Role Of Immunosuppressants in COVID Patients

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Abstract*— The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 in Wuhan city, Hubei province, China. This is the third and largest coronavirus outbreak since the new millennium after SARS in 2002 and Middle East respiratory syndrome (MERS) in 2012. Over 3 million people have been infected and the COVID-19 has caused more than 217 000 deaths. A concern exists regarding the vulnerability of patients who have been treated with immunosuppressive drugs prior or during this pandemic. Would they be more susceptible to infection by the SARS-CoV-2 and how would their clinical course be altered by their immunosuppressed state? This is a question the wider medical fraternity—including ophthalmologists, rheumatologists, gastroenterologist and transplant physicians among others—must answer. The evidence from the SARS and MERS outbreak offer some degree of confidence that immunosuppression is largely safe in the current COVID-19 pandemic. Preliminary clinical experiences based on case reports, small series and observational studies show the morbidity and mortality rates in immunosuppressed patients may not differ largely from the general population. Overwhelmingly, current best practice guidelines worldwide recommended the continuation of immunosuppression treatment in patients who require them except for perhaps high-dose corticosteroid therapy and in patients with associated risk factors for severe COVID-19 disease.

Index Terms*— Covid-19, SARS-CoV-2, coronavirus, immunosuppressive drugs, corticosteroid, mycophenolic acid, calcineurin inhibitors.

I. INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has a mild course in the majority of patients. However, approximately 14% of the patients require hospitalization for oxygen support and 5% is admitted to the intensive care unit (ICU) (World Health Organization (WHO), 2020).

COVID-19 has a triphasic course (Siddiqi and Mehra, 2020). In the first phase, patients have mild respiratory and systemic symptoms such as dry cough, malaise and fever. An adequate response of the innate and adaptive immune system can eliminate the virus and preclude disease progression to the next, more severe stages (Shi et al., 2020). In the second phase, viral multiplication and localized inflammation of the lung tissue occurs, causing viral pneumonia. A minority of patients with COVID-19 will undergo a transition into the third and most severe phase of illness: a syndrome of systemic hyper inflammation, also referred to as secondary hemophagocytic lymphohistiocytosis or the cytokine storm syndrome. These patients have high levels of pro-inflammatory cytokines, such as interleukin (IL)-2, IL-6, IL-7 and tumor-necrosis factor-α (TNF-α) (Huang et al., 2020; Zhang et al., 2020). In this phase, patients can develop acute respiratory distress syndrome (ARDS) and multiorgan failure, which are the main causes of mortality of COVID-19 (Cao et al., 2020; Du et al., 2020; Mehta et al., 2020; Ruan et al., 2020; Wu et al., 2020).

It is hypothesized that immunosuppressive drugs could be used to prevent and treat the hyperinflammatory phase of COVID-19.

Aim

To summarize the available evidence on the effect of immunosuppressive drugs on infection with SARS-CoV-2. The effects of immunosuppressive drugs on similar pandemic coronaviruses may resemble the effects on SARS-CoV-2. Thus, we also included studies on the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)

Methods

We conducted a PubMed search, without limitations for the publication date or publication type. In the search, the terms "COVID-19", "SARS", "MERS" (and multiple synonyms) were combined with search terms for the different classes of immunosuppressive drugs (see: Supplementary File 1). The last search was performed on 8 July 2020. All papers were screened by title and abstract, and the full text of potentially eligible papers was read. In addition, the reference list of all identified papers was screened manually. To be included in our review, studies had to meet the following inclusion criteria:

1. Studies concerning humans ≥ 18 years old, animals or cells infected with SARS-CoV-2, SARS-CoV or MERS-CoV.
2. In-vitro or in-vivo treatment with any of the following immunosuppressive drugs: calcineurin inhibitors (CNIs; cyclosporine (CsA), tacrolimus(TAC)), antimetabolites (like mycophenolic acid (MPA), azathioprine (AZA), methotrexate), mammalian target of rapamycin (mTOR) inhibitors (sirolimus, everolimus), corticosteroids (like methylprednisolone, hydrocortisone, prednisone, dexamethasone), cyclophosphamide, rituximab, alemtuzumab, IL-6 inhibitors (like tocilizumab), basiliximab, anakinra, dupilumab, brodalumab, secukinumab, ixekizumab, anti-TNF-α inhibitors (like infliximab), abatacept, belatacept, or eculizumab.
3. Data on one or more of the following outcome measures: viral load, viral replication, clinical outcome (e.g. mortality rate, ICU admission rate, length of hospital stay).
4. Study type: *in-vitro* study, animal study with control group, randomized controlled trial (RCT), cohort study with control group, case-control study.
5. Language: English.

We categorized the obtained information per immunosuppressive drug class. In addition, we sorted the data according to the type of outcome parameter, i.e. viral load or clinical outcome. The study protocol was registered in PROSPERO (registration number CRD42020181137).

Result

The database search yielded 1939 search hits, with 69 studies matching the inclusion criteria

Corticosteroids

Corticosteroids have a wide range of anti-inflammatory and immunomodulatory effects, including inhibition of the synthesis of pro-inflammatory cytokines, reduction of leucocyte trafficking, and induction of apoptosis of T-lymphocytes (Lansbury et al., 2019). In addition, corticosteroids might increase the sensitivity to vasopressors (Lansbury et al., 2019) and can be used to treat adrenal insufficiency, which is present in 20% of the patients with critical illness, and in up to 60% of those with sepsis and septic shock (Hui et al., 2018).

Viral Load

1. SARS-CoV-2: Five observational studies found that SARS-CoV-2 clearance time did not differ between steroid-treated patients and those not treated with steroids (Fang et al., 2020; Xu et al., 2020; Yuan et al., 2020; Zha et al., 2020; Zheng et al., 2020). Contrarily, three other observational studies report that steroid treatment was associated with a longer time until SARS-CoV-2 clearance (Chen et al., 2020; Ling et al., 2020; Xu et al., 2020). The risk of confounding was high in all these studies.
2. SARS-CoV: In a double-blinded RCT, patients with SARS were randomized to treatment with intravenous hydrocortisone (300 mg/day for 12 days) or placebo to study the effect of hydrocortisone on SARS-CoV clearance time (Lee et al., 2004). Patients with comorbidity, respiratory failure or symptoms for ≥ 5

days were excluded. In the second and third week of illness, the mean SARS-CoV RNA plasma levels were higher in the hydrocortisone group than in the placebo group, but the SARS-CoV clearance time did not differ between both groups. However, the risk of confounding is high, because high-dose methylprednisolone (500 mg/day) was given to 5 out of 10 patients in the hydrocortisone group and to 6 out of 7 patients in the placebo group. In a retrospective cohort study, steroid use was not associated with the viral load in lung tissue obtained at autopsy in 11 SARS patients (Mazzulli et al., 2004). However, the study may have been underpowered and the risk of confounding is high.

3. MERS-CoV: In an observational study in patients with MERS admitted to the ICU, steroid use was associated with a delay in MERS-CoV RNA clearance (Arabi et al., 2018). This study has a high risk of confounding.

Clinical Outcome

SARS-CoV-2: In an open-label RCT, patients with COVID-19 were randomized in a ratio of 1:2 to treatment with dexamethasone (6 mg daily, oral or intravenous, for a maximum of 10 days) at hospital admission or standard care (controls) (Recovery Collaborative Group et al., 2020). Age, sex, comorbidities and the requirement for respiratory support were similar in both groups. The median number of days since onset of symptoms was 8 days (IQR 5-13 days) for patients in the dexamethasone group and 9 days (IQR 5-13 days) for controls. Overall, the 28-day mortality rate was lower in dexamethasone-treated patients compared to controls (21.6 vs. 24.6%; age-adjusted RR 0.83, 95% CI 0.75–0.93). Similarly, dexamethasone use was associated with a lower 28-day mortality rate in the subgroups of patients requiring oxygen (RR 0.82, 95% CI 0.72–0.94) or invasive mechanical ventilation (RR 0.64, 95% CI 0.51–0.81) at randomization. Only in the subgroup of patients who were not treated with any type of respiratory support at randomization, there was no significant difference in the 28-day mortality rate between dexamethasone-treated patients and controls (RR 1.19, 95% CI 0.91–1.55). Dexamethasone use was also associated with a lower rate of invasive mechanical ventilation (RR 0.76, 95% CI 0.61–0.96) and shorter length of hospital stay (median 12 vs. 13 days).

MERS-CoV: One retrospective cohort study included MERS patients who were admitted to the ICU. Steroid use was associated with a higher 90-day mortality in a multivariable logistic regression model. However, in a Cox proportional hazard model, steroid use was not associated with a higher mortality rate (Arabi et al., 2018). This suggests that their statistical models did not adjust for all confounders. Another retrospective cohort study found that steroid use was associated with a higher mortality in hospitalized MERS patients (Alfaraj et al., 2019). It was not possible to assess the risk of bias in this study, because essential information is missing, such as baseline characteristics.

Effect of Steroid Dose

1. SARS-CoV-2: Two retrospective cohort studies found that a higher steroid dose was associated with death and prolonged time until viral clearance (Li S. et al., 2020), while use of low-dose steroids was not (Li S. et al., 2020; Li X. et al., 2020). Another retrospective cohort study found that each 10 mg increase in steroid dose was associated with a 4% increase in the risk of mortality after 28 days in COVID-19 patients with ARDS or sepsis (Lu et al., 2020). However, the steroid dose tended to be the highest in the patients who were most severely ill. Thus, there is significant risk of confounding by indication in these studies.

2. SARS-CoV: In one retrospective cohort study, a lower mean steroid dose was associated with a higher 28-day mortality and mechanical ventilation rate (Gomersall et al., 2004). Contrarily, another retrospective cohort study found that a higher mean steroid dose was associated with a higher mortality rate (Wei et al., 2009). Two other retrospective cohort studies found no association between steroid dose and clinical outcome (Ho et al., 2003; Hui et al., 2005). The first study found that the rate of ICU admission, mechanical ventilation or mortality after 3 weeks were not significantly different for patients treated with high-dose or low-dose steroids (Ho et al., 2003). The other study reports that steroid dose was not associated with the results of the 6-minute walking test or the severity of radiographic abnormalities at 6 months after SARS (Hui et al., 2005). In all of these studies, the risk of confounding (by indication) is high.

3. MERS-CoV: No studies available.

Timing of Steroid Administration

1. SARS-CoV-2: No studies available.
2. SARS-CoV: Two observational studies investigated whether the timing of steroid administration was associated with clinical outcomes in patients with SARS (Zhao et al., 2003; Jang et al., 2004). The first study reports that the time between onset of symptoms and start of steroids was not associated with the risk of ICU admission or mechanical ventilation (Jang et al., 2004). The second study found that early treatment with high-dose methylprednisolone might be beneficial for SARS patients (Zhao et al., 2003). In this study, treatment with methylprednisolone was started at different moments in the course of the disease: (A) if patients had not recovered after 14 days, (B) if symptoms or radiological abnormalities worsened, or (C) if fever persisted for ≥ 3 days after admission. The rate of mechanical ventilation was 7, 33, and 0%, respectively, and the mortality rate was 6, 12, and 0%, respectively. However, patients were not randomized to different treatment protocols, and consequently, baseline characteristics and disease severity were different. In both studies, the risk of confounding is high.

3. MERS-CoV: No studies available

Calcineurin Inhibitors (CNI): Cyclosporin A (CsA) and Tacrolimus (TAC)

CNIs (cyclosporine A (CsA) and tacrolimus (TAC)) are used to prevent rejection after organ transplantation and to treat autoimmune diseases, like inflammatory bowel disease. CsA and TAC inhibit T-cell activation. *In-vivo*, CsA and TAC form complexes with cyclophilins and FK506-binding proteins, respectively. These complexes prevent the phosphatase activity of calcineurin. As a result, the dephosphorylation of the nuclear factor of activated T cells is decreased (Carbajo-Lozoya et al., 2012; Ma et al., 2016).

In addition, cyclophilins, the binding proteins of CsA, catalyze the cis/trans isomerization of propyl peptide bonds. This is an essential step in correct folding of proteins, such as cellular and viral proteins (Ma-Lauer et al., 2020). This function of cyclophilin A is found to be essential for the replication of SARS-CoV-2 and other viruses belonging to the Nidovirales order (Carbajo-Lozoya et al., 2014).

Viral Load

1. SARS-CoV-2: No studies available.
2. SARS-CoV and MERS-CoV: Several *in-vitro* studies showed that CsA significantly inhibits the viral replication and the cytopathic effect (CPE: the virus-induced changes in host cells that cause cell death) of SARS-CoV and MERS-CoV in infected cells (Vero, Huh7, Calu-3, and human lung tissue) in a dose-dependent manner (de Wilde et al., 2011; Pfefferle et al., 2011; Carbajo-Lozoya et al., 2012; de Wilde et al., 2013; Li et al., 2018; Sauerhering et al., 2020). One of these studies found that a high concentration of CsA (15 μM) completely inhibited the CPE, without affecting the viability of the cells (de Wilde et al., 2013). Next to these *in-vitro* effects, CsA also inhibited MERS-CoV viral replication and reduced cellular apoptosis in *ex-vivo* cultures of bronchial and lung tissue (Li et al., 2018).

Similar to CsA, TAC inhibited the viral replication of SARS-CoV in Vero cells in a dose-dependent manner (Carbajo-Lozoya et al., 2012). In this study, high-dose TAC reduced SARS-CoV titers 11.112-fold after only 24 h (Carbajo-Lozoya et al., 2012).

Clinical Outcome

No studies matching the inclusion criteria.

Antimetabolites
Mycophenolic Acid (MPA)

Mycophenolic acid (MPA) and its prodrugs, mycophenolate mofetil (MMF) and mycophenolate sodium, are used in the treatment of autoimmune diseases and to prevent rejection in organ transplant recipients. MPA inhibits inosine-5'-monophosphate dehydrogenase, which leads to depletion of intracellular guanosine and deoxyguanosine nucleotides. This suppresses DNA synthesis and thus proliferation of T and B lymphocytes (Villarroel et al., 2009).

Viral Load

1. SARS-CoV-2: One *in-vitro* study found that MPA inhibits SARS-CoV-2 replication in VeroE6/TMPRSS2 cells (Kato et al., 2020). In another study (Han et al., 2020), human pluripotent stem cells (hPSC) were differentiated into lung organoids and then infected with SARS-CoV-2. In these lung organoids, MPA inhibited viral replication while the CPE of SARS-CoV-2 was still observed, even with high concentrations of MPA.
2. SARS-CoV: MPA does not inhibit the proteolytic activity of SARS-CoV PL^{pro} (Cheng et al., 2015) or SARS-CoV replication in Vero cells (Barnard et al., 2006).
3. MERS-CoV: Two studies showed that MPA effectively inhibits the proteolytic activity of the papain-like protease (PL^{pro}) of MERS-CoV (Cheng et al., 2015; Lin et al., 2018). PL^{pro} is responsible for the cleavage of nonstructural proteins, which are essential for viral maturation. Three other *in-vitro* studies showed that MPA significantly inhibited the replication and CPE of MERS-CoV in Vero cells (Chan et al., 2013; Hart et al., 2014; Shen et al., 2019). This effect was dose-dependent.

In contrast, an *in-vivo* study in marmosets infected with MERS-CoV found that the mean viral load in the lungs was higher in MMF-treated animals than in controls (Chan et al., 2015). However, since MERS-CoV does not cause lethal disease in marmosets, this animal model does not adequately resemble human MERS (Johnson et al., 2015).

Clinical Outcome

1. SARS-CoV-2: No studies available.
2. SARS-CoV: No studies available.
3. MERS-CoV: In a retrospective cohort study of 51 hospitalized patients with MERS, eight patients (16%) had received MMF as experimental treatment for MERS. Overall, 19 (37%) patients were admitted to the ICU and eventually died. All other patients survived. In univariate analysis, MMF treatment was associated with survival. However, MMF was given to the less severely ill patients and, seven of the eight MMF-treated patients (87.5%) were also treated with interferon beta (Al Ghamdi et al., 2016). Thus, there is significant risk of confounding.

Thiopurine Analogues

Thiopurine analogues (azathioprine (AZA) and 6-mercaptopurine (6MP)) are used as anticancer treatment, to prevent rejection in organ transplant recipients and as treatment of several chronic autoimmune diseases. AZA is a prodrug of 6MP. *In-vivo*, 6MP is converted into 6-thioguanine (6TG) which is incorporated into cellular DNA. This prevents further DNA replication (Chen et al., 2009).

Viral Load

1. SARS-CoV-2: No studies available.
2. SARS-CoV and MERS-CoV: 6MP and 6TG effectively inhibit the proteolytic activity of PL^{pro} of MERS-CoV and SARS-CoV in a dose-dependent manner (IC_{50} of 12 to 27 µM) in inhibition assays with peptide and fluorogenic substrates (Chou et al., 2008; Cheng et al., 2015; Lin et al., 2018). These results suggest that 6MP and 6TG can inhibit the replication of MERS-CoV and SARS-CoV *in-vitro*.

Clinical Outcome

No studies matching the inclusion criteria.

Mammalian Target of Rapamycin (mTOR) Inhibitors
Sirolimus (rapamycin) and everolimus are mTOR inhibitors. These drugs are used to prevent acute rejection in organ transplantation (Pfefferle et al., 2011). In higher doses, everolimus is also used as anticancer drug (Pfefferle et al., 2011). *In-vivo*, sirolimus and everolimus bind to the FK-binding protein 12 (FKBP-12). This sirolimus/everolimus-FKBP-12 complex binds to mTOR, a phosphatidylinositol kinase-related kinase. This inhibits protein synthesis, cell cycle progression and cell growth (Wullschleger et al., 2006).

Viral Load

1. SARS-CoV-2: No studies available.
2. SARS-CoV: No studies available.
3. MERS-CoV: One *in-vitro* study showed that sirolimus and everolimus dose-dependently reduce MERS-CoV infection in a hepatocyte derived cell line. For both drugs, the cytotoxicity was < 10% (Kindrachuk et al., 2015).

Clinical Outcome

No studies matching the inclusion criteria.

Anti-Cytokine Agents

Anti-Tumor-Necrosis-Factor-α (TNF- α) Agents

There are several types of anti-tumor-necrosis-factor-α (TNF-α) agents: infliximab, adalimumab and golimumab (monoclonal antibodies against TNF-α), certolizumab (TNF-α binding fragment of a monoclonal antibody) and etanercept (fusion protein composed of the extracellular portion of the TNF-receptor-2 and the Fc portion of immunoglobulin G1). Anti-TNF- α agents are used in the treatment of autoimmune diseases, like rheumatoid arthritis and inflammatory bowel disease. TNF-α is a pro-inflammatory cytokine that recruits neutrophils and monocytes to the area of inflammation and activates intracellular signaling in various cells of the immune system (Mitoma et al., 2018).

Viral Replication

No studies matching the inclusion criteria.

Clinical Outcome

1. SARS-CoV-2: No studies available.
2. SARS-CoV: In an animal study, mice infected with SARS-CoV were treated with an anti-TNF-α monoclonal antibody or an isotype-matched control antibody. In mice treated with the anti-TNF-α monoclonal antibody, the onset of weight loss and respiratory illness was delayed compared to controls. However, the mortality rate after 10 days was similar in both groups (Nagata et al., 2008).
3. MERS-CoV: No studies available.

Anakinra

Anakinra is an IL-1 receptor antagonist that is registered for the treatment of several autoinflammatory diseases, such as adult-onset Stills disease and familial Mediterranean fever (Cavalli et al., 2020).

Viral Replication

No studies matching the inclusion criteria.

Clinical Outcome

1. SARS-CoV-2: A retrospective cohort study (Cavalli et al., 2020) included patients with COVID-19 with moderate-to-severe ARDS and hyperinflammation (CRP ≥ 100 mg/L and/or ferritin ≥ 900 ng/mL). Patients treated with mechanical ventilation or other anti-inflammatory agents were excluded. Patients in the intervention group (n = 29) received high-dose anakinra (5mg/kg twice daily intravenously). The control group (n = 16) consisted of patients who retrospectively met the eligibility criteria for anakinra, but who presented to the hospital before the availability of the drug. The 3-week mortality rate was lower in anakinra-treated patients compared to controls (HR = 0.20, 95% CI 0.04–0.63, p = 0.009), but more anakinra-treated patients than controls still required mechanical ventilation after 3 weeks (17 vs. 6%). The percentage of patients that was discharged and had resumed normal activities after 3 weeks was not significantly different (45 vs. 44%). A limitation of this study is the significant risk of confounding. For example, there were significant differences between patients treated with anakinra and controls.
2. SARS-CoV: No studies available.
3. MERS-CoV: No studies available.

Tocilizumab and other IL-6 Inhibitors

IL-6 is an important pro-inflammatory cytokine that is involved in the acute phase response and differentiation and function of B and T cells (Mosharmovahed et al., 2020). Of the three commercially available IL-6 inhibitors (tocilizumab, sarilumab and siltuximab), tocilizumab is the most well-known (Khiali et al., 2020). Tocilizumab is a recombinant humanized anti-IL-6 receptor monoclonal antibody. Tocilizumab is used to treat several autoinflammatory diseases, like rheumatoid arthritis, giant cell arteritis and the chimeric antigen receptor T-cell induced cytokine storm syndrome (Khiali et al., 2020). Notably, Il-6 inhibitors were not available at the time of the SARS and MERS pandemics. The nine retrospective cohort studies matching the inclusion criteria are summarized in Table 3.

Retrospective cohort studies: IL-6 inhibitors in the treatment of patients with COVID-19.

Viral Replication

No studies matching the inclusion criteria.

Clinical Outcome

SARS-Cov-2: The nine studies matching the inclusion criteria report conflicting results. One retrospective cohort study found that treatment of COVID-19 patients with tocilizumab was associated with a higher mortality rate and higher prevalence of bacterial infection (Quartuccio et al., 2020). However, tocilizumab-treated patients were more severely ill than controls. They were also significantly older and more frequently received antivirals and glucocorticoids than controls.

In summary, one study found that tocilizumab was associated with a higher mortality rate, but this study has a high risk of confounding. Four observational studies found no effect of tocilizumab or sarilumab and four other studies showed a beneficial effect of tocilizumab.

2. SARS-CoV: No studies available.
3. MERS-CoV: No studies available.

Immunosuppressive Drugs without Studies Matching Eligibility Criteria

We could not identify any studies matching our inclusion criteria for the following drugs: abatacept, alemtuzumab, basiliximab, belatacept, brodalumab, cyclophosphamide, dupilumab, eculizumab, ixekizumab, methotrexate, rituximab, and secukinumab.

DISCUSSION

There are 30 studies meeting the inclusion criteria for our review that studied the effect of immunosuppressive drugs on infection with SARS-CoV-2. These studies investigated the effects of corticosteroids, mycophenolic acid (MPA), anakinra or tocilizumab.

The results of some clinical studies suggest that corticosteroids are beneficial in patients with COVID-19, especially in mitigating the effects of the cytokine storm. An RCT found that dexamethasone use was associated with a lower 28-day mortality rate, a shorter length of hospital stay and a lower prevalence of mechanical ventilation (Recovery Collaborative Group et al., 2020). The beneficial effect of dexamethasone was greatest in the most severely ill patients. For example, in patients treated with mechanical ventilation, dexamethasone reduced the risk of mortality with 36% (95% CI 19–49%), whereas dexamethasone had no effect on the mortality rate of patients who did not require oxygen support. The results of this RCT support our hypothesis that immunosuppressive drugs can be used to prevent and treat the hyperinflammatory phase of COVID-19.

CONCLUSION

Some immunosuppressive drugs may be beneficial in the treatment of COVID-19. MPA inhibits SARS-CoV-2 replication *in-vitro*. There are indications that corticosteroids and IL-6 inhibitors, like tocilizumab, can reduce mortality and prevent mechanical ventilation in patients with COVID-19. These results have to be confirmed in high-quality clinical trials before these drugs can be implemented as standard care. Based on the positive results of CNIs, mTOR inhibitors and thiopurine analogues in *in-vitro* studies with SARS-CoV and MERS-CoV, it would be interesting to investigate their effects on SARS-CoV-2 replication.

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