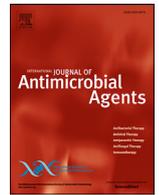




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The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality ☆☆☆

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ABSTRACT

Since December 2019, a viral pneumonia (COVID-19) from Wuhan, China has swept the world. Although the case fatality rate is not high, the number of people infected is large, and there are still a large number of patients dying. With the collation and publication of more and more clinical data, a large number of data suggest that there are mild or severe cytokine storms in severe patients, which is also an important cause of death. Therefore, the treatment of cytokine storm has become an important part of rescuing severe patients. Interleukin-6 (IL-6) plays an important role in cytokine release syndrome (CRS). If it can block the signal transduction pathway of IL-6, it is expected to become a new method for the treatment of severe patients. Tocilizumab is a blocker of IL-6R, which can effectively block IL-6 signal transduction pathway. So, tocilizumab is likely to become an effective drug for patients with severe COVID-19.

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Abbreviations: CRS, cytokine release syndrome; IL-6R, Interleukin-6 receptor; IL-6, Interleukin-6; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CTL, cytotoxic T lymphocyte; MTX, methotrexate; DMARDs, disease-modifying antirheumatic drugs; CAR-T, Chimeric Antigen Receptor T-Cell Immunotherapy; ALL, acute lymphoblastic leukemia; HLH, hemophagocytic lymphohistiocytosis; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; JAK, Janus kinase; STATA, signal transducer and activator of transcription; YAP, Yes-associated protein; AKT, phosphoinositide 3-kinase (PI3K)-protein kinase B; PI3K, also known as PKB phosphoinositide 3-kinase (PI3K)-protein kinase B; MAPK, mitogen-activated protein kinases.

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** **Specific author's contribution:** Chi Zhang and Zhao Wu searched the literature; Chi Zhang drafted the manuscript; Chi Zhang, Zhao Wu and Jia-Wen Li participated in the creation of figures and table; Hong Zhao and Gui-Qiang Wang provided the overall principle and direction of the study.

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1. Introduction

In December 2019, several patients with unexplained pneumonia appeared in Wuhan China, and a viral pneumonia sweeping the world is in the process of gestation. Several days later, the virus was identified as a new beta coronavirus and officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. As of March 14, 2020, the disease has caused 81026 infections in China, with a case fatality rate of 3.9% (3194/81026). A total of 55095 confirmed cases have been reported in other countries in the world, with a mortality rate of 4.1% (2238/55095), which is not much different from that in China. Although most patients present with mild symptoms and are not life-threatening, the number of deaths is still high due to the large population base.

The first COVID-19 pathology found bilateral diffuse alveolar injury with cytomyxoid fibroma exudate, and subsequent peripheral flow cytometry analysis found a decrease in CD4 and CD8 cells, but an increase in Th17 cell proportion [2]. Th17 cells are helper T cells differentiated from Th0 cells mainly stimulated by IL-6 and IL-23 [3]. A study to be published (Jing Liu et al.) incorporating COVID-19 from 40 patients (of which 13 were severe) suggests that severe cases show a sustained decrease in the proportion of lymphocytes compared with mild cases. In addition, CD8 T cells decreased and inflammatory cytokines (IL-6, IL-10, IL-2 and IFN- γ) increased in severe cases, in the peripheral blood.

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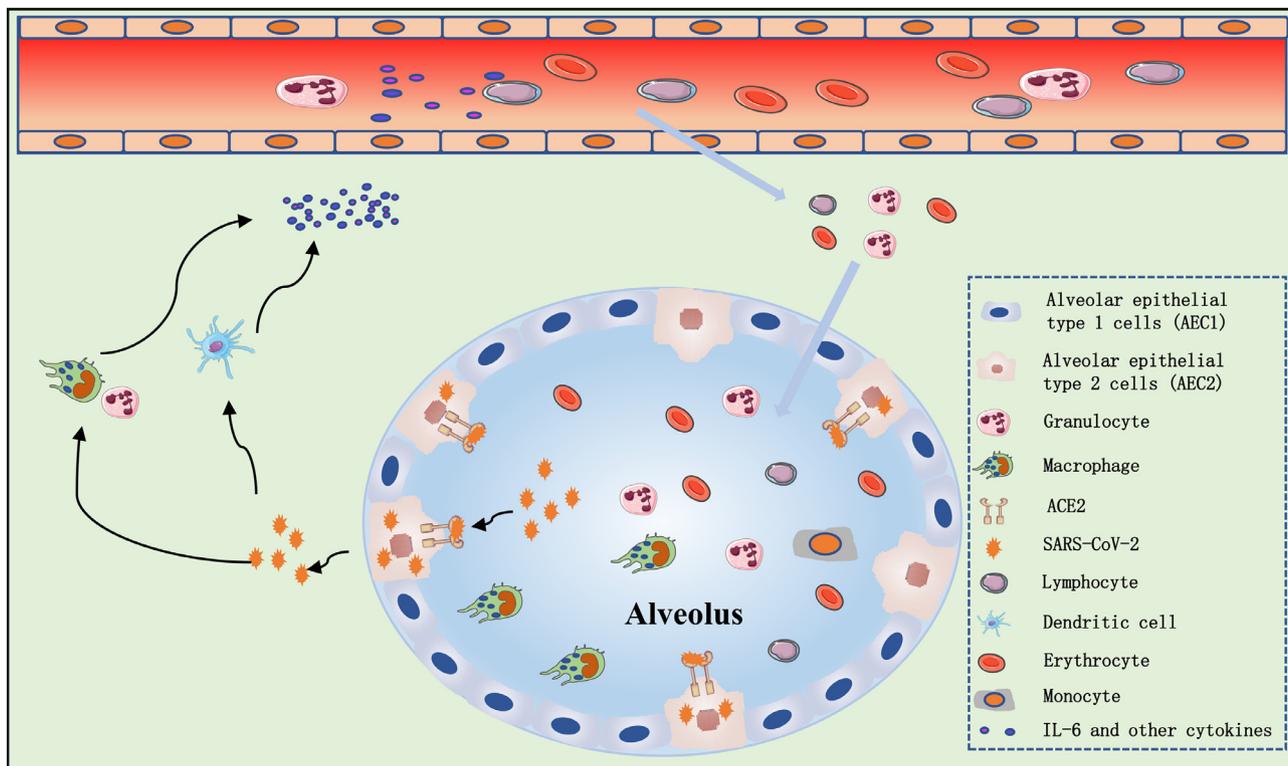


Fig. 1. Possible mechanism of cytokine release syndrome in severe COVID-19 patients. The SARS-CoV-2 infects alveolar epithelial cells (mainly Alveolar epithelial type 2 cells [AEC2]) through ACE2 receptor. The destruction of epithelial cells and the increase of cell permeability lead to the release of virus. The SARS-CoV-2 activate the innate immune system, macrophages and other innate immune cells not only capture the virus, but also release a large number of cytokines and chemokines including IL-6. Adaptive immunity is also activated by antigen presenting cells (mainly dendritic cells). T cells and B cells not only play an antiviral role, but also directly or indirectly promote the secretion of inflammatory cytokines. In addition, under the stimulation of inflammatory factors, a large number of inflammatory exudates and erythrocytes enter the alveoli, resulting in dyspnea and respiratory failure.

Taking together, we have a reasonable hypothesis that cytokine storms play an important role in severe cases, so neutralizing key inflammatory factors in CRS will be of great value in reducing mortality in severe cases.

2. Brief introduction of CRS

CRS is a systemic inflammatory response, which can be caused by infection, some drugs and other factors, characterized by a sharp increase in the level of a large number of pro-inflammatory cytokines [4–6]. CRS is more common in immune system-related diseases or immune-related therapy, such as CAR-T cell therapy, organ transplantation sepsis [7] and virus infection. The SARS-CoV-2 bind to alveolar epithelial cells, then the virus activates innate immune system and adaptive immune system, resulting in the release of a large number of cytokines, including IL-6. In addition, due to the role of these pro-inflammatory factors, vascular permeability increased, a large number of fluid and blood cells into the alveoli, resulting in dyspnea and even respiratory failure [8–10] (Fig. 1). The first gross examination report of a COVID-19 death autopsy suggests that the bronzed appearance of both lungs, and a large amount of gray-white viscous liquid overflow can be seen after incision [11].

3. IL-6 and the role in CRS

Interleukin-6 (IL-6) is an important member of the cytokine network and plays a central role in acute inflammation [12]. IL-6, discovered by Weissenbach in 1980 [13], is a multifunctional cytokine, which plays an important role in human metabolism, au-

toimmune cell differentiation, disease treatment and so on [14]. A brief introduction of IL-6 is shown in Fig. 2.

3.1. Structure and characteristics of IL-6

IL-6 is a small polypeptide consisting of four alpha helices. The molecular weight is 19–28kD, with 184 amino acid residues, usually in monomer form, with an isoelectric point of 5.0, glycosylation sites and two disulfide bonds. The gene encoding IL-6 is located on chromosome 7p¹⁵⁻²¹, including 4 introns and 5 exons [15].

IL-6 can be produced by almost all stromal cells and immune system cells, such as B lymphocytes, T lymphocytes, macrophages, monocytes, dendritic cells, mast cells and other non-lymphocytes, such as fibroblasts, endothelial cells, keratinocytes, glomerular Mesangial cells and tumor cells [16]. The main activators of IL-6 expression are IL-1 β and tumor necrosis factor (TNF- α) [14]. Of course, there are other ways to promote the synthesis of IL-6, such as Toll-like receptors, prostaglandins, adipokines, stress response and other cytokines [14].

In the early stage of infectious inflammation, IL-6 is produced by monocytes and macrophages stimulated by Toll-like receptors. In non-infectious inflammation, such as burns or traumatic injuries, it can also be produced by cells stimulated by Toll-like receptors. This acute IL-6 expression plays a central role in host defense by stimulating various cell populations.

3.2. Signal transduction pathway of IL-6

IL-6 plays a central role in cytokine storm. IL-6 is a multi-effective cytokine with anti-inflammatory and pro-inflammatory

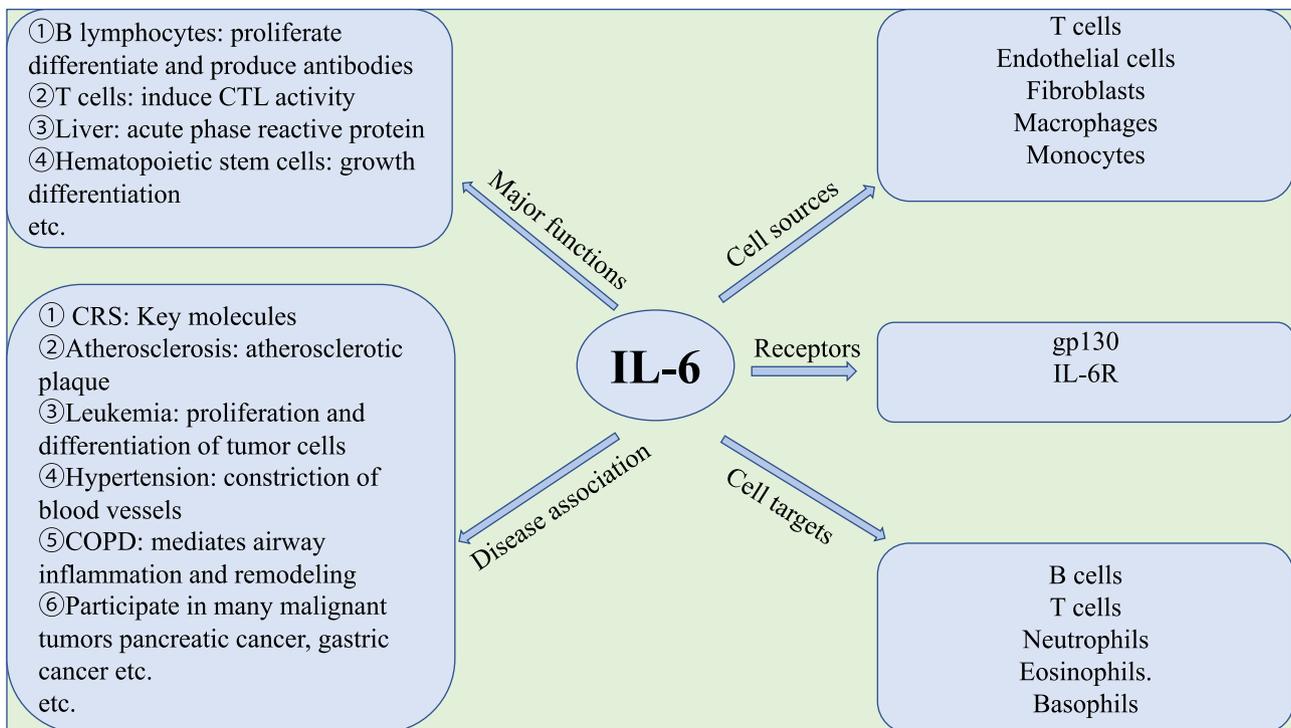


Fig. 2. Brief introduction of IL-6.

effects. There are three main pathways of IL-6 signal transduction [14,17] (Fig. 3): ①classical signal transduction (Fig. 3A), ②trans signal transduction(Fig. 3B) and ③trans presentation (Fig. 3C).

In the classical signal transduction pathway [18], IL-6 binds to its receptor IL-6R to form a complex, and then binds to the membrane protein gp130 to initiate intracellular signal transduction. IL-6R exists not only in transmembrane form, but also in soluble form. IL-6 binds to these two forms and then interacts with gp130 to trigger downstream signal transduction and gene expression [19–21]. In the trans signal transduction pathway, the binding affinity of sIL-6R to IL-6 is similar to that of IL-6R, and this complex binds to gp130, which initiates intracellular signal transduction. In the classical signal pathway, many cells cannot respond to IL-6 signal because they do not express IL-6R, but some of these cells can be stimulated by sIL-6R-IL-6 complex to respond to IL-6 signal and cause cell signal transduction [22,23]. The trans presentation signal is suppressed by extracellular sgp130, and sgp130 can form a complex with sIL-6R to prevent sIL-6R from binding to membrane-bound gp130 [16]. The next step is to activate the JAK-STAT (STAT1, STAT3 and, to a lesser extent, STAT5) pathway [22–25], in addition, RAS-RAF pathway [23,26], SRC-YAP-NOTCH pathway [27], and AKT-PI3K pathway [28,29] also being activated (Fig. 3D). So as to promote complex biological functions such as proliferation, differentiation, oxidative stress, immune regulation and so on [16].

3.3. Biological function

IL-6 can promote T cell population expansion and activation and B cell differentiation, regulate acute phase response, and affect the hormone-like properties of vascular disease, lipid metabolism, insulin resistance, mitochondrial activity, neuroendocrine system and neuropsychological behavior [14]. In addition, IL-6 promotes the differentiation of osteoclasts and angiogenesis, the proliferation of keratinocytes and glomerular membrane cells, and the growth of myeloma and plasmacytoma cells [14,25].

① Effect on B lymphocytes [30]: IL-6 can induce B cells to proliferate, differentiate and produce antibodies. IL-6 is especially needed when B cells are activated by antigen and differentiate into IgM, IgG and IgA antibodies. ② Effect on T lymphocytes [31]: IL-6 is the terminal helper factor of cytotoxic T lymphocyte (CTL), which can induce CTL activity and make immature thymocytes develop into CTL. In addition, IL-6 is a pro-inflammatory regulator of T cells. IL-6 can promote Th17 cell lineage and function, inhibit the induction of regulatory T cell (Treg), and promote the development of self-reactive pro-inflammatory CD4 T cell response. IL-6 combined with TGF- β can promote the development and function of Th17 cells, while Th17 cells are related to the occurrence and development of many self-inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus and so on [31–33]. ③ Effect on hepatocytes [34,35]: IL-6 is a strong inducer of acute phase reactive protein, which can induce hepatocytes to synthesize acute phase reactive protein at the gene transcription level, especially Serum amyloid A (SAA) and C-reactive protein (CRP). ④ Effect on hematopoietic stem cells [36]: IL-6 can cooperate with other cytokines to promote the growth of early bone marrow stem cells, enhance the differentiation of blood cells and promote their colony formation. ⑤Participate in the occurrence of immune abnormalities [37,38]: hypergammaglobulinemia, myocardial myxoma, bladder cancer, chronic rheumatoid arthritis and other patients are accompanied by abnormal increased levels of IL-6. ⑥Participate in the occurrence and development of cardiovascular diseases [39]: myocardial ischemia, coronary atherosclerosis, angina pectoris, congestive heart failure, hypertension and other patients are accompanied by abnormal increased levels of IL-6.

4. IL-6R antagonist Tocilizumab

The classical IL-6 signal is limited to the cells (macrophages, neutrophils, T cells, etc.) that express IL-6R, and plays a leading role in the low level of IL-6. The combination of IL-6 and cell-related IL-6R leads to gp130 homologous dimerization and initiates downstream pathway. However, when the level of IL-6 increases,

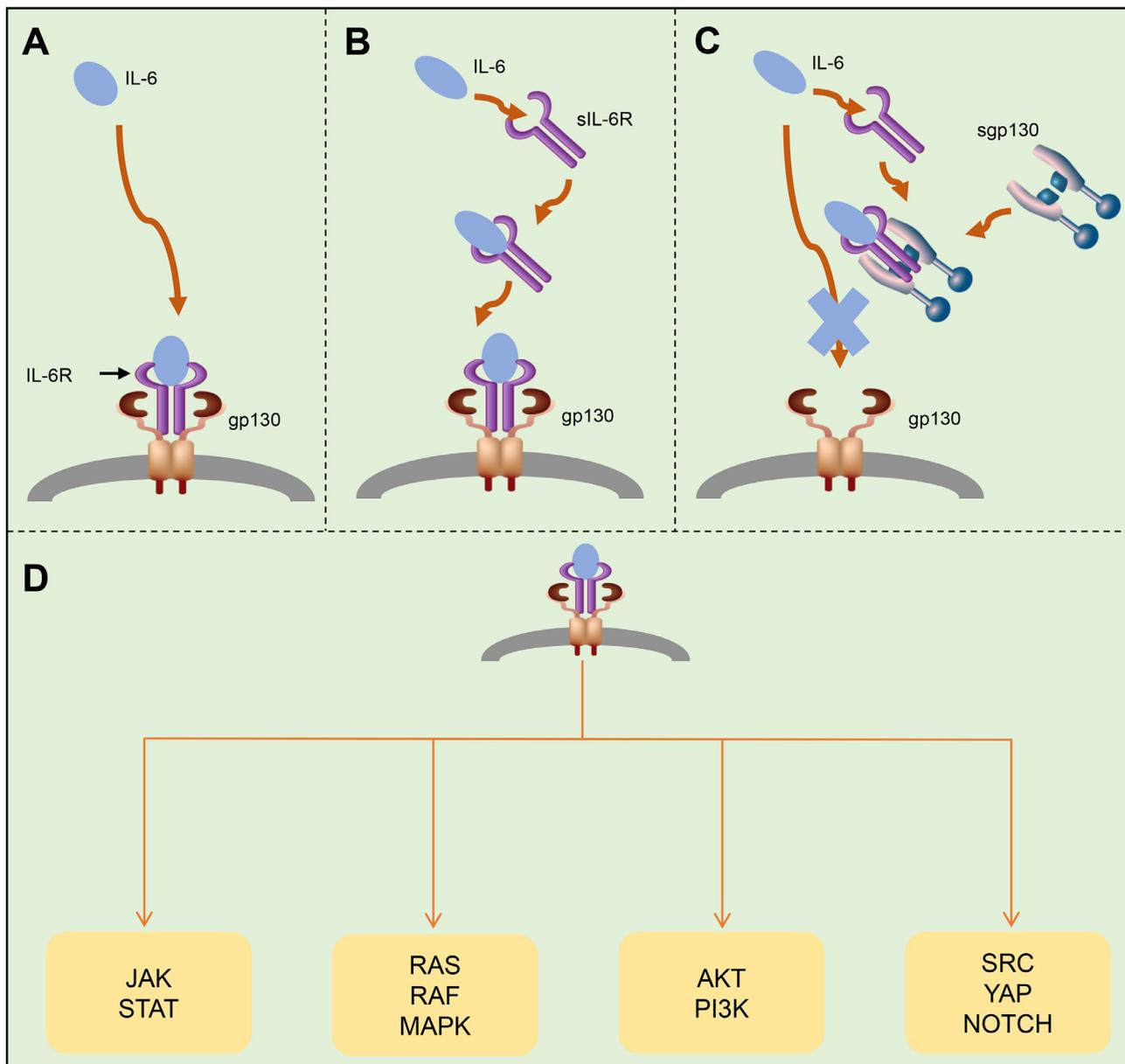


Fig. 3. Signal transduction pathway of IL-6. (A) classical signal transduction; (B) trans signal transduction; (C) trans presentation; (D) The next step is to activate the JAK-STAT (STAT1, STAT3 and, to a lesser extent, STAT5) pathway, in addition, RAS-RAF pathway, SRC-YAP-NOTCH pathway, and AKT-PI3K pathway also being activated. So as to promote complex biological functions such as proliferation, differentiation, oxidative stress, immune regulation and so on.

IL-6 signal is widely expressed, because gp130 is everywhere. The binding of tocilizumab with cell-related IL-6R and soluble IL-6R can inhibit classical and trans signals. So, it can inhibit CRS.

Tocilizumab is a recombinant humanized monoclonal antibody against human interleukin 6 (IL-6) receptor of immunoglobulin IgG1 subtype. Tocilizumab specifically binds soluble and membrane-bound IL-6 receptors (sIL-6R and mIL-6R) and inhibits sIL-6R and mIL-6R-mediated signal transduction. It has been approved for the treatment of rheumatoid arthritis [40] and systemic juvenile idiopathic arthritis [41]. In addition, it has also been reported that it plays a certain role in Castleman disease [42] and Crohn's disease [43]. It is worth noting that tocilizumab is effective in the treatment of severe CRS patients [44,45]. The recommended dose of tocilizumab is 8mg / kg intravenous drip every 4 weeks, for adult with rheumatoid arthritis, which can be used in combination with methotrexate or other anti-rheumatic drugs. When liver enzyme abnormality, neutrophil count and platelet count de-

crease, the dose of tocilizumab can be reduced to 4mg/kg. For systemic juvenile idiopathic arthritis (sJIA) patients, the dose of tocilizumab was 12mg/kg (body weight <30kg) and 8mg/kg (body weight ≥30kg). Intravenous drip every 2 weeks is recommended, and the drip time is more than 1 hour.

The safety of tocilizumab in 5 III phase double-blind controlled trials and its extended period was studied (The data come from the treatment of rheumatoid arthritis.) [46]. The total control population included all patients in the double-blind period of each core study from randomized grouping to the first change of treatment regimen or the completion of a 2-year treatment period. Among them, the double-blind control period of 4 studies was 6 months, and the other double-blind control period was 2 years. In these double-blind controlled trials, 774 patients received tocilizumab 4mg/kg combined with MTX (methotrexate) and 1870 patients with tocilizumab 8mg/kg combined with MTX or other DMARDs (disease-modifying antirheumatic drugs). A total of 288 patients

were treated with tocilizumab 8mg/kg alone. In a 6-month controlled trial, the incidence of infection events in patients with tocilizumab 8mg/kg + DMARD and placebo + DMARD was 127 cases/100 patient-year and 112 patient-year/100 patient-year, respectively. Among the total exposed population, the overall incidence of infection events in the tocilizumab + DMARD group was 108 cases / 100 patient year. The 6-month controlled trial also showed that the incidence of severe infection (bacteria, viruses and fungi) in the 8mg/kg + DMARD group was 5.3/100 patient-year, while that in the placebo + DMARD group was 3.9/ 100patient-year. In the monotherapy trial, the incidence of severe infection was 3.6cases/100 patient-year in tocilizumab group and 1.5cases / 100 patient-year in MTX group. With regard to the safety of tocilizumab in the treatment of patients with severe COVID-19, a preprinted study [47] was included in a study of 21 patients. The mean age was 56.8 ± 16.5 years, ranged from 25 to 88 years. There were no complications associated with tocilizumab and no history of illness deterioration or death. Overall, the risk of secondary infection with tocilizumab is not too high.

The largest clinical data [48] from China's Centers for Disease Control and Prevention show that of the 44672 confirmed cases included, 2683 (12.8%) were hypertension, 1102 (5.3%) were diabetes and 873 (4.3%) were other cardiovascular diseases. Among them, 1023 cases died, the crude death rate of unreported complications was 0.9%, and the mortality rate of patients with complications was much higher, 10.5% of patients with cardiovascular disease, 7.3% of patients with diabetes and 6.0% of patients with hypertension. There is some controversy about whether tocilizumab increases the risk of cardiovascular disease (CVD). Data from several randomized controlled trials (RCT) and real-world evidence (RWE) studies have been published. The Giles JT et al. study [49] included 3080 patients over 50 years old with more than one CVD risk factor for cardiovascular disease who met the diagnosis of active rheumatoid arthritis (1538 were treated with tocilizumab and 1542 were treated with etanercept). After an average follow-up of 3.2 years, 83 times of major adverse cardiovascular events (MACE) occurred in the tocilizumab group (5.4%), while 78 times of MACE occurred in the etanercept group (5.1%). The resulting hazard ratio (HR) was 1.05 (95%CI 0.77 - 1.43). The authors concluded that tocilizumab had a higher cardiovascular risk than etanercept. Interestingly, two RWE studies have come to a different conclusion from aforementioned RCT study. In the Kim SC et al. study [50], cardiovascular events in tocilizumab and Tumor Necrosis Factor Inhibitors (TNFi) were compared, and after strict propensity score matching, 9218 tocilizumab and 18810 TNFi were included. The cardiovascular incidence rate of tocilizumab was 0.52 per/100 person-years, and TNFi was 0.59 per / 100 person-years. The combined HR is 0.84 (95% CI 0.56 - 1.26). Another RWE study of Xie FL et al. [51] came to a similar conclusion that there was no significant difference in the risk of cardiovascular events associated with the use of tocilizumab compared with TNFi (HR[Medicare database]=0.79,95%CI 0.65–0.96; HR[MarketScanned database]=0.84, 95% CI 0.52–1.37). Although the conclusion of RCT study was slightly different from RWE studies, the 95%CI of all studies is not significant (that is, it contains 1), so tocilizumab increases cardiovascular events was insufficient.

In August 2017, the FDA of the United States approved tocilizumab for the treatment of CRS caused by CAR-T (Chimeric Antigen Receptor T-Cell Immunotherapy) therapy [52]. A 7-year-old girl with acute lymphoblastic leukemia (ALL) developed a severe cytokine storm after CAR-T treatment, and the subsequent treatment with tocilizumab dramatically improved her condition and did not affect the efficacy of CAR-T [44]. Another report reported that a male patient with ALL developed hemophagocytic lymphohistiocytosis (HLH) after treatment with blinatumomab. The patient developed severe respiratory failure and methemoglobinemia.

The subsequent treatment with tocilizumab successfully saved the patient's life [53].

SARS-CoV-2, SARS and MERS are coronaviruses, and CRS of varying degrees have occurred in severe patients with SARS [54–56] and MERS [57]. All of them had high expression of IL-6. Currently, a small sample clinical trial in China (Clinical trial registration ID: ChiCTR2000029765) has shown good efficacy in tocilizumab [47]. All 21 patients in the group met the criteria for severe or critical COVID-19 [58]. Among them, the severe criteria meet one of the following: shortness of breath, respiratory rate more than 30 beats / min; oxygen saturation was less than 93%, while resting; $\text{PaO}_2/\text{FiO}_2 \leq 300$ mmHg. Critical criteria meet one of the following: respiratory failure requiring mechanical ventilation; shock; and admission to ICU with other organ failure. After a few days of treatment, the fever patient's body temperature returned to normal (at the beginning, all 21 patients had a fever), and all other symptoms were significantly improved. 75% (15/20) of the patients reduced their oxygen intake, and one patient did not need oxygen. Imaging examination (CT scan) showed that 90.5% (19/21) of the patients had absorption of pulmonary lesions. Laboratory examination showed that the proportion of peripheral blood lymphocytes and C-reactive protein returned to normal. The deficiency is that only the level of IL-6 in peripheral blood before treatment with tocilizumab was reported (mean value 132.38 ± 278.54 pg/ml), but the level of IL-6 after treatment was not reported.

Finally, although from the analysis of COVID-19 's possible mechanism and small sample clinical data, tocilizumab has a better efficacy. However, from the point of view of pharmacoeconomics, we suggest that it should be used in critically ill COVID-19 patients with significantly elevated IL-6.

In conclusion, CRS occurs in a large number of patients with severe COVID-19, which is also an important cause of death. IL-6 is the key molecule of CRS, so IL-6R antagonist tocilizumab may be an important drug to save patients' lives.

Declarations

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Competing Interests: None.

Ethical Approval: Not required.

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