Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Current Research in Immunology 4 (2023) 100058

Contents lists available at ScienceDirect

Current Research in Immunology

journal homepage: www.sciencedirect.com/journal/current-research-in-immunology

Acute to post-acute COVID-19 thromboinflammation persistence: Mechanisms and potential consequences

Remy Martins-Gonçalves [a,b], Eugenio D. Hottz [c], Patricia T. Bozza [a,*]

[a] Laboratório de Imunofarmacologia, Instituto Oswaldo Cruz (IOC), Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil
[b] Programa de Imunologia e Inflamação, Universidade Federal do Rio de Janeiro, UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil
[c] Laboratório de Imunotrombose, Departamento de Bioquímica, Universidade Federal de Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Post-acute COVID-19 syndrome (PACS)
Platelet activation
Hypercoagulability
Thromboinflammation

ABSTRACT

Concerns for the long-term effects of COVID-19 infection have grown due to frequently reported persisting symptoms that can affect multiple systems for longer than 4 weeks after initial infection, a condition known as long-COVID-19 or post-acute COVID-19 syndrome (PACS). Even nonhospitalized survivors have an elevated risk for the development of thromboinflammatory-associated events, such as ischemic stroke and heart failure, pulmonary embolism and deep vein thrombosis. Recent findings point to the persistence of many mechanisms of hypercoagulability identified to be associated with disease severity and mortality in the acute phase of the disease, such as sustained inflammation and endotheliopathy, accompanied by abnormal fibrin generation and impaired fibrinolysis. Platelets seem to be central to the sustained hypercoagulable state, displaying hyperreactivity to stimuli and increased adhesive capacity. Platelets also contribute to elevated levels of thromboinflammatory mediators and pro-coagulant extracellular vesicles in individuals with ongoing PACS. Despite new advances in the understanding of mechanisms sustaining thromboinflammation in PACS, little is known about what triggers this persistence. In this graphical review, we provide a schematic representation of the known mechanisms and consequences of persisting thromboinflammation in COVID-19 survivors and summarize the hypothesized triggers maintaining this prothrombotic state.

1. Introduction

The emergence of SARS-CoV-2 had a generalized impact on global dynamics, affecting not only health systems but also society as a whole, disrupting supply chains, provoking economic instability and aggravating inequality and previous social vulnerabilities worldwide. Over the past years, massive research efforts have led to the development of surveillance for emerging variants of concern, effective immunizers and antivirals, and a better overall understanding of the physiopathology and progression of the disease, allowing for lower mortality and reduced impact on global dynamics. Although these efforts remain of the outmost importance, concerns for the long-term effects of COVID-19 infection have grown, as survivors of mild and severe infections can present a series of long persisting symptoms and sequelae affecting multiple systems, persistent inflammation, and elevated risk for the development of cardiovascular and thrombotic diseases, a condition that came to be recognized as post-acute COVID-19 syndrome (PACS) (Davis et al., 2023; Mantovani et al., 2022). In this graphical review, we summarize current evidence of persistent platelet and endothelial activation as part of the thromboinflammatory mechanisms underlying PACS, with emphasis on what is known (and still unknown) about the roles of thromboinflammatory persistence and hypercoagulability in PACS physiopathology.

2. Post-acute COVID-19 syndrome

Thromboinflammation is a key driver of acute COVID-19 pathophysiology and is characterized by an exacerbated inflammatory response that starts in the lungs and becomes systemic. This is accompanied by a highly prothrombotic state, with increased activation of the coagulation cascade and clot formation, evidenced by markedly elevated D-dimer levels and a high frequency of thrombotic events in hospitalized patients, reducing the chances of survival (Conway et al., 2022; Mizurini et al., 2021). The vast majority of the individuals who face SARS-CoV-2 survive the infection, and although the precise incidence of PACS is still unclear, mainly due to the difficulty in tracking

* Corresponding author.
E-mail addresses: pbozza@ioc.fiocruz.br, pbozza@gmail.com (P.T. Bozza).

https://doi.org/10.1016/j.crimmu.2023.100058
Received 28 December 2022; Received in revised form 19 March 2023; Accepted 5 April 2023
Available online 10 April 2023

nonhospitalized survivors, a systematic review found that the overall (inpatients and outpatients) median prevalence of symptom persistence at 60 days after initial infection was 72% (IQR 55–80%) (Nasserie et al., 2021).

Post-acute COVID-19 syndrome (PACS; long COVID) refers to the persistence of symptoms after 4 weeks of initial SARS-CoV-2 infection. Although more common in hospitalization survivors, even those who experienced mild acute COVID-19 can present a variety of long-lasting symptoms, including fatigue, muscle weakness, breathlessness, cough, headache, hypoxia, arrythmia, palpitations, impaired cognition/memory, loss of taste and smell, anxiety and depression, among others (Fig. 1).

One of the greatest causes of concern regarding PACS is the elevated risk for cardiovascular disorders (Fig. 1). Postdischarge ischemic heart failure, stroke and thrombotic events have been reported in survivors of severe and moderate COVID-19 infection (Patell et al., 2020). A study comparing over 150 thousand nonhospitalized, hospitalized and ICU-admitted COVID-19 survivors found that after one year, even those who were not hospitalized in acute COVID-19 present an increased risk for the development of cardiovascular disorders. Moreover, survivors of hospitalization have an excessive 1-year burden due to deep vein thrombosis (DVT) and pulmonary embolism (PE), pointing to the persistence of the thromboinflammatory state long after clearance of the infection (Xie et al., 2022).

3. Persistence of lung and systemic inflammation

Homeostasis disruption starts in the lungs (usually the first site of infection) as a result of the inflammatory response elicited by SARS-CoV-2 infection of ACE2-expressing alveolar type II pneumocytes (Fig. 2). Viral replication and tissue damage trigger resident macrophages and recruited monocytes to release chemokines, leading to mono/polymorphonuclear infiltration and elevated immune-metabolic activity in the lung parenchyma, with the production of proinflammatory mediators, such as TNF and IL-6 (Schultheiβ et al., 2022). Balanced and effective responses of incoming monocytes and neutrophils to promote viral clearance is critical for the progression of the infection. Exacerbated proinflammatory cytokine release, oxidative stress, DAMP-full forms of cell death (necro/pyroptosis) and release extracellular traps (NETosis) are all involved in the amplification of inflammation in acute COVID-19 (Ferreira et al., 2021; Veras et al., 2020), which may aggravate thromboinflammation leading to severe presentations.

Through F-FDG PET/CT scans of the lungs of COVID-19 pneumonia survivors, our group has recently demonstrated increased lung parenchyma density and glycolytic metabolic activity in survivors of moderate to severe COVID-19 pneumonia over 30–194 days after hospitalization (Rodrigues et al., 2022). This increased lung metabolic activity is suggestive of increased numbers of inflammatory cells accumulated through migration or local proliferation alongside cellular activation. Accordingly, COVID-19 survivors also exhibited increased systemic IL-6 accompanied by increased angiopoietin-1, ICAM and VCAM levels in circulation, markers of endothelial cell activation (Rodrigues et al., 2022), pointing to endotheliopathy as a feature of PACS. Consistently, several nonfibrotic radiographical abnormalities have also been described up to 12 months after initial infection (Faverio et al., 2022). Elevated levels of C-reactive protein, TNF, IL-6 and IL-1β also highlight and corroborate the persistence of systemic inflammation in patients with ongoing PACS.

Upon activation, endothelial cells release Weibel-Palade bodies, containing p-selectin and Von Willebrand Factor (VWF). VWF binds to exposed subendothelial collagen and lines the interior of the blood vessel, acting as a hub for coagulation factor activity and for the adherence of plasmatic fibrin (ogen), leukocytes and platelets, acting as a site of amplification of both inflammation and hypercoagulability (Fig. 2). Endothelial cell activation leads to increased production and release of E-selectin, which was found to be elevated in the plasma of PACS patients (Turner et al., 2022). Expression of selectins in the outer membrane facilitates the adhesion of circulating leukocytes to the vasculature. Collectively, these data suggest that systemic immune activation and lung parenchymal inflammation persist months after SARS-CoV-2 infection, with potential consequences to multiple organs and systems.

4. Dysfunctional fibrin (ogen) dynamics

Both intrinsic and extrinsic pathways of the coagulation cascade resolve in the proteolytic conversion of prothrombin (coagulation factor II) into active thrombin (factor IIa), a serine protease capable of converting soluble fibrinogen (factor I) into insoluble fibrin (factor Ia). Once formed, fibrin polymerizes and becomes crosslinked, forming a tear-

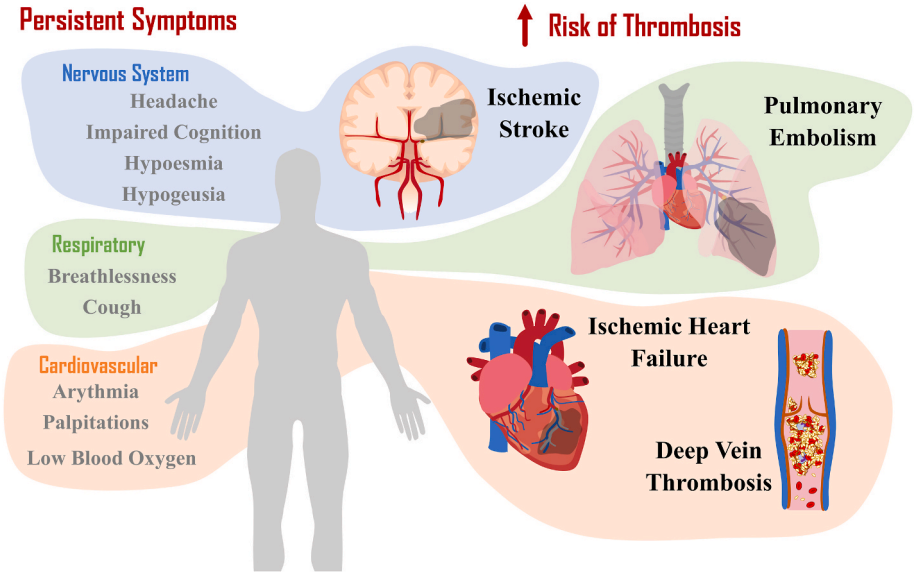

Fig. 1. – Post-acute COVID-19 syndrome. Survivors of both severe and mild cases of COVID-19 can have persisting symptoms, affecting multiple systems for longer than 4 weeks after initial infection, a condition known as Post-acute COVID-19 syndrome (PACS). Survivors of COVID-19 have an increased risk of developing cardiovascular diseases and thromboembolic events for at least one year after infection.

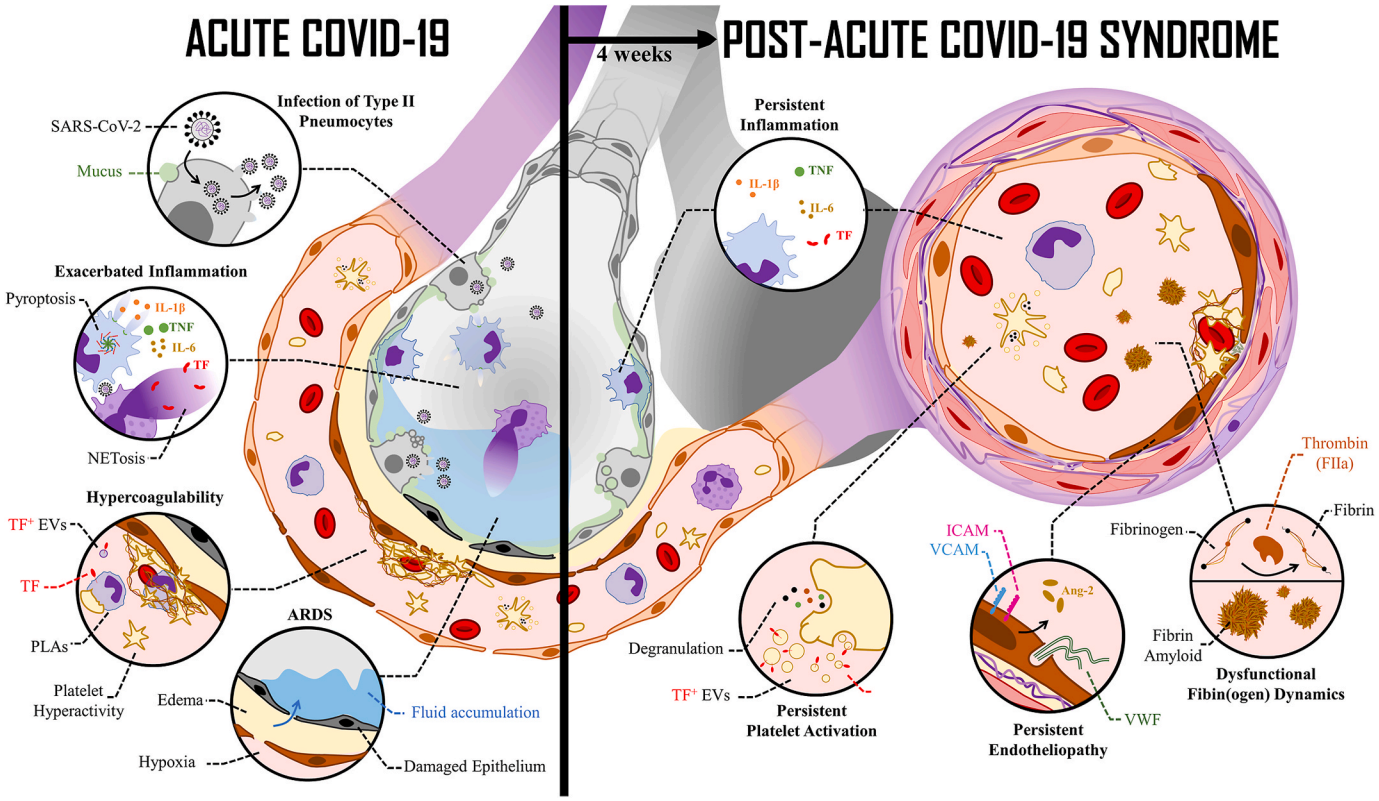

Fig. 2. – Thromboinflammatory mechanisms in acute disease and post-acute COVID-19 syndrome. Severe acute COVID-19 is characterized by an exacerbated inflammatory response in the alveoli (usually the first site of infection), leading to a hypercoagulable state and the development of acute respiratory distress syndrome (ARDS). Many thromboinflammatory mechanisms known to be associated with severity and mortality in acute COVID-19 have been shown to persist in individuals with ongoing PACS, including inflammation, elevated platelet activation and reactivity, endothelial cell activation and exacerbated fibrin clot formation associated with impaired fibrinolysis. NET – Neutrophil extracellular trap; PLAs – Platelet-leukocyte aggregates; ARDS - acute respiratory distress syndrome; VWF – von Willebrand factor.

resistant net capable of stabilizing the formation of the blood clot. Through fibrinolysis, the pathways that controls clot persistence and stability, crosslinked fibrin mesh is degraded by the serin protease plasmin into several fibrin degradation products (FDPs); among those, D-dimers are used in the clinic to evaluate clot formation/degradation.

Markedly elevated D-dimer levels are considered a hallmark of severe COVID-19, as their levels upon hospital admission are predictive of ICU admission and mortality (Violi et al., 2022). Fibrin conversion, polymerization and crosslinking occur at basal rates, and the fine regulation between fibrinogenesis and fibrinolysis controls hemostasis and prevents both abnormal clotting and bleeding. Increased D-dimer reflects the fibrinolysis rate necessary to prevent thrombus accumulation. However, both mild and severe COVID-19 patients also display circulating fibrin amyloid microthrombi, structures larger than one μm in cryosection, formed by residual fibrin aggregated with ordered β-sheet structures, and resistant to proteolysis (Grobbelaar et al., 2021; Pretorius et al., 2021).

Although not as markedly high as during severe-acute infection, elevated levels of D-dimer have been reported to persist for up to 4 months after COVID-19 survival, regardless of the severity of the acute phase (Martins-Gonçalves et al., 2022; Townsend et al., 2021). Additionally, individuals with post-acute COVID-19 syndrome can have increased plasmatic viscosity with the presence of fibrin amyloids (Fig. 2). These circulating microthrombi are enriched with α2-antiplasmin, an enzyme capable of inhibiting the proteolytic activity of plasmin, making the structures resistant to fibrinolysis and prone to accumulation (Pretorius et al., 2021), which is also found in higher concentrations in PACS patient's plasma (Turner et al., 2022).

5. Platelets drive PACS-associated hypercoagulability

Upon vascular damage, a series of simultaneous events involving cellular and molecular components is necessary for plugging of the vascular leakage, including release of ATP and VWF, exposure of adhesion molecules by the damaged endothelium, adhesion of platelets and leukocytes, activation of the coagulation cascade and formation of a fibrin mesh that stabilizes the clot. Similar to other prothrombotic viral infections, such as dengue and HIV, SARS-CoV-2 viral particles and COVID-19-associated inflammation induce many of these steps, promoting a pathological exacerbation of clot formation. However, after the acute phase, persistently elevated levels of D-dimer regardless of the severity or occurrence of thrombotic events in the acute phase of the disease indicate a persistent hypercoagulable state (Townsend et al., 2021; Martins-Gonçalves et al., 2022).

Besides being specialized in hemostatic control, platelets are immune-inflammatory cells that circulate in high concentrations in a "resting state", carrying granules underneath the outer membrane that are released upon activation. α-Granules contain platelet-derived growth factor (PDGF), platelet factor 4 (PF4), VWF, coagulation factors and p-selectin; while dense granules contain small molecules such as serotonin, Ca^{2+} and ATP/ADP. Platelet degranulation occurs upon the increase in intracellular calcium concentrations, which are kept low through efflux pump-dependent cAMP levels. G-protein coupled receptors on the platelet membrane, such as PAR-1 (agonized by thrombin) and P2Y receptors (agonized by purines), and ligand-gated cation channels, such as P2X receptors (agonized by purines), can promote a surge in intracellular Ca^{2+} and subsequent granule release.

In a recent study evaluating platelet activation and responsiveness during PACS, we have found that survivors of moderate to severe

COVID-19 pneumonia exhibit elevated platelet activation (Martins-Gonçalves et al., 2022), a feature previously observed in those acutely ill (Hottz et al., 2020) (Fig. 2). Platelets from PACS patients display increased surface expression of degranulation markers and elevated spontaneous release of granule products and thromboxane A_2 up to 6 months after symptom onset (Martins-Gonçalves et al., 2022). Platelets from survivors display increased adhesion capacity over a fibrinogen-coated surface, and when challenged with low/subthreshold concentrations of thrombin, uncapable of activation control platelets, those platelets from survivors degranulate, indicating that platelets circulate primed for activation in these individuals (Martins-Gonçalves et al., 2022). Similar features of platelet hyperreactivity have been also described in the acute phase of the disease (Manne et al., 2020). By using pharmacological approaches to investigate the pathways driving excessive platelet activation, we demonstrated that plasma from individuals with PACS activates platelets through mechanisms depending on purinergic receptors and integrin $\alpha_{IIb}\beta_3$ engagement, inducing an activation amplification program involving thromboxane A_2 synthesis and subsequent autocrine/paracrine platelet activation (Martins-Gonçalves et al., 2022). Nevertheless, new studies are necessary to identify the main triggers and pathological consequences of sustained platelet activation in PACS (Fig. 3).

Once activated, platelets are capable of expressing tissue factor (TF, coagulation factor III) on the surface, a serine protease mainly recognized for its role in extrinsic coagulation initiation by proteolytically activating coagulation factors VII and X, but also for signaling through protease-activated receptors (PARs) (Hottz et al., 2020). (Hottz et al., 2022). Once in the outer membrane, TF can be released bound to extracellular vesicles (EVs). Importantly, high concentrations of TF-bearing EVs in circulation correlate with disease severity and occurrence of thromboembolic events in severe COVID-19 patients, being proposed as a potential biomarker (Guervilly et al., 2021). Platelets seem to be one of the main sources of TF-bearing circulating EVs during COVID-19, with severe patients presenting increased concentrations of platelet-derived EVs with higher surface expression of TF (Moraes et al., 2022). Recently, we have reported the proteomic profile of circulating EVs from COVID-19 patients reflecting increased platelet degranulation, complement activation and fibrin clot formation (Moraes et al., 2022). A similar profile of circulating EVs was found to persist in individuals with PACS in our recent study. Survivors presented elevated levels of total and TF-bearing EVs in circulation, and although concentrations of EVs released from platelets were not changed compared to healthy donors, the surface expression of TF in platelet-derived EVs remained elevated up to 6 months after symptom onset (Martins-Gonçalves et al., 2022). These data suggest sustained EV-bound TF activity by continuous platelet activation in PACS as a potential mechanism of persisting hypercoagulability (Fig. 3).

Another feature of acute COVID-19 thromboinflammation that remains to be investigated in PACS is the capacity of platelets to drive inflammation and coagulation through interaction with circulating leukocytes. In severe COVID-19 patients, platelets have been shown to induce TF expression in monocytes in a p-selectin- and integrin $\alpha_{IIb}\beta_3$-dependent manner. Interestingly, both platelet and monocyte activation are amplified by platelet-induced TF expression and proteolytic activation of PAR-1/2, inducing proinflammatory cytokine release in the monocyte, especially TNF and IL-1β, in a thromboinflammatory amplification loop (Hottz et al., 2022). Both platelet activation and release of procoagulant extracellular vesicles are potentially involved in leukocyte immunoregulation and thromboinflammatory amplification during PACS, which deserves further in-depth investigation.

6. Hypothesis for thromboinflammation persistence

Although emerging evidence supporting the persistence of mechanisms of thromboinflammation associated with acute COVID-19 for long after the initial acute phase, the stimuli for this persistence are

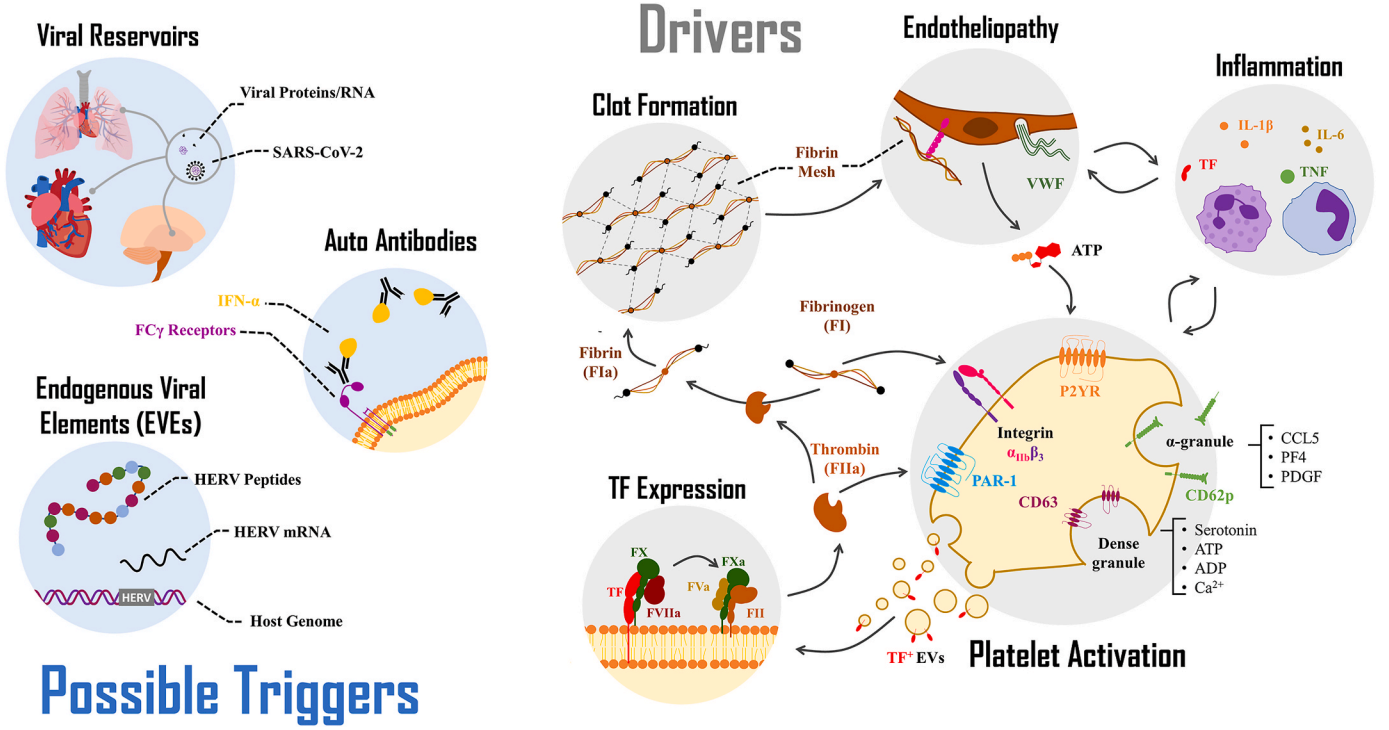

Fig. 3. – Possible stimuli and drivers of hypercoagulability. Some hypotheses have been proposed for the persistent hypercoagulable state found in COVID-19 survivors. These include unresolved inflammation, development of auto-antibodies, possible viral reservoirs or sources of viral RNA/proteins that could lead to activation of leukocytes and platelets, eliciting a continued inflammatory response. Regardless of the stimuli, individuals with PACS have persistent thromboinflammation, maintained by sustained endothelial cell and platelet activation and elevated fibrin clot formation. PF4 – Platelet Factor 4; CD62p – P-selectin; TF – Tissue Factor; VWF – von Willebrand Factor; PAR-1 – Protease activated receptor 1.

unknown. Among those triggers, failure to resolve inflammation may contribute to the persistently elevated levels of IL-1β, IL-6 and TNF in PACS (Schultheiβ et al., 2022). Another possible mechanism is the generation of cross-reactive autoantibodies (AutoAbs). High titers of AutoAbs, especially those targeting type I interferons, have been characterized in acute disease and correlate with severity and mortality (Wang et al., 2021). Although the role of AutoAbs in PACS has not yet been fully elucidated, the presence of anti–IFN–α antibodies at 2–3 months after symptom onset correlates with respiratory symptom persistence and plasma levels of IL-6 in PACS (Su et al., 2022).

Additionally, viral persistence in specific tissue reservoirs may contribute to systemic inflammation and immunothrombosis after clinical cure and hospital discharge. Viral replication in the alveoli is the first signal for the development of an immunologic response, and the presence of viral particles (or proteins or RNA) has been shown to contribute to many thromboinflammatory mechanisms, such as pyroptosis, NETosis and platelet activation. Autopsies of patients who died in the acute phase (peak approximately 10–14 days post infection) revealed that although the main site of burden is the lungs, viral RNA can be found in multiple non-respiratory tissues in early infection victims, including the myocardium, liver and central nervous system (CNS) (Stein et al., 2022). Importantly, evidence of SARS-CoV-2 RNA persistence in multiple anatomic sites, including the brain, long after (over 30 days) disease onset has been recently provided (Stein et al., 2022). In addition, viral RNA was found in stool and serum samples up to 4 months after initial infection (Phillips et al., 2021) and, in another study, approximately 30% of survivors had detectable levels of viral RNA and proteins in the lower gastrointestinal tract but not in nasopharyngeal swabs up to 6 months after infection (Gaebler et al., 2021). Long term studies are still necessary to unravel specific sites acting as viral reservoir and their roles to persisting symptoms in PACS.

In conditions of elevated chromatin activation and metabolic cellular activity, such as those elicited by severe systemic inflammation, several endogenous sequences of viral origin that were integrated into our ancestor's genome can be transcribed. Interestingly, several of these endogenous viral elements (EVEs), such as human endogenous retrovirus-K (HERV-K), have been shown to be reactivated in severe COVID-19 patients, correlating with clinical severity and levels of thromboinflammatory mediators (Temerozo et al., 2022). The retroviral RNA can be sensed by pattern recognition receptors, mimicking an infection by an exogenous virus (Bannert et al., 2018), and may contribute to the sustainment of the inflammatory milieu from acute to post-acute COVID-19. It is not known, however, whether HERV-K reactivation persists after patient recovery, potentially contributing to PACS-related inflammation pathophysiology. Further studies are still necessary to unravel the role of HERV-K and SARS-COV-2 long-term replication, as well as other potential mechanisms of persistent thromboinflammation and clinical outcomes in PACS patients.

CRediT authorship contribution statement

Remy Martins-Gonçalves: Conceptualization, Writing – review & editing, conceptualized, wrote, and edited the manuscript, prepared the illustrations. **Eugenio D. Hottz:** Conceptualization, Writing – review & editing, conceptualized, wrote, and edited the manuscript. **Patricia T. Bozza:** Conceptualization, Writing – review & editing, conceptualized, wrote, and edited the manuscript.

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.

Data availability

No data was used for the research described in the article.

Acknowledgments

The work of the authors is supported by CNPq/MCTI, Brazil; CAPES, Brazil; FAPERJ, Brazil; FAPEMIG, Brazil; and INOVA/FIOCRUZ, Brazil.

References

Bannert, N., Hofmann, H., Block, A., Hohn, O., 2018. HERVs new role in cancer: from accused perpetrators to cheerful protectors. Front. Microbiol. 9, 178. https://doi.org/10.3389/fmicb.2018.00178.

Conway, E.M., Mackman, N., Warren, R.Q., Wolberg, A.S., Mosnier, L.O., Campbell, R.A., Gralinski, L.E., Rondina, M.T., van de Veerdonk, F.L., Hoffmeister, K.M., Griffin, J. H., Nugent, D., Moon, K., Morrissey, J.H., 2022. Understanding COVID-19-associated coagulopathy. Nat. Rev. Immunol. 22, 639–649. https://doi.org/10.1038/s41577-022-00762-9.

Davis, H.E., McCorkell, L., Vogel, J.M., Topol, E.J., 2023. Long COVID: major findings, mechanisms and recommendations. Nat. Rev. Microbiol. 21, 133–146. https://doi.org/10.1038/s41579-022-00846-2.

Faverio, P., Luppi, F., Rebora, P., D'Andrea, G., Stainer, A., Busnelli, S., Catalano, M., Modafferi, G., Franco, G., Monzani, A., Galimberti, S., Scarpazza, P., Oggionni, E., Betti, M., Oggionni, T., De Giacomi, F., Bini, F., Bodini, B.D., Parati, M., Bilucaglia, L., Ceruti, P., Modina, D., Harari, S., Caminati, A., Intotero, M., Sergio, P., Monzillo, G., Leati, G., Borghesi, A., Zompatori, M., Corso, R., Valsecchi, M.G., Bellani, G., Foti, G., Pesci, A., 2022. One-year pulmonary impairment after severe COVID-19: a prospective, multicenter follow-up study. Respir. Res. 23, 65. https://doi.org/10.1186/s12931-022-01994-y.

Ferreira, A.C., Soares, V.C., de Azevedo-Quintanilha, I.G., Dias, S., da, S.G., Fintelman-Rodrigues, N., Sacramento, C.Q., Mattos, M., de Freitas, C.S., Temerozo, J.R., Teixeira, L., Damaceno Hottz, E., Barreto, E.A., Pão, C.R.R., Palhinha, L., Miranda, M., Bou-Habib, D.C., Bozza, F.A., Bozza, P.T., Souza, T.M.L., 2021. SARS-CoV-2 engages inflammasome and pyroptosis in human primary monocytes. Cell Death Dis. 7, 43. https://doi.org/10.1038/s41420-021-00428-w.

Gaebler, C., Wang, Z., Lorenzi, J.C.C., Muecksch, F., Finkin, S., Tokuyama, M., Cho, A., Jankovic, M., Schaefer-Babajew, D., Oliveira, T.Y., Cipolla, M., Viant, C., Barnes, C. O., Bram, Y., Breton, G., Hägglöf, T., Mendoza, P., Hurley, A., Turroja, M., Gordon, K., Millard, K.G., Ramos, V., Schmidt, F., Weisblum, Y., Jha, D., Tankelevich, M., Martinez-Delgado, G., Yee, J., Patel, R., Dizon, J., Unson-O'Brien, C., Shimeliovich, I., Robbiani, D.F., Zhao, Z., Gazumyan, A., Schwartz, R.E., Hatziioannou, T., Bjorkman, P.J., Mehandru, S., Bieniasz, P.D., Caskey, M., Nussenzweig, M.C., 2021. Evolution of antibody immunity to SARS-CoV-2. Nature 591, 639–644. https://doi.org/10.1038/s41586-021-03207-w.

Grobbelaar, L.M., Venter, C., Vlok, M., Ngoepe, M., Laubscher, G.J., Lourens, P.J., Steenkamp, J., Kell, D.B., Pretorius, E., 2021. SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. Biosci. Rep. 41, BSR20210611 https://doi.org/10.1042/BSR20210611.

Guervilly, C., Bonifay, A., Burtey, S., Sabatier, F., Cauchois, R., Abdili, E., Arnaud, L., Lano, G., Pietri, L., Robert, T., Velier, M., Papazian, L., Albanese, J., Kaplanski, G., Dignat-George, F., Lacroix, R., 2021. Dissemination of extreme levels of extracellular vesicles: tissue factor activity in patients with severe COVID-19. Blood Adv. 5, 628–634. https://doi.org/10.1182/bloodadvances.2020003308.

Hottz, E.D., Azevedo-Quintanilha, I.G., Palhinha, L., Teixeira, L., Barreto, E.A., Pão, C.R. R., Righy, C., Franco, S., Souza, T.M.L., Kurtz, P., Bozza, F.A., Bozza, P.T., 2020. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. Blood 136, 1330–1341. https://doi.org/10.1182/blood.2020007252.

Hottz, E.D., Martins-Gonçalves, R., Palhinha, L., Azevedo-Quintanilha, I.G., de Campos, M.M., Sacramento, C.Q., Temerozo, J.R., Soares, V.C., Dias, S.S.G., Teixeira, L., Castro, I.M.S., Righy, C., Souza, T.M.L., Kurtz, P., Andrade, B., Nakaya, H., Monteiro, R.Q., Bozza, F.A., Bozza, P.T., 2022. Platelet-monocyte interaction amplifies thromboinflammation through tissue factor signaling in COVID-19. Blood Adv. 6, 5085–5099. https://doi.org/10.1182/bloodadvances.2021006680.

Manne, B.K., Denorme, F., Middleton, E.A., Portier, I., Rowley, J.W., Stubben, C., Petrey, A.C., Tolley, N.D., Guo, L., Cody, M., Weyrich, A.S., Yost, C.C., Rondina, M. T., Campbell, R.A., 2020. Platelet gene expression and function in patients with COVID-19. Blood 136, 1317–1329. https://doi.org/10.1182/blood.2020007214.

Mantovani, A., Morrone, M.C., Patrono, C., Santoro, M.G., Schiaffino, S., Remuzzi, G., Bussolati, G., Covid-19 Commission of the Accademia Nazionale dei Lincei, 2022. Long Covid: where we stand and challenges ahead. Cell Death Differ. 29, 1891–1900. https://doi.org/10.1038/s41418-022-01052-6.

Martins-Gonçalves, R., Campos, M.M., Palhinha, L., Azevedo-Quintanilha, I.G., Abud Mendes, M., Ramos Temerozo, J., Toledo-Mendes, J., Rosado-de-Castro, P.H., Bozza, F.A., Souza Rodrigues, R., Hottz, E.D., Bozza, P.T., 2022. Persisting platelet activation and hyperactivity in COVID-19 survivors. Circ. Res. 131, 944–947. https://doi.org/10.1161/CIRCRESAHA.122.321659.

Mizurini, D.M., Hottz, E.D., Bozza, P.T., Monteiro, R.Q., 2021. Fundamentals in covid-19-associated thrombosis: molecular and cellular aspects. Front Cardiovasc Med 8, 785738. https://doi.org/10.3389/fcvm.2021.785738.

Moraes, E.C.S., Martins-Gonçalves, R., Silva, L.R., Mandacaru, S.C., Melo, R.M., Azevedo-Quintanilha, I., Perales, J., Bozza, F.A., Souza, T.M.L., Castro-Faria-Neto, H.C., Hottz, E.D., Bozza, P.T., Trugilho, M.R.O., 2022. Proteomic profile of procoagulant extracellular vesicles reflects complement system activation and platelet hyperreactivity of patients with severe COVID-19. Front. Cell. Infect. Microbiol. 12, 926352 https://doi.org/10.3389/fcimb.2022.926352.

Nasserie, T., Hittle, M., Goodman, S.N., 2021. Assessment of the frequency and variety of persistent symptoms among patients with COVID-19: a systematic review. JAMA Netw. Open 4, e2111417. https://doi.org/10.1001/jamanetworkopen.2021.11417.

Patell, R., Bogue, T., Koshy, A., Bindal, P., Merrill, M., Aird, W.C., Bauer, K.A., Zwicker, J.I., 2020. Postdischarge thrombosis and hemorrhage in patients with COVID-19. Blood 136, 1342–1346. https://doi.org/10.1182/blood.2020007938.

Phillips, S.P., Wei, X., Kwong, J.C., Gubbay, J., Schwartz, K.L., Majury, A., Groome, P.A., 2021. Duration of SARS-CoV-2 shedding: a population-based, Canadian study. PLoS One 16, e0252217. https://doi.org/10.1371/journal.pone.0252217.

Pretorius, E., Vlok, M., Venter, C., Bezuidenhout, J.A., Laubscher, G.J., Steenkamp, J., Kell, D.B., 2021. Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. Cardiovasc. Diabetol. 20, 172. https://doi.org/10.1186/s12933-021-01359-7.

Rodrigues, R.S., Motta Ribeiro, G., Barreto, M.M., Zin, W.A., de Toledo-Mendes, J., Martins, P.A.G., de Almeida, S.A., Basílio, R., Martins-Gonçalves, R., Hottz, E.D., Bozza, P.T., Bozza, F.A., Carvalho, A.R.S., Rosado-de-Castro, P.H., 2022. Increased lung immune metabolic activity in COVID-19 survivors. Clin. Nucl. Med. 47, 1019–1025. https://doi.org/10.1097/RLU.0000000000004376.

Schultheiß, C., Willscher, E., Paschold, L., Gottschick, C., Klee, B., Henkes, S.-S., Bosurgi, L., Dutzmann, J., Sedding, D., Frese, T., Girndt, M., Höll, J.I., Gekle, M., Mikolajczyk, R., Binder, M., 2022. The IL-1β, IL-6, and TNF cytokine triad is associated with post-acute sequelae of COVID-19. Cell Rep Med 3, 100663. https://doi.org/10.1016/j.xcrm.2022.100663.

Stein, S.R., Ramelli, S.C., Grazioli, A., Chung, J.-Y., Singh, M., Yinda, C.K., Winkler, C.W., Sun, J., Dickey, J.M., Ylaya, K., Ko, S.H., Platt, A.P., Burbelo, P.D., Quezado, M., Pittaluga, S., Purcell, M., Munster, V.J., Belinky, F., Ramos-Benitez, M.J., Boritz, E. A., Lach, I.A., Herr, D.L., Rabin, J., Saharia, K.K., Madathil, R.J., Tabatabai, A., Soherwardi, S., McCurdy, M.T., , NIH COVID-19 Autopsy Consortium, Peterson, K.E., Cohen, J.I., de Wit, E., Vannella, K.M., Hewitt, S.M., Kleiner, D.E., Chertow, D.S., 2022. SARS-CoV-2 infection and persistence in the human body and brain at autopsy. Nature 1–6. https://doi.org/10.1038/s41586-022-05542-y.

Su, Y., Yuan, D., Chen, D.G., Ng, R.H., Wang, K., Choi, J., Li, S., Hong, S., Zhang, R., Xie, J., Kornilov, S.A., Scherler, K., Pavlovitch-Bedzyk, A.J., Dong, S., Lausted, C., Lee, I., Fallen, S., Dai, C.L., Baloni, P., Smith, B., Duvvuri, V.R., Anderson, K.G., Li, J., Yang, F., Duncombe, C.J., McCulloch, D.J., Rostomily, C., Troisch, P., Zhou, J., Mackay, S., DeGottardi, Q., May, D.H., Taniguchi, R., Gittelman, R.M., Klinger, M., Snyder, T.M., Roper, R., Wojciechowska, G., Murray, K., Edmark, R., Evans, S., Jones, L., Zhou, Y., Rowen, L., Liu, R., Chour, W., Algren, H.A., Berrington, W.R., Wallick, J.A., Cochran, R.A., Micikas, M.E., Wrin, T., Petropoulos, C.J., Cole, H.R., Fischer, T.D., Wei, W., Hoon, D.S.B., Price, N.D., Subramanian, N., Hill, J.A., Hadlock, J., Magis, A.T., Ribas, A., Lanier, L.L., Boyd, S.D., Bluestone, J.A., Chu, H., Hood, L., Gottardo, R., Greenberg, P.D., Davis, M.M., Goldman, J.D., Heath, J.R., 2022. Multiple early factors anticipate post-acute COVID-19 sequelae. Cell 185, 881–895.e20. https://doi.org/10.1016/j.cell.2022.01.014.

Temerozo, J.R., Fintelman-Rodrigues, N., Dos Santos, M.C., Hottz, E.D., Sacramento, C. Q., de Paula Dias da Silva, A., Mandacaru, S.C., Dos Santos Moraes, E.C., Trugilho, M.R.O., Gesto, J.S.M., Ferreira, M.A., Saraiva, F.B., Palhinha, L., Martins-Gonçalves, R., Azevedo-Quintanilha, I.G., Abrantes, J.L., Righy, C., Kurtz, P., Jiang, H., Tan, H., Morel, C., Bou-Habib, D.C., Bozza, F.A., Bozza, P.T., Souza, T.M. L., 2022. Human endogenous retrovirus K in the respiratory tract is associated with COVID-19 physiopathology. Microbiome 10, 65. https://doi.org/10.1186/s40168-022-01260-9.

Townsend, L., Fogarty, H., Dyer, A., Martin-Loeches, I., Bannan, C., Nadarajan, P., Bergin, C., O'Farrelly, C., Conlon, N., Bourke, N.M., Ward, S.E., Byrne, M., Ryan, K., O'Connell, N., O'Sullivan, J.M., Ni Cheallaigh, C., O'Donnell, J.S., 2021. Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. J. Thromb. Haemostasis 19, 1064–1070. https://doi.org/10.1111/jth.15267.

Turner, S., Naidoo, C.A., Usher, T.J., Kruger, A., Venter, C., Laubscher, G.J., Khan, M.A., Kell, D.B., Pretorius, E., 2022. Increased levels of inflammatory molecules in blood of Long COVID patients point to thrombotic endotheliitis. https://doi.org/10.1101/2022.10.13.22281055.

Veras, F.P., Pontelli, M.C., Silva, C.M., Toller-Kawahisa, J.E., de Lima, M., Nascimento, D.C., Schneider, A.H., Caetité, D., Tavares, L.A., Paiva, I.M., Rosales, R., Colón, D., Martins, R., Castro, I.A., Almeida, G.M., Lopes, M.I.F., Benatti, M.N., Bonjorno, L.P., Giannini, M.C., Luppino-Assad, R., Almeida, S.L., Vilar, F., Santana, R., Bollela, V.R., Auxiliadora-Martins, M., Borges, M., Miranda, C.H., Pazin-Filho, A., da Silva, L.L.P., Cunha, L.D., Zamboni, D.S., Dal-Pizzol, F., Leiria, L.O., Siyuan, L., Batah, S., Fabro, A., Mauad, T., Dolhnikoff, M., Duarte-Neto, A., Saldiva, P., Cunha, T.M., Alves-Filho, J.C., Arruda, E., Louzada-Junior, P., Oliveira, R.D., Cunha, F.Q., 2020. SARS-CoV-2–triggered neutrophil extracellular traps mediate COVID-19 pathologySARS-CoV-2 directly triggers ACE-dependent NETs. J. Exp. Med. 217, e20201129 https://doi.org/10.1084/jem.20201129.

Violi, F., Pignatelli, P., Vestri, A.R., Spagnoli, A., Cipollone, F., Ceccarelli, G., Oliva, A., Amitrano, M., Pirro, M., Taliani, G., Cangemi, R., Lichtner, M., Pugliese, F., Falcone, M., D'Ardes, D., Venditti, M., Mastroianni, C.M., Loffredo, L., ADA GROUP, 2022. The ADA (Age-D-Dimer-Albumin) Score to Predict Thrombosis in SARS-CoV-2. Thromb Haemost. https://doi.org/10.1055/a-1788-7592.

Wang, J., Pendurthi, U.R., Yi, G., Rao, L.V.M., 2021. SARS-CoV-2 infection induces the activation of tissue factor-mediated coagulation via activation of acid sphingomyelinase. Blood 138, 344–349. https://doi.org/10.1182/blood.2021010685.

Xie, Y., Xu, E., Bowe, B., Al-Aly, Z., 2022. Long-term cardiovascular outcomes of COVID-19. Nat. Med. 1–8. https://doi.org/10.1038/s41591-022-01689-3.