Levels of von Willebrand factor antigen and von Willebrand factor cleaving protease (ADAMTS13) activity predict clinical events in chronic heart failure

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Summary

Decreased activity of ADAMTS13, the von Willebrand factor (VWF) cleaving protease, was recently reported in cardiovascular diseases and in hepatic failure. Chronic heart failure (CHF) is characterised by abnormalities of left ventricular function accompanied by the failure of the liver and dysregulation of endothelial activation. Therefore, the aim of our study was to measure ADAMTS13 activity in CHF, and determine the prognostic value of VWF and ADAMTS13 on major clinical events in CHF.

ADAMTS13 activity (measured by FRETS-VWF73 substrate) was decreased in CHF (n = 152, left ventricular ejection fraction <45%), and it correlated negatively with B-type natriuretic peptide (BNP) NYHA (New York Heart Association) classes, markers of synthetic capacity of the liver and endothelial dysfunction (all $p<0.005$). Both, high VWF:Ag levels (hazard ratio [HR] 1.52, 95% confidence interval [CI] 1.189–1.943), and low ADAMTS13/VWF:Ag ratios (HR 0.70, 95% CI 0.58–0.84) independently and significantly predicted short-term (1 year follow-up) clinical adverse events in heart failure (HF).

Decreased activity of ADAMTS13 with concomitant high VWF:Ag levels is a significant independent predictor of clinical events in CHF. The levels of the two molecules may integrate the impaired synthetic capacity of the liver and the disturbed endothelial regulation and can therefore be a useful tool to predict clinical events in CHF.

Keywords

Heart failure, ADAMTS13, von Willebrand factor, endothelial dysfunction, hepatic failure

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Introduction

The clinical syndrome of chronic heart failure (CHF) is characterised by abnormalities of left ventricular function and neurohormonal regulation, which are accompanied by effort intolerance, fluid retention, decreased longevity and severe failure of most of the organs. Endothelial injury is a well known complication of CHF (1). It is demonstrated by impaired endothelium-mediated vasodilation, but it also refers to a proinflammatory and prothrombotic state. The elevated level of von Willebrand factor (VWF) in CHF was described earlier (2, 3), and as expected, strong correlation was found between VWF and the markers of disease severity (4). Chong et al. (5) and Chin et al. (6) reported that high levels of VWF were associated with a worse outcome in CHF, although the size of the studies did not allow any detailed analysis.

The VWF is released from endothelial cells in an ultra large form (ULVWF), which distinguishes itself not only by molecular weight but also by the ability to aggregate platelets in conditions of high shear stress (7, 8). The plasma metalloprotease ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motif, 13) cleaves prothrombotic ULVWF into less active multimers. Significantly reduced levels of ADAMTS13 activity were found in physiological states, such as pregnancy

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and the neonatal period, as well as several pathological conditions, in spite of the absence of clinically overt thrombotic events (9). The measurement of ADAMTS13 activity has helped separate disease entities of thrombotic microangiopathies (TMA) on the base of molecular etiology (10).

The gene for ADAMTS13 has been reported to be actively expressed in the liver stellate cells (11, 12) suggesting that the liver may be a source of plasma ADAMTS13. ADAMTS13 activity was shown to be decreased in patients with severe hepatic disease (13–15). Furthermore, ADAMTS13 expression was recently shown by unstimulated human platelets, and the expression increases upon thrombin stimulation (16). In addition, human endothelial cells have also been shown to express ADAMTS13 mRNA and constitutively synthesise and release the protein (17), while the ADAMTS13 expression of human renal podocytes and endothelium were also detected (18).

Since CHF is a proper model disease to study disturbed endothelial-, renal- and hepatic function together with the constant inflammation we hypothesised that ADAMTS13 activity and VWF antigen levels are altered in CHF. Here in this study we report for the first time, that decreased ADAMTS13 activity is associated with increased severity of heart failure. Furthermore, the VWF level and the ratio of ADAMTS13/VWF:Ag has independent predictive power for severe clinical events in CHF. In addition, data from our well-characterised CHF cohort allowed us to conclude that low ADAMTS13 activity is linked to impaired hepatic synthetic capacity and endothelial dysfunction in CHF.

Methods

Study cohort

The study was carried out in accordance with the Helsinki Declaration at the IIIrd Department of Internal Medicine, Semmelweis University, based on a study protocol approved by the highest Ethical Committee of Hungary. Consecutive patients with clinical signs of CHF referred to trans-thoracic echocardiography were considered for inclusion. All patients with <45% left ventricular ejection fraction who provided written informed consent were included independently of the etiology of the disease from the out- or inpatient cardiology departments. Patients with co-existing malignant or acute infectious conditions were not included. A total of 152 patients (112 men, 40 women) were enrolled between February 2005, and July 2006. The full clinical record of the patients was registered at inclusion with the detailed physical status and routine laboratory tests. All patients were on optimised heart failure treatment, 58.6% took loop diuretics, 53.9% were on ACE-inhibitors and 52% took β-blockers. All patients were contacted after 1-year from study entrance. For patients alive one year after inclusion, all major clinical events (re-hospitalisation due to worsening of heart failure [HF], or lack of it) were registered. Information on mortality (with specific cause of death) was collected from hospital database, medical staff or family members.

Blood samples were taken after 6 hours of fasting between 8 and 10 am by antecubital veinpuncture into native, EDTA- and sodium citrate anticoagulated tubes. The samples were processed to obtain aliquots of serum and plasma and later stored at –70 °C until further analysis.

Determination of ADAMTS13 activity

The fluorigenic substrate, FRETS-VWF73, was purchased from Peptides International (Louisville, KY, USA) and applied for the determination of ADAMTS13 enzyme activity according to the protocol provided by the supplier with minor modifications. Briefly, citrated plasma was diluted 1:20 in assay buffer (5 mmol/l Bis-Tris, 25 mmol/CaCl2, 0.005% Tween 20, pH 6.0) and mixed with 5 μmol/l FRETS-VWF73 substrate solution (20 μl each, in white 384-well plates. Fluorescence was measured at 37°C every 2 minutes for 1 hour in Chameleon microplate reader (Hidex, Turku, Finland) equipped with a 340 nm excitation and a 460 nm emission filter. The reaction rate was calculated by linear regression analysis of fluorescence over time. A two-fold dilution series of normal human plasma (mixed from citrated plasma samples of 10 healthy blood donors) was applied as standard curve, 100% ADAMTS13 activity was set at the reaction rate observed in the 1:20 diluted sample. The intra-assay variation coefficient was <5%, the inter-assay coefficient of variation (CV) % was 6–9% (measured at 60 and 100% activity levels). In some plasma samples, we also measured ADAMTS13 enzyme activity with the collagen-binding assay, as described by Gerritsen et al. (19). The results of the FRETS-VWF73 assay showed good agreement with that of the collagen-binding method (20).

Determination of immunoreactive von Willebrand factor levels (VWF:Ag)

VWF:Ag was measured by ELISA according to Cejka (21), using primary and HRP (horseradish peroxidase) labelled secondary polyclonal rabbit anti-human VWF antibodies (DakoCytomation, Glostrup, Denmark) and TMB (tetramethylbenzidine) substrate. Three dilutions of the samples were applied into the ELISA wells. Washing buffer (0.01M PBS with 0.1% Tween 20, pH 7.4) was used for the dilution. Optical density reading was carried out using Infiniti 200M (Tecan Trading AG, Switzerland), and the Magellan software of the instrument was used for calculations, applying Four Parameter Marquardt curve fitting. WHO VWF:Ag Standard was used for calibration and the results of different plasma dilutions (after multiplying) were averaged only if the OD values were in the measurement range (typically between 0.064–1.673). Intra- and inter assay CVs were 7.1 % and 10.6% for Dade Behring normal control plasma (when the values of the two dilutions were evaluated individually).

Determination of other laboratory parameters

Levels of NT-proBNP (Biomedica ELISA kit (Cat No. BI-20852)), serum TNF-alpha (R&D System high sensitivity ELISA kit (Cat No. HSTA00C)), and serum IL-6 (R&D System high sensitivity ELISA kit (Cat No.HS600B)) were measured according to the manufacturer's instructions. Plasma CT-proET-1 (C-terminal-pro-endothelin-1) levels, referring to ET-1 were measured by a novel sandwich immunoluminometric assay using 2 polyclonal antibodies (BRAHMS AG, Hennigsdorf, Germany), as previously described (22). Standard laboratory parameters were measured by Roche Integra 800 (clinical chemistry, CRP), or by Cell-Dyn 3500 hematology analyser (complete blood count).

Table 1: Basic clinical characteristic of the study cohort.

(n)	All patients (152)	With event* (63)	Event-free (89)	p-value**
Male (%)	112 (73.7%)	44 (69.8)	68 (76.4)	0.365
Age (years)	67 (58.6–76.6)	68.8 (59–76)	69.17 (58.2–77.2)	0.978
NYHA (n, I/II/III/IV)	30/51/53/18	4/19/27/13	26/33/25/5	0.0002
NT-proBNP (pmol/ml)	0.86 (0.41–1.92)	1.2 (0.57–0.32)	0.63 (0.36–1.40)	0.001
CT-proET-1 (pmol/l)	106 (73–152)	143 (85.6–186)	92.6 (65.7–126)	<0.0001
LV-EF (%)	34 (27–40)	30 (23–38)	35 (29–41)	0.003
Peripheral oedema (%)	64 (42.1%)	34 (54.0)	30 (33.7)	0.013
Heart rate (1/min)	80 (70–94)	80 (70–96)	80 (65.5–92)	0.333
Diastolic BP (Hgmm)	80 (70–95)	75 (60–80)	80 (70–80)	0.099
BMI (kg/m2)	26.4 (24.4–30.9)	26.6 (24.0–32.0)	26.3 (24.4–30.6)	0.956
Se. sodium (mmol/l)	140 (138–143)	139 (135–142)	141 (139–143)	0.001
Se. creatinine (μmol/l)	97 (78–119)	108 (87–165)	91 (74–112)	0.001
Se. bilirubin (μmol/l)	13.4 (9.0–19.6)	14.6 (9.9–21)	12.12 (8.9–19.2)	0.202
AST (U/l)	23 (18–33)	23 (19–32)	24 (18–34)	0.768
ALT (U/l)	24 (18–36)	25.5 (17–38)	23 (18–36)	0.959
ALP (U/l)	80.5 (66–105.6)	88 (69–120)	78 (65–103)	0.156
Gamma-GT (U/l)	70.4 (34–126)	79 (37–143)	62 (28–102)	0.076
HGB (g/l)	141 (131–152)	138 (123–152)	143 (132–154)	0.128
PLT (10^9/l)	190 (161–231)	198.5 (168–233)	187 (159–216)	0.296
CRP (mg/l)	6.1 (2.8–15.3)	8.01 (2.9–20.8)	5.11 (2.67–12.15)	0.074
Albumin (g/l)	41 (38–44)	40 (38–44)	41 (38–44)	0.256
Total protein (g/l)	71.1 (7.7)	72 (67–77)	71.5 (66.5–76.5)	0.728
IL-6 (pg/ml)	9.9 (5.5–16.3)	11.4 (8.34–15.53)	8.16 (4.18–16.23)	0.008
TNF-alpha (pg/ml)	2.5 (1.6–4.1)	3.01 (1.96–5.18)	2.2 (1.41–3.62)	0.023
ADAMTS13 act. (%)	76.7 (27.7)	70.6 (48.4–94.9)	79.2 (61.4–95.2)	0.049
VWF:Ag(%)	182.0 (142.5–277.0)	215.0 (156.0–330.0)	165.0 (128.0–226.0)	0.002
ADAMTS13/VWF	0.38 (0.25–0.60)	0.29 (0.20–0.43)	0.45 (0.34–0.66)	<0.0001

Numbers in the table represent median (interquartile range) or numbers of patients (percentage). *Event represents all cause mortality or re-hospitalisation due to the progression of the HF, during the first year of follow up. **Mann-Whitney test or Perason Chi-square test.
NYHA – New York Heart Association, NT-proBNP – N-terminal pro-Brain-Natriuretic-Peptide, CT-proET-1 – C-Terminal pro-Endothelin-1, LV-EF – Left Ventricular Ejection Fraction, BP – Blood Pressure, BMI – Body Mass Index, AST – Aspartate Aminotransferase, ALT – Alanine Transaminase, ALP – Alkaline Phosphatase, Gamma-GT – Gamma Glutamyl Transpeptidase, HGB – Haemoglobin, PLT – Platelets, CRP – C-Reactive Protein, IL-6 – Interleukin-6, TNF – Tumor Necrosis Factor, ADAMTS13 – A Disintegrin And Metalloprotease with Thrombospondin type 1 motif 13, VWF – von Willebrand Factor.

Statistical analysis, power calculations

For descriptive purposes the values of each measurement are given as median and 25th-75th percentile, or as numbers (percent), since most of the variables were not normally distributed, except ADAMTS13 activity. Non-parametric tests were used for group comparisons; continuous variables between two groups were compared with Mann-Whitney U test, whereas categorical variables were compared with Pearson's χ^2 test. Spearman rank order correlation coefficients were calculated for estimation of interrelations between ADAMTS13 activity, VWF:Ag and other variables. The association between NYHA classes and plasma activity of ADAMTS13, and VWF:Ag level were calculated using Kruskal-Wallis ANOVA by ranks test.

Multivariate Cox proportional hazard models were fitted to assess the effect of ADAMTS13 activity/ VWF:Ag, and VWF:Ag on CHF event free survival. Survival times were measured from inclusion in the study until both of the endpoints studied, all-cause mortality or rehospitalisation due to CHF. The studied patient characteristics and laboratory markers together with the logarithm transformed variants of the continuous variables were pre-evaluated using a multitude of univariate Cox regressions. The best predictors by their chi-square values of likelihood ratio tests were noted and the plain or logarithmic variants of the predictors were selected for later inclusion in the multivariate models. One predictor was included in the multivariate models from each group of variables of clinically justified pathological pathways to adjust for the known effects and the studied variables. The results of the Cox regression models are presented as hazard ratios standardised on 1 SD (standard deviation) range of the predictors (if not otherwise stated), the corresponding

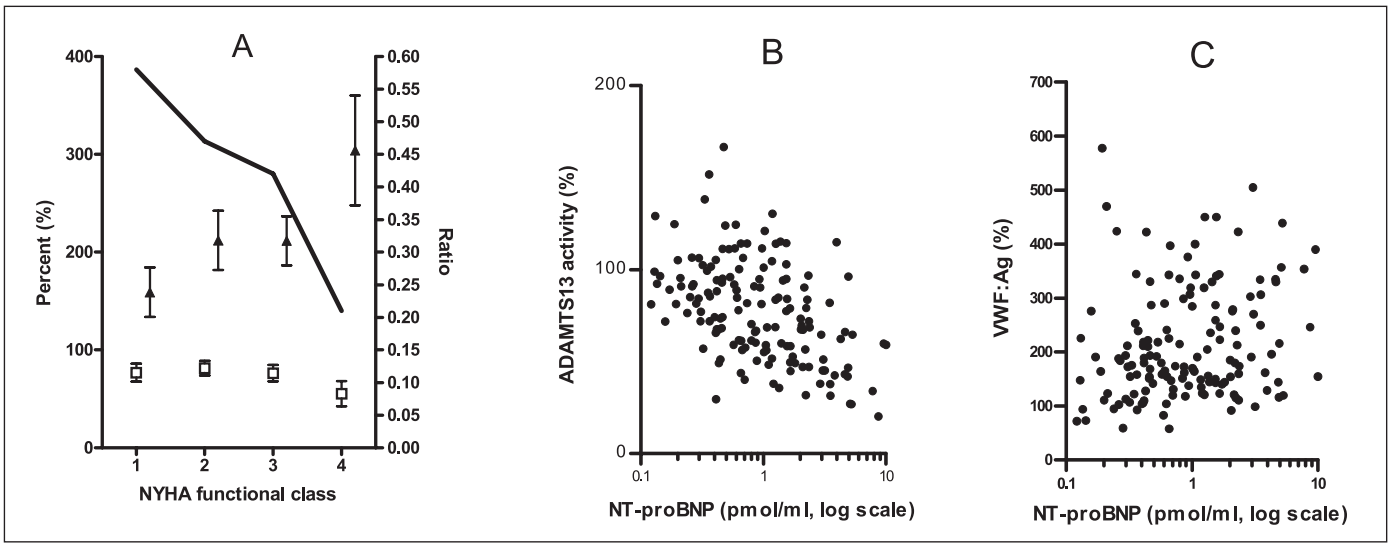

Figure 1: Panel A: Association of ADAMTS13 activity (□) and von-Willebrand factor antigen levels (▲) with severity of chronic heart failure (mean, 95% confidence interval, both on left Y-axis, n = 152). Results of Kruskal-Wallis ANOVA: ADAMTS13 F = 3.98, p = 0.009; VWF:Ag F = 6.89, p = 0.0001. The ADAMTS13 activity/VWF:Ag ratio is indicated by solid line (right Y-axis). Panel B and C: Correlation between NT-proBNP levels and ADAMTS13 activity (r = -0.41, p<0.0001) or von Willebrand factor antigen concentrations (r = 0.21, p = 0.010).

95% confidence intervals (CI) and the chi-square and p-values of likelihood ratio tests. Age was considered as a time-dependent covariate.

The power of our study to detect the observed differences in ADAMTS13/VWF ratio between the 'event-free' and the 'with event' patient group, at a Type I Error rate of 0.05, was 95.5%.

Statistical analyses were carried out using the software STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA), GraphPad Prism 4.03 (GraphPad Software, San Diego, CA, USA) and SPSS 13.01 (Apache Software Foundation, USA). Two tailed p-values were calculated and the significance level was put at a value of p<0.05.

Results

Characteristics of the patient cohort

The basic clinical characteristics of the patient cohort are given in Table 1. The aetiology of heart failure was ischaemic/non-ischaemic in 60/40% of the patients. All of the patients had manifest left ventricular systolic dysfunction (LVEF <45%), however, disease severity spanned from NYHA class I to IV, and NT-proBNP levels from 0.129 to 11.298 pmol/ml. There were 47 outpatients (31%) and 105 (69%) in-hospital patients. Thus, our cohort included patients with mild, moderate and severe HF. The cohort was extensively characterised in order to get detailed information for heart function, salt- and water-homeostasis, hepatic-, renal-, haemopoetic- and endothelial dysfunction, inflammation (Table 1). The mean ADAMTS13 activity was 76.7% (SD 27.7) in the CHF cohort showing normal distribution (Shapiro-Wilks test for normality p=0.72). One patient, who had large anterior acute myocardial infarction 5 years before enrolment into the study, had 1% ADAMTS13 activity with blocking anti-ADAMTS13 antibodies (measured repeatedly in consecutive samples taken >3 years apart) without any history of TTP (thrombotic thrombocytopenic purpura) or low platelet counts. Severely decreased (<10%) ADAMTS13 activity levels were not observed in other cases. Low ADAMTS13 activity levels were not associated with decreased platelet counts in the CHF cohort.

The clinical and laboratory parameters of patients with or without clinical events (all-cause mortality or re-hospitalisation) during the first 365 days of follow up are given in Table 1. Event free survival, among other variables, was associated with moderate disease and lower NT-proBNP levels, significantly higher ADAMTS13 activity, decreased VWF:Ag level and higher ADAMTS13/VWF ratio.

Association of decreased ADAMTS13 activity and increased VWF:Ag levels with disease severity in heart failure

As presented in Figure 1 low ADAMTS13 activity (p = 0.009) and high VWF:Ag levels (p = 0.0001) were associated with increasing NYHA functional class with the lowest ADAMTS13 activity levels in NYHA class IV. Accordingly, the ratio of ADAMTS13/VWF:Ag decreased across NYHA classes reaching a median as low as 0.21 in NYHA IV in contrast to 0.58 in NYHA I. Confirming the association of ADAMTS13 activity and VWF:Ag concentrations with severity of heart failure is the observation presented in panels B and C in Figure 1., showing the highly significant correlations of ADAMTS13 activity and VWF:Ag with NT-proBNP levels.

Prediction of clinical events by high VWF:Ag levels and low ADAMTS13/VWF ratio

In univariate models low ADAMTS13 activity, high VWF:Ag levels and their ratio were all significant predictors of clinical events (data not shown). In the multivariable Cox models after adjustments for key clinical covariates (age, BMI, serum sodium levels, diastolic blood pressure, heart rate, haemoglobin and cre-

atinine level), the standardised hazard ratio for VWF:Ag was 1.52 (CI: 1.189–1.943), and for ADAMTS13/VWF:Ag 0.70 (CI: 0.579–0.845) (Table 2). The chi-square value of ADAMTS13/VWF:Ag (15.742) is twice, and thus markedly higher than that of VWF:Ag (10.194), indicating that the ratio has substantially higher predictive power as compared to the VWF antigen levels. ADAMTS13 activity levels were not significant predictors of clinical events in the adjusted models, indicating that its primary association with disease severity is dependent on important clinical variables and may be a result of covariance. Therefore, utilising our well-characterised CHF cohort we searched for laboratory and clinical correlates of ADAMTS13 activity levels.

Correlations of ADAMTS13 activity, VWF:Ag levels and their ratio with the laboratory and clinical parameters

Table 3 shows the Spearman rank correlation coefficients of ADAMTS13 activity, VWF:Ag levels and their ratio with the clinical and laboratory parameters. Both studied factors correlated significantly with the parameters of disease severity, like NYHA classes, NT-proBNP, and the presence of peripheral oedema. Significant (even after multiplying the p-values according to Bonferroni's rule) correlation was found between ADAMTS13 and the synthetic function of the liver (albumin, total protein) and a marker of endothelial dysfunction (CT-proET-1), but ADAMTS13 activity did not show significant correlation with inflammatory markers and liver enzymes. VWF:Ag correlated strongly with the signs of renal function and inflammation (acute phase reaction and IL-6). The ratio of ADAMTS13/VWF:Ag showed highly significant correlations with the same biomarkers of the above mentioned pathways as ADAMTS13 activity.

Table 2: Final multivariable models of high VWF:Ag levels (A) and low ADAMTS13/VWF:Ag ratios (B) for prediction of clinical events (all-cause mortality or re-hospitalisation due to worsening of HF) in patients with chronic heart failure.

A

	HR	95% CI	Chi-square	p-value
VWF	1.520	1.189–1.943	10.194	0.001
Creatinine	1.177	0.9171.511	1.582	0.208
Heart rate	1.008	0.995–1.021	1.377	0.241
BMI	1.044	0.830–1.314	0.134	0.714
Diastolic BP	0.798	0.621–1.026	3.167	0.075
Sodium	0.867	0.639–1.169	0.876	0.349
Haemoglobin	0.761	0.606–0.956	5.185	0.023
Age (year)	1.002	0.999–1.005	1.642	0.440

B

	HR	95% CI	Chi-square	p-value
ADAMTS13/VWF	0.700	0.579–0.845	15.742	<0.0001
Creatinine	1.219	0.946–1.571	2.229	0.135
Heart rate	1.010	0.997–1.024	2.118	0.146
BMI	1.108	0.883–1.390	0.764	0.382
Diastolic BP	0.847	0.657–1.093	1.654	0.198
Sodium	0.870	0.650–1.163	0.865	0.352
Haemoglobin	0.786	0.630–0.981	4.275	0.039
Age (year)	1.002	0.999–1.005	2.025	0.363

Hazard ratios /1SD increase or decrease (if not otherwise stated) with their 95% CI, chi-squares and p-values of likelihood tests are presented.
VWF – von Willebrand Factor, BMI – Body Mass Index, BP – Blood Pressure, ADAMTS13 – A Disintegrin And Metalloprotease with Thrombospondin type 1 motif 13.

Table 3: Correlation between ADAMTS13 activity, von Willebrand antigen levels, and their ratio and clinical variables in patients with chronic heart failure (n=152).

	ADAMTS13		VWF:Ag		ADAMTS13/VWF	
	r	p-value	r	p-value	r	p-value
Age	–0.27	0.0009	0.16	0.043	–0.28	0.0003
NYHA	–0.23	0.005	0.33	<0.0001	–0.34	<0.0001
NT-proBNP	–0.44	<0.0001	0.21	0.010	–0.41	<0.0001
CT-proET-1	–0.29	0.0003	0.31	<0.0001	–0.44	<0.0001
BMI	0.25	0.002	0.08	0.29	0.04	0.649
Se. sodium	0.003	0.97	–0.30	0.0002	0.22	0.006
Se. creatinine	–0.18	0.023	0.26	0.001	–0.28	0.0003
Se. bilirubin	–0.20	0.013	0.03	0.71	–0.15	0.074
Albumin	0.25	0.003	–0.34	<0.0001	0.36	<0.0001
HGB	0.14	0.09	–0.02	0.79	0.07	0.39
PLT	0.06	0.30	–0.03	0.72	0.06	0.46
CRP	–0.14	0.09	0.37	<0.0001	–0.31	<0.0001
Total protein	0.28	<0.001	–0.07	0.384	0.19	0.018
ADAMTS13			–0.17	0.042	-	-

Spearman rank correlation coefficients with p-values are presented.

Discussion

The novel observation reported in this study is, that ADAMTS13 activity is decreased in chronic heart failure. Low ADAMTS13 activities are accompanied with high VWF:Ag levels and their ratio gradually decrease with worsening HF. Furthermore, both, high VWF:Ag levels and low ADAMTS13/VWF:Ag ratios independently and highly significantly predict short-term clinical adverse events in HF. ADAMTS13 activity alone is non-significant predictor of clinical events in the adjusted models (data not shown). Levels of ADAMTS13 activity correlates with important clinical variables including markers of synthetic capacity of the liver and endothelial dysfunction.

Multiple mechanisms accounting for the moderately decreased ADAMTS13 activities may dominate in CHF. First, the impaired hepatic synthetic capacity may be linked to decreased production of the protease, since the liver is one of the sources of ADAMTS13 (11, 12). Undernutrition is one of the hallmark characteristics accompanying the progression of heart failure (23), which can lead to hypoproteinamia as well. Second, since endothelial cells are also major sources of ADAMTS13 (17), the chronic endothelial activation may also be one reason for decreased activity. Supporting this assumption is our observation on the strong inverse correlation between endothelin-1 and ADAMTS13 levels. Third, increased consumption may also lead to decreased activity of ADAMTS13, since the endothelial dysfunction in CHF results in elevated secretion of immature VWF:Ag (2, 3), and it was reported that secondary consumption of ADAMTS13 may occur (24).

Several potential mechanisms, suggested by literature data (25–28), were also considered as potential co-variates of ADAMTS13 activities in our study. Inhibition of ADAMTS13 by the inflammatory cytokine IL-6 was shown in *in vitro* studies (25). Stimulated by these findings we investigated whether IL-6 and TNF-alpha plasma levels are related to ADAMTS13, but no significant correlations were observed. The role of inflammation in the regulation of ADAMTS13 activity was further investigated by its relation to acute phase reactants (26). The same was the case when analysing the relationship with serum creatinine levels and age: neither showed independent association with ADAMTS13 activity in multiple regression models (data not shown). Furthermore, none of our patients suffered from disseminated intravascular coagulation (DIC) as evidence by the lack of decreased fibrinogen levels (data not shown). Therefore, the inactivation of ADAMTS13 by thrombin or plasmin (as presented in patients with sepsis induced DIC [28]) in CHF is most probably not present.

In complete agreement with Chin et al. (6) we report here a significant prediction of clinical events in CHF by increased VWF:Ag levels. Increased VWF antigen levels are related to endothelial damage, an important contributing factor to progression of HF. However, the ratio of ADAMTS13/VWF:Ag seems to have much higher predictive power as compared to that of VWF antigen levels alone in the standardised hazard models (Table 2). Since ADAMTS13 activity is strongly related to hypoproteinemia and endothelial damage, it is tempting to speculate that the ratio represents multiple pathophysiological pathways in CHF in an integrated manner.

A large body of evidence supports an important prognostic value of decreased ADAMTS13 activity in cardiovascular diseases. Bongers et al. (29) reported increased risk of ischaemic stroke in patients with low ADAMTS13 levels. In patients after myocardial infarction, Cox hazard analysis revealed that the early increase of VWF levels and VWF/ADAMTS13 ratio, and the early decrease of ADAMTS13 levels were significant predictors of future thrombotic events during the 1-year follow up period (30). The same group also reported significantly lower ADAMTS13 antigen levels in patients with unstable angina compared to stable angina and chest pain syndrome (30). Crawley et al. (31) and Chion at al. (32) also reported an association of ADAMTS13 with the risk of myocardial infarction. In line with these observations are our results presented in this study on the significant prediction of clinical events by ADAMTS13/VWF:Ag ratio. Since this ratio correlated with multiple important laboratory and clinical variables measured and registered in our study, it is tempting to speculate that it may represent increased risk in an integrated manner. Supporting this hypothesis is that according to the results of the adjusted Cox models the ADAMTS13/VWF:Ag ratio is independent predictor of clinical events.

Our results on the increased levels of VWF:Ag together with decreased ADAMTS13 due to the high thrombotic risk may explain that patients with CHF have an increased rate of venous thromboembolism and stroke, as well as recurrent ischaemia and infarction (33). The causes of death in CHF are largely cardiac in origin, and usually result from lethal arrhythmias, but both epidemiological and autopsy results suggest that acute intra-coronary thrombotic occlusion may be the probable triggering event (34, 35). CHF patients can therefore be regarded to be at high risk of thrombosis-related complications. Indeed, the components of Virchow's triad are fulfilled in CHF, with abnormalities of flow (poor cardiac function), vessel wall (endothelial dysfunction/damage) and blood constituents, with significant abnormalities of haemostatic factors and platelet function (36, 37).

It should be noted that the level of decrease in ADAMTS13 activity did not reach those regarded as severely decreased activity (<10%), and indeed, it was not related with decreased platelet numbers (Table 3). Rather, the decrease of ADAMTS13 activity was mild, and seemingly regulated by and connected to mechanisms related to disease severity, hepatic function and inflammation.

What is known about this topic?

– Chronic heart failure is characterised by constant endothelial dysregulation.
– vWF:Ag levels are elevated in CHF and predict clinical events.

What does this paper add?

– Decreased activity of ADAMTS13 with concomitant high vWF:Ag levels is a significant independent predictor of clinical events in CHF.
– Low ADAMTS13 activity is related to decreased hepatic synthetic capacity and endothelial dysfunction.

A potential limitation of our study is the application of the FRETS-VWF73 rapid fluorigenic assay for the measurement of ADAMTS13 activity. This assay is not the most widely used assay in studies on ADAMTS13 activity. However, according to literature data (38) and our observations (39), both, the widely used assays by detecting VWF multimeric structure by its ligand-binding activities and the FRETS-VWF73 assay exhibit reliable results to detect low, moderately reduced as well as normal level of ADAMTS13 activity.

To obtain more information on the role of ADAMTS13 in cardiovascular diseases, and particularly on increased thrombotic risk, is not only a mechanistic interest, because preparations of recombinant ADAMTS13 might have a potential application in the control of this risk (24, 40). Further prospective studies are needed to provide more causative results on the prognostic role of the VWF-ADAMTS13 pathway in these diseases before such supplementary therapies may be indicated.

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Conflict of interest

Dr Kunde and Dr Papassotiriou are employees of BRAHMS AG, Hennigsdorf, Germany that commercialises immunoassays and has developed the CT-proET-1 assay, for which it owns patent rights. The present study was not financed by BRAHMS AG. The remaining authors report no conflicts.

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