Editorial

COVID-19: viral infection, endotheliopathy and the immuno-inflammatory response. . . is it time to consider a standard (non-immunized) plasma therapy approach to maintain homeostasis?

1. Introduction

Severe novel Coronavirus Disease 2019 (COVID-19) is characterized by severe pneumonia, requiring hospitalization in Intensive Care Units (ICUs). Respiratory and multi-organ failure most often occur secondarily (median 10 days after the onset of symptoms) [1]. Transient viremia is documented in severe forms [2] but death can occur even after viral clearance. Thromboembolic complications, including deep vein thrombosis and pulmonary embolism, are also reported [3]. Finally, with skin lesions similar to vasculitis or vasomotor disorders COVID-19 is described as an infectious disease with vascular manifestations [4]. SARS-COV-2 infection relies on direct endothelial dysfunction by downregulation of ACE2 and studies of COVID-19 pathophysiology highlighted inflammatory and vascular mechanisms [5]. All these may be treated by new therapeutic approaches, but not necessarily with new drugs. Here, we discuss these mechanisms and how therapeutic non-immunized plasma (i.e. not specifically collected in COVID-19 convalescent donors) could be integrated into treatment protocols.

2. COVID-19 endotheliopathy and coagulopathy

Accumulated data have placed endotheliopathy and coagulopathy as key factors in the COVID-19 pathophysiology with a critical tipping point at days 6-8 after onset [5].

In patients who died from SARS-CoV-2, pathological analyses highlighted a broad endothelial infection with reactive vasculitis. No evidence for a bacterial superinfection or a direct myocardial tropism of the virus was reported [6–8]. All the cells expressing angiotensin-2 converting enzyme (ACE2) receptor are potential targets of SARS-CoV-2 [9]. Since endothelial cells with ACE2 receptors are ubiquitous, this COVID-19 vasculitis is identified not only in pulmonary capillaries but also in remote organs. The vascular endothelium represents an exchange surface estimated between 280 and 350m^2. In case of a viral infection, endothelium reacts by synthesis and release of many factors involved in the modulation of angiogenesis, inflammation, hemostasis, and vascular permeability [10]. The endothelium is lined with a glycocalyx network which regulates the interaction of plasma solutes, red blood cells, platelets and leukocytes [11]. Glycocalyx and endothelial cells are first in the line of defense in systemic inflammation and Acute Respiratory Distress Syndrome (ARDS) as in severe COVID-19

patients. Whatever the underlying etiology, ARDS is characterized by an endotheliopathy which activates two pathophysiological pathways: inflammation and microvascular thrombogenesis. The first is maintained by activated endothelial cells, which massively release cytokines, inflammatory mediators and increase neutrophil adhesion (E-selectin, cadherin, Neutrophil Extracellular Traps). Microvascular thrombogenesis is caused by several mechanisms that disrupt the hemostatic balance. This include the disruption of the protein C pathway and the over-expression of Tissue Factor (TF), as well as the secretion of unusually large Von Willebrand Factor multimers. Microthrombi are prevalent in ARDS and are associated with multiple organ failure (MOF) [12].

In severe sepsis, several studies have shown that a drop in protein C activation accompanies degradation of the glycocalyx [13]. Among other endothelial mechanisms, expression of TF, absent from endothelial cells and monocytes under physiological conditions, is induced by many pro-inflammatory molecules. High levels of TF in patients with ARDS are associated with increased mortality [14]. Along with triggering clot formation, TF induces other modifications such as expression of adhesion molecules and decreases in pro-fibrinolytic activity.

In severe COVID-19, endotheliopathy and coagulopathy are evident from the commonly observed laboratory parameters: thrombocytopenia, pro-inflammatory cytokines, fibrin degradation products, D-dimers, elevated PT and aPTT [15]. Several studies reported patients who died from COVID-19 with criteria for disseminated intravascular coagulation [16], or a state of hypercoagulability and systemic inflammation with several biomarkers of endothelial dysfunction and microvascular thrombosis: increased CRP, Von Willebrand factor, D-dimers, abnormal thromboelastogram [17].

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SARS-CoV-2 immunopathogenesisTo understand why endotheliopathy and coagulopathy are so prevalent in severe COVID-19, it is necessary to study the pathogenesis of SARS-COV-2 infection and its interactions with the immune system.

The cytopathic effect of SARS-CoV-2 and the induced immune response may differ depending on the infected cells and host factors. First, a cytokine storm is reported in severe forms of COVID-19
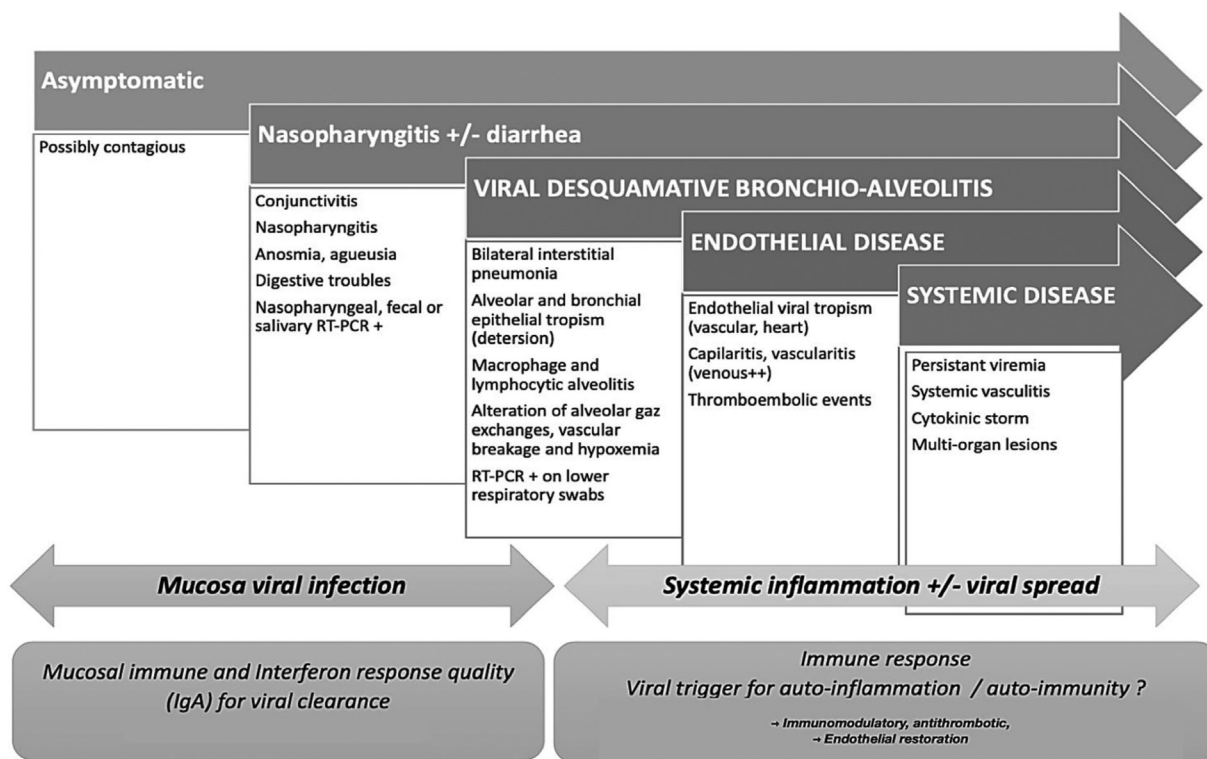
Fig. 1. Pathogenesis of COVID-19 time phases and potential therapeutic approaches.

with high levels of IL-2, IL-6, IL-7, granulocyte-colony stimulating factor (GCSF), monocyte chemoattractant protein (MCP) 1, and TNFα [18,19]. In an in vitro model of SARS-CoV-1, a very closely related virus to SARS-COV-2, it was possible to induce overexpression of blood coagulation genes, in particular those involved in vasoconstriction, platelet aggregation, vascular permeability, fibrin, and the coagulation pathways, generating a pro-coagulant profile. SARS-CoV-1 infection leads to an increased expression of the Toll-like receptor 9 (TLR-9) which is a mediator involved in the activity of autoimmune diseases such as lupus or scleroderma characterized by vascular dysfunction [20].

Secondly, neutralizing antibodies against SARS COV-2 (IgM and IgG) target the spike protein receptor-binding domain. They appear early (from day 6-14) in both mild and severe forms of COVID-19 and can be associated with positive or negative outcome. In fact, some severe COVID-19 patients present a high level of neutralizing antibodies contemporaneously with SARS-CoV-2 detected viremia [21]. Junqueira et al. identified an inflammation pathway mediated by monocytes which are infected by opsonization via their receptors of Fcγ antibodies. This is another way of SARS-COV2 infection ACE2 independent that affects monocytes which consequences are inflammation and pyroptosis [22].

Finally, markers of vasculitis and thrombophilia are identified in COVID-19: glomerulonephritis with anti-cytoplasmic neutrophil antibodies (ANCA), cryofibrinogen and anti-phospholipid antibodies [23]. Although the link between autoimmunity and SARS-COV-2 infection is not clearly established, these studies show that there is a correlation.

3. Discussion and treatment perspectives

As proposed by Bonaventura et al. [5], the hypothesis is that one of the secondary aggravation mechanisms of COVID-19 culminate in systemic thrombotic endotheliopathy, associating direct viral toxicity to the vascular endothelium, hypercoagulation

and dysregulation of inflammatory responses. Immune adaptive response to SARS-CoV-2 could also play a deleterious role, which remains to be determined. Given the complex pathophysiology of SARS-CoV-2 infection, it may benefit from a multimodal therapeutic approach at each stage of COVID-19 disease (Fig. 1).

Endotheliopathy could benefit from a therapeutic approach combining restoration of the vascular endothelium, immunomodulatory and anticoagulant molecules. In this perspective, plasma by itself could be a viable alternative therapy but not limited to immune or convalescent plasma. In fact, most blood and plasma donors now have significant anti-SARS-CoV-2 antibodies titers whether in post infection or post vaccination, but antibody levels substantially and gradually declined after vaccination or infection. Moreover, a recent meta-analysis does not support the routine clinical use of convalescent plasma in patients with Covid-19 [24,25]. However, plasma is also a complex biological product, acting as a physiological extracellular environment and composed of hundreds of molecules. Its efficacy could be much broader than isolated immunoglobulins, by repairing damaged endothelium and mitigating coagulation imbalances. Knowledge on the subject is limited, and scattered, distributed through studies and management of different diseases, but converging on an excessive host response to endothelial injury.

Early plasma administration in patients with traumatic hemorrhagic shock helps restoration of the endothelial wall glycocalyx and reduces blood transfusion needs [26]. Studies have shown that administration of glycosaminoglycans, natural components of the endothelial glycocalyx, have in vitro and in vivo positive effects on its reconstruction [27]. Likewise, albumin intake seems to limit erosion of glycocalyx. Other plasma molecules probably come into play through their protective function as anti-apoptotic, anti-inflammatory and/or anti-oxidative stress effect [28].

In a similar way, although Ebola infection is classified as a hemorrhagic fever patients manifest hemorrhages. In fact, they manly develop multiorgan failure (MOF) and two mechanisms

appear to be essential in the disease outcome: disseminated intravascular coagulation (DIC) and endotheliopathy. The latter does not result from direct viral cell damage but is the consequence of massive release of tissue factor and pro-inflammatory cytokines/chemokines [29]. During the 2015 Ebola outbreak, repeated high doses of plasma over 24-48H in a few patients was accompanied by a clinical and biological improvement [30]. Similarly, several studies for management of patients with septic shock show clinical improvement with inflammatory, immune and endothelial parameters normalization [31,32]. Interestingly, plasma exchange seems not to be necessary and Straat et al. studied the effect of the transfusion of 12 ml/kg (i.e. high doses but not more than crystalloid volumes usually perfused per day to severely ill patients) of fresh frozen plasma, without exchange, prior to an invasive procedure in critically ill patients who presented a coagulopathic but non-bleeding state. This team showed that fresh frozen plasma transfusion decreased syndecan-1 and factor VIII levels, suggesting a stabilized endothelial condition, possibly by increasing ADAMTS13, which is capable of cleaving vWF [33]. Concerning COVID-19, Keith et al. also discuss plasma transfusion as treatment of severe forms [34]. This team and others successfully used early and high doses of plasma exchange to treat severe COVID-19 patients, in a manner similar to use in patients with sepsis complicated by MOF or in microthrombotic angiopathy diseases. They showed improvement of clinical outcome, laboratory findings and cytokines levels [35,36]. Indeed, plasma is the only currently available product that contains a mixture of many molecules involved in the regulation of immuno-inflammation, coagulation, endothelial activation and glycocalyx restoration.

To be successful, the key points of non-immunized plasma therapy are probably time and dose. First, treating without delay, in particular without waiting for late signs of organ failure is likely to be critical. Furthermore, 1 or 2 units of plasma, as used in trials of immune plasma, is probably not enough to be efficient in treating endotheliopathy and coagulopathy affecting a system composed of liters of blood and kilometers of endothelium. Plasma is iso-osmotic, thus, crystalloid fluid therapy could advantageously be replaced by plasma. Depending on patient-specific variables, high doses of plasma (i.e. 12 mL/Kg) could be transfused over 24-48 hours.

About limitations and potential complications of plasma therapy, we can anticipate transfusion related acute lung injury (TRALI), allergic reaction, and transfusion-associated circulatory overload edema (TACO). However, these complications are uncommon and no death associated with plasma transfusion occurred between 2013 and 2018 in France. Concerning TRALI, the risk is limited by selection of male donors, or nulliparous women and women negative for anti-HLA antibodies [37].

Finally, accumulated data place endotheliopathy and coagulopathy as key factors in the COVID-19 pathophysiology. Given these pathophysiologic mechanisms and knowledge about plasma benefits in traumatic, severe sepsis and other conditions, we hypothesize that early and high doses of plasma will be efficient and to prevent evolution of COVD-19 into MOF and critical illness. Actually, several controlled trials using plasma exchange to treat hospitalized COVID-19 patients are registered (ClinicalTrials.gov).

Disclosure of interest

The authors declare that they have no competing interest.

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