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Biochemical indicators of coronavirus disease 2019 exacerbation and the clinical implications

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) has sparked a global pandemic, affecting more than 4 million people worldwide. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause acute lung injury (ALI) and even acute respiratory distress syndrome (ARDS); with a fatality of 7.0 %. Accumulating evidence suggested that the progression of COVID-19 is associated with lymphopenia and excessive inflammation, and a subset of severe cases might exhibit cytokine storm triggered by secondary hemophagocytic lymphohistiocytosis (sHLH). Furthermore, secondary bacterial infection may contribute to the exacerbation of COVID-19. We recommend using both IL-10 and IL-6 as the indicators of cytokine storm, and monitoring the elevation of procalcitonin (PCT) as an alert for initiating antibacterial agents. Understanding the dynamic progression of SARS-CoV-2 infection is crucial to determine an effective treatment strategy to reduce the rising mortality of this global pandemic.

1. Introduction

Since its first emergence in December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel human coronavirus (HCoV), has spread across the world at an alarming rate. SARS-CoV-2 was named by the International Committee on Taxonomy of Viruses (ICTV) based on the phylogenetic similarity to SARS-CoV [1,2]. Coronavirus Disease 2019 (COVID-19), the disease caused by SARS-CoV-2, is far more extensive than the previous HCoV-diseases, with the number of confirmed patients worldwide has currently exceeded 4.0 million [3–5].

After the outbreak of COVID-19, angiotensin-converting enzyme 2 (ACE2) was promptly been identified as the functional receptor of SARS-CoV-2 [2]. ACE2 is mainly distributed on the surface of well-differentiated airway epithelial cells (especially ciliated cells), and the respiratory tract has shown to be a predominant channel for HCoV to invade the human body [6]. SARS-CoV-2 can cause severe lower respiratory tract infection, resulting in acute lung injury (ALI), acute respiratory distressed syndrome (ARDS), and potentially death [7]. A retrospective study of 1099 COVID-19 patients from Zhong Nanshan’s group showed, 91.1 % of the patients developed pneumonia and 3.4 % of the patients developed ARDS [8]. While Chen and colleagues reported that over 17 % of the patients with COVID-19 in Wuhan developed ARDS [9]. The fatality rate worldwide has currently reached 7.0 % [3], and respiratory failure has been considered to be the leading cause (69.5 %) of SARS-CoV-2 related death [10]. In order to provide some implications for clinical therapy and drug exploration, we reviewed COVID-19 related clinical studies by searching the keywords “SARS-CoV-2”, “COVID-19” and “2019-nCoV” on Sinomed, Pubmed, Embase, WHO, and medRxiv, to explore the possible influencing factors behind COVID-19 exacerbation and related biochemical indicators.

2. Lymphopenia

Several clinical studies have shown that lymphopenia occurs frequently in COVID-19 patients, manifested as the decrease of total T cells, CD4+ T cells and CD8+ T cells in the peripheral blood; with the degree of decline related to the severity of the disease. Compared with patients with mild symptoms, a higher proportion of severe or deceased patients had lymphopenia [10–14], which is similar to the observation during SARS-CoV infection [15]. According to the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7) published by National Health Commission of China [16], an autopsy report of a patient who died from COVID-19 indicated (1) the spleen atrophied

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significantly, with apparent focal hemorrhage and necrosis; (2) lymphocytes in the lymph nodes were depleted and necrotized; and (3) the number of CD4+ T and CD8+ T cells in spleen and lymph nodes decreased significantly. These pathological characteristics coincide with that of patients who died of SARS-CoV infection [17]. Furthermore, compared with SARS in 2003, severe COVID-19 tends to have a higher viral load and a longer virus-shedding period [18,19]. Since the RNA sequence of SARS-CoV was found in the macrophages and aggregated T cells of lymph nodes [20], the direct impairment from SARS-CoV-2 may be an important cause of lymphopenia in patients with COVID-19.

The severe damage in lymphoid organs by SARS-CoV-2 may relate to the ineffective control of the virus infection during the early stage, which has been seen in SARS-CoV infection [21]. Type I interferon (IFN), including IFN-α and IFN-β, plays a critical role in prohibiting viral infections and initiating adaptive immune responses; including in SARS-CoV infection [21,22]. Type I IFN can inhibit virus replication in vitro as well as in macaques and mice [23–26]. However, SARS-CoV may delay or evade innate immune response through early antagonism on IFN response, in which IFN can only be detected after SARS-CoV reached a high titer [7,27,28]. Present evidence implied that the viral load of SARS-CoV-2 peaks at the time of presentation, even earlier than the time to peak of SARS-CoV [29]. We suspected that similar to SARS-CoV, SARS-CoV-2 may escape from the immune response through the “physical” cover of double-membrane vesicles (DMV) and the “chemical” interfere of the proteins encoded by itself [30,31].

The clearance of the virus mainly depends on cellular immunity [32]. Innate antiviral signaling is initiated upon the recognition of pathogen-associated molecular patterns (PAMPs) by specific pattern recognition receptor (PRR) molecules expressed on host cells [33,34]. This ultimately leads to the activation of transcriptional factors, primarily interferon regulatory factor (IRF) and nuclear factor-kappaB (NF-κB), for the induction of type I IFN and other proinflammatory mediators [33,34]. RIG-I like receptors (RLR) and Toll-like receptors (TLR) are two types of PRR that can recognize viral PAMP [34]. SARS-CoV encoded structural proteins (such as N protein and M protein), nonstructural proteins (ns), and accessory proteins (like ORF3b and ORF6) to antagonize the recognition and signal transduction [31]. N protein inhibits IFN-β synthesis by inhibiting the activation of IRF-3 and NF-κB [35]; M protein prevents gene transcription of IFN-β by inhibiting the activation of IRF-3/IRF-7 transcription factors [36]; other proteins such as ns1, ns3, ORF3b and ORF6 etc. can also antagonize IFN responses [37–43]. Recently, a team from the University of Chicago identified that the protein ns15 from SARS-CoV-2 is 89% identical to the ns15 found in SARS-CoV [44]. Since ns15 acts as an endoribonuclease for the virus’s double-stranded RNA, inhibition of ns15 could slow viral replication [45]. Most recently, Kim et al. reported SARS-CoV-2 can also express ORF7a, 3a, 8, 6, and 7b [46], indicating that targeting these proteins may help to recover the inhibition of SARS-CoV-2 infection at an early stage.

In addition to lymphopenia, SARS-CoV-2 may also induce T cell exhaustion [47]. Expression of immune-checkpoint molecules such as programmed cell death 1 (PD-1) and T cell immunoglobulin and mucin domain-3 (TIM-3), accompanied by the elevation of anti-inflamatory cytokines, is known to be the common indicators of T cell depletion [48]. Wang’s team has observed that up-regulated PD-1 and TIM-3 between COVID-19 patients and healthy individuals, and between prodromal stages and overtly symptomatic stages among COVID-19 patients; which was also associated with the increased levels of anti-inflammatory IL-10 [47]. The expression of inhibitory immune receptors T-cell immunoglobulin and ITIM domain (TIGIT) and the CD94/NK group 2 member A (NKG2A) on CD8+ T cells was increased significantly in COVID-19 patients [49,50]. However, our current knowledge about T cell (mainly CD8+ T cell) depletion gained from the research of tumors and chronic viral infection, such as HIV and HBV. It is generally believed that this phenomenon occurs when the body has been stimulated by antigens for a “long” period of time, which is not in line with acute viral infection [48,51]. Therefore, it is not clear that T cell exhaustion is actually accruing in SARS-CoV-2 infection.

Since SARS-CoV-2 can escape from the host immunity by inhibiting the production of type I IFN during the early stage of infection, an artificial supplement of IFN-α/β during the early stage of COVID-19 infection may help to relieve injuries caused by the virus. However, we do not recommend applying glucocorticoids (GKS) in the early stage, as early use of immunosuppressive medications may exacerbate the virus’s damage to the body.

3. Cytokine storm

Although the direct damage from the viruses contributes to the initiation of the disease, the cytokine storm caused by HCoV plays a vital role in the development of ALI and ARD [7]. Cytokines are a group of small molecular proteins secreted by immune and tissue cells that includes interleukin (IL), colony-stimulating factor (GFS), IFN, tumor necrosis factor (TNF), growth factor (GF) and chemokines. Cytokines participate in immune responses through the activation of multiple signaling pathways, such as JAK-STAT, TRAF-NF-kB, TRAP-1, and IRAK-NF-kB [32]. The term “cytokine storm” appeared for the first time in an article relating to graft-versus-host disease in 1993 [52] as an interpretation for mechanisms of immune diseases or chronic inflammation. Gradually, scientists found that viruses, including cytomegalovirus, streptococci, influenza virus, and SARS-CoV, can also evoke cytokine storms [53–56]. During viral infection, PRRs activate IRF and NF-kB pathways by recognizing PAMP, then promote the release of pro-inflammatory cytokines and chemokines from infected local epithelial cells or macrophages. These cytokine-activated macrophages and virus-infected dendritic cells (DC) lead to a broader immune response, attracting more inflammatory cells to inflammatory sites, releasing a large number of cytokines, which eventually, results in a cytokine storm. Excessive cytokines may spill into the circulatory system and cause systemic cytokine storms, resulting in multiple organ dysfunction [7,57].

Previously, it has been found that the serum levels of pro-inflammatory cytokines [IFN-γ, IL-1, IL-6, IL-12, and transforming growth factor-β (TGF-β)], and chemokines (CCL2, CXCL9, CXCL10, and IL-8) in SARS-CoV-infected patients were higher than those in healthy individuals. While the level of anti-inflammatory cytokine IL-10 in severe patients was significantly lower than that in healthy controls [58–60]. At the beginning of the epidemic, Huang et al. reported that the plasma levels of IL2, IL7, GSCF, CXCL10, MCP1, MIP1A, and TNF-α were higher in COVID-19 patients in the intensive care unit (ICU) than those of COVID-19 patients outside the ICU [61]. Furthermore, the relatively higher level of serum ferritin was also seen in deceased patients comparing with discharged patients, and in severe patients comparing with mild patients [62,63]. Thus, the pattern of cytokine storm in COVID-19 patients was thought to be similar to secondary hemophagocytic lymphohistiocytosis (sHLH) [64–66]. Unlike the cytokines profile of SARS patients, COVID-19 patients have a remarkable increase of anti-inflammatory cytokines IL-10 (Table 1), which has been often seen in HLH patients in previous studies [61,67,68]. Compared with other increased cytokines, increased IL-10 was frequently associated with IL-6 in patients with severe disease(Table 1) [13,69,47,61], suggesting both IL-10 and IL-6 are the indicators of COVID-19 exacerbation.

IL-6, a kind of pleiotropic cytokine, is expressed by immune cells such as DC, monocytes, macrophages, B cells, and subsets of activated T cells, as well as by non-immune cells such as fibroblasts, epithelial cells, and keratinocytes [74–77]. It can activate JAK/STAT, Ras/ERK/C/EBP, and PI3 K/Akt pathways through IL-6R and signal-transducing receptor gp130 [78,79]. IL-6 also regulates B cell proliferation and differentiation and induces T cell differentiation [80–82]. Although IL-6 has been proposed to be involved in the repair of lung injury in the mouse model of influenza A H1N1 virus [83], it contributes to the
pathogenesis of inflammatory or autoimmunity diseases [84,85], and excessive production of IL-6 leads to serious disease progression in viral infection [86,87].

IL-10 is also a pleiotropic cytokine that limits and terminates inflammatory responses through inhibiting antigen-presenting cells (APC), pro-inflammatory cytokines production, and T cell response [88]. Various subpopulations of CD4⁺ helper T (Th) cells, including Th1, Th2, and Th17, produce IL-10, as an important self-regulatory function of Th cells during infections [89–92]. Although reducing IL-10 levels could improve the resistance to infection in some animal models, the weakening control of inflammatory responses may cause significant damage to the hosts at the same time [93]. Thus, IL-10 plays a central role in maintaining a balance between damage and protection.

IL-6 induces naïve T cells to differentiate into Th2 cells and Th17 cells, and the activated Th2 and Th17 cells produce IL-10 that regulates the immune response [94–98]. (Fig. 1). The similarity in the signaling network between IL-10 receptors and IL-6R suggests the common biological process among IL-10 and IL-6 [99]. It has been reported that IL-6 cooperated with IL-10 to eliminate pathogens and modulate the cellular immune response in the patient infected with the H1N1 influenza virus, but the produced immunosuppressive environment delayed virus clearance or increase secondary infection [86,100]. Pathological findings of a deceased COVID-19 patient showed elevated IL-6 and IL-10 with increased highly proinflammatory CCR4⁺CCR6⁺Th17 in peripheral CD4⁺T cells [101]. Rapid accumulation of proinflammatory cytokines stimulates the over-reaction of anti-inflammatory response, resulting in severe immune injury in patients. Chinese Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7) suggests the increase of IL-6 as an indicator for critical patients [16]. We recommend measuring both IL-10 and IL-6 as the signs of the cytokine storm intensification in COVID-19 patients.

Great attention has been paid to the cytokine storm in COVID-19 infection to address the immediate need for reducing the rising mortality. Anti-inflammatory medications are commonly used for the management of cytokine storm, which includes GCs, intravenous immune globulin (IVIG), and inflammatory cytokines antagonists (such as IL-1R antagonists, IL-6R antagonists, and JAK inhibitors). However, GCs may slow down the clearance of the coronavirus [102]. High-dose IVIG at the early stage of clinical deterioration was reported to be successful in three cases [103], and an ongoing randomized controlled trial (RCT) of IVIG in severe COVID-19 patients (NCT04261426) may provide more authoritative evidence of this treatment.

Since IL-1β and IL-6 are the major cytokines involved in the pathogenesis of sHLH [65], the IL-6R antagonist (tocilizumab and sarilumab) and IL-1R antagonists (anakinra) may be effective choices. A retrospective study of continuous intravenous infusion of anakinra in five patients established a rapid serologic and subsequent clinical improvement in adult patients with sHLH [104] and a randomized, placebo-controlled phase III trial indicated that anakinra reduced mortality in sepsis patients with features of sHLH [105]. Tocilizumab, which attracts more attention compared with anakinra, was found to improve fever, hypoxemia, lung lesions, and CRP levels in most of the severe COVID-19 patients with no obvious adverse reactions in retrospective studies, but several patients still aggravated [106,107]. Serum level of IL-6 after tocilizumab therapy tended to further spike initially, and then decrease, while the patients who failed treatment exhibited a persistent and dramatic increase of IL-6 [107,108]. Nevertheless, we cannot draw any firm conclusions on the efficacy and safety of anakinra or tocilizumab due to the limited sample size and retrospective method of the present evidence. Further studies are required to determine the efficacy and safety of these medications.
probability of potential secondary infection, and to find an appropriate biochemical indicator for monitoring the therapeutic effect. Clinical trials evaluating anakinra (NCT04341584, NCT04324021, NCT04339712, and NCT04330638) and tocilizumab (ChiCTR2000029765, ChiCTR2000030796, ChiCTR2000030894, NCT04317092, NCT04335071, and NCT04320615 et al) in the treatment of COVID-19 are ongoing across the world.

4. Elevation of procalcitonin (PCT)

Secondary bacterial infection has been found to further exacerbate viral pneumonia, which is also related to the imbalance of T cells and plasma cytokines [109]. Bacterial coinfection is known to be associated with approximately 40% of viral respiratory tract infections requiring hospitalization [110]. Early detection of bacterial infection and timely intervention will help to alleviate the deterioration of COVID-19. Procalcitonin (PCT), released by bacterial infectious tissues under the irritation of pro-inflammatory cytokines, is a more specific marker of serious bacterial infection compared to C-reactive protein (CRP) and IL-6 [111]. PCT-based strategy has been applied to guide antibiotic use in ICU or emergency wards, since the serum PCT levels in patients with severe bacterial infections are much higher than those with simple viral infections or non-specific inflammatory diseases [111–113]. Anti-microbial interventions are encouraged in patients with lower respiratory tract infection who has the value of PCT > 0.25 ng/mL or > 0.5 ng/mL, for they are likely or highly-likely respectively, to undergo a bacterial infection [113,114].

Multiple studies have shown that PCT levels increased in critical or deceased COVID-19 patients. (Table 2) Li et al. [115] reported that 90.5% of deceased patients had elevated PCT levels (0.11 – 75 ng/mL), including PCT > 0.25 ng/mL in 12 patients and PCT > 0.5 ng/mL in 9 patients, and the PCT value increased from the admission to death. In another retrospective study of 1099 COVID-19 patients, higher proportions of PCT > 0.5 ng/mL were found in severe cases than mild cases (13.7% vs 3.7%) [8]. Patients who reached primary composite endpoint (including admission to an ICU, the use of mechanical ventilation, or death) had a higher proportion of PCT > 0.5 ng/mL than patients without end-point events (24.0% vs 3.9%) [8]. The correlation between increased PCT and severe COVID-19 was also observed in pediatric patients [116]. These findings, although preliminary, suggested that bacterial infection may promote the aggravation of COVID-19. Timely measuring PCT as a sign of secondary bacterial infection for the decision of antibacterial agents use is critical for COVID-19 patients. The aggravation of COVID-19 ascribed to the secondary bacterial infection and the adverse events caused by unreasonable use of antibiotics could be reduced. The most effective selection of the antibiotic agents should be based on bacterial culture. In consideration of the dysregulation of the immune system in severe COVID-19 patients, antibacterial agents with immunomodulatory effects, such as macrolides (erythromycin and clarithromycin) might be a better option. Previous studies have shown that in addition to antibacterial effects, these drugs can also alleviate inflammatory reactions by reducing the accumulation of pro-inflammatory cytokines and regulating neutrophil function and apoptosis [117–120].

Table 2

| Studies | Patients’ categories | Number of patients | PCT v<sub>100</sub> ng/mL (n, %) | P value |
|----------------|---------------------|--------------------|--------------------------|--------|
| Li et al [115] | Dead | 21 | 0.36 (0.13, 1.91) | > 0.05 |
| Zhang et al [10] | Dead | 69 | 0.3 (0.1–1.1) | > 0.1 |
| Chen et al [121] | Dead | 96 | 0.33 (0.14–0.65) | > 0.25 |
| Guan et al [8] | Severe | 173 | NA | > 0.50 |
| Severe | 926 | NA | NA | 19, 3.7 |
| Xiong et al [122] | Severe | 21 | 0.33 ± 0.27 | NA |
| Severe | 18 | 0.13 ± 0.11 | NA |
| Yuan et al [123] | Severe | 31 | 0.05 (0.01–2.1) | NA |
| Severe | 192 | 0.01 (0.01–0.4) | NA |
| Zhang et al [124] | Severe | 50 | 0.1 (0.06–0.3) | NA |
| Non-severe | 68 | 0.05 (0.03–0.1) | NA |
| Huang et al [61] | ICU | 12 | 0.1 (0.1–0.4) | NA |
| Non-ICU | 27 | 0.1 (0.1–0.1) | NA |
| Wang et al [125] | ICU | 36 | NA | 27, 75.0 |
| Non-ICU | 102 | NA | NA |
| Non-ICU | 40 | 0.11 (0.08–0.16) | NA |
| Mild | 95 | 0.04 (0.03–0.06) | NA |
| General | 96 | 0.11 (0.06, 0.20) | NA |
| Qi et al [63] | Severe | 286 | 0.1 (0.0–0.2) | NA |
| Non-severe | 166 | 0.05 (0.03–0.09) | NA |
| Li et al [126] | Critically severe | 16 | 0.44 ± 0.512 | NA |
| Severe | 56 | 0.14 ± 0.353 | NA |
| Moderate | 60 | 0.08 ± 0.279 | NA |
| Wang et al [73] | SpO₂ < 90 % | 14 | 0.13 (0.13–0.15) | NA |
| SpO₂ ≥ 90 % | 55 | 0.13 (0.13–0.15) | NA |
| Shi et al [129] | With cardiac injury | 82 | 0.27 (0.10–1.22) | NA |
| Without | 234 | 0.06 (0.03–0.10) | NA |
| Wu et al [130] | With ocular symptoms | 12 | NA | 8, 66.7 |
| Without | 25 | NA | 7, 28.0 |
| Qiu et al [116] | Moderate pediatric | 19 | 0.32 ± 0.19 | NA |
| Mild pediatric | 17 | 0.15 ± 0.13 | NA |
| Sun et al [131] | Pediatric | 8 | 0.085 (0.05, 0.128) | NA |
| Xia et al [132] | Pediatric | 20 | NA | 16, 80 |

*Number of patients with the record of PCT. aPCT values in different studies were expressed as different forms, including mean ± SD, median (IQR), and median (mix-max). bPCT value ≥0.05. cP-value of the comparison between the PCT value of two groups. dP-value of the comparison between the proportion of the patients with increased PCT value in two groups. The tests with a P-value of < 0.05 are considered statistically significant.
5. Conclusions

The ongoing COVID-19 pandemic is threatening the world with substantial mortality. SARS-CoV-2 could cause lymphopenia and cytokine storm, together with secondary bacterial infections, adding to the deterioration of the disease in severe cases of COVID-19. Understandings of the dynamic process of SARS-CoV-2 infection would help to make an appropriate decision on treatment strategies. We recommend measuring both IL-10 and IL-6 as a sign of intensification of cytokine storm in severe COVID-19 cases, though test IL-10 is not routinely carried out in the clinic setting. We also recommend using serum PCT level to guild the initiation of antibacterial agents in patient with COVID-19. Although the selection of immunomodulators is challenging, previous retrospective studies have revealed distinct immune regulators between the early and late stages of SARS-CoV-2 infection. For instance, supplementation of type I IFN, instead of GCs, at the early stage of infection may help to control the virus and relieve injury. IL-1R and IL-6 antagonists could potentially be the effective choices for managing cytokine storm. However, these preliminary results would need to be validated from multiple ongoing RCTs. Due to the rapid nature of COVID-19 pandemic, global collaboration in research and information sharing is ultimately needed.

Declaration of Competing Interest
None.

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