6)	
G2	147.6 (48.0)	0.589	182.0 (60.6)	0.335
G3	134.7 (58.6)		203.6 (80.2)	

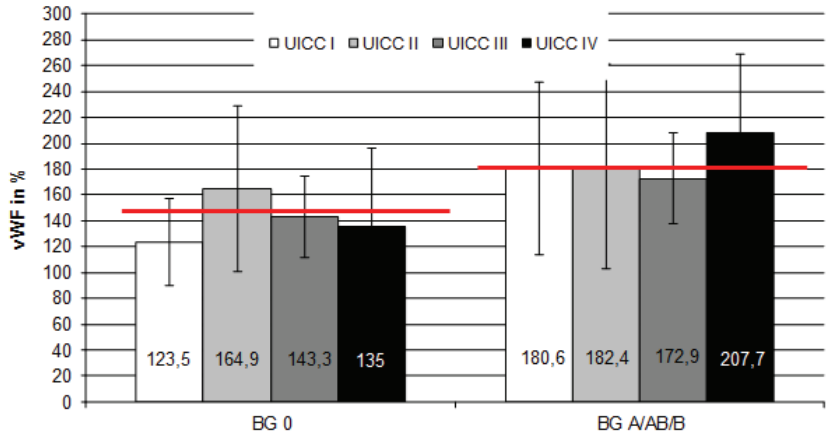

Figure 4. UICC stages and their mean plasma vWF levels separated for blood group 0 and A/AB/B. Upper normal ranges are marked by a red line (BG 0: 145%, BG A/AB/B: 180%).

Table 5. Patients with normal CEA values (<5ng/ml) showed lower levels of vWF than patients with elevated CEA-levels (≥ 5ng/ml) but significance was missing (0.080).

	CEA < 5	CEA ≥ 5	P-Wert
n total	39	21	
n BG 0	13	6	
Mean vWF in %	164.1	196.7	0.080
SA	64.3	68.6	
Median vWF in %	149.0	202.0	

Palade bodies of endothelial cells and released in case of endothelial damage or by the presence of inflammatory cytokines [27, 28]. ABO blood group is a major determinant of plasma vWF levels resulting in significantly lower plasma levels in persons with BG 0 compared to BG A/AB/B and therefore different normal ranges for BG 0 and BG A/AB/B exist [7, 8, 12]. Earlier studies described increased vWF plasma levels in patients with CRC compared to controls, whereat the highest levels were observed in CRC patients with metastases [18, 19]. The suggested explanation is a possible involvement of vWF in the building of tumour-cell embolies favoring the development of metastases [29-31]. In this study we performed a detailed analysis of vWF within tumour as well as corresponding healthy mucosa and plasma of CRC patients. Results were mainly correlated with histopathological characteristics of the patients for analyzing the usefulness of vWF as a clinical marker in CRC.

Analyzing the vWF positive stained cells within paraffin embedded tissue sections we could not detect significant differences between tumour and healthy mucosa. Furthermore vWF positive cells in tumour and corresponding healthy mucosa did not correlate. The vWF concentration within tumour tissues was independent of different histopathological tumour characteristics and did not correlate with UICC stages. Even in earlier studies a correlation of microvessel density (MVD; measured either by factor VIII or vWF staining) resulted in inconsistent findings. There are studies describing a positive, others a negative end even others no correlation between MVD and infiltration depth of CRC [32-34]. Also conflictive results have been described for MVD and infiltration of regional lymph nodes and distant metastases [32, 33, 35, 36]. Possibly these discrepant results are rooted in different counting and staining procedures or in selection of patients [37]. Because of these conflicting results the usefulness of evaluation of vWF in tumor tissues is in our opinion questionable.

In contrast we found concordant results in the analysis of plasma vWF. CRC patients presented with elevated vWF plasma levels compared with healthy controls. Such an association was also reported for CRC by others [18, 19] as well for other kinds of cancers [13-19]. Interestingly, statistical significance was reached in BG A/AB/B but not in BG 0, possibly caused by the small number of patients with BG 0 (n=23). In contrast to studies by Wang and Damin their previously described correlation of increasing plasma levels in a stage dependent manner was not reproducible in our patients [18, 19]. Furthermore a correlation with single histopathological tumour characteristics-like T, N, M, L, V categories- was not convincing and even some pa-

tients with disseminated tumour disease presented with mean plasma levels below the upper border of normal range. A correlation of vWF plasma levels with the standard tumor marker CEA resulted in elevated mean vWF levels in patients with elevated CEA, but this correlation was not significant. We could also identify age as a factor influencing vWF levels in CRC patients: patients over 60 years presented with significant higher vWF plasma levels compared with patients below 60 years. Interestingly, the previously in healthy persons described ranking of plasma vWF levels depending on the blood groups (0<A<B<AB) [8, 12, 38, 39] was highly significant reproducible in our CRC patients (p=0.012). This leads to the idea that vWF levels are less influenced by cancer than by determinant blood groups.

We could demonstrate that not only malignancy but mainly blood group and age influence vWF plasma levels in CRC patients. According to the literature even more clinical conditions – like myocardial infarction, diabetes mellitus, liver disease and acute infections- influence vWF plasma levels [9-11]. Because many factors – besides cancer- influence the vWF plasma levels, vWF is not a useful clinical marker in CRC.

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