

Role of von Willebrand Factor and ADAMTS-13 in the Pathogenesis of Thrombi in SARS-CoV-2 Infection: Time to Rethink

Nuccia Morici^{1,2} Maurizio Bottiroli³ Roberto Fumagalli^{3,4} Claudia Marini¹ Marco Cattaneo^{5,6}

¹ Intensive Cardiac Care Unit and De Gasperis Cardio Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

² Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy

³ Department of Anesthesiology, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

⁴ Università degli Studi Milano-Bicocca, Milan, Italy

⁵ Dipartimento di Scienze della Salute, Università Degli Studi di Milano, Milan, Italy

⁶ Unità di Medicina 2, ASST Santi Paolo e Carlo, Milan, Italy

Address for correspondence Nuccia Morici, MD, Dipartimento Cardioracovascolare, Intensive Coronary Care Unit and De Gasperis Cardio Center, ASST Grande Ospedale Metropolitano Niguarda, Piazza Ospedale Maggiore, 3, Milan 20162, Italy (e-mail: nuccia.morici@ospedaleniguarda.it).

Thromb Haemost

There is growing evidence supporting the idea that vascular occlusions in pulmonary and systemic circulation are among the most severe and common causes of poor outcome in COVID-19 patients.^{1–7} These vascular events appear to be mostly caused by local formation of thrombi, rather than by venous thromboembolism, which likely form as a consequence of a thromboinflammatory process, triggered by viral infection-induced endothelial damage and cytokine storm, with consequent activation of hemostasis leading to thrombus formation and boosting inflammation further.⁸ Among the several hemostasis players that are implicated in thromboinflammation, von Willebrand factor (VWF) has a leading role.^{9–11} VWF plays two important roles in normal hemostasis: it carries factor VIII and mediates platelet-vessel wall and platelet-to-platelet interaction, especially at high shear, through its binding to the platelet membrane glycoprotein (GP) Ib and GPIIb/IIIa.¹² VWF is a multimeric protein that is released into the circulation from endothelial stores in a highly thrombogenic form, characterized by the presence of ultra-large multimers. Under normal circumstances, these ultra-large multimers are cleaved by the protease ADAMTS-13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) and, consequently, the high thrombogenicity of released VWF is reduced.^{12–14} Conditions that are associated with congenital or autoantibody-induced ADAMTS-13 deficiency are characterized by increased incidence of thrombotic complications, which may be very severe and potentially fatal, as in thrombotic thrombocytopenic purpura (TTP).^{15,16} Acquired deficiency of ADAMTS-13 is associated

also with systemic disorders, including severe inflammatory diseases and sepsis.^{9,10,17} Considering that COVID-19 is a severe inflammatory disease, it is plausible that it is associated with acquired ADAMTS-13 deficiency and, hence, increased thrombogenicity of VWF.

To test this hypothesis, we measured ADAMTS-13, VWF levels, and other relevant tests in plasma from six patients admitted to the intensive care unit of ASST Grande Ospedale Metropolitano Niguarda in Milan, Italy. Patients' consent was obtained on admission. All tests were performed by means of commercial kits: in particular, VWF assays and ADAMTS-13 measurement were done by HemosIL AcuStar (Werfen, Milano, Italy). Main clinical characteristics and laboratory findings of the patients are reported in **Table 1**. Their mean (\pm standard deviation) age was 62 ± 5 years. Two patients had cardiovascular risk factors (**Table 1**). No thrombotic events were diagnosed in 5 patients, while subclavian and axillary veins thrombosis was detected in one; two patients had bleeding events: rectorrhagia and a bleeding complication after a tracheotomy procedure.

Factor VIII, D-dimer, fibrinogen, VWF:Ag (antigen), VWF:RCo (ristocetin cofactor activity), and VWF:CB (collagen-binding activity) were significantly increased in all patients, confirming the results of a previous study (with the exception of VWF:CB, which had not been measured).¹⁸ These results reflect a severe inflammatory state, as all the tested proteins are known to be acute phase reactants and D-dimer increases in inflammatory conditions. The original finding of our study is that 5 of the 6 tested patients had plasma

received
May 9, 2020
accepted
May 20, 2020

© Georg Thieme Verlag KG
Stuttgart · New York

DOI <https://doi.org/10.1055/s-0040-1713400>
ISSN 0340-6245.

Table 1 Clinical characteristics and main laboratory examinations of the six included patients

	Reference values	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age		63	65	54	59	65	68
Sex		Female	Male	Female	Male	Male	Male
Ethnicity		Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Hispanic
Hypertension		No	No	No	Yes	Yes	No
Diabetes		No	No	No	Yes	No	No
Prior cardiovascular disease		No	No	No	Yes	No	No
Prior antithrombotic treatments		No	No	No	No	No	No
ABO blood group		O	O	AB	O	A	O
Chronic kidney disease		No	No	yes	No	No	No
Clinical presentation							
Fever		Yes	Yes	Yes	Yes	Yes	Yes
Cough		Yes	No	Yes	No	Yes	No
Dyspnea		No	Yes	Yes	No	Yes	No
Gastrointestinal disorders		No	No	No	Yes	No	No
Syncope		No	Yes	No	Yes	No	No
Disease severity status		Critical	Critical	Critical	Critical	Critical	Critical
Invasive mechanical ventilation		Yes	Yes	Yes	Yes	Yes	Yes
In-hospital death		Yes	No	No	No	Yes	No
Cerebrovascular events		No	No	No	No	No	No
Deep vein thrombosis		No	No	No	No	No	Yes
Pulmonary artery occlusion		No	No	No	No	No	No
Acute coronary syndrome		No	No	No	No	No	No
Vascular occlusion in other districts		No	No	No	No	No	No
Bleeding events		Yes	No	No	No	No	Yes
Peak creatinine, mg/dL	0.5–1.0	2.9	2.7	3.1	1.0	1.1	2.2
Peak blood urea nitrogen, mg/dL	18–48	249	226	220	109	53	140
FVIII, U/dL	51–147	279	316	308	229	240	259
VWF:Ag, U/dL	40–165	772	735	568	455	763	511
VWF:RCo, U/dL	41–151	451	496	470	339	496	448
VWF:CB, U/dL	45–174	612	683	502	397	623	485
ADAMTS-13, %	45–138	24	37	30	56	44	33
Anti-ADAMTS-13, U/mL	< 12	< 12	< 12	< 12	Not tested	< 12	18
IL-6, pg/mL	< 7	7,152	Not tested	Not tested	42	90	Not tested
C-reactive protein, mg/dL	0–0.5	24	45	45	30	31	19

Abbreviations: ADAMTS-13, a disintegrin and metalloprotease with a thrombospondin type 1 motif, number 13; FVIII, factor VIII; IL-6, interleukin 6; VWF:Ag, von Willebrand factor antigen; VWF:CB von Willebrand collagen-binding activity; VWF:RCo, von Willebrand factor ristocetin cofactor activity.

ADAMTS-13 levels lower than 45%, the lower limit of the normal range: 3 of them had ADAMTS-13 levels around 30%, possibly preventing the effective cleavage of VWF multimers, thus favoring the formation of local thrombi in the circulation. Up to one-third of patients with sepsis have been shown to have ADAMTS-13 levels < 50%,¹⁷ while patients with septic shock and ADAMTS-13 levels < 30% had increased risk of mortality.¹⁹ Plasma levels of antibodies against

ADAMTS-13 were normal in our patients, with the exception of one, who displayed mild elevation of the antibody titer (► **Table 1**). This observation reinforces the hypothesis that low ADAMTS-13 levels in inflammation and sepsis are not caused by autoimmunity, but by consumption by the large amounts of VWF released in the circulation by endothelial cells, and/or by cleavage by cell-derived proteases.¹⁷ It is interesting to note that the VWF:RCo to VWF:Ag ratio was

slightly decreased in our COVID-19 patients, as it has already been shown in another study,¹⁸ while the VWF:CB to VWF:Ag ratio was close to unity. The correspondence between VWF activity tests and immunological determinations of VWF is probably not optimal when the amount of protein present is extremely high; the better correspondence with VWF:Ag that was displayed by VWF:CB, compared with VWF:RCo, is likely a reflection of the greater sensitivity of VWF:CB to very high molecular weight multimers,²⁰ which are likely increased in our patients with low ADAMTS-13 levels. Unfortunately, we could not measure VWF multimers directly.

The mild–moderate decrease in plasma ADAMTS-13 levels in COVID-19 patients is not expected to cause a thrombotic microangiopathy of similar severity as that observed in TTP, as testified also by the very mild reduction in platelet count displayed by our patients. However, it could contribute to the pathogenesis of thrombi. Indeed, the association of low ADAMTS-13 plasma levels and thrombosis has already been shown in previous studies: while the association with thrombotic microangiopathies different from TTP, myocardial infarction, and stroke is well established,²¹ that with venous thromboembolism is less clear.²² This is in accordance with the role of high molecular weight multimers of VWF in supporting platelet–vessel wall interaction especially at high shear rates that are encountered in the arterial circulation.^{12,23} These considerations open the question of what could be the most effective treatment of VWF-mediated thrombotic episodes in COVID-19. Heparin, whether unfractionated or low molecular weight, is likely the best therapeutic option to prevent venous thromboembolism and, as a matter of fact, is recommended for thromboprophylaxis in all critically ill medical patients at risk.²⁴ The question for COVID-19, which is characterized by a high degree of hypercoagulability,^{6,18} is whether doses of heparin higher than those generally recommended are necessary, which is being currently addressed by several randomized clinical trials (NCT04372589, NCT04367831, NCT04345848, NCT04366960). Heparin prophylaxis might also limit the severity of disseminated intravascular coagulation that is observed in COVID patients,⁶ albeit not very frequently. In contrast, heparin is unlikely the ideal treatment for microthrombosis in arterioles of the lungs and other organs of COVID patients,⁸ for which treatment aimed at restoring the balance between VWF and ADAMTS-13 (infusion of fresh-frozen plasma or recombinant ADAMTS-13 concentrate), at inhibiting VWF–platelet interaction (infusion of caplacizumab), or, more unspecifically, at inhibiting platelet function, could have more promising results. Other targets that play an important role in thromboinflammation should also be considered in COVID-19.⁸

Limitations

The clinical relevance of the results of our study is limited by its very small sample size. However, they are intended as hypothesis-generating only, supporting a pathophysiological concept that may foster further investigation of the throm-

boinflammatory process that characterizes COVID-19. A study enrolling a much larger patient cohort would be required to draw well-founded conclusions as to the potential pathogenic role of VWF/ADAMTS-13 unbalance in this disorder.

Conflict of Interest

None declared.

References

- 1 Wang T, Chen R, Liu C, et al. Attention should be paid to venous thromboembolism prophylaxis in the management of COVID-19. *Lancet Haematol* 2020;7(05):e362–e363
- 2 Wang J, Hajizadeh N, Moore EE, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): a case series. *J Thromb Haemost* 2020. Doi: 10.1111/jth.14828
- 3 Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost* 2020;18(05):1094–1099
- 4 Danzi GB, Loffi M, Galeazzi G, Gherbesi E. Acute pulmonary embolism and COVID-19 pneumonia: a random association? *Eur Heart J* 2020;41(19):1858
- 5 Rotzinger DC, Beigelman-Aubry C, von Garnier C, Qanadli SD. Pulmonary embolism in patients with COVID-19: time to change the paradigm of computed tomography. *Thromb Res* 2020;190:58–59
- 6 Thachil J, Tang N, Gando S, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost* 2020;18(05):1023–1026
- 7 Driggin E, Madhavan MV, Bikdeli B, et al. Cardiovascular considerations for patients, health care workers, and health systems during the coronavirus disease 2019 (COVID-19) pandemic. *J Am Coll Cardiol* 2020;75(18):2352–2371
- 8 Cattaneo M, Bertinato EM, Bircocchi S, et al. Pulmonary embolism or pulmonary thrombosis in COVID-19? Is the recommendation to use high-dose heparin for thromboprophylaxis justified?. *Thromb Haemost* 2020. Doi: 10.1055/s-0040-1712097
- 9 Chen J, Chung DW. Inflammation, von Willebrand factor, and ADAMTS13. *Blood* 2018;132(02):141–147
- 10 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(01):100–106
- 11 Denorme F, Vanhoorelbeke K, De Meyer SF. von Willebrand factor and platelet glycoprotein Ib: a thromboinflammatory axis in stroke. *Front Immunol* 2019;10:2884
- 12 South K, Lane DA. ADAMTS-13 and von Willebrand factor: a dynamic duo. *J Thromb Haemost* 2018;16(01):6–18
- 13 Plaimauer B, Zimmermann K, Völkel D, et al. Cloning, expression, and functional characterization of the von Willebrand factor-cleaving protease (ADAMTS13). *Blood* 2002;100(10):3626–3632
- 14 Dong JF, Moake JL, Nolasco L, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood* 2002;100(12):4033–4039
- 15 Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001;413(6855):488–494
- 16 Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med* 1998;339(22):1585–1594
- 17 Levi M, Scully M, Singer M. The role of ADAMTS-13 in the coagulopathy of sepsis. *J Thromb Haemost* 2018;16(04):646–651

- 18 Panigada M, Bottino N, Tagliabue P, et al. Hypercoagulability of COVID-19 patients in intensive care unit. A report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost* 2020. Doi: 10.1111/jth.14850
- 19 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(06):R273
- 20 Ng C, Motto DG, Di Paola J. Diagnostic approach to von Willebrand disease. *Blood* 2015;125(13):2029–2037
- 21 Masias C, Cataland SR. The role of ADAMTS13 testing in the diagnosis and management of thrombotic microangiopathies and thrombosis. *Blood* 2018;132(09):903–910
- 22 Calabrò P, Gagnano F, Golia E, Grove EL. von Willebrand factor and venous thromboembolism: pathogenic link and therapeutic implications. *Semin Thromb Hemost* 2018;44(03):249–260
- 23 Moake JL, Turner NA, Stathopoulos NA, Nolasco LH, Hellums JD. Involvement of large plasma von Willebrand factor (vWF) multimers and unusually large vWF forms derived from endothelial cells in shear stress-induced platelet aggregation. *J Clin Invest* 1986;78(06):1456–1461
- 24 Kahn SR, Lim W, Dunn AS, et al. Prevention of VTE in nonsurgical patients. *Antithrombotic Therapy and Prevention of Thrombosis, 9th: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines*. *Chest* 2012;141(02):e195S–e226S