Received: 25 June 2019 | Accepted: 18 November 2019

DOI: 10.1111/jth.14686

ORIGINAL ARTICLE

# Von Willebrand factor and ADAMTS13 impact on the outcome of *Staphylococcus aureus* sepsis

Marijke Peetermans[1] | Severien Meyers[1] | Laurens Liesenborghs[1] | Karen Vanhoorelbeke[2] | Simon F. De Meyer[2] | Christophe Vandenbriele[1] | Marleen Lox[1] | Marc F. Hoylaerts[1] | Kimberly Martinod[1] | Marc Jacquemin[1] | Thomas Vanassche[1] | Peter Verhamme[1]

[1]Department of Cardiovascular Sciences, Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

[2]Laboratory for Thrombosis Research, University of Leuven campus Kulak Kortrijk, Kortrijk, Belgium

**Correspondence**
Peter Verhamme, Department of Cardiovascular Sciences, Center for Molecular and Vascular Biology, University of Leuven, Herestraat 49, Box 480, 3000 Leuven, Belgium.
E-mail: peter.verhamme@kuleuven.be

**Funding information**
Research Foundation Flanders

## Abstract

**Background:** Previous clinical evidence correlates levels of von Willebrand factor (VWF) and its cleaving protease ADAMTS13 with outcome in septic patients. No previous studies addressed if VWF and ADAMTS13 affected the outcome of *Staphylococcus aureus* sepsis.

**Objectives:** We studied the role of VWF and ADAMTS13 in *S. aureus* sepsis both in patients and in mice.

**Methods:** VWF levels and ADAMTS13 activity levels were measured in plasma samples from 89 *S. aureus* bacteremia patients by chemiluminescent assays and were correlated with clinical sepsis outcome parameters. In wild-type mice and mice deficient in VWF and ADAMTS13, we investigated the outcome of *S. aureus* sepsis and quantified bacterial clearance and organ microthrombi.

**Results:** In patients with *S. aureus* bloodstream infections, high VWF levels and low ADAMTS13 activity levels correlated with disease severity and with parameters of inflammation and disseminated intravascular coagulation. In septic mice, VWF deficiency attenuated mortality, whereas ADAMTS13 deficiency increased mortality. Bacterial clearance was enhanced in VWF-deficient mice. The differences in mortality for the studied genotypes were associated with differential loads of organ microthrombi in both liver and kidneys.

**Conclusions:** In conclusion, this study reports the consistent relation of VWF, ADAMTS13 and their ratio to disease severity in patients and mice with *S. aureus* sepsis. Targeting VWF multimers and/or the relative ADAMTS13 deficiency that occurs in sepsis should be explored as a potential new therapeutic target in *S. aureus* endovascular infections.

KEYWORDS

ADAMTS13 protein, disseminated intravascular coagulation, sepsis, *Staphylococcus aureus*, von Willebrand factor

Manuscript handled by: Marcel Levi

Final decision: Marcel Levi, 18 November 2019

  

## 1 | INTRODUCTION

During bloodstream infections, activation of the hemostatic system is part of the host response to the pathogen. Widespread endothelial activation by inflammatory cytokines further promotes a prothrombotic state.[1-3] Many bacteria also directly activate the coagulation system or exhibit specific interactions with the vascular wall.[4,5]

Activated endothelial cells release von Willebrand factor (VWF) from Weibel-Palade bodies. VWF forms high molecular weight multimeric molecules that remain temporarily associated with the vessel wall. The (ultra) large VWF multimers trigger the adhesion of platelets and platelet-leukocytes conjugates, but also of bacteria such as *Staphylococcus aureus*.[6,7] Under normal conditions, this VWF is rapidly cleaved by ADAMTS13, a VWF-specific protease.[8] Inflammatory and septic states have been associated with reduced ADAMTS13 activity.[9-12] Indeed, a large body of clinical data confirms that during sepsis, VWF levels and multimer size tend to increase,[13-18] whereas ADAMTS13 activity decreases.[15,16,18-24] In many cases, these changes correlate with outcome and organ failure.[14,16,22-29]

This imbalance between VWF and ADAMTS13 was hypothesized to contribute to the development of microvascular thrombosis and multiorgan failure in sepsis.[30] However, animal studies using models for Gram-negative or polymicrobial sepsis, such as cecal ligation and puncture or lipopolysaccharide injection, have yielded conflicting results on the contribution of VWF and ADAMTS13 to the course of sepsis.[31]

Up to one-half of bloodstream infections are caused by Gram-positive bacteria, with *S. aureus* being the second most frequently isolated single pathogen.[32,33] *S. aureus* is highly specialized to interact with platelets, the coagulation system, fibrin, and subendothelial matrix proteins.[5-7,34-36] In addition, *S. aureus* can also bind directly to VWF using its von Willebrand binding protein (vWbp), a feature that has been proved crucial for its adhesion to the vessel wall.[6,7,36] *S. aureus* can form small bacteria-platelet-fibrin microaggregates, which will stick avidly to VWF exposed on the vessel wall.[7,34,37]

Because of these specific features of *S. aureus*, we aimed to investigate the role of VWF and its cleaving protease ADAMTS13 in *S. aureus* bloodstream infection as a relevant addition to the conflicting literature data on VWF and ADAMTS13 in Gram-negative sepsis.

**Essentials**

- Conflicting data on the contribution of von Willebrand Factor (VWF) and ADAMTS13 to sepsis outcome.
- We studied the role of VWF and ADAMTS13 in *S. aureus* sepsis both in patients and in mice.
- VWF/ADAMTS13 ratio in bacteremia patients correlate with a more severe disease state.
- *Vwf*−/− mice had enhanced bacterial clearance and reduced mortality rates and organ microthrombi.

## 2 | METHODS

### 2.1 | Patient samples

Plasma samples from patients with a diagnosis of *S. aureus* bacteremia were collected between March 2013 and April 2016 in the University Hospitals Leuven as baseline samples of a clinical trial (NCT01911624).[38] Patients provided informed consent for study procedures including coagulation testing on plasma samples. The clinical trial was approved by the institutional review board. In this trial, patients with renal or hepatic failure or severe thrombocytopenia were not included.

VWF:Ag and ADAMTS13 activity were measured by chemiluminescent immunoassays using an AcuStar according to the manufacturer's instructions.[39] A plasma pool (Visucon, Affinity Biological Inc) was used undiluted and diluted threefold in diluent buffer (Werfen) as quality controls. In addition to VWF:Ag levels, VWF collagen binding (VWF:CB), and VWF ristocetin-triggered GPIb binding activity (VWF:RCo) were also detected. These data were available for 89 patients (VWF, n = 88; ADAMTS13, n = 89; VWF/ADAMTS13 ratio, n = 87). Other laboratory and clinical parameters included: hospital stay, acute physiology assessment and chronic health evaluation (APACHE) II score, simplified acute physiology score (SAPS) score, weighted Charlson index, activated partial thromboplastin time, prothrombin time, platelet count, fibrinogen, D-dimer (and calculated disseminated intravascular coagulation score), white blood cell count, neutrophilia, C-reactive protein (CRP), creatinine, and estimated glomerular filtration rate chronic kidney disease epidemiology formula (CKD-EPI).

### 2.2 | Murine infection model

#### 2.2.1 | Mouse strains

All animal experimental procedures were approved by the Ethics Committee of the KU Leuven. Five- to 11-week old mice of the same sex were housed in standard conditions with free access to food and water ad libitum. Knock-out mice for *Vwf* and *Adamts13* in C57BL/6 background were available from our in-house breeding program.

#### 2.2.2 | *S. aureus* sepsis models

Bacteria were grown overnight in tryptic soy broth, washed, and diluted in phosphate-buffered saline. Mice were injected via the tail vein with *S. aureus* Newman, using an inoculum of $5 \times 10^7$ colony

forming units (CFU). Preliminary experiments showed that an infectious inoculum of $2 \times 10^6$ CFU of *S. aureus* Newman administered via tail vein injection to wild-type mice did not cause severe symptoms, whereas $2\text{-}6 \times 10^7$ CFU resulted in signs of sepsis, and a 10-fold higher inoculum of $5 \times 10^8$ CFU led to mortality in wild-type mice within 120 hours.

C57Bl/6 wild-type (WT), VWF, or ADAMTS13 homozygous knockout (*Vwf−/−*, *Adamts13−/−*) mice were used. These were observed four times daily to assess disease score and mortality during a 10-day follow-up period. If the disease score was above a predefined threshold of 9 points, mice were euthanized and this time point was used as a proxy for mortality. Infection with the same inoculum of an isogenic mutant of *S. aureus* Newman, lacking the vWbp (*S. aureus* del vwb) was compared with infection with the parent *S. aureus* strain.

In a second set of experiments, mice were infected with the same inoculum of *S. aureus* Newman, and blood samples were taken on citrate (3.2% sodium citrate, 10% vol/vol) on days 1, 3, and 5 after infection. In a final set of experiments, mice were euthanized at 24 hours postinfection via heart punction. Liver and kidneys were collected for histology.

We also performed these experiments with the addition of an injection of either 3500 U/kg of recombinant human ADAMTS13 (rhADAMTS13, kindly provided by Baxalta [research project H16-32463]) or vehicle [NaCl 0.9%] (final volume, 90-120 μL). The treatment was randomly assigned and the observations and sample collections were carried out by blinded operators.

### 2.2.3 | Blood analysis

Neutrophil and platelet numbers were analyzed in a cell counter (Cell-Dyn 3700, Abbott) on blood samples of days 1, 3, and 5 after induction of *S. aureus* bacteremia.

We also analyzed interleukin (IL)-6 levels in these plasma samples, using a commercial mouse IL-6 ELISA kit (mIL-6 ELISA Ready-SET-Go!, eBioscience).

Cell-free DNA was measured using Sytox Green Dye (Life Technologies) and a standard curve of lambda-DNA (Fisher Scientific), as previously described.[40]

In a subset of 24-hour sepsis experiments, a blood sample was taken 1 hour after infection and used for quantitative plating on blood agar to determine *S. aureus* bacterial load (CFU/mL) in blood.

### 2.2.4 | Histology

Paraffin-embedded tissue samples of kidneys and liver were used to prepare 10-μm-thick sections. Routine histopathologic stainings with hematoxylin-eosin and Martius Scarlet Blue were performed. The load of microthrombi per surface area of liver or kidney tissue was scored. We also performed a modified Gram staining on tissue sections (Brown-Hopps staining).

## 2.3 | Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0b (GraphPad Software). Correlations among VWF, ADAMTS13, or the VWF/ADAMTS13 ratio and the clinical and laboratory parameters were assessed, using Pearson *r* coefficients.

For the sepsis experiments with 10-day follow-up, survival curves were compared using Mantel-Cox test, with Gehan-Breslow-Wilcoxon posttest to compare curves in pairs. The data for weight and disease score at each time point were compared among the three groups with ANOVA, with unpaired *t*-test as post-test. If not normally distributed, Kruskal-Wallis test and Mann-Whitney test were used.

# 3 | RESULTS

## 3.1 | Patients

In patients with *S. aureus* bloodstream infection, we found VWF:antigen (Ag) plasma levels of 332 ± 114% (mean ± standard deviation) and ADAMTS13 activity levels of 49 ± 18% (Figure 1A,B). Of note, no significant correlation existed between VWF and ADAMTS13 (Pearson $r = -.1684$, $P = .1168$). Levels for the VWF:CB and VWF:RCo activity were 254 ± 102% and 299 ± 118%, respectively. Strong correlations ($r \geq .25$; $P \leq .01$) were observed between VWF:Ag levels and VWF:RCo, VWF:CB, D-dimers, fibrinogen, and CRP (Figure S1A1-2, 4-6). VWF levels also correlated with APACHE II score ($r = .2204$, $P = .0427$) and borderline with SAPS II score ($r = .1901$, $P = .0796$) (Figure S1A3). ADAMTS13 activity levels strongly inversely correlated with D-dimers, CRP, and with neutrophil count ($r = -.2315$, $P = .0442$) and SAPS II score ($r = -.2282$, $P = .0346$) (Figure S1B2-3, 5-6). Borderline significant correlations were found between ADAMTS13 and VWF:RCo ($r = -.2037$), APACHE II ($r = -.1996$), weighted Charleston index ($r = -.197$), prothrombin time ($r = -.2077$), and leukocytes ($r = -.261$).

We calculated the ratio of VWF:Ag to ADAMTS13 activity levels (Figure 1C). This VWF:Ag/ADAMTS13 ratio showed strong correlations to D-dimers ($r = .4208$), leukocytes ($r = .3299$), and CRP ($r = .4105$) (Figure 1D-I). Additionally, the VWF:Ag/ADAMTS13 ratio correlated with diseases scores (APACHE II, $r = .2764$; SAPS II, $r = .275$).

## 3.2 | Mice

To investigate if VWF levels and VWF multimerization grade (regulated by ADAMTS13) can modulate the outcome in *S. aureus* infection, we studied *S. aureus* sepsis in a mouse model.

During a 10-day follow-up experiment, we observed increased weight loss and mortality in *Adamts13−/−* compared with WT mice. On the other hand, *Vwf−/−* mice had improved survival and exhibited fewer sepsis symptoms than WT or *Adamts13−/−* mice (Figure 2).

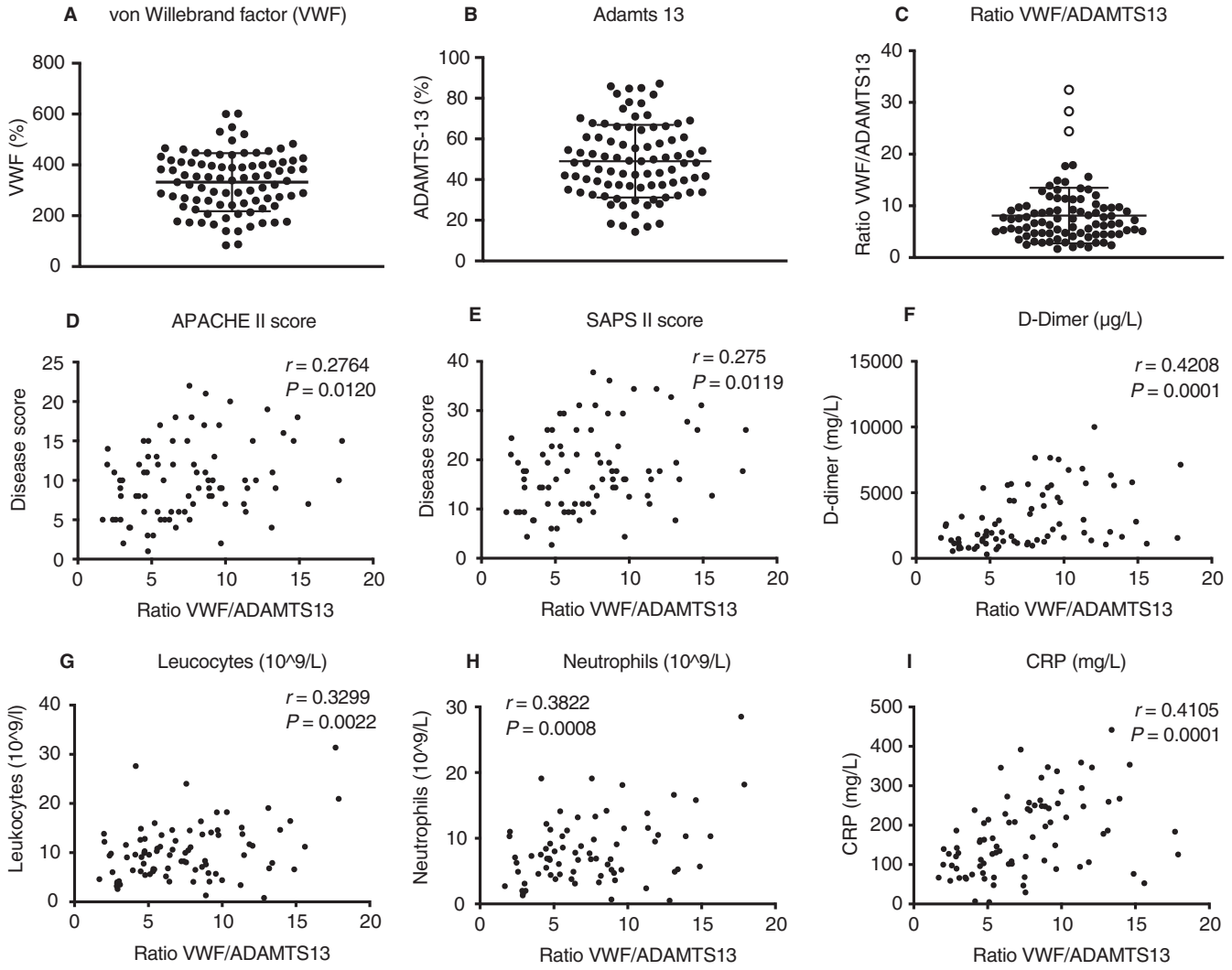


**FIGURE 1** von Willebrand factor (VWF) to ADAMTS13 ratio correlates with disease severity, inflammation and disseminated intravascular coagulation score in patients with *S. aureus* bacteremia. (A) VWF antigen levels. (B) ADAMTS13 activity levels at diagnosis of *S. aureus* bacteremia. (C) Ratio of VWF to ADAMTS13 (arbitrary units). Three outliers (open circle) were not included in the correlation analysis. (D-I) Correlation between the VWF/ADAMTS13 ratio and different parameters: APACHE II disease score (D), SAPS II score (E), D-dimers as parameter of coagulation activation (F), leukocytes (G), neutrophils (H), and C-reactive protein (CRP) as parameter of inflammation (I). Pearson *r* coefficients and the corresponding *P* values are shown on the graphs

When we analyzed bacterial loads 1 hour after infection, lower numbers of bacteria were present in the circulation of *Vwf*−/− mice (2.78 log, interquartile range [IQR] 2.57-2.94 vs 3.19 log, IQR 3.05-3.30 or vs 3.26 log, IQR 3.23-3.29 for WT or *Adamts13*−/−, respectively, n = 8-10 per group, $P < .001$ for pairwise comparison of *Vwf*−/− to WT or to *Adamts13*−/−) (Figure 3).

We investigated if the different genotypes, apart from a different severity of infection and mortality, would also show differences in platelet consumption. We therefore performed an additional set of sepsis experiments in which we took blood samples of mice at days 1, 3, and 5 after infection with *S. aureus*. At 3 and 5 days postinfection, *Vwf* knock-out mice had normal or increased platelets counts, whereas *Adamts13* knock-out mice developed severe thrombocytopenia (Figure 4).

There were no significant differences in neutrophilia on days 3 and 5, but *Vwf* knock-out mice had numerically higher mean neutrophil counts, compared with WT or *Adamts13* knock-out mice. No significant differences in cell-free DNA were found among the three groups of mice on day 1; however, *Adamts13*−/− mice had higher cell-free DNA values on day 3 compared with WT or to *Vwf*−/− mice (Figure 5). IL-6 levels were elevated in all mice, but no differences between groups were observed (data not shown).

In another set of experiments, mice were euthanized at 24 hours after infection to evaluate kidney and liver pathology. More microthrombi were present in these end organs in *Adamts13*−/− mice, compared with *Vwf* knock-out mice (Figure 6).

We performed the same sepsis studies using an *S. aureus* strain that lacks the bacterial vWbp, a coagulase that also mediates specific direct binding of *S. aureus* to VWF. In these experiments, the increased mortality in *Adamts13*−/−, compared with WT mice was still observed ($P < .05$). Hence, even in the absence of the specific

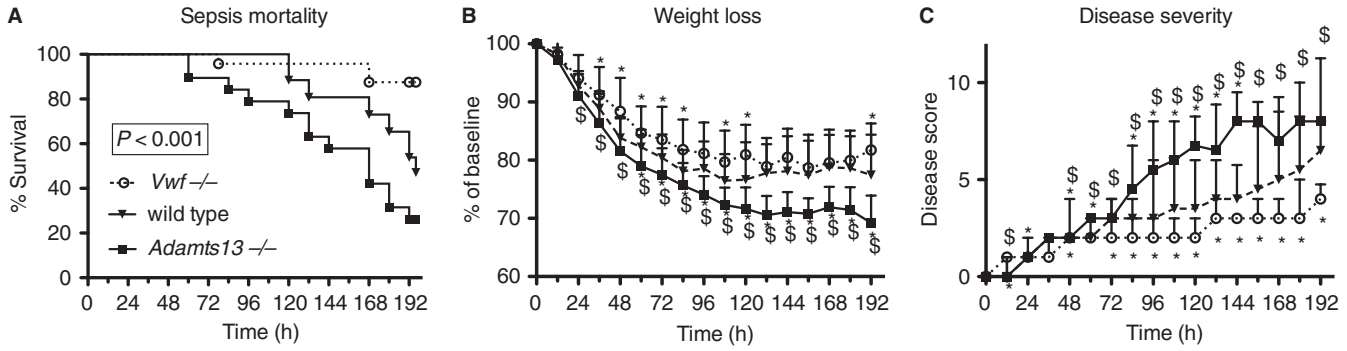


**FIGURE 2** von Willebrand factor (VWF) and ADAMTS13 influence outcome in murine *S. aureus* sepsis. (A) Survival curves, (B) weight loss, and (C) disease severity scores of mice after bloodstream infection with *S. aureus* Newman ($5 \times 10^7$ CFU). We compared mice of three different genotypes: wild-type (n = 26), *Vwf* knock-out (*Vwf*−/−) (n = 24), or Adamts13 knock-out (*Adamts13*−/−)(n = 19). $P < .001$ for the difference between survival curves, $P < .05$ for *Vwf*−/− vs wild-type, $P < .05$ for *Adamts13*−/− vs wild-type, and $P < .0001$ for *Adamts13*−/− vs *Vwf*−/−. Weight is shown as mean and standard deviation, disease scores as median and IQR. *Indicates a significant difference between knock-out mice (*Adamts13*−/− or *Vwf*−/−) and wild-type. $^{\$}$Indicates a significant difference between *Adamts13*−/− and *Vwf*−/− groups

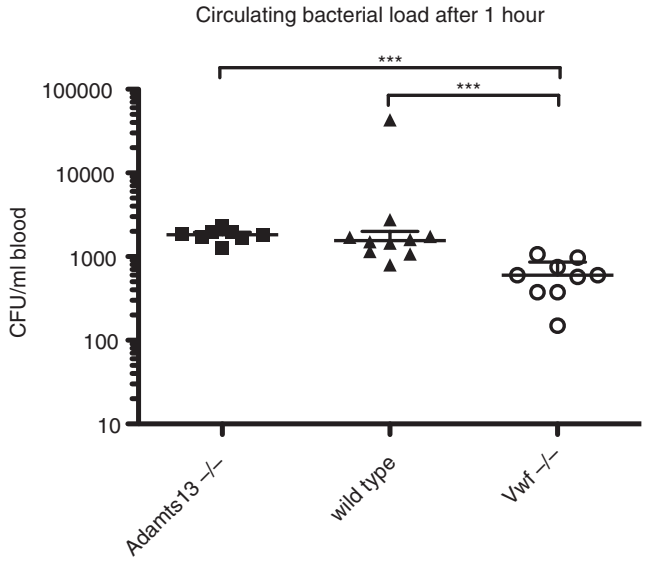


**FIGURE 3** Initial clearance of *S. aureus* bacteremia. One hour after injection of the same inoculum of $5 \times 10^7$ CFU of *S. aureus*, we performed quantitative cultures of blood samples of mice from different genotypes. The remaining circulating bacterial load is lower in Vwf knock-out mice, compared with wild-type or to *Adamts13* knock-out mice. ***$P < .001$

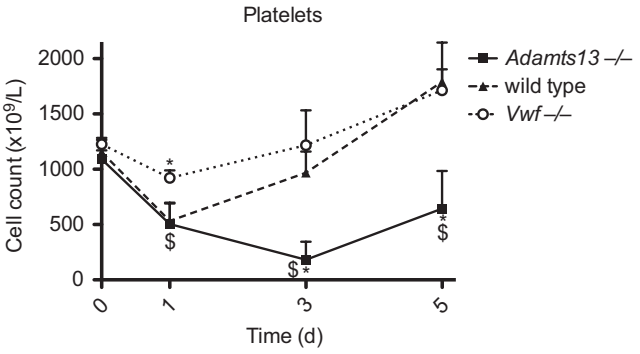


**FIGURE 4** Severe thrombocytopenia during *S. aureus* sepsis in ADAMTS13 knock-out mice. Platelet counts were assessed on the day of *S. aureus* injection, and on days 1, 3, and 5 thereafter. *Indicates a significant difference between knock-out mice (*Adamts13*−/− or *Vwf*−/−) and wild-type. $^{\$}$Indicates a significant difference between *Adamts13*−/− and *Vwf*−/− groups (n = 6-7 per group)

bacterial vWbp, the presence of ultra large VWF strings resulting from ADAMTS13 deficiency still promotes worse outcome of *S. aureus* bloodstream infection (Figure 7A). In addition, also when using ADAMTS13-deficient mice, the presence or absence of the *S. aureus* vWbp did not influence sepsis outcome (Figure 7B).

We then performed the *S. aureus* sepsis studies (using WT *S. aureus* Newman) in mice that received a dose of rhADAMTS13 or vehicle control to study whether we could rescue these mice from the development of widespread organ microthrombi. When using *Adamts13*−/− mice that received supplementation with rhADAMTS13, we indeed observed less liver microthrombi (Figure 8). However, rhADAMTS13 did not reduce microthrombi in WT mice.

## 4 | DISCUSSION

We present the first study that specifically addresses the role of VWF in *S. aureus* sepsis in both patients and mice. *S. aureus* is a frequent cause of bloodstream infection that exhibits many specialized interactions with the vessel wall and VWF. Our data confirm that a profound deregulation of VWF and ADAMTS13 is observed in patients with *S. aureus* bacteremia and that this is associated with a more severe disease state and pronounced inflammation. In a mouse model, we observed that low VWF levels protect against mortality of *S. aureus* sepsis, possibly because of a decrease in microvascular thrombosis in end organs. In contrast, ADAMTS13-deficient animals showed high mortality, and more microvascular thrombosis, which could be rescued by the administration of recombinant ADAMTS13.

In our patient population of septic patients, a severely disturbed VWF/ADAMTS13 ratio was observed. Interestingly, we found no correlation between ADAMTS13 activity levels and VWF antigen levels.

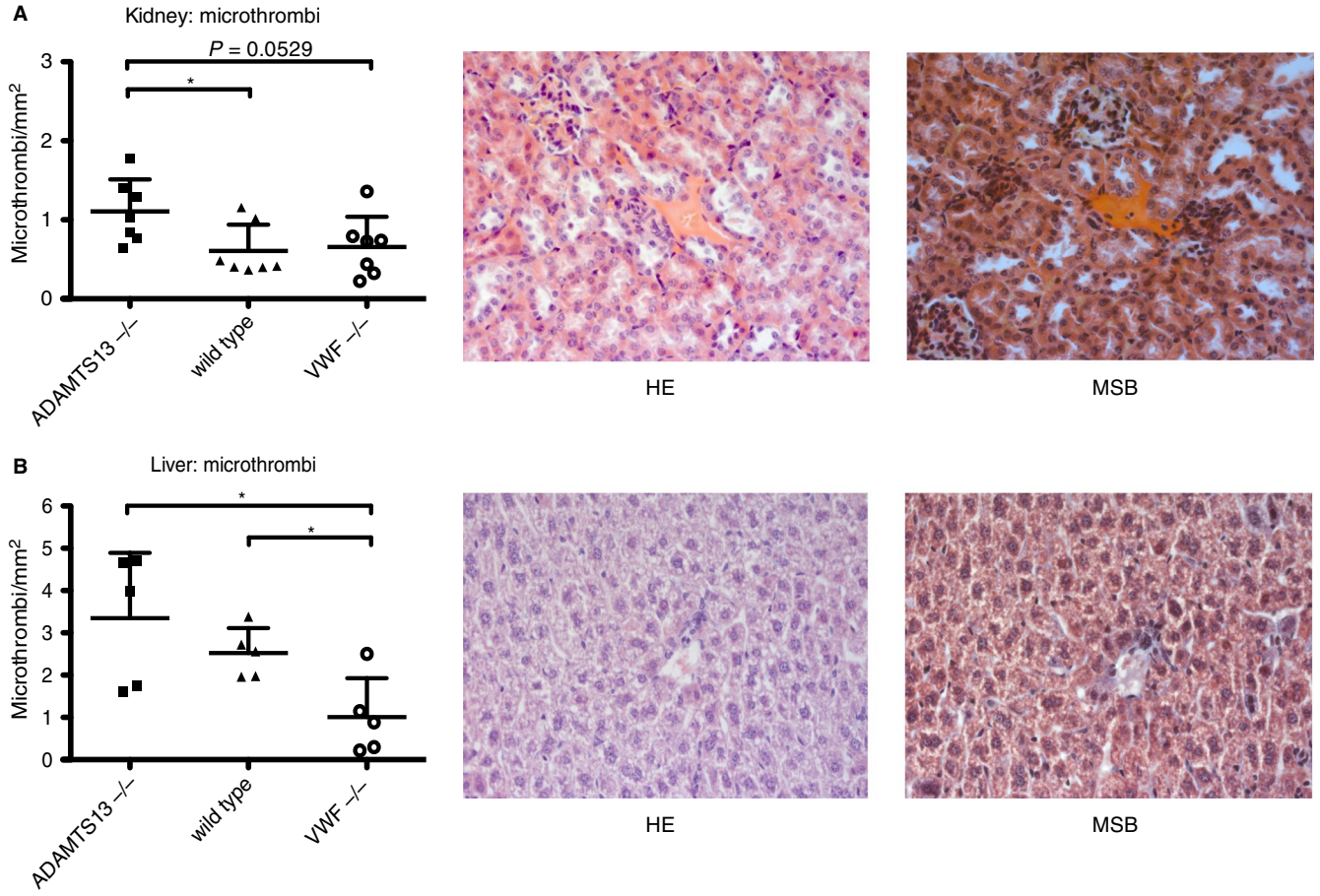


**FIGURE 5** More end-organ microthrombi in ADAMTS13 knock-out mice. The burden of microthrombi in (A) kidneys and (B) liver of *Adamts13*−/−, wild-type, and *Vwf*−/− mice were compared: *Adamts13*−/− mice had significantly more microthrombi, both in liver and kidney sections. *$P < .05$. An example of a microthrombus in kidney and in liver is shown for a wild-type mouse on hematoxylin-eosin (HE) and Martius Scarlet Blue (MSB) staining

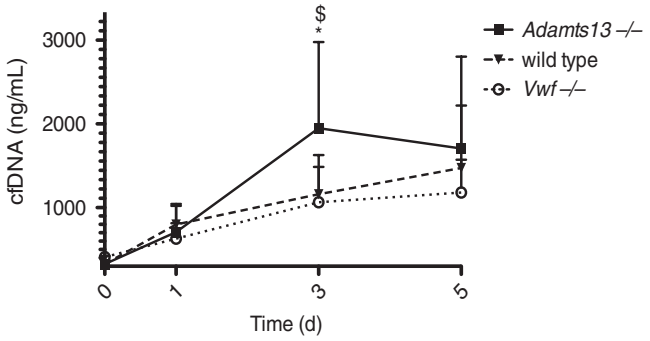


**FIGURE 6** Analysis of cell-free DNA in plasma of mice after *S. aureus* bacteremia. Samples were analyzed on days 1, 3, and 5 after injection of $5 \times 10^7$ CFU of *S. aureus* Newman. Day 0 control values are from control mice of the respective genotypes. *Indicates a significant difference between *Adamts13*−/− and wild-type. $^{\$}$Denotes a significant difference between *Adamts13*−/− and *Vwf*−/− groups. (n = 15 *Adamts13*−/−, 26 wild-type, and 16 *Vwf*−/−)

However, the use of the VWF/ADAMTS13 ratio augmented the correlation with the laboratory parameters of coagulation, inflammation, and end-organ failure, suggesting that a disbalance of VWF multimers release and cleavage plays a role in end-organ damage.

The relative ADAMTS13 deficiency, with observed values of approximately 50%, may affect hemostatic balance in septic patients, even when these are higher than the values encountered in patients with TTP. In vitro flow experiments have shown that at 25% of normal ADAMTS13 concentrations, ultra large VWF multimers are only cleaved half as well.[41] Functional deficiency of ADAMTS13 in sepsis may already occur at plasma ADAMTS13 antigen levels above 25%. Indeed, inflammatory cytokines, bacterial products, and white blood cell enzymes can result in release of large amounts of ultra large VWF from endothelial cells.[42,43] Furthermore, a proinflammatory environment will inhibit ADAMTS13 synthesis[44] and action,[45] and finally, plasmin, thrombin, and elastin can cleave and inactivate ADAMTS13.[46,47] In our patient plasma samples, the ADAMTS13 activity levels did not correlate with plasmin-α2-antiplasmin complex levels (data not shown).

Although the high values of VWF and low values of ADAMTS13 in patients do not prove their causal role in the course of sepsis, there is a biological plausibility, which we investigated in our animal model. When manipulating the host background, we showed that disease severity paralleled the VWF/ADAMTS13 ratio in mice, consistent with patient data. A strength of our work is the use of a model for monomicrobial Gram-positive bacteremia, a

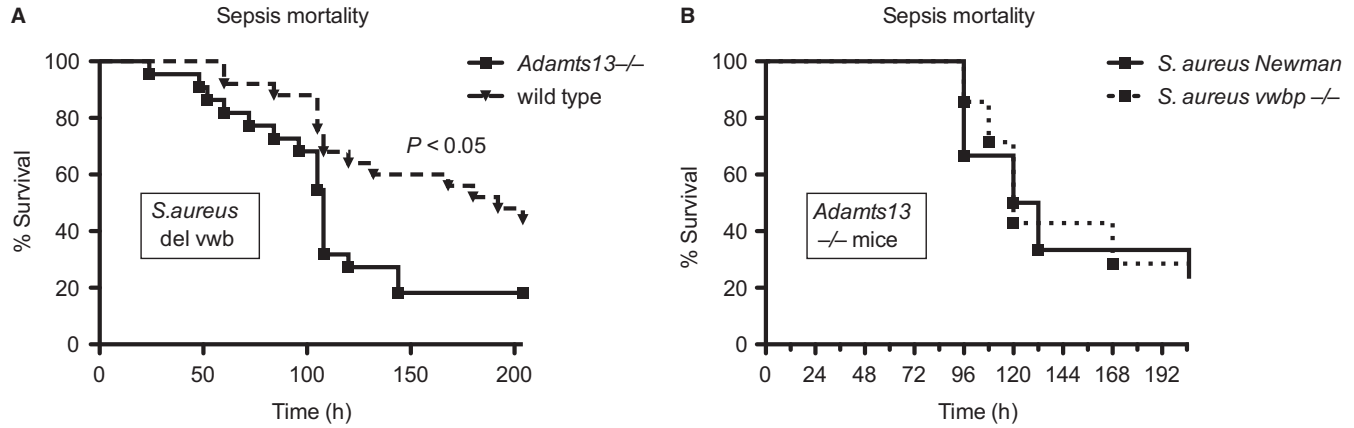


**FIGURE 7** The role of the specific VWF-binding protein of *S. aureus* is limited in sepsis and ADAMTS13 deficiency. (A) Survival of mice after bloodstream infection with $5 \times 10^7$ CFU of a *S. aureus* Newman mutant strain lacking the specific VWF-binding protein (*S. aureus* vwbp−/−). We compared wild-type (n = 25) and Adamts13 knock-out (*Adamts13*−/−) (n = 22) mice, and observed increased mortality, as seen in the experiments with the parent *S. aureus* Newman strain. (B) Survival of ADAMTS13-deficient mice after bloodstream infection with $5 \times 10^7$ CFU of *S. aureus* Newman (n = 6) or an isogenic mutant strain lacking the specific VWF binding protein (*S. aureus* vwbp−/−) (n = 7)

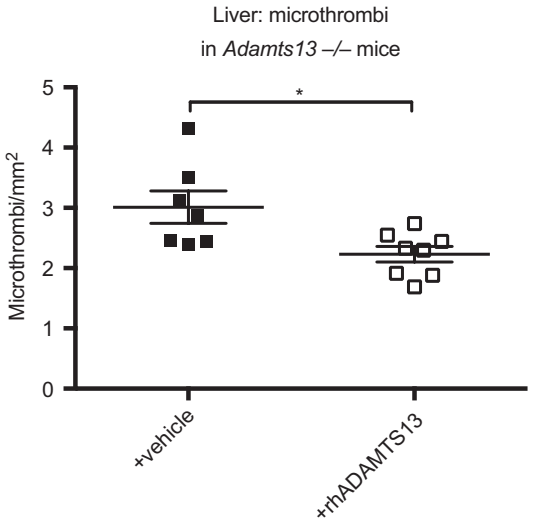


**FIGURE 8** ADAMTS13-deficient mice can be partly rescued from widespread microthrombosis by the administration of recombinant ADAMTS13. The burden of microthrombi in the liver of *Adamts13*−/− mice, injected with either recombinant human ADAMTS13 (rhADAMTS13, 3500 U/kg), or with vehicle control, were compared: *Adamts13*−/− mice, supplemented with rhADAMTS13 (n = 8), had significantly less microthrombi than control mice (n = 7/8). *Denotes $P < .05$

frequently encountered entity in patients that is overlooked in most current animal models of sepsis. However, we admit that the bacterial loads of *S. aureus* and other pathogens, which are needed to induce severe sepsis in mice, are higher than those present in septic patients.[48,49] In other animal models for bacterial sepsis, a lower mortality was noticed in VWF knock-out mice in two studies using cecal ligation and puncture (CLP),[50,51] but opposite findings of increased mortality in VWF knock-out mice have also been reported.[52] To further complicate available experimental evidence, ADAMTS13 knock-out mice had similar survival as WT mice after CLP[51] and did not show signs of thrombotic microangiopathy. Even though we could reduce organ microthrombi in ADAMTS13-deficient mice by administering rhADAMTS13, we could not prove a similar rescue effect in WT mice. One could hypothesize that the changes in our mouse model might be more pronounced than those observed in patients because of the high bacterial load used in animal models, and possibly not amenable to salvage with rhADAMTS13 therapy.

The observed decrease in platelet count in parallel with end-organ microthrombosis is consistent with the human phenotype of sepsis-associated thrombocytopenia and disseminated intravascular coagulation.[53] Previous experimental investigations of microvascular thrombogenicity, using light/dye-induced thrombosis in cremaster venules[54,55] or ferric chloride-induced thrombosis in mesenteric arterioles,[56] have shown dependence on VWF for endotoxin injection but not for CLP sepsis. These models of isolated direct vascular injury do not allow to capture all the players relevant in sepsis. Similarly, our group has previously identified a central role of the vWbp protein of *S. aureus* to adhere to an inflamed mesenteric vessel wall that exposes multimeric VWF.[7] ADAMTS13 knock-out mice expose ultra large VWF multimers; however, a role for its binding partner vWbp was not evident in *S. aureus* sepsis in these mice, probably reflecting the presence of several redundant mechanisms for adhesion of microthrombi, activated platelets, and *S. aureus* to VWF exposed on the vessel wall in septic conditions. Also, comparing ADAMTS13 deficient with WT mice, the importance of this single host factor was far greater than the single bacterial factor vWbp. Nonetheless, this does not exclude a role of vWbp in primary adhesion in other diseases such as infective endocarditis.

In conclusion, the levels of ADAMTS13 and VWF correlate with the outcome of *S. aureus* sepsis, both in patients and in a mouse model. We could show increased bacterial retention and more microthrombi in organs of ADAMTS13 knock-out mice, whereas a

protective effect on sepsis outcome and microthrombotic complications was observed in VWF knock-out mice. Interestingly, in septic patients, ADAMTS13 activity levels are frequently diminished, whereas VWF levels tend to increase, causing an imbalance between VWF and its cleaving protease.

Because (relative) ADAMTS13 deficiency worsens outcome of *S. aureus* sepsis, the question arises if substitution of ADAMTS13 could revert this. In the past, similar rationale has driven research on activated protein C and antithrombin III as adjunctive treatments for sepsis. However, compilation of the available evidence does not support their efficacy, and both are associated with bleeding complications.[57,58] Interestingly, ADAMTS13 supplementation has no or minimal effect on bleeding.[59-62] The potential role of ADAMTS13 repletion in ameliorating outcome, as shown for stroke and myocardial ischemia,[59-62] and in our experiments with ADAMTS13-deficient mice, should be tested in more detail in sepsis models. We and others hypothesize that the cleavage of the highly adhesive ultra large VWF on endothelium by ADAMTS13, could reduce recruitment of platelets, neutrophils, and bacteria, and microthrombosis in end organs during sepsis.[30] ADAMTS-13 could be delivered as injection of the recombinant protein, or via adeno-associated viral expression.[30] Alternatively, rather than cleaving the ultra large VWF, one could inhibit its interactions with platelets, by blocking the platelet GPIb-binding site on VWF with the nanobody caplacizumab.[63] The further study of ADAMTS13 and other molecules that reduce the adhesive and prothrombotic phenotype of an activated endothelial cell layer in sepsis, is warranted.

## ADDENDUM

M. Peetermans participated in the study design, performed experiments, analyzed data results, and drafted the manuscript. S. Meyers performed experiments, analyzed data results, and partially drafted and critically read the manuscript. L. Liesenborghs participated in the study design, performed experiments, and critically read the manuscript. K. Vanhoorelbeke and S. F. De Meyer participated in the study design and critically read the manuscript. C. Vandenbriele, M. F. Hoylaerts, K. Martinod, and T. Vanassche critically read the manuscript. M. Lox conducted the animal experiments. M. Jacquemin provided the VWF and ADAMTS13 levels measured with the AcuStar and critically read the manuscript. P. Verhamme participated in study design, partially drafted the manuscript, and critically read the manuscript.

### ACKNOWLEDGEMENTS

PV and TV are senior clinical investigator of the Research Foundation Flanders. MP, SM, and LL are recipients of a fellowship funded by the Research Foundation Flanders.

### CONFLICTS OF INTEREST

The authors have no conflicts of interest.

### ORCID

*Severien Meyers* https://orcid.org/0000-0003-3481-4793
*Laurens Liesenborghs* https://orcid.org/0000-0001-5616-8548
*Kimberly Martinod* https://orcid.org/0000-0002-1026-6107
*Thomas Vanassche* https://orcid.org/0000-0002-7404-8918
*Peter Verhamme* https://orcid.org/0000-0001-8698-2858

### TWITTER

*Peter Verhamme* @CMVB_KULeuven

### REFERENCES

1. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood*. 2003;101(10):3765-3777.
2. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol*. 2013;13(1):34-45.
3. Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med*. 2013;369(9):840-851.
4. Lemichez E, Lecuit M, Nassif X, Bourdoulous S. Breaking the wall: targeting of the endothelium by pathogenic bacteria. *Nat Rev Microbiol*. 2010;8(2):93-104.
5. Liesenborghs L, Verhamme P, Vanassche T. *Staphylococcus aureus*, master manipulator of the human hemostatic system. *J Thromb Haemost*. 2018;16(3):441-454.
6. Pappelbaum KI, Gorzelanny C, Grassle S, *et al*. Ultralarge von Willebrand factor fibers mediate luminal *Staphylococcus aureus* adhesion to an intact endothelial cell layer under shear stress. *Circulation*. 2013;128(1):50-59.
7. Claes J, Vanassche T, Peetermans M, *et al*. Adhesion of *Staphylococcus aureus* to the vessel wall under flow is mediated by von Willebrand factor-binding protein. *Blood*. 2014;124(10):1669-1676.
8. Ruggeri ZM. Von Willebrand factor, platelets and endothelial cell interactions. *J Thromb Haemost*. 2003;1(7):1335-1342.
9. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. *Blood*. 2001;98(9):2730-2735.
10. Loof AH, van Vliet HH, Kappers-Klunne MC. Low activity of von Willebrand factor-cleaving protease is not restricted to patients suffering from thrombotic thrombocytopenic purpura. *Br J Haematol*. 2001;112(4):1087-1088.
11. Scully M, Liesner R, Burgess C, Lawrie A, Machin S. ADAMTS 13 in non-thrombotic thrombocytopaenic purpura conditions. *Br J Haematol*. 2008;141(2):262-265.
12. Moore JC, Hayward CP, Warkentin TE, Kelton JG. Decreased von Willebrand factor protease activity associated with thrombocytopenic disorders. *Blood*. 2001;98(6):1842-1846.
13. McGill SN, Ahmed NA, Christou NV. Increased plasma von Willebrand factor in the systemic inflammatory response syndrome is derived from generalized endothelial cell activation. *Crit Care Med*. 1998;26(2):296-300.
14. Kremer Hovinga JA, Zeerleder S, Kessler P, *et al*. ADAMTS-13, von Willebrand factor and related parameters in severe sepsis and septic shock. *J Thromb Haemost*. 2007;5(11):2284-2290.
15. Nguyen TC, Liu A, Liu L, *et al*. Acquired ADAMTS-13 deficiency in pediatric patients with severe sepsis. *Haematologica*. 2007;92(1):121-124.
16. Bongers TN, Emonts M, de Maat MP, *et al*. Reduced ADAMTS13 in children with severe meningococcal sepsis is associated with severity and outcome. *Thromb Haemost*. 2010;103(6):1181-1187.
17. Reuken PA, Kussmann A, Kiehntopf M, *et al*. Imbalance of von Willebrand factor and its cleaving protease ADAMTS13 during systemic inflammation superimposed on advanced cirrhosis. *Liver Int*. 2015;35(1):37-45.

18. Claus RA, Bockmeyer CL, Budde U, *et al*. Variations in the ratio between von Willebrand factor and its cleaving protease during systemic inflammation and association with severity and prognosis of organ failure. *Thromb Haemost*. 2009;101(2):239-247.
19. Reiter RA, Varadi K, Turecek PL, Jilma B, Knobl P. Changes in ADAMTS13 (von-Willebrand-factor-cleaving protease) activity after induced release of von Willebrand factor during acute systemic inflammation. *Thromb Haemost*. 2005;93(3):554-558.
20. Ono T, Mimuro J, Madoiwa S, *et al*. Severe secondary deficiency of von Willebrand factor-cleaving protease (ADAMTS13) in patients with sepsis-induced disseminated intravascular coagulation: its correlation with development of renal failure. *Blood*. 2006;107(2):528-534.
21. Bockmeyer CL, Claus RA, Budde U, *et al*. Inflammation-associated ADAMTS13 deficiency promotes formation of ultra-large von Willebrand factor. *Haematologica*. 2008;93(1):137-140.
22. Hyun J, Kim HK, Kim JE, *et al*. Correlation between plasma activity of ADAMTS-13 and coagulopathy, and prognosis in disseminated intravascular coagulation. *Thromb Res*. 2009;124(1):75-79.
23. Martin K, Borgel D, Lerolle N, *et al*. Decreased ADAMTS-13 (A disintegrin-like and metalloprotease with thrombospondin type 1 repeats) is associated with a poor prognosis in sepsis-induced organ failure. *Crit Care Med*. 2007;35(10):2375-2382.
24. Peigne V, Azoulay E, Coquet I, *et al*. The prognostic value of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13) deficiency in septic shock patients involves interleukin-6 and is not dependent on disseminated intravascular coagulation. *Crit Care*. 2013;17(6):R273.
25. Rubin DB, Wiener-Kronish JP, Murray JF, *et al*. Elevated von Willebrand factor antigen is an early plasma predictor of acute lung injury in nonpulmonary sepsis syndrome. *J Clin Invest*. 1990;86(2):474-480.
26. Calfee CS, Gallagher D, Abbott J, Thompson BT, Matthay MA, Network NA. Plasma angiopoietin-2 in clinical acute lung injury: prognostic and pathogenetic significance. *Crit Care Med*. 2012;40(6):1731-1737.
27. Kayal S, Jais JP, Aguini N, Chaudiere J, Labrousse J. Elevated circulating E-selectin, intercellular adhesion molecule 1, and von Willebrand factor in patients with severe infection. *Am J Respir Crit Care Med*. 1998;157(3 Pt 1):776-784.
28. Ware LB, Conner ER, Matthay MA. von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. *Crit Care Med*. 2001;29(12):2325-2331.
29. Fukushima H, Nishio K, Asai H, *et al*. Ratio of von Willebrand factor propeptide to ADAMTS13 is associated with severity of sepsis. *Shock*. 2013;39(5):409-414.
30. Levi M, Scully M, Singer M. The role of ADAMTS-13 in the coagulopathy of sepsis. *J Thromb Haemost*. 2018;16(4):646-651.
31. Bockmeyer CL, Reuken PA, Simon TP, *et al*. ADAMTS13 activity is decreased in a septic porcine model. Significance for glomerular thrombus deposition. *Thromb Haemost*. 2011;105(1):145-153.
32. Camargo LF, Marra AR, Pignatari AC, *et al*. Nosocomial bloodstream infections in a nationwide study: comparison between solid organ transplant patients and the general population. *Transpl Infect Dis*. 2015;17(2):308-313.
33. Luzzaro F, Ortisi G, Larosa M, Drago M, Brigante G, Gesu G. Prevalence and epidemiology of microbial pathogens causing bloodstream infections: results of the OASIS multicenter study. *Diagn Microbiol Infect Dis*. 2011;69(4):363-369.
34. Peetermans M, Verhamme P, Vanassche T. Coagulase activity by *Staphylococcus aureus*: a potential target for therapy? *Semin Thromb Hemost*. 2015;41(4):433-444.
35. Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. *J Thromb Haemost*. 2011;9(6):1097-1107.
36. Bjerketorp J, Jacobsson K, Frykberg L. The von Willebrand factor-binding protein (vWbp) of *Staphylococcus aureus* is a coagulase. *FEMS Microbiol Lett*. 2004;234(2):309-314.
37. Vanassche T, Kauskot A, Verhaegen J, *et al*. Fibrin formation by staphylothrombin facilitates *Staphylococcus aureus*-induced platelet aggregation. *Thromb Haemost*. 2012;107(6):1107-1121.
38. Peetermans M, Liesenborghs L, Peerlinck K, *et al*. Targeting coagulase activity in *Staphylococcus aureus* bacteraemia: a randomized controlled single-centre trial of staphylothrombin inhibition. *Thromb Haemost*. 2018;118(5):818-829.
39. Verfaillie CJ, De Witte E, Devreese KM. Validation of a new panel of automated chemiluminescence assays for von Willebrand factor antigen and activity in the screening for von Willebrand disease. *Int J Lab Hematol*. 2013;35(5):555-565.
40. Hampson P, Dinsdale RJ, Wearn CM, *et al*. Neutrophil dysfunction, immature granulocytes, and cell-free DNA are early biomarkers of sepsis in burn-injured patients: a prospective observational cohort study. *Ann Surg*. 2017;265(6):1241-1249.
41. Dong JF, Whitelock J, Bernardo A, Ball C, Cruz MA. Variations among normal individuals in the cleavage of endothelial-derived ultra-large von Willebrand factor under flow. *J Thromb Haemost*. 2004;2(8):1460-1466.
42. Chignard M, Balloy V, Renesto P. Leucocyte elastase-mediated release of von Willebrand factor from cultured endothelial cells. *Eur Respir J*. 1993;6(6):791-796.
43. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004;104(1):100-106.
44. Cao WJ, Niiya M, Zheng XW, Shang DZ, Zheng XL. Inflammatory cytokines inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells. *J Thromb Haemost*. 2008;6(7):1233-1235.
45. Chen J, Fu X, Wang Y, *et al*. Oxidative modification of von Willebrand factor by neutrophil oxidants inhibits its cleavage by ADAMTS13. *Blood*. 2010;115(3):706-712.
46. Crawley JT, Lam JK, Rance JB, Mollica LR, O'Donnell JS, Lane DA. Proteolytic inactivation of ADAMTS13 by thrombin and plasmin. *Blood*. 2005;105(3):1085-1093.
47. Lam JK, Chion CK, Zanardelli S, Lane DA, Crawley JT. Further characterization of ADAMTS-13 inactivation by thrombin. *J Thromb Haemost*. 2007;5(5):1010-1018.
48. Peters RP, van Agtmael MA, Gierveld S, *et al*. Quantitative detection of *Staphylococcus aureus* and *Enterococcus faecalis* DNA in blood to diagnose bacteremia in patients in the intensive care unit. *J Clin Microbiol*. 2007;45(11):3641-3646.
49. Henry NK, McLimans CA, Wright AJ, Thompson RL, Wilson WR, Washington JA 2nd. Microbiological and clinical evaluation of the isolator lysis-centrifugation blood culture tube. *J Clin Microbiol*. 1983;17(5):864-869.
50. Corken A, Russell S, Dent J, Post SR, Ware J. Platelet glycoprotein Ib-IX as a regulator of systemic inflammation. *Arterioscler Thromb Vasc Biol*. 2014;34(5):996-1001.
51. Lerolle N, Dunois-Larde C, Badirou I, *et al*. von Willebrand factor is a major determinant of ADAMTS-13 decrease during mouse sepsis induced by cecum ligation and puncture. *J Thromb Haemost*. 2009;7(5):843-850.
52. Kasuda S, Matsui H, Ono S, *et al*. Relevant role of von Willebrand factor in neutrophil recruitment in a mouse sepsis model involving cecal ligation and puncture. *Haematologica*. 2016;101(2):e52-e54.
53. Vanderschueren S, De Weerdt A, Malbrain M, *et al*. Thrombocytopenia and prognosis in intensive care. *Crit Care Med*. 2000;28(6):1871-1876.
54. Patel KN, Soubra SH, Bellera RV, *et al*. Differential role of von Willebrand factor and P-selectin on microvascular thrombosis in endotoxemia. *Arterioscler Thromb Vasc Biol*. 2008;28(12):2225-2230.

55. Patel KN, Soubra SH, Lam FW, Rodriguez MA, Rumbaut RE. Polymicrobial sepsis and endotoxemia promote microvascular thrombosis via distinct mechanisms. *J Thromb Haemost*. 2010;8(6):1403-1409.
56. Chauhan AK, Walsh MT, Zhu G, Ginsburg D, Wagner DD, Motto DG. The combined roles of ADAMTS13 and VWF in murine models of TTP, endotoxemia, and thrombosis. *Blood*. 2008;111(7):3452-3457.
57. Allingstrup M, Wetterslev J, Ravn FB, Moller AM, Afshari A. Antithrombin III for critically ill patients: a systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med*. 2016;42(4):505-520.
58. Marti-Carvajal AJ, Sola I, Gluud C, Lathyris D, Cardona AF. Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients. *Cochrane Database Syst Rev*. 2012;12:CD004388.
59. Cai P, Luo H, Xu H, *et al*. Recombinant ADAMTS 13 attenuates brain injury after intracerebral hemorrhage. *Stroke*. 2015;46(9):2647-2653.
60. Zhao BQ, Chauhan AK, Canault M, *et al*. von Willebrand factor-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. *Blood*. 2009;114(15):3329-3334.
61. Denorme F, Langhauser F, Desender L, *et al*. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. *Blood*. 2016;127(19):2337-2345.
62. De Meyer SF, Savchenko AS, Haas MS, *et al*. Protective anti-inflammatory effect of ADAMTS13 on myocardial ischemia/reperfusion injury in mice. *Blood*. 2012;120(26):5217-5223.
63. Peyvandi F, Scully M, Kremer Hovinga JA, *et al*. Caplacizumab reduces the frequency of major thromboembolic events, exacerbations and death in patients with acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2017;15(7):1448-1452.


## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Peetermans M, Meyers S, Liesenborghs L, et al. Von Willebrand factor and ADAMTS13 impact on the outcome of *Staphylococcus aureus* sepsis. *J Thromb Haemost*. 2020;18:722–731. https://doi.org/10.1111/jth.14686