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Letter to the Editors-in-Chief



Thrombosis post COVID-19 vaccinations: Potential link to ACE pathways

To the editor,

We read with interest the recent report which described an absence of hypercoagulable state in healthy volunteers receiving the BNT162b2 mRNA SARS-CoV-2 vaccination [1].

“Campello E, Simion C, Bulato C, et al. Absence of hypercoagulability after nCoV-19 vaccination: An observational pilot study [published online ahead of print, 2021 Jun 25]. *Thromb Res.* 2021;205:24-28. doi:<https://doi.org/10.1016/j.thromres.2021.06.016>”

Cerebral vein sinus thrombosis has been reported in SARS-CoV-2 infection and is associated with thrombocytopenia [2]. It has also been reported as a rare adverse effect after ChAdOx1 vaccination (AstraZeneca) with an incidence of 0.22–1.75 per 100,000 person-years with a slightly higher incidence in women [3].

Recently, a case series was reported in relationship with the BNT162b2 mRNA SARS-CoV-2 vaccination [4]. The authors postulated mechanisms such as very high spike protein levels, a high number of activated platelets and aberrant complement activation which in rare unison, results in thrombosis.

We would like to highlight some key features of the mRNA vaccines which would protect from thrombosis compared to getting COVID-19 infection. One of the key features is that the spike protein produced by the mRNA vaccines are trans-membrane anchored [5] and not released into the circulation like in active SARS-CoV-2 infection and hence does not have the potential to interact with systemic endothelial cells. Moreover, the spike protein produced through these vaccines have proline mutations which affects its capability to bind to ACE2 as is unable to adapt to its shape [5]. The immune response with production of neutralizing antibodies against the spike protein will also be protective against any systemic effects of spike protein interactions with ACE2.

The higher risk for CVT after ChAdOx1 vaccination could be associated with the production of the wild-type spike protein. However, in these cases a mechanism similar to autoimmune heparin-induced thrombocytopenia (HIT) has been proposed [6], rather than a direct effect of spike protein. Spike protein driven mechanisms in these cases include cross reactivity of the anti-spike antibody produced with platelets, or adenoviral vector entry into the platelets with an aberrant expression of spike protein on platelet surface [6].

It has also recently been seen that there is an imbalance between venous and arterial thrombosis in mRNA vaccines (25–30% venous and 70–75% arterial) which is not seen with ChAdOx1 vaccination (52% arterial, 48% venous) [7].

A direct systemic interaction of the spike protein with ACE2 receptors on the platelets cannot be ruled out. A recent report described the existence of ACE2 receptors and TMPRSS2, a serine protease required for protein priming on platelets. This suggests that binding of the spike protein to platelet ACE2 could trigger platelet activation and formation of leukocyte-platelet aggregates [8].

One of the possible reasons for this systemic interaction could be genetic variants of the ACE2 receptor which make it more sensitive to interactions with the spike protein. Genetic variants of the ACE2 receptor (K26R, T92I) have been reported to increase binding to the spike protein. The recombinant K26R and T92I mutant ACE2 protein has a higher affinity for the spike protein-receptor binding domain through its effect on N90-linked glycan and N-glycosylation, respectively [9].

In patients with ACE2 polymorphisms, the higher risk of thrombosis could also be secondary to inhibition of ACE2 (due to stronger link with spike protein) in the renin-angiotensin-system which would lead to unopposed activation of ACE1 pathways and formation of Angiotensin II (Ag II). Angiotensin II-induced hypertension is accompanied by enhanced thrombosis in microvascular arterioles mediated through angiotensin (AT), AT2 receptor (onset of thrombosis) and AT4 receptor (flow cessation). Besides the activation of AT1 receptor which leads to atherothrombosis, activation of AT2 and AT4 receptor pathways leads to microvascular thrombosis [10] (Fig. 1).

Several factors can increase the expression of ACE2, including hypertension, diabetes and obesity, which also could increase the severity of COVID-19 infection.

Genetic polymorphisms affecting the expression of ACE can be a contributing factor too. For example, ACE polymorphisms, insertion, allele I or deletion, allele D of a 287-base pair Alu repeat sequence in intron 16 have been described. The D/D homozygotes have 65% higher ACE levels, I/D heterozygotes 31% more ACE when compared to I/I homozygotes [11]. The D/D homozygotes lead to a higher risk of COVID-19 related infection [11], pulmonary embolism [12] and mortality [13]. The presence of ACE D/D polymorphism would lead to higher availability of ACE relative to ACE2 and this difference will be exaggerated in the presence of spike proteins interacting with ACE2. This could lead to an unopposed activation of the renin-angiotensin-aldosterone system favouring the generation of angiotensin II (AgII), with its deleterious downstream effects on AT1R, leading to hypertension and macrovascular thrombosis. Additionally, angiotensin II is known to lead to microvascular thrombosis through activation of platelet activation, aggregation and thrombosis pathways through its effects on AT2R and AT4R [10] (Fig. 1).

To understand the putative susceptibility factors of thrombosis with these vaccines, we recommend evaluation of the direct impact of the spike 2

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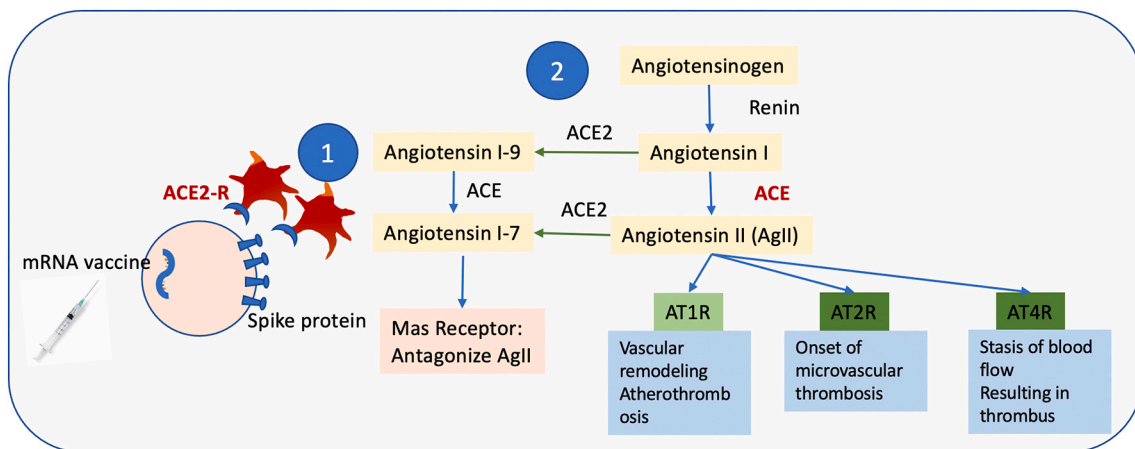


Fig. 1. Figure showing the likely mechanisms of increased susceptibility to thrombosis: 1. ACE2 receptor mutation leading to increased sensitivity to interaction with spike protein and a decrease in AgII-Mas pathway with unopposed AgII-AT1-4R receptor signaling; 2. ACE receptor mutation leading to higher propensity towards activation of the AgII pathway relative to Mas receptor and downstream activation of AgII Type 1 (AT1R), Type 2(AT2R) and Type 4 receptors (AT4R) leading to higher propensity for macrovascular (AT1R) and microvascular thrombosis (AT2 & AT4).

protein on the platelets, the renin-angiotensin-system and thrombosis pathways. The impact of ACE and ACE2 genetic polymorphisms on these systems and the risk of thrombosis need further evaluation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] E. Campello, C. Simion, C. Bulato, et al., Absence of hypercoagulability after nCoV-19 vaccination: an observational pilot study, *Thromb. Res.* 205 (2021) 24–28, <https://doi.org/10.1016/j.thromres.2021.06.016>.
- [2] D.D. Cavalcanti, E. Raz, M. Shapiro, S. Dehkharghani, S. Yaghi, K. Lillemo, E. Nossek, J. Torres, R. Jain, H.A. Riina, A. Radmanesh, P.K. Nelson, Cerebral venous thrombosis associated with COVID-19, *AJNR Am. J. Neuroradiol.* 41 (2020) 1370–1376.
- [3] J B Schulz P Berlitz HC Diener , et al, the DGN SARS-CoV-2 Vaccination Study Group. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv2021.04.30.21256383; doi: 10.1101/2021.04.30.21256383.
- [4] B.E. Fan, J.Y. Shen, X.R. Lim, T.M. Tu, C.C.R. Chang, H.S.W. Khin, J.S. Koh, J.P. Rao, S.L. Lau, G.B. Tan, Y.W. Chia, K.Y. Tay, S. Hameed, T. Umapathi, K.H. Ong, B.M.R.V. Prasad, Cerebral venous thrombosis post BNT162b2 mRNA SARS-CoV-2 vaccination: a black swan event, *Am. J. Hematol.* (2021 Jun 16), <https://doi.org/10.1002/ajh.26272>.
- [5] K.S. Corbett, D.K. Edwards, S.R. Leist, et al., SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness, *Nature* 586 (2020) 567–571, <https://doi.org/10.1038/s41586-020-2622-0>.
- [6] D. Tsilingiris, N.G. Vallianou, I. Karampela, M. Dalamaga, Vaccine induced thrombotic thrombocytopenia: the shady chapter of a success story, *Metabol. Open.* 11 (2021 Sep), 100101, <https://doi.org/10.1016/j.metop.2021.100101>.
- [7] D.M. Smadja, Q.Y. Yue, R. Chocron, O. Sanchez, Louet A. Lillo-Le, Vaccination against COVID-19: insight from arterial and venous thrombosis occurrence using data from VigiBase, *Eur. Respir. J.* 58 (2021) 2100956, <https://doi.org/10.1183/13993003.00956-2021>.
- [8] S. Zhang, Y. Liu, X. Wang, et al., SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19, *J. Hematol. Oncol.* 13 (2020) 120, <https://doi.org/10.1186/s13045-020-00954-7>.
- [9] K. Suryamohan, D. Diwanji, E.W. Stawiski, et al., Human ACE2 receptor polymorphisms and altered susceptibility to SARS-CoV-2, *Commun. Biol.* 4 (2021) 475, <https://doi.org/10.1038/s42003-021-02030-3>.
- [10] E.Y. Senchenkova, J. Russell, L.D. Almedia-Paula, et al., Angiotensin II-mediated microvascular thrombosis, *Hypertension* 56 (2010) 1089–1095.
- [11] H. Zheng, J.J. Cao, Angiotensin-converting enzyme gene polymorphism and severe lung injury in patients with coronavirus disease, *Am. J. Pathol.* 190 (2020) 2013–2017, <https://doi.org/10.1016/j.ajpath.2020.07.009>.
- [12] C. Calabrese, A. Annunziata, A. Coppola, P.C. Pafundi, S. Guarino, V. Di Spirito, V. Maddaloni, N. Pepe, G. Fiorentino, ACE gene I/D polymorphism and acute pulmonary embolism in COVID19 pneumonia: a potential predisposing role, *Front Med (Lausanne)* 7 (2021) 631148, <https://doi.org/10.3389/fmed.2020.631148>.
- [13] M. Bellone, S.L. Calvisi, ACE polymorphisms and COVID-19-related mortality in Europe, *J. Mol. Med. (Berl.)* 98 (2020) 1505–1509.

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