

# Spike Sars-COV-2 Protein as Procoagulant Factor and Vaccine Class Effect Hypotesys

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## Abstract

This work is written in actual situation by which some covid vaccine are under deeply investigation related some rare cases of thrombosis. It is really interesting to observe the literature that have reported in first and second – third wave of covid-19 disease a relationship with increase thrombosis in the most severe patients. So it is possible to say that there is a procoagulant property of covid-19 virus. But this property is related to all virus particle or it can be mediated or due by the spike-protein? And if there is this relationship it was a real good feature di project vaccine based on this protein?

To investigate in this direction can be a good instrument to better understand some unclear aspect of this Vaccine rapid production.

**Keywords:** Thrombosys • COVID-19• Spike-protein • Procoagulant factor • Epidemiology • Incidence • Vaccine • Class effect • Screening procedure pre vaccination.

## Introduction

Usually many years are needed to project and test a new vaccine but to fight the actual dangerous covid-19 pandemia was needed to introduce new vaccine or gene manipulation product with similar effect in a reduced time (like about 1 year). This methodology is not so good to verify the effect in long time (5-10 year), and also for some kind of vaccine other problems seem to contrast this vaccine campaign.

Observing the media and also some scientific literature some rare cases of thrombosis is reported and also some countries of advanced nations stopped this asking to EMA clarify in order to start. What it is interesting is that among the many kind of thrombosis it was observed in some cases a particular Kind of the pathology: cerebral venous sinus thrombosis [1]. So the question is why this form of pathology is more common?

Thrombosis is pathology especially of the elderly and its incidence increase with the age. It can be due by genetic or autoimmune disorder. We must remember also that neoplastic disorder associated with increase of TNF and increase of procoagulant production. RNA virus cell infection produce

stimulation of the so called innate immune sensor with hyperactivation of the immune response Interleukins (ILs), cytokine, TNF and other mediator of inflammation and with clotting disorder. In addition of the therapeutic use of heparin in severe COVID 19 with clotting problem

According to Augusto Di Castelnuovo et al. 2021 Heparin in COVID-19 Patients is associated with reduced hospital mortality: the Multicenter Italian CORIST Study; "In-hospital heparin treatment was associated with a lower mortality, particularly in severely ill COVID-19 patients and in those with strong coagulation activation. The results from randomized clinical trials are eagerly awaited to provide clear-cut recommendations. "But this effect due by the coronavirus is linked to the SPIKE protein? [2]. and if yes it was a good thing to project virus vaccine based on this protein?

Is there a class effect for the vaccine involved with this mechanism?

## Materials and Methods

With an observational effects some relevant biomedical literature from PUBMED or other database of open literature is analyzes.

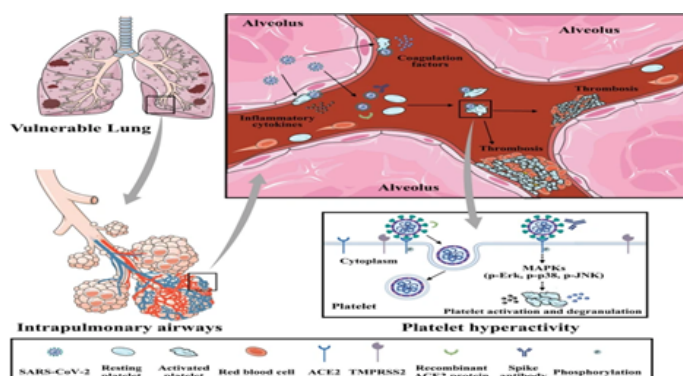
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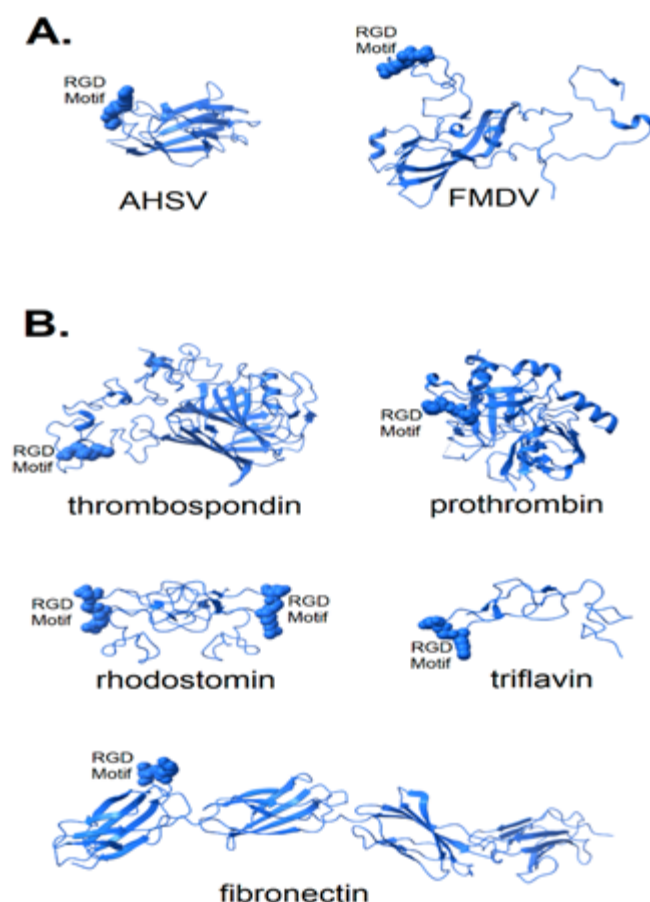
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Some Figures 1-2 presented help to show the meaning of the text.

An experimental project hypothesis submitted in order to produce a global conclusion.



**Figure 1.** Short diagrams showing that SARS-CoV-2 activates platelets and promotes blood clotting in COVID-19. A global diagram showing SARS-CoV-2 from alveoli binds and activates platelets, which promotes thrombosis and inflammatory response in capillaries, thus contributing to the development of diffuse intravascular coagulation and acute respiratory distress syndrome. The -CoV-2 spike protein binds to ACE2 and ACE2 phosphorylate, which leads to activation of MAPK signal transmission (Erk SARS phosphorylation, p-38 and JNK), subsequent platelet activation, and release of coagulation factors and secretion of inflammatory cytokines. The interaction between SARS-CoV-2 spike protein and platelet ACE2 leads to platelet activation which is suppressed by recombinant human ACE2 protein and monoclonal anti-spike antibodies. Modified of Si Zhang et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19



**Figure 2.** Structure of known integrin-binding proteins: (A) Virion proteins known to bind integrins through an RGD motif (shown in space-fill) include

(right) foot and mouth disease virus capsid protein (5neu—this RGD motif is highly flexible prior to integrin-binding, but structurally stabilized when bound to integrin image is from a co-crystal of the capsid protein and integrin with integrin structure removed to make visible the RGD domain); (left) African horse sickness virus (1ahs top domain of capsid protein VP7). (B) Other proteins known to bind integrin through an RGD motif: thrombospondin (1ux6); prothrombin (3u69); rhodostomin (4rqg) and triflavin (1j2l) are disintegrins, small toxins from snake venom with high affinity to integrins; and fibronectin (1fnf—domains 6–10) an extracellular matrix protein with an integrin-binding RGD motif in its 10th domain. Figure 2: Structure of known integrin-binding proteins: (A) Virion proteins known to bind integrins through an RGD motif (shown in space-fill) include (right) foot and mouth disease virus capsid protein (5neu—this RGD motif is highly flexible prior to integrin-binding, but structurally stabilized when bound to integrin image is from a co-crystal of the capsid protein and integrin with integrin structure removed to make visible the RGD domain); (left) African horse sickness virus (1ahs top domain of capsid protein VP7). (B) Other proteins known to bind integrin through an RGD motif: thrombospondin (1ux6); prothrombin (3u69); rhodostomin (4rqg) and triflavin (1j2l) are disintegrins, small toxins from snake venom with high affinity to integrins; and fibronectin (1fnf—domains 6–10) an extracellular matrix protein with an integrin-binding RGD motif in its 10th domain.

## Results

According to Si Zhang et al: “SARS-CoV-2 and its Spike protein directly enhanced platelet activation such as platelet aggregation, PAC-1 binding, CD62P expression,  $\alpha$  granule secretion, dense granule release, platelet spreading and clot retraction in vitro, and thereby spike protein enhanced thrombosis formation in wild-type mice transfused with hACE2 transgenic platelets, but this was not observed in animals transfused with wild-type platelets in vivo. Further, we provided evidence suggesting that the MAPK pathway, downstream of ACE2, mediates the potentiating role of SARS-CoV-2 on platelet activation, and that platelet ACE2 expression decreases following SARS-COV-2 stimulation. SARS-CoV-2 and its Spike protein directly stimulated platelets to facilitate the release of coagulation factors, the secretion of inflammatory factors, and the formation of leukocyte-platelet aggregates” [3].

According to K A Bauer et al. “These studies demonstrate that TNF is able to provide a substantial net procoagulant stimulus to the hemostatic mechanism, and suggest that this cytokine may be a mediator of certain hypercoagulable states in humans” [4].

Mahmoud B Malas et al state that “Thrombo Embolism rates of COVID-19 are high and associated with higher risk of death. Robust evidence from ongoing clinical trials is needed to determine the impact of thromboprophylaxis on TE and mortality risk of COVID-19” [5].

According to European Medicine Agency Pharmacovigilance Risk Assessment Committee (PRAC). Related covid disease: EMA report on Coagulation abnormalities in context of COVID-19 2021-03-12 (last update) Information overview v1.2 Mechanistic and pathophysiological aspects “The local mediator and link between inflammation, coagulation abnormalities and microthrombosis is reported to be neutrophil extracellular traps (NETs), in a process dubbed NETosis (reports predate COVID-19). This mediation occurs at both levels, local (microvascular) and systemic (macrovascular), for reasons including that NETs can enter vessels and cause platelet activation (through Toll-like receptors, TLR), factor V activation and thrombin generation. NETs are large extracellular, web-like structures of former cytosolic and granule proteins that assemble on a scaffold of chromatin, and these have been described to kill extracellular pathogens (Lee and Grinstein 2004)” [6].

Lee Makowski et al. “Although ACE2 angiotensin converting enzyme (2) is considered the primary receptor for CoV-2 cell entry, recent reports suggest that alternative pathways may contribute. This paper considers

the hypothesis that viral binding to cell-surface integrins may contribute to the high infectivity and widespread extra-pulmonary impacts of the SARS-CoV-2 virus. This potential is suggested on the basis of the emergence of an RGD (arginine-glycine-aspartate) sequence in the receptor-binding domain of the spike protein. RGD is a motif commonly used by viruses to bind cell-surface integrins. Numerous signaling pathways are mediated by integrins and virion binding could lead to dysregulation of these pathways, with consequent tissue damage. Integrins on the surfaces of pneumocytes, endothelial cells and platelets may be vulnerable to CoV-2 virion binding. For instance, binding of intact virions to integrins on alveolar cells could enhance viral entry. Binding of virions to integrins on endothelial cells could activate angiogenic cell signaling pathways; dysregulate integrin-mediated signaling pathways controlling developmental processes; and precipitate endothelial activation to initiate blood clotting. Such a procoagulant state, perhaps together with enhancement of platelet aggregation through virions binding to integrins on platelets, could amplify the production of microthrombi that pose the threat of pulmonary thrombosis and embolism, strokes and other thrombotic consequences. The susceptibility of different tissues to virion integrin interactions may be modulated by a host of factors, including the conformation of relevant integrins and the impact of the tissue microenvironment on spike protein conformation. Patient-specific differences in these factors may contribute to the high variability of clinical presentation. There is danger that the emergence of receptor-binding domain mutations that increase infectivity may also enhance access of the RGD motif for integrin binding, resulting in viral strains with ACE2 independent routes of cell entry and novel integrin-mediated biological and clinical impacts. The highly infectious variant, B.1.1.7 (or VUI 202012/01), includes a receptor-binding domain amino acid replacement, N501Y, that could potentially provide the RGD motif with enhanced access to cell-surface integrins, with consequent clinical impacts" [7].

"There has been increasing reports associating the coronavirus disease 2019 (COVID-19) with thromboembolic phenomenon including ischemic strokes and venous thromboembolism. Cerebral venous thrombosis (CVT) is a rare neurovascular emergency that has been observed in some COVID-19 patients. Nine studies and 14 COVID-19 patients with CVT were studied. The median age was 43 years (IQR=36-58) and majority had no significant past medical conditions (60.0%). The time taken from onset of COVID-19 symptoms to CVT diagnosis was a median of 7 days (IQR=6-14). CVT was commonly seen in the transverse (75.0%) and sigmoid sinus (50.0%); 33.3% had involvement of the deep venous sinus system. A significant proportion of patients had raised D-dimer (75.0%) and CRP levels (50.0%). Two patients reported presence of antiphospholipid antibodies. Most patients received anticoagulation (91.7%) while overall mortality rate was 45.5%" [8].

Prof David Werring, Professor of Clinical Neurology, UCL Institute of Neurology, UCL, said "Although the number of people diagnosed in the UK with cerebral venous thrombosis after receiving the Astra Zeneca COVID-19 vaccine has increased to 22 cases, this is among 18 million receiving the vaccine. So the absolute risk of CVST after this vaccine remains extremely low (about 1.5 per million) and it's not clear if this is any higher than the usual expected incidence of CVST (probably around 5 to 15 per million people per year, though the figures vary as it can be difficult to diagnose in some cases).

"However, emerging evidence suggests that some cases of post-vaccination CVST have unusual features, including low blood platelets, male sex (CVST is typically more common in females), a usually rare type of antibody to platelet-factor 4 (PF-4), and a high risk of severe clots. This raises the possibility that the vaccine could be a causal factor in these rare and unusual cases of CVST, though we don't know this yet, so more research is urgently needed.

"CVST can reduce drainage of blood into the cerebral veins, leading to a rare type of stroke. Common symptoms of CVST include severe, often progressive, headache over hours to days, sometimes with seizures or

stroke symptoms (such as weakness of the face, arm or leg, or disturbances of vision or speech) [9].

### Experimental project hypothesis

In order to verify the hypothesis of work related the topic of this article it is necessary verify for all kind of vaccine based on covid-19 Spike Protein and the incidence and prevalence of thrombosis of all kinds. Of all the thrombosis it must be verified how many are central venous thrombosis. After this data it is needed to verify the incidence of this kind of thrombosis related the CVT in covid-19 severe disease.

A similarity in this data is not due to coincidence, but make possible to think about the relationship between SPIKE PROTEIN and increased thrombotic risk. (Why in the same region?).

### Other fact to be valued:

Group A 1.000.000 patient to be vaccinated with every kind of covid vaccine

Group B 1.000.000 patient to be vaccinated but before tested for platelet blood level and for second level clotting test. (Cerebral venous sinus thrombosis is rare, with estimated 3-4 cases per million annual incidences in adults)

## Discussion

It is clear by literature that in covid-19 disease there is an increase of prothrombotic signals. This can be related or to the full virus or by one part of this or by the increased inflammatory reaction of human body. (Like TNF as procoagulant factor and other factors effect on platelet activation). Coagulation disorder in COVID-19 has led to recommendations for the administration of anticoagulants to reduce the risk of complications related to thrombosis. Most studies on the treatment of this disease have confirmed the use of unfractionated or low molecular weight heparin [10,11].

In rare cases of side effects of the Covid vaccine, a low level of platelets has been found [9]. Not in all patient vaccinated it is observed the same effect. There is a strange increased number of central venous thrombosis versus all other thrombosis. Numerous signaling pathways are mediated by integrins and virion binding: "RGD (arginine-glycine-aspartate) sequence in the receptor-binding domain of the spike protein. RGD is a motif commonly used by viruses to bind cell-surface integrins. Numerous signaling pathways are mediated by integrins and virion binding could lead to dysregulation of these pathways, with consequent tissue damage. Integrins on the surfaces of pneumocytes, endothelial cells and platelets may be vulnerable to CoV-2 virion binding binding of intact virions to integrins on alveolar cells could enhance viral entry [7].

## Conclusion

Binding of virions to integrins on endothelial cells could activate angiogenic cell signaling pathways; dysregulate integrin-mediated signaling pathways controlling developmental processes; and precipitate endothelial activation to initiate blood clotting. Such a procoagulant state, perhaps together with enhancement of platelet aggregation through virions binding to integrins on platelets, could amplify the production of microthrombi that pose the threat of pulmonary thrombosis and embolism, strokes and other thrombotic consequences. "There is a similarity in thrombosis due by covid-19 and some rare event post some covid-19 vaccination.

All this evidence requires to submit a relevant question: to use spike protein model to produce a vaccine is really the right solution. It is possible that a class effect can be observed? And before to use this vaccine it is needed to test patient for platelet level, coagulation factor level and first level and second level tests like protein C and S deficiency, factor V Leiden, D-dimer, antithrombin abnormality and other factors that can be relevant

(smoke, an estrogen and a progestin pill, chronic inflammatory disease).

## Conflict of Interest Statement

No

## Ethical consideration

Respected all international rules

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