

THE ROLE OF ADAMTS13 IN THE COAGULOPATHY OF SEPSIS

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Abstract

The interaction between platelets and the vessel wall is mediated by various receptors and adhesive proteins, of which von Willebrand factor is most prominent. The multimeric size of von Willebrand factor is an important determinant of a more intense platelet-vessel wall interaction, and is regulated by the von Willebrand factor cleaving protease, ADAMTS13. A deficiency in ADAMTS13 leads to higher concentrations of ultra-large von Willebrand factor multimers and pathological platelet-vessel wall interactions, in its most typical and extreme form leading to thrombocytopenic thrombotic purpura (TTP), a thrombotic microangiopathy characterized by thrombocytopenia, non-immune haemolysis, and organ dysfunction. Thrombotic microangiopathy associated with low levels of ADAMTS13 may be a component of the coagulopathy observed in patients with sepsis. Here we review the potential role of ADAMTS13 deficiency and ultra-large von Willebrand factor multimers in sepsis, and their relationship with sepsis severity and prognosis. In addition, we discuss the possible benefit of restoring ADAMTS13 levels or reducing the effect of ultra-large von Willebrand multimers as an adjunctive treatment in patients with sepsis.

Keywords:

ADAMTS13, thrombotic microangiopathy, von Willebrand factor, sepsis, coagulation

Introduction

In normal circumstances platelets flow uninterrupted along the vascular endothelium without adhering or aggregating. However, upon disruption of the integrity of the vessel wall a rapid and multifaceted interaction between circulating platelets, endothelial cells and subendothelial structures occur.[1] As a result, platelets adhere to the vessel wall, are activated and form aggregates with each other, leading to microvascular obstruction and cessation of bleeding. This interaction between platelets and the vessel wall is mediated by cellular receptors on the platelet surface and endothelial cells, including integrins and selectins, and by adhesive proteins, such as von Willebrand factor and fibrinogen.

Von Willebrand factor is a large polymer of disulfide-linked subunits, reaching a molecular mass ranging from about 500 kDa to more than 10,000 kDa, which translates to coiled molecules up to 1250 nm long (thereby equaling the size of platelets). Larger von Willebrand factor multimers have much stronger hemostatic competency than smaller molecules as they contain relatively large number of the sites that mediate interactions between the endothelial cells, subendothelial matrix and platelet receptors.[2]

The size of von Willebrand factor multimers is regulated by ADAMTS13, also known as von Willebrand factor-cleaving protease. ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type I repeats-13) is a protease capable of cleaving ultra-large multimers of von Willebrand Factor (VWF), thereby decreasing VWF's prohemostatic properties.[3] Decreased levels of ADAMTS13 lead to a reduced capability to degrade ultra-large von Willebrand factor multimers. The ensuing increase in platelet-vessel wall interaction can lead to a thrombotic microangiopathy, clinically manifesting as a syndrome with multiple organ dysfunction, most prominently in the brain and kidneys, but potentially affecting all organs. Ongoing consumption of platelets will result in thrombocytopenia, and microvascular obstruction may cause red cell fragmentation (non-immune hemolytic anaemia).[4]

The most typical and extreme form of thrombotic microangiopathy is represented by thrombotic thrombocytopenic purpura (TTP), caused by an almost complete deficiency of ADAMTS13.[5] TTP is rare, with an annual incidence in the Western world of 2 per million.[6] It may be caused by a congenital deficiency in ADAMTS13 or acquired antibodies against the protease, the latter being most common. The trigger for the formation of auto-antibodies against ADAMTS13 is incompletely understood, but is hypothesized to be a combination of genetic predisposition and acquired factors, such as infection or pregnancy.[7] Other forms of thrombotic microangiopathy, such as haemolytic-uremic syndrome (HUS), or the thrombotic microangiopathy associated with malignant hypertension may also be

associated with lower levels of ADAMTS13.[8-10] However, this occurs to a more moderate extent and, in some cases, ADAMTS13 levels may be normal.[11]

Sepsis may be another clinical condition in which thrombotic microangiopathy may occur, and this is often associated with decreased levels of ADAMTS13.[12] We will describe how thrombotic microangiopathy can be a component of the coagulopathy that occurs in patients with sepsis, the role and prognostic significance of ADAMTS13 in sepsis and severe infection, as well as potential therapeutic implications.

Thrombotic microangiopathy in sepsis

Sepsis is now defined as a dysregulated host response to infection that results in life-threatening organ dysfunction.[13] Patients with sepsis almost always have coagulation abnormalities.[14] Hemostatic changes may range from subtle activation of coagulation that can only be detected by molecular assays for clotting factor activation to a more serious activation of coagulation that can be picked up by a drop in platelet count and slight elongation of global clotting assays.[15] In its most excessive form, overt disseminated intravascular coagulation (DIC) may occur, associated with diffuse microvascular thrombosis in small and moderate-size vessels and simultaneous widespread hemorrhage.[16, 17]

Patients with sepsis may also display signs of thrombotic microangiopathy, causing further consumption of platelets, and non-immune haemolysis. Indeed, the occurrence of schistocytes in the blood film is not uncommon in patients with sepsis. Thrombotic microangiopathy per se does not lead to excess thrombin generation and fibrin formation and will not affect levels of coagulation factors. However, since septic patients may present with a combination of DIC, characterized by platelet activation and fibrin generation, and thrombotic microangiopathy, it is difficult to distinguish between these mechanisms in practice.

Similar to other types of thrombotic microangiopathy, that associated with sepsis is caused by an enhanced platelet-vessel wall interaction. A crucial factor in the pathogenesis of this enhanced platelet-vessel wall interaction is thought to be the release of (ultra-large) von Willebrand factor multimers as a result of inflammation-induced endothelial cell perturbations. Von Willebrand factor is an acute phase protein that is markedly upregulated and released during systemic inflammation.[18]

In addition to very high levels of von Willebrand factor antigen and von Willebrand factor propeptide (indicating substantial release of the protein), ultra-large von Willebrand factor multimers are found in the blood of septic patients and correlate with disease severity. [19] Apart from playing a role as a ligand between platelets and the (sub)endothelium, ultra-large von Willebrand factor may also play a role in further attracting leucocytes to the injured endothelium, facilitating complement activation, and promoting adhesion of microorganisms

to the surface of the vessel wall.[20] Neutrophil extracellular traps (NETs) are released in response to infection and inflammation, with an association between disease severity and mortality.[21, 22] In TTP, NET levels are related to the platelet count and disease remission/exacerbation. Their role in local thrombosis, associated with platelet aggregation and systemic thrombosis is well documented. In TTP, treatment with ADAMTS 13 replacement during plasma exchange is associated with a reduction in detectable levels of NETs.[23]

The concentration of (ultra-large) von Willebrand factor multimers in patients with sepsis was inversely correlated with the plasma level of ADAMTS13. Several studies have also confirmed the association between low ADAMTS13 levels and sepsis severity (*q.v.*). [18, 19] Hypothetically, the inflammation-mediated massive release of von Willebrand factor from the endothelium consumes and depletes the available concentration of ADAMTS13, leading to insufficient cleavage capacity and control of von Willebrand factor multimeric size.[24] Other factors that may contribute to the reduction in plasma activity of ADAMTS13 in patients with sepsis are proteolytic cleavage by neutrophil elastase, thrombin, or plasmin (which are all being generated during sepsis), and inhibition of the metalloprotease by pro-inflammatory cytokines, such as interleukin (IL)-6. [25, 26] Furthermore, competitive inhibition of ADAMTS13 binding to von Willebrand factor caused by high levels of thrombospondin-1 during severe inflammatory states may (theoretically) contribute to the failure to adequately regulate cleavage of ultra-large von Willebrand factor multimers.[27]

Experimental studies lend evidence to the above described mechanisms. In a porcine *E. coli* sepsis model decreased levels of ADAMTS13 were related to increased ultra-large von Willebrand factor multimers in plasma and deposition of clots rich in von Willebrand factor and platelets in kidneys leading to tubular damage.[28] Interestingly, in von Willebrand factor deficient mice subjected to abdominal sepsis caused by cecal ligation and puncture, no reduction in ADAMTS13 levels was observed (in contrast to a marked reduction in wild-type mice), supporting the hypothesis that the ADAMTS13 deficiency in sepsis is due to consumption by massive release of ultra-large von Willebrand factor multimers.[29, 30] In addition, pretreatment of endotoxemic mice with inhibitors of neutrophil elastase or deficient in plasminogen attenuated the reduction of ADAMTS13, indicating that proteolytic cleavage of ADAMTS13 during systemic inflammation may play a role as well.[31]

ADAMTS13 levels in patients with sepsis

Several studies have focused on ADAMTS13 levels in patients with sepsis. Up to one third of patients with sepsis have ADAMTS13 levels that are <50% of normal.[18, 24, 32, 33] One study reported that about 15% of patients had severely reduced (<10%) ADAMTS13 levels,

however, it should be noted that this study was done in Japanese patients who have a relatively high frequency of the ADAMTS13 p.P475S polymorphism (allele frequency approximately 10%), which is urea-sensitive. As ADAMTS13 levels in this study were measured with a urea-based assay, these much reduced levels may have been spuriously low.[24] Studies in septic children also report decreased ADAMTS13 levels in the majority of cases, with the deficiency strongly correlating to a more severe coagulopathy.[34-36] Low levels of ADAMTS13 in sepsis are associated with reduced concentrations of cleaved von Willebrand factor.[12] Low levels of ADAMTS13 are also seen more frequently in patients with overt DIC and are strongly correlated to more severe renal insufficiency.[24, 37] The decrease in ADAMTS13 levels appears to be clearly associated with severity and increasing organ failure scores.[37] Interestingly, plasma levels of ADAMTS13 were significantly lower (mean levels 31%) in patients with sepsis compared to patients with other systemic inflammatory conditions with a non-infective aetiology (mean levels 56%).[38]

Malaria can also present with features of thrombotic microangiopathy. Malaria-infected patients have higher von Willebrand factor and lower ADAMTS13 levels. The proposed mechanism behind the drop in ADAMTS13 activity in malaria is endothelial activation caused by parasitized erythrocytes.[39, 40] In addition, free haemoglobin may interfere with von Willebrand factor multimeric size by oxidizing the ADAMTS13 cleavage site in von Willebrand factor.[41]

One study compared ADAMTS13 activity in patients suffering from severe or uncomplicated falciparum malaria in Bangladesh. In the severe malaria group (with a mortality rate of 23%) ADAMTS13 activity was statistically reduced (mean activity 23%) in comparison to uncomplicated malaria patients (mean activity 56%).[42] Similarly, a recent study demonstrated low levels of ADAMTS13 in patients infected with *Burkholderia pseudomallei* (melioidosis), a common cause of severe Gram negative infection in Asia.[43] Mean ADAMTS13 levels in these patients were 31% and were associated with enhanced von Willebrand factor release and high levels of ultra-large von Willebrand factor multimers.

Prognostic significance of ADAMTS13

A strong association is reported between the magnitude of decrease in ADAMTS13 levels in patients with sepsis and an adverse outcome. Significantly lower ADAMTS13 levels are seen at the time of intensive care admission in eventual non-survivors.[32, 35, 44] Patients with ADAMTS13 plasma concentrations $\leq 50\%$ had an approximate 10% higher risk of death compared with patients who present with no or only mild reduction in ADAMTS13 levels.[34, 45] One recent study demonstrated an approximate 50% lower survival rate in patients (mortality 59%) with septic shock and ADAMTS13 levels $< 30\%$, compared with patients

having higher levels of ADAMTS13 (mortality 28%).[46] The predictive value of ADAMTS13 deficiency for mortality was as powerful as APACHE II or similar risk scores.[47]

ADAMTS13 as a therapeutic target in sepsis

Microvascular thrombotic obstruction may be relevant in the pathogenesis of organ dysfunction in patients with sepsis it may be suggested that targeting platelet-vessel wall interaction in sepsis needs further exploration. In view of the central role of ultra-large von Willebrand factor multimers and the regulatory function of ADAMTS13 in regulating von Willebrand factor multimeric size, this may hypothetically be an interesting point of impact for a potentially effective (adjunctive) therapy in septic patients.[48] It should be emphasized, however, that so far there is no solid evidence indicating that restoration of ADAMTS13 and/or von Willebrand factor multimeric size will result in a clinically meaningful improvement of the outcome of patients with sepsis.

Currently, the cornerstone of treatment for ADAMTS13 deficiency in TTP is plasma exchange, which has clearly been shown to have a major benefit on survival.[5] The evidence base for plasma exchange in sepsis is however inconclusive.[49] [see across] Potentially, and in contrast to classical TTP, plasma administration rather than plasma exchange may be effective in sepsis as there is no need to remove an auto-antibody. However, the volume of plasma needed to sufficiently increase ADAMTS13 levels may be a limiting factor.

Recombinant human ADAMTS13 was tested in murine and rat models of thrombotic microangiopathy. In mice with targeted disruption of the ADAMTS13 gene and challenged with recombinant von Willebrand factor, the administration of recombinant ADAMTS13 attenuated tubular damage, resulted in less myocardial injury and reduced the number of animals with neurological symptoms.[50] Similarly, in a rat model of ADAMTS13 inhibition by a polyclonal antibody and administration of von Willebrand factor, treatment with recombinant ADAMTS13 prevented the occurrence of thrombocytopenia, haemolytic anaemia and formation of platelet/von Willebrand factor-rich clots in brain and kidneys.[51] In addition to recombinant ADAMTS13, gene therapy options have been explored. Recently, adeno-associated virus (AAV)-mediated expression of recombinant ADAMTS13 effectively regulated von Willebrand factor multimer size and protected ADAMTS13 knockout mice challenged with shiga toxin against thrombocytopenia and death.[52] It remains to be established, however, whether restoration of ADAMTS13 levels in sepsis would result in an improvement of clinically relevant outcomes.

An alternative to restoring ADAMTS13 levels is targeting ultra-large von Willebrand factor multimers. Agkisacucetin is a non-enzymatic platelet glycoprotein (GP) Ib receptor blocker

derived from snake venom.[53] It effectively inhibits binding of von Willebrand factor to platelets. It has a good antithrombotic potential in phase I clinical studies and is currently being evaluated in a randomised controlled phase II study in patients with acute coronary disease. So far, there is no documented experience, however, in experimental or clinical sepsis. Alternatively, caplacuzimab is a humanised nanobody aimed at the platelet GP1b binding site of von Willebrand factor.[54] Early human studies showed potent inhibition of platelet-aggregation; a phase II clinical trial in patients with TTP showed promising results despite a small increase in haemorrhagic complications.[41] Also this compound has not yet been evaluated in septic conditions. Lastly, von Willebrand factor-platelet binding can be affected by aptamers (oligonucleotides). However, aptamers have limited systemic bioavailability and very short elimination half-lives, which may hamper their clinical application.[55]

Targeting von Willebrand factor multimeric size can also be achieved by N-acetylcysteine (NAC) that is well known for its use as a mucolytic agent in lung disease. NAC reduces the disulfide bond within ultra-large von Willebrand factor multimers, thereby decreasing their platelet binding potential.[56] A possible mechanism is that ultra-large von Willebrand factor multimers bear a high resemblance to polymeric mucins. *In vitro* and *in vivo* experiments, using purified human VWF and injecting NAC in ADAMTS13^{-/-} as well as in wild-type mice, demonstrated a reduction in ultra-large von Willebrand factor multimers. Of note, administration of NAC resulted in significantly smaller thrombi in ADAMTS13-deficient mice, although it did not prevent all thrombus formation. A clinical trial evaluating the potential use of NAC in TTP is ongoing (<http://www.clinicaltrials.gov>; ClinicalTrials.gov Identifier: NCT01808521). A recent Cochrane systematic review did not, however, suggest any outcome benefit for NAC in sepsis.[57]

Conclusion

Increased platelet-vessel wall interaction, in its most extreme form manifesting as thrombotic microangiopathy, may be a relevant factor in the pathogenesis of sepsis and its related coagulopathy. Thrombotic microangiopathy in sepsis may be facilitated by ultra-large von Willebrand factor multimers that could be increased due to (secondary) ADAMTS13 deficiency. ADAMTS13 deficiency strongly correlates with the severity of sepsis and predicts an adverse outcome. Hypothetically, restoring ADAMTS13 levels and/or reducing ultra-large von Willebrand factor multimers may be effective as adjunctive treatment in patients with sepsis, however, there is no experimental or clinical evidence supporting that suggestion so far. Ultra-large von Willebrand factor multimers and ADAMTS13 may be potentially interesting targets for (adjunctive) treatment in patients with sepsis, either aiming at restoring ADAMTS13 levels or blocking platelet-von Willebrand factor inhibition. Eventually,

randomised controlled studies in patients with sepsis evaluating laboratory markers of thrombotic microangiopathy and clinically relevant outcomes, including organ dysfunction and mortality, are required to establish a role of restoration ADAMTS13 function in patients with sepsis.

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