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

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Supplementary data

Supplementary information:

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Design and Methods

ADAMTS13/VWF balance was analyzed in groups of patients with SIRS (i) following extracorporeal cardiopulmonary circuit, (ii) severe sepsis of various causes. Stages of disease severity and organ failure were defined by APACHEII-score, SAPSII-score, and SOFA-score [1, 2]. After institutional ethical approval and written informed consent, three groups of ICU-patients were studied:

(1) Twenty four patients who underwent *elective* cardiac surgery with a low risk of developing post-operative organ dysfunction as indicated by an EURO-score (European System for Cardiac Operative Risk Evaluation) of <6 [3] and a maximum post-operative ICU stay of two days (92% with one day). Plasma samples were taken within the first 24h after ICU admission and normalized for hemodilution. These data were used as ICU controls.

(2) Twenty two patients after *non-elective* on-pump cardiac surgery with a high risk of developing organ dysfunction (EURO-score ≥6) [3]. Plasma samples were taken on five consecutive post-operative days and normalized for hemodilution.

(3) Eleven patients who met the criteria for severe sepsis or septic shock according to the ACCP/SCCM Consensus Conference [4]. 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 three groups of patients on the first day on the ICU and biochemical variables determined on the first and last day (discharge or death) on ICU are presented in **Table 1** of the main document.

ADAMTS13 activity was determined by the collagen-binding method using recombinant VWF as substrate [5]. This assay had provided highly reliable results in multicenter studies [6]. Auto-inhibitory factors of ADAMTS13 were excluded by dilution experiments.

VWF antigen (VWF:Ag) was measured by an enzyme-linked immuno-sorbent assay [7]. For VWF multimer analysis, samples were diluted to obtain a final concentration ranging from 0.05 to 0.1 U/mL and separated by gel electrophoresis (60V, 16°C, 15h, 1.2% LGT-Agarose), detected by VWF-antibody conjugated with horseradish peroxidase (Dako Hamburg, Germany) and visualised by enhanced chemiluminescence (ECL detection kit; Amersham, Freiburg, Germany). The gels were recorded by a CCD camera and evaluated by AIDA software [8].

The ISTH-score for overt DIC [9] was calculated with the following modifications: fibrinogen concentration was not included as it is often increased in systemic inflammation; for D-dimer concentration the following cut-off values were used: 0-0.25 µg/mL = 0 points, 0.25-2.5 µg/mL = 2 points, > 2.5 µg/mL = 3 points [10].

Statistical analysis: The non-parametric Mann-Whitney-U test and ANOVA were applied for comparison between patient groups as well as between survivors and non-survivors. *P* values <0.05 were considered significant. The results are presented as medians and $1^{st}/3^{rd}$ quartile.

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