

## Autologous Platelet-Rich Plasma: A Potential Therapy to Mitigate Severe COVID-19 Manifestations

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The Chinese Ministry of Health's Chinese Health Protection Centers reported the appearance of COVID-19, a group of pneumonia, in Wuhan toward the end of 2019. Common symptoms found are sore throat, headache, pain, diarrhea, skin lesions, and eye irritation. The immune regulatory system may become dysfunctional as a result of SARS-COV-2, leading to an uncontrolled inflammatory response. These events will then result in lymphocyte activation and dysfunction, lymphopenia, an increase in pro-inflammatory cytokine productions, and abnormalities in granulocytes and monocytes, all of which are symptoms of cytokine release syndrome (CRS). CRS may result in shock, acute respiratory distress syndrome, organ failure, respiratory failure, and even death in COVID-19 patients. In order to avoid this, activated autologous platelet-rich plasma (aaPRP) therapy was created to treat CRS. aaPRP therapy is replicable in a short time, easy, and at a low cost. The numerous growth factors in aaPRP have anti-inflammatory properties that reduce the production of IL-1, IL-6, and TNF. The use of aaPRP treatment may lessen the need for invasive mechanical breathing, avoid alopecia and sepsis, and lessen pulmonary fibrosis. According to the findings of the phase I/II study that were conducted, it shows that aaPRP was given through intravenously and did not cause side effects in COVID-19 patients. To fully comprehend the aaPRP treatment for COVID-19 patients, however, more research is required. This review will go into discuss about the pathophysiology of COVID-19 as well as potential areas for aaPRP therapy.

**Keywords:** COVID-19; Platelet-Rich Plasma; Severe Covid; Therapy COVID-19.

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In 31 December 2019, Chinese Department of Health released a press statement regarding the emergence of a string of pneumonia cases in Wuhan that are connected to a local seafood market.<sup>1</sup> According to the local health commission, some patients came to the hospital with atypical

pneumonia symptoms such as headache, pain, diarrhea, skin lesions, and eye irritation which evolves overtime as the disease progresses.<sup>2,3</sup>

Early studies reported laboratory biomarkers with deviation towards viral infection and inflammation: leukopenia, neutrophilia,

increased prothrombin time (PT), and hypoalbuminemia.<sup>4</sup> Coagulation parameters showed an increase in fibrin degradation: elevation of fibrin degradation product (FDP) such as D-dimer.<sup>4,5</sup> A drastic increase in pro-inflammatory cytokines further causes cytokine release syndrome (CRS) in severe cases.<sup>5</sup>

Improved understanding about immunologic therapies over the last decade has enabled clinicians to conduct researches and trials about the potential usage of immunomodulatory drug, targeting the pathophysiologic mechanisms of severe clinical manifestation. Take for example Tocilizumab (TCZ), a monoclonal antibody which specifically blocks IL-6 receptor which is an important inflammatory cytokine.<sup>6-8</sup> Although IL-6 may prove itself to be beneficiary in acute response to infections, dysregulated over secretion of IL-6 may cause lethal hyperinflammation.<sup>6</sup> Tocilizumab works by inhibiting the entire receptor complex which prevents signal transduction triggering B and T cells, and is proven in clinical trials effective for preventing and mitigating severe COVID-19 symptoms.<sup>6-9</sup>

However, such immunomodulatory modalities are costly and not widely available. An alternative modality which utilizes activated autologous platelet-rich plasma (aaPRP) may be the answer to the lack of an affordable and widely-available treatment to severe COVID-19.<sup>10,11</sup> This paper aims to review the detailed mechanism of COVID-19 pathophysiology and possible sites of treatment by aaPRP.

### **Thrombopathogenesis**

After entering the body through the respiratory system, direct viral entry was initiated by adhesion between receptor-binding domain (RBD) and ACE-2 receptor (hACE-2) after cleaving by TMPRSS2, cathepsin L, or furin.<sup>12,13</sup> Macrophages will recognize S1 and S2 as pathogen-associated molecular patterns (PAMPs) of SARS-CoV-2 via the toll-like receptor 2 (TLR-2) pattern recognition receptors concurrently occurring during viral entry (PRRs).<sup>14,15</sup> This triggers innate inflammatory response through production of chemokines and pro-inflammatory cytokines.<sup>14,16</sup>

Dysregulation of inflammatory mediators due to efficient evasion of IFN-I by various non-structural proteins (NSPS) of SARS-CoV-2

inhibits production of interferon-stimulated genes and its antiviral effects.<sup>17</sup> This immunopathology allows 'free replication' of the pathogen until a more adaptive immune response kicks in. Massive buildup of mucus within the lungs may create consolidation, dyspnea, up to ARDS.<sup>18</sup>

Immunopathogenesis due to non-structural proteins of SARS-CoV-2 further triggers both local and systemic immunothrombosis within the patient.

### **Platelet-Rich Plasma (PRP)**

Platelet-rich plasma (PRP) is a cutting-edge intervention classified under regenerative medicine, which includes other forms of treatment modalities such as gene therapies, cell-based therapies, and tissue engineering.<sup>10</sup>

PRP is a platelet concentrate which consists of plasma and platelets. Platelet and plasma were closely involved in cell recruitment, multiplication and specializes for healing. aaPRP was obtained from the centrifugation of patient's blood samples. Due to its healing nature, aaPRP therapy is usually used to induce faster healing in soft tissue, bones, and osteoarthritis.<sup>19,20</sup> During at the COVID-19 pandemic, aaPRP therapy was developed for additional therapy for COVID-19 patients with immune dysregulation. aaPRP therapy may reduce clinical severity in COVID-19 patients, thus minimizing ventilation needs, relieve lung fibrosis, and reduce hair loss often seen in COVID-19 syndrome.<sup>21-23</sup>

### **aaPRP Therapy in COVID-19**

Researches had shown that SARS-CoV-2 may cause disruption of normal immune response, triggering an uncontrolled inflammatory response. This phenomenon may give rise to an increase of pro-inflammatory cytokines production, triggering the occurrence of Cytokine Release Syndrome (CRS). The development of lymphocyte activation and dysfunction, lymphopenia, an increase in pro-inflammatory cytokine productions, and abnormalities in granulocytes and monocytes are all indications of CRS. Moreover, cytokine storm would lead to the hyper-activation of NF- $\kappa$ B in IL-6 AMP, which in COVID-19 patients results in deadly symptoms such shock, acute respiratory distress syndrome, organ failure, respiratory failure, and death. Due to its ability to create massive disruption within the organ system, it

is imperative to find ways to reduce symptoms or all in all prevent COVID-19 patients from experiencing CRS and cytokine storm.<sup>24</sup>

Various treatments have been developed to prevent cytokine storms such as mesenchymal stem cells (MSCs) therapy, a therapy involving various kinds of growth factors, trophic factors, and cytokines that has anti-inflammatory and immunomodulating effects. However, MSC therapy is considered quite expensive because the production is quite difficult and can only be done in special laboratories licensed by the Ministry of Health. In addition, autologous MSC therapy needed patient's own cells to be cultured for 2-3 weeks to increase the amount of MSC so that it may be quantically suitable for COVID-19 therapy.<sup>21</sup>

aaPRP therapy has advantages because it is produced in a short time, easy, and at a cheaper cost. In research carried out by Karina *et al.* (2021) using autologous activated PRP (aaPRP) found that the use of aaPRP as additional therapy decrease the occurrence of CRS in severe COVID-19. After administration of aaPRP, a considerable reduction in CRP and lymphocyte levels was followed by a marginal rise in neutrophil, neutrophil lymphocyte ratio (NLR), and lymphocyte C-reactive protein ratio (LCR) values. In terms of safety, administration of aaPRP does not induce serious side effects and is clinically safe to use. aaPRP is known to reduce the need for invasive mechanical ventilation, prevent pulmonary fibrosis, alopecia post COVID-19 infection, pulmonary fibrosis, accelerates the regeneration of damaged lung cells, and prevents sepsis.<sup>22</sup>

#### **Mechanism of aaPRP Therapy**

When COVID-19 is severe, hyperinflammation is a symptom that the patient needs intensive care unit (ICU) treatment.<sup>25</sup> Inflammation begins with the occurrence of primary infection. Inflammatory cytokine production increases dramatically in response to white blood cell activation. White blood cells will then be attracted to the infection location by inflammatory signals.<sup>26</sup> The inflammatory cytokines IL-2, IL-6, monocyte chemoattractant protein-1 (MCP-1), protein-1a inflammatory macrophage (MIP-1A), and tumor necrosis factor-beta (TNF-beta) may be discovered to be elevated in severe cases. In this situation, the body will try to decrease the anti-inflammatory cytokines.<sup>21,27</sup>

aaPRP is known to have several substances that exerts anti-inflammatory effect. epidermal growth factor (EGF), basic fibroblast growth factor (BFGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF- $\beta$ ), vascular endothelial growth factor (VEGF), and insulin-like growth factor 1 (IGF-1) are the aaPRP growth factor's. These growth factors are generated by the platelets to inhibit the production of IL-1, IL-6, and TNF by the synoviocytes. TGF- $\beta$  functions as an immunosuppressant throughout the inflammatory phase, preventing the production of cytokines that promote inflammation. Moreover, aaPRP posses anti-inflammatory components such interleukin-1 receptor antagonist (IL-1RA), which can stop IL-6 from being released.<sup>21</sup>

#### **aaPRP Making Process for COVID-19**

Eight BD vacutainer citrate tubes with a 1.2% sodium citrate buffer and 3 ml of blood each are used to collect blood samples. Next the blood will be centrifuged for 10 minutes with a speed of 1000rpm (188 x g) to plasma separate in red blood cells. The plasma that is separated is then moved carefully into 2 15ml tubes. After that, plasma is centrifuged for 10 minutes with a speed of 3000rpm (1690 x g). Platelet Poor Plasma (PPP) located at the top is removed by aspiration until the remaining 3 ml and is homogenized so that only leaves an aaPRP. Add calcium activator solution next to promote the development of fibrin clots. The removal of formed fibrin lumps allows for the isolation of autologous activated PRP.<sup>21</sup>

#### **aaPRP Administration**

A blood transfusion set was used to intravenously administer aaPRP after diluting it in 100 mL of 0.9% sodium chloride for 10 to 15 minutes. The patient was given an aaPRP therapy 3 times, namely on day 1, 3, and 5 after the patient was transferred to ICU.<sup>21</sup> Based on the clinical trial phase I/II study, it shows that giving aaPRP through intravenously known safely given and not found side effects in COVID-19 patients even in various patients with pathological conditions.<sup>28,29</sup>

#### **CONCLUSION**

Patients treated in the ICU showed the occurrence of leukopenia, neutrophilia, increased prothrombin time, hypoalbuminemia,

and increased D-dimer. On the other hand, there is also an increase in fibrin degradation products (FDP) accompanied by a simultaneous increase in D-Dimer and fibrinogen and the increase in pro-inflammatory cytokines which will cause CRS occurrence. Various treatments were developed to overcome these conditions, one of which was aaPRP therapy. In terms of production, aaPRP is cheaper, easier, and may be produced in a short time. aaPRP contains several anti-inflammatory growth factors that can suppress overregulation of pro-inflammatory cytokines, thereby preventing the occurrence of CRS and cytokine storm. In addition, aaPRP therapy has the benefit of preventing pulmonary fibrosis and alopecia. aaPRP therapy was given intravenously and clinical trials had found minimal to no side effects in COVID-19 patients.

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