COVID-19 Diagnosis and Coronary Artery Thrombosis

Subjects: Cardiac & Cardiovascular Systems

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Coronavirus disease 2019 is characterized by its severe respiratory effects. Data early on indicated an increased risk of mortality in patients with cardiovascular comorbidities. Early reports highlighted the multisystem inflammatory syndrome, cytokine storm, and thromboembolic events as part of the disease processes. The role of neutrophil extracellular traps (NETs) is explored in the pathogenesis of the disease. The structure and anatomy of the virus are pivotal to its virulence in comparison to other α and β Coronaviridae (HCoV-229E, HCoV-OC43, HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1). In particular, the host interaction and response may explain the variability of severity in patients. Angio tensin-converting enzyme 2 (ACE2) activation may be implicated in the cardiovascular and throm bogenic potential of the disease. The virus may also have direct effects on the endothelial lining affecting hemostasis and resulting in thrombosis through several mechanisms.

Keywords: SARS-CoV-2 infection ; COVID-19 ; coronary artery thrombosis

1. Structure and Genomics of SARS-CoV-2

The structure and genetics of SARS-CoV-2 are crucial to understand disease pathophysiology and to develop drugs or vaccines. These features are also relevant for their implementation and diffusion among people. SARS-CoV-2 has a spherical geometrical form with a single strand of positive-sense RNA [1][2][3] and relies on multiple components for cell adhesion and replication: different surface spike glycoproteins (S) which allow the adhesion to the cell membrane and induce antibody neutralization, an envelope protein membrane (E), structural membrane proteins such as M-protein (M), and the positive-sense single-stranded RNA with its structurally associated proteins such as nucleoprotein (N) [4][5][6][7][8][9][10][11].

SARS-CoV-2 has peculiar genomic variations compared to SARS-CoV and MERS-CoV, resulting in a more virulent virus. For instance, a single-nucleotide mutation in the S protein (N501T) enhances the viral binding to angiotensin-converting enzyme type 2 (ACE2) receptors of host cells [12].

Biologists and geneticists have worked to identify six strains of SARS-CoV-2 [13][14]. G and GR variants are more common in Europe, while GH is frequently found in North America.

2. SARS-CoV-2 Host Interaction

2.1. Host Cell Interaction

Transmission of SARS-CoV-2 occurs via inhalation of respiratory microdroplets from individuals infected with SARS-CoV-2. Once in the host, SARS-CoV-2 enters the cell using methods common to other viruses [15]. The spike protein (S) binds the virus to the ACE2 receptor on the surface of the cell [16].

TMPRSS2 is fundamental for viral entry into target cells and spread in the infected host, but an additional system for cell entry is the S protein which can use the endosomal cysteine proteases CatB/L. Hofmann et al. [17] demonstrated that TMPRSS2 activity was only inhibited, but not eliminated using camostat mesylate, reflecting a residual S protein priming by CatB/L.

A special concern is related to temperature influence on viral replication. This can be effective throughout the airway tract ranging from 30–32 °C in the nose to 37 °C in the deeper airways. Considering the abundant replication of SARS-CoV-2 in the nose, it may be assumed that S protein is fine-tuned in this anatomical region. Many studies investigated this feature dedicating their observations to spike protein mutations [18][19][20][21]. Mutation S^{G614} became predominant within four months of the beginning of the pandemic. A high-level load in the upper airways is suggestive of more transmissibility. This has been ascribed to protein stability, increased level of the open spike conformation, and a more efficient proteolytic

activation of the S protein. Two particular mutations of spike proteins are related to variants Asp (D) or Gly (G) at residue 614. For both strains, infectivity remained largely stable at 33 °C. At 37 °C, both viruses deteriorated, but the decline was faster for the S^{D614} strain. It is worth noting that, at 37 °C, its infectivity was 10-fold (day 3 p.i.) to 35-fold (day 4 p.i.) lower than at 33 °C ($p < 0.0001$), while the S^{G614} virus was 2.7–7-fold less infectious at 37 °C versus 33 °C ($p = 0.02$). This indicates that mutation S^{G614} has a key role in the stability of S protein at 37 °C [22].

The pH may have a specific role in spike stability [22]. The lumen of the bronchi (pH ~ 7.5) is less acidic than the nasal cavity (pH ~ 6.3). The latter pH (6.3) has a more stabilizing effect than the former (pH 7.5). This was tested for almost all pseudoviruses. Between pH 7.5 and 8.0, no significant difference in spike stability was observed. One exception concerned the test on SARS-S, which had the highest stability at pH 7.5 and a lower infectivity at pH 6.3 ($p = 0.0014$). A similar pH influence was noted in the two variants of SARS-2-S.

2.2. Host Response: How NETs Interfere

The host may exhibit distinctive clinical features of severe COVID-19 following SARS-CoV-2 infection. The extreme inflammatory response elicited in the host by SARS-CoV-2 has recently aroused great interest, with particular emphasis on the excessive activation of NETs, cytokine storm, and sepsis. Multi-organ damage is caused by the combination of these three factors.

The role of neutrophils in COVID-19 disease severity has been well studied. Evidence suggests that neutrophil activators such as IL-8 and G-CSF and effectors including resistin, lipocalin-2, and hepatocyte growth factor are early expressed biomarkers in patients with the severe form of COVID-19. Furthermore, a substantial link in the relationship between high levels of immature granulocytes/neutrophils and increased mortality [23] was disclosed.

SARS-CoV-2 can cause the release of neutrophil extracellular traps (NETs) by neutrophils [24]. In a landmark paper, Brinkmann anticipated the role of NETs [25], which embody not only chromatin fibers but also enzymes such as neutrophil elastase, cathepsin G, and myeloperoxidase [26][27]. NETs represent an outpost against infections with the specific action of immobilizing and degrading bacteria, fungi, viruses, being a critical effector mechanism for containing infections [28]. However, the nonunique role of NET in immunity has been revealed, with a dual effect, pro- or anti-inflammatory [29][30]. Aggregates of NETs reduce inflammation, leading to the degradation of cytokines and chemokines [31]. Regarding the tissue damage due to NETs, it was revealed during infection with Escherichia coli that an interaction between NETs and platelets caused tissue damage [32]. Patients with COVID-19 experience a high level of NETs in plasma [33][34][35], correlated with a greater severity of the disease [35], evidenced by the occurrence of critical lung damage and microvascular thrombosis [34].

Concern about vascular occlusion caused by NETs involves several target tissues: lung [36], kidney, liver [37], and heart. This suggests that the thrombotic effects of NETs could be responsible for the systemic and harmful effects present in critically ill patients with COVID-19. A synergistic role with NET was also evoked by the activation of the complement system. In patients with COVID-19, it has been disclosed that inhibition of C3 [38] and C5 [39] reduced NET release. Marked coagulation dysregulation is the cause of a worse prognosis in COVID-19 [40][41][42], and both NETs and complement proteins are associated with these thrombotic events [39]. A new frontier in COVID-19 therapy stems from research on the triple complement–NET coagulation interaction.

Genetics has taught people that abundant NET formation in patients with COVID-19 is sustained by higher transcriptional level [43]. Investigators hypothesized that the transcriptional increase assets may be related to a negative regulatory mechanism of the host's immune response of natural killer cells (NK) and T cells, with a consequential reduction in the antiviral response [17]. The main cause of this altered response results in the clinically more severe forms of COVID-19, in which both circulating and lung neutrophils have been found to release high levels of NET. There is evidence that this phenomenon is exacerbated by a direct action induced by SARS-CoV-2 in favoring the release of NETs [35]. Furthermore, this NET release is linked to PAD-4 levels [35]. PAD4 plays a pivotal role in the constitution of NETs, which is due to the hypercitrulination process of histones, with consequent decondensation of chromatin caused by PAD [44].

The spectrum of work of neutrophils activated by SARS-CoV-2 is broad since they can induce apoptosis of A549 cells of the pulmonary epithelium and myocardial tissue [45][46], thus strengthening the role played by neutrophils in COVID-19 immunopathology and other infections from coronavirus [35].

3. COVID Infection and Cardiovascular Implications

Clinical evidence underlines that those with cardiovascular diseases are at risk or have a more severe illness due to SARS-CoV-2 infection than the general population [47]. Patients with coronary artery disease (CAD) or impaired left-ventricular function have increased risk of developing major cardiac injury, requiring hospitalization or intensive treatments, as they have pre-existing alterations of the renin–angiotensin–aldosterone system with upregulation of ACE receptors. The increased number of ACE receptors upon the surface cell makes them more prone to virus entry as this receptor is used as a gateway [48][49].

Exogenous ACE-2 activation limits thrombus formation and platelet aggregation, as well as attachment to vessels [50][51]. Elevated values of ACE-2 are related to an increased susceptibility to SARS-CoV-2 infection and are generally considered a COVID-19-specific negative prognostic factor [52][53]. Plasma ACE-2 and angiotensin peptides levels may also indicate the progress of treatment and the RAAS state during COVID-19. Earlier studies established that a soluble form of recombinant human ACE-2 (rhACE-2; APN01 (0.4 mg/kg, IV, BID for 7 days), GSK2586881: 0.4 mg/kg, IV, BID for 3 days) neutralized excessive SARS-CoV virus and enhanced the protective cellular action of ACE-2 in ARDS patients [54][55]. ACE inhibitors (ACEi) upregulate ACE-2 expression on the cell surface, and this may improve the survival rate in COVID-19 patients [49], maintaining Ang II degradation, which can decrease AT1R activation.

Myocardial injury is a major contributor of mortality in COVID. In a study per-formed in hospitals in Wuhan, China, a high percentage mortality (70%) was reported in patients with high cTnI levels. Acute inflammation stimulus triggered by SARS-CoV-2 infection is embedded in atherosclerotic plaque development and progression [56]. This problem in SARS-CoV-2 is directly related to an acute inflammatory stimulus, triggered by virus infection. Development and destabilization of atherosclerotic plaque may induce acute myocardial infarction (AMI). These data are confirmed by many studies, particularly those performed in China [57][58][59][60]. A particular role in ischemic heart disease is represented by the so-called “cytokine storm” [61]. Proinflammatory cytokines elicited from endothelial cells cause a change in homeostatic functions and may result in endothelial impairment, subsequent destabilization of the atherosclerotic plaque, and thrombosis. Cytokines such as IL-1α, IL-1β, IL-6, and TNF-α can perturb all of the protective functions of the normal endothelium and potentiate the pathological processes.

The pathophysiological mechanism of a cytokine storm is centered on the autoinduction of proinflammatory cytokine IL-1.

IL-1 can induce its own gene expression, precipitating an amplification that leads to a cytokine storm [62][63][64]. IL-1 induces also the expression of other proinflammatory cytokines including TNF-α. The invasion of IL-1 and leucocytes can elicit the production of chemoattractant molecules including chemokines that provoke the penetration of inflammatory cells into tissues [65]. In the meantime, IL-1 stimulates the production of IL-6. IL-6 is a 27 kDa cytokine involved in a variety of immune and inflammatory responses. Plasma levels are generally very low. During acute infection, a large variety of cells including macrophages, as well as B and T lymphocytes, increase the production of IL-6. In addition to local effects, IL-6 provides a proximal stimulus to the acute phase response. IL-6 induces the synthesis of fibrinogen, the precursor of clots, PAI-1, a major inhibitor of the endogenous fibrinolytic mediators, and C-reactive protein, an inflammation biomarker strictly linked to COVID-19 [66]. During infection, the endothelium becomes activated, resulting in a loss of barrier function, expression of adhesion molecules such as soluble ICAM-1 (intercellular adhesion molecule 1) and soluble VCAM-1 (vascular cell adhesion molecule 1), release of VWF that allows binding of platelets, and expression of TF that activates the coagulation system.

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