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Solid Organ Transplantation in the Era of COVID-19

Edited by
Macé M. Schuurmans and René Hage

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About the Editors

Macé M. Schuurmans

Macé M. Schuurmans, MD has been the Medical Director of the Zurich Lung Transplant and adult Cystic Fibrosis Program since January 2020. He has degrees Pulmonology, Internal Medicine, Sleep Medicine, and Epidemiology, as well as work experience in the three Swiss German-speaking University Hospitals and at the Tygerberg Academic Hospital, Cape Town, South Africa. His research interests are optimization of clinical management of cystic fibrosis and lung transplant patients including allograft dysfunction, viral infections, gastrointestinal complications and adherence/work integration issues, and smoking prevention and smoking cessation.

René Hage

René Hage, MD, graduated at the Erasmus University of Rotterdam, Netherlands. He completed his training as a pulmonologist at the University Medical Centre Utrecht. Dr. Hage has twenty years of experience in pulmonology and has since 2020 been a staff member at the Division of Pulmonology at the University Hospital Zurich (USZ) in Switzerland, with special interest in lung transplantation and cystic fibrosis.

Preface to "Solid Organ Transplantation in the Era of COVID-19"

The impact of COVID-19 on solid organ transplantation was expected to be major since many healthcare systems were struggling to deal with the surges in SARS-CoV-2 infections. This affected routine patient care by limiting resources, had an impact on patient selection and donor organ availability and led to variable evolutions of the patient who had already received a solid organ transplant. The first three articles of this issue deal with the impact of COVID-19 on lung transplant recipients. The first article reports on lung transplant activity in a large Brazilian hospital, showing the challenges and achievements under partially extreme conditions. The second article evaluates the longer-term outcomes of lung transplant recipients who survived COVID-19 in a small sample. Dr. Domingo Franco-Palacios from Detroit presented their experience in a larger series of 64 SARS-CoV-2 infections, reporting a moderately high mortality rate, despite the use of the latest treatments available at the time.

The outcomes of COVID-19 in the unvaccinated liver- and kidney-transplant recipients were studied in 103 consecutive cases by Hailey Hardgrave et al. The classic risk factors known previously, such as hypertension, diabetes and obesity, appeared not to be valid risk factors for the worst outcomes among the transplant recipients; instead, in people over 60 years of age, the use of Belatacept and cylosporine were associated with mortality. Ricardo Wesley Alberca et al., from Brazil, analyzed COVID-19 severity and mortality among liver, heart and kidney recipients and found that the heart and kidney transplant recipients not only had an increase in several COVID-19 severity-associated biomarkers, but also required more intensive care resources and had a higher mortality rate in contrast to liver transplant recipients.

Together with my co-editor Dr. René Hage, we summarized the knowledge concerning COVID-19-related lung fibrosis, describing the two different pathways and phenotypes at the time. Previously, weevaluated the potential role of transplant drugs against SARS, MERS and COVID-19 and summarized, in a systematic review, the evidence concerning COVID-19 in patients with solid organ transplantation in April and May 2020. We then described the outcomes of 18 lung transplant recipients with COVID-19 after reporting on our first case; a woman with SARS-CoV-2 and Norovirus Co-Infection.

A multi-system inflammatory syndrome (MIS-C) diagnosis was made in a pediatric heart transplant recipient and his course with prolonged virus detection and lack of IgG response are discussed by Dr. Bibhuti B. Das. Ryan J. Winstead and colleagues examined the influence of remdesivir on the cycle threshold in 30 kidney transplant recipients and were not able to show evidence of a more rapid decline in viral load in those transplant patients who received the drug.

We would like to thank all the authors for their excellent contributions to this topic and are confident that the topics mentioned here will stimulate further research to attempt to understand the best approach to COVID-19 in the context of solid organ transplantation.

Macé M. Schuurmans and René Hage
Editors

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Editorial

COVID-19: Impact on Lung Transplant Activity at a Large Brazilian Hospital

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The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in late 2019, and has caused a huge number of hospitalizations and deaths worldwide. Until now, there have been more than 500 million diagnosed cases and more than 6 million deaths, according to the Johns Hopkins coronavirus center [1]. Organ transplantation, like most other surgical procedures, and especially lung transplantation, has been extensively affected by the pandemic [2]. At our center, the Heart Institute of the Hospital das Clínicas in São Paulo, we experienced a unique situation brought on by the pandemic that resulted in the decline of lung transplant procedures in 2020 and 2021.

The Hospital das Clínicas of the Faculty of Medicine of the University of São Paulo (HC-FMUSP, São Paulo, Brazil) is the largest hospital complex in Latin America, serving mainly the Brazilian public health service, the Unified Health System (SUS, Brasília, Brazil). This complex occupies an area of 600,000 m^2, with 2400 hospital beds spread throughout eight institutes: the Central Institute (ICHC, São Paulo, Brazil), the Psychiatry Institute (IPq, São Paulo, Brazil), the Orthopedics and Traumatology Institute (IOT, São Paulo, Brazil), the Medical Rehabilitation Institute (IMREA, São Paulo, Brazil), the Children's Institute (ICr, São Paulo, Brazil), the Heart Institute (InCor, São Paulo, Brazil), the Radiology Institute (InRad, São Paulo, Brazil) and the Cancer Institute (ICESP, São Paulo, Brazil) [3].

In times of normalcy, the ICHC houses most of the medical specialties of the HC-FMUSP complex. The department of thoracic surgery, and its subdivisions (including the lung transplant group), are housed at the Heart Institute (InCor, São Paulo, Brazil). However, the COVID-19 pandemic led to an extraordinary and historic mobilization, with the ICHC becoming a COVID-19 exclusive institute, and its several specialties temporarily relocated to the other institutes. Moreover, the clinical staff was divided into "COVID" and "non-COVID" sectors in order to reduce the intra-hospital spread of the disease. With these measures, the HC-FMUSP complex was able to increase the number of ICU beds in the ICHC from 83 to 300 and create 500 new infirmary beds[3]. Nonetheless, all non-urgent and non-cancer related hospitalization and surgical procedures were interrupted, including the lung transplant program.

This arrangement lasted from April to October 2020 and encompassed the first wave of COVID-19 that hit Brazil. During the final months of 2020 and the first semester of 2021 progress was slow, primarily due to the second and third waves. It was only in the second half of 2021, with mass vaccination, that the lung transplant program slowly began to recover.

As a result, between March 2020 and March 2022 (a two-year period) only 43 lung transplants were performed in our center. In the first year of the pandemic affecting Brazil (March 2020 to February 2021) only 14 transplants were performed by us and in the second year (April 2021 to March 2022) only 29 transplants were possible due to the COVID-19 impact on our health care system. This meant a significant decrease from the 39 transplants performed in 2019 alone [4]. Of those 43 procedures, four (9.3%) were done for patients with irreversible pulmonary fibrosis associated with COVID-19.

Patients with acute respiratory distress disorder (ARDS) do not usually need lung transplantation, so we reserve this treatment as a last resort for specific cases. Nevertheless, during this pandemic a series of cases were reported worldwide regarding chronic ARDS patients who received lung transplantations approximately four to six weeks after being diagnosed with COVID-19. These patients had shown no improvement in pulmonary function and all the other organs were functionally preserved. Following those reports, the Toronto Lung Transplant Group published in an editorial ten directives that should be considered before proposing lung transplantation as a therapy for respiratory failure due to advanced COVID-19 affecting the lungs [5]. In a nutshell the criteria included: 1. Age below 65; 2. No other organic dysfunction besides the lungs; 3. Allow enough time for recovery (typically four to six weeks); 4. Radiological evidence of irreversible lung damage; 5. Patients awake and conscious to understand the full scope of the proposed treatment; 6. Patients should be able to participate in physical rehabilitation; 7. Fulfill the regular requirements for a lung transplantation; 8. Negative PCR for SARS-CoV-2; 9. Be treated in a center with experience in high risk transplantation; 10. The center should have access to a broad donor pool and low waiting-list mortality.

Based on these directives from the Toronto Lung Transplant Group, the Technical Board of Thoracic Organs of the State of São Paulo presented its revised criteria for lung transplant in COVID-19 patients in April 2021 [6]: 1. Negative PCR for SARS-CoV-2 in lower respiratory tract sample; 2. Age below 65; 3. Irreversible lung damage after 6 weeks; 4. Body mass index (BMI) before hospital admission between 17 and 27; 5. Patients are hemodynamically stable; 6. Absence of active bacterial or fungal infection; 7. Patients awake and conscious to understand the full scope of the proposed treatment or being accompanied by guardians able to understand the situation and willing to be evaluated by nursing and psychology staff; 8. Green light for transplantation by social services staff and have no history of drug or tobacco use; 9. Able to participate in physical rehabilitation; 10. Transesophageal echocardiogram with no anomalies and ejection fraction greater than 50%; 11. Lack of coronary artery obstructions (with the exception of those which may be managed by catheter interventions); 12. No other organ dysfunction beside the lungs; 13. Provide full consent for the procedure to the lung transplant team; 14. Give the lung transplant team full autonomy to suspend the procedure in accordance with the evaluation of a multi-professional group.

From this point on, we received a series of cases both from inside and outside the HC-FMUSP complex for lung transplant evaluation. Cases from the HC-FMUSP complex were assessed in conjunction with the team responsible for the patient's care. Those from other hospitals were evaluated via telemedicine. If those patients fulfilled the requirements for lung transplantation, they would be transferred to the InCor for further evaluation. In addition to this, the processes of donor selection, organ procurement and operative tactics had to be adapted to the COVID-19 situation. The extended criteria were applied regarding recipient selection, as well as testing with PCR for SARS-CoV-2 and chest tomography. Only the organ procurement was done in the usual way, consisting of evaluation, perfusion and organ removal [6].

Furthermore, the lung transplant group took the lead in extracorporeal membrane oxygenation (ECMO) canulation in COVID-19 patients. This was implemented initially for ventilatory disorders, but ultimately due to pulmonary hypertension associated with low lung compliance, as a bridge to recovery or even lung transplantation. Throughout this period, 24 COVID-19 patients were canulated for venovenous ECMO. Of those, nine were successfully decannulated and four were subjected to lung transplantation.

During the surgical procedure in patients with irreversible pulmonary fibrosis associated with COVID-19, central venoarterial ECMO was implemented as intraoperative care, while maintaining peripheral venovenous ECMO at a low flow. The surgery was initiated on the side with a lower perfusion score and reperfusion of the lungs was done slowly and gradually. The pressure of the pulmonary arteries was controlled by decreasing or increasing the circulatory assistance during the release of clamps from the pulmonary

artery and left atrium. Throughout the transplantation, a cell saver was used and blood transfusion, replacement of fibrinogen and prothrombic factors had to be provided more often than in the pre-COVID-19 era. By the end of the transplant procedure, in the event of preserved biventricular function, the central venoarterial assistance would be removed and the ECMO circuit maintained by circulating a saline solution. If there was still a need for respiratory assistance after the transplant procedure, peripheral cannulation was maintained. Only one of the patients required this in the transplant period reported here. An extensive review of hemostasis parameters was performed and pleural drains were placed in both pleurae, in line with the usual practice, anteriorly and posteriorly [6,7].

Since COVID-19 patients subjected to lung transplantation had a long history of ICU stay and even previous use of ECMO, patients remained critically ill in the early post-operative days and were treated in the ICU. After transplantation we introduced the immunosuppressant therapy following our center's routine, and adjusted it with each patient's needs and acceptance. In addition to all the medications required for regular transplant recipients, early dialysis was often necessary, as well as additional antibiotics and antifungal medication for treatment of previous colonization or new infections. Tracheostomy proved to be an important tool as it allowed the patient to eat, walk and exercise [6]. We had a 100% survival rate for the 30th pos-operative day, with the main complications being fungal infections in the early posttransplant phase.

Besides the impact the COVID-19 pandemic had on our surgical routine, it is also important to note its effects on the patients that are awaiting lung transplantation and those who already had received a lung transplant. Between March 2020 and March 2022, we reported 15 cases of COVID-19 in patients awaiting lung transplantation. Two of those patients died as a result of the infection or its complications, representing almost a 50% increase in our annual death rate of patients awaiting lung transplantation. Prior to COVID-19 we had about 4.5 deaths per year on the waiting list.

Moreover, as of March 2020, we had 213 post-lung transplant patients in follow-up, with a mean age of 46, being 51.8% male and 48.2% female. During this period, 29 (13.6%) patients were diagnosed with COVID-19. Of those, 23 patients were diagnosed, and received supervised treatment at our service and 6 had an external follow-up. Nine patients died (31.03%) as a result of this infection.

Lung transplantation in the midst of the pandemic proved to be a major challenge. Not only did we face the task of adapting our services to the most significant global health crisis since the 1918 Spanish Flu, but also regarding the search for viable donors and, most importantly, to prevent mortality among severely ill patients, who are either on the waiting list or have an acute disease that progressed to a chronic form of COVID-19 with respiratory failure.

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Article

COVID-Related Chronic Allograft Dysfunction in Lung Transplant Recipients: Long-Term Follow-up Results from Infections Occurring in the Pre-vaccination Era

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Abstract: Introduction: We report on characteristics and lung function outcomes among lung transplant recipients (LTRs) after COVID-19 with infections occurring in the first year of the coronavirus pandemic prior to introduction of the vaccines. Methods: This was a retrospective study of 18 LTRs who tested positive for SARS-CoV-2 between 1 February 2020 and 1 March 2021. The mean age was 49.9 (22–68) years; 12 patients (67%) were male. Two patients died due to severe COVID-19. Results: During the study period, there were 18 lung transplant recipients with a community-acquired SARS-CoV-2 infection. In this cohort, seven had mild, nine had moderate, and two had severe COVID-19. All patients with mild and moderate COVID-19 survived, but the two patients with severe COVID-19 died in the intensive care unit while intubated and on mechanical ventilation. Most patients with moderate COVID-19 showed a permanent lung function decrease that did not improve after 12 months. Conclusion: A majority of LTRs in the current cohort did not experience an alteration in the trajectory of FEV1 evolution after developing SARS-CoV-2 infection. However, in the patients with moderate COVID-19, most patients had a decline in the FEV1 that was present after 1 month after recovery and did not improve or even deteriorated further after 12 months. In LTRs, COVID-19 can have long-lasting effects on pulmonary function. Treatment strategies that influence this trajectory are needed.

Keywords: chronic lung allograft dysfunction; community-acquired respiratory viral infection; CLAD hypothesis

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1. Introduction

Community-acquired respiratory virus (CARV) infections pose a significant challenge among lung transplant recipients (LTRs). The rate of infection among LTRs is much higher than in other solid organ recipients due to the direct exposure of the lung to the potentially hostile external environment. Other risk factors contributing to the infection risk are severe immunosuppression, the blunted cough reflex due to lung denervation, poor lymphatic drainage, and impaired mucociliary clearance as a result of ischemic injury to the bronchial mucosa and narrowing of the bronchial anastomosis [1]. Compared to bacterial respiratory infections, viral infections initially lead to less severe symptoms, but then lead to a greater worsening of the lung function [2]. In LTRs, CARV can lead to both acute and chronic allograft dysfunction [3–8].

The mechanisms behind allograft dysfunction are only partly understood, hampering the establishment of adequate treatment. It has been suggested that symptomatic respiratory viral infections after lung transplantation elicit immune responses to lung self-antigens by inducing circulating exosomes that contain lung-associated self-antigens [9]. CARV infections may activate alloimmune responses, leading to post-CARV chronic lung allograft dysfunction (CLAD) [7].

Since the first successful human lung transplantation in 1983 [10], the overall 5-year survival rates in lung transplantation are still only approximately 50–70%, which is considerably worse compared to other solid organ transplantations, even after significant improvements in donor selection, organ preservation, perioperative management, and better treatment of post-operative complications [11].

CLAD is the leading cause of death beyond the first year after lung transplantation. Currently, there is no medical treatment that can cure CLAD. Several treatments have been introduced in an attempt to slow the progression of CLAD such as azithromycin, pravastatin, montelukast, extracorporeal photopheresis, and total lymphoid irradiation [12–16].

Symptomatic respiratory viral infections were shown to be independently associated with CLAD [7]. In LTRs, CLAD is a progressive and in most patients irreversible process, and a major cause of long-term allograft failure and death. CLAD has two different main phenotypes called bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS). About 70% of LTRs with CLAD have the BOS phenotype.

In CLAD-BOS, there is a persistent decline in the forced expiratory volume in 1 s (FEV1) associated with an obstructive ventilatory defect; whereas in CLAD-RAS, there is a restrictive defect with an increased FEV1/forced vital capacity (FVC) ratio or a decrease in FVC or total lung capacity (TLC). It was observed in a prior study that CLAD-RAS could develop after COVID-19 [17]. Mahan et al. showed a significant loss of lung function in 18 LTRs (40.9%), of which 3 patients (5.6%) developed CLAD-RAS. Prior studies have shown a strong association between respiratory viral infections and the development of CLAD in which symptomatic viral infections demonstrated a stronger relationship with CLAD [17–20].

Data on long-term effects of CARV due to infection with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in LTRs are scarce. Infection by SARS-CoV-2 can be highly variable in disease severity, ranging from mild upper respiratory distress to fulminant viral pneumonitis with multi-organ failure and death. In this study, we evaluated the impact of SARS-CoV-2 infection in LTRs during the first year after the infection.

2. Methods

This was a single-center, retrospective-chart-review study. The study population consisted of consecutive adult LTRs $\geq$ 18 years of age with COVID-19 at the University Hospital Zurich, Switzerland, in which formal informed consent was given. The infection with SARS-CoV-2 was demonstrated using real-time reverse transcriptase polymerase chain reaction (RT-PCR).

Patients with COVID-19 were classified as "mild" when clinical symptoms consisted of mild constitutional symptoms, fever, or a dry cough. Patients were classified as "moderate" COVID-19 when the clinical symptoms including dyspnea with or without hypoxia and where chest imaging was abnormal (infiltrates and/or ground-glass opacities). The baseline characteristics of these patients were published previously [21]. Full recovery was defined as two negative SARS-CoV-2 RT-PCR tests at least 24 h apart along with the resolution of symptoms. Patients with acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), or cardiac failure were classified as "severe" COVID-19.

All spirometric testing was conducted in the lung transplant clinic using a Geratherm respiratory spirometer (Geratherm Medical AG). The spirometry analysis was performed according to the Standardization of Spirometry by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) [22]. CLAD-BOS and CLAD-RAS were defined according to the classification by Verleden [12]. CLAD-BOS stage 0 was defined as FEV1 > 90% of baseline, BOS stage 1 as FEV1 decline of 66–80% of baseline, BOS stage 2 as FEV1 decline of 51–65%, and BOS stage 3 as an FEV1 decline $\leq$ 50% of baseline. CLAD-RAS was defined as a total lung capacity (TLC) decline > 10% or an FEV1/FVC > 0.70.

Using the pre-infection baseline FEV1 ($FEV1_{pre}$) and post-infection FEV1 ($FEV1_{post}$), we calculated the change in lung function as follows: $(FEV1_{pre} - FEV1_{post})/FEV1_{pre}$.

3. Statistical Analysis

Descriptive statistics were performed; the main data are summarized in Tables 1–4. The results are reported as the mean with the range and categorical variables were calculated as counts (*n*) and percentages (%).

Table 1. Non-vaccinated lung transplant recipients with mild and moderate COVID.

Pat	Age, m/f	FEV1 Pre	FVC Pre	FEV1 1m	FEV1 3m	FEV1 6m	FEV1 12m	%FEV1 Δ 1m	%FEV1 Δ 12m	%FVC Δ 12m
1	56, f	2800 (104%)	2810 (96%)	2880 (113%)	2770 (111%)	2820 (113%)	NA	+2.9%	NA	+1.1%
2	22, f	1710 (57%)	2250 (86%)	1810 (61%)	1880 (63%)	2050 (69%)	2030 (68%)	+16%	+16%	+16.4%
3	27, m	2750 (71%)	3610 (79%)	2640 (69%)	2730 (71%)	2500 (65%)	2490 (66%)	−4.0%	−9.45%	−1.98%
4	64, m	2010 (56%)	2470 (58%)	2050 (66%)	2030 (66%)	2010 (60%)	1910 (57%)	+1.99%	−4.98%	+18.2%
5	34, m	1520 (33%)	3030 (54%)	NA	1340 (30%)	1190 (27%)	1120 (25%)	NA	−26.3%	−8.99%
6	19, f	2400 (62%)	2340 (73%)	NA	NA	2840 (79%)	2730 (77%)	NA	+13.8%	+17%
7	67, f	1660 (89%)	2380 (105%)	NA	1530 (83%)	1650 (89%)	1500 (68%)	NA	−9.64%	−4.39%

Abbreviations: m = male; f = female; FEV1 pre = forced expiratory volume in 1 s pre-COVID; FEV1 1m, 3m, 6m, 12m = forced expiratory volume in 1 s after 1, 3, 6, and 12 months, respectively; %FEV1 Δ 1m, 12m = $(FEV1_{pre} - FEV1_{post})/FEV1_{pre}$ 1 month or 12 months after recovery from COVID, respectively; FVC = forced vital capacity; %FVC1 Δ 12m = $(FVC_{pre} - FVC_{post})/FVC_{pre}$ 12 months after recovery from COVID; NA = not applicable.

Table 2. Non-vaccinated lung transplant recipients with moderate COVID.

Pat	Age, m/f	FEV1 Pre	FVC Pre	FEV1 1m	FEV1 3m	FEV1 6m	FEV1 12m	%FEV1 Δ 1m	%FEV1 Δ 12m	%FVC Δ 12m
1	28, m	3430 (69%)	4970 (81%)	3330 (61%)	3160 (58%)	3610 (66%)	3680 (68%)	−2.92%	+7.29%	+1%
2	48, m	3000 (89%)	3730 (90%)	NA	2570 (72%)	2730 (77%)	2840 (80%)	NA	−5.33%	−10%
3	38, m	1610 (46%)	3210 (74%)	NA	1100 (31%)	1320 (39%)	1360 (39%)	NA	−15.5%	−2.2%
4	68, m	2430 (79%)	3090 (76%)	2370 (80%)	1980 (64%)	2660 (86%)	2140 (74%)	−2.47%	−11.9%	−12.4%
5	66, m	2180 (78%)	2930 (81%)	1980 (71%)	2330 (82%)	1560 (55%)	1300 (47%)	−9.17%	−40.4%	−20.6%
6	49, f	1430 (52%)	1700 (53%)	1500 (55%)	1480 (54%)	NA	1440 (50%)	+4.90%	+0.70%	+13.7%
7	68, m	3160 (97%)	4420 (103%)	3010 (92%)	3320 (105%)	NA	NA	−4.75%	NA	NA
8	64, f	2450 (89%)	3340 (94%)	2020 (76%)	NA	2340 (85%)	1910 (70%)	−17.6%	−22.0%	−37.4%
9	63, m	4530 (127%)	4990 (107%)	NA	3440 (96%)	4250 (120%)	3940 (111%)	NA	−13.0%	−10.8%

Abbreviations: Pat = patient number; m = male; f = female; FEV1 pre = forced expiratory volume in 1 s pre-COVID; FEV1 1m, 3m, 6m, 12m = forced expiratory volume in 1 s after 1, 3, 6, and 12 months, respectively; %FEV1 Δ 1m, 12m = $(FEV1_{pre} - FEV1_{post})/FEV1_{pre}$ 1 month or 12 months after recovery from COVID, respectively; FVC = forced vital capacity; %FVC1 Δ 12m = $(FVC_{pre} - FVC_{post})/FVC_{pre}$ 12 months after recovery from COVID; NA = not applicable.

Table 3. Pre-COVID chronic lung allograft dysfunction (CLAD) and donor-specific antibody (DSA) monitoring (mild COVID).

Pat	CLAD Pre-COVID	DSA Pre-COVID	DSA Post-COVID < 3 mo.	DSA Post-COVID 3–6 mo.	DSA Post-COVID 6–12 mo.
1	BOS 0	Neg	DQ2 MFI-2124	Neg.	Neg.
2	BOS 0	Neg.	DQ6 MFI-4224	DQ6 MFI-1827	Cw5 MFI-826 DR52 MFI-1207 DQ6 MFI-1278
3	BOS 0p	DQ8 MFI-3949	N/A	N/A	DQ8 MFI-1417
4	BOS 0	DQ2 MFI-2260	No data	DQ2 MFI-572	No data
5	BOS 3	DQ2 MFI-6628 DP1 MFI-1280	Neg.	N/A	DQ2 MFI-7222 DP1 MFI-2264
6	BOS 0	Neg.	Neg.	Neg.	Neg.
7	BOS 0p	Neg.	N/A	Neg.	Neg.

Table 4. Pre-COVID chronic lung allograft dysfunction (CLAD) and donor-specific antibody (DSA) monitoring (moderate COVID).

Pat	CLAD Pre-COVID	DSA Pre-COVID	DSA Post-COVID < 3 mo.	DSA Post-COVID 3–6 mo.	DSA Post-COVID 6–12 mo.
1	BOS 1	Neg.	Neg.	N/A	Neg.
2	BOS 0	Neg.	Neg.	N/A	Neg.
3	BOS 3	Neg.	N/A	Neg.	Neg.
4	BOS 1	Neg.	Neg.	N/A	Neg.
5	BOS 3	DR18 MFI-1802 DR51 MFI-610 DQ2 MFI 24,766	DR18 MFI-1308 DQ2 MFI 20,201	DR18 MFI-1538 DR51 MFI-875 DR52 MFI-888 DQ2 MFI 18,100	DR18 MFI-1250 DQ2 MFI 15,223
6	BOS 0p	Neg.	N/A	N/A	Neg.
7	BOS 1	Neg.	Neg.	N/A	Neg.
8	BOS 1	Neg.	Neg.	N/A	Neg.
9	BOS 0	Neg.	Neg.	Neg.	Neg.

4. Ethical Considerations

The study was granted approval by the Zurich branch of the Swiss Medical Ethics Committee (Swissethics No. 2021-00293).

5. Results

During the study period, there were 18 episodes of SARS-CoV-2 infection among the LTRs, all of which were community-acquired. The lung function data are shown in Table 1 (mild COVID-19) and Table 2 (moderate COVID-19). The mean age was 49.9 (22–68) years; 12 of the LTRs (67%) were male.

In the group of patients with mild COVID-19, the mean C-reactive protein level (CRP) was 29.8 (4–77) mg/L; while in moderate COVID-19, the mean CRP was 58.2 (4.8–140) mg/L. In mild COVID-19, the mean creatinine level (119 μmol/L, range 60–166) was less than in moderate COVID-19 (mean creatinine 231 μmol/L, range 17–809). In both the mild and moderate COVID-19 patients, there was no obesity (mean body mass index 22.4 and 26.3 kg/m^2, respectively) observed. All patients were under chronic triple immunosuppressive therapy, including prednisone in all (100%) of the patients. In the mild COVID-19 patients, this included cyclosporine A in one (14%), tacrolimus in six (86%) rapamycin in one

(14%), and mycophenolate mofetil in six (86%) of the patients. In the moderate COVID-19 patients, the immunosuppression included cyclosporine A in three (33%), tacrolimus in four (44%), and everolimus in one (11%) of the patients. During the active infection with SARS-CoV-2, in all patients mycophenolate mofetil was then discontinued as part of our standard practice.

Although we discontinued mycophenolate mofetil, most patients did not develop donor-specific antibodies (DSA), as shown in Tables 3 and 4.

The pre-transplant diagnosis was cystic fibrosis (CF) in four (57%), chronic obstructive pulmonary disease (COPD) in one (14%), and interstitial lung disease (ILD) in two (29%) patients in the group of patients with mild COVID-19, while in the group of moderate COVID-19 patients, this was CF in two (22%), COPD in four (44%), ILD in two (22%), and pulmonary arterial hypertension in one (11%) of the patients.

Two LTRs died due to severe COVID-19; these patients were intubated and therefore there were no post-COVID lung function data available. Since the only two patients with severe COVID-19 did not survive the infection, they are not shown in a separate table. The median age was 58.5 years (range 56–61); both were male with a mean BMI of 31.2 kg/m^2. Both patients had very high CRP levels (mean 302 mg/L, range 199–406) and chronic kidney failure (mean creatinine 208, µmol/L, range 202–213). Both had interstitial lung disease as the pre-transplant diagnosis. Empiric antibiotic treatment was standard in all patients, both ambulatory and hospitalized. As the evolution of COVID-19 was favorable in most patients, additional microbiology samples were not indicated. Only in severe cases were additional samples performed; these patients were intubated and finally died.

6. Discussion

This retrospective study in LTRs with COVID-19 showed lung function decline after COVID-19 in most patients with moderate COVID-19. In most patients with mild COVID-19 evolution, the lung function evolution was not affected. Patients who showed lung function decline after COVID-19 in the first month did not recover in the following year, and in this group of patients, most showed a further lung function deterioration. After 12 months, 10 patients (56%) showed a decreased FEV1 as compared to pre-COVID FEV1 measurements, with a FEV1 range of −4.98% to −40.4%. In four patients (n = 22%), the FEV1 decrease was 5–10%, three patients (n = 17%) lost $\geq$ 10–20%, and three patients (n = 17%) lost $\geq$ 20%. Most patients did not develop DSA even one year post-COVID.

Although CLAD-BOS was frequently diagnosed after moderate COVID-19, we had no patients with CLAD-RAS or a mixed phenotype. These results were in line with a retrospective multicenter study that collected data from three Dutch transplant centers and included 74 LTRs that showed a significantly lower lung function that remained significantly lower compared to the pre-COVID-19 values [23].

The so-called wild type of the SARS-CoV-2 was first demonstrated in China at the end of 2019. Relevant virus mutations were the Alpha variant (B.1.1.7, first demonstrated in the United Kingdom in September 2020), followed by the Beta variant (mutation E484K, first seen in South Africa in May 2020), the Gamma variant (P.1, initially detected in Brazil, in November 2020), the Delta variant (B.1.617.2, initially detected in India in May 2021), and the Omicron variant (November 2021).

Although a genotyping PCR was not initially performed at our hospital, the above-described patients were studied between 1 February 2020 and 1 March 2021 and probably mainly suffered from the wild type, Alpha variant, Beta variant, or the Gamma variant of SARS-CoV-2 based on the predominant strains detected in this period.

The numbers of affected patients at that time (beginning of the pandemic) were relatively small. The second wave of the pandemic in Switzerland was in October 2020 [24]. At the beginning of the pandemic's spread in Switzerland, specifically in March 2020, there were only 3000 COVID-19 patients diagnosed despite widespread testing, but this rapidly increased to over 500,000 in January 2021 [25].

At the time of this study, patients had not yet received the vaccinations because they were not yet available. The approval of the first COVID-19 mRNA vaccine, called BNT162b2 (Pfizer BioNTech) [26], was on 19 December 2020, followed by the COVID mRNA vaccine by Moderna, which was approved on 12 January 2021 in Switzerland [27]. Moreover, at this time there were no clear guidelines on how to deal with immunosuppression in LTRs with COVID-19 and vaccination uptake was slightly delayed due to prioritization of elderly persons at the beginning of the vaccine roll-out.

We now know that vaccination in lung transplant recipients is a key strategy that reduces the risk of severe COVID-19 and hospitalization [28]. Vaccination is also now considered as an indirect treatment in the prevention of CLAD BOS in LTRs due to SARS-CoV-2 infection. Unfortunately, two problems in vaccination of LTRs have become evident. The first problem is that LTRs have a blunted humoral and cellular immune response after COVID-19 vaccination [29]. The second is a shorter duration of the protective effects of the vaccine [29–32].

A weaker immune response was demonstrated in immunosuppressed transplant recipients who received the trivalent influenza vaccine; these patients showed significantly lower antibody titers [33]. Another study also showed a weaker response in immunosuppressed heart transplant recipients after pneumococcal vaccination [34]. In LTRs, the standard therapy is a triple immunosuppression in which most patients receive a combination of a calcineurin inhibitor (CNI), a mycophenolate derivative such as mycophenolate mofetil (MMF), and a corticosteroid (typically prednisone or prednisolone) [35]. All types of immunosuppressive drugs have different mechanisms of action that when combined will severely blunt the immune response. CNI blocks T-cell activation and proliferation, MMF impairs the proliferation of B and T lymphocytes and increases apoptosis, while corticosteroids mainly affect T lymphocytes by impairing their development, survival, activation, and migration [36].

Our study had some obvious limitations, namely the small number of patients, the single-center experience, and the retrospective design of the study. The data should therefore be interpreted with caution; for firm conclusions, further studies are needed.

In other viral infections in LTRs, acute rejection and chronic lung allograft dysfunction are well-known complications as well [37]. Allograft dysfunction is not only caused by direct effects of viral replication, but also by immunologically mediated lung injury [37]. The exact mechanisms are only partially understood.

In conclusion, this study suggested a potential relationship between SARS-CoV-2 infection and CLAD. More specifically, our hypothesis was that the risk of the development of CLAD-BOS was higher in the LTRs with moderate COVID-19 compared to those with mild COVID-19. Moreover, the decline in FEV1 could already be seen as soon as 1 month after COVID, with an additional FEV1 deterioration in the following months.

In LTRs, emphasis on prevention of COVID-19 by minimizing exposure and widespread use of vaccinations is certainly warranted because the increased severity of SARS-CoV-2 infections appears to increase the risk of CLAD development. The exact role of vaccination in LTRs requires further studies that include long-term follow-up data on FEV1 evolutions for different clinical situations because the current serological data show a suboptimal antibody response in these immunosuppressed patients. Thus, the protective effects regarding COVID-19 severity and disease course are not well studied to date.

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Outcomes of COVID-19 in a Large Cohort of Lung Transplant Recipients: A Retrospective Study

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Abstract: *Background:* Early reports of COVID-19 in lung transplant recipients (LTRs) showed high hospitalization and mortality rates. However, the outcomes of COVID-19 in LTRs since the advent of newer therapies and vaccines have been poorly defined. *Methods:* We evaluated the risks for SARS-CoV-2-related hospitalization and mortality in a cohort of LTRs at the Henry Ford Lung Transplant Program in Detroit, Michigan during the study period March 2020–March 2022. Univariate logistic regression, followed by multivariable modeling were performed to estimate the odds ratio (OR) with 95% confident intervals (CI). *Results:* Sixty-four laboratory-confirmed SARS-CoV-2 infections were identified in 59 patients. For the primary analysis of the hospitalization and mortality risks, we included these 59 patients with symptomatic COVID-19. SARS-CoV-2 infections were confirmed with real-time polymerase chain reaction (RT-PCR) from a nasopharynx swab. The mean age (±STD) was 61 (±12), 63% were males, 27% were African Americans, and the time from lung transplant to COVID-19 was 5.5 (±4.8) years. Thirty-four (57.6%) patients were hospitalized, and the inpatient mortality rate was 24% (8/34). A multivariable analysis showed that patients with a higher baseline forced expiratory volume (FEV1) were less likely to be hospitalized (OR = 0.91 and 95% CI 0.87–0.98, $p = 0.02$). Seventy-five percent (75%; 6/8) of patients on invasive mechanical ventilation died, compared with only 8% mortality rate in those without mechanical ventilation (OR = 36.0 and 95% CI 4.2–310.4, $p < 0.01$). Although a trend toward a higher risk of death was observed in those infected during the Alpha ($p = 0.17$) and Delta ($p = 0.22$) waves, no significant risk was detected after adjusting for other covariates. Five LTRs were diagnosed with COVID-19 twice. Thirty of the sixty-four COVID-19 cases (46.8%) occurred in LTRs that had received at least two doses of any of the available mRNA vaccines at a median of 123 days (IQR 98–164 days) after vaccination. Twelve of the thirty (40%) were hospitalized, and four patients (33%) died during their hospitalizations. *Conclusions:* In our LTR population, the hospitalization and mortality rates associated with COVID-19 were high despite the increased use of new therapies. Vaccine-breakthrough infections were common and were associated with poor outcomes. Studies are needed to determine optimal prevention and therapeutic strategies to improve COVID-19 outcomes in LTRs.

Keywords: COVID-19; lung transplant recipients; immunocompromised; breakthrough infections

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1. Introduction

Few centers across the globe have reported on the outcomes of COVID-19 in lung transplant recipients (LTRs) [1–6]. Most reports relate to the early period following the declaration of the COVID-19 pandemic in March 2020 by the World Health Organization. These initial reports, predominately of hospitalized LTRs with short-term follow-ups, showed high hospitalization and mortality rates associated with COVID-19. The mortality rate of COVID-19-related acute respiratory distress syndrome (ARDS) requiring invasive

mechanical ventilation was significantly higher in LTRs than in the immunocompetent patient population (75–100% versus 20–40%, respectively) [7–9], even if compared with non-immunosuppressed patients with severe refractory hypoxic respiratory failure on venovenous extracorporeal membrane oxygenation (vvECMO). In a registry of 1900 solid organ transplant (SOT) recipients with COVID-19, including over 150 LTRs, the morbidity and mortality rates were higher for LTRs compared with other SOT recipients [10].

Since these early reports, the modalities used for the treatment and prevention of COVID-19 have rapidly evolved. Remdesivir, an antiviral agent administered to hospitalized patients with moderate illness who did not require hospitalization into an intensive care unit (ICU), was shown to shorten the time to clinical recovery [11,12]. In patients with COVID-19-related pneumonia and hypoxic respiratory failure, landmark studies of corticosteroid therapy demonstrated significant improvement in outcomes and reduced mortality [13]. Favorable outcomes were reported with the use of immunomodulators, such as tocilizumab and baricitinib, in addition to corticosteroids in patients with severe and critical COVID-19 [12,14]. The use of anticoagulation to prevent venous thromboembolism (VTE); high quality critical care, including treatment of secondary infections; the support of other organs; and the avoidance of further damage by the ventilator or self-induced lung injury are standard of care. Venous thromboembolism prevalence was found to be higher in COVID-19 patients than in other ICU patient populations [15]. In noncritically ill patients with COVID-19, an initial strategy of therapeutic-dose anticoagulation with heparin was associated with better outcomes [16]. These modalities were incorporated into guidelines endorsed by several medical societies for the treatment of COVID-19, utilizing a tiered approach based on the severity of illness [17]. Additionally, the modification of immunosuppression (corticosteroid augmentation and the discontinuation of cell-cycle inhibitors) was recommended in SOT recipients (SOTRs) [18,19].

In December 2020, messenger RNA vaccines received an Emergency Use Authorization in the United States of America. The effectiveness of these vaccines was noted to be suboptimal in SOTRs due to the immunosuppressant therapies used to prevent rejection and an attenuated immune response [20]. Vaccine effectiveness was further compromised by the emergence of variants of SARS-CoV-2 [21]. Other therapies aimed to prevent serious illness in this high-risk population became available over time, including different compositions of monoclonal antibodies and oral antivirals [22].

However, the effect of these new modalities of therapy on the outcomes of COVID-19 in the LTR population is incompletely understood. We described the clinical course and outcomes of COVID-19 in 59 LTRs, as well as the incidences of vaccine-breakthrough infections from March 2020 to March 2022 at our institution. The primary outcome of interest for our study was examining the risk of COVID-19-related hospitalization and inpatient mortality.

2. Methods

2.1. Study Design

This was a single-center retrospective cohort study that included all adult LTRs at the Henry Ford Transplant Institute, who were symptomatic and tested positive for SARS-CoV-2 with a real-time polymerase chain reaction (RT-PCR) test via a nasopharyngeal swab from March 2020 to March 2022. This study was approved by the Henry Ford Health System Institutional Review Board (#14948) and consent was waived.

2.2. Data Collection and Definitions

Patient information was obtained by review of the electronic health records (EHR). Demographics, clinical characteristics, comorbidities, date and indication of transplantation, date of COVID-19 diagnosis, disease severity, and management were evaluated. COVID-19 vaccination data were obtained from the EHR and the Michigan Care Improvement Registry (MCIR), which reports COVID-19 vaccine administration. All patients were followed until 31 March 2022.

"COVID-19 severity" was defined as per the National Institutes of Health (NIH) COVID-19 guidelines [23]. "Vaccine-breakthrough infection" was defined as having COVID-19 diagnosed at least 14 days after the administration of a COVID-19 vaccine, i.e., a minimum of 2 doses of an mRNA vaccine [21]. The periods of activity in the United States of America (USA) of the various SARS-CoV-2 variants were based on the Center for Disease Control and Prevention (CDC) timeline [23]. The approximate periods of activity for the variants were: wild-type (March 2020–December 2021); Alpha (January 2021–April 2021); Delta (May 2021–December 2021); and Omicron (January 2022 onwards).

2.3. Statistical Analysis

The patients' characteristics and clinical conditions were summarized. The mean (SD) was used for the continuous variable, and percentage was used for the categorical and binary variables for all patients. Non-normally distributed numerical variables were summarized as medians with interquartile range.

The data were compared between two groups of patients with symptomatic COVID-19 (hospitalized versus non-hospitalized) and based on the vital statuses of inpatients (dead versus alive). The chi-square test/Fisher exact test were used for categorical variables and a two-sample t-test was used for continuous variables to study the univariate effect of the variables on each outcome of interest. Stepwise logistic regression was preformatted to study the risk/association to hospitalization. Variables would retain in the model if there was a significant effect with p-value < 0.05 after adjusting for the other covariates with estimation of odds ratio (OR) and its 95% confident intervals (CI), where OR < 1 and 95% CI < = 1 indicates a proactive effect for hospitalization, while OR > 1 and 95% CI > 1 indicates a risk for hospitalization. A similar analysis was performed to study the risk/association to inpatient death.

The statistical analysis was performed with SPSS version 25.0 (IBM Corp, Armonk, NY, USA).

2.4. COVID-19 Management

The decision to hospitalize LTRs and the level of care needed were made by the lung transplant team based on disease severity. In general, LTRs were hospitalized if they had hypoxia or severe symptoms (tachypnea with a respiratory rate of >30 breaths/minute, chest pain, dyspnea, etc.) suggestive of a progression to respiratory failure and clinical instability. The standard of care of hospitalized COVID-19-infected LTRs was based on the disease stage and severity, using institutional protocols that were consistent with NIH treatment guidelines [17]. Patients with mild or moderate disease and unchanged baseline oxygenation were treated as outpatients. The general recommendation was symptomatic treatment, to isolate, hydrate well, and communicate with the LT coordinators if symptoms worsened. Immunosuppression management changed over the course of the pandemic, with a discontinuation of the cycle-cell inhibitors in all LTRs with any severity of COVID-19 during the Delta variant period, and in hospitalized LTRs regardless of the circulating variant at the time of a COVID-19 diagnosis. In patients with mild and moderate COVID-19 that were not hospitalized, a virtual visit was conducted 1 week after the diagnosis of COVID-19 to confirm clinical improvement or the need of hospitalization in case of disease progression. At follow-up visits, the decision to resume the cell-cycle inhibitor was made on average 31 days after discontinuation in 9 patients who did not require hospitalization. Additional therapy with monoclonal antibodies was utilized as they became available in outpatients to prevent disease progression.

For hospitalized patients with mild-to-moderate COVID-19, the administration of intravenous remdesivir was considered within 7 days of symptom onset. In patients with COVID-19-related pneumonia and hypoxia, the corticosteroid dose was increased. In addition, for patients in the ICU with high levels of inflammatory markers and oxygen therapy requiring non-invasive or invasive mechanical support, tocilizumab or baricitinib were considered on a case-by-case basis.

3. Results

3.1. Patient Characteristics, Clinical Course, and Management

A query of all 170 LTRs routinely followed-up with our program identified 59 LTRs with symptomatic COVID-19 from March 2020 to March 2022: a cumulative incidence of 34.7%. Five LTRs were diagnosed with SARS-CoV-2 twice and their disease severities varied, with only one patient hospitalized twice during each COVID-19 diagnosis. The most common symptoms of COVID-19 at presentation were dyspnea, nausea, vomiting, fever, diarrhea, and cough. A CT scan of the chest was available in 27 of 34 (79%) cases of hospitalized patients. Bilateral ground-glass opacities (GGO) were present in 70% of cases, and single-lung GGO was present in 30%. The time from the COVID-19 diagnosis to CT chest scan was a median of 2 days (IQR 0–11 days).

Sixty-four laboratory-confirmed SARS-CoV-2 infections were identified in these 59 patients, of whom 34 (57.6%) were hospitalized. Most patients in this cohort underwent bilateral LT for idiopathic interstitial pneumonia (38%) or chronic obstructive pulmonary disease (20.6%). Other indications included fibrotic interstitial lung disease associated with connective tissue diseases, sarcoidosis, pulmonary artery hypertension, cystic fibrosis, and e-cigarette- or vaping-use-associated lung injury (in one patient, the first case ever described of LT for this indication in the USA [24]). Three patients underwent dual organ transplants with bilateral LT (one heart–lung and two liver–lung), and another patient underwent redo bilateral LT for advanced bronchiolitis obliterans syndrome.

The baseline characteristics of thirty-four hospitalized and twenty-five non-hospitalized patients are summarized in Table 1. The median age at diagnosis was 64 years, 62% were males, 66% Caucasians, and 27% were African Americans. The two groups were generally comparable, except hospitalized patients were more likely to be women and African Americans. The median FEV1 (mL) prior to COVID-19 was lower in hospitalized patients at 1675 (IQR 1440–2100) compared with 2400 (IQR 1620–2790) in non-hospitalized patients ($p = 0.01$) (Supplementary Materials).

The maintenance immunosuppression regimen for most LTRs consisted of a combination of corticosteroids and a calcineurin inhibitor. A cell-cycle inhibitor was part of the regimen in 47% and rapamycin in 13% of patients at baseline. Most patients (77%) were receiving azithromycin for bronchiolitis obliterans syndrome prophylaxis. The mean time post-LT to COVID-19 diagnosis was 5.4 years (±4.8). No cases were reported in newly transplanted patients (<6 months post-LT). All cases were acquired through community exposure to cases of COVID-19 within their households. The largest number of COVID-19 cases occurred during the period of Delta variant activity (19 patients; 10 LTRs required hospitalization). Monoclonal antibodies (casirivimab-imdevimab, bamlanivimab-etesivimab, and bebtelovimab or sotrovimab), based on CDC recommendations, were administered to 14 patients at the outpatient setting. Two of them required hospitalization and there were no deaths.

Of the 34 LTRs with COVID-19 that were hospitalized for hypoxic respiratory failure secondary to SARS-CoV-2 infection, one patient was hospitalized twice for separate infection episodes.

The standard of care of hospitalized COVID-19-infected LTRs was based on institutional protocols. Frequently used therapies in our hospital include augmented corticosteroids, remdesivir, tocilizumab or baricitinib, supplemental oxygen, anticoagulation, and supportive care, as clinically indicated. Intravenous remdesivir was only administered to hospitalized patients (55%, 19/34), and IV tocilizumab was administered in four cases of critical COVID-19 without contraindications (two patients in the ICU did not receive IV tocilizumab due to an active infection). Baricitinib was used in only one patient.

Table 1. Baseline characteristics of hospitalized and non-hospitalized lung transplant recipients with COVID-19.

Variable	*Response*	*All N = 59*	*Non-Hospitalized N = 25*	*Hospitalized N = 34*	† *p-Value*
Gender	F	22 (37%)	6 (27%)	16 (73%)	0.07
	M	37 (63%)	19 (51%)	18 (49%)	
Age	Mean ± SD	61.42 ± 12.27	61.48 ± 9.77	61.38 ± 13.97	0.98
Race	African American	16 (27%)	3 (19%)	13 (81%)	0.04
	Caucasian	39 (66%)	21 (54%)	18 (46%)	
	Others	4 (7%)	1 (25%)	3 (75%)	
BMI	Mean ± SD	28.25 ± 7.74	27.89 ± 6.13	28.52 ± 8.82	0.76
Reason for LT					0.31
	Idiopathic interstitial pneumonia	23 (39%)	10 (43%)	13 (57%)	
	COPD	12 (20%)	6 (50%)	6 (50%)	
	Other fibrotic ILD	8 (14%)	3 (38%)	5 (63%)	
	Sarcoidosis	8 (14%)	1 (13%)	7 (88%)	
	PAH	3 (5%)	1 (33%)	2 (67%)	
	Cystic fibrosis	2 (3%)	2 (100%)	0 (0%)	
	EVALI	1 (2%)	0 (0%)	1 (100%)	
	Re-Do Transplant	1 (2%)	1 (100%)	0 (0%)	
LT type	Bilateral Lung	52 (88%)	23 (44%)	29 (56%)	0.72
	Single Lung	4 (7%)	1 (25%)	3 (75%)	
	Liver/Lung	2 (3%)	1 (50%)	1 (50%)	
	Heart/Lung	1 (2%)	0 (0%)	1 (100%)	
Post Op Month	Mean ± SD	65.76 ± 57.96	53.92 ± 41.98	74.47 ± 66.61	0.15
FEV1_mL	Mean ± SD	2031.86 ± 835.91	2352.40 ± 935.51	1796.18 ± 675.67	0.01
CLAD	None	30 (51%)	18 (60%)	12 (40%)	0.08
	BOS Stage 1	9 (15%)	2 (22%)	7 (78%)	
	BOS Stage 2	11 (19%)	2 (18%)	9 (82%)	
	BOS Stage 3	6 (10%)	4 (67%)	2 (33%)	
	RAS	3 (5%)	1 (33%)	2 (67%)	
Comorbidities					0.33
	HTN	35 (60%)	16 (46%)	19 (54%)	
	DM	28 (48%)	12 (43%)	16 (57%)	
	BMI > 30	19 (32%)	7 (37%)	12 (63%)	
	CKD 3 or higher	39 (66%)	16 (41%)	23 (29%)	

LT, lung transplant; BOS, bronchiolitis obliterans syndrome; CKD, chronic kidney disease; CLAD, chronic allograft dysfunction following the International Society for Heart and Lung Transplantation 2002 classification; COPD, chronic obstructive pulmonary disease; EVALI, e-cigarette- or vaping-use-associated lung injury; ILD, interstitial lung disease; IQR, interquartile range; LTRs, lung transplant recipients; PAH, pulmonary artery hypertension, RAS, restrictive allograft syndrome. One patient had typical clinical and radiological features of COVID-19 with a negative RT-PCR test, but subsequently developed positive SARS-CoV2 antibodies. † $p < 0.05$ significant.

An ICU level of care was necessary in 14 LTRs (14/59, 24%), and eight of these patients (8/14, 57%) required invasive mechanical ventilation for severe hypoxemia. No LTRs received extracorporeal membrane oxygenation support. Cell-cycle inhibitors were discontinued in all cases of LTRs requiring hospitalization.

3.2. Vaccine Breakthrough

Vaccination with a minimum of two doses of a COVID-19 mRNA vaccine (BNT162b2 or Pfizer-BioNTech, and mRNA-1273 or Moderna) were recommended for all our LTRs. Fifty patients in this cohort completed a two-shot vaccination series (Pfizer: 33 patients; Moderna: 17 patients), and nine patients were unvaccinated at the time of the data censoring. COVID-19 occurred in 30 patients who received at least a two-doses series of an mRNA vaccine at a median of 123 days (IQR 98–164 days) after vaccination. Twelve of the thirty patients (40%) were hospitalized and four of these patients died (overall mortality: 13.3%; inpatient mortality: 33%). The three patients that required invasive mechanical ventilation died. Of

the 50 vaccinated patients, 31 received a third dose. Of the 31 patients that received a third dose, 19 had breakthrough infections (61.2%), and two patients died.

3.3. Outcomes

The patients were followed for a median of 150 days (IQR 68–369). The result of the multivariable analysis showed that patients with a higher baseline forced expiratory volume (FEV1) were 9% less likely to be hospitalized (OR = 0.91 and 95% CI 0.87–0.98, $p = 0.02$). The overall mortality was 13% (8/59), with an inpatient mortality of 23.5% (8/34), and 75% of those mechanically ventilated died (6/8). The mortality rate was 8% (28/34) in patients hospitalized without treatment with mechanical ventilation (OR = 36.0 and 95% CI 4.2–310.4, $p < 0.01$). Of the eight patients that died during the study period, four were patients with vaccine-breakthrough COVID-19. The cause of death in seven patients was septic shock and multiorgan failure. One patient died from antibody-mediated rejection 2 months following a COVID-19 infection. Common critical COVID-19 complications were sepsis, invasive mechanical ventilation, and acute kidney injury (Acute Kidney Injury Network stage 3). Bacterial and mold infections were commonly identified.

The characteristics of patients that died and those who survived are shown in Table 2. The patient demographics; underlying comorbidities; time from LT, FEV1, and CLAD; and presumed type of variant were comparable in both groups. Most deaths (four patients) occurred during the period of Delta variant activity. Although there was a trend of lower mortality observed during the Alpha wave (9%), and a higher mortality rate during the Delta wave (36%), no significant risk was detected after adjusting for the other covariates.

Table 2. Factors associated with mortality among hospitalized patients.

Variable	*Response*	*Hospitalized N = 34*	*Dead N = 8*	*Alive N = 26*	*p-Value*
Gender	F	16 (47%)	4 (25%)	12 (75%)	0.85
	M	18 (53%)	4 (22%)	14 (78%)	
Age	Mean ± SD	61.38 ± 13.97	66.63 ± 8.78	59.77 ± 14.99	0.23
Race	African American	13 (38%)	3 (23%)	10 (77%)	0.91
	Caucasian	18 (53%)	4 (22%)	14 (78%)	
	Others	3 (9%)	1 (33%)	2 (67%)	
BMI	Mean ± SD	28.52 ± 8.82	31.09 ± 9.67	27.74 ± 8.58	0.35
Reason for LT	IIP	13 (38%)	4 (31%)	9 (69%)	0.85
	EVALI	1 (3%)	0 (0%)	1 (100%)	
	Other fibrotic ILD	5 (15%)	1 (20%)	4 (80%)	
	COPD	6 (18%)	2 (33%)	4 (67%)	
	Sarcoidosis	7 (21%)	1 (14%)	6 (86%)	
	PAH	2 (6%)	0 (0%)	2 (100%)	
LT type	Bilateral Lung	29 (85%)	7 (24%)	22 (76%)	0.85
	Single Lung	3 (9%)	1 (33%)	2 (67%)	
	Liver/Lung	1 (3%)	0 (0%)	1 (100%)	
	Heart/Lung	1 (3%)	0 (0%)	1 (4%)	
Post Op Month	Mean ± SD	74.47 ± 66.61	74.75 ± 42.78	74.38 ± 73.11	0.99
FEV1_mL	Mean ± SD	1796.18 ± 675.67	1761.25 ± 455.02	1806.92 ± 737.66	0.87
CLAD					0.63
	BOS Stage 1	7 (21%)	2 (29%)	5 (71%)	
	BOS Stage 2	9 (26%)	2 (22%)	7 (78%)	
	BOS stage 3	4 (12%)	0 (0%)	4 (100%)	
	RAS	2 (6%)	0 (0%)	2 (100%)	

Table 2. *Cont.*

Variable	*Response*	*Hospitalized N = 34*	*Dead N = 8*	*Alive N = 26*	*p-Value*
Comorbidities					0.33
	HTN	3 (9%)	1 (33%)	2 (67%)	
	DM	2 (6%)	0 (0%)	2 (100%)	
	BMI > 30	3 (9%)	2 (67%)	1 (33%)	
	CKD 3 or higher	24 (71%)	5 (21%)	19 (79%)	
Medical ward		20 (59%)	0 (0%)	20 (100%)	<0.01
ICU		14 (41%)	8 (57%)	6 (43%)	<0.01
IMV		8 (24%)	6 (75%)	2 (25%)	<0.01
SARS-CoV-2 wave					
First_wave		10 (29%)	2 (20%)	8 (80%)	0.75
Alpha_wave		11 (32%)	1 (9%)	10 (91%)	0.17
Delta_wave		11 (32%)	4 (36%)	7 (64%)	0.22
Omicron_wave		4 (12%)	1 (25%)	3 (75%)	0.94
Breakthrough	N = 30	12 (40%)	4 (33%)	9 (75%)	0.43

BMI, body mass index; IIP, idiopathic interstitial pneumonia; PAH, pulmonary arterial hypertension; BOS, bronchiolitis obliterans syndrome; CKD, chronic kidney disease; CLAD, chronic allograft dysfunction following the International Society for Heart and Lung Transplantation 2002 classification; FEV1, forced expiratory volume in 1 s; IMV, invasive mechanical ventilation; RAS, restrictive allograft syndrome; SD, standard deviation.

4. Discussion

In this retrospective case study of 59 LTRs with symptomatic SARS-CoV-2 infections, we noted a high overall mortality rate of 13%, an inpatient mortality of 24%, and a 75% mortality rate in those mechanically ventilated.

A higher FEV1 at baseline was found to be protective against hospitalization with an OR = 0.91 (95% CI 0.87–0.98, $p = 0.02$). The risk for severe COVID-19 and a high mortality rate in LTRs have been demonstrated in previous reports [25–28]. A study across 68 ICUs in different regions of the United States found a 28-day ICU mortality of 40% in severe COVID-19 cases in 98 SOTRs (including four LTRs) [26]. In a registry from the University of Washington of 1900 SOT cases, including over 150 LTRs with at least 28-days follow-up after SOT, the mortality rate was 15% regardless of the season (these data were collected from the spring to fall of 2020). Over a third of hospitalized patients required an ICU level of care [10]. In the largest single-center study to date that included 32 LTR patients with severe COVID-19, the reported overall mortality rate was 47% (with a 100% mortality rate in those requiring invasive mechanical ventilation) [1]. In a study of 11 LTRs hospitalized for COVID-19, the ICU admission rate was 45%, and the mortality rate was 71% in those requiring mechanical ventilation [5].

All deaths in our study occurred in hospitalized LTRs with severe or critical COVID-19. Of the eight patients that died, COVID-19 was the cause of death in seven of the patients, and one patient died from antibody-mediated rejection two months following the COVID-19 infection. Six of the eight patients on invasive mechanical ventilation died. No deaths were identified in non-hospitalized LTRs with mild or moderate COVID-19 symptoms. Although the immunosuppression in LTRs may play a role, the key determinants of severity and mortality in SARS-CoV-2-infected LTRs are advanced age and underlying comorbidities. The comorbidities frequently seen in LTRs are arterial hypertension, renal failure, and cardiovascular diseases, which are also risk factors for adverse outcomes in COVID-19 in the general population.

Although our mortality rate was consistent with previous reports, our cohort had a larger proportion of African Americans (27%) compared with that in other studies. Previous reports suggested that the African American population is more severely affected by COVID-19, which was attributed to a higher prevalence of underlying comorbidities, as well as social inequalities resulting in less access to the health care system, especially at the beginning of the pandemic [29]. Most COVID-19 cases and deaths occurred during the

period of Delta variant activity, and generally paralleled the patterns of COVID-19 reported in the state of Michigan.

Furthermore, our report highlighted the risk of vaccine-breakthrough infections (30/64, 46.8% of the described cases) and the risk of COVID-19-related hospitalization and death, even in LTRs with a series of two doses of a COVID-19 vaccine. The hospitalization and mortality rates in our 30 LTRs with vaccine-breakthrough infections were 40% (12/30) and 13% (4/30), respectively. In comparison, a recent study of breakthrough COVID-19 infections in 14 LTRs that received two doses of an mRNA vaccine reported an 85.7% hospitalization and a 0% mortality rate at 4 weeks [28]. The favorable outcomes reported in that study may be a consequence of a younger cohort of patients (median age: 54 years) and milder disease at the time of hospitalization, as only 50% of the patients had clinical features of lower respiratory tract infection. Moreover, breakthrough COVID-19 infections in our cohort occurred at a median of 123 days post-vaccination when the vaccine-induced immunity is expected to wane. COVID-19 vaccination is an important strategy in preventing severe disease, hospitalization, and death; however, immune responses to vaccination are impaired in LTRs [20]. Less than a quarter of LTRs developed protective levels of antibodies after two or three doses of mRNA vaccines in studies that measured IgG antibody titers against domains of the SARS-CoV-2 spike protein to assess the serological response [30–33]. Similarly, cell-mediated immune responses are suboptimal in LTRs [34]. Factors that affect poor immune responses in LTRs include the use of cell-cycle inhibitors, such as mycophenolic acid; old age; induction therapy; and a regimen combination of tacrolimus plus mycophenolic acid with/without steroids [35]. The type of vaccine and optimal number of COVID-19 mRNA vaccine doses still need to be determined [20]. Most guidelines currently recommend four doses of an mRNA vaccine for transplant recipients, as well as the vaccination of close contacts [36,37].

The limitations of this study included its retrospective design, single-center data, absence of the genotyping of variants, and lack of autopsies to support cause of death. However, the study had several strengths including the large number of LTRs with COVID-19 diagnosed during the early and late periods of the pandemic when more modalities of therapy became available. In addition, the study described one of the largest cohorts of LTRs with vaccine-breakthrough infections.

5. Conclusions

We confirmed and expanded on the results from previous studies of COVID-19 infections in LTRs. The mortality from COVID-19 remained high in the LTR population despite the use of newer modalities of therapy. A higher FEV1 at baseline with a difference of at least 100 mL was shown to protect against hospitalization. Breakthrough COVID-19 infections are common in vaccinated LTRs and can result in severe disease. Additional studies are needed to determine the optimal vaccination strategies in the LTR population. Risk mitigation strategies including social distancing and masking during periods of high transmission, and prompt diagnosis and treatment are important. The long-term effects of COVID-19 on lung allografts remain unknown.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/transplantology3030026/s1, Table S1: Baseline characteristics of lung transplant recipients with COVID-19 based on level of care; Table S2: Characteristics of 59 lung transplant recipients with symptomatic COVID-19.

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Article

SARS-CoV-2 Infection of Unvaccinated Liver- and Kidney-Transplant Recipients: A Single-Center Experience of 103 Consecutive Cases

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Abstract: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was declared a pandemic in March 2020. Its reported impact on solid-organ-transplant-recipient morbidity and mortality has varied. The aim of this study was to present the effect of transplant status, patient comorbidities and immunosuppression modality on the survival of solid-organ-transplant recipients who contracted SAR-CoV-2 during the pre-vaccination era, at a single academic transplant center. Patients (n = 103) were assessed for 90-day mortality. A univariate analysis identified an age of over 60 years (HR = 10, $p = 0.0034$), Belatacept (HR = 6.1, $p = 0.022$), and Cyclosporine (HR = 6.1, $p = 0.0089$) as significant mortality risk factors; Tacrolimus was protective (HR = 0.23, $p = 0.022$). Common metabolic comorbidities (hypertension, diabetes, obesity) did not stand out as risk factors in our patient cohort. This study on the unvaccinated is expected to facilitate a paired comparison of outcomes in transplanted patients who contracted SARS-CoV-2 during the latter period of the pandemic, when broad SARS-CoV-2 vaccination and novel antibody treatments became broadly available.

Keywords: solid-organ transplant; COVID-19; unvaccinated; immunosuppression

1. Introduction

In January 2020, a novel coronavirus now known as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was first identified in Wuhan City, China [1]. The World Health Organization announced SARS-CoV-2 as a Public Health Emergency Concern and declared the viral outbreak a pandemic in March 2020 [2]. Exactly two years since, the U.S. has had more than 79 million confirmed SARS-CoV-2 cases and almost one million fatalities [3]. During the same period, over 452 million cases and 6 million SARS-CoV-2-related deaths have been reported globally [4].

As has been previously discussed by this research group and others, individuals that have received liver and kidney transplants are at a significantly heightened risk for morbidity and mortality from SARS-CoV-2 infection compared to the general population [5–14]. Liver- and kidney-transplant recipients have higher rates of diabetes, obesity, hypertension, and cardiovascular disease, which have all been identified as risk factors for severe SARS-CoV-2 complications in early reports at our institution and by others [6,8,11,15–18].

Early anecdotal experience [8] and later reports have reported a higher mortality risk among kidney-transplant recipients following SARS-CoV-2 infection compared to the liver-transplant recipients [19], with both groups having higher hospitalization and intensive-care-unit-admission rates. These early observations were debated in the later periods of the pandemic. This perhaps reflects the higher quality of care and closer surveillance of the transplant patients compared to the general population as well as the better understanding of the disease pathophysiology and effective treatments as the pandemic evolved, among other reasons [6,7,9,11,12,14,15,17,20,21].

Despite the plethora of published reports, the role of immunosuppression in SARS-CoV-2 severity in post-transplant patients remains unclear: Standard immunosuppression could potentially suppress the immune system's capacity to mount a sufficient response to neutralize the viral insult, modulate systemic inflammatory storm, or suppress viral replication [5,6,9,11,13,15,20–27]. By convention, most transplant clinicians modify the maintenance of immunosuppression in transplant patients infected by SARS-CoV-2, frequently by decreasing or even discontinuing antimetabolites [6,12,13,15,23,24,27]. The international society for heart and lung transplantation has officially recommended consideration for using mycophenolate mofetil, mTOR inhibitors, and azathioprine in transplant patients with moderate to severe SARS-CoV-2 [28]. Virus-targeted immunotherapies, i.e., monoclonal antibodies (MABs) and convalescent plasma have emerged as potential treatments. Studies have reported a decrease in hospitalization need and mortality rates following the use of MABs in high-risk groups, such as the immunocompromised transplant recipients [29–31].

This study aimed to study the SARS-CoV-2-specific mortality and associated risk factors of a cohort of 103 consecutive unvaccinated solid-organ-transplant recipients that were transplanted at a single academic transplant center, using a prospectively populated institutional SARS-CoV-2 transplant registry.

2. Materials and Methods

2.1. Study Inclusion

At the onset of the pandemic, we sought to build and populate a registry of all transplant recipients who contracted the disease, after obtaining Institutional Review Board exemption [8]. The study included all consecutive adult solid-organ-transplant recipients 18 years of age or above who had previously received a solid-organ transplant in our institution (liver, kidney or both) and tested positive for SARS-CoV-2 between 1 February 2020 and 18 February 2021. Subjects were included regardless of the elapsed time between transplantation and the positive SARS-CoV-2 test. All patients had functioning grafts at the time of enrollment. A positive SARS-CoV-2 diagnosis was determined via either a positive polymerase chain reaction or a positive antigen test [9]. The subjects were either completely unvaccinated or less than 2 weeks from their last vaccination.

2.2. Database Creation

As already described in our preliminary reports, an institutional Research Electronic Data Capture database was created, populated by all consecutive eligible de-identified subjects [8,18]. The collected data included patient demographic characteristics, comorbidities, transplant details, immunosuppression regimen, and SARS-CoV-2-specific treatment and outcomes [8,18]. Patients were followed for a 90-day period from the time of diagnosis [8,18].

2.3. Statistical Analysis

Subjects were divided into groups of survivors and fatalities at the end of the 90-day follow-up period. Categorical variables were reported as the number and percentage of the total group (%) and compared using the Fisher's exact test [32,33]. Continuous variables were reported as a median and interquartile range (lower quartile, upper quartile) and compared using the Wilcoxon rank sum test [8,32,33]. A univariate Cox regression model

was performed on the above-discussed variables and a Kaplan–Meier survival curve was constructed by age group [32].

3. Results

A total of 103 patients were enrolled, with 76 kidney-transplants recipients, 23 liver-transplant recipients, and 4 simultaneous liver–kidney-transplant (SLK) patients. There was a total of 10 90-day mortalities and 93 surviving patients. Patient demographic information, transplant type, comorbidities, and immunosuppression-regimen descriptions are shown in Table 1. Age, gender, transplant type, and comorbidities were statistically similar between the groups. There was a statistically significant difference ($p < 0.001$) between the median age of 67 and 52 in the dead and survivor groups, respectively. Significant differences also existed between groups in terms of immunosuppression regimens, namely Tacrolimus ($p = 0.037$) and Cyclosporine ($p = 0.029$).

Table 1. Patient Demographics, Transplant Type, Comorbidities, and Immunosuppression. Age reported as Median (IQR); analyzed with Wilcoxon rank sum test. Categorical variables reported as n (%); analyzed with Fisher's exact test.

	Deaths N = 10 (%)	**Survivors N = 93 (%)**	**Total N = 103 (%)**	**Mortality Rate (%)**	***p* Value**
Age	**67 (62, 70)**	**52 (42, 59)**	**54 (42, 62)**		**<0.001**
Gender					>0.900
Male	6 (60.0)	52 (56.0)	58 (56.3)	10.3	
Female	4 (40.0)	41 (44.0)	45 (43.7)	8.9	
Transplant Type					0.600
Liver	1 (10.0)	22 (24.0)	23 (22.3)	4.3	
Kidney	9 (90.0)	67 (72.0)	76 (73.8)	11.8	
SLK	0	4 (4.3)	4 (3.9)	0.0	
Total	10	93	103	9.7	
Comorbidities					
HTN	10 (100.0)	69 (74.0)	79 (76.7)	12.7	0.110
Diabetes	7 (70.0)	37 (40.0)	44 (42.7)	15.9	0.094
Obesity	0 (0)	16 (17.2)	16 (15.5)	0	0.354
Coronary Artery Disease	2 (20.0)	8 (8.6)	10 (9.7))	20.0	0.250
Immunosuppression					
Tacrolimus	6 (60.0)	82 (88.0)	88 (85.4)	6.8	0.037
Cyclosporine	3 (30.0)	5 (5.4)	8 (7.8)	37.5	0.029
Prednisone	7 (70.0)	48 (52.0)	55 (54.4)	12.7	0.300
MMF	7 (70.0)	66 (71.0)	77 (70.9)	9.1	>0.900
Sirolimus	1 (10.0)	5 (5.4)	6 (5.8)	16.7	0.500
Belatacept	2 (20.0)	3 (3.2)	5 (4.9)	40.0	0.073
Azathioprine	0	3 (3.2)	3 (2.9)	0.0	>0.900

SLK, simultaneous-liver kidney transplant; MMF, mycophenolate mofetil.

A univariate Cox regression model was performed for ages greater than 60 and immunosuppression regimen, shown in Table 2. Patients aged >60 were associated with a higher hazard ratio (HR) (HR = 10, $p = 0.0034$), as well as Cyclosporine (HR = 6.1, $p = 0.0089$) or Belatacept for the immunosuppression maintenance (HR = 6.1, $p = 0.022$), contrary to Tacrolimus (HR = 0.23, $p = 0.022$). No significant mortality risk or benefit was seen in patients taking prednisone, MMF, Sirolimus, or Azathioprine.

Table 2. Univariate Cox Regression Model of Selected Variables.

	Beta	HR	95% CI	*p* Value
Age > 60	**2.30**	**10.00**	**(2.10–48.00)**	**0.003**
Immunosuppression				
Tacrolimus	−1.50	0.23	(0.06–0.81)	0.022
Cyclosporine	1.80	6.10	(1.60–24.00)	0.009
Prednisone	0.72	2.10	(0.53–7.90)	0.300
MMF	−0.05	0.95	(0.25–3.70)	0.950
Sirolimus	0.59	1.80	(0.23–14.00)	0.570
Belatacept	1.80	6.10	(1.30–29.00)	0.022
Azathioprine	−17.00	3.90×10^{-8}	(0-Inf)	1.000

MMF, mycophenolate mofetil.

A Kaplan–Meier survival curve and the associated life table are shown in Figure 1 and Table 3, respectively. No SARS-CoV-2-related deaths within 90 days post-infection occurred in the youngest (20–51) age group. For the rest of the groups, deaths occurred 2 to 45 days post-SARS-CoV-2 diagnosis. The oldest patient group (aged ≥ 72) had the least survival probability (75%) compared to the rest (reference 20–51 years; $p < 0.001$).

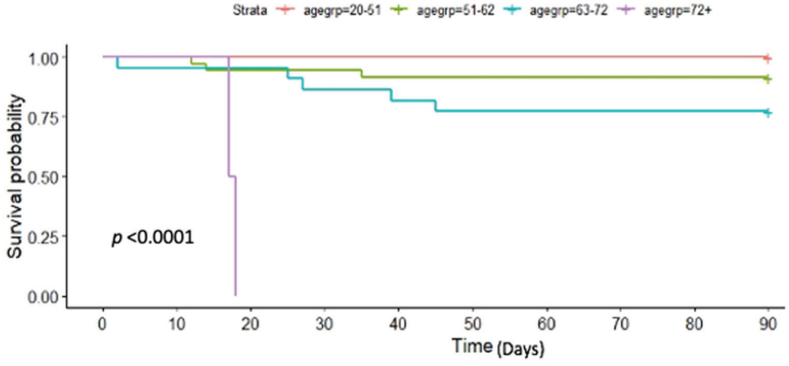

Figure 1. Kaplan-Meier survival curve of unvaccinated SARS-CoV-2 positive solid organ transplant recipients, stratified by age groups (years): 20–51, 51–63, 63–72, >72. Patient survival was inferior in the oldest age group ($p < 0.0001$).

Table 3. Life Table by Age Group.

	Number at Risk			
Age Group (years)	**0-Days**	**30-Days**	**60-Days**	**90-Days**
21–51 years	44	44	44	44
51–62 years	35	33	32	32
63–72 years	22	19	17	17
72+ years	2	0	0	0
	Survival (%)			
Age Group (years)	**0-Days**	**30-Days**	**60-Days**	**90-Days**
21–51 years	100	100	100	100
51–62 years	100	94.29	91.43	91.43
63–72 years	100	86.36	77.27	77.27
72+ years	100	0	0	0

4. Discussion

During the study period, 103 solid-organ-transplant patients were diagnosed with SARS-CoV-2 at our institution, with a 9.7% SARS-CoV-2-specific mortality rate within three months of diagnosis. This finding was similar to our early institutional experience and to reports by others during the first year of the pandemic, before vaccinations had

become broadly available [7–9,11,17,18]. In our cohort, most of the infected patients were kidney-transplant recipients, which aligned with the higher prevalence of this transplant subgroup. Similar to our preliminary reports [8,18], the kidney-transplant-recipient SARS-CoV-2 mortality rate was 11.8% vs. 4.3% among the liver-transplant recipients, with a calculated relative risk of 2.7 (95% CI 0.36–20.3). There were no reported deaths among the 4 combined liver–kidney-transplant recipients who had tested positive for SARS-CoV-2.

Hypertension and diabetes were present in 12.7% and 15.9% of the deaths. Despite early reports by others, these comorbidities were not associated with increased mortality in our cohort. SARS-CoV-2 mortality increased with advancing age, a finding described in general population outcomes [34].

Mirroring the practice of decreasing or discontinuing MMF in the presence of a viral infection, such as Cytomegalovirus, the antimetabolite dose was decreased or held for two weeks from the time of SARS-CoV-2 diagnosis. Our study failed to demonstrate any significant MMF effect on SRS-CoV-2-related mortality. However, more than 60% of patients who were taking MMF at the time of diagnosis had this medication held or decreased.

In our patient cohort, Tacrolimus demonstrated a protective effect (HR = 6.1, $p = 0.022$), an observation already reported by others [35]. A meta-analysis of 11 cohort studies investigating the impact of immunosuppression on SARS-CoV-2 suggested that Tacrolimus usage did not impact mortality or SARS-CoV-2 infection severity [36]. In our cohort, only eight (7.8%) patients had been on Cyclosporine at the time of the SARS-CoV-2 infection, three of whom died. In the univariate Cox regression, Cyclosporine was associated with a 6.1 (95% CI 1.6–24) death risk, contrary to a favorable 0.23 (95% CI 0.064–0.81) when using Tacrolimus as a Calcineurin inhibitor. These findings do not necessarily imply causation and should therefore be interpreted with caution; the findings may be attributed to the small patient sample and/or lack of control of confounding variables, including, but not limited to, the underlying indication for the switch to Cyclosporine from Tacrolimus, which has been the standard of care in our institution.

Two out of five (60%) patients who had been on Belatacept at the time of SARS-CoV-2 diagnosis eventually succumbed to the disease (HR = 6.1, $p = 0.022$). The literature is largely limited to case studies on the impact of Belatacept on SARS-CoV-2 outcomes. As a T-cell co-stimulation inhibitor, Belatacept is theorized as a potential mitigator of the cytokine storm caused by SARS-CoV-2 infection; however, it has also been shown to potentially increase the risk of severe opportunistic infections [37,38]. Similar to Cyclosporine, it remains unclear if this apparent positive correlation of Belatacept with severe SARS-CoV-2 infection reflects causation; since Belatacept is a choice often reserved for patients intolerant to CNIs and/or with a significant cardiovascular burden or recent cardiac events, there might be confounders that have not been identified in this small population sample, such as the underlying indication of the patient being switched to Belatacept. Like in the case of Cyclosporine, it may be the underlying comorbidities that led to the immunosuppression-regimen switch rather than the immunosuppression choice *per se*, as the factors impacting the disease outcome.

As scientific evidence evolved along with the pandemic progression, treatment for SARS-CoV-2 for both inpatients and outpatients at this institution changed over the course of this study, in alignment with the federal guidelines and transplant organizations' recommendations. Monoclonal-antibody therapy was recommended for SARS-CoV-2-positive transplant recipients managed in the outpatient setting and became available near the end of the study period in December 2020. A total of 21 (20.38%) patients in this study received monoclonal-antibody therapy. Remdesivir and convalescent plasma were also used for inpatients meeting certain criteria. A total of nine (8.74%) patients received Remdesivir, and five (4.85%) received convalescent plasma. While the impact of these treatments was not analyzed as part of this portion of the study, it is reasonable to consider that their use may have mitigated the mortality in these patients, particularly towards the latter stages of the cohort, when antibody treatments became standardized and broadly available, particularly for the higher-risk subgroups.

These data were collected over a period when SARS-CoV-2 vaccination was not widely available, therefore providing an opportunity to assess the viral infection fatality in our immunosuppressed population prior to the broad implementation of SARS-CoV-2 vaccines.

Age cohorts stood out as remarkable predictors of outcome and provided for a more robust analysis. No patients died in the 20–51-year age group and patients in the >72-year group had the least survival probability (75%, $p < 0.001$). Other studies have found age to be one of the most important factors in predicting SARS-CoV-2 mortality. This study, combined with data from existing works, is perhaps suggestive of the need to provide more robust, earlier intervention in the older transplant population [39–42]. Novel treatments such as MABs, antiviral agents, and most importantly preventative measures, could prove particularly life-saving in this older group of unvaccinated SARS-CoV-2-positive transplant recipients.

Statistical limitations existed in this study due to the small sample size. This led to severe model instability when a multivariate Cox regression analysis was attempted, as well as some instability of the univariate regression model. Model instability is particularly prevalent in fields with zero covariates in the fatality group. An extension of this study is currently ongoing to capture a larger study population in the attempt to build a stable model for analysis.

5. Conclusions

Our SARS-CoV-2 transplant registry demonstrated an almost 10% death rate in the early pandemic era, when vaccinations were not yet available and MAB treatment options were still evolving. Despite a trend for the kidney-transplant recipients being more susceptible to severe disease, particularly at the outset of the pandemic, this did not reach significance, while age prevailed as the mortality predictor, increasing the death hazard by a factor of 10 over the age of 60. Tacrolimus immunomodulation was protective in our patient sample. However, these findings should be interpreted with caution, since they could be inherent to the well-known limitations of a small sample size and retrospective study bias. Randomized trials are needed to elucidate the various immunosuppression modalities' impact on disease progression. This pilot study, which was conducted in a highly endemic area of the disease and on a patient population with overall morbidity and mortality among the highest in the United States, may provide the control group for future high-quality propensity-score-matched studies.

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transplantology

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Communication

COVID-19 Severity and Mortality in Solid Organ Transplantation: Differences between Liver, Heart, and Kidney Recipients

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Abstract: The infection by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can generate a wide spectrum of clinical manifestations ranging from asymptomatic to severe respiratory and systemic disease with coagulation disorder named coronavirus disease 2019 (COVID-19). Patients with comorbidities have been identified as risk groups for severe COVID-19, also having a higher death risk. Previous reports have conflicting results regarding if solid organ transplant recipients present an increased risk for COVID-19. Nevertheless, previous investigations failed to distinguish between different organs received or made a longitudinal investigation on those patients. We recruited 39 solid organ transplant recipients: 25 kidney transplant recipients, 7 heart transplant recipients, and 7 liver transplant recipients and 25 age-matched non-transplant COVID-19 patients without comorbidities (control group) and compared daily laboratory data in addition to performing survival analysis. Heart and kidney transplant recipients presented an increase in several COVID-19 severity-associated biomarkers, such as neutrophil-to-lymphocyte ratio and thrombocytopenia, in comparison to the control group and liver transplant recipients. Heart and kidney transplant recipients also presented an increase in the need for intensive care and invasive mechanical ventilation during the disease's course. Importantly, heart and kidney transplant recipients presented a higher mortality rate in comparison to liver transplant recipients and non-transplant recipients. In our cohort, heart and kidney transplant recipients presented a difference in clinical characteristics and survival rate in comparison to liver transplant recipients. Further investigation involving immune response to SARS-CoV-2 in solid organ recipients should consider and separate patients according to the organ grafted.

Keywords: transplant recipients; COVID-19; SARS-CoV-2; inflammation; survival

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1. Introduction

The coronavirus disease 2019 (COVID-19) is a respiratory and systemic disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). SARS-CoV-2 can infect multiple organs, including lungs, heart, liver, and kidney [1].

Several risk factors are associated with an increased risk for severe COVID-19, such as respiratory disorders [2] and metabolic diseases [3]. Transplantation is an established treatment for end-stage organ diseases, and patients commonly receive immunosuppressive therapy to prevent organ rejection [4]. There is conflicting data in the literature regarding the impact of COVID-19 on solid organ transplant (SOT) recipient patients. Some reports suggest that immunosuppressive therapy reduces the severity of the COVID-19-associated inflammation, while other reports did not observe such an effect, reporting similar inflammation to non-SOT patients [5–8]. Previous reports have identified increased lethality in

SOT recipients, comparing the survival rate with that of the general population, which could be influenced by the difference in treatments and associated comorbidities [9]. A case-controlled study concluded that SOT patients were not at greater risk during COVID-19, the immunosuppressive treatment did not influence the outcome of COVID-19 [10], and SOT patients did not present an increase in respiratory failure or cytokine production [11].

The SOT recipients may also respond differently to COVID-19 due to associated comorbidities, drugs used to prevent organ rejection, or the organ transplanted [12]. The prevalence of SOT patients varies among COVID-19 reports, which could be affected by the susceptibility to SARS-CoV-2 or the general prevalence of those patients in the population [13].

Several case reports have investigated the COVID-19 outcome in solid organ transplant recipients. Nevertheless, no longitudinal comparison between different organ recipients has been made to this moment. Therefore, we performed a longitudinal investigation on COVID-19 course and survival analysis in SOT patients (recipients of heart, kidney, and liver) with over one and a half years post transplant, in a single-center investigation during the same period.

2. Materials and Methods

Patients were at the "Hospital das Clínicas" of the Medical School of the University of São Paulo (HCFMUSP). SARS-CoV-2 RNA was detected by a reverse-transcriptase polymerase chain reaction in nasopharyngeal swab samples. In a cohort of 397 patients, 39 were solid organ transplant recipients: 25 kidney transplant recipients (KIDNEY), 7 heart transplant recipients (HEART), and 7 liver transplant recipients (LIVER). Two patients from the LIVER group received the transplant from a living donor. All patients underwent solid organ transplants more than 18 months prior to SARS-CoV-2 infection. The control group consisted of 25 non-transplant recipients without comorbidities diagnosed with COVID-19 during the same period (CONTROL).

Exclusion criteria for all groups were the presence of other comorbidities except for systemic arterial hypertension (SAH) and type 1 and 2 diabetes mellitus (DM). All patients with SAH underwent daily use of losartan (50 mg) for SAH control. Cyclosporine and tacrolimus levels on the serum were monitored and within the reference levels during COVID-19 (cyclosporine: 100–300 ng/mL and tacrolimus: 5–20 ng/mL). During hospitalization, COVID-19 patients received systemic and standard treatment. All patients received antibiotics (azithromycin) and anticoagulants. Part of the patients received antivirals (oseltamivir) and the other part received systemic corticosteroids (dexamethasone), as depicted in Supplementary Table S1. This study was approved by the Ethics Committee of HCFMUSP (No. 30800520.7.0000.0068-2020) and followed the 2013 revision of the Declaration of Helsinki and Istanbul. The Ethics Committee waived the need for written informed consent for its retrospective observational nature. EDTA blood samples were collected daily during hospitalization. Statistical analyses were performed using the Kruskal–Wallis test with Dunn's multiple comparisons and survival analyses were performed with the log-rank test for trend with GraphPad Prism-8 software (GraphPad Inc., San Diego, CA, USA).

3. Results

In our cohort of 397 patients, 39 were SOT recipients and sub-grouped according to the organ grafted/received: liver (n = 7), kidney (n = 25), and heart (n = 7). These three groups of SOT recipients did not differ concerning age and number of years post transplant (Table 1). However, the KIDNEY and HEART groups presented an increased hospitalization time in comparison to the CONTROL and LIVER groups, while the hospitalization time was comparable between the latter two groups (Table 1). Importantly, the outcomes were also markedly disparate. Most of the heart transplant patients (85.7%) required intensive care and invasive mechanical ventilation. This was also the outcome for 44% of the kidney transplant patients and 32% of the CONTROL group. This is in sharp contrast with the liver transplant recipients, none of whom evolved to this outcome. In summary, in

our cohort, heart transplant patients were at a significantly higher risk of severe respiratory injury and assisted mechanical ventilation than patients of the CONTROL and LIVER transplant groups but not significantly different from the KIDNEY group (Table 1).

Table 1. Patients' characteristics on admission.

	Control (N = 25)	Liver (N = 7)	Kidney (N = 25)	Heart (N = 7)	Reference Values	*p*-Value
Sex (Male/Female)	16/9	5/2	14/11	4/3	-	-
Age (Years)	**62.5 ± 2.3 ***	**66.5 ± 1.5**	**52.3 ± 2.4 ***	**50.1 ± 5.5**	-	0.005
Years Post Transplant	-	6.7 ± 1.6	7.1 ± 1.5	5.5 ± 1.6	-	0.86
Hospitalization Time (Days)	**14.4 ± 1.0**	**11.8 ± 1.3**	**18.5 ± 1.2 *,#**	**26.0 ± 1.2 *,#**	**-**	**<0.001**
Needed ICU Care (%)	**32**	**0**	**44**	**85.7**	**-**	**-**
LABORATORY DATA						
Leukocytes ($\times 10^3$/mm^3)	8.0 ± 1.8	6.0 ± 2.4	6.8 ± 1.2	11.5 ± 5.7	04–11	0.977
Patients with Leukocytosis (%)	20	42.8	16	42.8		0.19
Neutrophils ($\times 10^3$/mm^3)	5.2 ± 1.4	4.9 ± 1.9	5.5 ± 1.2	10.0 ± 5.4	2.5–7.5	0.979
Patients with Neutrophilia (%)	24	14.2	52	57.1		0.07
Lymphocytes ($\times 10^3$/mm^3)	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.1	0.5 ± 0.2	1.5–3.5	0.885
Patients with Lymphopenia (%)	100	100	100	100		-
Neutrophil-to-Lymphocyte Ratio (NTL)	7.5 ± 1.8	7.7 ± 1.5	9.4 ± 2.6	16.6 ± 5.5	4–11	0.532
Patients with ↑ NTL (%)	**20**	**0**	**52 #**	**85.7 *,#**		**<0.001**
Platelets ($\times 10^3$/mm^3)	251.7 ± 22.6	159.3 ± 39.5	239.3 ± 17.9	239.8 ± 36.1	150–400	0.291
Patients with Thrombocytopenia (%)	0	14.2	14.2	14.2		0.06
Patients with ↑ ALT (%)	8	14.2	16	0		0.61
Aspartate Aminotransferase (AST) (U/L)	69 ± 31.1	34.6 ± 4.3	41.3 ± 11.0	25.3 ± 1.4	<37	0.714
Patients with ↑ AST (%)	20	14.2	32	0		0.29
Direct Bilirubin (mg/dL)	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.02	0.2 ± 0.9	<0.3	0.243
Patients with ↑ Direct Bilirubin (%)	16	14.2	32	57.14		0.12
Indirect Bilirubin (mg/dL)	0.13 ± 0.05	0.14 ± 0.06	0.04 ± 0.0	0.14 ± 0.01	0.1–0.6	0.338
Patients with ↑ Indirect Bilirubin (%)	0	0	0	0		-
Creatinine (mg/dL)	**0.8 ± 0.1 ***	**2.1 ± 0.5**	**2.2 ± 0.2 ***	**2.4 ± 1.2**	**0.7–1.2**	**0.003**
Patients with ↑ Creatinine (%)	**20**	**100**	**100**	**100**		**>0.001**
Urea (mg/dL)	**34.3 ± 8.9**	**84.6 ± 12.1**	**95.1 ± 11.7**	**146.7 ± 37.3 ***	**10–50**	**0.003**
Patients with Uremia (%)	**12**	**100**	**100**	**100**		**<0.001**
C-Reactive Protein (CRP) (mg/L)	165.8 ± 41.4	57.8 ± 24.2	106.4 ± 43.2	67.6 ± 30.2	<5.0	0.419
Patients with ↑ CRP (%)	100	100	100	100		-
TRANSPLANT MOTIVE						
Cancer		2	3			
Hepatitis C Cirrhosis		3				
Alcoholic Cirrhosis		2				
Diabetes Mellitus			7			
Hypertension			7			
Glomerulopathy			3			
Polycystic Kidney Disease			2			
Chronic Interstitial Nephritis			2			
Idiopathic Dilated Cardiomyopathy						
Cardiac Insufficiency						
Chagas Cardiomyopathy						
Other			2			

↑ = increased * $p < 0.01$ difference statistically significant in relation to the CONTROL group. # $p < 0.01$ difference statistically significant in relation to the LIVER group. **Bold** is used to highlight values with statistically significant alterations. Reference values from Divisão de Laboratório Central do HC/FMUSP. Values presented as % or mean ± SEM.

We then searched for laboratory markers taken on admission that could correlate with the adverse outcome. Neutrophil, lymphocyte counts, and the neutrophil-to-lymphocyte ratio (NTL) are considered COVID-19-associated severity biomarkers. Although no marked differences in the cell counts were detected among the groups (Table 1), the percentage of patients with elevated NTL was significantly higher in the HEART and KIDNEY groups, especially the latter (86%) (Table 1). Additionally, noteworthy is the observation that none of the liver transplant recipients had abnormal NTL (Table 1). All groups were lymphopenic on admission (Table 1). Interestingly, renal function tests (urea and creatinine levels) were abnormal in all patients of the three SOT groups but in only a small fraction of the CONTROL ($\leq$20%) (Table 1). This translated into a trend for higher urea and creatinine levels in the SOT recipients, especially in the HEART and KIDNEY groups (Figure 1F,G).

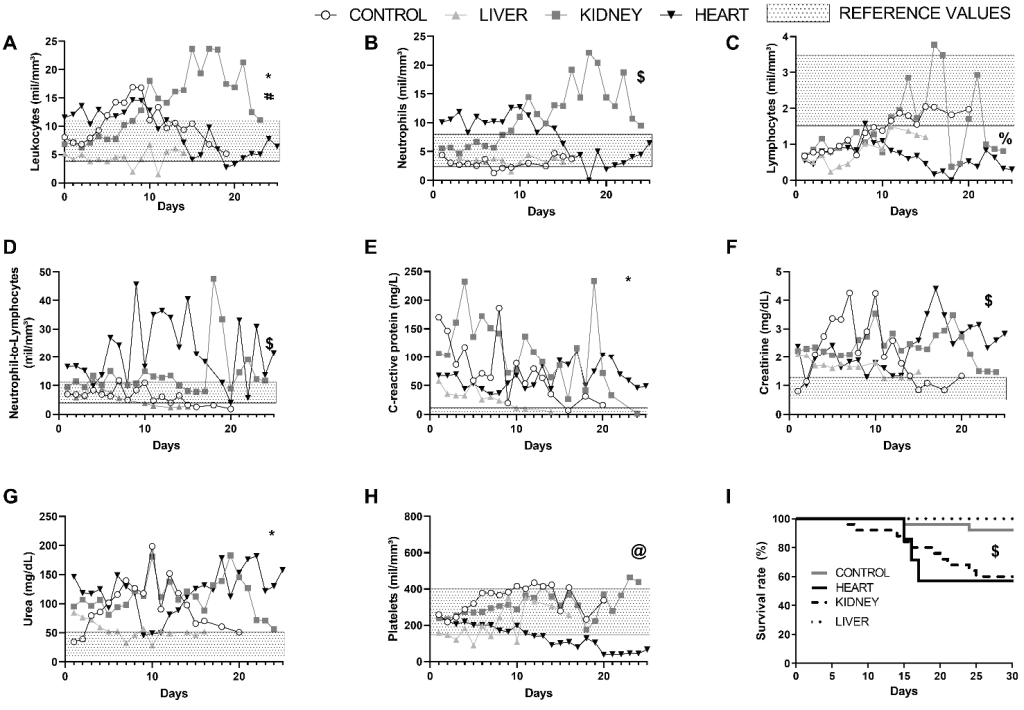

Figure 1. Daily clinical features of COVID-19 patients of the CONTROL, LIVER, KIDNEY, and HEART groups. (**A**) Leukocytes, (**B**) neutrophils, and (**C**) lymphocyte counts, (**D**) ratio of neutrophils-to-lymphocytes, (**E**) C-reactive protein, (**F**) creatinine and (**G**) urea levels, (**H**) platelet count, and (**I**) survival analysis. CONTROL, non-SOT recipients with COVID-19; LIVER, liver transplant recipients with COVID-19; KIDNEY, kidney transplant recipients with COVID-19; HEART, heart transplant recipients with COVID-19. * $p < 0.05$ difference from LIVER to all other groups. # $p < 0.05$ difference from KIDNEY and HEART. $ $p < 0.05$ difference from CONTROL and LIVER to all other groups. % $p < 0.05$ difference from HEART in comparison to CONTROL and KIDNEY. @ $p < 0.05$ difference from HEART in comparison to all other groups. Statistical analysis: In (**A**–**G**) was used Kruskal–Wallis test with Dunn's multiple comparisons was used and in (**H**), Log-rank test for trend. Data were collected between 1 May 2020 and 31 July 2020.

We were not able to detect differences in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels among the groups: few patients presented ALT and AST values over the upper limit of the reference values. (Table 1). The same holds true for the indirect and direct bilirubin levels as well as the platelets counts: few patients presented alterations of these biomarkers (Table 1). On the other hand, CRP levels were comparably elevated in all groups (Table 1).

Several investigations on COVID-19 patients have focused on a single-point analysis, usually at hospital admission, which can be affected by the time elapsed since the SARS-CoV-2 infection. Previously, we identified that longitudinal data from hospitalization to SARS-CoV-2 clearance and hospital discharge could provide valuable information and a better comprehension of COVID-19 [2]. Therefore, we performed a daily comparison of laboratory data from the hospitalization day until the hospital discharge.

We verified that the LIVER group presented a lower number of leukocytes during all hospitalization time compared with the three other groups (Figure 1A). Periods of leukocytosis were evident in the CONTROL and HEART groups but especially in the KIDNEY group (Figure 1A). Figure 1B shows that, except for the CONTROL group, the leukocyte changes reflected, in most part, the fluctuation in neutrophils counts, the LIVER and HEART group presenting low counts and the KIDNEY group presenting a steady

increase. In the CONTROL group, the leukocyte changes were due mostly to the steady increase in lymphocytes number, while these cells numbers varied little in the HEART and LIVER groups but peaked transitorily in the KIDNEY group, thereby also contributing to their leukocytosis (Figure 1B). Consistent with this, the KIDNEY and HEART groups were the ones that presented a more frequently increased NTL ratio; remarkably, there was no elevation of the NTL ratio in the LIVER group (Figure 1D,E). Thus, the KIDNEY and HEART groups were the ones that presented the most consistently evident biomarkers of severity. Eosinophils and monocytes did not show alterations during the hospitalization period in any of the groups (data not shown). On the other hand, the HEART group was the only one to exhibit a significant reduction in platelet counts during the disease course (Figure 1H).

Regarding the renal function, again, the KIDNEY and HEART groups presented the most prominent alterations in urea and creatinine levels, with the LIVER group showing only mild and transitory alterations, while the CONTROL group showed substantial but transitory alterations. Finally, the CRP was altered most of the time in all groups, with milder values in the LIVER group.

Importantly, the survival analysis indicated a statistically better prognosis in the CONTROL and LIVER groups compared to the KIDNEY and HEART groups (Figure 1I).

4. Discussion

Numerous risk factors have been associated with severe COVID-19 and an increased risk of death [3]. A nationwide investigation reported an increase in incidence, severity, and mortality in SOT patients with COVID-19 [14]. A recent cohort study in SOT and non-SOT patients with COVID-19 identified that SOT patients did not present an exacerbated inflammatory response in comparison to non-SOT but presented a tendency for a higher mortality rate [15]. In contrast, another report identified a low mortality rate in SOT and patients on the solid organ transplant waiting list but a higher mortality rate for hospitalized patients [16]. A caveat from previous reports is that patients received different treatments [17], while in our cohort, the patients were hospitalized in the same period at the service, receiving standard care for COVID-19 patients.

Infections represent a serious mortality cause in kidney, heart, and liver transplant patients, especially within the first year after the transplant [18]. Respiratory infections, in particular, can generate different disease manifestations and/or severity, according to the organ received, and present differences in severity between adults and children [19]. We did not identify any secondary bacterial infection in this cohort, which could drastically alter the laboratory data.

Azzi et al. raised the hypothesis that SARS-CoV-2 infection could differ depending on the type of organ transplanted [7,20,21]. Although previous manuscripts identified recipients of different organs in their cohorts, patients were not classified according to the organ grafted, and no comparison between the different SOTs was carried out [10,22]. In our cohort, SOT patients received the organ transplant over one year prior to COVID-19 infection and were classified according to the organ received, presenting significant differences in several inflammatory markers on the first hospitalization and during the COVID-19 disease course. During COVID-19, patients regularly present an increase in circulating leukocytes, with an increase in neutrophils and a reduction in lymphocytes, characterizing an immune dysregulated and hyper-inflammation condition [23]. In our investigation, liver transplant recipients neither presented the leukocytosis found in all other groups nor the neutrophilia of the heart and kidney transplant recipients, suggesting a less severe COVID-19 [24]. Heart transplant recipients also presented significant lymphopenia, a biomarker associated with severity and lethality during COVID-19 [23,25]. Due to the importance of neutrophils and lymphocytes in the COVID-19 pathogenesis, the NTL ratio is widely used as a severity biomarker [2,25]. In our cohort, the HEART and KIDNEY groups presented increased NTL compared with the LIVER and CONTROL groups, further stressing the higher severity of COVID-19 in those groups. Consistent with

this, CRP serum levels were elevated in the CONTROL, KIDNEY, and HEART groups compared with the LIVER group.

Elevated serum creatinine levels correlate with renal injury by COVID-19 and with a poor prognosis [25]. The KIDNEY group, as expected, as well as the HEART group presented persistently and markedly elevated urea and creatinine levels during the hospitalization period. Noteworthy, the LIVER group presented normal or slightly elevated urea and creatinine levels most of the time, while in the CONTROL group, there was a trend for elevated urea levels. These results are in contrast with previous reports and meta-analyses, which showed that all SOT patients appear to present an increase in the need for intensive care and mortality. This is due, at least in part, to carrying out the analysis separately to each of the three types of SOT recipients [26].

Hypercoagulation is another important factor contributing to COVID-19 mortality. There was a trend for decreased platelet count only in the HEART group, indicating a more severe COVID-19-induced coagulation dysfunction [25].

Overall, these analyses indicate that SOT patients may present significant differences in the course of COVID-19, especially regarding severe inflammation and mortality. Apparently, liver transplant recipients would display a more benign disease course. In fact, most of the liver recipients (86%) were on a single immunosuppressive regimen (tacrolimus), while heart and kidney recipients were on a triple-drug regimen (Supplementary Table S1). The liver allograft is more immune privileged than other solid organs commonly transplanted, endowing lower risk of rejection and less immunosuppressive regimens [27,28]. The usage of immunosuppressive treatment has been demonstrated to increase the cycle threshold in the reverse-transcriptase polymerase chain reaction used to identify the SARS-CoV-2 RNA in nasopharyngeal swab tests [29] Therefore, it is also conceivable that it also impacts the SARS-CoV-2 infection course [29] since tacrolimus has been shown in vitro to reduce non-SARS-CoV-2 coronavirus [30]. On one side, the tolerance state could be perturbed by the inflammatory response of COVID-19 (an issue that was not examined here), and on the other side, severe immunosuppressive regimens could impair the anti-SARS-CoV-2 immune response, favoring virus replication and spread, which ultimately would trigger an unbalanced or exaggerated immune reactivity. The liver transplant patients appear to suggest that moderate immunosuppression favors the control of the hyper-inflammation and benefits from the SARS-CoV-2-infected patient. The several previous reports of the COVID-19 clinical course in SOT patients did not analyze the different transplants separately, which may partially explain the conflicting results regarding the clinical characteristics and mortality rate [10,11,14–17,31].

These preliminary results should be confirmed in larger cohorts and with other SARS-CoV-2 variants. One advantage of our small cohort is that all patients were from the first Brazilian wave of COVID-19, when presumably only one viral strain was circulating in Brazil, and underwent the same clinical approach.

Our university hospital is currently a reference center for moderate to severe cases of COVID-19; hence, these results may not represent the profile in asymptomatic and mild SARS-CoV-2-infected SOT-recipient patients. Our preliminary study is the first to compare different organ transplant receivers in the Brazilian population; nonetheless, further investigations in other solid and non-solid organ transplants are necessary to understand the COVID-19 immune response in these populations.

5. Conclusions

Our data indicate that heart and kidney recipient patients present an increase in COVID-19-associated inflammatory biomarkers during the disease course and lower survival rates in comparison to non-SOT patients and liver recipient patients. Further investigations should analyze the differential effects of COVID-19 in larger cohorts of specific organ transplant patients.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/transplantology2030030/s1, Table S1: Solid organ transplant treatment previous to COVID-19.

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Review

COVID-19-Associated Lung Fibrosis: Two Pathways and Two Phenotypes, Lung Transplantation, and Antifibrotics

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Abstract: COVID-19 can be associated with lung fibrosis. Although lung fibrosis after COVID-19 is a relatively rare finding, the mere fact that globally a very large number of patients have had COVID-19 leads to a significant burden of disease. However, patients with COVID-19-associated lung fibrosis have different clinical and radiological features. The aim of this review is to define the different phenotypes of COVID-19-associated lung fibrosis, based on the medical literature. We found that two phenotypes have emerged. One phenotype is COVID-19-related acute respiratory distress syndrome (CARDS); the other phenotype is post-COVID-19 pulmonary fibrosis (PCPF). Both phenotypes have different risk factors, clinical, and radiological features, and differ in their pathophysiological mechanisms and prognoses. A long-term follow-up of patients with pulmonary complications after COVID-19 is warranted, even in patients with only discrete fibrosis. Further studies are needed to determine the optimal treatment because currently the literature is scarce, and evidence is only based on small case series or case reports.

Keywords: lung transplantation; SARS-CoV-2; fibrosis; phenotype hypothesis

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1. Introduction

Pulmonary transplant physicians are confronted with a new type of lung transplant referral, linked to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in which the clinical condition is named Coronavirus Disease 2019 (COVID-19), and the respiratory tract is the primary site of infection and of subsequent complications.

A small number of COVID-19 survivors suffer from COVID-19-related pulmonary fibrosis as a long-term consequence. Although COVID-19-related pulmonary fibrosis is a relatively rare disease, even a small percentage of the COVID-19 survivors affected by this condition can pose a significant healthcare problem due to the very large number of COVID-19 patients worldwide. Patients with COVID-19-related pulmonary fibrosis typically suffer from significant physical impairments and are at higher risk of death after COVID-19 when compared to patients without interstitial lung disease [1]. The burden of disease of COVID-19-related end stage lung disease, therefore, may be larger than previously assumed.

It is difficult to predict which patients will develop COVID-19-related pulmonary fibrosis, but known risk factors include male sex, a lung function with a forced vital capacity (FVC) of <80% predicted, and obesity [1]. So far two different phenotypes of COVID-19-related pulmonary fibrosis have emerged, both showing different clinical behaviors, risk factors, radiologic characteristics, and prognoses [2]. The COVID-19-related acute respiratory distress syndrome (CARDS) is a well-known condition leading to end stage lung disease. The other condition has been termed post-COVID-19 pulmonary fibrosis (PCPF). Both conditions appear to have different pathophysiological pathways, which could potentially be influenced by new treatments.

In this article, we summarize the features of the two different phenotypes of COVID-19-related pulmonary fibrosis based on the relatively few number of studies available in the medical literature. A narrative literature search was performed from 26 September 2021 to 19 May 2022, using the following databases: MEDLINE, EMBASE, Cochrane Library, and Google Scholar. Keywords included "fibrosis", "post-COVID", "ARDS", and "lung transplantation". The search was filtered for adults older than 18 years. The reference list of identified articles was searched for additional relevant studies. Possible treatment options, including lung transplantation, are discussed as well.

2. What Is the Risk of Pulmonary Fibrosis in COVID-19?

Many patients who have survived COVID-19 report dyspnea as a persistent symptom after recovery. Dyspnea has been reported in over 40% of patients after recovery from COVID-19 [3]. In many of these patients, dyspnea can be attributed to extrapulmonary effects, including cardiovascular, neurological, and muscular dysfunction. Dyspnea can also be related to persistent pulmonary lesions after COVID-19 and can lead to substantial disability, even after initial recovery from COVID-19. Sometimes dyspnea and pulmonary alterations after COVID-19-related lung disease are associated with a dependency on supplemental oxygen. Persistent pulmonary lesions, including ground-glass opacities, consolidations, and reticulations, have been described in twenty percent of patients at 6 months after hospitalization for COVID-19 pneumonia [4]. One study showed that risk factors in patients who had not recovered after COVID-19 pneumonia, were older age, male sex, a longer in-hospital stay, and a lower PaO_2/FiO_2 ratio at admission [4]. These patients also showed more severe chest computed tomography (CT) scan abnormalities at hospital admission [4]. In the other two highly pathogenic coronaviral diseases, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), persistent pulmonary lesions have been reported as well. In SARS, one study completed a 15-year follow-up, showing pulmonary lesions on chest CT scans initially in 9.4% of patients, which diminished to 3.2% after one year and remained stable thereafter [5]. Studies in MERS patients showed comparable data [6].

3. Pulmonary Fibrosis in Lung Transplant Recipients after COVID-19

Follow-up data on the outcomes among lung transplant recipients who survived COVID-19 are scarce. Persistent post-COVID-19 parenchymal opacities (n = 29, 65.9%) could be demonstrated in chest CT in a majority of the lung transplant recipients who survived COVID-19 [7]. Significant loss of lung function was also observed in this population (n = 18, 40.9%), in which three patients (5.6%) developed chronic lung allograft dysfunction (CLAD), all three with the restrictive allograft syndrome (RAS) phenotype [7]. These patients typically had low absolute lymphocyte counts ($<0.6 \times 10^3$/dl) and elevated ferritin levels (>150 ng/mL) [7]. Generally, the association between respiratory viral infections and the development of CLAD is suggested to be stronger in the case of symptomatic viral infections [8–11]. In one study asymptomatic respiratory viral infections were not associated with a significant decline in lung function [11,12]. If this also holds true for SARS-CoV-2 infections currently is unknown. In immunocompetent patients, pulmonary fibrosis four months after COVID-19 has been shown to be associated with the severity of illness [13]. In lung transplant recipients, however, these data are still lacking.

4. Interstitial Disease Patterns: CARDS

The two main phenotypes of pulmonary complications in patients with COVID-19 are acute respiratory distress syndrome (ARDS) related to COVID-19 (CARDS), and post-COVID-19 pulmonary fibrosis (PCPF) [2,14]. The underlying interstitial patterns are described in Table 1.

Table 1. Different aspects of the two phenotypes of COVID-19-associated lung fibrosis.

	COVID-19-Related ARDS (CARDS)	**Post-COVID-19 Pulmonary Fibrosis (PCPF)**
clinical features	7–14 days after initial infection secondary pulmonary hypertension +++	12–16 weeks after initial infection secondary pulmonary hypertension +
mortality 90 days	30–50%	unknown
risk factors	mechanical ventilation, VILI, hyperoxia, prolonged hypoxia, increased BMI, elderly patients, possibly thromboembolism and hypercoagulability, possibly NETS	profound dyspnea, higher respiratory rate, comorbid hypertension, ICU admission, hyperoxia, prolonged hypoxia, elderly patients, possibly thromboembolism and hypercoagulability, possibly NETS, higher CRP levels, lymphocytopenia, neutrophilia, eosinopenia, lower baseline IFN-γ and MCP-3
biomarkers	IL-6 moderately increased persistent deactivation of key immune cells, e.g., reduced surface expression of the mHLA-DR	cytokine-driven: TGF-β and IL-1β longer telomere lengths appear to be protective; this genomic biomarker estimates the balance of profibrotic and antifibrotic susceptibilities
restrictive ventilatory defect	++	+++ (rib cage shrinkage)
pneumothorax	+++	++
pathophysiology	severe pulmonary infiltration/edema and endothelitis	inflammation leading to impaired alveolar homeostasis, alteration of pulmonary physiology resulting in pulmonary fibrosis
radiological features	rapid progression of bilateral air space opacities, with consolidations with lower lobe predominance, with anteroposterior gradient. Chest CT with rapid progression involving all 5 lobes in a patient with COVID-19 should increase concern for ARDS. Predilection for dense consolidation in the dependent posterior lower lobes with relative sparing of the anterior or non-dependent areas. In survivors, after several months from initial CT, lower lobes are spared from fibrotic changes while new fibrotic changes with traction bronchiectasis may appear in the previously spared upper lobes	

ARDS = Acute Respiratory Distress Syndrome, CARDS = COVID-19-related ARDS, CRP = C-reactive protein, CT = computed tomography, IFN-γ = interferon gamma, IL-1β = Interleukin-1 beta, MCP-3 = monocyte chemoattractant protein 3, mHLA-DR = monocytic human leukocyte antigen-DR, NETS = neutrophil extracellular traps, PCPF = post-COVID-19 pulmonary fibrosis, TGF-β = Tumor Growth Factor beta, VILI = mechanical ventilation-induced lung injury. Frequency of occurrence: + (rare), ++ (associated), +++ (frequent). Table modified from Ref [2].

4.1. Clinical Features and Mortality

CARDS typically occurs early (usually within 14 days after initial symptoms) in the disease course of COVID-19 with patients becoming critically ill due to the rapid onset of respiratory failure. CARDS is diagnosed when a patient has a confirmed SARS-CoV-2 infection and develops ARDS, according to the Berlin 2012 ARDS diagnostic criteria [15]. These criteria include (1) new or worsening acute respiratory failure within 1 week of a known clinical insult, (2) bilateral opacities, not fully explained by effusions, lobar/lung collapse, or nodules, and (3) respiratory failure not fully explained by cardiac failure or fluid overload.

Compared to ARDS from other causes, CARDS has a worse outcome. Bellani et al. reported hospital mortality in ARDS patients of 34.9% for mild, 40.3% for moderate, and 46.1% for severe ARDS [16]. In CARDS, mortality of 52.4% has been reported [17].

4.2. Risk Factors

Risk factors for the development of CARDS and progression from CARDS to death included older age, neutrophilia, organ, and coagulation dysfunction (e.g., higher lactate dehydrogenase and D-dimer) [17]. High fever ($\geq$39 °C) was associated with a higher likelihood of CARDS development but a lower likelihood of death [17]. Treatment with methylprednisolone decreased the risk of death [17]. The main causes of death in CARDS are respiratory failure (53%) followed by combined respiratory and cardiac failure (33%), while myocardial damage and circulatory failure were shown in 7% of patients [17].

4.3. Radiology

In CARDS, the pre-existing typical radiological COVID-19 pneumonia features with bilateral, lower lung predominant, and multifocal lesions, become progressively consolidative. The typical rounded opacities, termed "COVID balls", increase in extent and density, and evolve into fibrotic bands [18]. Typical chest CT findings in COVID-19 pneumonia are classified as typical, atypical, and indeterminate, as defined by the Radiological Society of North America (RSNA) expert consensus statement [19]. In CARDS, the chest CT features are similar to ARDS from other etiologies [20]. Importantly, in survivors of CARDS, the amount of irreversible fibrosis should not be overrated. The presence of the initial consolidation seems to protect against the subsequent development of fibrosis. The fibrotic lung changes that are seen in survivors are predominantly present in the anterior or non-dependent lobes of the lungs [20]. The posterior or dependent portions of the lungs are thus, relatively preserved. This is clinically relevant because areas that initially show consolidations have potentially reversible alterations and should not be over-interpreted as fibrosis [20]. In addition, areas that initially resemble fibrosis and traction bronchiectasis can potentially be reversible as well after the resolution of the air space opacities [20]. Excellent examples of chest CT features have been described by Gosangi et al. [20]. Complications of CARDS are ventilatory-associated lung injury, leading to lung tension cysts, pneumomediastinum, pneumopericardium, pulmonary interstitial emphysema (PIE), and pneumothorax [20].

4.4. Pathophysiology

In CARDS, the pathological feature of ARDS is diffuse alveolar damage (DAD). A cytokine storm has been suggested to initiate and promote lung fibrosis progression and severity. A profibrotic macrophage response of the SARS-CoV-2 infection also triggers lung fibrosis. It has been shown that macrophages in COVID-19 express genes associated with profibrotic functions [21]. In ARDS and multiple organ failure, the cytokine storm is thought to be the predominant mechanism leading to tissue damage [22]. In the pulmonary interstitium, there is not only excessive deposition of extracellular matrix (ECM) but there are also changes in the structure and composition of the ECM [23]. Moreover, in reaction to injury of the alveolar epithelial cells, type II alveolar epithelial (AT II) cells proliferate and differentiate into type I alveolar (AT I) cells [24,25]. Aging and loss of AT II cells are involved in the pathogenesis of lung fibrosis, and AT II is highly associated with fibrosis in virus-infected patients [24].

5. Interstitial Disease Patterns: PCPF

5.1. Clinical Features and Mortality

Patients typically suffer from postviral exertional dyspnea, with persistent fibrotic changes on chest CT. Although some risk factors are known, this group of patients can be diverse as shown in different case reports and case series published to date [26–32].

5.2. Risk Factors

In a prospective study of 173 patients with COVID-19, evidence of pulmonary fibrosis was observed in 90 patients (52%) at 3-month CT follow-ups [26]. Risk factors were pulmonary consolidation (odds ratio [OR] = 2.84), severe disease (OR 2.40), and a higher CT severity score (OR 1.10) at admission [26]. Of 62 patients who underwent chest CT scans again at 6 months of follow-up, in 41 patients (66.1%) the fibrosis remained unchanged, whereas in 21 patients (33.9%) a radiological improvement was documented [26]. In addition, older age, cigarette smoking, high-dose systemic corticosteroid use, and long-term mechanical ventilation were risk factors in another study [27]. The study of Han et al. showed that in older patients, high-dose systemic corticosteroid use and mechanical ventilation are risk factors as well [28]. Aging may cause a shift to a more profibrotic and irreversible senescent phenotype of fibroblasts [29].

Other risk factors were higher C-reactive protein (CRP) and lower lymphocyte counts [30–32].

5.3. Radiology

The fibrotic changes include traction bronchiectasis, honeycombing, parenchymal bands, and interlobar septal thickening (IST) [26]. Nabahati et al. did not see any patients with progressive pulmonary fibrosis at the 6-month chest CT follow-up [26]. For this study cohort, a longer follow-up has not yet been published.

Two other studies demonstrated fibrotic abnormalities in the 6-month chest CT follow-up of 35% and 32% of patients, respectively [27,28]. Importantly, studies are difficult to compare because of different patient characteristics at diagnosis. It has been suggested that PCPF's course could be similar to other well-documented forms of postviral pulmonary fibrosis, such as those occurring after SARS, MERS, or influenza H1N1 infections [33]. Although in SARS patients, fibrosis could be demonstrated in more than 50% of patients after an average of 37 days, only 5% of patients continued to show fibrotic changes after a 15-year follow-up [33].

5.4. Pathophysiology

The key site of SARS-CoV-2 infection is the angiotensin converting enzyme 2 (ACE2). To enter the human host cell, the virus uses the spike "S" protein, which results in binding to ACE2. In human cells, the ACE2 gene expression is the highest in nasal epithelial cells and decreases throughout the lower respiratory tract, including epithelial cells of the trachea, bronchi, and alveolar cells. In normal human cells, the function of ACE2 is converting angiotensin II (Ang II) into angiotensin 1–7 (Ang 1–7), in order to regulate the cardiovascular system and blood pressure. Ang II has a fibrotic effect by upregulating the level of a pro-fibrotic cytokine named transforming growth factor-1β (TGF-β), which transforms fibroblasts into myofibroblasts and promotes extensive collagen deposition [24,34]. On the contrary, Ang 1–7 has an anti-fibrotic effect. When the spike "S" protein of the SARS-CoV-2 down-regulates the level of ACE2, it increases the level of Ang II and decreases the level of Ang 1–7, resulting in promoting inflammation and pulmonary fibrosis [24].

6. How Should We Treat COVID-19-Related End Stage Lung Disease?

The cause of COVID-related end stage lung disease still remains unclear, and more studies are needed to build our understanding of why some patients develop lung fibrosis, and other patients do not. This question is important, as these patients will require long-term medical care and the number of patients is considerable. Moreover, the long-term behavior of fibrotic changes is still unknown, as most studies have a relatively short follow-up period, which is an inherent problem when dealing with a relatively new virus. Although the long-term pulmonary consequences of COVID-19-related pulmonary fibrosis remain speculative, the large number of individuals affected by COVID-19-related fibrosis could lead to a worldwide healthcare challenge of unprecedented magnitude [35]. Another unanswered question is whether other variants of SARS-CoV-2 will influence disease severity. The omicron variant of SARS-CoV-2 (PANGO lineage B.1.1.529) was reported on

24 November 2021 and has been associated with a more proximal adherence in the airways, in contrast to the delta variant, which has a more distal distribution in the airways. The omicron variant replicates significantly less efficiently than other SARS-CoV-2 variants in both nasal turbinates and lungs and induces substantially attenuated lung pathology [36].

Currently, there is no consensus on the use of antifibrotics in patients with COVID-19-related end stage lung disease. Only two drugs (pirfenidone and nintedanib) are used to treat idiopathic pulmonary fibrosis (IPF). Both drugs have been approved by both the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) and can decrease the rate of pulmonary fibrosis progression. Pamrevlumab (FG-3019), a new antifibrotic drug for intravenous use, is currently being investigated in phase 2 trials and has shown promising results in patients with IPF in the phase 2 PRAISE trial [37]. It is a monoclonal antibody against connective tissue growth factor (CTGF) in IPF. However, in COVID-19-associated pulmonary fibrosis, there are no published studies on pamrevlumab yet.

Pirfenidone is an orally administered pyridine with combined anti-inflammatory, antioxidant, and antifibrotic properties. The mechanism of action includes the inhibition of fibroblast proliferation, but details have not yet been fully determined. Nintedanib is an inhibitor of multiple receptor tyrosine kinases and was initially developed as an antiproliferative and anti-angiogenic drug for cancer treatment.

IPF is defined as a spontaneously occurring (idiopathic) specific form of chronic fibrosing interstitial pneumonia associated with a pattern of Usual Interstitial Pneumonia (UIP) on imaging or histology [38]. It has been shown that both pirfenidone and nintedanib also show efficacy in non-IPF patients with progressive fibrosis [39]. This was shown both for nintedanib (INBUILD) and for pirfenidone [40,41]. The INBUILD study showed a reduction in FVC decline of about 60% compared to placebo. For both antifibrotic drugs, pirfenidone and nintedanib, a meta-analysis showed a reduced decline in the forced vital capacity (FVC) in patients with idiopathic pulmonary fibrosis (IPF) and also non-IPF patients [39]. As suggested by Wells et al., there may be a common pathway in non-IPF disease with IPF-like disease progression [42]. Based on this hypothesis, antifibrotics might be effective in COVID-19-associated lung fibrosis in an early stage. The similar cytokine profile in both IPF and COVID-19 also suggests a common pathway [43]. Although it is currently uncertain to which extent COVID-19-associated lung fibrosis will be progressive, the early treatment seems prudent considering the autopsy study in ARDS patients, showing that a longer disease duration led to a higher risk of lung fibrosis [44]. Using a score to assess the risk of progression to severe disease may help in the timing of treatment escalation [45]. Another argument for antifibrotic treatment early in the disease course is that previous coronavirus outbreaks have been associated with substantial postviral lung fibrosis and physical impairment [46]. In one interventional study of 30 patients with COVID-19 requiring mechanical ventilation, the patients were treated with nintedanib, showing no significant differences in 28-day mortality compared to the control group without nintedanib, but it showed significantly shorter lengths of mechanical ventilation and lower percentages of high-attenuation in computed tomography volumetry [47].

The results of four ongoing trials investigating antifibrotic drugs in COVID-19-associated lung fibrosis probably will shed some light on this important and urgent question [48–51]. Interestingly, the mTOR inhibitor rapamycin, a well-known drug in transplant medicine, has been suggested to have both antiviral action and antifibrotic properties. The latter is also known for the treatment of lymphangioleiomyomatosis [46].

In severe lung fibrosis, only lung transplantation as the ultimate therapy could be an option for selected patients. The number of COVID-19-related lung transplantations is relatively small but increasing. In the United States, a query of the United Network for Organ Sharing showed that as of 30 April 2021, only 78 lung transplantations had been performed (50 for CARDS and 28 for PCPF) [14], increasing to 299 (183 for CARDS and 107 for PCPF) as of 31 January 2022 [52]. In Europe, the Eurotransplant consortium reported only 21 patients undergoing lung transplantation for COVID-19-related end stage lung

disease [14]. In the scientific literature, only a limited number of case reports and case series have been published so far with limited follow-up data. The difficult question is whether patients benefit from lung transplantation in COVID-19-related end stage lung disease and how their perioperative and long-term disease course differs in comparison to lung transplant experiences with other fibrotic lung diseases. Since COVID-19 frequently affects multiple organ systems the short-term and long-term outcomes may differ. ECMO is being used increasingly for COVID-19 patients with improved outcomes [53–55]. In the case reports and case series, early outcomes have been acceptable for these marginal lung transplant recipient candidates, although Cypel et al. warned about publication bias in the context of a new disease [56]. The ideal "transplant window" is transplantation that is not too early (e.g., in patients that may recover spontaneously or due to medical therapy) and not too late (e.g., in extremely debilitated patients with muscle wasting, critical illness polyneuropathy, and myopathy, lacking good potential for recovery and rehabilitation, relevant pulmonary hypertension with right-sided heart failure, or even with multi-organ failure). Some potential for recovery has been described in patients with severe COVID-19-related ARDS [55]. Unfortunately, regarding the risk factors for COVID-19-associated fibrosis, many patients will be excluded from being transplant candidates due to comorbidities, and/or secondary complications such as renal dysfunction, muscle wasting, or multiple organ failure while in the intensive care unit (ICU) [56]. Importantly, the patients should be completely free of the coronavirus infection to prevent harboring the virus and developing a relapse of the infection later, especially during chronic triple immunosuppressive therapy after lung transplantation. Another important issue is that there is a general consensus described in the latest consensus document of the International Society of Heart and Lung Transplantation (ISHLT), that patients should be awake and able to discuss the lung transplantation and provide consent for the procedure [57,58]. The enormous impact of lung transplantation on quality of life should be fully understood. Waking up after intubation for an acute viral illness and being informed that lung transplantation has been performed, implicating a life with chronic triple immunosuppression and potential complications ahead, can lead to a severe psychotrauma, that can be too difficult to deal with [56]. The ten considerations that should be carefully evaluated when assessing a patient with COVID-19-associated lung transplantation have been published in 2020. In very experienced high-volume centers some criteria may be disregarded on a case-by-case basis. As mentioned by Lepper et al., the case series of lung transplantation for COVID-19 described by Bharat et al. showed that many patients had a complicated intraoperative and postoperative course, including mass transfusions, continued extracorporeal support, re-thoracotomy, primary graft dysfunction, and prolonged postoperative stay in the ICU [54,59]. However, in this case series, patients did not necessarily fulfill all requirements as mentioned by some other authors [54,56]. Defining the ideal recipient in this situation remains difficult, in which increased in-hospital SARS-CoV-2 transmission, and depleting healthcare resourses should also be taken into account. Those patients who have a risk of imminent death and those who are on mechanical ventilation or ECMO should have priority if there are no absolute or important relative contraindications or major risk factors.

An unanswered question is whether patients with pre-existing lung fibrosis should be vaccinated with the SARS-CoV-2 vaccination. Prevention of COVID-19 seems to be the best strategy. However, acute exacerbations of idiopathic pulmonary fibrosis after the SARS-CoV-2 vaccination have been described, and this could be underreported as many cases will not necessarily be published in the medical literature [60]. Another study described the development of pulmonary fibrosis in a previously healthy patient after the SARS-CoV-2 vaccination [61]. VigiAccess, the global pharmacovigilance database of the World Health Organization (WHO), reports 679 patients with ARDS, 346 patients with lung consolidation, 159 patients with organizing pneumonia, and 280 patients with pulmonary fibrosis, related to Comirnaty (Pfizer) COVID-19 vaccination. In relation to the Spikevax (Moderna) vaccination, ARDS was reported in 679 patients, interstitial lung disease in

678 patients, idiopathic pulmonary fibrosis in 56 patients, acute lung injury in 12 patients, and idiopathic interstitial pneumonia in 3 patients [62].

7. Prognosis in Patients with COVID-19-Associated Lung Fibrosis

COVID-19 survivors were shown to have significant functional and radiological abnormalities after 4 months, which were attributed to small-airways and lung parenchymal disease [63]. Radiological abnormalities were associated with more severe or critical diseases [63]. Another study with a 6-month follow-up showed fibrotic changes in more than one-third (40 of the 114 patients, 38%) of survivors after severe COVID-19 pneumonia [28].

A recent meta-analysis including 70 studies with a median follow-up of 3 months, showed fibrotic changes in one-third of patients, whereas no significant resolution was observed in fibrotic changes [64]. Others have observed radiological evidence of lung injuries suggestive of lung fibrosis, but with a reversible component, thus not being the classical fibrotic changes known to us previously in other fibrotic lung diseases. It is, therefore, difficult to differentiate reversible lung injuries from irreversible pulmonary fibrosis, raising the question under what circumstances and criteria antifibrotic therapy is truly indicated [14].

8. Conclusions

The two described phenotypes should be used to classify the type of COVID-19-associated lung fibrosis in order to better define the evolution of these conditions and determine the appropriate treatment strategy and the timing of lung transplant evaluation and listing. With additional data in this rapidly evolving field, the two phenotypes may be defined more clearly and the multiple treatment options can be used optimally based on an increasing body of evidence. Long-term fibrotic complications remain a major concern contributing to morbidity and mortality.

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transplantology

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Review

Transplant Drugs against SARS, MERS and COVID-19

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Abstract: There is an urgent need to develop drugs and vaccines to counteract the effects of the new coronavirus SARS-CoV-2 and adequately treat the corona virus disease (COVID-19). As these drugs are still under investigation, research also focuses on existing medication with proven effectiveness in other coronaviral diseases. The advantages of existing therapeutic drugs that are currently approved (for other indications) are the known safety profile, general availability and relatively lower costs involved in extending the purpose to a new disease. Calcineurin inhibitors (CNI) are drugs that have shown effectiveness in several coronaviral diseases, and are well-known and widely used drugs in transplant medicine. The aim of this narrative review is to present the current evidence of CNI in coronaviral diseases, the biophysiology of CNI and to suggest possible ways to study CNI as a new treatment option for COVID-19. We searched original papers, observational studies, case reports, and meta-analyses published between 2000 and 2020 in English in the PubMed database and Google Scholar using the keywords: (coronavirus), (treatment), (MERS), (SARS), (COVID-19), (tacrolimus), (ciclosporin), (cyclosporin) AND (calcineurin inhibitor). We excluded studies in patients with clear indications for immunosuppressive therapy. Additionally, we searched in the preprint servers and the World Health Organization bulletin. Ten studies were identified and included. Calcineurin inhibitor therapy has been suggested to be effective for coronaviral diseases in different settings. The results are summarized in a table. CNI should be investigated as a first treatment option based on evidence of direct antiviral effects and its properties preventing severe systemic hyperinflammation, as has been observed in COVID-19 with predominantly pulmonary immunopathological changes.

Keywords: immunosuppression; treatment; hypothesis; cytokine storm syndrome; hyperinflammation; tacrolimus; FK506; cyclosporine

1. Introduction

Coronaviruses (CoV) are among the frequent pathogens causing the common cold. They have a single-stranded RNA genome, that is coiled within the virion. In electron microscopy they show spikes protruding from the virion envelope with a crown-like shape, which lead to the name "coronavirus".

They belong to the order of the Nidovirales, and within this order, the coronaviruses have been studied in great detail because of their zoonotic transmission since the 21st century, causing life-threatening infections in humans, their societal and economic impact, unusual features of their pathogenesis, and the complexity of their molecular biology [1]. The coronaviruses are classified into two main subfamilies: the Torovirinae and the Coronavirinae, the latter being subdivided into the genera Alpha-, Beta-, Gamma-, and Deltacoronavirus [1]. The Alpha- and Betacoronaviruses include the seven Coronavirus serotypes, of which there are four (CoV-NL63, -HKU1, -E229, -OC43) with a low pathogenicity, causing mild upper respiratory tract infections. The other three serotypes are highly dangerous viruses, such as the Severe Acute Respiratory Syndrome

Coronavirus-1 (SARS-CoV-1) causing SARS, the Middle East Respiratory Syndrome coronavirus (MERS-CoV) causing MERS, and the novel SARS-CoV-2 causing Coronavirus Disease-19 (COVID-19). So far, Gamma- and Deltacoronaviruses have been discovered mostly in avian species [1]. The Gamma- and Deltacoronaviruses cause economically important diseases of livestock, poultry, and laboratory rodents [2].

1.1. Pathophysiology in Coronaviral Infections

All coronaviruses have a different antigenicity, depending on the spike (S-) protein of the virus. In contrast to influenza viruses, the S-proteins in coronaviruses are very stable. To enter the human cell, coronaviruses use different human cell surface peptidases. Both SARS-CoV and SARS-CoV-2 use the human angiotensin-converting enzyme-2 (ACE-2), which functions as a receptor for the virus. This receptor is widely expressed in a number of organs including pulmonary tissue, as well as in monocytes and macrophages [3]. Recent studies also demonstrated that both SARS-CoV and SARS-CoV-2 also can use lectins to enter the cell. Moreover, SARS-CoV-2 can use neuropilin-1, which is strongly expressed by endothelial cells and epithelial cells facing the nasal cavity [4–6].

Entering the cytoplasm by the receptor, the virus uncoats and starts replicating in the human cell. The exact pathophysiology responsible for the unusually high morbidity and mortality following CoV infections with high pathogenicity, are incompletely understood. Important mechanisms could be the virus-induced direct cytopathic effects, as well as the viral evasion of the host immune system.

1.2. Morbidity and Mortality of Coronaviruses

A dysregulated immune system, resulting in an overshooting inflammatory response, contributes to morbidity and mortality. Mortality rates in MERS, SARS and COVID-19 are around 35%, 9% and 5% of infected individuals, respectively. Nevertheless, the number of infected patients has never been so large as in the current COVID-19 pandemic. The total number of patients suffering from MERS was 2400, from SARS 8300, and from COVID-19 (so far) passes 17 million [7]. MERS spread to 27 countries, SARS to 30 countries and COVID-19 represents currently a global threat of increasing magnitude. Symptoms of these lethal coronaviruses differ.

MERS is a disease predominantly affecting the lower respiratory tract, which in most patients leads to pneumonia. Clinical manifestations are fever, malaise, chills, myalgia, cough, dyspnea, diarrhea, vomiting, and abdominal pain. In severely ill patients dyspnea is severe with acute respiratory failure, renal failure, and shock. As in SARS-CoV-2, there is a high incidence in older patients. Predictors of poor outcome include age above 60 years, male gender, diabetes mellitus, chronic lung disease and chronic renal disease, low albumin level and progressive lymphocytopenia [8]. MERS-CoV infections can be asymptomatic in 12.5–25% of patients [8].

SARS can present with hypoxia, cyanosis, fever, dyspnea and acute respiratory failure. The WHO case definition (2003) includes the following: (1) fever higher than 38 °C or history of such in the past 2 days, (2) radiological evidence of new infiltrates consistent with pneumonia, (3) chills, cough, malaise, myalgia, or known history of exposure, and (4) positive test for SARS-CoV by one or more assays.

In SARS patients, neutralizing antibodies are detected 2–3 weeks after the onset of disease, and 90% of patients recover without hospitalization [2]. About 10% of SARS patients develop severe respiratory failure after 5–7 days following infection, with interstitial pneumonia characterized by progressive diffuse alveolar damage.

In COVID-19, most frequent co-morbidities are hypertension, cardiovascular disease, diabetes, and obesity [9]. Age appears to be the strongest predictor of COVID-19 related death. Clinical manifestations of COVID-19 include fever, malaise, myalgia, non-productive cough, dyspnea, nausea, vomiting and diarrhea. Gastrointestinal symptoms can be the first manifestation of COVID-19, especially in patients with immunosuppressive drugs. Olfactory and/or gustatory dysfunctions have been reported in 64% to 80% of patients [10].

COVID-19 can progress to severe organ dysfunction of the heart, brain, lung, liver, kidney, and coagulation system [10], and can lead to myocarditis, cardiomyopathy, ventricular arrhythmias, and hemodynamic instability [10]. In severe infection, patients may develop acute cerebrovascular disease and encephalitis [10]. Hypercoagulopathy leading to both venous and arterial thromboembolic events occur in 10% to 25% in hospitalized patients, and in ICU patients with COVID-19 in 31–59% [10]. Approximately 72% of non-surviving COVID-19 patients had hypercoagulopathy [9].

SARS-CoV-2 also can induce vascular damage, and pre-existing endothelial dysfunction combined with the direct assault of SARS-CoV-2 on the vascular system may account for a high mortality of COVID-19 patients [9].

Hospitalized patients with COVID-19 need ICU treatment in approximately 17–35% of patients, most commonly due to hypoxemic respiratory failure requiring intubation and mechanical ventilation [10].

About 4–32% of patients are completely asymptomatic. However, it is unclear which of the following three scenarios are represented in these reports: (1) truly asymptomatic infection by individuals who never develop symptoms, (2) transmission by individuals with very mild symptoms, or (3) transmission by individuals who are asymptomatic at the time of transmission but subsequently develop symptoms [10].

1.3. Treatment of Coronaviruses

There is an urgent need to develop therapeutic drugs and vaccines against SARS-CoV-2 for the treatment and prevention of COVID-19, respectively. As these drugs still are under investigation, research also focuses on existing drugs with proven effectiveness in other (corona-)viral diseases. Sometimes this is referred to as "repurposing". Using currently approved drugs for other indications reduces time, costs and safety issues. Calcineurin inhibitors (CNI) showing favorable effects in multiple coronaviruses, thereby replacing the "one-drug-for-one-bug" paradigm. They are well-known, already existing drugs in transplant medicine used for solid organ transplant (SOT) recipients, and are also prescribed in rheumatology, dermatology and ophthalmology.

1.4. Scope of This Review

This review highlights the current evidence of CNI as pan-coronaviral inhibitors, including the current understanding of the biophysiological characteristics of CNI influencing the viral behavior in the human host. We also provide an outlook on what aspects should be considered when investigating this transplant medicine approach for the treatment of immunocompetent patients suffering from COVID-19.

2. Methods

We searched original papers, observational studies, case reports, and meta-analyses published between 2000 and 2020 in English in the PubMed database and Google Scholar using the keywords: (coronavirus), (treatment), (MERS), (SARS), (COVID-19), (tacrolimus), (ciclosporin), (cyclosporin) AND (calcineurin inhibitor). In addition to the commonly used preprint servers for COVID-19 research (bioRxiv and medRxiv, arXiv, Research Square, www.preprints.org, Open Science Framework, and the WHO Bulletin) we searched all available preprint servers mentioned in the preprint server directory ASAPbio registry (49 entries, last updated in January 2020) without indexing in Google Scholar for additional papers with the same search criteria as mentioned above. Namely, the following preprint servers were searched: Autorea, Cell Sneak Peek, Journal of Medical Internet Research preprints, Neuroimage Clinical First Look, Preprints with The Lancet, Social Science Research Network, Surgery Open Science first look, Therapoid, ViXra. We searched for additional references in the bibiographies of the detected papers to obtain additional references relating to the main topic. We also tried to obtain pre-clinical and clinical safety data from the two pharmaceutical companies that currently market the two main approved CNI drugs without responses.

We excluded studies in patients with clear indications for immunosuppressive therapy, such as solid organ transplant recipients or rheumatological patients, since we aimed to investigate the CNI in immunocompetent patients.

3. Results

Ten studies were included. Calcineurin inhibitor therapy has been documented to be effective in various coronaviral diseases both in vitro as well as in vivo. So far, no data in immunocompetent patients on effects of CNI in human SARS-CoV-2 infections have been published. The results are shown in Table 1.

Table 1. Coronaviral serotypes and treatment with calcineurin inhibitors.

Coronaviral Serotype Studies in Humans	**CNI**	**Remarks**	**Ref. No.**
MERS-CoV	Tac	renal transplant recipient on tacrolimus survived	[11]
MERS-CoV	CsA	inhibition of viral replication	[12]
Coronaviral Serotype Studies in Animals	**CNI**	**Remarks**	**Ref. No.**
feline CoV	CsA	inhibition of viral replication in dose-dependent manner	[13]
turkey CoV	CsA	enhanced virus titers in kidney	[14]
Coronaviral Serotype Studies In Vitro	**CNI**	**Remarks**	**Ref. No.**
MERS-CoV	CsA + IFN-α	inhibition of viral replication	[15]
MERS-CoV, SARS-CoV	ALV	inhibition of viral replication	[16]
SARS-CoV, CoV-229E	CsA	inhibition of viral replication SARS-CoV replication impaired, but not fully blocked (1–5% of cells remained SARS-CoV positive, even in high CsA concentrations)	[17]
CoV-NL63, CoV-229E, SARS-CoV	CsA	inhibition of viral replication	[18]
SARS-CoV, CoV-NL63, CoV-229E	Tac	inhibition of viral replication	[19]
CoV-NL63	CsA-d	inhibition of viral replication by CsA derivatives (Alisporivir, NIM811)	[19]
SARS-CoV-2	CsA	potent antiviral activity in SARS-CoV-2, cyclophillin depedent (and calcineurin independent)	[20]

ALV = alisporivir, CNI = calcineurin inhibitor, CoV = coronavirus, CsA = cyclosporin A, CsAd = cyclosporine A derivatives, IFN-α = interferon alpha, MERS = Middle East respiratory syndrome, Tac = tacrolimus.

The available studies with in vivo data on SARS-CoV, MERS-CoV and SARS-CoV2 include studies performed on animal models of coronavirus-related diseases. SARS-CoV replication has been studied in mice, Syrian golden and Chinese hamsters, civet cats, and non-human primates [21], and MERS-CoV in mice, camelidae and non-human primates [21]. These animal studies investigated protease inhibitors, monoclonal and polyclonal antibodies, convalescent plasma, interferons, ribavirin, lopinavir/ritonavir in both SARS-CoV and MERS-CoV [21], but to our knowledge there are no animal data on SARS-CoV and SARS-CoV-2 addressing CNI therapy. Exceptions are the feline and turkey CoV, which do not have the possibility of spillover into human hosts [13,14]. The other exception is alisporivir, a nonimmunosuppressive cyclophillin inhibitor (CsA analog) in SARS-CoV, with strong in vitro dose-dependent antiviral properties against SARS-CoV-2 [16].

4. Discussion

So far, the COVID-19 pandemic has led to more than 17 million SARS-CoV-2 infected patients and over 700000 deaths [7]. The number of cases is still, or again, on the rise in many countries and there currently is no geographic region where the pandemic seems totally under control.

4.1. COVID-19 in Solid Organ Transplant Recipients

Surprisingly, in solid organ transplant (SOT) recipients, a relatively low number of patients have been reported, in case reports or small case series [22]. Symptoms of COVID-19 in SOT recipients often can be atypical, such as gastrointestinal (i.e., diarrhea, anorexia, and upper abdominal discomfort) or neurological (i.e., delirium), and therefore this diagnosis needs a high index of suspicion [23].

Whilst SOT recipients require life-long administration of immunosuppressive drugs in order to minimize alloreactivity and preserve solid organ allograft function, severe infections related to immunosuppression are feared. Based on this, SOT recipients have been considered to belong to the vulnerable population for SARS-CoV-2 infections and severe consequences of COVID-19 were expected.

Paradoxically, SOT recipients with SARS-CoV-2 infections have shown a relatively benign course of disease, most of them with a favorable outcome within a short timeframe. D'Antiga (Italy) published on March 20, 2020 the first descriptive analysis of clinical observations in SARS-CoV-19 positive transplant patients and suggested that unlike common viral agents (e.g., adenovirus, influenza, respiratory syncytial virus), infection with SARS-CoV-19 might not lead to a worse general condition in immunosuppressed patients [24–26]. Another study showed that transplant status was not associated with COVID-19 mortality [27].

This is in sharp contrast to many immunocompetent COVID-19 patients, in whom a subset develop severe COVID-19 which is associated with a high mortality rate.

Moreover, the number of SOT recipients with COVID-19, described in several case reports and some case series, is relatively low compared to the number of immunocompetent COVID-19 patients. Although definite numbers of SOT recipients with COVID-19 have not been reported by the transplant societies, our own estimation is that there are less than 1000 patients. However, these numbers are still increasing as the pandemic is ongoing. Long-term consequences of COVID-19 in SOT recipients cannot be estimated yet due to the relatively short follow-up duration of a few months.

4.2. Cytokine Storm Syndrome (CSS)

In the severe COVID-19 phase, the pathophysiological response is a cytokine-mediated systemic hyperinflammation, called cytokine storm syndrome (CSS) or cytokine release syndrome (CRS). CSS is a life-threatening emergency associated with high mortality. It was first described in renal allograft recipients [28], receiving the anti-T-cell antibody muromonab-CD3 (OKT3), an immunosuppressive drug [28]. These patients developed systemic reactions that closely resembled the symptoms induced by the injection of pure recombinant cytokines [28], which was related to a massive release of highly biologically active mediators [28]. In CSS, laboratory results demonstrate pancytopenia (anemia, leukocytopenia, thrombocytopenia), coagulation disorders, elevated serum creatinine, liver enzymes, C-reactive protein (CRP) and hyperferritinemia.

The presence of hyperferritinemia seems to play a relevant pathophysiological role in CSS. In autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE) and the anti-phospholipid syndrome, it is a well-known feature [29]. H-ferritin has been shown to suppress myeloid cells, and also affects lymphoid cells by suppressing the proliferation of T-cells and impair the maturation of B-cells [30]. Moreover, ferritin may favor the loss of tolerance and the onset of autoimmunity [30]. Ferritin can be also a pro-inflammatory signaling molecule [29]. Hyperferritinemia has been associated with different CSS-related conditions such as macrophage activation syndrome (MAS) and septic shock [29].

CSS is clinically characterized by persistent fever, lymphadenopathy, hepatosplenomegaly, central nervous system (CNS) abnormalities with multiple organ failure (MOF), disseminated intravascular coagulation (DIC), renal and/or cardial insufficiency, and shock.

In COVID-19, the CSS spectrum of respiratory symptoms is wide, and can be mild (cough, mild dyspnea) to severe (severe dyspnea, with progression to Acute Respiratory Distress Syndrome (ARDS) or Acute Lung Injury (ALI), with a fulminant post-ARDS pulmonary fibrosis. The pulmonary complications in CSS has an aerogenic and a vascular route. The first is by aerogenic SARS-CoV-2 transmission which leads to SARS-CoV-2 reaching the ACE-2 receptors in the alveolar epithelial cells. This results in the downregulation of ACE-2 expression and increasing the angiotensin level, leading to increased pulmonary capillary permeability and pulmonary edema [31]. The second is by the blood circulation, where SARS-CoV-2 reaches the lung again, interacting with the ACE-2 receptors on the surface of alveolar capillary endothelial cells, where it attacks the capillary endothelium. The resultant

immune responses further aggravate lung injury by the CSS [31]. These cytokines include interleukin (IL)-1β, IL-1Rα, IL-2, IL-10, fibroblast growth factor (FGF), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), interferon-γ-inducible protein (IP10), monocyte chemo attractant protein (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), platelet derived growth factor (PDGF), tumor necrosis factor (TNFα) and vascular endothelial growth factor (VEGF) [32]. Moreover, in severe COVID-19, there is a reduction of natural killer (NK) cells, CD4+ and CD8+ T-lymphocytes and IFN-γ expression in CD4+ T-lymphocytes. In this phase, SARS-CoV-2 infection leads to a reduction and functional exhaustion of T cells [33] and by the above described mechanisms, SARS-CoV-2 is hijacking our immune defense systems.

These inflammatory factors may be among the leading causes in the rapid worsening of COVID-19. Similar to SARS-CoV and MERS-CoV, in SARS-CoV-2 increased amounts of proinflammatory cytokines in serum were associated with pulmonary inflammation and extensive lung damage [34]. Moreover, compared to patients with less severe disease, patients requiring ICU admission had higher serum cytokine concentrations than those that did not require ICU admission, suggesting that the cytokine storm was associated with disease severity [34]. However, regarding the role of cytokine storm in COVID-19, it still is not clear which cytokine(s) plays a critical role in the initiation of severe COVID-19 [35].

Another major unanswered question is why most COVID-19 patients with CSS are elderly patients, and CSS is extremely rare in young COVID-19 patients. One explanation might be that aging is associated with mild elevated levels of local and systemic pro-inflammatory cytokines, including IL-6, IL-8, TNF-α, IL-13, IFN-γ, as well as acute phase proteins. This chronic mild inflammation in aging, so-called "inflamm-aging" [36], results in an increased risk of a cytokine storm in some critical elderly patients with COVID-19 infection [36].

4.3. Cytokine Storm Syndrome in Other Diseases

The cytokine storm syndrome has been described in infectious and non-infectious diseases, and is not unique to COVID-19. Cytokine profiles can be slightly different, dependent on the cause of the CSS, as has been reviewed by Gao et al. [31]. It has also been observed in other viral infections (SARS-CoV, MERS-CoV, Avian H5N1 Influenza and the Gram-negative bacterium *Francisella tularensis*), graft-versus-host disease, autoimmune diseases (SLE, systemic juvenile idiopathic arthritis), hematologic conditions (hemophagocytic lymphohistiocytosis) and medications [37]. It is possible that increased levels of proinflammatory cytokines in older people are responsible for a more severe course of the disease or a particular aspect of immunosenescence [38].

Although the human immune response against SARS-CoV-2 remains poorly defined, it has been suggested that calcineurin inhibitors, used chronically in many solid organ recipients, may play a protective role in patients with COVID-19 [39–41].

4.4. The Calcineurin/NF-AT Signaling Pathway

To understand the mechanisms of CNIs, the interaction between intracellular calcineurin and nuclear factor of activated T cells (NF-AT) is important (Figure 1). In resting cells, NF-AT proteins are hyperphosphorylated and are localized in the cytoplasm. On activation, NF-AT proteins undergo rapid dephosphorylation by calcineurin. The dephosphorylated NF-AT proteins then translocate into the nucleus, where they regulate gene transcription. NF-ATs regulate a large number of inducible genes in immune cells, including cytokines, cell-surface receptors, and chemokines [42].

Dephosphorylating NF-AT by calcineurin is a calcium-dependent process, and as soon as the calcium signals cease, it leads to rephosphorylation, initiating NF-AT to return from its active state in the nucleus to its inactive state in the cytoplasm.

4.5. Two Calcineurin Inhibitors in Clinical Use

Currently, cyclosporin A (CsA) and tacrolimus (FK506) are CNIs used in the clinical setting. CsA was approved by the FDA in 1983. It is a cyclic polypeptide, derived from the fungus

Tolypocladium inflatum [43]. Tacrolimus (also known as FK506), used since 1995, is a macrolide antibiotic, isolated from the soil bacterium *Streptomyces tsukabensis*, with quite a similar mechanism of action as CsA [44], which will be discussed later.

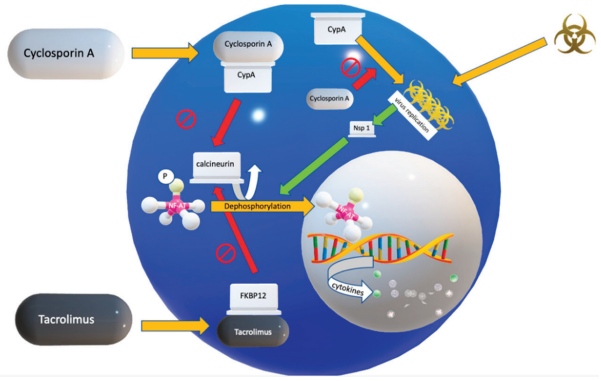

Figure 1. Pathogenesis of COVID-19 and the suggested actions of cyclosporin and tacrolimus. Upon binding of a CoV protein to cyclophilins, the Calcineurin/nuclear factor of activated T cells (NF-AT) pathway is activated, via the coronaviral non-structural protein-1 (Nsp) leading to a systemic cytokine storm. In addition, cyclophilin A (CypA) probably stimulates CoV replication. Cyclosporin A inhibits viral replication. Adapted from Tanaka et al., *Viruses* **2013**, *5*, 1250–1260; doi:10.3390/v5051250.

4.6. Mechanism of Action of Cyclosporin A (CsA)

As a calcineurin inhibitor, cyclosporin A (CsA) binds to cyclophilins (Cyps). Cyps are the binding partner proteins of CsA, which, as host cell receptors for CsA, mediate the immunosuppressive action of CsA, by inhibiting calcineurin (Figure 1) [11]. Cyclophilins belong to the immunophilins, and the most abundant cyclophilin is Cyclophilin A (CypA), which is widely distributed in almost all tissues and accounts for 0.1–0.4% of the total protein content in a cell [45]. It also is abundant in the cytosolic extract from lymphocytes, and has a high affinity for CsA. CypA acts as an acceleration factor in protein folding and assembly [45].

Inflammatory stimuli [46,47], oxidative stress [46,48] and activated platelets [49] result in cellular secretion of CypA. Following stimulation with reactive oxygen species (ROS), CypA was detectable at the plasma membrane of vascular smooth muscle cells within 30 min of stimulation [46]. When CsA inhibits Cyps, several important effects occur, which are summarized below:

(a) Preventing the Cytokine Storm Syndrome

First of all, as described before, CNIs block the translocation of NF-AT from the cytosol into the nucleus. Inhibiting the NF-AT dephosphorylation inhibits expression of NF-AT dependent genes. This is thought to be the major mechanism in preventing the systemic cytokine storm syndrome in COVID-19. Blockade of NF-AT into the nucleus prevents transcription of genes that encode for cytokines such as interleukins (IL) [11], and in this way inhibits the pro-inflammatory pathway.

(b) Direct Antiviral Effect of CsA

CsA may have a direct antiviral effect, in which Cyps might play a critical role in replication of coronaviruses [11]. Although in vitro data do not necessarily imply effects in vivo, there are numerous in vitro data suggesting antiviral properties of CsA in other non-coronaviral diseases. Through blocking the interaction of cellular cyclophilins with viral proteins and inhibiting viral RNA synthesis, it inhibits replication in hepatitis B virus, hepatitis C virus (HCV), HIV virus, influenza A virus, West Nile virus, Rift Valley fever virus, and Zika virus [50]. In HCV, clinical trials have shown that even non-immunosuppressive derivatives of CsA still can potently suppress HCV viral load in patients [51,52].

(c) Indirect Effect of CsA in Cardiovascular Complications of COVID?

CypA might be interesting for the understanding and possibly treatment of cardiovascular morbidity in COVID-19. CypA is also a growth factor for vascular smooth muscle cell (VSMC) under oxidative stress [45] and in this way plays a crucial role in cardiovascular disease. Whether this mechanism is relevant in COVID-19, and whether CsA could play a protective role in this context, is currently unknown. Nevertheless, it would be interesting to investigate CypA as a biomarker in cardiovascular complications of COVID-19. Alternatively, it might explain the elevated risk of COVID-19 patients for cardiovascular comorbidity, since these patients have elevated reactive oxygen species (ROS), which induce secretion of CypA from VSMC.

(d) Direct Antifibrotic Effect of CsA

Pulmonary fibrosis is one of the major complications in COVID-19 patients [38], with acute respiratory distress syndrome (ARDS) being the main cause of post-COVID pulmonary fibrosis. Similar cytokine profiles in idiopathic pulmonary fibrosis (IPF) and COVID-19 suggest analogous pathomechanisms in both diseases. Interestingly, cytokine overexpression in IPF, COVID-19 and SARS/MERS all show elevated IL-1B, IL-6, MCP1, TNF-α and TGF-β [38]. Therefore, drugs useful in the treatment of IPF could also be beneficial for COVID-19 patients [38]. CsA might have a direct antifibrotic action, as has been demonstrated in patients with antisynthetase syndrome associated interstitial lung disease, who were refractory to corticosteroids, but improved on CsA [53,54]. In tacrolimus, the combination with methylprednisolone pulse therapy showed to mitigate acute exacerbations (AE) of IPF, prevented re-AE IPF, and contributed to a better prognosis compared to steroid monotherapy [55]. Studies have shown that CsA is superior to the corticosteroid monotherapy in terms of prognosis for IPF [56,57].

This feature of CsA may be an important aspect to consider when attempting to prevent post-COVID-19 pulmonary fibrosis, although further studies are still needed to elucidate the magnitude of the effect.

4.7. Mechanism of Action of Tacrolimus

Tacrolimus (FK506) is another important calcineurin inhibitor, known from transplant medicine. In contrast to CsA, which binds to the immunophilin Cyp, tacrolimus binds to the immunophilin called FK-506 binding protein (FKBP12) (Figure 1). Due to tacrolimus, the FKBP forms a complex with the calcium-dependent phosphatase named calcineurin and inhibits the activity of calcineurin. Similar to CsA, tacrolimus has also been shown to have a favorable activity as antifibrotic agent, for example in patients with idiopathic inflammatory myopathy or antisynthetase syndrome- interstitial lung disease (ILD) [53,58]. Interestingly, in animal experiments, upregulated FK506-binding protein 10 (FKBP10) in bleomycin-induced lung fibrosis and IPF improved with knockdown of FKBP10, attenuating collagen secretion [59]. Although no firm conclusions can be drawn yet, tacrolimus also might have an anti-fibrotic activity in COVID-19.

Although clinical trials are still awaited, preliminary clinical experience reports suggest that tacrolimus is protective for liver transplant recipients, but so far not for other organs, for example kidneys. Concerning this observation in tacrolimus, an intriguing question is, if tacrolimus has a different degree of protection against CSS effects in various organs. In other words, is there possibly a specific organ effect, beyond the pharmacokinetics? The various cytokines may play different roles in solid organs. The most important cytokines resulting in CSS in the liver, may be different from the cytokines that are the most important ones in the kidney or lung. This is however still speculative. Nevertheless, in other (non-coronaviral) diseases, the different CNIs (CsA and tacrolimus), when compared to each other, were shown to be discordant in respect to the antiviral effects. This could be the result of the different cytokine profiles addressed by the different CNIs, leading to protection of different organs. Moreover, ACE2 is abundantly present in the lung epithelial cells, and CNIs only have antiviral effects after infection of the target cells, when the virus replication starts in the

cytoplasm. Different ACE2 density could contribute to differences in organ protection by CNIs, called the immunolocalization of ACE2. Another study suggested a different receptor repertoire potentially involved in the SARS-CoV-2 infection at the epithelial barriers and in the immune cells [60]. As mentioned before, the understanding of the pathogenesis is still incomplete.

4.8. Alternative Drugs to Inhibit the Cytokine Storm Syndrome

Among alternative drugs that may have the ability to inhibit the systemic hyperinflammation in COVID-19 are IL-6 blockers (Tocilizumab, Sarilumab), IL-1 blocker (Anakinra, Canakinumab), heparins (low molecular weight and unfractionated heparin), intravenous immunoglobulins (IVIG), hyperimmune immunoglobulins (neutralizing antibodies), JAK inhibitors (Ruloxitinib, Bariticinib), corticosteroids (methylprednisolone, dexamethasone), statins and recombinant human angiotensin-converting enzyme 2 (rh ACE2). These are discussed elsewhere [61].

Interestingly, there is another CypA inhibiting drug, without immunosuppressive activity, named alisporivir [16,62,63]. It is a non-immunosuppressive analogue of CsA with strong Cyps inhibition properties. Alisporivir has reached phase three clinical development for the treatment of COVID-19 [63].

Preclinical data show strong antiviral and cytoprotective properties of alisporivir in various models of coronavirus infection, including SARS-CoV-1, MERS-CoV and SARS-CoV-2 [63]. Nevertheless, an important question remains if alisporivir can also inhibit the cytokine storm syndrome, as it has no immunosuppression activity.

5. Conclusions

Calcineurin inhibitors have been proven to be effective in a number of coronaviral diseases and other related conditions. The most important CNIs used in the clinical context are cyclosporin and tacrolimus. They block the calcineurin pathway by forming complexes with immunophilins, being cyclophilin for cyclosporine A, and FKB12 for tacrolimus. These immunophilins prevent calcineurin from dephosphorylating the NF-AT transcription factor. This results in the inhibition of the transcription of genes encoding for cytokines, decreasing the risk of CSS.

Paradoxically, the CNIs that are crucial to solid organ transplantation and render SOT recipients more susceptible to opportunistic infections, appear to also have the ability to suppress the cytokine storm syndrome in COVID-19. In this regard it would not be rational to follow the guidelines of the Massachusetts General Hospital (Boston, MA, USA), which advises to consider decreasing tacrolimus/cyclosporin by 50% in solid organ transplant recipients with COVID-19, and in critical illness in liver and kidney transplant recipients to stop all immunosuppression except for prednisolone.

In the search of effective treatment options for the novel coronavirus SARS-CoV-2, CNIs should be evaluated as a first line treatment option because of the suggested direct antiviral effects as and its potential to suppress the severe systemic hyperinflammation state and thus reduce the disease severity of COVID-19 [64]. Based on the known CNI effects in various coronaviral diseases, they are likely to be effective in multiple coronaviral serotypes (including SARS-Cov-2) and, as multitarget agents, may more effectively reduce the likelihood of developing viral resistance as compared to other strategies. If CNIs can be proven to be effective also for previously immunocompetent patients with moderate to severe COVID-19, then they may be an easy and affordable option for the rapid management of the COVID-19 patients in many parts of the world, since these drugs are affordable and already quite widely available.

6. Outlook

Given the unique mechanism in mitigating the cytokine storm syndrome, the calcineurin/NF-AT signaling pathway presents an attractive target for therapeutic drug development for prevention of severe COVID-19. Currently the research of CNI for SARS-CoV-2 infection and prevention of severe COVID-19 disease is still limited. Therefore, the results of the clinical TACROVID trial from

Barcelona, Spain are urgently awaited and currently pending. This trial investigates the clinically important question of tacrolimus in patients with COVID-19, with in one arm treating patients with methylprednisolone pulses 120 mg/day for three consecutive days (if they were not previously administered) with tacrolimus at the necessary dose to achieve plasma levels of 8–10 ng/mL, versus the other arm with usual care including all necessary treatments with the exception of CNIs [65].

Recruitment has also started for the study in which cyclosporine is clinically tested in patients with COVID-19 requiring oxygen supplementation but not requiring ventilator support [60]. This trial is a phase 1 safety study to determine the tolerability, clinical effects, and changes in laboratory parameters of short course oral or IV cyclosporine administration [66].

Major questions that remain open should be addressed in research, and the TACROVID trial, the American cyclosporine study (and probably an alispirovir trial in the near feature) will likely shed more light on this issue [65,66]. In our opinion, clinically relevant questions comprise those mentioned in Table 2.

Table 2. Proposed research questions on treatment with calcineurin inhibitors.

Research Question	Possible Answers in Literature	Refs.
Which patients with COVID-19 could benefit from the addition of CNI to the standard therapy	• The inclusion criteria of the TACROVID trial could be helpful. They include COVID-19 infection confirmed by PCR, new onset radiological infiltrates, respiratory failure (PaO2/FiO2 < 300 or satO2/FiO2 < 220), C-reactive Protein > 100 mg/L and/or D-Dimer > 1000 µg/L and/ or Ferritin > 1000 ug/L.	[65]
Does CypA play a role in cardiovascular morbidity in COVID-19 patients?	• Could CypA be a marker for cardiovascular morbidity in COVID-19 patients?	[45]
How to screen for patients with a high risk of progression to more severe stages of COVID-19 and thus merit pharmacological interventions	• Several scoring systems are available, such as the AIFELL score, which includes an altered sense of smell/taste, inflammation (C-reactive protein ≥ 30 mg/L), radiological infiltrates, fever (≥38.0 °C), elevated lactate dehydrogenase (LDH) levels (>400 U/L) and lymphocytopenia (absolute count < 1.45 G/L). The score is calculated by counting the number of criteria met at initial presentation in the emergency room, whereas each criterion equals one point (Score range 0 to 6 points).	[67]
Which patients with COVID-19 should be excluded from CNIs?	• life expectancy ≤ 24 h, glomerular filtration ≤ 30 mL/min/1.73 m^2, leukopenia ≤ 4000 cells/µL. (exclusion criteria in TACROVID trial)	[65]
CNI monotherapy or combination therapy with either a corticosteroid, an antimetabolite (Mycophenolate)	• dexamethasone led to a lower 28-day mortality among those who were receiving either invasive mechanical ventilation or oxygen alone at randomization but not among those receiving no respiratory support. Would it also improve the effect of CNIs?	[68]
Alternative immunomodulatory drugs?	• Rapamycin (m-TOR inhibitor)? Probably yes • Rapamycin (m-TOR inhibitor)? No • Many other immunomodulatory drugs are reviewed elsewhere	[69] [70] [71]
Alisporivir as non-immunosuppressive cyclophilin inhibitor?	• Inhibition (in vitro) of SARS-CoV-2 in literature • However, when not immunosuppressive, does it protect against cytokine storm? Or only protection against the cytopathic effect?	[62,63]

From a general point of view there is a vast experience with CNI in transplantation medicine including dosing regimens and experience with achieving specific drug levels by therapeutic drug monitoring. This experience can be beneficial when, within a short time frame, a CNI-based immunosuppression should be established, which also takes into account comorbidity (renal function, other medication/interactions). Which target drug levels should be used is another open question. Based on the experience with SOT recipients, similar drug levels should probably be targeted as for maintenance of immunosuppression in such patients. Whether in certain situations augmentation of immunosuppression may be wise as a second step would have to be evaluated as well. In addition to the immunosuppressive strategy the most appropriate marker for disease activity measurement in these COVID-19 patients will have to be determined in the context of the immunosuppressive therapy (CRP, Procalcitonin (PCT), certain Interleukins, differential white blood cell count, etc). Besides dose and monitoring of the immunosuppression, the duration of continuation of this therapy will have to be evaluated in addition to the effects on viral load and potentially observed prolonged viral shedding. The best evidence is probably derived from dual or triple immunosuppressive regimens in SOT, therefore the combination of two immunosuppressive drugs which certainly includes a CNI because of its pleiotropic effects (including antiviral effects) is likely to be a promising pharmacological strategy to prevent severe COVID-19. As a potential predictor of severe disease course, the AIFELL

score may be considered [67]. The triage score relies on disease markers that at an early stage indicate whether a more severe disease progression may be expected.

Although there remain many open questions, CNI should be investigated as a first treatment option, based on evidence of direct antiviral effects and its properties preventing CSS, as has been observed in COVID-19 with predominantly immunopathological changes of the respiratory tract.

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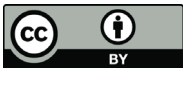

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Review

COVID-19 in Patients with Solid Organ Transplantation: A Systematic Review

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Abstract: The novel coronavirus, SARS-CoV-2, is causing a pandemic of unknown precedent, with huge healthcare challenges and worldwide disruptions to economic and social life. Lung transplant recipients and other solid organ transplant (SOT) recipients are immunosuppressed, and therefore are generally considered at an increased risk for severe infections. Given the current gap in knowledge and evidence regarding the best management of these patients, we conducted a systematic review of studies on SARS-CoV-2 infections and Coronavirus Disease 2019 (COVID-19) in SOT recipients, to evaluate the association between immunosuppression in these patients, SARS-CoV-2 infection and COVID-19 outcomes. The focus was the severity of the disease, the need for mechanical ventilation and intensive care unit (ICU) admissions, and rate of death. The literature search was conducted repeatedly between 16 March and 8 April 2020. We searched original papers, observational studies, case reports, and meta-analyses published between 2019 and 2020 using two databases (PubMed, Google Scholar) with the search terms: [transplant OR immunosuppression] AND [COVID-19 OR SARS-CoV-2]. Further inclusion criteria were publications in English, French, German and Italian, and reference to humans. We also searched the reference lists of the studies encountered. From an initial search of PubMed and Google Scholar, 19 potential articles were retrieved, of which 14 were excluded after full-text screening (not being case reports or case series), leaving 5 studies for inclusion. No further studies were identified from the bibliographies of retrieved articles. Based on the limited research, no firm conclusions can be made concerning SOT recipients, but the current evidence suggests that immunosuppression is most likely associated with a better outcome of SARS-CoV-2 infection and COVID-19 because it prevents hyperinflammation (cytokine storm) in this particular population. There is a need for further research that would allow results to be adjusted for other factors potentially impacting COVID-19 severity and outcome.

Keywords: SARS-CoV-2; coronavirus; immunosuppression; tacrolimus; corticosteroids; mycophenolate mofetil; hyperinflammation; cytokine storm; pandemic; transplantation

1. Introduction

The emergence of the novel coronavirus, which started in the last quarter of 2019 in Wuhan (China), and its rapid spread around the world, have caused a pandemic of global concern and impact [1]. The virus was first termed 2019-nCOV, and the International Committee on Taxonomy of Viruses subsequently named it "Severe Acute Respiratory Syndrome Coronavirus 2" (SARS-CoV-2).

The novel coronavirus has a presumptive zoonotic origin [2]. According to the Emergency Committee of the World Health Organization (WHO), the 2019-nCOV was declared a public health

emergency of international concern (PHEIC) on 30 January 2020 [3]. The WHO named the disease caused by SARS-CoV-2 "COVID-19". As yet, effective treatment against SARS-CoV-2 is absent.

1.1. Known Coronaviruses with Fairly Benign Outcomes

Coronaviruses infecting humans are not new. The wide range of possible hosts includes birds, pets, bats, farm animals and camels. Currently there are seven coronavirus species causing disease in humans. In four of these, called 229E, OC43, NL63 and HKU1, respiratory symptoms predominantly consist of self-limiting common cold symptoms, causing a respiratory or gastrointestinal disease. Infections with the strain 229E can be associated with fever and cough in 10−20% of cases. The illness usually lasts between 2 and 18 days [2]. Patients affected by the strain OC43 have the same symptoms as those affected by the 229E strain. Infections with NL63, a strain known since 2004 and initially described in the Netherlands, cause typically mild symptoms, whereby it primarily is observed in young children, elderly and immunocompromised patients with prior respiratory illnesses. In children it can also cause obstructive laryngitis (croup) [4]. However, a subtype of NL63 has been associated with severe lower respiratory tract infection in hospitalized children in China [5]. The HKU1 strain was discovered in 2005 in Hong Kong, causing relatively mild respiratory symptoms in children, but it is also associated with a high incidence of seizures and has also been found in a patient with meningitis [4,6,7]. In contrast to the benign outcomes in the general population, in lung transplant recipients these viruses can cause acute febrile illnesses, and may even persist for up to several months in some individuals, making concurrent infection with another virus difficult to interpret [8]. In lung transplant recipients, a viral respiratory tract infection (VRTI) is associated with chronic lung allograft dysfunction (CLAD). In the first year after lung transplantation, coronavirus in particular is associated with increased risk of CLAD development [9]. In a study by Magnusson, in a total of 125 lung transplant recipients with VRTI, 19.2% (n = 21) had a coronavirus infection. The coronavirus subspecies were OC43 in 7.2% (n = 9), 229E in 5.6% (n = 7), NL63 in 3.2% (n = 4) and HKU1 in 0.8% (n = 1) [9]. Another study showed that coronaviruses have an important role among patients with underlying conditions and in transplanted patients [10]. In healthy children, human coronaviruses were detected in 3.3% (n = 11), in healthy adults in 12% (n = 6), in health care workers in 12.8% (n = 86), in patients after renal transplantation in 20.3% (n = 30), in children with heart diseases in 24.7% (n = 44) and in patients after stem cell transplantation in 24.3% (n = 44) [10].

1.2. The Highly Pathogenic Coronaviruses

The other three coronavirus species are zoonotic in origin and have been associated with severe, life-threatening respiratory disease outbreaks. The first was Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), leading to an outbreak in 2002 and 2003 in Guangdong Province (China). It was initiated by a zoonotic transmission (likely from bats via palm civets), and infected 8098 people, leading to an overall case fatality rate of 11% [11]. This was followed by the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) outbreaks in 2012 (Saudi Arabia) and 2015 (South Korea), probably originating from bats via dromedary camels. Unlike SARS, the infection with MERS-CoV is generally mild in healthy individuals, but very severe in patients with underlying comorbidities, such as chronic lung diseases, diabetes, renal failure and a weakened immune system. It infected 2994 people, with a case fatality rate of 34% [12]. The third zoonotic coronavirus is the recent 2019 novel coronavirus SARS-CoV-2, which originated in Wuhan (South China). The recent outbreak of the SARS-CoV-2 has been linked to the Huanan Seafood Wholesale Market in Wuhan. This market sold a variety of both live and dead animals of wild and domesticated origin in over one thousand stalls. There is some debate about whether this market is the true origin of the outbreak, but it certainly was one area of early transmission in the 2020 pandemic.

1.3. Route of Transmission

Based on our current knowledge, spreading of the SARS-CoV-2 occurs from person to person via respiratory droplets (defined as particles > 5 µm). Risk factors are close contact (≤2 m), especially over a prolonged time (generally considered to be >15 min), and direct contact with infectious secretions like sputum or blood [13]. A fecal−oral transmission appears likely but has not been proven yet [14]. Not only can SARS-CoV-2 be found in feces, but also stool samples can remain positive even when samples from the respiratory tract have become negative [15].

The gastrointestinal symptoms in some patients with COVID-19 may be explained by the extended persistence and shedding in the gastrointestinal tract.

Infection with SARS-CoV-2 can lead to a disease called COVID-19 that predominantly affects the lungs. The impact of COVID-19 in immunocompromised patients after solid organ transplantation (SOT) is largely unknown, as only a small number of such infections have occurred so far, and detailed reports are still awaited.

1.4. Severity Stages of COVID-19

COVID-19 can have various stages of severity. Siddiqi et al. proposed three stages of COVID-19 severity [16] (Figure 1):

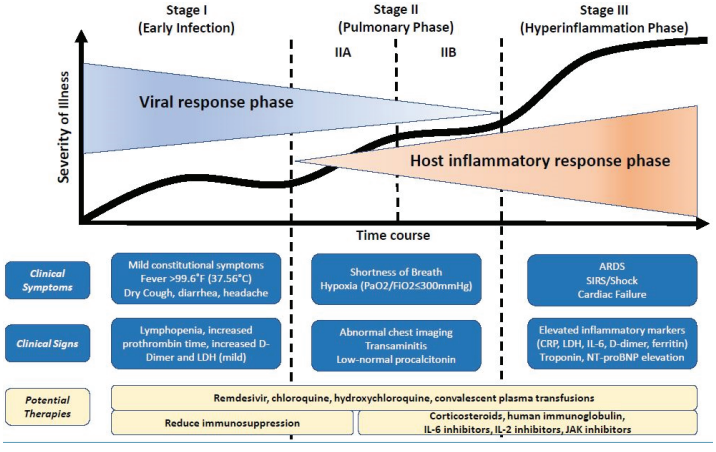

Figure 1. Classification of COVID-19 Disease States and Potential Therapeutic Targets. Legend: The figure shows three escalating phases of disease progression with COVID-19, with associated signs, symptoms and potential phase-specific therapies. ARDS = Acute respiratory distress syndrome; CRP = C-reactive protein; IL = Interleukin; JAK = Janus Kinase; LDH = Lactate Dehydrogenase; SIRS = Systemic inflammatory response syndrome. (From: Siddiqi HK, Mehra MR. COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal. *J Heart Lung Transplant*. 2020 [in press], DOI:https://doi.org/10.1016/j.healun.2020.03.012).

Stage I (early infection) can include mild constitutional symptoms—fever, dry cough, diarrhea, headache—with laboratory examination revealing lymphocytopenia, increased prothrombin time, increased D-dimer and mild Lactate Dehydrogenase (LDH) elevation.

Stage II (pulmonary phase) can be subdivided into IIa (without hypoxia) and IIb (with hypoxia, defined as a PaO_2/FiO_2 of < 300 mmHg). In Stage II disease, patients develop a viral pneumonia, with cough, fever and possibly hypoxia. Radiologic imaging shows bilateral infiltrates or ground-glass opacities. Laboratory tests reveal increasing lymphocytopenia, along with elevated transaminases. At this stage, most patients with COVID-19 should be hospitalized.

Stage III (systemic hyperinflammation) is characterized by Acute Respiratory Distress Syndrome (ARDS), Systemic Inflammatory Response Syndrome (SIRS)/shock, and/or cardiac failure. In the laboratory examination there are elevated inflammatory markers (C-reactive Protein, LDH, Interleukin-6, D-dimer, ferritin), and an elevation of troponin and N-Terminal-pro-Brain Natriuretic Peptide (NT-proBNP).

1.5. Diagnosis

According to the guidelines of the International Society of Heart and Lung Transplantation (ISHLT) concerning patients with cardiothoracic transplant, routine testing of asymptomatic patients is not recommended [17]. An asymptomatic patient who has been in contact with a confirmed case of COVID-19 should be advised to undergo home quarantine for 2 weeks, and testing for SARS-CoV-2 is only indicated if symptoms occur (or otherwise as per local public health guidelines). Testing for SARS-CoV-2 in patients with symptoms of COVID-19 (fever, cough, headaches, myalgia, fatigue, nasal congestion, sudden anosmia, diarrhea, etc.) should be treated like any other patient considered at increased risk of developing severe disease, as per local guidelines [17].

A real-time polymerase chain reaction (RT-PCR), or sequencing of respiratory or blood samples using "primers" based on the viral RNA sequence, indicates whether a person is currently infected. In a study on detection of SARS-CoV-2 in different types of specimens from 205 patients, the virus was detected in 93% (n = 14) of patients where bronchoalveolar lavage fluid was sampled, in 72% (n = 75) of sputum samples, in 63% (n = 5) of nasal swabs, in 46% (n = 6) of bronchoscopic brush biopsies, and in 32% (n = 126) of pharyngeal swabs. Further, in feces specimens, the virus was detected in 29% of cases (n = 44). In blood, SARS-CoV-2 could be detected in only 1% (n = 3), whereas in none of the patients could the virus be detected in urine [18]. Blood samples should be stored for subsequent analysis, for example for antibody testing. Recently, in collaboration between the U.S. Department of Health and Human Services, the U.S. Department of Defense, and the company Cepheid, a new COVID-19 molecular diagnostic test, allowing SARS-CoV-2 detection within 45 min, has been approved by the U.S. Food and Drug Administration (FDA) for point-of-care detection in emergency use for COVID-19. A further benefit of this rapid diagnostic test is that it only requires one minute hands-on time to perform it, reducing the exposure time of the laboratory personnel to potentially virus-containing samples. However, the question remains unanswered as to how this test compares to the widely used RT-PCR test. There have been concerns related to potential false negative results.

Serologic diagnosis by detection of specific antibodies (immunoglobulin M, immunoglobulin G) is currently being introduced. Timelines for the appearance and persistence of these immunoglobulins are currently not well established.

In this paper, we review COVID-19 in solid organ transplant (SOT) recipients, most of whom are under long-term dual- or triple-drug immunosuppressive therapy.

2. Methods

The literature search was conducted repeatedly between 16 March and 8 April 2020. We searched original papers, observational studies, case reports and meta-analyses published between 2019 and 2020, using two databases (PubMed, Google Scholar) with the search terms: [transplant OR immunosuppression] AND [COVID-19 OR SARS-CoV-2]. Further inclusion criteria were publications in English, French, German and Italian, and reference to humans. We also searched the reference lists of the studies encountered.

3. Results

From an initial search of PubMed and Google Scholar, 19 potential articles were retrieved, of which 14 were excluded after full-text screening (not being case reports or case series), leaving 5 studies for inclusion. No further studies were identified from the bibliographies of retrieved articles.

Table 1. Main references of original papers and case reports (excluding reviews, meta-analyses or commentaries).

Author, Ref. and Date	Solid Organ Transplanted, Year of Transplant	Age (y) Sex (m/f)	IS Rx	Symptoms	Temp SpO_2 CRP CT Chest	Severity Stage (Siddiqi)	Treatment	Outcome
Steinack et al. [19]	Lung, 2019	55 f	Tac MMF Pred	Nausea Vomiting Diarrhea Dry cough Rhinorrhea	38.9 96% 77 mg/L 3 nodular lesions	IIA	piperacillin/tazobactam iv	survived hospitalzation 12 days
Li et al. [20]	Heart, 2003	51 m	Tac MMF	intermittent fever chills fatigue poor appetite diarrhea	38.5 99%–> 75% 18.6 mg/L GGO (bilat.)	IIA –> IIB	levofloxacin iv ribavirin iv moxifloxacin iv ganciclovir iv IVIG methylprednisolone iv moxifloxacin po umifenovir Tac and MMF stopped from day 7–13	survived, hospitalization 27 days, no mechanical ventilation or ECMO, CT at discharge improved (residual lesions).
Li et al. [20]	Heart, 2017	43 m	Tac MMF	fever (2 days) fatigue poor appetite	38.5 normal SpO_2? 13.4 mg/L GGO (bilat.)	IIA	ceftriaxone iv ganciclovir iv moxifloxacin po umifenovir	survived, hospitalization 11 days, no mechanical ventilation or ECMO
Zhu L et al. [21]	Kidney, 2008	52 m	Tac MMF Pred	fatigue dyspnea chest pain tightness nausea loss of appetite abdominal pain dry cough fever headache	38.9 96% 30.2 mg/L GGO (bilat.)	IIA	umifenovir methylprednisolone interferon-α inh. IVIG Biapenem iv pantoprazole Tac stopped from day 6–11, reintroduced with 50% reduction for 7 days, followed by normal doses MMF stopped from day 6–11, reintroduced by normal dose after 12 days pred stopped during hospitalization	survived, hospitalization 12 days, no mechanical ventilation or ECMO

Table 1. *Cont.*

Author, Ref. and Date	Solid Organ Transplanted, Year of Transplant	Age (y) Sex (m/f)	IS Rx	Symptoms	Temp SpO_2 CRP CT Chest	Severity Stage (Siddiqi)	Treatment	Outcome
Guillen E et al. [22]	Kidney, 2016	50 m	Tac Everol Pred	fever vomiting	37.4 98% 13.2 mL/L unilateral –> bilat. infiltrate	IIA–> IIB	ceftriaxone azithromycin lopinavir/ritonavir HCQ TAC and everol temporarily stopped due to interactions interferon-β intubation and MV	
Qin J et al. [23]	Liver, 2020	37 m	Tac Pred	unknown	Temp, SpO_2 and CRP unknown GGO	IIB	Tac and Pred maintained, gradually titrated to lower doses HFOT Oseltamivir rh-GCSF IVIG	survived, hospitalization 2 months (including liver transplantation)
Gandolfini I, et al. [24]	Kidney, 2010	75 m	Tac Pred MMF	Fever Cough Myalgia Dyspnea	38.0 SpO_2 and CRP unknown GGO	IIB	NIV HCQ Lopinavir-ritonavir or darunavir-cobicistat	died
Gandolfini I, et al. [24]	Kidney, 2019	52 f	Tac Pred MMF	Fever Cough Myalgia Dyspnea	39.0 SpO_2 and CRP unknown GGO	IIB	NIV HCQ Lopinavir-ritonavir or darunavir-cobicistat Colchicine	alive

3.1. Lung Transplant Recipients

Steinack et al. reported a 55-year-old woman who underwent a bilateral lung transplantation 5 months prior to infection. The lady was under therapy with tacrolimus, mycophenolate mofefil and prednisolone (Table 1) [19]. The patient presented with gastrointestinal symptoms (nausea, vomiting, diarrhea) and only minor respiratory symptoms (dry cough and rhinorrhea). Initially, she had fever and normal oxygen saturation breathing room air. Stool specimens detected a Norovirus infection, and virus PCR testing of the nasal swab returned positive for SARS-CoV-2. There were only minimal consolidations on chest computed tomography (CT) imaging, without any associated ground-glass opacities. She recovered on empiric intravenous antibiotic treatment without the use of additional antiviral agents, whilst continuing preexisting Cytomegalovirus (CMV)-prophylaxis with valganciclovir. There were no signs of allograft dysfunction in the 6-week follow-up.

3.2. Renal Transplant Recipients

There are three case reports describing a COVID-19 with stages IIA and IIB (Table 1) [21,22,24]. In the case report by Guillen et al., the first patient was a 50-year-old man under tacrolimus, everolimus and prednisone therapy [22]. He presented to the hospital with fever and vomiting, without other symptoms. After 5 days, the patient, who initially was sent home, returned to the emergency department with persistent fever and cough, but without gastrointestinal symptoms. At that time, he was afebrile and had a normal oxygen saturation. Because of a unilateral infiltrate on chest X-ray (CXR), a community-acquired pneumonia was considered. However, he tested positive on naso- and oropharyngeal swabs for SARS-CoV-2. He was treated with lopinavir/ritonavir, but worsened clinically with disease progression on CXR showing bilateral infiltrates, requiring intubation with mechanical ventilation. The final outcome of the patient has not yet been communicated.

Zhu et al. [21] described a 52-year-old man on immunosuppressive therapy with tacrolimus, mycophenolate mofetil, and prednisone. He presented with fatigue, dyspnea, chest tightness, chest pain, nausea, loss of appetite, intermittent abdominal pain and occasional dry cough. He developed fever and the chest CT showed bilateral ground-glass opacities, suggesting the presence of COVID-19 pneumonia. Immunosuppression was completely stopped, and treatment with methylprednisolone (40 mg daily, intravenously), intravenous immunoglobulins (5 g on the first day and 10 g/day for the next 11 days), biapenem, pantoprazole, and Interferon (IFN)-α (5 million units daily by atomization inhalation) was started. A follow-up chest CT showed massive improvement later, and the patient was finally discharged from hospital.

Gandolfini et al. [24] described two stage IIB COVID-19 renal transplant cases requiring non-invasive ventilation. Both patients were on tacrolimus, steroids and mycophenolate mofetil, presenting with fever and dyspnea on admission, with CT showing bilateral ground-glass opacities. The first patient developed abrupt worsening of his respiratory conditions and died 5 days after admission to the hospital, before he could be intubated. The second patient was stabilized and treated with colchicine after initially receiving retroviral therapy and hydroxychloroquine.

3.3. Liver Transplant Recipients

In liver transplant recipients, only one case report with COVID-19 stage IIB has been published (Table 1) [23]. A 37-year-old man underwent a liver transplant 3 months previously. He was under immunosuppressive therapy with tacrolimus and glucocorticoids. Two days after transplantation, he had a persistent fever and chest CT showed a minor pleural effusion. His sputum showed gram-positive cocci and gram-negative bacilli. After 9 days, his chest CT was repeated and, due to the COVID-19 outbreak, he was sampled for SARS-CoV-2 and found positive. He required high-flow oxygen therapy, and additionally tacrolimus and glucocorticoids were gradually titrated to lower doses. After 24 days, his fever subsided, and tacrolimus was increased due to acute cellular rejection. The patient was discharged without any signs of multisystem organ failure during hospitalization.

3.4. Heart Transplant Recipients

Recently, two heart transplant recipients from China with COVID-19 have been reported: one patient with a moderately severe (stage IIB) and another with a mild (stage IIA) presentation (Table 1) [20]. The first patient was a 51-year-old man on maintenance immunosuppressive therapy with tacrolimus and mycophenolate mofetil. He initially presented with fever, chills, fatigue and poor appetite, as well as diarrhea. He had a normal oxygen saturation initially, but then his clinical condition worsened, and his saturation decreased to 75% without supplemental oxygen. This was improved after giving oxygen via a face-mask. He was treated with intravenous human gammaglobulin (10 g/day) and methylprednisolone (80 mg/day) for 5 days, while immunosuppression was stopped. The initial ground-glass opacities in the chest CT showed significant improvement after therapy, and the patient was discharged from the hospital.

The second patient was a 43-year-old man with tacrolimus and mycophenolate mofetil maintenance immunosuppression, who was admitted having suffered from fever for 2 days with fairly discrete lung lesions on his chest CT (stage IIA). His clinical situation deteriorated, and he suffered from severe fatigue and poor appetite. There were no further complications and he could be discharged from the hospital.

3.5. Consequences for the Pre- and Post-Transplantation Practice

3.5.1. What Is New in the COVID-19 Pandemic?

Viral diseases in the past have motivated researchers to generate algorithms for donor screening, in order to prevent the use of organs from potentially infected donors, and also to improve recipient management, in order to reduce the chances of viral transmission and disease among recipients [25]. Some of the emerging viruses in the past (SARS-CoV, MERS, etc.) were only limited to a certain geographic area, thus not severely hampering the transplantation/donation procedure as a whole. The current COVID-19 pandemic is of unprecedented magnitude. The virus is highly contagious, crossing borders all over the world. There are over 3,500,000 confirmed cases and over 245,000 deaths, affecting 206 countries [26], and probably many more undiagnosed people with COVID-19. Unfortunately, the widespread occurrence of the virus has a great impact on SOT, requiring preventive and possibly therapeutic measures.

3.5.2. Restrictions Concerning Donors, Recipients and Transplantation Centers

Not only does the pandemic restrict the number of potential organs available due to infected donors, but it may also affect recipients on the waiting list or just before transplantation. Donor screening for the presence of the SARS-CoV-2 virus or evidence of disease (COVID-19) is highly recommended, which may lead to possible delays in organ procurement and organ transplantation, depending on testing availabilities. In addition, the large number of COVID-19 patients requiring specialist care, including intensive care unit (ICU) resources, certainly competes with the efforts to transplant severely ill patients in order to enable survival and increase quality of life. The pandemic is therefore restricting the capacity for transplantation in many hospitals. This is also due to the transformation of many general or specialized intensive care units (ICUs) into specialized COVID-19 ICUs with strict isolation measures, and also due to shortages of health care workers relating to COVID-19 care requirements. In addition to the scarcity of ventilator capacity in ICUs, many hospitals have shut down their routine outpatient checkups in order to prevent further spread of the infection, resulting in impaired or absent capacity for evaluating patients for possible SOT. These factors will decrease both the number of potential donors and SOT recipients all over the world. On the other hand, there may be hospitals still evaluating candidates and performing transplantation procedures, thanks to sufficient ICU bed availability. Depending on the resources available, the waiting list mortality may suffer under these circumstances. In these centers, donor organ procurement and transplantation can

possibly be increased, when other centers decide to shut down their SOT programs of solid organ transplantations due to the requirements for COVID-19 care.

3.5.3. Shutdown in Phases: Different Consequences for Different Organs

A phased approach to new transplant activity during the COVID-19 pandemic has been proposed by Kumar et al. [25]. In this article, a reduction of 25%, 50% or 75% in transplant activity depends on the risk tolerance, hospital capacity and degree of virus activity in the jurisdiction [25]. This has different consequences for each type of organ. For example, a 25% reduction in transplant activity corresponds to priority level "elective", which means that there will be no living donor kidney transplantation, but non-urgent lung transplantation activity will be continued. A 100% reduction of the health system occurs if facilities are overwhelmed with COVID-19 patients, with no ICU capacity. In that situation, severe shortages of health care personnel lead to a halt of all living and deceased donor transplant activity.

The same authors also propose a classification of 25%, 50%, 75% and 100% reduction in ambulatory transplant checkups, with the corresponding levels of medical service at the transplant center.

3.5.4. Risks for Recipients

To the best of our knowledge, until now there have been no cases of donor-to-recipient transmission of SARS-CoV-2. On the other hand, recipients run the risk of nosocomial SARS-CoV-2 infections during the pandemic. Asymptomatic patients infected with SARS-CoV-2 can spread the virus. It is currently unknown whether asymptomatic individuals are only asymptomatic initially after contracting the infection, or if they remain asymptomatic throughout the course of the SARS-CoV-2 infection. In spite of the asymptomatic carrier state, these individuals may transmit the virus, although the exact mechanism of acquiring and transmitting the virus requires further study [27,28]. Even in a convalescent patient, a high sputum viral load has been demonstrated, raising concerns about prolonged viral shedding of SARS-CoV-2 after recovery [28].

3.5.5. Risks for Health Care Workers in General

In Italy, health care workers with COVID-19 were reported to be 8.9% of all COVID-19 patients (2026 of 22512 people, respectively). In comparison, the 2002–2003 SARS epidemic led to 8422 probable cases, with 916 deaths in 29 countries, affecting health care workers in approximately 30% of all SARS infections. As has been demonstrated for SARS, peak viral loads were reached at 12–14 days of illness, when patients were probably hospitalized, explaining the relatively high number (n = 174, 17%) of health care workers testing positive for the SARS virus. Another aspect that merits more attention is the huge amount of psychological stress among medical and paramedical team members, associated with risks of burnout, insomnia, anxiety, distress, depression or post-traumatic stress disorder (PTSD) [29,30]. This aspect, however, is not the focus of this review, also due to the currently limited data relating to this issue.

3.5.6. Health Care Workers with Pregnancy

The elevated risk of COVID-19 in health care workers may also involve pregnant transplant team members. Data on pregnant COVID-19 patients are very limited. The clinical presentation in pregnant women was similar to those reported for non-pregnant adult patients who developed COVID-19 pneumonia [25]. Currently, there is no evidence for intrauterine infection caused by vertical transmission in women who develop COVID-19 pneumonia in late pregnancy [31]. Another study with 15 pregnant patients with COVID-19 pneumonia showed no worse clinical outcome in terms of CT imaging features of COVID-19 pneumonia. Nevertheless, these patients had only a mild type of COVID-19 pneumonia. There was no neonatal asphyxia, neonatal death, stillbirth or abortion, but 4/15 patients still were pregnant at the end of the study, and the final outcome of this population

has not been reported yet [32]. However, it is noteworthy that the maternal immune system in early pregnancy is very sensitive, and for the fetus this is an important stage of organ development [33].

In the H1N1 2009 influenza viral infections, the SARS outbreak in 2003, and the MERS outbreak in 2012, there were high incidences of maternal and infant complications, such as spontaneous abortion, premature delivery, intrauterine growth retardation, tracheal intubation, admission to intensive care unit, renal failure and disseminated intravascular coagulation (DIC) [33–36]. In the SARS outbreak, 57% of the women during the first trimester had spontaneous abortions, likely a result of the hypoxia during SARS-related acute respiratory distress [36].

In a case-control study to determine the effects of SARS on pregnancy, comparing ten hospitalized pregnant and 40 hospitalized non-pregnant women with the SARS infection in Hong Kong, the maternal mortality rate with SARS was 30%, compared to 0% in the non-pregnant group [37]. In pregnant women with SARS-CoV during the 2002–2003 epidemic, there were no cases of vertical transmission of the virus documented [11].

3.5.7. Health Care Workers in Transplant Teams

Any transplant team member with symptoms of a viral infection should undergo the appropriate testing, and avoid exposure to patients as long as symptoms persist or while the test result is pending. For transplant team members, there are also risks during exposure to transplant recipients potentially spreading viral infections with larger quantities of virus (super-shedders or super-spreaders) and/or prolonged viral shedding [38].

4. Discussion

We reviewed the currently available evidence on various aspects of SARS-CoV-2 infections and COVID-19, relating to SOT recipients and the transplant teams involved. Some special aspects need to be discussed.

4.1. The Potentially Protective Effect of Immunosuppression Relating to COVID-19 Stage III, a Hypothesis Based on Preliminary Observations in SOT

With respect to the COVID-19 case fatality in patients with chronic immunosuppression after SOT, both a higher incidence of disease and mortality could be expected. Surprisingly, this has not been the case so far. Until mid April, only seven documented cases in patients with SOT had been reported, whereas SARS-CoV-2 had by then resulted in more than 2,000,000 infections worldwide. In Italy, patients with COVID-19 (irrespective of clinical stage) required ICU admission in 12% of the total SARS-CoV-2 positive cases presenting with any kind of symptom and sampled for virus material, and 16% of all hospitalized patients [39]. In China, case fatality was 49.0% in critical cases (1023 of 2087), and in Italian patients it has been reported to be high as 7.2%, although final numbers from the ongoing coronavirus crisis are still pending [39].

One possible explanation for these unexpectedly low numbers could be that immunosuppression in SOT patients protects against the dramatic elevation of pro-inflammatory cells in the presence of COVID-19. It possibly mitigates the hyperinflammation (“cytokine storm”) that can be observed in immunocompetent patients with COVID-19 Stage III. There is some evidence that this is true for the calcineurin inhibitor tacrolimus (see below). Secondly, a cytopathic effect due to the virus could play an important role. Viruses can kill the human cells in which they reproduce, leading to cellular damage in the infected organs. The question of whether immunosuppressive therapy also can mitigate the viral cytopathic effect remains unanswered. Reports on autopsy in COVID-19 patients are extremely rare. Tian et al. described the pathologic findings in two COVID-19 patients, showing edema and prominent proteinaceous exudates, vascular congestion, and inflammatory clusters with fibrinoid material and multinucleated giant cells. Reactive alveolar epithelial hyperplasia and fibroblastic proliferation (fibroblast plugs) is indicative of early organization [40].

The mechanism of a possible beneficial effect of immunosuppressive therapy remains unclear. One paradigm could be the effect of immunosuppressive therapy on both the innate and adaptive immunity to SARS-CoV-2. The innate immune response includes cells such as IL-1, -2, -3, -6, TNF-α and IFN-γ, trying to protect the human cells from infection and to eliminate the virus, occurring well before the adaptive immunity becomes activated. The adaptive immunity has two major divisions, which are the antiviral B-cell (antibody mediated) and T-cell immune response. The antibody-mediated response binds to free viral particles, in order to block infection of the host cells. This part of the immune response, however, has more importance in preventing reinfection, which is currently the focus in developing vaccines against SARS-CoV-2. In contrast, the T-cell division of the adaptive immunity is much more important for resolution of the virus than the B-cell response. T-cells are needed for recognizing and destroying SARS-CoV-2 infected cells and the coordination of the whole machinery of the inflammatory response. An overshoot of this inflammatory response could lead to organ damage (cytokine storm, hyperinflammation).

In most patients with SOT, the maintenance immunosuppression includes calcineurin inhibitors (CNIs, namely tacrolimus or cyclosporine), an antiproliferative agent (mycophenolate mofetil (MMF) or azathioprine) and low-dose corticosteroids (prednisone or prednisolone) as maintenance therapy.

The CNIs impair upregulation of (among others) interleukin (IL)-2, thereby reducing the proliferation, maturation and survival of T-cells, impairing an effective immune response. They also inhibit IL-4, TNF-α and IFN-γ. Corticosteroids also reduce the expression of many molecules that are needed in the immune response, such as IL-1, -2, -3, -6, TNF-α and IFN-γ. The antiproliferative agents diminish the clonal expansion of the alloreactive T-cells.

In this way, a cytokine storm could possibly be prevented in SOT patients. Therefore, immunosuppression is probably not a risk factor, but rather beneficial in this population, although the number of observations is very low, not allowing definitive conclusions yet. Even in patients with a lung transplantation, who generally have a more profound immunosuppressive therapy compared to patients with other SOT, a higher risk of incidence and severity of COVID-19 has not been observed so far. Moreover, in the described cases, none of the patients had signs of a major acute or chronic allograft dysfunction, another known complication of respiratory viral infections, particularly in lung transplant recipients. On the other hand, by blocking the above-mentioned important components of the antiviral innate immune response, one would expect the incidence (not severity) of (mild) COVID-19 to be increased, or at least be equal to that among immunocompetent individuals. On the contrary, in the medical literature there is a surprisingly low number of case reports on SOT patients with COVID-19. This could be an under-reported group of patients, or the low number may be related to the fact that these patients have been aware of their susceptibility to infections since being transplanted, and thus act more prudently in the context of the pandemic than the non-transplanted population for whom these measures are largely new and not yet routine behavior. However, more studies concerning these questions and a longer follow-up are needed to draw more firm conclusions concerning these aspects of the pandemic.

What do we learn from studies in other coronaviruses? As seen in MERS, there is a potential role for tacrolimus [41]. One case report described two renal transplant recipients who tested positive for MERS CoV. The patient under tacrolimus had a full recovery, whereas the other patient, who was not on this treatment, did not survive the infection [42]. In vitro, in studies of the pathways of the viral replication of coronavirus, tacrolimus effectively inhibited the viral replication of SARS-CoV, coronavirus NL63 and 229E [43]. This was confirmed with a tacrolimus derivative in another laboratory study [44]. Although these studies are not specific to COVID-19, evaluation of tacrolimus could be interesting in the treatment of COVID-19.

Mycophenolate mofetil as a potential therapy for MERS and SARS-CoV has also been studied. Although in laboratory studies it seemed to inhibit both MERS-CoV and SARS-CoV, in an animal experiment with marmosets it showed high viral loads with more severe or even fatal disease [45–47].

The role of corticosteroids in SARS-CoV is not conclusive. They were widely used during the SARS-CoV outbreak, but can promote viral rebound and acute respiratory distress syndrome [48]. Importantly, in animal experiments with dexamethasone, it was suggested that in pigs with SARS-CoV infection, dexamethasone could reduce the early pro-inflammatory response, but a prolonged administration could promote viral replication [49]. In a human study that separated SARS-CoV patients into four treatment groups, the best response was seen in the group receiving early high-dose corticosteroids [50].

4.2. Atypical Symptoms in SOT Patients with COVID-19

A remarkable observation is that SOT patients frequently have gastrointestinal symptoms as part of COVID-19. These symptoms have been described in immunocompetent COVID-19 patients, but they appear to be rare (3−5%) [51,52]. Gastrointestinal symptoms from COVID-19 in SOT patients cannot be explained as yet, but may be related to the immunosuppressive treatment, or to the co-medication possibly altering the intestinal microbiome and thus modifying the intestinal reactivity to the viral infection. Again, the number of observations of COVID-19 patients with these manifestations is too small for firm conclusions.

4.3. Secondary Effects of COVID-19 on SOT Patients

In transplantation medicine, COVID-19 has had a noticeable negative influence both on the ambulatory and the hospitalized patients with SOT, with strong psychological effects and increased need for psychological support from transplant physicians, transplant psychiatrists and psychologists, in addition to the somatic effects. This negative influence may become even more obvious as time passes, and the missed follow-up appointments may potentially influence the course of the disease in the coming months. The total impact in this area will only be fully understood in the near future when studies address these issues.

In summary, although further research is urgently needed to give a clearer picture of the impact of SARS-CoV-2 and COVID-19 on the SOT community, the currently available limited data suggest a reduced immediate impact of COVID-19 in respect to severity of disease, most likely due to "protective" immunosuppression. Based on this preliminary observation, we expect a milder disease severity and probably a better outcome in patients with SOT in a population, because they are typically well aware of the risks of viral (and other) infections and thus practice prevention strategies more rigorously, due to knowledge they have acquired prior to the current coronavirus pandemic. In the absence of definitive medical treatment protocols, many treatments have been suggested. Although it is too early for results of large clinical trials, generally in the above-described case reports with COVID-19 stage IIA or IIB, MMF initially is stopped, tacrolimus is reduced, methylprednisolone iv is started, and empiric broad-spectrum antibiotics are given. Hydroxochloroquine or lopinavir-ritonavir/darunavir-cobicistat was given as off-label therapy in some patients. Intravenous gammaglobulins are an alternative treatment for patients at risk of infection-triggered rejection, in whom the immunosuppressive treatment cannot be escalated due to increased drug-related adverse events or fear of increased viral replication.

Whilst more definitive and clinically proven treatments are awaited, the above described treatments can be helpful in the short term and may be reassuring for the SOT community.

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Abbreviations

bilat.	bilateral
CRP	C-reactive protein
CT chest	computed tomography of the chest
ECMO	extracorporal membrane oxygenation
everol	everolimus
GGO	ground-glass opacity
HFOT	high flow oxygen therapy
HCQ	hydroxochloroquine
IS Rx	immunosuppressive therapy
iv	intravenous
IVIG	intravenous immunoglobulin G
MMF	mycophenolate mofetil
MV	mechanical ventilation
NIV	non-invasive ventilation
po	per os
Pred	Prednisone
rh-GCSF	recombinant human granulocyte colony-stimulating factor
Tac	tacrolimus
inh.	inhalation

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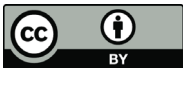

transplantology

MDPI

Case Report

Clinical Characteristics, Treatments and Outcomes of 18 Lung Transplant Recipients with COVID-19

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Abstract: We report clinical features, treatments and outcomes in 18 lung transplant recipients with laboratory confirmed SARS-CoV-2 infection. We performed a single center, retrospective case series study of lung transplant recipients, who tested positive for SARS-CoV-2 between 1 February 2020 and 1 March 2021. Clinical, laboratory and radiology findingswere obtained. Treatment regimens and patient outcome data were obtained by reviewing the electronic medical record. Mean age was 49.9 (22–68) years, and twelve (67%) patients were male. The most common symptoms were fever (n = 9, 50%), nausea/vomiting (n = 7, 39%), cough (n = 6, 33%), dyspnea (n = 6, 33%) and fatigue (n = 6, 33%). Headache was reported by five patients (28%). The most notable laboratory findings were elevated levels of C-reactive protein (CRP) and lactate dehydrogenase (LDH). Computed Tomography (CT) of the chest was performed in all hospitalized patients (n = 11, 7%), and showed ground-glass opacities (GGO) in 11 patients (100%), of whom nine (82%) had GGO combined with pulmonary consolidations. Six (33%) patients received remdesivir, five (28%) intravenous dexamethasone either alone or in combination with remdesivir, and 15 (83%) were treated with broad spectrum antibiotics including co-amoxicillin, tazobactam-piperacillin and meropenem. Four (22%) patients were transferred to the intensive care unit, two patients (11%) required invasive mechanical ventilation who could not be successfully extubated and died. Eighty-nine percent of our patients survived COVID-19 and were cured. Two patients with severe COVID-19 did not survive.

Keywords: SARS-CoV-2; viral infection; hyperinflammation; cytokine storm syndrome; dexamethasone; remdesivir; hemodialysis

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1. Introduction

In the last decades, life expectancy in lung transplant recipients (LTRs) has improved. According to data of the International Society of Heart and Lung Transplant registry, including 260 lung transplantation centers with 69,200 adult LTR worldwide, infections remain the leading cause of death within the first year after lung transplantation [1]. Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) has affected solid organ transplant (SOT) recipients as well as immunocompetent patients and continues to claim lives globally. Data on COVID-19 in LTRs are scarce, mostly reported in case reports and small case series. One study reported 17 LTR with COVID-19 in a series of 90 SOT recipients, but the LTR were not analyzed separately [2]. Verleden et al. studied a total of 10 LTR with COVID-19 [3]. Another study performed a telephone survey including 41 LTR with COVID-19 [4].

Surprisingly, with respect to the chronic immunosuppression in SOT recipients, the expected higher incidence and mortality of COVID-19 in this group has not been widely observed. One hypothesis is that the use of calcineurin inhibitors in this group of patients

mitigates the severe hyperinflammation (cytokine storm syndrome) and may thus contribute to less morbidity and mortality. This retrospective study of consecutive LTRs with COVID-19 from the Zurich Lung Transplant Center aims to describe the features of the disease by analyzing the clinical, laboratory and radiology characteristics and the outcome of these patients.

2. Methods

2.1. Study Population, Setting, and Clinical Data

The study population consists of consecutive adult LTR recipients ≥18 year of age with COVID-19 at the University Hospital Zurich, Switzerland, in which formal informed consent was given. No patient had to be excluded due to lack of informed consent or age <18 years. For the diagnosis of COVID-19, a laboratory confirmation of SARS CoV-2 by real time reverse transcriptase polymerase chain reaction (RT-PCR) was required, irrespective of clinical signs and symptoms. The data comprised demographics, clinical, laboratory and radiology characteristics as well as treatments and outcomes. The data were abstracted from the electronic medical record of the patients. The long-term immunosuppression was recorded. We also documented comorbidity including hypertension, diabetes, cardiovascular disease, malignancy and chronic kidney disease.

Severity of disease was classified according to Siddiqi [5]. The risk stratification by the AIFELL score was documented [6]. Data on antiviral, antibiotic and anti-inflammatory therapy were recorded, as well as clinical treatment setting (normal ward, intermediate care, and intensive care). Additionally, we recorded the need for oxygen therapy (normal breathing without additional oxygen, oxygen therapy with nasal cannula, non-rebreather face mask, high flow oxygen therapy, non-invasive ventilation, invasive mechanical ventilation or extracorporeal membrane oxygenation). Full recovery was defined as two negative SARS CoV-2 RT-PCR tests at least 24 h apart along with resolution of symptoms and clinical syndrome, and in case of hospital discharge without the need for additional oxygen therapy.

2.2. Laboratory and Radiology Assessment

Diagnosis of COVID-19 was confirmed by RT-PCR using nasopharyngeal swabs. Laboratory investigations included complete blood count (hemoglobin, leucocytes and platelets) with differential blood count including eosinophils, neutrophils, and lymphocytes. The chemistry panel included the renal function, liver enzymes, and the C-reactive protein (CRP). Radiology data included computed tomography (CT) scan of chest in all hospitalized patients.

2.3. Statistical Analysis

Descriptive statistics were performed. Main data are summarized in tables. Results were reported as mean with the range, and categorical variables were calculated as counts (n) and percentages (%).

2.4. Ethical Consideration

The study was granted approval by the Zurich branch of the Swiss Medical Ethics Committee (Swissethics, No. 2021-00293).

3. Results

3.1. Clinical Patient Characteristics

Between February 2020 and March 2021, a positive SARS-CoV-2 infection was demonstrated by PCR in eighteen LTRs. The median age was 49.9 years, and most patients were male (n = 12, 67%). The demographic and clinical characteristics are summarized in Table 1. The mean time since transplantation was 5.5 years, with cystic fibrosis and COPD being the most common pretransplant underlying disease (n = 6, 33% and n = 5, 28%, respectively).

Table 1. Patient characteristics according to COVID-19 disease severity.

	Mild (Siddiqi I) n = 7	**Moderate (Siddiqi IIA,B) n = 9**	**Severe (Siddiqi III) n = 2**
Age, mean yrs., (range)	41.3 (19–64)	54.7 (28–68)	58.5 (56–61)
Male sex (%)	3 (43%)	7 (78%)	2 (100%)
BMI, mean (kg/m^2)	22.4	26.3	31.2
Pretransplant diagnosis			
Cystic fibrosis	4 (57%)	2 (22%)	0
COPD	1 (14%)	4 (44%)	0
ILD	2 (29%)	2 (22%)	2 (100%)
PAH	0	1 (11%)	0
Comorbidities (%)			
Hypertension	2 (29%)	6 (56%)	2 (100%)
Chronic kidney disease	2 (29%)	4 (44%)	2 (100%)
Diabetes	2 (29%)	3 (33%)	1 (50%)
Cardiovascular disease	1 (14%)	3 (33%)	2 (100%)
Malignancy	0	4 (44%)	1 (50%)

BMI = body mass index.

The most common symptoms were fever (n = 9, 50%), nausea/vomiting (n = 7, 39%), cough (n = 6, 33%), dyspnea (n = 6, 33%) and fatigue (n = 6, 33%). Headache was reported by five patients (28%), and anorexia by three patients (23%). Only one patient (6%) reported altered sense of smell and taste (Table 2). Among the comorbidities, 10 patients (56%) had hypertension, six (33%) diabetes mellitus, six (33%) cardiovascular disease, eight (44%) chronic kidney disease and five (28%) a history of malignancy. Among the immunosuppressive drugs, 18 patients (100%) were long-term treated with prednisone, 15 patients (83%) had mycophenolate mofetil (MMF), 10 patients (56%) were on tacrolimus treatment, six patients (33%) had cyclosporine A, one patient (6%) had everolimus and one (6%) had rapamycin. Severity stages were classified according to Siddiqi, ranging from stage I to III. Seven (39%) patients had mild disease (stage I), five (28%) had moderate disease without hypoxemia (stage IIA), four (22%) with hypoxemia (stage IIB), while two (11%) were categorized as severe (stage III).

3.2. Laboratory and Radiological Features

The most notable laboratory findings were elevated levels of CRP, ferritin and D-dimers.

Computed Tomography (CT) of the chest was performed in all hospitalized patients, and showed ground-glass opacities (GGO) in 11 out of 11 (100%) patients, of whom nine (82%) had GGO combined with pulmonary consolidations. In two patients (18%), there was also a small pleural effusion, in which a thorax drainage was not indicated. One patient (9%) had a pneumothorax, which required insertion of a chest tube.

3.3. Therapeutic Intervention

Treatment data are mentioned in Tables 3 and 4: Six (33%) patients received remdesivir, five (28%) intravenous dexamethasone either alone or in combination with remdesivir, and 15 (83%) were treated with broad-spectrum antibiotics including co-amoxicillin, tazobactam-piperacillin, and meropenem. One patient was treated with COVID-19 convalescent plasma (CCP). Four (22%) patients were transferred to the intensive care unit, and two patients required invasive mechanical ventilation who could not be successfully extubated and died.

Table 2. Symptoms, signs and laboratory values.

	Mild (Siddiqi I) n = 7	**Moderate (Siddiqi IIA,B) n = 9**	**Severe (Siddiqi III) n = 2**
Symptoms			
Fever	2 (29%)	7 (78%)	0
Cough	1 (14%)	5 (56%)	0
Dyspnea	0	4 (44%)	2 (100%)
Sore throat	0	0	0
Fatigue	1 (14%)	4 (44%)	1 (50%)
Anorexia	1 (14%)	2 (22%)	0
Diarrhea	1 (14%)	2 (22%)	0
Nausea/vomiting	3 (43%)	3 (33%)	1 (50%)
Altered sense of smell	0	0	0
Altered sense of taste	0	1 (11%)	0
Headache	2 (29%)	3 (33%)	0
Rhinorrhea	1 (14%)	1 (11%)	0
Vital signs			
Temperature (°C)	38.4 (37.9–38.8)	37.3 (35.9–39.2)	38.3 (36.8–39.8)
Heart rate (bpm)	118	82 (69–129)	88 (81–95)
Oxygen saturation, %	96	96.4 (95–99)	92 (90–93)
Laboratory values, mean (range)			
CRP (mg/l)	29.8 (4–77)	58.2 (4.8–140)	302 (199–406)
Hemoglobin (g/l)	135 (104–172)	121 (96–152)	113 (99–127)
Thrombocytes (G/l)	201 (153–313)	186 (126–365)	174 (172–176)
Leucocytes (G/l)	6.41 (3.67–10.6)	6.67 (4.99–9.38)	11.6 (5.9–17.3)
Neutrophils (G/l)	4.89 (3.26–8.03)	5.29 (2.04–7.49)	10.4 (4.1–16.1)
Eosinophils (G/l)	0.067 (0–0.14)	0.024 (0–0.08)	0 (0–0)
Lymphocytes (G/l)	0.95 (0.17–1.25)	0.60 (0.18–1.58)	1.29 (0.47–2.1)
ASAT (U/l)	42.7 (18–69)	29.9 (14–44)	44 (39–49)
ALAT (U/l)	47.3 (11–90)	24.7 (12–39)	16 (9–23)
LDH (U/l)	383 (309–450)	486 (5–636)	801 (500–1101)
Bilirubin (µmol/l)	17.7 (5–34)	8.1 (4–15)	N/A
Creatinine (µmol/l)	119 (90–166)	231 (17–809)	208 (202–213)
Creatinin kinase (U/l)	46 (43–49)	113 (25–525)	92 (69–114)
Blood Group (A, B, AB, 0)			
A (%)	3 (43%)	7 (78%)	0
B (%)	0	1 (11%)	0
AB (%)	0	0	0
0 (%)	4 (57%)	1 (11%)	2 (100%)
AIFELL Score at presentation	1.5 (1–2)	3.2 (2–4)	4.5 (4–5)

Table 3. Treatment strategies.

	Mild (Siddiqi I) n = 7	**Moderate (Siddiqi IIA,B) n = 9**	**Severe (Siddiqi III) n = 2**
Immunosuppression			
Prednisone	7 (100%)	9 (100%)	2 (100%)
Mycophenolate mofetil	6 (86%)	7 (78%)	2 (100%)
Cyclosporine A	1 (14%)	3 (33%)	2 (100%)
Tacrolimus	6 (86%)	4 (44%)	0
Everolimus	0	1 (11%)	0
Rapamycin	1 (14%)	0	0
Treatment			
Remdesivir	0	6 (67%)	0
Augmentin	3 (43%)	0	1 (50%)
Ceftriaxone	0	0	0
Tazobactam/piperacillin	1 (14%)	3 (33%)	1 (50%)
Meropenem	0	3 (33%)	1 (50%)
Vancomycin	0	1 (11%)	0
Dexamethasone	0	3 (33%)	2 (100%)
Treatment setting			
Ambulant	6 (86%)	0	0
Hospital, normal ward	1 (14%)	7 (78%)	0
Hospital, intermediate care	0	0	0
Hospital, intensive care	0	2 (22%)	2 (100%)
Hospitalization (days)	7 (7–7)	22 (3–44)	20 (19–20)
Oxygenation			
Normal, room air	7 (100%)	5 (56%)	0
Oxygen, nasal cannula	0	3 (33%)	
Oxygen, non-rebreather	0	0	0
Oxygen, HFOT	0	1 (11%)	0
Non-invasive ventilation	0	0	0
Mechanical ventilation	0	0	2 (100%)
ECMO	0	0	0
Outcome			
Alive	7 (100%)	9 (100%)	0
Dead	0	0	2 (100%)

ECMO = extracorporeal membrane oxygenation; HFOT = high-flow oxygen therapy.

Table 4. Patient characteristics, treatments and outcome.

Patient Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Date of COVID-19	10/ 2020	10/ 2020	03/ 2020	10/ 2020	10/ 2020	11/ 2020	11/ 2020	11/ 2020	12/ 2020	05/ 2020	11/ 2020	12/ 2020	01/ 2021	12/ 2020	01/ 2021	01/ 2021	01/ 2021	01/ 2021
Demographic																		
Age (years)	56	28	56	48	38	22	68	66	27	64	49	68	64	34	19	61	67	63
male/female	m	m	f	m	m	f	m	m	m	m	f	m	f	m	f	m	f	m
BMI, kg/m2	31	27.4	21.9	19.3	19.6	20.3	42.8	27.1	19.5	27.7	18.9	22.4	27.4	14.9	15.2	31.4	37.5	31.8
Transplant Data																		

Table 4. *Cont.*

Patient Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Transplant year	2019	2019	2019	2019	2006	2019	2015	2020	2014	2018	2016	2012	2016	2016	2020	2016	2010	2018
Previous disease	ILD	PAH	COPD	CF	CF	CF	COPD	IPF	CF	IPF	Pl.par.Fi	COPD	COPD	CF	CF	ILD	ILD	COPD
Comorbidities																		
Hypertension	1	1		1					1	1	1	1	1			1		1
Diabetes					1		1	1						1		1	1	
Cardiovascular disease	1											1	1			1	1	1
Malignancy	1				1		1	1			1							
Chronic kidney disease	1				1				1	1	1	1	1			1		
Immunosuppression																		
Prednison	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MMF	1	1	1	1		1	1			1	1	1	1	1	1	1	1	1
Ciclosporine	1			1								1				1	1	1
Tacrolimus		1	1			1	1		1	1	1		1	1	1			
Everolimus					1													
Certican/rapamy									1									
Risk stratification																		
Siddiqi Stage	III	IIA	I	IIB	IIB	I	IIA	IIA	I	I	IIA	IIB	IIB	I	I	III	I	IIA
AIFELL Score	4	1	2	4	3		3	4			2	4	4	1		5		
Radiology																		
Examination (CT/CXR)	CT	CT	CT	CT	CT		CT	CT			CT	CT	CT			CT		CXR
GGO	1	1	1	1	1		1	1			1	1	1			1		
Consolidation	1			1	1			1			1	1	1			1		1
Pleural Effusion				1			1											
Pneumothorax	1																	
Treatment																		
Antiviral																		
Remdesivir				1	1 HD dose			1				1 HD dose	1					1
Antibiotics																		
Azithromycin																		
Augmentin									1					1		1	1	
Ceftriaxon		1																1
Tazobac	1		1					1			1	1						
Meropenem	1				1		1						1					
Vancomycin													1					
Corticosteroids																		
Prednison	1																	
Dexamethasone	1							1					1			1		1
CCP																	1	
Treatment Setting																		
Ambulant						1			1	1				1	1		1	
Hosp. normal ward		1	1		1		1	1			1	1						1
Hosp. ICU	1			1									1			1		
No. of Hosp. Days	20	3	7	43	44		9	26			3	36	7			19		23
Oxygenation																		
Normal breathing no O2		1	1			1	1	1	1	1	1			1	1		1	1
Oxygen nasal cannula					1							1	1					
Oxygen HFOT				1														
Mechanical ventilation	1															1		
Outcome																		
Alive		1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1
Dead	1															1		

BMI = body mass index; CCP = COVID convalescent plasma; CT = computed tomography of the chest; CXR = chest x-ray; GGO = ground-glass opacities; HD dose = hemodialysis dose; HFOT = high flow oxygen therapy; Hosp. = hospitalization; ICU = intensive care unit; ILD = interstitial lung disease; IV = intravenous; MMF = mycophenolate mofetil; No. = number; PAH = pulmonary arterial hypertension; Pl.par Fib = pleuroparenchymal fibroelastosis.

3.4. Remdesivir in Patients with Impaired Renal Function

Remdesivir, a therapy originally developed to treat hepatitis C and Ebola, is used in treating selected patients with COVID-19. In three (17%) patients, due to an impaired renal function, remdesivir formally was contraindicated. One of them was on chronic intermittent hemodialysis, the other two had an eGFR < 30 mL/min. In patients with hemodialysis (HD), to the best of our knowledge, there are no clinical data or guidelines how to treat these patients with remdesivir. At the University Hospital of Zurich, remdesivir in HD patients, starts with 200 mg on the first day of HD, followed by 100 mg on the third and fifth day.

On the first day, the maximal concentration (Cmax) is measured immediately after the first remdesivir infusion, followed by measurements of Cmax after 2–3 h. On the second day, the minimal concentration, Cmin, is measured 24h after the first remdesivir dose. On day three, Cmin is measured before and after dialysis, and Cmax immediately after the second dose and also three hours later. On the fourth day, Cmin is measured 24h after the second dose (trough). On the fifth day, Cmin is measured before HD and after HD. The Cmax is measured after the infusion of the last dose, as well as 3 h later. Currently, the Cmax values are experimentally and help to find the area under the curve (AUC), after which dose adjustments can be considered. However, in case the dose is not in the target range, dosage adjudgments are empirical. Additionally, in patients with renal failure but without HD, remdesivir treatment consists of three doses: On the first day 200 mg are given followed by remdesivir 100 mg every 48 h (remdesivir on day 1, 3 and 5).

3.5. Autopsy in a Patient with COVID-19 with Siddiqi Stage III Disease

One 56-year-old patient with a bilateral lung transplantation 2 years ago due to interstitial lung disease, probably IPF, died 16 days after being diagnosed with COVID-19 by detection of SARS-CoV-2 infection. The cause of death was a severe ARDS. He presented in the emergency department of our hospital, reporting fever, acute dyspnea and a non-productive cough. On admission, he had a temperature of 39.5 °C (auricular), a heart rate of 95/min., an oxygen saturation of 93% without supplemental oxygen, a blood pressure of 146/95 mmHg and a normal heart- and lung auscultation. Among the relevant comorbidities, the patient suffered from an impaired renal function due to calcineurin inhibitors, worsened by the acute viral infection (at admission eGFR 16 ml/min., previously 30 mL/min.), and had been diagnosed with central and subsegmental pulmonary embolism four months before admission. He was on coumarin treatment for this. CT of the chest showed a right-sided consolidation in the upper lobe, with concomitant right-sided GGO, without signs of air trapping. After four days, the chest CT showed severe progression with new bilateral infiltrates in the lower lobes, and progression of GGO and bilateral crazy-paving pattern). Due to severe ARDS the patient needed intubation for invasive mechanical ventilation. He was treated with dexamethasone 6 mg iv. for 10 days (after 10 days, prednisone 15 mg was continued), remdesivir and pragmatically with meropenem, levofloxacin, and amphotericin. The immunosuppression with ciclosporin was continued; however, MMF was discontinued due to lymphocytopenia and thrombocytopenia. Six days later, still on mechanical ventilation support, he developed an obstructive shock due to a total left-sided tension pneumothorax, probably due to barotrauma (Figure 1a–e). Initially, he was treated with chest tube drainage (20 Ch.), sand after two days a second chest tube (28 Ch.) was inserted, followed by another chest tube (28 Ch.) two days later due to insufficient lung expansion. Other complications in this patient were atrial fibrillation, protracted thrombocytopenia and renal failure requiring hemofiltration.

In the bronchoalveolar lavage (BAL), performed immediately after intubation, PCR of SARS-CoV-2 was positive. In the BAL, no microorganisms were grown, and the Aspergillus antigen (galactomannan) was negative (index < 0.5). Bronchoscopy showed no endobronchial mucus retention (Figure 1f,g). In addition, the serological Aspergillus antigen was negative (index < 0.5), so was the PCR for Bordetella pertussis and parapertussis. Chlamydia pneumoniae and psittaci, Legionella pneumophila and other species, as well as

Mycoplasma pneumoniae were all negative in the BAL. The aerobic and anaerobic samples from blood cultures were negative. Microbiological investigations of the vena jugularis and arteria radialis catheters were negative. The patient still had considerable subcutaneous emphysema (Figure 1h) developed progressive multiorgan failure and after 16 days in the intensive care unit, treatment was discontinued after the unanimous decision of the medical team and the family of the patient.

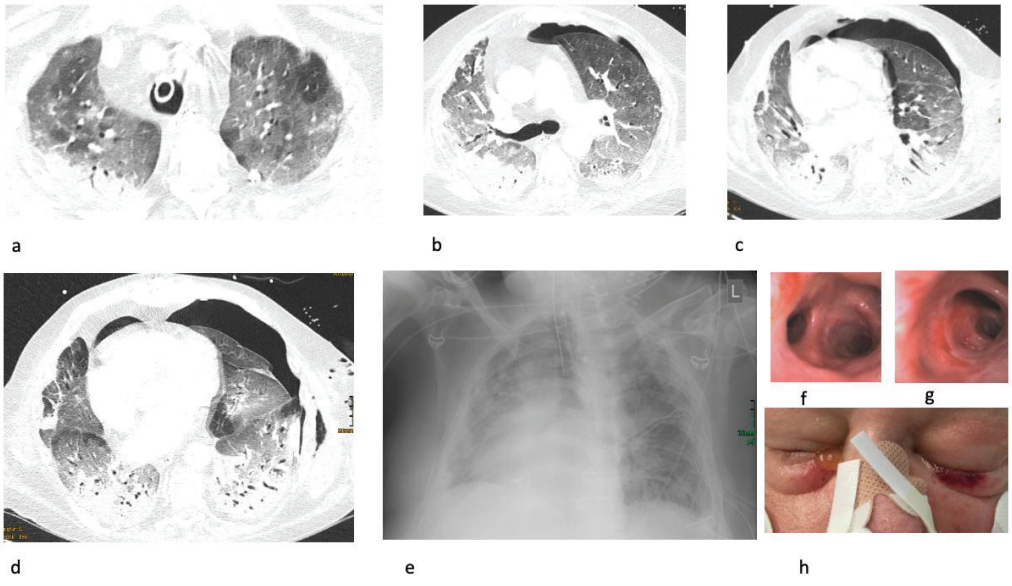

Figure 1. CT scan, chest X-ray, bronchoscopy images, and subcutaneous emphysema. CT scan showing ARDS, GGO and pneumothorax (**a**–**d**); chest X-ray after pneumothorax drainage (**e**); bronchoscopy without endobroncial mucus retention (**f**,**g**); massive subcutaneous emphysema (**h**).

The patient died that same day, and an autopsy was performed. The post mortal SARS-CoV-2 PCR was still positive, and the SARS-CoV-2 concentration in the tracheobronchial secretion showed 56 million SARS-CoV-2 copies/mL. Pathology examination further showed the typical COVID-19 diffuse endothelitis in both lungs and in the epicardial and intramyocardial blood vessels. Capillaries in heart and lung showed diffuse peripheral thrombi with fibrine and leucocytes, which are also well-known COVID-19 findings. In the lung parenchyma, there was extensive multifocal hemorrhagic infarction, and multifocal acute bronchopneumonia, as well as diffuse alveolar damage (DAD). The multifocal acute bronchopneumonia suggested bacterial superinfection, although (with ongoing broad-spectrum antibiotics and amphotericin B) no bacteria or Aspergillus could be cultured in any of the samples. An additional finding in the autopsy was an early stage bowel necrosis and a centrilobular hepatic necrosis.

4. Discussion

In this case series, 18 LTRs with different severity degrees of COVID-19 have been described. Although we have a small number of patients and statistics are descriptive, there tends to be a male predominance with elderly patients with a more severe COVID-19 stage (IIB and III). The extrapulmonary symptoms were predominant in most of the LTRs in our case series.

As has been seen in immunocompetent patients as well, in our case series the LTRs had a more severe COVID-19 stage with a higher body mass index (BMI). The mortality in severe COVID-19 (stage III) was 100%, and these patients were not only older and had

higher BMI values, but they also all had the other COVID-associated main risk factors, namely hypertension, chronic kidney disease and cardiovascular disease. Moreover, the patients with severe COVID-19 also had a higher CRP value, lower hemoglobin, higher leucocytes, neutrophils, and higher LDH values. In contrast to the literature reports on immunocompetent patients with COVID-19, the transaminases in our patients, did not show relevant elevations.

Most patients had blood group A, followed by 0. Both patients with severe COVID-19 had blood group 0.

The AIFELL score, a triage tool used to assess risk in COVID-19 patients, was also low in the Siddiqi stage I, higher in stage II and the highest in stage III patients. All patients were treated with long-term immunosuppressive drugs in the pre-COVID stage, including prednisone in all patients, and in most patients MMF and a calcineurin inhibitor. The most common calcineurin inhibitor was tacrolimus. Six patients (two of them had chronic hemodialysis) have been treated with remdesivir, and five with dexamethasone. One patient was treated with COVID-19 convalescent plasma.

Only 6 out of 18 patients were treated in the ambulatory setting, all other patients were hospitalized, with a mean hospitalization duration of 20 days.

In the following paragraphs, we will discuss some of the above-mentioned special aspects of our case series.

4.1. COVID-19 Severity: The Siddiqi Stages

COVID-19 in most patients in our case series was classified as mild or moderate, according to the Siddiqi classification. The Siddiqi stages classify COVID-19 disease states and potential therapeutic targets [5]. This classification has three escalating phases. Stage I (early infection), characterized by a viral response phase, with clinical symptoms including mild constitutional symptoms, fever, a dry cough, and laboratory shows lymphopenia. In this phase, immunosuppression should be reduced, and excess systemic steroids be avoided. In Stage II (pulmonary phase), there is both a viral response phase and a host inflammatory response phase. Clinical symptoms include dyspnea without hypoxia (IIA) and with hypoxia (IIB), and chest imaging is abnormal (infiltrates), laboratory values showing elevated transaminases, and procalcitonin is generally low or normal. In this stage, mycophenolate should be reduced, according to the ISHLT guidance document [7]. In Stage III (hyperinflammation phase), there is solely a host inflammatory response, with acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS) and cardiac failure, and laboratory values show elevated inflammatory markers (CRP, LDH, Interleukin-6, D-dimer, ferritin), troponin, and N-terminal pro-b-type natriuretic peptide (NT-proBNP). In Stage III, mycophenolate should also be discontinued [7]. Patients in Stage II and III are likely to be the patients that would benefit most of the continued use of calcineurin inhibitors, attenuating the hyperinflammation (cytokine storm).

4.2. SARS-CoV-2 Infection: Extrapulmonary Manifestations

Although infection in the respiratory system is the most important manifestation of COVID-19, there are also important extrapulmonary disease manifestations. In our case series, the extrapulmonary manifestations were the predominant symptoms. Extrapulmonary COVID disease can result in gastrointestinal, cardiovascular, renal and neurological morbidity. Pathophysiologically, there is severe microvascular thrombosis and inflammation, resulting from vascular endothelial dysfunction [8,9]. Interestingly, endothelial dysfunction is also present in the high-risk population for severe COVID-19, in particular patients with hypertension, obesity and diabetes [8]. These comorbidities were also relevant in the patients in our case series, especially in the two patients with severe COVID-19 who did not survive. The patient in which autopsy was performed also showed extensive endothelial dysfunction, as has been described above.

In the literature, most COVID-19 fatalities in immunocompetent patients were observed in elderly men with the aforementioned comorbidities. Although the exact patho-

physiology of endothelial dysfunction in COVID-19 needs further clarification, the scientific evidence suggests that COVID-19 targets endothelial cells [10].

4.3. SARS-CoV-2 Infection: Pulmonary Manifestations

The autopsy report on our patient showed an important pulmonary component of COVID-19. Inflammation is a key component of SARS-CoV-2 infection. Research has shown that there are similarities between the acute respiratory distress syndrome (ARDS) and the acute radiation syndrome (ARS) [11]. The ARS, being a public health emergency, occurs after exposure to high doses of radiation and leads, as in ARDS, to a cytokine storm, with remarkably similar pathophysiology, including increased pro-inflammatory molecules and decreased other anti-inflammatory molecules [11]. Medical treatment strategies for ARS could be helpful in the clarification of the COVID-19 induced ARDS [11]. Among the medical countermeasures in ARS are growth factors, antioxidants, anti-inflammatory agents, anti-fibrotic drugs, RAS-targeted approaches, and treatment for vascular injury such as statins. These approaches are potentially of interest since treatment options for COVID-19 are still very limited and there is no cure for COVID-19. If all available treatment strategies fail to prevent post-COVID-19 lung fibrosis, lung transplantation may be the only curative option in selected severe cases without signs of improvement over weeks or months. Patients recovering from severe COVID-19, especially after ARDS, have a high risk of developing pulmonary fibrosis [12].

4.4. Liver Function Test Abnormalities

In our case series, the liver function (both transaminases and bilirubin levels) at presentation and during the course of the disease was unremarkable, even in the severe cases. This is in contrast with the literature on immunocompetent patients with COVID-19, in whom liver function test abnormalities are associated with a severe course of the SARS-CoV-2 infection [13]. In a meta-analysis, including 3428 patients from 20 retrospective studies, liver dysfunction was significantly higher in critically ill patients with unfavorable outcomes in COVID-19 [14,15], as could also be observed in other coronaviral diseases (SARS and MERS) [16–18]. Studies show the incidence of liver injury ranging from 58–78%, presenting with elevated transaminases and bilirubin levels [19,20]. Autopsy studies show mild lobular and portal activity along with microvascular stenosis [14,21–23]. In case of hepatic involvement in COVID-19, this can be a direct cytopathic effect such as in hyper-inflammation (cytokine storm) and sepsis, or a drug-induced liver injury. Interestingly, cholangiocytes have a higher ACE2 receptor expression, which makes the liver a potential target for SARS-CoV-2 [15]. In the literature, a higher proportion of liver enzyme elevation was observed in patients receiving lopinavir/ritonavir treatment (56.1% vs. 25%) [24], but in our case series none of the LTR received this drug treatment. However, one study in patients with COVID-19 showed liver injury in 10–13% of patients treated with remdesivir [25]. We did not observe this in our cohort (data on evolution of liver enzymes not shown).

4.5. CRP as Marker of Disease Activity in COVID-19

In our cases the patients with the highest CRP values within 3 days of disease onset were those that had the most severe COVID-19 disease evolution. All patients with a CRP level $\geq$199 mg/L were treated in the ICU and died. CRP is a marker of COVID-19 disease activity. It is a plasma protein that is produced in the liver. Various mediators of inflammation, such as Interleukin (IL-)6, can induce CPR production. Elevation of CRP levels in COVID-19 are associated with the severity of COVID-19. Compared to the erythrocyte sedimentation rate (ESR), CRP levels were significantly greater during early periods of severe COVID-19 cases and were shown to be a more sensitive biomarker in reflecting disease development [26,27]. In a retrospective study, the majority of patients with severe COVID-19 showed significantly higher CRP levels as in the non-severe COVID-19 patients (57.9 mg/L vs. 33.2 mg/L, $p < 0.001$) [28]. Another retrospective study showed

that CRP can effectively assess disease severity and predict outcomes in patients with COVID-19, with an increased risk of progression to a higher severity stage in patients with CRP levels >41.8 mg/L [29–31]. CRP values were found to be a more reliable indicator for earlier identification of case severity than CT scans alone [26].

4.6. COVID-19 and ABO Blood Group

Here, we could show no clear influence of ABO blood group on outcome. The two fatal cases had blood group 0. Several studies showed an association between ABO blood groups and the risk of SARS-CoV-2 pneumonia [32].

Data from Wuhan (medRxiv, preprint, not peer-reviewed) including 2173 COVID-19 patients, showed that blood group A was overrepresented in COVID-19 compared with non-A blood groups. In contrast, patients with blood group O showed a significantly lower risk for the infection compared with non-O blood groups [33].

Similar results were shown by the Presbyterian hospital in New York, including 1559 patients with COVID-19 [34].

Gérard et al. studied the ABO blood group in patients with COVID-19, by comparing the patients (n = 1888) possessing anti-A in their serum (i.e., those of B and O blood groups) and those who did not (i.e., those of A and AB blood groups) to the control cohort (n = 3694) [35]. They found significantly less COVID-19 in patients with anti-A in serum (i.e., B and O blood groups) compared to those lacking anti-A (i.e., A and AB blood groups), showing a possible protective effect of anti-A. Surprisingly, Gérard et al. also found a difference between anti-A from O and anti-A from B. The anti-A from O showed an underrepresentation in COVID-19 and anti-A from B an overrepresentation, indicating that anti-A from O is more protective than anti-A from B [35]. This important difference could be related to the isotype of antibodies, being anti-A isotype IgM in serum from blood group B patients, but IgGs in blood group O serum. Although several studies have shown a higher risk of SARS-CoV-2 infection for certain blood groups, one meta-analysis has shown that there was no clear correlation between blood groups and the severity of COVID-19 [36].

4.7. AIFELL Score

The AIFELL score, developed by Levenfus et al., is a clinical prediction score used for triage purposes to assess risk and provide guidance of further diagnostic and therapeutic steps in patients suspected to have COVID-19 [6]. It can be easily applied in emergency wards, and also has an interactive website (www.aifell.net) correlating the AIFELL score to the above-mentioned Siddiqi stages. In this way, it can be used to select probable COVID-19 cases for hospitalization. Unfortunately, this score has not been evaluated in LTRs yet, but seems promising in this group of patients as well. Altered smell and/or taste is one aspect assessed by the AIFELL score, and this disease feature was only noted in one patient in this case series. Although this symptom appears to be fairly frequent in the general population, it was hardly noted in LTRs.

4.8. Remdesivir in Patients with Impaired Renal Function

In this case series, six (33%) patients were treated with remdesivir, of these three had kidney failure (two were on chronic intermittent hemodialysis), which is generally considered a contraindication for remdesivir treatment.

Remdesivir (GS-5734) is a nucleoside analog with a broad antiviral activity to RNA viruses and is also still under investigation for the treatment of Ebolavirus (EBOV, the primary indication), MERS and SARS-CoV-1 [37]. As the first approved drug for COVID-19, remdesivir remains somewhat controversial in the treatment of COVID-19. Both the SOLIDARITY trial [38] and the ACTT-1 trial [39] did not show a significant survival benefit. Nevertheless, in the ACTT-1 trial, remdesivir was shown to be superior to placebo in shortening the time to recovery in adults who were hospitalized with COVID-19, and showed evidence of lower respiratory tract infection [39]. In the ACTT-1 trial, the median

recovery time improved from 15 to 10 days in patients with remdesivir compared to placebo, respectively. The lower 14-day mortality rate in the remdesivir treatment group may indicate a beneficial effect, although it was not statistically significant [39]. However, that study was not powered to evaluate mortality, and therefore mortality should be further evaluated in larger studies with different stages of COVID-19. Reducing the time to recovery by 31%, remdesivir may therefore help to reduce the number of inpatient days, with potential positive effects on hospital costs and capacity issues during the pandemic [37]. Taking the results of both trials together, it might be concluded that treating patients "relatively late" in the course of the disease, remdesivir will not improve the mortality rate in patients with COVID-19. Defining the right timing for the use of remdesivir in COVID-19 still needs further studies. Both trials had a very heterogeneous study population, making firm conclusions on remdesivir treatment difficult. If effective at all, remdesivir should probably be given early in the disease process. Currently, studies with remdesivir treatment in COVID-19 accounting for disease severity and measuring viral load of SARS-CoV-2 are still awaited. Patients with high viral loads are possibly the best candidates to treat with the antiviral drug remdesivir, but this still needs to be proven.

An important issue, especially relevant in the LTR population, is the question of how to treat patients with an impaired renal function. As a prodrug, remdesivir is predominantly metabolized by hepatic enzymes with hydrolase activity [40,41]. Routine monitoring of liver function tests is recommended, and remdesivir should be discontinued in patients with alanine aminotransferase (ALAT) $\geq$10 times the upper limit of normal. The proposed standard dosage is 200 mg as a single dose on day 1, followed by 100 mg once daily. In patients with an eGFR $\leq$30 mL/min and in patients with renal replacement therapies, remdesivir is not recommended.

However, a multicenter, retrospective study reviewing hospitalized patients with SARS-CoV-2 who received remdesivir, showed that this remdesivir treatment was not significantly associated with increased acute kidney injury (AKI) at the end of treatment in patients with an eGFR <30 mL/min compared to patients with an eGFR $\geq$30mL/min [42]. The advice not to prescribe remdesivir in patients with an eGFR $\leq$30 mL/min can be understood in the light of the paucity of clinical data in this group of patients. In animal experiments, remdesivir has been shown to be nephrotoxic in monkeys and rats; however, these doses were 2.1–3.5 fold higher than the doses used in the treatment of SARS-CoV-2 for humans [42]. Nevertheless, in the study of Ackley et al., there was a significantly higher mortality rate in patients with an eGFR <30 mL/min., attributed to the older age, more comorbidities, more frequent use of vasopressors or inotropes, and more frequent use of mechanical ventilation [42].

One case report on a LTR with an initially normal renal function who experienced renal failure after initiation of remdesivir for the treatment of COVID-19 is of concern [43]. In this patient, serum remdesivir concentrations were undetectable, but there were elevated levels of the remdesivir metabolite (GS-441524), suggesting that the metabolite could be responsible for the renal failure in this patient [42,43]. Although it should be mentioned that renal disease is a predictor of COVID-19 related mortality [44], and more than one-fourth of patients with an eGFR <30 mL/min require mechanical ventilation due to COVID-19 related respiratory failure [42], a potential renal toxicity from the accumulation of remdesivir active metabolites requires further study.

4.9. COVID-19 Convalescent Plasma (CCP)

One of our patients in this case series was treated with convalescent plasma collected from recovered COVID-19 patients (CCP). Currently, add-on CCP, in addition to remdesivir and dexamethasone, can be considered in patients with an immune-deficient state, such as after SOT receiving immunosuppressive therapy, in HIV/AIDS, after aplasia-inducing chemotherapy before neutrophils recovery. This is a passive immune therapy, that currently being evaluated in clinical trials. It has been shown that most individuals with laboratory-diagnosed SARS-CoV-2 infection develop not only measurable antibody responses, but

also neutralizing antibodies [45]. The neutralizing antibody levels decline within the first 3 months following diagnosis, which suggests the collection of convalescent plasma with high neutralizing antibody concentrations may be optimally performed within a short time window after the infection has resolved [45]. Studies on CCP suggest improved clinical outcomes including radiological resolution, reduction in viral loads and improved survival. Most data relating to CCP treatment comes from the non-transplant population. The study of Duan et al. showed rapidly increasing neutralizing antibodies, and significantly improved clinical symptoms along with an increase of the oxyhemoglobin saturation within 3 days. In addition, improvements of lymphocyte counts, decreased C-reactive protein and various degrees of resolution of lung lesions in the radiological examinations were observed [46].

Moreover, there was no evidence of clinical hyperimmune responses after CCP treatment in a case series with 20 critically ill patients and 20 controls [47]. In a large series of 5000 hospitalized adults with severe or life threatening COVID-19, with 66% requiring intensive care unit treatment, the transfusion of CCP showed that the mortality rate was not excessive, and suggested that transfusion of CCP is safe in hospitalized patients with COVID-19 [48]. In that series, the incidence of all serious adverse events (SAEs) in the first four hours after CCP was <1%, including a low mortality rate (0.3%) [48]. Among the SEAs were mortality (n = 4), transfusion-associated circulatory overload (TACO; n = 7), transfusion-related acute lung injury (TRALI; n = 11), and severe allergic transfusion reactions (n = 3) [48]. In an uncontrolled case series of five critically ill patients with COVID-19 and ARDS, CCP treatment showed clinical improvement, and in four patients, ARDS resolved at 12 days after CCP, and three patients were weaned from mechanical ventilation within 2 weeks of treatment [49]. Although this treatment is promising, data on LTRs with COVID-19 treated with CCP are still lacking. Our patient who received CCP survived COVID-19 and did not show clear adverse events from the CCP treatment. However, caution with CCP in LTRs is certainly advisable, since CCP in immunosuppressed patients has also been associated with the emergence of new SARS-CoV-2 variant populations in these patients. These viral mutants are more likely to arise in immunocompromised patients, as they have a higher viral burden, increasing the opportunity for variant selection [50]. Therefore, CCP use for COVID-19 in LTRs could give rise to SARS-CoV-2 mutations. This has been observed in immunosuppressed patients treated with CCP for COVID-19 [51].

This hypothesis was confirmed in animal experiments, showing that CCP resulted in antibody-resistant SARS-CoV-2 variants, including the E484K mutation associated with vaccine resistance [52]. A comparable mechanism with emergence of resistant variants has been observed in immunocompromised patients with influenza infections who received long-term oseltamivir treatment [53]. The emergence of SARS-CoV-2 variants may lead to infections in COVID-19 vaccinated LTRs or in those who have survived COVID-19.

4.10. Respiratory Co-Infections

In our study, 15 patients (83%) were treated with broad-spectrum antibiotics including co-amoxicillin, tazobactam-piperacillin and meropenem, in order to prevent or treat respiratory co-infections. In all 18 patients, we found no evidence of respiratory co-infections, although these infections cannot be completely ruled out.

Estimations of the prevalence of co-infections among COVID-19 patients range from 0% to 45% [54]. Most of the co-infections occur within the first 4 days after infection[55], more commonly in SOT recipients [55,56].

In SOT recipients with COVID-19, respiratory secondary co-infections have been addressed in a large number of studies, showing bacterial, viral and fungal secondary infections [57]. Bacterial secondary co-infections were due to Gram negative bacteria, including Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumanni, Klebsiella pneumoniae, Morganella morganii, and Stenotrophomonas maltophilia, as well as Gram positive bacteria, including Enterococcus faecalis, Staphylococcus aureus, and Streptococcus haemolyticus [57].

Several authors reported viral secondary co-infections due to Cytomegalovirus (CMV) infection [58]. Although a secondary co-infection with influenza virus was demonstrated in a liver transplant recipient, this has not been described in LTR yet [59]. Aspergillus fumigatus [60] and Aspergillus niger [61] are also known to cause secondary co-infection in LTR.

While bronchoscopy is not available or advisable in routine care of COVID-19 patients, it is an option in mechanically ventilated ICU patients, providing microbiological samples from broncho-alveolar lavage or bronchial wash samples. Alternatively, samples can be obtained from (blind) tracheal aspirates.

4.11. Immunosuppressive Therapy during COVID-19

In LTRs affected by COVID-19, the optimal management of immunosuppression still remains an unanswered question. Our patients in this case series were all on long-term immunosuppressive therapy. Immunosuppression causes lymphopenia, being a risk factor for severe COVID-19. Moreover, mycophenolate as well as mTOR inhibitors, can impair the immune response to viral (and bacterial) infections. Therefore, in COVID-19, mycophenolate often will be reduced or discontinued, as we also did during the SARS-CoV-2 infection. The risk of decreasing or pausing mycophenolate should be weighed against the risk of transplant rejection. This is in line with the current recommendation of the International Society of Heart and Lung Transplantation (ISHLT), advising to hold mycophenolate mofetil, mTOR inhibitors or azathioprine in the context of hospital admission with moderate/severe COVID-19 [7].

On the other hand, calcineurin inhibitors (CNIs) may prevent or attenuate the cytokine storm, by inhibiting interleukin (IL)-6 and IL-1 pathways, and therefore are maintained in most patients. In our 18 patients, we did not reduce or discontinue CNI.

4.12. Hospital Admission Rate

The clinical spectrum of COVID-19 in LTRs is broad, ranging from mild infection of the upper respiratory tract to severe acute respiratory distress syndrome with multiorgan failure and death as demonstrated here in this single center case series. The impact of maintenance immunosuppression in LTRs on COVID-19 severity, remains to be defined.

In our case series, 67% (n = 12) of patients with COVID-19 were hospitalized. One meta-analysis, studying the hospital admission rate in solid organ transplant recipients with COVID-19, showed that the hospital admission rate in these patients is significantly higher (81%) as compared to the general population [57]. However, the higher admission rates may rather reflect the defensive treatment strategy in these vulnerable patients, in whom careful clinical monitoring in a hospital setting is preferred since the respiratory deterioration in COVID-19 patients frequently is rapid and escalation of therapy is easiest in the hospital setting. In some instances, when patients fear COVID-19 deterioration, they might prefer inpatient treatment and monitoring, but sometimes also the opposite can be seen when patients fear hospitalization due to COVID-19 related overcrowding of hospitals with limited resources or they fear contracting COVID-19 in the hospital setting. The latter has been observed frequently during the early phases of the pandemic, with a general avoidance of the hospitals due to accumulation of severe cases. It should also be mentioned that studies comparing these admission rates are difficult to compare due to differences in comorbidities in LTRs compared to the general population.

5. Conclusions

This study reflects the experience with COVID-19 in LTRs during the first two disease waves in Switzerland and describes clinical, laboratory, radiology features and clinic outcomes. Severe disease was shown in two patients, who did not survive. The study highlights the exceptionally high rate of non-pulmonary symptoms in comparison to immunocompetent patients, and suggests that comorbidity as well as elderly and overweight patients have a higher risk of non-favorable outcomes. Laboratory values suggesting

a dismal outcome are elevated CRP and LDH, while liver functions remained normal in all stages of COVID-19 severity. In LTRs, remdesivir and CCP can be considered as treatment options depending on the disease stage. Additionally, in patients with chronic renal insufficiency, remdesivir could be considered using an adapted dosage. The rate of hospitalization in this population is relatively high, several explanations have been discussed above.

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Case Report

SARS-CoV-2 and Norovirus Co-Infection after Lung Transplantation

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Abstract: Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is spreading as a pandemic in 2020. Few reports on infections in thoracic transplantation have been published so far. We present a case of COVID-19 in a 55-year old female lung transplant recipient infected 5 months posttransplant, who additionally was co-infected with a Norovirus. Respiratory and gastrointestinal symptoms were observed without need of therapeutic escalation except for antibiotic therapy. We observed a moderate disease evolution likely due to triple immunosuppression.

Keywords: Lung Transplantation; COVID-19; SARS-CoV-2; Coronavirus; norovirus

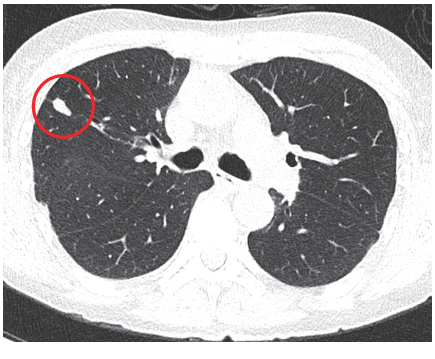
1. Introduction

In late 2019, a novel coronavirus, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged from Wuhan, China [1]. This virus leads to the coronavirus disease 2019 (COVID-19), currently causing a pandemic with worldwide severe economic and healthcare consequences [2]. Among the most dominant clinical characteristics are fever, cough and fatigue, whereas gastrointestinal symptoms are rather uncommon. Critical disease conditions may be caused by severe and sustained systemic inflammatory responses (hyperinflammation or "cytokine storm") and a cytopathic effect, leading to acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis that is hard to correct, coagulation dysfunction and multiple organ failure. The exact viral host factors that influence the pathogenesis are still being investigated. The SARS-CoV-2 infection binds to human host cell receptors, using human angiotensin-converting enzyme 2 (hACE2), although there are more factors influencing susceptibility to infection and disease progression. Elderly people (>65 years of age) with underlying diseases such as hypertension, chronic obstructive pulmonary disease (COPD), diabetes and cardiovascular disease seem more susceptible to an infection and prone to serious outcomes of this viral disease [3]. Since the spread of SARS-CoV-2 around the world, there have only been a few case reports describing COVID-19 in patients after solid organ transplantation (SOT).

We present a case of COVID-19 in a lung transplant (LTX) recipient presenting with fever and gastrointestinal symptoms in our emergency department.

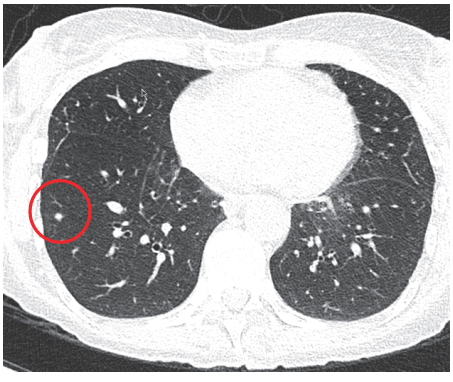
2. Case Report

A 55-year-old female with advanced chronic obstructive pulmonary disease (COPD) underwent a successful bilateral LTX 5 months earlier. Induction therapy (basiliximab) was used, and the patient was started on a standard immunosuppressive regime (cyclosporine—C2 target level 1200–1500 µg/L after 48 h), 1.0 g bid mycophenolate mofetil, and i.v. methylprednisolone according to our standard protocol. In March 2020 she presented with vomiting, diarrhea and a 38.9 °C fever in our emergency department. In the recent months and weeks, she had an uneventful post-transplant course except for a switch from cyclosporine to tacrolimus because of strongly variable trough levels. A recent surveillance bronchoscopy showed no evidence of acute cellular rejection and a stable allograft function with an FEV1 of 104% predicted based on standard triple immunosuppressive therapy (tacrolimus, mycophenolate and prednisolone). At the time of presentation, she had a new onset of mild respiratory symptoms consisting of a dry cough and rhinorrhea. Due to the fever, respiratory symptoms and ongoing SARS-CoV-2 pandemic, diagnostic sampling included blood, feces, and urine and a nasal swab for SARS-CoV-2. The nasopharyngeal swab for SARS-CoV-2 was positive, prompting hospitalization on a special isolation ward. Feces were positive for norovirus after having been negative 2 months earlier. The stool specimen tested negative for SARS-CoV-2. The serological antibody test for SARS-CoV-2 IgG und IgM was negative 14 days after the positive nasopharyngeal swab test. The main results at presentation included CRP 77 mg/L, leukocytosis (9.9 g/L), neutrophilia (8.96 g/L) and a marked declining lymphocytopenia (0.52 g/L, previously 1.3 g/L). The arterial blood gas analysis was within normal limits, thus no oxygen supplementation was given initially. Initial serum interleukin (IL-)6 was slightly elevated 4.5 pg/mL (Ref < 3.1) and decreased subsequently. Soluble IL-2-receptor (sIL-2R) was 394 pg/mL (Ref < 477) at presentation, increased up to 2778 on day 6 and decreased to normal levels (327) on day 15. IgG was in the lower normal range with 7.1 g/L (7–16). Five days after admission, CRP dropped to 5.7 mg/L (Ref < 5 mg/L). Bronchoalveolar lavage was not performed due to the favorable clinical evolution and the lack of respiratory deterioration. Chest computed tomography (CT) imaging revealed, at initial presentation, three small new solid nodular consolidations without predominant ground-glass opacities or pleural effusions (Figure 1a–c). These findings had not been observed 3 months earlier in a routine CT examination. The consolidations diminished over time and some of them disappeared completely in a follow up chest CT 6 weeks later (Figure 1d–f).



(**a**)

Figure 1. *Cont.*



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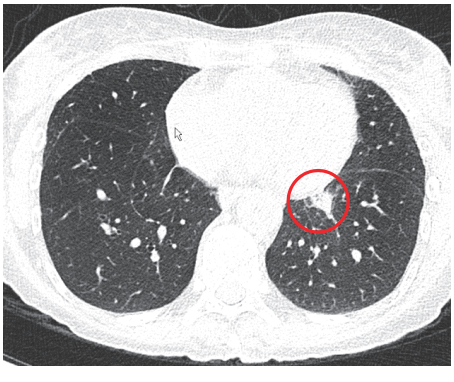

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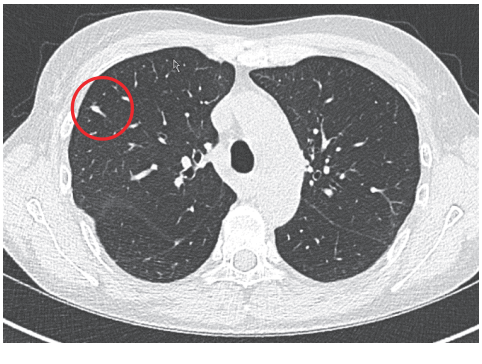
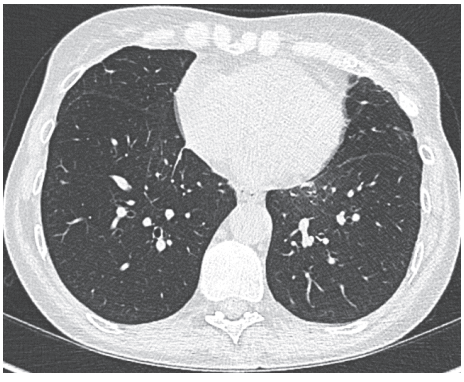
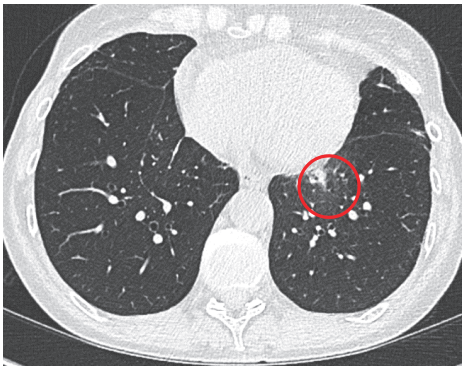


(**d**)

Figure 1. *Cont*.



(**e**)



(**f**)

Figure 1. (**a**–**c**) new pulmonary consolidations in the chest computed tomography (CT) on March 18th that were not visible in a chest CT 3 months before. (**d**–**f**): All of the consolidations resolved partially or completely in the follow-up chest CT 6 weeks later.

The patient was treated empirically with intravenous piperacillin/tazobactam 4.5 g every 8 h for 10 days. We administered neither lopinavir/ritonavir nor hydroxychloroquine, the drug regimen for COVID-19 used predominantly in China and Italy at that time and also the drug regimen for severe cases at our hospital then. This treatment was withheld due to the fairly stable condition of the patient without signs of respiratory deterioration in the first hours of hospitalization and due to concerns about potential drug interactions in this LTX recipient. The clinical evolution was favorable, with the normalization of temperature, stool frequency and no further vomiting. The dry cough and rhinorrhea were resolved within a week. Spirometry remained stable throughout. After three consecutive negative nasal and pharyngeal swabs for SARS-CoV-2, the patient was discharged on day 12 and quarantined at home, with additional empiric amoxicillin/clavulanic acid for 7 days. Lung function continues with steadily increasing allograft function with an FEV1 of 113% predicted on day 201 posttransplant.

3. Discussion

We observed a SARS-CoV-2 infection presenting with fever, respiratory and gastrointestinal symptoms in a severely immunosuppressed LTX recipient with good evolution during hospitalization on intravenous empiric antibiotics. We did not administer the drug regimen for COVID-19 recommended

at that time, which included lopinavir/ritonavir and hydroxychloroquine due to potential drug–drug interactions and absence of deterioration during hospitalization and also because of emerging evidence of the antiviral combination lacking therapeutic benefits [4]. The special features of this SARS-CoV-2 infection in this LTX recipient were: (1) predominant gastrointestinal symptoms at presentation with a diagnosis of norovirus co-infection and fairly mild respiratory symptoms, despite some new and atypical findings on chest imaging that showed regression in the follow-up chest CT 6 weeks later, (2) a moderately elevated pro-inflammatory response (no "cytokine storm" or hyperinflammation) during early infection, probably blunted by immunosuppression, and (3) a delayed increase in sIL-2R, which normalized after admission.

We assume the positive outcome in this case was due to profound immunosuppression, probably because this averted most inflammatory responses, with an initially reduced cytokine storm and a lack of progression of pulmonary infiltrates, both of which are typically observed in SARS-CoV infection of immunocompetent patients [5,6]. The inflammatory immune reaction was limited to a mildly increased IL-6 at presentation and a sIL-2R peak after 1 week, which returned to reference values one week later. The observed inflammation markers were only slightly raised when compared to the reported cytokine data in immunocompetent COVID-19 patients. This modified inflammatory response correlates with the mild clinical course, since cytokines like IL-6 and sIL-2R are described as useful markers to estimate the severity of COVID-19 [7]. The pathogenetical role of IL-6 in COVID-19 manifestations is still not fully understood and there is currently a lack of evidence of the beneficial therapeutic impact of IL-6 inhibitors. The current multicenter randomized controlled trial of tocilizumab (IL-6 receptor blockade, licensed for cytokine release syndrome) in patients with severe pneumonia due to SARS-CoV-2 and elevated IL-6 may shed more light on this topic. The N-protein of SARS-CoV seems responsible for the cytokine dysbalance and for the inflammatory-mediated acute lung injury [8]. The lower inflammatory response relies on glucocorticoids mitigating the N-protein of SARS-CoV-2-induced pulmonary inflammation and modulating the involved cytokines. In the future, the harm and benefit of corticosteroid treatment needs to be carefully considered in patients after SOT with SARS-CoV-2 infection. In immunocompetent patients, the routine administration of high doses of corticosteroids are currently discussed controversially due to concerns that steroids might even exacerbate lung injury by facilitating viral replication in patients with SARS-CoV-2-associated lung injury [9], as previously shown in influenza pneumonia [10]. Another therapeutic approach supports immunosuppressive therapy by aiming to reduce hyperinflammation in particular by using the mechanisms of action observed for tacrolimus in similar settings. It targets the inhibition of viral replication and, accordingly, leads to a reduction of virus titer [11,12]. Anti-inflammatory and anti-cytokine drugs seem to be a promising therapeutic approach in controlling viral damage, assuming that SARS-CoV-2 invades endothelial cells and induces endothelial inflammation, resulting in microvascular dysfunction with a subsequent pro-coagulant state and ischemia in essential organs like the heart, lung, kidney, liver and intestine, which also explains the worse clinical outcome of patients with pre-existing cardiovascular disease [13]. In LTX recipients, respiratory viral infections have been shown to play a major role in acute and chronic allograft dysfunction. Higher sIL-2R levels released from activated B-cells and monocytes have been found in patients suffering from neoplastic, infectious and autoimmune diseases, but also in SOT recipients affected by allograft rejection [14]. Although these results are suggestive of imminent allograft dysfunction in our patient, the most recent results from the follow-up visit 6 weeks after hospital discharge demonstrated a continuously increasing lung function and the chest CT scan showed no evidence of chronic allograft dysfunction, as judged by the lack of detection of air trapping in expiration images. Of course, a longer follow-up of the patient is needed to allow for a final assessment of this aspect, since the onset of complications may be delayed by months. This case of COVID-19 has an atypical pulmonary presentation in the initial chest CT, showing three small nodular consolidations without predominant ground-glass opacities, which is in contrast to the already published cases in the literature (Figure 1a–c). We attribute this to the suppressed immune system and the thereby modified or reduced cytokine storm, which might explain the mild clinical course of the disease, as mentioned

above. The follow-up chest CT showed diminished or completely dissolved consolidations as a sign of recovery from the modified pulmonary involvement of COVID-19 (Figure 1d–f).

In order to understand the disease evolution and to choose the optimal time point in the disease course for initializing therapy, COVID-19 illness was classified recently into Stages I–III, representing increasing grades of severity [15]. Our patient would be placed between early infection (Stage I: mild symptoms, lymphocytopenia, dry cough and diarrhea) and the beginning of the pulmonary phase (Stage II A: abnormal chest imaging).

Viral diseases can have atypical presentations, as has been observed for the different clinical manifestations of SARS-CoV-2 infection and COVID-19 [2,16,17]. SOT recipients should therefore remain cautious avoiding infection [6,18]. Due to atypical presentations, an infection with SARS-CoV-2 may also lead to delay in diagnosis if there is not a high index of suspicion initially for SARS-CoV-2. A kidney transplant recipient, initially suffering from gastrointestinal symptoms, but then developing respiratory symptoms within 48 h, has recently been described [18].

An important feature was the clinical presentation with vomiting, diarrhea and fever up to 38.9 °C. Fever and diarrhea seem to be common initial symptoms in SOT, which has also been reported in SOT recipients under immunosuppressive therapy [19–21]. Until today, SARS-CoV-2-infected patients after SOT are scarce compared to the worldwide incidence of COVID-19 [22–25]. We suspect an underestimated number of SARS-CoV-2 infections in SOT due to atypical clinical presentations thus often not qualifying the patient for SARS-CoV-2 testing in many centers. Moreover, the prudent behavior of LTX recipients and other SOT recipients due to the fear of infectious disease may also explain the low number of COVID-19 cases among these patients.

The stool specimen of our patient was positive for norovirus. Norovirus might increase gastrointestinal wall permeability, resulting in diarrhea and leading to a secondary SARS-CoV-2 infection by fecal–oral transmission [26]. However, this hypothesis cannot be proven, especially since diarrhea is a common manifestation of COVID-19 in immunocompetent and solid organ transplant recipients. Based on the current knowledge we cannot determine the route of infection of this patient since no index patient could be found, and because the patient presented with both gastrointestinal and respiratory symptoms.

4. Conclusions

We describe a fairly mild COVID-19 case in a severely immunosuppressed LTX recipient with good evolution on empiric intravenous antibiotics only. Immunosuppressive therapy might have averted the cytokine storm and the progression of pulmonary disease typically observed in some SARS-CoV-2 infections of immunocompetent patients. Diarrhea is described as a common symptom, even as an isolated manifestation in COVID-19. We assume that immunosuppression may modify the clinical presentation of COVID-19 after SOT. Immunosuppression, in particular tacrolimus, may avert the strong immunological reactions and therefore prevent some of the sequalae of SARS-CoV-2. For this reason, typical respiratory symptoms consisting of a cough and shortness of breath may not always be observed in these patients, thus creating new challenges for infection control and preventing the spread of the disease. This is the beginning of a new infectious era, leading to a global health crisis wherein potential harms need to be anticipated as soon as possible. The increasing SARS-CoV-2 transmission and resultant emerging pandemic are still beyond human control because of the altered biologic characteristics that provide SARS-CoV-2 its virulence and thus this virus poses a real challenge for future drug development. Physicians treating COVID-19 should be encouraged to include their patients in randomized controlled trials in order to gain the clinical evidence so urgently needed in the care of patients suffering from severe COVID-19. Further studies are required to confirm the abovementioned hypothesis before immunosuppression may be safely proposed as a supportive treatment approach in immunocompetent and SOT recipients.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
COVID-19	Coronavirus Disease 2019
SOT	Solid Organ Transplantation
LTX	Lung Transplantation
COPD	Chronic Obstructive Pulmonary Disease
IL-6	Interleukin 6
sIL-2R	Soluble Interleukin 2-Receptor
CT	Computed Tomography

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Case Report

Presentation of SARS-CoV-2 in a Pediatric Heart Transplant Recipient with Multiple Underlying Comorbidities

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Abstract: A six-year-old heart transplant recipient with additional significant co-morbidities, including severe hypoxic-ischemic injury, gastrostomy, tracheostomy, and mechanical ventilation dependency, encountered SARS-CoV-2 infection. The patient received tacrolimus and mycophenolate to prevent graft rejection, presented initially with SARS-CoV-2 positive and presumed pseudomonas aeruginosa pneumonia. Twenty-three days later, the patient presented with fever recurrence with evidence for systemic inflammation, which resolved rapidly with high-dose methylprednisolone. Interestingly, while IgM to SARS-CoV-2 was present, IgG was not detected even three months after his first positive test for SARS-CoV-2. The author discusses potential immune mechanisms that might have affected the course of multi-system inflammatory syndrome children (MIS-C) in this patient.

Keywords: SARS-CoV-2; COVID-19; myocarditis; pediatric heart transplantation

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1. Introduction

There is little data on clinical characteristics and outcomes with SARS-CoV-2 infection in children and adults with an immunocompromised status after heart transplantation [1–5]. Higher mortality was associated with COVID-19 in adult transplant recipients with right ventricular dysfunction, arrhythmias, thromboembolic events, and markedly elevated cardiac biomarkers [5]. It is not easy to extrapolate adult outcomes to pediatric heart transplant recipients because of higher comorbidities such as hypertension, obesity, and diabetes in adult patients. A six-year-old heart transplant recipient with additional significant co-morbidities, including severe hypoxic-ischemic injury, gastrostomy, tracheostomy, and mechanical ventilation dependency, presented with multi-system inflammatory syndrome children (MIS-C) with evidence for systemic inflammation and masquerade as acute cardiac allograft rejection that resolved rapidly with high-dose steroid tretament.

2. Case Report

A six-year-old boy was diagnosed with left ventricular noncompaction cardiomyopathy with severe heart failure and underwent heart transplantation at nine months. His postoperative course was complicated by severe hypoxic-ischemic injury, gastrostomy, and tracheostomy, and he remained on mechanical ventilation. He had no clinical rejection evidence with maintenance immunosuppression (tacrolimus and mycophenolate sodium) and was doing well. He encountered SARS-CoV-2 infection and presented with fever and cyanosis. His chest-X ray (CXR) showed bilateral perihilar infiltrations (Figure 1A). Pseudomonas aeruginosa was isolated from tracheal aspirate and was sensitive to Imipenem, Amikacin, and Ofloxacin. The polymerase chain reaction for the viral respiratory panel was negative for other viral pathogens. His tracheal aspirate for aspergillus filament and pneumocystis carinii were negative. He was admitted to the hospital for supportive care and treated with intravenous Imipenem, and discharged home to complete a ten-day course with oral Ofloxacin. His father was also positive for SARS-CoV-2. Both were quarantined at home as per the center for disease control (CDC) guidelines. His father tested negative

for SARS-CoV-2 after initial positive test subsequently, but the patient continued to be positive at three weeks.

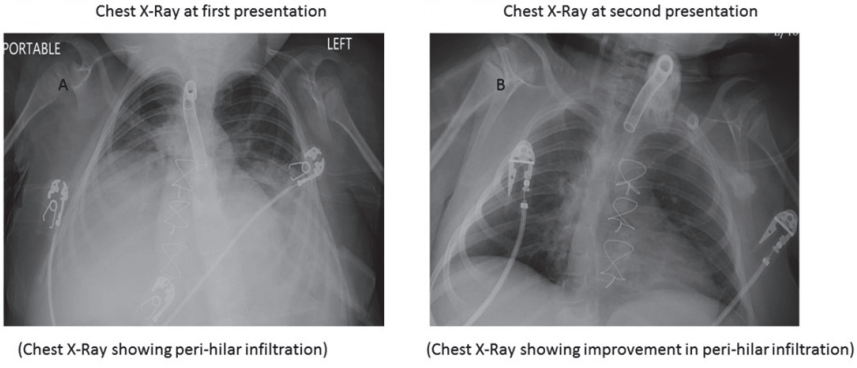

Figure 1. (**A**): Chest X-Ray (CXR): (Initial presentation) Perihilar infiltrates (Expiratory film) vs. (**B**): Second CXR with improvement in infiltrations.

The patient had a fever (38.3 °C) recurrence 23 days after initial positive SARS-CoV-2 and presumed pseudomonas pneumonia. He was hemodynamically stable but noted to have tachycardia (180 beats/min), frequent premature ventricular contractions, cyanosis (oxygen saturation was in the 80 s) for which he was admitted to the cardiac intensive care unit. A repeat CXR (Figure 1B) showed improved lung infiltrations compared to the previous one (Figure 1A). His electrocardiogram showed sinus tachycardia, and non-specific ST changes (Figure 2A) compared to his baseline (Figure 2B). His laboratory tests revealed a white blood cell count of 2.8×10^9/L, hemoglobin 10.5 g/L, platelets 161×10^9/L, and 23% of lymphocytes on the differential count. A complete metabolic panel showed no end-organ damage with normal creatinine and liver enzymes. His C-reactive protein was 88.45 mg/L (<10.01 mg/L), erythrocyte sedimentation rate 88 mm/h (2–34 mm/h), pro-calcitonin 1.5 ng/L (<0.05 ng/L), sedimentation rate 88 mm/h (2–34 mm/h), ferritin 35 ng/mL (22–322 ng/mL), brain natriuretic peptide 4218 pg/mL (0–124 pg/Ml), and troponin 1.2 ng/mL (<0.1 ng/mL). His echocardiogram showed normal biventricular systolic function and normal coronaries. His repeat blood, endotracheal aspirate, and urine cultures were negative. His repeat tracheal swab was negative for adenovirus, metapneumovirus, enterovirus, influenza A and B virus, parainfluenza type1-4 virus, bordetella pertussis, parapertusis, chlamydia, and mycoplasma. The differential diagnoses were presumed SARS-CoV-2 myocarditis or acute cardiac allograft rejection. He was treated with intravenous methylprednisolone 10 mg/kg/dose twice daily for three days. After starting the steroid, within 48 h, his heart rate returned to its baseline (60 s–70 s/min), and his inflammatory markers decreased significantly (erythrocyte sedimentation rate 32 mm/h, C-reactive protein 19 mg/L, and procalcitonin 0.07 ng/mL). His daily troponin trended downward, and at the end of the third day of the steroid pulse, troponin was 0.05 ng/mL, and brain natriuretic peptide was 52 pg/mL. His repeat echocardiogram showed no change in function. No changes to his immunosuppression regimen were made. His tacrolimus trough level was 4.8 ng/mL. He was discharged home four days after hospitalization on his home immunosuppression regimens and oral prednisone, which was tapered over two weeks and stopped. On follow-up after two weeks, he was doing well without recurrence of fever or tachycardia, and inflammatory markers and troponin were normal. His repeat tests for SARS-CoV-2 were positive at six weeks and then three months from his initial positivity. His CD4, CD8, CD3, CD19 cell counts, CD4:CD8 ratio, and immunoglobulin levels were within the normal limit. His serology returned positive for SARS-CoV-2 IgM, but IgG was negative three months from his first positive test for SARS-CoV-2. He became finally SARS-CoV-2 negative four months after his initial positive test and underwent

cardiac magnetic resonance (CMR) imaging as a part of surveillance that showed no myocardial edema, myocardial perfusion defects, or regional wall motion abnormalities at rest (Figure 3A). There was no evidence of delayed enhancement to suggest myocardial fibrosis or scarring (Figure 3B).

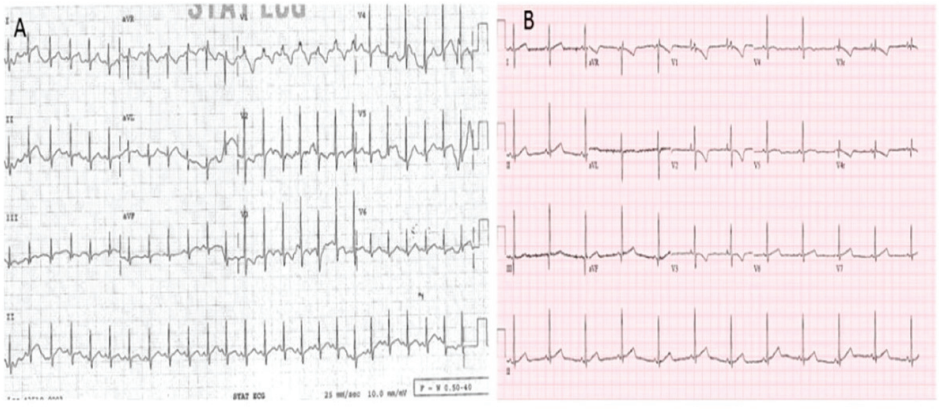

Figure 2. (**A**): A 12-lead ECG showing sinus tachycardia (150 bpm) and non-specific ST changes vs. baseline ECG (**B**): sinus rhythm with heart rate 70 bpm.

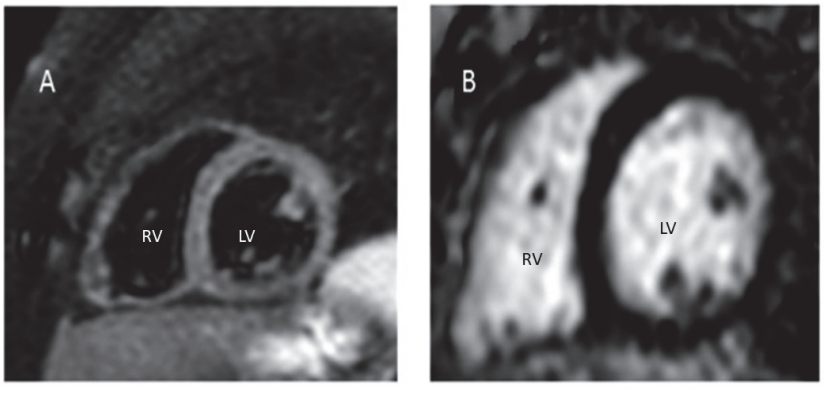

Figure 3. Short-axis T2-weighted image through the left ventricle base showing no early (**A**) or late-gadolinium enhancement (**B**).

3. Discussion

The patient described in this report satisfies multi-system inflammatory syndrome children (MIS-C) diagnosis criteria as defined by the CDC [6] with a history of SARS-CoV-2 infection recently, fever for more than 24 h, cardiac arrhythmia, and hypoxemia. This case is interesting because of the dilemma of whether this is a presentation of MIS-C or acute cardiac allograft rejection. Myocarditis appears less likely given the cardiac function is normal by echocardiogram. The intriguing part is that the full clinical manifestation of MIS-C can be masked while on immunosuppression [7] due to its presumed pathomechanism of hyperimmune response. This case probably proves the hypothesis that MIS-C represents a continuum of phenotypic severity from mild organ involvement to severe life-threatening complications such as cardiogenic shock. He responded dramatically to high-dose steroids despite the severe neurological injury, tracheostomy, and mechanical ventilation dependency. The diagnosis is unlikely to be myocarditis as the response to

steroids is usually not as dramatic in the case of myocarditis as in this case. In addition, the dramatic improvement in inflammatory biomarkers following intravenous steroid reinforces the diagnosis of MIS-C.

The clinical presentation after SARS-CoV-2 depends upon the immune response of the host. Whether immunosuppression alters the predisposition to acquiring infection with SARS-CoV-2 or if the disease implications are modified for better or worse remains uncertain. The SARS-CoV-2 virus hijacks the host cell machinery and can translate its proteins facilitating viral replication—the viral proteins and RNA released to lead to an interferon-dependent viral response. The unbalanced and excessive pro-inflammatory response can lead to MIS-C. On the other hand, an effective immune response to the Spike protein of SARS-CoV-2 helps contain the viral illness. Due to immunosuppressive treatment, transplant recipients are expected to be particularly susceptible to infection and a severe clinical COVID-19. Treatment of other viral infections in transplant patients often includes a reduction in immunosuppression. However, no current guidelines recommend the optimal approach to managing the treatment of SARS-CoV-2 infection. The case reports and small case series [1–5,7,8] published that have shown that the maintenance immunosuppression regimen is continued in these patients if COVID-19 is mild or asymptomatic. However, in renal transplantation patients, a steroid-sparing immunosuppression regimen is favored [9]. The immunosuppression medications may contribute to possible prolonged infectivity [8]. In this patient, his serological response to SARS-CoV-2 returned with no IgG response but IgM positive, and he remained positive for SARS-CoV-2 for three months. A normal serologic response (IgG positive) in adult solid organ transplant recipients with COVID-19 has been reported previously [10]. Given a lack of clear evidence on the immunological response to SARS-CoV-2 in the immunocompromised patient, the serological finding, as in this case, has implications for vaccine usefulness. It is unclear if immunocompromised patients will generate the intended immune response and need further study.

The case emphasizes the need to collect information further and study the impact of SARS-CoV-2 in different populations. It also demonstrates the potential clinical similarities between MIS-C and acute cardiac allograft rejection in a pediatric heart transplant recipient.

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Institutional Review Board Statement: Not applicable. Our IRB allow a single case report.

Informed Consent Statement: Informed consent is obtained from patient's father.

Data Availability Statement: Data sharing is not applicable.

Conflicts of Interest: The author has no conflict of interest to disclose.

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Brief Report

Effect of Remdesivir on COVID-19 PCR Positivity and Cycle Threshold in Kidney Transplant Recipients

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Abstract: Information regarding Coronavirus disease 2019 in the transplant population is lacking. Recently it has been suggested that cycle threshold values obtained on polymerase chain reaction tests may serve as a marker of disease severity with lower values (i.e., higher viral load) being associated with higher mortality. This study was done to assess the impact of remdesivir use on the time to a negative COVID-19 PCR as well as the degree of change between two Ct's based on treatment. A total of 30 kidney transplant patients with a new diagnosis of COVID-19 were assessed. Serial PCR results were followed from the time of diagnosis then every 2–4 weeks until negative. In patients who received remdesivir immediately after COVID-19 confirmation compared to no remdesivir, time to negative PCR was not statistically different with a median duration of 57 days in both groups ($p = 0.369$). The change in the Ct between the first and the second PCR test was also not statistically different between groups with a median change of 18.4 cycles in the remdesivir group and 15.7 cycles without remdesivir ($p = 0.516$). The results of this small single-center analysis suggest that remdesivir may not be beneficial in shortening time to a negative COVID-19 PCR.

Keywords: COVID-19; transplant; remdesivir; kidney; PCR; infection

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1. Introduction

Coronavirus disease 2019 (COVID-19) has an increased incidence and risk of severe infection among immunocompromised patients [1]. Transplant patients are also known to frequently have delayed clearance or prolonged shedding of respiratory viruses [2]. With this in mind, therapy that may reduce the severity and/or duration of illness are crucial. Currently, remdesivir is the only FDA approved anti-viral drug for COVID-19 although the evidence supporting its benefit is uncertain based upon published trials [3]. Recently it has been suggested that cycle threshold values (Ct) obtained on polymerase chain reaction (PCR) tests may serve as a marker of disease severity with lower values (i.e., higher viral load) being associated with higher mortality [4]. Similarly, reduction in SARS-CoV-2 viral load with treatment is now considered a valid surrogate marker of treatment efficacy [5]. This letter describes our center's experience with using remdesivir and/or reduced anti-metabolite dosing in an attempt to expedite clearance of the virus, as indirectly measured by serial PCR Ct testing. Patients with severe leukopenia (WBC < 3.0) were treated with elimination of anti-metabolite while the remainder received a 50% reduction in anti-metabolite dose [6].

2. Materials and Methods

This is a retrospective analysis using the electronic medical record. A total of 30 kidney transplant patients with a new diagnosis of COVID-19 as confirmed by PCR testing were included in this analysis. Patients were diagnosed between 31 March 2020—4 September 2020. Remdesivir was indicated in all patients for a (+) COVID-19 PCR and symptoms requiring presentation to receive care per clinical judgement of the transplant nephrologist. Serial PCR results were followed from the time of diagnosis and then every 2–4 weeks until becoming negative. Student's T-testing was performed on parametric continuous data, and Wilcoxon Signed Rank testing was employed when data were not guaranteed to be parametric. The chi-square or fisher's exact test was used for categorical data. Statistical analysis was performed using JMP PRO version 15.0.

3. Results

These data are presented in Table 1. In patients who received remdesivir immediately after COVID-19 confirmation compared to no remdesivir, the time to negative PCR was not statistically different with a median duration of 57 days in both groups (p = 0.369). The change in the Ct between the first and the second PCR test was also not statistically different between groups with a median change of 18.4 cycles in the remdesivir group and 15.7 cycles without remdesivir (p = 0.516). The median time between two PCRs in the remdesivir group was 23 days and 27 days in the no remdesivir group.

Table 1. Baseline Variables and Outcomes.

	Remdesivir (12)	**No Remdesivir (18)**	***p*-Value**
Baseline Variables			
Age (years)	57.5 [50, 67.8]	56 [41.5, 66]	0.582
Time from Transplant (months)	60 [19.5, 61]	71 [10, 115.5]	0.320
LDH (units/L)	245 [213, 299]	245 [193.5, 428]	1.0
Ferritin (ng/mL)	866 [198, 2237.8]	1843 [488, 2379.5]	0.430
WBC (10 e^9/L)	4.5 [1.6, 5.9]	5.5 [3.2, 7.4]	0.328
GFR (mL/min)	67 [54.5, 101.3]	53 [22, 76.5]	0.047
pO2 (%) [a]	97 [94, 98]	97 [95, 98.5]	0.878
CRP (mg/dL)	4.7 [2, 15.1]	0.95 [0.5, 6.1]	0.076
Anti-metabolite stopped	9 (75%)	8 (44%)	0.141
Initial Ct	19.2 ± 2.3	21.6 ± 1.9	0.568
Outcomes			
Time to (-) PCR (days) [c]	57 [46, 127]	57 [31, 101.5]	0.369
Change in Ct from PCR 1 to PCR 2 (Cycles) [b,c]	18.4 [9, 22.4]	15.7 [5.6, 17.9]	0.516

All medians compared using Wilcoxon rank sums for non-parametric data; [a] Missing 9 data points in "no remdesivir" arm; [b] 7 patients in "remdesivir" arm and 5 patients in "no remdesivir" arm; [c] SARS-CoV-2 PCR testing was performed using the following assays based on which reagents were available at the time: TaqPath COVID-19 Combo Kit (ThermoFisher), cobas SARS-CoV-2 Test (Roche), Xpert Xpress SARS-CoV-2 (Cepheid), or a lab developed test adapted from the CDC SARS-CoV-2 assay.

4. Discussion

The Adaptive COVID-19 Treatment Trial (ACTT-1) demonstrated a reduced time to recovery with use of remdesivir [7]. The World Health Organization recently published their interim results of antiviral drugs for COVID-19. In this analysis, the authors describe 2750 patients who were randomized to treatment with remdesivir. They concluded that none of the drugs assessed, including remdesivir, had an effect on overall mortality, initiation of ventilation, or length of stay [3]. While these large studies utilized clinical endpoints, little is known about the effect of remdesivir on viral load. Clearance of virus with negative or high Ct PCR is often of increased importance regarding disposition and isolation requirements, immunosuppression regimens, and transplant listing [8]. The results of this small single-center analysis suggest that remdesivir may not be beneficial in shortening

time to a negative COVID-19 PCR. As far as the change between two cycle thresholds after beginning remdesivir, our data suggest no difference, however our sample is small and likely underpowered. Our data are inadequate to determine drug efficacy; and is limited by a small sample size. Nevertheless, it suggests that an unnecessary admission or prolonged hospitalization for the sole purpose of remdesivir administration may not warranted.

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