Cyclosporine and COVID-19: Risk or favorable?

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic is declared a global health emergency. COVID-19 is triggered by a novel coronavirus: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Baseline characteristics of admitted patients with COVID-19 show that adiposity, diabetes, and hypertension are risk factors for developing severe disease, but so far immunosuppressed patients who are listed as high-risk patients have not been more susceptible to severe COVID-19 than the rest of the population. Multiple clinical trials are currently being conducted, which may identify more drugs that can lower mortality, morbidity, and burden on the society. Several independent studies have convincingly shown that cyclosporine inhibit replication of several different coronaviruses in vitro. The cyclosporine-analog alisporivir has recently been shown to inhibit SARS-CoV-2 in vitro. These findings are intriguing, although there is no clinical evidence for a protective effect to reduce the likelihood of severe COVID-19 or to treat the immune storm or acute respiratory distress syndrome (ARDS) that often causes severe morbidity. Here, we review the putative link between COVID-19 and cyclosporine, while we await more robust clinical data.

KEYWORDS
clinical research/practice, immunosuppressant - calcineurin inhibitor (CNI), immunosuppression/immune modulation, infection and infectious agents - viral, infectious disease, organ transplantation in general

1 | INTRODUCTION

In recent months, the coronavirus disease 2019 (COVID-19) pandemic has stressed healthcare systems worldwide and the World Health Organization has declared it a global health emergency. Patients receiving immunosuppressive treatment, for example, due to organ transplantation or autoimmune disease, are instructed to isolate at home because of a presumed higher risk of more serious disease and possible death. COVID-19 is triggered by a novel coronavirus identified in December 2019 in Wuhan, China and has been named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Two drugs have shown promising effect on COVID-19 patients. Remdesivir proved in a recent trial to reduce time to recovery but had no effect on mortality.[1] Dexamethasone has in a preprint been shown to reduce 28-day mortality in a subgroup of patients.[2] Dexamethasone and remdesivir obviously target COVID-19 differently, which highlights the complex pathogenesis of the disease. SARS-CoV-2 induces a variety of symptoms such as fever, myalgia, dry cough, loss of smell, and in some patients progresses to

Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; FKBP, FK506 binding protein; ICU, intensive care unit; IL-2, interleukin-2; MERS-CoV, Middle East respiratory syndrome coronavirus; NFAT, nuclear factor of activated T cells; Nsp1, nonstructural protein 1; RCT, randomized clinical trial; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

a more severe disease requiring admittance to intensive care units (ICUs). The first clinical characteristics from China showed that a more severe disease outcome defined as need for ICU admittance or death was associated with the presence of comorbidities, particularly diabetes, obesity, hypertension, cardiovascular disease, and chronic kidney disease.[3-7] There exist only very limited data on disease severity in transplanted patients treated with calcineurin inhibitors in combination with other drugs, such as prednisolone or mycophenolate mofetil. However, the first sparse available data indicate that transplanted patients have no increased risk of severe disease despite of multiple and diverse comorbidities. Li et al[8] report of 2 cases of COVID-19 in heart transplant recipients. One had severe disease and both patients recovered. In 2 heart transplant recipients with COVID-19 in the United States, 1 patient died from multiorgan failure and the other patient had mild disease and was discharged on day 8.[9] In 4 kidney transplant recipients from Wuhan, China, it was stated that all patients benefited from reduction of immunosuppressants, because all 4 patients had mild disease.[10] A 50-year-old COVID-19 patient in Spain, treated with tacrolimus because of previous kidney transplantation, needed treatment in the ICU on day 12.[11] These early case reports have now been supported by 3 cohort studies from transplanted patients in Europe.[12-14] In a study from Spain, 29 kidney transplant recipients with COVID-19 were evaluated. One group of patients (n = 6) were reduced in calcineurin inhibitor dose and the second group (n = 23) either continued their usual cyclosporine dose or were switched from tacrolimus to cyclosporine. The first group had a mortality of 50% and the second group only 12.5%, thus supporting the idea that continuous use of cyclosporine might be beneficial in COVID-19 patients. However, the study is observational and not a blinded randomized clinical trial (RCT) and both groups were also treated with multiple non-protocolized drugs: two thirds of the patients were given high-dose steroid, one third were given intravenous immunoglobulin, one third an interleukin-6 (IL-6) inhibitor, and all patients were given hydroxychloroquine, thus making it difficult to draw definite clinically conclusions from these interesting observations from Rodriguez-Cubillo et al.[15]

The transplantation society currently recommends close attention to patients with medication-induced lymphopenia, but no specific instructions on antirejection regimen exist because of the current lack of evidence.[16] Romanelli et al[17] have suggested that clinicians must consider pausing immunosuppressants in transplanted patients with COVID-19, which is a common strategy in transplanted patients with infections. This suggestion may in part be based on data from the epidemic of Middle East respiratory syndrome (MERS) as some case reports of kidney transplant recipients infected with MERS died.[18,19]

The aforementioned COVID-19 clinical observations may be premature, but they all indicate that immunosuppression and use of calcineurin inhibitors impose no increased risk for severe disease, and we speculate that calcineurin inhibitors may protect from severe disease and ultimately death because transplanted patients often have a high prevalence of other risk factors such as hypertension, diabetes, and obesity. However, there may exist other explanations to why COVID-19 seems to be mitigated in transplant recipients, for example, that this patient group is particularly compliant with hygiene recommendations and preventive measures. Another explanation could be the easier access to hospitals and thus increasing numbers of detected COVID-19 infected with mild symptoms, which would otherwise not be found in the general population thus diluting the case fatality rate.

Computational methods looking into host–virus interactions and possible antiviral drug targets with repurposed drugs suggest tacrolimus among others as a potential drug against COVID-19.[20,21] A letter by Russel et al suggests that there is tantalizing in vitro evidence for cyclosporine as an anti-coronavirus agent as well as a potential disease-modifying role through inhibition of severe acute respiratory syndrome (SARS) coronavirus-mediated IL-2 induction and authors advocate that a trial of cyclosporine should be considered in the event of a future SARS epidemic.[22] These promising pilot data require appropriate power in larger studies before immunosuppression can be considered a low risk or maybe even protective for severe COVID-19 but here we review how cyclosporine may influence COVID-19.

2 | COVID-19

Six coronaviruses have previously been shown to cause human disease but 4 of these are considered low pathogenic coronaviruses (229E, HKU1, OC43, and NL63) that causes mild upper respiratory tract infections,[23] in contrast to the highly pathogenic β-coronaviruses: SARS[24,25] and MERS that both cause severe lower airway infection and fatal viral pneumonia.[23,26] Back in 2002-2003, SARS infected more than 8000 causing at least 774 deaths with a case fatality rate of approximately 10%. MERS proved even more deadly causing around 2500 cases and approximately 900 deaths and a case fatality rate of approximately 35%.[6] Both SARS and MERS have a broad spectrum of symptoms ranging from flulike symptoms to acute respiratory distress syndrome (ARDS).[27]

SARS-CoV-2 is also a β-coronavirus, it shares 79% homology with SARS[28,29] and has 96% sequence identity with bat coronavirus (BatCoV RaTG13).[29-31] To understand the pathogenesis of COVID-19 it is important to discriminate between the tissue damage induced by the pathogen and the indirect and later effects caused by the immune response, which on one hand is required in order to eradicate the virus but also may induce significant organ damage. The main target for SARS-CoV-2 is the lungs like SARS. Severe pneumonia develops and is often associated with massive inflammatory cell infiltration (lymphocytes, macrophages, and neutrophils) and elevated proinflammatory cytokine/chemokine responses resulting in acute lung injury and ARDS.[23] The incubation period of SARS-CoV-2 is typically 2-7 days[32] before the patients present with fever, cough, dyspnea, fatigue, and myalgia,[4,32-34] which may be accompanied by rhinorrhoea, pharyngalgia, anosmia, ageusia, and diarrhea.[6] The median age of hospitalized patients has been reported to be

around 49-63 years old in China.[4,7,34-36] A remarkable high number of patients below 70 years of age without significant comorbidities are requiring admittance in the ICU with ventilator support, with a median age of 61 years in the United Kingdom.[37] A recent study showed that of patients admitted to ICU 61.1% had ARDS, 44.4% had arrhythmia, and 30.6% had shock.[35] The high frequency of ARDS may be the cause for admittance to ICU but other studies investigating hospitalized patients with no ICU admittance report ARDS in 17%-29% of all hospitalized patients[33,34] and a case fatality rate of 3.6%-6.8%.[33,38] Early data from China showed a mortality rate of 11%-16% among hospitalized patients.[4,7,34] The late but typically abrupt onset of severe COVID-19 with hypoxemia and dyspnea requires hospital admission and some rapidly progressed to ARDS that eventually would lead to septic shock, metabolic acidosis, coagulation dysfunction, and multiple organ dysfunction syndrome.[6,32,35] It remains to be shown if these symptoms are similar or reduced in immunocompromised patients because of inhibition of the cytokine response as previously shown for parainfluenza virus.[39] Data from 21 solid organ transplant recipients from Switzerland showed almost similar clinical presentation; however, remarkably the severity and complication rates were not higher than in the general population with 19% developing ARDS, 9.5% died, and 23% needed ICU admission.[13] Another Spanish study including 33 kidney transplant recipients showed that 52% were admitted to ICU, 6% needed mechanical ventilation, and 6% died.[12] The study concluded that the severity of COVID-19 was not different in immune compromised patients. Although these findings are in opposition to a small American cohort study reporting more severe outcome in transplant recipients with 34% ICU admission and 24% death of hospitalized cases.[40]

Severity of COVID-19 is related to the onset of lower respiratory tract disease and pneumonia that typically occur 7-10 days after onset. This seems slightly delayed compared with influenza virus pneumonia.[32,41,42] Lymphocytopenia has been suggested to be a negative predictor of disease progression and prognosis, whereas newer data suggest that elevated D-dimer, C-reactive protein, viral load, and low albumin are poor prognostic signs. None of these markers have been reproduced consistently and they all need to be validated in larger ongoing clinical trials. The most common finding on computed tomography scan was bilateral pneumonia and ground-glass opacities[33] that were associated with more severe disease manifestations. Other radiological findings, such as interlobular septal thickening, pulmonary consolidations, and the so-called white lung due to atelectasis and pleural effusion, were also associated with severe COVID-19.[32,43] A comprehensive Chinese study showed that older age and lower CD4-T-cell count were associated with ICU admission and ARDS, and highly elevated IL-6 levels in ICU patients were suggestive of a cytokine storm and an inappropriate host-inflammatory response in patients with severe disease.[36] Indeed, patients admitted to the ICU had higher levels of numerous cytokines when compared to patients not in the ICU.[4] It has been speculated that the reason why children suffer from less severe disease during this pandemic is because of the role of the thymus gland and thus the difference in cytokine expression.[44] On the other hand, the overactive immune reaction may be due to impaired viral clearance again highlighting that appropriate immune system activity is required at all times. Postmortem studies of COVID-19 patients showed diffuse alveolar damage, cellular fibromyxoid exudates, desquamation of pneumocytes, and hyaline membranes in the lung equivalent to ARDS.[45,46] They also report of a hyperactive state with overactivated T cells and suggest that most of the lung injury seems to be caused by severe immune injury.

3 | CALCINEURIN INHIBITORS AND CORONAVIRUS REPLICATION

The outbreak of SARS, MERS, and now COVID-19 has demonstrated human vulnerability to coronavirus epidemics. Neither vaccines nor therapeutics are available against human or animal coronaviruses and most of the drugs used in ongoing clinical trials to treat COVID-19 have been selected because they repress coronavirus replication in vitro. One of the consequences of the SARS epidemic was an increase in efforts to understand coronavirus replication and identify additional possible targets for antiviral therapy including calcineurin.[47] Pfefferle et al investigated coronavirus-host interactions using a genome-wide yeast-two hybrid screening and identified the coronavirus nonstructural protein 1 (Nsp-1) and several immunophilins as important in virus-host response[48] and Luo et al[49] report that the nucleocapsid protein of SARS-CoV bind to cyclophilin A.

Cyclosporine and tacrolimus are the most used calcineurin inhibitors in daily clinical practice for prevention of alloimmune response in transplantation.[50,51] Both compounds suppress the immune system and the main action is prevention of interleukin-2 (IL-2) production in T cells.[52] Cyclosporine and tacrolimus are chemically distinct molecules that bind to the intracellular immunophilins cyclophilin[52-55] and FK506 binding protein (FKBP)-12,[56] respectively, and this calcineurin-inhibitor-immunophilin complex inhibits the phosphatase activity of calcineurin that regulates nuclear translocation and subsequent the activation of nuclear factor of activated T cells (NFAT). NFAT inhibition will eventually lead to the block of transcription of cytokines and thus inhibition of T cell activation[48,52-55,57-59] (Figure 1).

Several independent studies have shown that coronavirus replication and growth depend on active immunophilin pathways. This has been shown for several human coronaviruses including HCoV-NL63,[60,61] and HCoV-229E.[62,63] However, studies silencing specific immunophilins only show partial effect thus indicating a more complex mechanism of action.[53,64,65] The immunophilin pathway is inhibited by both tacrolimus and cyclosporine. Cyclosporine at noncytotoxic concentrations induces a strong inhibition of the replication of several coronaviruses including SARS-CoV[48,64] (EC_{50} 3.3 μmol/L), MERS-CoV,[66] and HCoV-229E in vitro.[64] Cyclosporine alone or in combination with interferon alpha also inhibit replication of MERS in human ex vivo explant cultures.[67] Tacrolimus in low noncytotoxic concentrations inhibits the growth of numerous human coronaviruses including SARS-CoV (EC_{50} 6.9 μmol/L) in vitro.[60]

Tacrolimus   Cyclosporine

FKBP-Calcineurin   Cyclophillin-Calcineurin   Nucleocapsid/Nsp1-Cyclophillin

NFAT

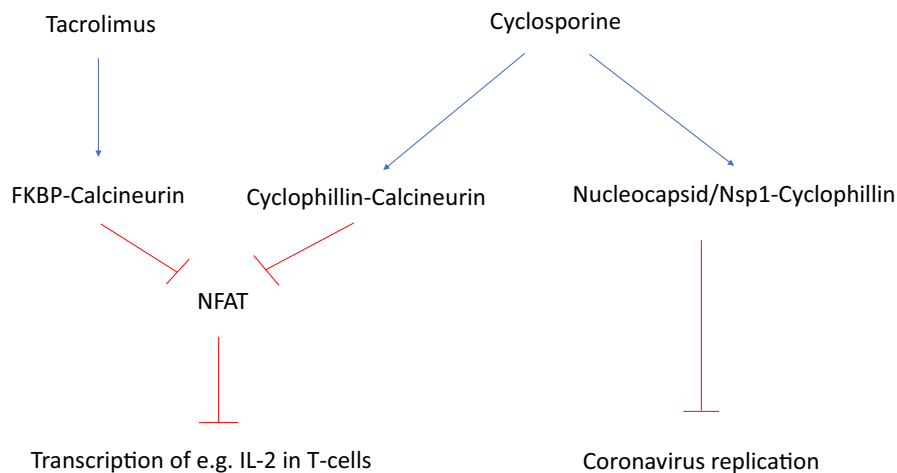Transcription of e.g. IL-2 in T-cells   Coronavirus replication

FIGURE 1 Overview of the 2 most commonly used calcineurin inhibitors and the possible effects on COVID-19. FKBP, FK506 binding protein; IL-2, interleukin-2; NFAT, nuclear factor of activated T cells; Nsp1, nonstructural protein 1 [Color figure can be viewed at wileyonlinelibrary.com]

Moreover, several cyclosporine and tacrolimus analogs, which are pharmaceutical agents similar to either cyclosporine or tacrolimus but designed to lack the immunosuppressive effect, including the drug alisporivir, have demonstrated potent suppressive effects on replication of multiple coronaviruses in vitro such as SARS-CoV (EC_{50} 1.3-8.3 µmol/L),[68] MERS-CoV (EC_{50} 1.5-4.0 µmol/L),[68] HCoV-NL63 (EC_{50} 0.8 µmol/L),[61] and HCoV-229E (EC_{50} 1.37-2.77 µmol/L),[62] and now 2 independent studies have found that alisporivir inhibits SARS-CoV-2 in vitro with EC_{50} of 4.9 µmol/L[69] and 0.46 µmol/L,[70] respectively. Alisporivir was tested in vivo but was unable to diminish morbidity or mortality in an in vivo mouse model, which highlights that in vitro findings have a hard time being extrapolated to the clinical setting.[68] Noteworthy, cyclosporine and derivatives have also in vivo been shown to be effective against other virus, such as influenza virus,[71] HIV, and hepatitis C virus, and usage in clinical studies of hepatitis C-infected adults showed promising results[72-75] but has not had any clinical implications and warrants further studies. Still the in vivo effect and particularly clinical effects of calcineurin inhibitors on SARS-CoV-2 have yet to be confirmed.

The in vitro findings on coronavirus replication and beneficial effects in other virus diseases in vivo are intriguing, but the obvious question is whether the dosage of cyclosporine required to attain efficient inhibition of SARS-CoV-2 in the lungs is safe and tolerable. The cyclosporine concentration required to inhibit virus replication exceeds by far the serum concentrations that typically are well below 200 ng/mL.[76-78] This implies that the dosage used to treat most patients with cyclosporine is too low to effectively eradicate the virus. One of the challenges is to obtain sufficient tissue concentration, as the main virus load is in the airways and lungs and not in serum and the concentration of cyclosporine in the lungs is lower than in serum. Moreover, the required dosage for actively treating patients with severe COVID-19 would be 3-6 fold higher, which in turn would cause severe adverse and possible toxic effects especially nephrotoxicity.[79,80] Moreover, the free available fraction and particularly the local tissue concentration of cyclosporine in the lungs would be too low to induce a substantial inhibition of virus replication. Peak concentration in serum cyclosporine could in theory reach levels that approximate antiviral concentrations but the

only way to reach high local tissue concentrations would be through cyclosporine inhalation. Inhaled cyclosporine has been tested in animals,[81] healthy volunteers, and lung transplant recipients[82,83] and the lung concentration of inhaled cyclosporine is 3 times higher than when systemically administered.[81] It is generally well tolerated, although a few cases of transient reduced forced expiratory volume in the first second (FEV1) following inhalation have been reported. Inhaled cyclosporine is not available as routine treatment and cannot be advised at this moment for COVID-19 patients as there is no human in vivo proof of an antiviral effect.

The Immunonephrology Working Group of the European Renal Association–European Dialysis and Transplant Association has published recommendations for the management of patients with immune-mediated kidney disease during this current pandemic, and authors point out that patients with mild COVID-19 might continue low dose of cyclosporine because of the in vitro evidence of inhibition of coronavirus replication.[84] Moreover, a recent comment supports the idea that cyclosporine might be the drug-of-choice during the COVID-19 pandemic for kidney transplant recipient because of the in vitro evidence and thus providing an "old" alternative to the routine rejection regimens.[85] However, rejection rates are higher in patients on cyclosporine compared to tacrolimus in kidney,[86] heart,[87] and liver transplant recipients.[88] The defining evidence would be to investigate cyclosporine treatment in hospitalized patients with COVID-19. There is reason to believe that it would be safe to treat this patient group with low dose cyclosporine (<3 mg/kg). Previous studies have shown that cyclosporine is safe to use in critically ill patients with severe infections, inflammatory diseases and even circulatory vulnerable patients, and high-dose cyclosporine (4.5-8.3 mg/kg) is well tolerated in steroid-refractory ulcerative colitis.[89] Moreover, a meta-analysis showed that cyclosporine had a beneficial effect on mortality for Stevens-Johnson syndrome and toxic epidermal necrolysis[90] where rapid onset of treatment response as in COVID-19 is warranted. In a randomized, double-blinded, placebo-controlled trial cyclosporine improved lung function when given to severe asthma patients for 3 months and significantly lowered exacerbation rate compared to the placebo group and was well tolerated in patients with severe asthma.[91] In a multicenter, double-blind, randomized trial of bolus

injection of cyclosporine was tested in patients with an acute anterior ST-segment elevation myocardial infarction (STEMI) who were undergoing primary percutaneous coronary intervention. Cyclosporine did not result in improved clinical outcomes compared to placebo; however, interestingly, the authors did not find any significant difference in the safety profile between the two treatment groups thus indicating that cyclosporine treatment in these circulatory unstable patients was well tolerated.[92] In a randomized controlled trial of cyclosporine plus intravenous immunoglobulin (IVIG) treatment or IVIG alone to children with Kawasaki disease found that combined treatment with cyclosporine and IVIG reduced the incidence of coronary artery abnormalities. Authors report no difference of adverse effects in the 2 groups and concludes that cyclosporine treatment is safe and well tolerated.[93] This is of particular interest because of the observed increased incidence of Kawasaki disease during the COVID-19 pandemic.[94] One case report from Japan of a 10-year-old boy with a mycoplasma pneumoniae lung abscess with laboratory indication of cytokine storm was treated with cyclosporine, which suppressed the hypercytokinemia.[95] Based on the presented findings, all of these studies show that cyclosporine is safe to give to a broad range of critical ill patients and we believe that it is safe to investigate cyclosporine in a placebo or dexamethasone controlled trial of COVID-19 patients requiring admittance to hospital.

We cannot recommend switching antirejection regimen during COVID-19, as the available data reviewed here are not sufficient to recommend replacing tacrolimus with cyclosporine during severe COVID-19. However, we do suggest that revised guidelines should recommend continuing cyclosporine to patients during COVID-19 except in cases of renal failure, severe leucopenia, or high serum cyclosporine levels. A switch from tacrolimus to cyclosporine would at this point be based purely on positive observational data with a putative benefit for COVID-19 morbidity but with a possible higher risk of rejection and we warrant controlled studies to test whether this switch is advisable or not.

4 | CONCLUSION

Remdesivir and dexamethasone are the only drugs available with proven effect on COVID-19, although more efficient therapy is warranted and most patients are still only receiving supportive care including oxygen and empirical antibiotic therapy to prevent secondary infections. Currently, more than 200 new clinical trials are registered at clinicaltrials.org testing various treatments, for example, angiotensin-converting enzyme inhibitors, serine protease inhibitors, IL-6 inhibitors, Janus kinase (JAK)-inhibitors, interferons, antivirals, or azithromycin. To the best of our knowledge, no clinical controlled trial is being conducted to test the effect of any calcineurin inhibitor. Several groups have found in vitro evidence of cyclosporine mediated inhibition of replication of several coronaviruses including SARS and MERS and the cyclosporine-analog alisporivir inhibits SARS-CoV-2 in vitro. Because of the limited number of patients and quickly contained SARS

and MERS epidemics, these compounds have never been tested in a clinical setting before. We are still awaiting robust data from COVID-19 patients actively treated with calcineurin inhibitors due to transplantation or autoimmune disease but so far there is no evidence that use of cyclosporine possesses an additional risk for severe COVID-19 in addition to the comorbidities such as diabetes, smoking, hypertension, and obesity that often coexist in these patients. More controversial but not less intriguing is the putative impact of cyclosporine on severe COVID-19, which ultimately should be tested in a RCT in hospitalized patients during this current pandemic to determine whether cyclosporine could reduce the need for ICU admittance and high oxygen demand.

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