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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic coronavirus that caused an outbreak of uncommon viral pneumonia called coronavirus disease 2019 (COVID-19), which originally emerged in Wuhan, China, in December 2019 (https://covid19.who.int/).

With the pandemic already in its second year, the disease’s impact on the respiratory tract is becoming increasingly obvious. Indeed, it is currently well established that, in addition to the staggering cytokine storm and lung inflammation associated with the infection, thrombotic complications, including microvascular, venous, or arterial thrombosis, and cardiovascular manifestations significantly contribute to the disease severity, leading to morbidity, multi-organ failure, and mortality.¹

Platelets, the small anucleated cellular fragments derived from their megakaryocytes precursors and traditionally linked to thrombosis and hemostasis, are also key players that mediate inflammation, infectious diseases, and immune response.²

Upon exposure to invading pathogens, platelets contribute to the immunity either directly by cytokine production and antimicrobial peptides release; or indirectly by amplifying the immune response through interaction with neutrophils, monocytes, and lymphocytes.³,⁴ However, the immunothrombosis triggered during infections may adversely impact immunological and hemostatic processes; thus leading to adverse clinical outcomes.⁵

Several recent reports highlighted the association of coagulopathy events and COVID-19 severity; revealed by elevated levels of D-dimers and fibrin-degradation products and hyperactivated platelets in critically ill patients with COVID-19.⁶,⁷ However, how platelets interact with the SARS-CoV-2 remains controversial. Such controversy is undoubtedly triggering an exciting debate among researchers in the SARS-CoV-2–related coagulopathy field during this pandemic.
The recently published conflicting results of this interaction as well as its implication for better management of COVID-19–related thrombotic complications will be debated here.

2 | PLATELETS ARE HYPERACTIVATED IN COVID-19

While the pandemic is progressing worldwide, numerous studies have reported that patients with COVID-19 demonstrated platelet activation, aggregation, and platelet-leukocyte aggregate formation, thus highlighting the essential role of platelets during SARS-CoV-2 infection and immunopathology.\(^8\)–\(^10\)

Compared to donors not infected with SARS-CoV-2, platelets isolated from patients with COVID-19 were hyperactivated when exposed to platelet agonists, such as thrombin, ADP, and collagen.\(^9,11\) Such hypersensitivity could be partially due to increased mitogen-activated protein kinase signaling pathway activation and thromboxane synthesis.\(^9,11\)

Additionally, platelet-derived microvesicles can be involved in thrombosis, by providing anionic phospholipids, and support coagulation cascade.\(^12\) Similarly, the release of neutrophil extracellular traps (NET), called NETosis, requires platelets and may participate to thrombosis during SARS-CoV-2 infection.\(^13\) NETs are networks of extracellular fibers of decondensed chromatin carrying histones and antimicrobial peptides.\(^14\) They also bind blood cells and generate a procoagulant and prothrombotic scaffold.\(^15,16\) In fact, several studies have identified NETs as important components of micro- and macrovascular thrombi\(^17–19\) and bronchoalveolar lavage fluid\(^20\) in patients with COVID-19, even after virus clearance from the lungs.

Moreover, upon SARS-CoV-2 infection, activated platelets release immune and inflammatory molecules including platelet-derived growth factor, platelet factor 4 (PF4), RANTES, serotonin, soluble P-selectin (sP-selectin), and soluble glycoprotein VI (sGPVI)\(^8,9,11,21,22\); and express a plethora of immune receptors, including CD40L, Toll-like receptor, and the Fc receptor for the IgG (Fc\(\gamma\)RIIA).\(^23,24\)

A platelet transcriptome study conducted by Manne et al.\(^9\) has revealed transcriptional changes in patients with COVID-19 distinct from those reported in other viral infections.

Cytokine storm is an umbrella term encompassing several disorders of immune dysregulation characterized by constitutional symptoms, systemic inflammation, and multiorgan dysfunction that can lead to multiorgan failure if inadequately treated.\(^25\) This phenomenon, also known as hypercytokinemia, is a hallmark of COVID-19, leading to the accumulation of chemokines, cytokines, and several soluble factors, which may activate platelets and other inflammatory cross-talk pathways.\(^11,26\) Such activation triggers platelet adhesion to the subendothelium and results in thrombus formation, subsequently inducing ischemia and pulmonary embolism.\(^27\)

Other viruses can also trigger cytokine storm, including herpesviruses, such as herpes simplex virus, and other influenza viruses, such as H5N1.\(^25\)

Platelets are also known to be directly activated by certain viruses. Indeed, in response to direct infection by dengue and influenza viruses, megakaryocytes upregulate interferon-\(\alpha\) genes.\(^28,29\)

Similarly, simplex virus-1 can activate platelet aggregate formation and thrombosis using the previously generated opsonizing antibodies and their interaction with the Fc\(\gamma\)RIIA.\(^30\) Recently, a key role of platelet-mediated immunothrombosis in COVID-19 that signals through Fc\(\gamma\)RIIA and the C5a–C5a receptor pathway has been identified, revealing the role of platelet hyperactivation in complications associated with SARS-CoV-2 infection.\(^31\)

However, unlike for other viruses, mechanisms underlying the direct interaction of SARS-CoV-2 and platelets and/or megakaryocytes remain a true controversy. Indeed, two recent independent studies conducted in China resulted in conflicting findings. Zhang et al.\(^{21}\) reported a possible activation of platelets and megakaryocytes directly by SARS-CoV-2 as evidenced by the presence of the virus mRNA in platelets of some patients with COVID-19, while a most recent study revealed that platelet activation occurs through an angiotensin-converting enzyme (ACE2)-independent mechanism.\(^32\)

3 | DOES SARS-COV-2 INTERACT DIRECTLY WITH PLATELETS?

SARS-CoV-2 is a positive-sense single-stranded RNA virus related to a number of naturally occurring betacoronaviruses.\(^32\)

ACE2, the negative regulator of the renin-angiotensin system, has been recognized as the entry receptor for the SARS-CoV-2 infected host cells.\(^33\)

There is a little to no expression of ACE2 on most immune cells, including CD4\(^+\) T cells, CD8\(^+\) T cells, natural killer T cells, B cells, regulatory T cells, T helper 17 cells, monocytes, dendritic cells, and granulocytes.\(^34\) On the contrary, this receptor is strongly expressed by alveolar epithelial cells, nasopharyngeal airway epithelial cells, and vascular endothelial cells, as well as lung macrophages.\(^35\) Such virus tropism certainly explains the prevailing respiratory symptoms associated with the disease.

Another potential cellular entry process has been proposed for the viral invasion, using the transmembrane serine protease-2 (TMPRSS2), which is essential for the cleavage of the SARS-CoV-2 S protein, thus allowing the fusion of viral and cell membrane and the virus internalization by the cell.\(^36\)

Following the onset of the COVID-19 pandemic and considering its staggering proinflammatory feature, most studies have focused their interest on the expression of ACE2 on immune cells, but a little attention was given to platelets and their megakaryocytes precursors until a few research groups explored such expression on these cells.

Using RNA sequencing (RNA-seq), reverse transcriptase polymerase chain reaction, and western blot analyses, Manne et al.\(^9\) did not reveal any ACE2 or TMPRSS2 in CD45-depleted platelets collected from either patients with COVID-19 or healthy subjects. Using similar approaches, concomitant work by Zaid et al.\(^{11}\) also
reported that there is no detection of ACE2 on platelets derived from patients with COVID-19 nor from healthy volunteers.

Consistent with these reports, a more recent retrospective survey of plasma samples from a cohort of 62 patients with severe and nonsevere COVID-19 revealed an increased thrombosis and high levels of sP-selectin and sGPVI as well as RANTES and PF4 release during platelet activation. However, the characteristics and mechanisms of the direct SARS-CoV-2–platelet interaction are yet to be elucidated.22

In contrast, an independent study carried out by Zhang et al.21 has shown a strong expression of ACE2 and TMPRSS2 mRNA and protein on platelets from healthy individuals and mice. Moreover, using in vitro assays and in vivo ACE2 transgenic mice, the same group ascertained their findings and reported that the SARS-CoV-2 virus and its spike protein induce direct platelet activation.

Another aspect of platelet–SARS-CoV-2 interaction was recently reported by Koupnova et al., which reported that SARS-CoV-2 initiates programmed cell death in platelets. Indeed, based on platelet RNA analysis by ARTIC v3 sequencing for SARS-CoV-2, transmission electron microscopy and immunofluorescence, this group showed that SARS-CoV-2 virions became internalized when they were attached to microparticles, bypassing the need for ACE2. Such internalization leads to rapid digestion, apoptosis, necroptosis, and extracellular vesicle release, thus contributing to dysregulated immunity and thrombosis.37

4 | TAKING TOGETHER THESE DISCORDANT FINDINGS ON WHETHER PLATELETS EXPRESS ACE2 AND/OR TMPRSS2, HOW COULD WE ARGUE IN FAVOR OF ONE OR THE OTHER STATEMENT?

Considering that investigations related to this topic were conducted in different parts of the world, the first possible explanation to such discrepancy would be ethnicity. Indeed, studies carried out by Manne et al.9 and Zaid et al.11 included individuals from North Africa and North America, while the cohort studied by Zhang et al.21 was from Asia. However, in our opinion, this argument would not be valid given that Shen et al.22 recently investigated patients from Asia as well. This group investigated in vitro SARS-CoV-2 infection in human platelets and their megakaryocyte cell line progenitor MEG-01.

According to this study, the presence of SARS-CoV-2 RNA in both MEG-01 cells and supernatant suggested that the virus may infect and reproduce in megakaryocytes despite insufficient efficiency; nevertheless, no viral particles were localized in MEG-01 cells as revealed by electron microscopy and immunofluorescence assay (IFA). The authors speculated that platelets may not support SARS-CoV-2 duplication; a fact that was echoed by Zaid et al.11 and Bury et al.38 Additionally, the lack of ACE2 expression on platelets and megakaryocytes was also shown by western blot and IFA in the study of Shen et al.22 Similarly, ACE2 and TMPRSS2 RNA were not detectable in a previous microarray-based integrated plateletomics study that mainly included healthy Black subjects.39

Besides the ethnicity argument, conflicting findings may be attributed to different technical approaches used to isolate RNA platelets in different investigations. Both studies conducted by Manne et al.9 and Zaid et al.11 used CD45-depleted washed platelets, a step that eliminated any remaining leukocytes from platelet preparation.

On the contrary, using gel-purified platelets, Zhang et al.21 confirmed the absence of white cells by using CD14 marker in their platelet preparation, a step that would probably leave a residual contamination by CD14-nonexpressing white cells, such as lymphocytes and natural killer cells, thus explaining ACE2 detection in their preparation. Moreover, on the animal level, the same group demonstrated that the administration of SARS-CoV-2 spike protein in K18 hACE2 transgenic mice induces platelet hyperactivation and aggregation; however, the expression of this receptor either on platelets or megakaryocytes of these mice remains to be solved and needs to be further investigated, with still the main focus on how these findings in mice could be translated into humans.39 It is also worth highlighting that a previous RNA-seq analysis documented the lack of expression of ACE2 and TMPRSS2 by platelets and megakaryocytes in mice.40

Despite the reported differences regarding the direct SARS-CoV-2–platelet interaction, it is worth noticing that all studies converge toward the same finding that platelets are activated during COVID-19; and some of them further ascertain that the virus RNA can be found within platelets.28 All these data advocate in favor of a potential ACE2-independent mechanism that SARS-CoV-2 might use for possible binding and/or entry into platelets.

Using RNA-seq analysis, Shen et al.22 showed unchanged levels of glucose-regulated protein (GRP 78), ADAM1, cathepsin L, GRP1, and asialoglycoprotein 1 in platelets between intensive care unit (ICU) and non-ICU patients with COVID-19 and healthy individuals; and reported increased CD147 and kringle-containing transmembrane protein 1 and reduced neuropilin 1 levels in patients as well as in MEG-01 cells upon SARS-CoV-2 incubation. These data suggest a marked alteration of megakaryocyte and platelet transcriptomic profile, reflecting a similar finding to dengue virus infection.28

Emerging evidence suggested CD147 as a potential receptor for SARS-CoV-2 and its overexpression is associated with certain diseases, such as chronic obstructive pulmonary disease, asthma, that represent risk factors associated with complications during the COVID-19 pandemic.28 Nonetheless, the binding of SARS-CoV-2 to CD147 is to be uncovered and the role of this receptor in SARS-CoV-2 infection remains disputable.41

In contrast, in their report related to SARS-CoV-2 directly interacting with platelets via ACE2, Zhang et al.21 also supported a direct interaction of CD147, SARS-CoV-2, and the spike protein.

Based on recent structural studies, CD26 was suggested to be another SARS-CoV-2 receptor.9 Such statement needs to be further investigated since the expression of this receptor on platelets...
is still debatable. Indeed, previous platelet RNA-seq and proteomic analysis suggest that neither platelets nor megakaryocytes express CD26, under physiological or infectious conditions. In contrast, the RNA abundance of 14 receptors and cofactors, including CD26, in human platelets and megakaryocytes was explored based on the RNA-seq data reported in earlier studies and revealed the expression of CD26 on these cells, though at very low levels. Together, these findings could be a hint of a possible CD26-SARS-CoV-2 direct interaction but does not provide tangible data to support a binding solely through this receptor.

In addition to all discrepancy arguments cited above, it is worth mentioning that during this pandemic, there was a rapid and large volume of new COVID-19 data published in a very short time in the quest to disseminate this new information and insights, thus helping containing the virus spread worldwide.

Therefore, considering that the technical barrier for COVID-19-related studies was lowered and the well-intended change of the publication process, the field is more likely to be inconsistent and needs to be revisited experimentally to clarify (Figure 1).

5 | CONCLUSIONS AND RECOMMENDATIONS

As the pandemic is still wreaking havoc across the globe, more studies are carried out and the understanding of the COVID-19 pathophysiology is continuously evolving, shedding more light on still enigmatic mechanisms underlying platelet hyperactivation during SARS-CoV2 infection. Following the cytokine storm triggered by the virus infection, platelets’ reactivity may be a critical step in the inflammatory and prothrombotic response, named immunothrombosis.

How the SARS-CoV-2–platelet interaction takes place is still obscure, and therefore more studies are warranted to uncover such mechanisms, taking into consideration ethnicity and gearing toward the expression of potential alternative SARS-CoV-2 entry receptors or pathways other than the previously established ACE2 receptor and the spike priming serine protease TMPRSS2.

Besides the uneven health care system efficiency in different countries, compiling clinical data worldwide demonstrated an unequal burden of this disease among certain populations, therefore urging the research community to explore a probable population-based differential expression of SARS-CoV-2 key receptors on the surface of platelets and/or other immune cells.

Moreover, as the megakaryocytes are the platelet precursors and considered the cargo carrying all the molecules and factors necessary to platelets’ function before their release into circulation, all future studies should explore the SARS-CoV-2 and platelet interaction without losing sight of behavior differences between these two interdependent entities.

RELATIONSHIP DISCLOSURE

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Both authors wrote and edited the manuscript. YZ is supported by the Cheikh Zaid Foundation.
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How to cite this article: Zaid Y, Guessous F. The ongoing enigma of SARS-CoV-2 and platelet interaction. Res Pract Thromb Haemost. 2022;6:e12642. doi:[10.1002/rth2.12642](https://doi.org/10.1002/rth2.12642)