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The study on the treatment of Xuebijing injection (XBJ) in adults with severe or critical Corona Virus Disease 2019 and the inhibitory effect of XBJ against SARS-CoV-2

To the Editor,

The sudden outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) rapidly became a global pandemic, marked the third introduction of a virulent coronavirus into the human society [1]. Although our understanding of coronaviruses has undergone a huge leap after two precedents, the effective approaches to treatment and epidemiological control are still lacking. Xuebijing injection (XBJ) is a traditional Chinese medicine, whose main components are hydroxysafflor yellow A, paeoniflorin oxide, Ligusticum chuanxiong lactone I and paeoniflorin, *etc* [2], and it has many pharmacological activities include activating circulation, removing blood stasis, and clearing away toxins [3]. It can be used for systemic inflammatory response syndrome induced by infection, and also be used to treat the period of organ function damage in Multiple Organ Dysfunction Syndrome (MODS). Its efficacy in severe areas such as sepsis and MODS has been widely recognized by clinical experts.

In 2019, a randomized controlled trial on the efficacy of XBJ *versus* placebo for critically ill patients with severe community acquired pneumonia (SCAP) was conducted in 33 top three hospitals in China and 710 adult patients were enrolled. The results showed that adding XBJ on the basis of routine anti-infective treatment, the 28-day mortality of patients with severe pneumonia complicated with sepsis could be reduced by 8.8 %, significantly improving pneumonia severity index (PSI), shortening mechanical ventilation time by 5.5 days and intensive care unit (ICU) length of stay by 4 days, and there was no significant difference in the incidence of adverse events [3]. Combined with the pharmacological effects of XBJ and its evidence-based medicine in critical fields such as SCAP, sepsis and MODS, it was speculated that XBJ could be used as a new therapeutic drug that could benefit patients with severe or critical Corona Virus Disease 2019 (COVID-19). In our study, a total of 11 adult patients with severe or critical COVID-19 hospitalized in ICU of Dongguan people's Hospital of Guangdong Province from February 10, 2020 to March 10, 2020 were included. Patients were included if (1) they matched the diagnostic criteria for COVID-19 pneumonia [4], (2) diagnostic criteria of community-acquired pneumonia [5], (3) PSI was grade III-V, or oxygenation index $\leq$300 mmHg, (4) age $\geq$18 years. The study protocol has been approved by the Ethic Committee of Dongguan people's Hospital of Guangdong Province (Number: DRYA2020-006). And we analyzed the clinical data of patients with severe or critical COVID-19 treated with XBJ, which all the patients received XBJ (normal saline 100 mL + XBJ 100 mL, every 12 h for 7 days). XBJ, specification 10 mL/ampule, packaging 10 ampules/container, concentration 500 mg/mL (Supplemental material Table 1). The results showed that the PSI grade and PSI score at day 7 were significantly better than those at day 1 ($P < 0.05$), which was similar to the results of the above study. What's more, the effects of XBJ against SARS-CoV-2 *in vitro* by CPE assay and plaque assay were measured. The results showed that XBJ was able to protect cells from virus-induced cell death and inhibit the average size and plaque number in XBJ-treated cells in a dose-dependent manner. The SI index of XBJ reached to 40.06 on SARS-CoV-2 (Supplemental material Fig. 2). The results suggested that XBJ might be a key parameter on influencing the viral activity, which also needed to be validated *in vivo*.

The majority of patients with severe COVID-19 develop dyspepsia and/or hypoxaemia one week after onset, and the number of lymphocytes in peripheral blood often decreases significantly. Our study included 11 patients with severe or critical COVID-19, most of them had epidemiological history and basic diseases (including hypertension, diabetes, coronary heart disease, *etc*.) (Supplemental material Table 2). It is suggested that patients with basic diseases such as hypertension, diabetes and coronary heart disease are at high risk for the development of severe or critical diseases. Most patients had varying degrees of dyspnea on admission, and laboratory measurements showed that oxygenation index, partial pressure of carbon dioxide in artery ($PaCO_2$) and lymphocyte count were low, consistent with previous studies [6]. The included cases met the diagnostic criteria of severe or critical COVID-19. According to the clinical guidance for COVID-19 pneumonia diagnosis and treatment, all patients were given bed rest, oxygen therapy (patients with severe were treated with high-flow nasal canula (HFNC), patients with critical were treated with invasive mechanical ventilation), atomized interferon-α, Lopinavir/ritonavir and organ function support, plus XBJ (Supplemental material Table 3). XBJ may improve lung injury in patients with severe or critical COVID-19, but further research is needed to determine.

Cytokine Storm (CS) is an important node in the transformation of COVID-19 from mild to severe or critical, and it is also a cause of severe or critical death. Improving the immune function of patients and reducing the storm of inflammatory factors are two key links in the treatment of patients with severe COVID-19. The results of this study showed that XBJ could improve the oxygenation index, $PaCO_2$ and lymphocyte count of patients with severe or critical COVID-19 at day 7, and all of them were cured and discharged from hospital (Supplemental material Table 2). The mechanism remains to be studied. We speculated that XBJ could enhance and consolidate immunity and detoxification ability. However, XBJ had little effect on white blood cell (WBC), neutrophil count, C-reactive protein (CRP) and procalcitonin (PCT) in patients with severe or critical COVID-19 at day 7, which was estimated to be related to the small sample size of this study.

It is reported that highly pathogenic coronaviruses such as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) cause fatal pneumonia, which is mainly associated with rapid virus replication, massive inflammatory cell infiltration and elevated proinflammatory cytokine/chemokine responses. Although the pathophysiology of fatal pneumonia caused by highly pathogenic coronaviruses has not been completely understood, recent studies suggest a crucial role of cytokine storm in causing fatal pneumonia. Early studies have shown that increased amounts of proinflammatory cytokines (eg, IL-6, IP-10, and

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MCP-1) in serum of SARS patients, similar in the serum of MERS patients with increased concentrations of proinflammatory cytokines (IFN-γ, TNF-α, IL-15, and IL-17) [7]. Chaolin Huang et al. reported the cytokine storm in the NCIP patients in ICU than those in non-ICU patients [8]. Therefore, the expression of inflammatory cytokines in the serum of COVID-19 patients was determined after XBJ treatment (Supplemental material Fig. 1). The results showed that the expression of TNF-α, IP-10, MIP-1β, RANTES in the COVID-19 patients was dramatically elevated during day 2 and day 3 after virus infection, whereas the protein expression of these inflammatory cytokines was significantly inhibited by XBJ treatment during day 7 and day 8, which indicated that XBJ could inhibit the inflammation of the COVID-19 patients *via* inhibiting the inflammatory mediators expression. Moreover, the results showed that XBJ inhibited the release of TNF-α, IL-6, MIP-1β, RANTES, and IP-10 induced by SARS-COV-2 in Huh-7 cells (Supplemental material Fig. 3) in a dose-dependent manner.

A centralized monitoring study on the clinical safety of XBJ was conducted in 93 medical institutions. A total of 31913 patients using XBJ were included. The incidence of adverse reactions was reported to be 0.3 % (96 patients), belonging to the occasional grade. And they are all adverse reactions that have been stated in the drug instructions. The main manifestations were skin pruritus, rash, chest tightness and fever *etc*. All adverse reactions were mild and disappeared or relieved after drug withdrawal or symptomatic treatment. The results showed that XBJ was safe under the condition of reasonable clinical use. In this study, no adverse reactions such as allergic reaction and organ function damage were reported, and the biochemical indexes such as CTN-I, ALT, AST and SCr were monitored before and after treatment, and there was no significant difference, which preliminarily proved that XBJ was safe (Supplemental material Table 4).

In conclusion, our results revealed that XBJ may improve lung injury in patients with severe or critical COVID-19. Moreover, XBJ could significantly protect cells from SARS-CoV-2-induced cell death and inhibit the average size and plaque number *in vitro*. The anti- SARS-CoV-2 effect was attributed to the blocking of the proliferation of virus, and inhibiting the upregulated expression of pro-inflammatory cytokines induced by SARS-CoV-2. These findings warrant further evaluation of XBJ as a potential agent for SARS-CoV-2 treatment and provide information to further reveal the mechanisms.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.105073.

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