Inflammation-associated ADAMTS13 deficiency promotes formation of ultra-large von Willebrand factor

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ABSTRACT

In a prospective, longitudinal study, we investigated the association between decreased ADAMTS13 activity and impaired hemostasis, as well as organ dysfunctions in patients with systemic inflammation due to extracorporeal cardiopulmonary circuit or with severe sepsis. Similar to negative acute phase proteins, ADAMTS13 activity declined stepwise according to the extent of inflammatory responses. A marked imbalance between ADAMTS13 activity and VWF antigen level was associated with the appearance of ultra-large VWF multimers in plasma, with organ dysfunction and lethality. Our data support the view that systemic inflammation results in an ADAMTS13 deficiency which activates hemostasis.

Key words: ADAMTS13, extracorporeal circulation, sepsis, systemic inflammation, organ dysfunction, platelets, thrombotic microangiopathy.

Citation: Bockmeyer CL, Claus RA, Budde U, Kentouche K, Schneppenheim R, Lösche W, Reinhart K, Brunkhorst FM. Inflammation-associated ADAMTS13 deficiency promotes formation of ultra-large von Willebrand factor. Haematologica. 2008 Jan; 93:(1)137-140. DOI: 10.3324/haematol.11677

Introduction

Systemic inflammatory response syndrome (SIRS) denotes the complex findings in patients with systemic activation of the innate immune response by infectious or non-infectious insults. The pathophysiology of SIRS involves release of cytokines, activation of endothelial cells and neutrophils. The concomitant activation of coagulation factors and blood platelets may result in disseminated intravascular coagulation (DIC) and may also contribute to organ dysfunction.[1] ADAMTS13 is the principal physiological modulator of the size and function of von Willebrand factor (VWF) in plasma.[2] In cases of severe ADAMTS13 deficiency such as thrombotic thrombocytopenic purpura (TTP), ultra-large VWF multimers (ULVWF) appear in plasma causing platelet activation and subsequent thrombotic microangiopathy. This may be associated with severe organ failure.[3] Decreased ADAMTS13 activity is described in sepsis[4] and mild or severe SIRS, such as strenuous exercise or cardiac surgery.[5, 6] An acquired deficiency of the plasma protease ADAMTS13 is one of the mechanisms that may contribute to platelet activation in SIRS and sepsis. The aim of our study was to contribute further evidence of a possible role for an imbalance between ADAMTS13 and VWF in SIRS and sepsis-associated organ failure. Therefore, we measured ADAMTS13/VWF balance in groups of patients with SIRS and sepsis of various causes, stages of disease severity and organ failure.[7,8]

Design and Methods

After obtaining institutional ethical approval and written informed consent, three groups of Intensive Cure Unit (ICU)-patients were studied: (i) twenty four patients who underwent *elective* cardiac surgery with a low risk of developing post-operative organ dysfunction. These data were used as ICU controls; (ii) Twenty two patients after *non-elective* on-pump cardiac surgery with a high risk of developing organ dysfunction;[9] plasma samples were taken on five consecutive post-operative days; and (iii) eleven patients with severe sepsis or septic shock.[10] Plasma samples were obtained on a daily basis until discharge or death. A total number of 133 patient days were evaluated. Demographic and clinical data obtained on the first day on the ICU and bio-

Funding: the study was supported in part by a grant from the Thuringian Ministry of Science and Arts (TMWFK, project B-309-00014) and by the Centre for Clinical Research, Jena (IZKF), subproject 4.8. CLB has received financial support from the 'Förderverein' Friedrich-Schiller University, Jena (Loder-Grant for young investigators). Neither the sponsors nor any pharmaceutical company were involved in study design, data collection, data analysis and interpretation, in the preparation of the manuscript or the decision for submission.
Manuscript received May 3, 2007. Manuscript accepted August 9, 2007.
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The online version of this article contains a supplemental appendix.

Table 1. Characteristics of patients with SIRS and sepsis.

Patient group	*ICU-controls*	*Non-elective on-pump cardiac surgery*	*Sepsis: Survivors*	*Sepsis: Non-Survivors*
N (female)	24 (4)	22 (6)	5 (3)	6 (2)
Age (*yrs.*)	65 (58/69)	70 (60/75)	47.0 (47/59)	66.5 (51/72)
EURO-Score	3 (2/4)	9 (6/11)‡‡‡	-	-
APACHE II-Score	9.5 (8/12)	24 (20/29)‡‡	22.0 (15/22)‡‡	25.0 (21/29)‡‡
SAPS II-Score	21 (19/27)	32 (26/49)‡‡	39.0 (36/48)‡‡	47.0 (38/67)‡‡
ISTH-Score (mod.)	0.5 (0/1)	2 (2/2)‡	4 (4/5)‡‡	4 (4/5)‡‡
SOFA-Score	5 (4/6)	10 (8/11)‡‡	13 (11/14) ‡‡	12 (10/15)‡‡
ICU-LOS (*days*)	1 (1/2)	9 (6/12)‡‡‡	19.0 (12/27)‡‡‡	14.5 (11/28)‡‡‡
Hospital LOS (*days*)	13.5 (12/16)	18 (12/23)	34.0 (15/44)‡‡ §	-
Laboratory data within 24h after admission				
ADAMTS13 (%)	41 (29/48)	32 (28/36)	20 (11/21)‡‡	20 (12/28)‡‡
VWF:Ag (%)	175 (129/193)	282 (243/375)‡‡‡	344 (234/350)*, ‡‡‡	566 (396/800)‡‡‡
Platelets (Gpt/L)	166 (128/192)	161 (130/189)	57 (43/66)‡‡‡	99 (91/123)‡‡
Interleukin-6 (pg/mL)	293 (202/652)	314 (235/362)‡	278 (193/4318)‡	475 (319/698)‡
Procalcitonin (ng/mL)	0.56 (0.47/2.67)	10.6 (3.9/17.9)‡	10.0 (8.3/27.1)*, ‡	3.9 (0.5/6.4)‡
Laboratory data at day of discharge or death				
ADAMTS13 (%)	n.d.	40 (30/51)*	37 (33/43)*	20 (8/28)
VWF:Ag (%)	n.d.	486 (386/598)	353 (353/356)	581 (525/795)
Platelets (Gpt/L)	n.d.	106 (83/148)	134 (106/589)*	99 (35/147)
Interleukin-6 (pg/mL)	n.d.	280 (135/433)	159 (97/230)**	5012 (412/12389)§
Procalcitonin (ng/mL)	n.d.	4.6 (2.7/10.0)	0.5 (0.2/0.6)**, §	3.0 (2.2/5.3)

*EURO: European System for Cardiac Operative Risk Evaluation; APACHE: acute physiology and chronic health evaluation; SAPS: simplified acute physiology score; ISTH: International Society of Thrombosis and Haemostasis; ICU: Intensive care unit; LOS: length of stay. ‡p<0.05 vs. ICU-controls. ‡‡p<0.01 vs. ICU-controls. ‡‡‡p<0.001 vs. ICU-controls. * p<0.05 vs. non-survivor. ** p<0.01 vs. non-survivor. §p<0.05 vs. non-elective on-pump cardiac surgery. Data are given as median (1./3. Quartil) or as absolute values.*

chemical variables determined on the first and last day (discharge or death) on ICU are presented in Table 1. For additional information analyzing ADAMTS13 activity, VWF antigen (VWF:Ag), VWF multimer analysis and calculation of ISTH-score for overt DIC see online supplementary information.

Statistical analysis

The non-parametric Mann-Whitney-U test and ANOVA were performed to compare patient groups as well as between survivors and non-survivors. p values <0.05 were considered significant. The results are presented as medians and 1st/3rd quartile.

Results and Discussion

As shown in Table 1, disease severity (APACHEII and SAPSII-score) and severity of organ dysfunction (SOFA-score) as well as the modified ISTH-score gradually increased on ICU day 1 when comparing ICU controls, non-elective cardiac surgery patients, and patients with severe sepsis. There were no differences between survivors and non-survivors. A similar trend was found for VWF:Ag, IL-6 and procalcitonin, while ADAMTS13 was lowest in sepsis and highest in ICU-controls on day 1. In all septic patients and in all patients with non-elective cardiac surgery ADAMTS13 was below the lower limit of normal (LLN, 40%).[9] This was found in 33% of the ICU-controls. There were significant differences in platelet counts among the three patient groups on ICU day 1. A comparison of data obtained on the last day of ICU showed that non-surviving septic patients had significantly lower ADAMTS13 activity and higher IL-6 levels (approximately 15-30-fold) compared to survivors of sepsis or patients with non-elective cardiac surgery. No differences were found in VWF:Ag level and platelet count. There were significant time-dependent changes between the first and the last ICU day in patients with non-elective cardiac surgery in terms of VWF:Ag (1.7-fold increase) and platelet count (MWU, p<0.01) while the other variables remained relatively unchanged. However, we observed a significant increase in ADAMTS13 activity and platelet count in septic patients and a decrease in IL-6 and procalcitonin levels in survivors, but not in non-survivors (ANOVA p<0.01, Table 1). In addition, in non-survivors, we found an inverse course of IL-6 and VWF:Ag (increase) with ADAMTS13 activity (decrease). When all patients and ICU controls were considered, there was a decrease of ADAMTS13 levels to values <30% on 143 and values <10% on 19 out of 267 patient days. Using pooled patient data within 24 hours of ICU admission, further associations between ADAMTS13 activity, disease severity and organ dysfunction could be determined. ADAMTS13 gradually decreased as SOFA-score increased (Figure 1). At moderate organ dysfunction (SOFA-score <7) the median activity was above the *Lower Limit of Normal* of 40%, and was as low as 20% when SOFA-score was >13. A similar association was found among ADAMTS13, IL-6, or procalcitonin. Patients with IL-6 or procalcitonin levels within the third tertiles had significantly lower ADAMTS13 activities compared with patients in the first tertiles (Figure 1). The presence of ULVWF is a condition for severe organ dysfunction in TTP (see Figure 2).[2] Presence of ULVWF was found in septic and

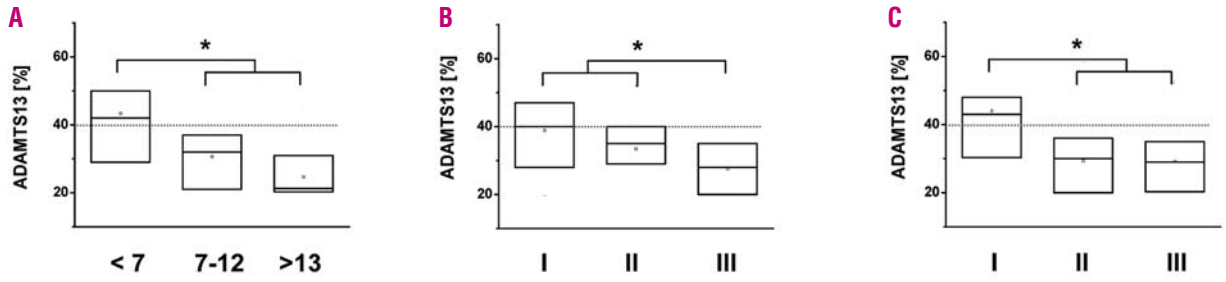

Figure 1 (right). ADAMTS13 activity, inflammation and organ dysfunction in ICU-patients. Association of ADAMTS13 with SOFA-Score (A), interleukin-6 (B) and procalcitonin (C) in ICU-controls, patients after non-elective cardiac surgery and patients with sepsis within 24 hrs of admission to ICU according to SOFA-score: <7 (n=20), 7-12 (n=26), >13 (n=11). The study population was divided into tertiles of plasma levels of IL-6 (I: 94.9 [31.0/136.9], II: 298.0 [203.5/316.0], III: 691.0 [492.2/921.7] pg/mL) and of procalcitonin (I: 0.43 [0.30/0.50], II: 5.02 [2.37/6.79], III: 19.1 [11.3/27.1] ng/mL; values below the detection level of the assay were rated with 0.3 ng/mL). ANOVA analysis was performed to test whether increasing SOFA-Score and increasing concentrations of pro-inflammatory proteins are associated with decreased ADAMTS13 activity (ANOVA testing, * $p<0.05$).

non-elective cardiac surgery patients. ULVWF was found in 75% of analyzed patients with SIRS-associated organ dysfunction and in all septic patients (Figures 2A, C and E). ULVWF disappeared in a surviving septic patient, but persisted in a non-survivor (Figure 2). The disappearance of ULVWF was accompanied by a substantial increase of ADAMTS13 activity over time in the surviving patient to *Lower Limit of Normal* while it successively decreased in the non-surviving patient with a final drop to non-detectable levels (<5%) two days prior to death (Figures 2A-D). Infusion of fresh frozen plasma (FFP) resulted in a transient increase of ADAMTS13 activity (Figures 2A and C). As proof of principle we analyzed pooled patient data for an inverse relation between ADAMTS13 activity and presence of ULVWF. ADAMTS13 activity was significantly lower at days with ULVWF compared with days without ULVWF (Figure 3A). To prove an association between ADAMTS13 activity and activation of coagulation we divided pooled patient data into those with ADAMTS13 activity above and below 30%. Figure 3B demonstrates that low activity was associated with a significantly higher DIC-score. The relation between low ADAMTS13 activity

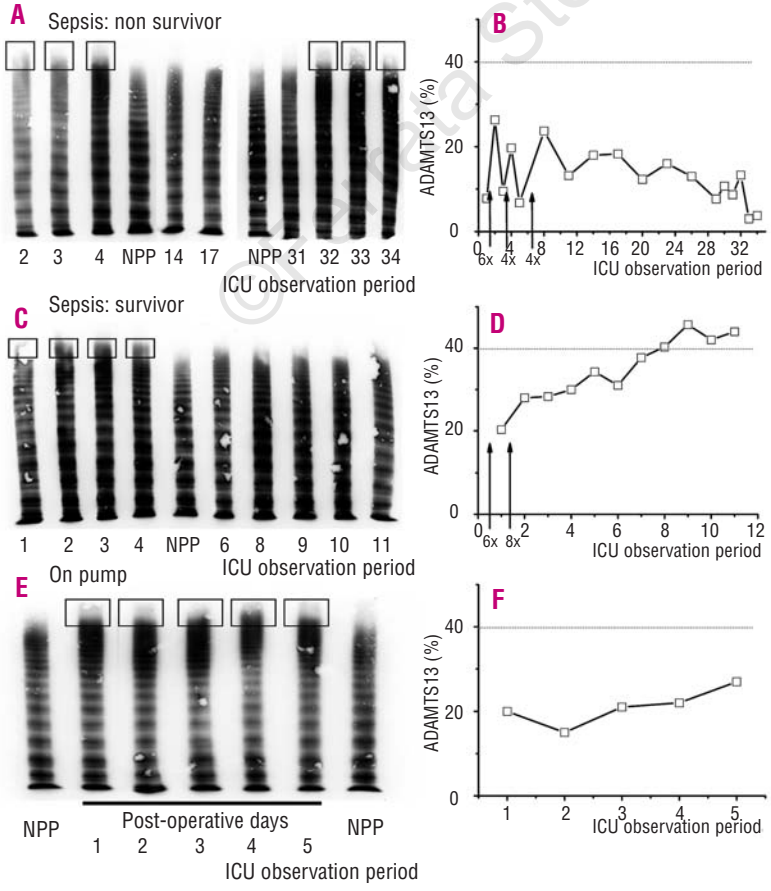

Figure 2. ADAMTS13 activity, VWF:Ag levels and appearance of ULVWF in ICU-patients.VWF multimer patterns and ADAMTS13 activity in a non-surviving (A,B), a surviving patient (C,D), both with severe sepsis and in a patient after non-elective on-pump surgery as a model for non-infectious SIRS (E,F).[14] Numbers of fresh frozen plasma (FFP) applications are indicated by arrows. ULVWF multimers were detected in sepsis and in patients after non-elective cardiac surgery (=non infectious SIRS) as indicated by boxes.

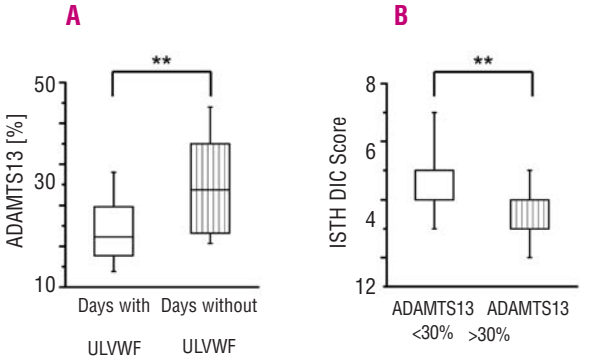

Figure 3. ADAMTS13 and hypercoagulopathy in patients with severe sepsis. In patients with severe sepsis, detection of ULVWF was associated with a diminished ADAMTS13 activity (A), while an ADAMTS13 activity < 30% was associated with an elevated ISTH-DIC-score (B).

and disturbed hemostasis was also clearly seen by an evaluation of changes in platelet counts. In all patients and ICU-controls, ADAMTS13 activity was significantly lower on days with a decrease in platelet count >30% compared with days with minor changes or with an increase in platelet count (27 [21/29] vs. 37 [33/40]%, $p<0.01$). Therefore, low ADAMTS13 activity and presence of ULVWF contribute to both activation of coagulation and platelets resulting in thrombocytopenia. In conclusion, we demonstrated that an acquired, diminished ADAMTS13 activity is not only restricted to pediatric patients with sepsis and adults with sepsis-associated DIC. Recently published data documented a higher incidence of acute renal dysfunction accompanied by highly active VWF in patients with residual ADAMTS13 activity <20%.[4,10-12] Given this, we can conclude: (i) severe deficiency of ADAMTS13 results in consumption of platelets and is not restricted to genetic mutations or auto immune diseases; (ii) ADAMTS13 and VWF interact to form a fine-tuned ADAMTS13/VWF system; and (iii) there is association of an acquired ADAMTS13 deficiency with the severity of inflammatory host response that is independent of its origin. Given the continuing high mortality in patients with SIRS and sepsis, assessment of ADAMTS13 activity and detection of ULVWF may be of major clinical relevance. This may lead to changes in patient management since plasma exchange using enzyme-containing plasma preparations such as FFP may restore the capacity to cleave ULVWF in the circulation, as shown in patients with high risk developing veno-occlusive disease.[13] This therapeutic approach also needs to be tested for sepsis in controlled prospective studies.

Authors' Contributions

CLB and RAC performed biochemical experiments and were responsible for experimental analysis and data interpretation. UB provided experimental assistance for the presentation of ULVWF and participated in analysis of ADAMTS-13 activity. KK provided plasma samples of TTP patients and RS recombinant VWF. WL and KR provided substantial and helpful comments throughout the study. FMB had the original idea for the study and interpreted clinical data. All authors participated in the interpretation of results, and reviewed and approved the final version. The authors reported no potential conflicts of interest.

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