Received: 22 June 2020 | Accepted: 8 July 2020
DOI: 10.1111/jth.15013

# COVID-19-associated coagulopathy—Hypothesis: Are children protected due to enhanced thrombin inhibition by higher $\alpha_2$-Macroglobulin macroglobulin (α2-M)?

We are overwhelmed by scientific publications on clinical observations, virology, and epidemiology of SARS-CoV-2 infections. There is a growing body of evidence that hypercoagulability complicates COVID-19, probably contributing to poor prognosis. This should stimulate further research into the pathogenesis of this procoagulant type of disseminated intravascular coagulation (DIC), and therapeutic options.[1]

Viral infections may have different effects on the coagulation system. While, eg, Marburg virus causes a severe bleeding phenotype, in COVID-19 particularly hypercoagulability with elevated D-dimer concentration and strong involvement of endothelium, vessels, and solid organs are evident. A higher risk for acquiring COVID-19 is associated with blood group A, which is connected with increased von Willebrand factor,[2] possibly enhancing hypercoagulability. This kind of disorder has been denoted as thromboinflammation,[3] with thrombin as a central pathophysiological player. In this scenario, the complex pathophysiology with interactions of the body´s innate immune response with different connected systems such as coagulation, fibrinolysis, kallikrein-kinin system, complement, and other mediators of immunity (particularly cytokine storm) are not fully understood. It is important to consider that a critical balance between (proteolytic) activation and natural inhibitor mechanisms normally regulates these systems and their interactions. For instance, in the coagulation system, natural inhibitors like antithrombin III (AT III), tissue factor pathway inhibitor (TFPI), and the thrombomodulin/protein C (PC) system should control activators like tissue factor and thrombin.[1]

The COVID-19 pandemic raises burning questions. For instance, children's role in the pandemic is still a puzzle. Several important observations in COVID-19[4,5] show age-dependent differences in the clinical course; children often have milder disease than adults do and deaths have been extremely rare. Whereas the attack rate in children appears to correspond to that in adults, it is obvious that children are less frequently diagnosed having lower susceptibility to infection, lower propensity to show clinical symptoms, or both.[6] The reasons for the relative resistance of children remain obscure. It was suggested that maturational changes; more active innate immune response; or differences in the distribution, maturation, and functioning of viral receptors may play a role.[6] Immune responses in adults are generally slower, less coordinated, and less efficient, making adults and particularly the elderly more susceptible to all infectious agents, due to a process denoted as immunosenescence.

Endothelial cells play a central role in inflammation and their alteration may trigger many involved mediator systems. The endothelial lesion in COVID-19 may thus culminate in a final, destructive phase of inflammation, sending a diverse army of cells and cytokines to fight invaders and mop up the debris of battle.[2] It might be reasonable to assume that children are more likely to possess a "healthier" immune response and endothelial cells than adults, particularly than older adults with hypertension, diabetes, or vascular diseases. However, is this the whole story concerning children's obviously more favorable course of COVID-19? We think, in order to understand this advantage, we have to explore more closely the complex interactions of the general host defense systems, particularly those against bleeding or thrombosis and pathogen invasion, because thrombin as key activator of blood coagulation, platelets, and endothelial cells may have profound impact on innate immunity.

What is different in children compared to adults and older people? There are age-related fluctuations in the physiological control circuits such as coagulation, fibrinolysis, and the complement system. The plasma level of the "versatile" and unique inhibitor α2-Macroglobulin (α2-M) is particularly very high in childhood, more than 200% higher compared to adults.[7-9] The coagulation system in children is quantitatively different from adults. Many questions remain about the true nature of the age-related differences in the proteins themselves, and how these differences are regulated has remained a total mystery.

Interestingly, α2-M is a phylogenetically very old inhibitor. All animals and plants have immune systems that protect them from a diversity of pathogens. In the plasma of vertebrates and members of several invertebrates, α2-M is an abundant protein.[10] It catches with its cage-like structure a great variety of activated proteins such as thrombin and immune mediators by "Venus flytrap" and "snap-trap" mechanisms,[11] thus keeping them away from their targets. Trapped peptidases are still active, but have restricted access to their substrates such as fibrinogen due to steric hindrance. While AT III is the predominant antithrombin, α2-M accounts for about 25% of the antithrombin activity of plasma, abolishing clotting activities of thrombin in the fibrinogen test, but not impairing its esterase activity with synthetic substrates.[12] α2-M regulates proteolysis in complex biological processes, such as nutrition, signalling, transport of hormones, and tissue remodelling, but also defends the host organism against external attacks. One might assume that a protein preserved over five hundred million years

should have a major function, and indeed, α2-M appears to be particularly important in contributing as well to thrombin inhibition and attenuation of immune reactions.

Background and trigger to our hypothesis is an old case report from 1973 of a family with hereditary AT III deficiency.[13] The 34-year-old father suffered from recurrent deep vein thromboses (DVT) and pulmonary embolisms. Examining immunological AT III in eight family members, we found a reduction to 45% in the father and to 50% in his 12-year-old son. The activity determined with the 1973 available two-step clotting-test "progressive antithrombin" was 50% in the father and in contrast 75%, ie, in the normal range, in the 12-year-old son. For α2-M, which is an additional antithrombin as mentioned above, an age-appropriate normal low value (301 mg/dL) was found in the father and an almost twice as high value of 541 mg/dL in the son. Two other young siblings also had high α2-M values of 723 and 540 mg/dL, and normal AT III values by both methods and we assumed a kind of "compensation mechanism." About 10 years later, the son experienced a DVT in his mid-20s. We made a similar observation of this "mechanism of compensation" in another family with hereditary AT III deficiency.

Comparing progressive antithrombin activity and plasma concentrations of three thrombin inhibitors (AT III, α2-M, heparin cofactor II), a strong positive correlation was found[14] in unselected adult patients and a selected low AT III activity patient group. The included three hereditary AT III deficient patients had a mean age of 41 years. The authors suggested that AT III is the main inhibitor in normal plasma and more effective than α2-M in inhibiting fibrin formation, but confirmed that α2-M or possibly other inhibitors might contribute to the total progressive antithrombin activity in human plasma. It is well known that the first thrombotic manifestation in hereditary AT III deficiency occurs usually not before the third decade of life, suggesting that there are protective mechanisms in place for the young. In adult patients with AT III deficiency, α2-M levels are high; however, the study of families did not include enough younger patients for evaluation. Nevertheless, there is good reason to assume that the lower risk of thromboembolic complications in AT III-deficient children may be in part due to the protective effect of elevated α2-M levels during childhood.[15,16]

Our hypothesis is that similarly during SARS-CoV-2 infection the higher α2-M level in childhood may contribute to the more favorable course of COVID-19 in children. Interestingly, α2-M has been identified on the luminal surface of endothelial cells,[17] and the localization of α2-M at the surface of the vessel wall suggests that this protease inhibitor may protect the vascular endothelium, which may be of particular importance in the "endothelitis" COVID-19.

This would lead us back to the question how we could control the hyper-procoagulant process in COVID-19. We feel that heparin is not enough to stop this process.[3] We would like to propose that we should take advantage of the fact that inhibitors can act in a cooperative way in order to counteract an overshooting activation of defense systems. If this is true, the patients would not need further activators, eg, fibrinolytics; rather, native proteins and inhibitors, such as AT III, PC, and maybe even α2-M could be used. There is an important difference to inherited coagulation defects like hemophilia, where very low levels of factor VIII are sufficient to prevent spontaneous bleeding. COVID-19, like acute respiratory distress syndrome (ARDS), is a dynamic process with high turnover and critical importance of the abovementioned native proteins, for which lower normal levels might be not sufficient. Having in mind the significance of any progress in treating COVID-19, but also the old, huge, and still unresolved problem of treating inflammatory DIC, eg, in sepsis and ARDS, killing many more people than COVID-19 so far has, further research is urgently warranted.

## CONFLICTS OF INTEREST

No authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

W. Schramm wrote the first draft; all authors contributed to the concept, literature search, and conclusions, and edited the final version.

Wolfgang Schramm[1]
Rainer Seitz[2]
Lutz Gürtler[3]

[1]*Rudolf Marx Stiftung, Ludwig-Maximilians University (LMU), Munich, Germany*
[2]*Paul-Ehrlich-Institut, Langen, Germany*
[3]*Max von Pettenkofer Institut, Ludwig-Maximilians University (LMU), Munich, Germany*

**Correspondence**
Wolfgang Schramm, Rudolf Marx Stiftung, Ludwig-Maximilians University (LMU), Munich, Germany.
Email: Wolfgang.Schramm@med.uni-muenchen.de

## REFERENCES

1. Seitz R, Schramm W. DIC in COVID-19: Implications for prognosis and treatment? *J Thromb Haemost*. 2020;18(7):1798-1799.
2. Schleef M, Strobel E, Dick A, et al. Relationship between ABO and Secretor genotype with plasma levels of factor VIII and von Willebrand factor in thrombosis patients and control individuals. *Brit J Haematol*. 2004;128:100-107.
3. JacksonSP,DarboussetR,SchoenwaelderSM.Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. *Blood*. 2019;133:906-918.
4. Ludvigson JF. Systematic review of covid-19 in children shows milder cases and a better prognosis than adults. *Acta Paediatr*. 2020;109(6):1088-1095.
5. Lu X, Zhang L, Du H, et al. SARS-Cov-2 Infection in children. *N Engl J Med*. 2020;382:1663-1665.
6. Davies NG, Klepac P, Liu Y, et al. Age-dependent effects in the transmission and control of COVID-19 epidemics. *Nature Med*. 2020. https://doi.org/10.1038/s41591-020-0962-9
7. Ganrot PO, Schersten B. Serum Alpha 2 Makroglobulin concentration and its variation with age and sex. *Clin. Chim. Acta*. 1967;15:113-120.
8. Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O. Reference distributions for a2-macroglobulin: a comparison of a large cohort to the world's literature. *J Clin Lab Analysis*. 2004;18:148-152.

9. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. *Blood*. 1992;80(8):1998-2005.
10. Armstrong PB, Quigley JP. Alpha-2-makroglobulin: an evolutionary conserved arm of the innate immune system. *Dev Comp Immunol*. 1999;23(4-5):275-290.
11. Garcia-Ferrer I, Marrero A, Gomis-Rüth FX, Goulas T. α2-Macroglobulins: structure and function. In: Harris J, Marles-Wright J, eds. *Macromolecular protein complexes. Subcellular biochemistry* (vol 83). Cham, Switzerland: Springer; 2017:149–174.
12. Rinderknecht H, Geokas MC. Role for alpha 2 makroglobulin in haemostatic balance. *Nat New Biol*. 1972;239:116-117.
13. Schramm W, Fateh A, Marx R. Hereditärer antithrombin III Mangel. In: editiones Roche Basel, ed. *Thrombophilie: XVIII. Hamburger symposium über Blutgerinnung*. Grenzach-Wyhlen, Germany: Hoffman-La Roche; 1975.
14. Abildgaard U, Fagerhol K, Egeberg O. Comparison of progressive antithrombin activity and the concentrations of three thrombin inhibitors in human plasma. *J Clin Lab Investig*. 1970;26:349-354.
15. Kremers R, Bloemen S, Al Dieri R, et al. Alpha-2-macroglobulin is a major determinant of a lower thrombin generation in infants and children compared to adults. *Blood*. 2013;122(21):2344.
16. Mitchell L, Piiovella F, Ofosu F, Andrew M. Alpha 2 Makroglobulin may provide protection from thromboembolic events in Antithrombin III deficient children. *Blood*. 1991;78:2299-2304.
17. Becker CG, Harpel PC. α2-Makroglobulin on human vascular endothelium. *J Exp Med*. 1976;144:1-9.

Received: 2 June 2020 | Accepted: 4 June 2020
DOI: 10.1111/jth.14965

# Letter in response to article "Systematic review of viscoelastic testing (TEG/Rotem) in obstetrics and recommendations from the women's SSC of the ISTH"

To the Editors,

We read with interest this article giving recommendations for using TEG/Rotem technology in obstetrics. While this systematic review comments on the variability that can be found in these tests, we feel it does not emphasize strongly enough the essential component of quality control for these tests.

Amgalan et al[1] quoted data from our earlier published studies[2] and noted that the use of lyophilized plasma for external quality assessment (EQA) rather than whole blood may explain the lack of consistency shown in our studies as the devices are not designed to assess plasma. We have demonstrated previously that where plasma is the usual testing matrix, lyophilized material used in our EQA programs is commutable,[3] however we acknowledge that point of care (POC) testing is generally designed to test fresh whole blood and it is not possible to provide like for like material for EQA exercises for POC devices. It is therefore more difficult to quality control POC testing but we can use lyophilized samples to demonstrate test precision and to compare centers' results. We cannot however use lyophilized material to ensure accuracy. The basic premise of EQA is that if the same sample is tested by all users similar results should be achieved and limits for this can be applied. The UK National External Quality Assessment Scheme for Blood Coagulation (NEQAS BC) has been providing an EQA program for Rotem delta and TEG5000 devices for more than 5 years. To our knowledge only UK NEQAS BC, ECAT Foundation which is the Dutch EQA program and CAPs (College of American Pathologists) the U.S. EQA program provide EQA for these devices across the globe and all use lyophilized plasma.

UK NEQAS BC provides lyophilized citrated plasma samples for these devices as fresh whole blood is insufficiently stable for distributing samples to all our users across the UK and in Europe. TEG/Rotem tests require the presence of red cells and platelets to achieve the clot firmness parameters to be fully generated. However, the use of plasma, which obviously lacks red cells, and platelets does allow the assessment of the initial burst of coagulation which gives the early parameters of reaction time (R time) in TEG devices and clotting time (CT) in Rotem technology. The TEG 5000 requires pipetting of the patients' (or EQA) samples and the Rotem delta requires pipetting of samples and reagents for these tests. Recent advances from both manufacturers have seen the introduction of the Rotem Sigma and TEG6, which are both cartridge-based technology. These new technologies do not require any pipetting and there is a suggestion that this new approach should improve precision of testing and this would be evident in a reduction in the coefficients of variance (CVs) of these tests. UK NEQAS BC has introduced an EQA program for Rotem Sigma and the TEG6 devices. Again, the sample type is lyophilized citrated plasma and the EQA assessment is applied to the R time for TEG6 and the CT for Rotem Sigma.

The EQA program we provide involves centers testing one sample per survey and three surveys per year. We ask users to test for 30 minutes and if no clotting is achieved in this time they should return results of "flatline" or "no clotting achieved." The sample types provided have been either from a normal donor or normal donor plasma spiked with

Manuscript handled by: David Lillicrap
Final decision: David Lillicrap, 4 June 2020