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COVID-19 in heart transplant recipients: a multi-center analysis of the Northern Italian outbreak

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Abstract

Aims

The aim of this study was to assess the clinical course and outcomes of all heart transplant recipients affected by COVID-19 who were followed at the leading heart transplant centers of Northern Italy.

Method and Results

Since February 2020, we enrolled all 47 cases (79% male) in a first cohort of patients, with a mean age of 61.8 ± 14.5 years, who tested positive for SARS-CoV-2, out of 2676 heart transplant recipients alive before the onset of the COVID-19 pandemic at 7 heart transplant centers in Northern Italy. To date, 38 patients required hospitalization while 9 remained self-home quarantined and 14 died. Compared to the general population, prevalence (18 vs 7 cases per 1000) and related case fatality rate (29.7 vs 15.4%) in heart transplant recipients were doubled. Univariable analysis showed older age (p=0.002), diabetes mellitus (p=0.040), extracardiac arteriopathy (p=0.040), previous PCI (p=0.040), CAV score (p=0.039), lower GFR (p=0.004), and higher NYHA classes (p=0.023) were all significantly associated with in-hospital mortality. During the follow-up two patients died and a third patient has prolonged viral-shedding alternating positive and negative swabs.

Since 1st July 2020, we had 6 new patients who tested positive for SARS-CoV-2, 5 patients asymptomatic were self-quarantined, while 1 is still hospitalized for pneumonia. A standard therapy was maintained for all, except for the hospitalized patient.

Conclusion

The prevalence and mortality of SARS-CoV-2 should spur clinicians to immediately refer heart transplant recipients suspected as having SARS-CoV2 infection to centers specializing in the care of this vulnerable population.

Keywords

COVID-19 and Heart Transplant Recipients; Heart Transplantation; SARS-CoV-2; Immunosuppressive Therapy

Abbreviations

ALT	Alanine aminotransferase
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase
BMI	Body mass index
CAV	Cardiac allograft vasculopathy
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus Disease 19
CRP	C-reactive Protein
ECLS	Extracorporeal life support
GFR	Glomerular filtration rate
ICU	Intensive Care Unit
iLVEDV	Indexed left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
NYHA	New York Heart Association
PCI	Percutaneous coronary intervention
PCT	Procalcitonin

RT-PCR	Reverse transcriptase-polymerase chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome-Coronavirus-2
sPAP	Systolic pulmonary artery pressure
TAPSE	Tricuspid annular plane systolic excursion
WBC	White blood cells

Introduction

The worldwide SARS-CoV-2 pandemic has created unprecedented challenges for public health, demanding exceptional efforts for the successful management and treatment of affected patients. Northern Italy was the first region in Western Europe to experience a large outbreak of COVID-19 which has since rapidly spread throughout the world. As of the end of June 2020, more than 70% of the total amount of positive patients nationally and almost 80% of deaths occurred in this area (1).

In this context, information on the incidence and clinical characteristics of COVID-19 in heart transplant recipients are still limited (2, 3). Challenges remain in fully characterizing SARS-CoV-2 infection in this population given the scant literature available on the prevalence, clinical presentation, outcomes, and relationship between immunosuppressive therapy and severe forms of the disease.

Chronically immunosuppressed patients may have a greater viral burden, resulting in heightened infectivity and eventual worse prognosis. On the other hand, the interaction between immunomodulators and severe infection pathogenesis is still not fully understood. An abnormal immune response to the infection consisting of elevated cytokine production and perpetuation of a systemic inflammatory state may play a crucial role in the course of COVID-19 infection (4). Additionally, the lung damage observed in the severe forms of the disease may be related to hyperactivation of the immune response rather than the viral infection itself. Heart transplant patients represent a unique cohort of chronically immunosuppressed subjects in which SARS-CoV-2 may stimulate an unpredictable clinical course of infection. Scientific literature is still lacking organized and multi-institutional reports on this especially vulnerable population (2,3,5).

To date, this study provides the most comprehensive data on SARS-CoV-2 infection prevalence and clinical characteristics in heart transplant recipients. Here, we sought to identify clinical variables and therapeutic strategies associated with a worse outcome, focusing on immunosuppressive therapy modulation and its possible interactions with anti-inflammatory medication and antiviral drugs.

Methods

Study oversight

It is declared that every reasonable effort was made to obtain informed consent to participate in this study. Notably, the use of data for scientific and research purposes is already included in the informed consent agreements used at the participating centers. The local ethics committees approved of the study design, consent process, and review and analysis of the data.
We also guarantee the respect of anonymity and professional secrecy and use the collected data and the statistical analysis solely for the scientific purposes granted in accordance with the law in force (GDPR).

Study population

We designed a retrospective, observational, multicenter study across 7 leading heart transplant centers of Northern Italy: Milan, Padua, Bergamo, Bologna, Turin, Verona, Udine. All heart transplant recipients with positive nasopharyngeal RT-PCR tests for SARS-CoV-2 at these centers were included in this study.

Data sources

We obtained medical records and compiled data for hospitalized patients and outpatients with laboratory-proven COVID-19 infection. We extracted data on recent exposure history, clinical symptoms or signs, and laboratory findings on admission from electronic medical records. All laboratory testing and radiologic assessments were performed according to standard of care. Pertinent information was compiled into a unique dataset and forwarded to the data-processing coordination center at the University of Padua. A team of experienced heart transplant clinicians abstracted and reviewed all data. If the core data were missing, requests for clarification were sent back to each group's Principal Investigator for completion.

Study outcomes

Primary outcomes were COVID-19 infection prevalence and case fatality rate in heart transplant patients. Secondary outcomes were rate of hospitalization, ICU admissions, and hospital length of stay.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD). The Student's t-test for unpaired data or the Mann-Whitney U test were used to compare parametric and non-parametric continuous variables respectively (normal distribution was assessed by the Kolmogorov-Smirnov test). Categorical variables were presented as relative and absolute frequency. $\chi 2$ analysis or the

Fisher exact test were used to compare categorical variables where appropriate. A two-sided p-value of less than 0.05 was considered significant.

All statistical analyses were performed using SPSS software version 20 (IBM Corp., Armonk, NY,). Data are updated to July 1st, 2020.

Results

Out of 2676 heart transplant recipients alive before the onset of the COVID-19 pandemic at the end of February 2020, 47 patients were included in this analysis as part of a first cohort of patients. Among them, 2 patients had received combined heart-kidney transplants and 1 patient had undergone re-transplantation. This preliminary series reports baseline patient characteristics, features of COVID-19 onset and hospital course, laboratory results, and outcomes (Table 1).

Cohort 1 Analysis

Baseline and Risk Factors

All collected data are presented in Table 1. Mean age was 61.8 ± 14.5 years and mean time from heart transplantation was 10.5 ± 8.7 years; 79% of patients were male. The most frequent risk factors were arterial hypertension (64%) and dyslipidemia (47%). Mean BMI was 25.27 ± 5.80 kg/m^2 with 17% meeting the BMI criteria for obesity. Impaired renal function was present in the majority of subjects, with a mean eGFR of 48.17 ± 32.14 ml/min (calculated with CKD-EPI) and 13% of patients had end-stage kidney disease requiring hemodialysis; 91% of cases were in NYHA class I-II. The most common immunosuppressive regimen consisted of cyclosporine combined with mycophenolate mofetil. Almost 50% of patients received corticosteroids. Heart allograft function, assessed by last available echocardiogram, was preserved in all subjects.

COVID-19 Onset

All heart transplant recipients enrolled had a positive RT-PCR nasopharyngeal swab test for SARS-CoV-2 with 93% of being symptomatic. The most frequent symptom was fever (87%), followed by cough (70%) and shortness of breath (70%). Mean duration of symptoms was 9.80 ± 7.31 days. In febrile patients, the mean fever peak was 38.2 ± 0.6°C and fever lasted for a mean of 7.5 ± 3.8 days. In 72% of cases, radiographic signs of pneumonia were present. These data are summarized in Table 2.

In Hospital stay

Pertinent data are summarized in Tables 2-3. Of 47 subjects 38 (81%) required hospitalization, with a mean length of stay of 17.79 ± 10.70 days; 4 (9%) patients required intensive care unit stay. Mean O_2 saturation at admission was 92 ± 6%. Thirteen (28%) patients developed ARDS. Non-invasive and invasive ventilation were required in 15 (32%) and 2 (4%) patients, respectively. Only 1 patient required a tracheostomy for failure of weaning from ventilatory support, and 1 patient was treated with nitric oxide. Pronation therapy was performed for 3 patients. Vasopressor support with noradrenaline was required in 3 patients due to septic shock; ECLS was never used. Major adverse events observed were superimposed bacterial infection requiring targeted antibiotic treatment (11%) and sepsis (9%). During hospitalization, allograft function was assessed by laboratory and echocardiographic monitoring which did not show evidence of myocardial dysfunction or injury (Table 2). For this analysis we report the lowest echocardiographic LVEF and the peak troponin I levels. Mean LVEF during hospitalization was 59.1 ± 6.7% and comparable to that measured in the last pre-COVID-19 echocardiogram (p=0.201). No clinically significant allograft rejections were observed.

Of the 38 hospitalized patients, 14 (37%) died and 24 (63%) have been discharged home. Cause of death for the deceased patients was respiratory failure in all cases with the exception of one who died due to multi-organ failure.

Pharmacological Treatment

More than 80% of patients received hydroxychloroquine, while antiretroviral therapy with ritonavir/lopinavir was used 50% of the subjects. Prophylaxis with broad spectrum antibiotics was administered in 83% of cases, mainly in the form of beta-lactamases and macrolides (Table 1). One patient received a monoclonal antibody against IL-6 (tocilizumab). A corticosteroid administration as bolus medication was performed in 10 patients (21%).

Doses of immunosuppressive drugs were reduced in all 38 (100%) hospitalized patients. Mycophenolate mofetil dose was decreased in 57% of patients, everolimus in 25%, cyclosporine in 18% tacrolimus in 5%, and azathioprine in all patients receiving this drug. Thirteen (28%) patients received anticoagulation with low molecular weight heparin. Laboratory findings during hospitalization are shown in Table 3.

Non-hospitalized SARS-CoV-2 positive heart transplant recipients:

Nine patients in our cohort were either asymptomatic or had mild symptoms. These patients did not require hospitalization, but were instructed to self-quarantine at home according to a protocol which included frequent telephone monitoring by the corresponding heart transplant team. Compared to reminder of the population, the non-hospitalized patients exhibited significant differences in mean age (p=0.002), eGFR (p<0.001), and prednisone use (p=0.033) (Table 1). Cyclosporine and mycophenolate mofetil were the most common immunosuppression regimen, and 67% of patients in this group also received corticosteroids. At COVID-19 onset, 5 (71%) recipients were symptomatic, presenting mostly with fever (78%) and cough (67%); median oxygen saturation at onset was 98 ± 1%. Hydroxychloroquine was administered to 3 (33%) patients, ritonavir/lopinavir to 2 (22%), and antibiotic prophylaxis to 4 (44%). Unlike hospitalized patients, 4 (44%) home self-quarantined patients continued their immunosuppressive regimen without adjustment. In the remainder of the non-hospitalized subgroup, mycophenolate mofetil was reduced in 50% of the patients, everolimus in 50%, while the only patient receiving tacrolimus had his dosage halved. Cyclosporine and prednisone doses were unchanged. All patients had a rapid resolution of symptoms and did not require further treatment.

Univariable survival analysis:

Univariable analysis (Table 1) of baseline characteristics showed older age (p=0,002), diabetes mellitus (p=0.040), peripheral vascular disease (p=0.040), previous PCI (p=0.040), CAV score (p=0.039), lower eGFR (p=0.004) and higher NYHA class (p=0.023) were all significantly associated with in-hospital mortality. Chronic immunosuppressive therapy was similar in survivors and non-survivors. Symptoms at COVID-19 onset were similar with the exception of shortness of breath (p=0.004). In the subgroup of deceased patients, radiographic signs of pneumonia (p=0.005) and ARDS (p<0.001) were more frequent with a lower O_2 saturation at admission (p<0.001) and lowest value during hospitalization (p<0.001). During hospitalization, non-survivors required non-invasive ventilation at higher rates (p<0.001). Immunosuppressive therapy adjustment and use of anti-inflammatory and antiretroviral drugs were similar between survivors and patients who succumbed to COVID-19. We observed a significant difference in length of hospital stay (23.2±8.9 vs 8.5±6.2 days, p<0.001) between survivors and non-survivors.

Early-Mid Term follow up analysis:

During the follow-up two patients died and a third patient has prolonged viral-shedding alternating positive and negative swabs in absence of symptoms and not requiring hospitalization. An asymptomatic patient previously self-home quarantined with no therapy modifications and

negativized swab test, died due to CMV pneumonia. The second patient, who was treated by reducing the immunosuppressive regimen and discharged home with standard therapy, two months later was re-hospitalized due to cardiogenic shock for suspected allograft rejection. No endomyocardial biopsy was performed. He was treated with thymoglobulin infusion but he was complicated with pneumonia and cholecystitis and died due to septic shock. He had negative nasopharyngeal swab test for SARS-CoV-2. All the other patients are doing well and are strictly monitored.

Cohort 2 Analysis

From 1st July 2020, we had 6 new patients with a positive test for SARS-CoV-2 . Five patients are self-quarantined, 1 is still hospitalized for pneumonia. Patients at home maintained immunosuppressive therapy unchanged and antibiotic therapy was not administered if they were afebrile. The hospitalized patient had fever and bilateral interstitial pneumonia for which immunosuppressive therapy was reduced and prophylactic antibiotic therapy was added along with supplemental oxygen therapy. Neither antiviral drugs nor hydroxychloroquine were administered (Table 4).

Discussion

We present the largest retrospective and first Italian multicenter case series of heart transplant patients with COVID-19. We enrolled totally 53 patients with documented SARS-CoV-2 infection amongst 2676 heart transplant recipients alive at the onset of COVID-19 pandemic from 7 leading heart transplant centers in Northern Italy. The close collaboration between the participating centers enabled the collection of data from a unique cohort of heart transplant recipients experiencing the infection early in the SARS-CoV-2 pandemic. The timing of infection permits to analyze unique aspects of the clinical course and outcomes of this disease. Furthermore, by assessing the clinical conditions at admission, chronic immunosuppressive therapy and its modulation, supportive measures during hospitalization and specific COVID-19 treatments, we were able to identify baseline characteristics and medical interventions that could impact patients' prognosis.

To date, in the Italian regions considered (Lombardy, Veneto, Emilia-Romagna, Piedmont, and Friuli Venezia Giulia), the prevalence of the disease in the overall population accounts for 7 cases per 1000 people (1). The median age of all Italian positive cases with COVID-19 is 61 years and 54% of those affected are female (1). Among deaths, the median age was 82 (0-109) years and 57%

were male. Arterial hypertension (66%), diabetes mellitus (30%) and ischemic heart disease (28%) were the most common pre-existing conditions. More than 60% of the deaths had 3 or more diseases at the time of infection. Fever (75%), along with dyspnoea (73%) and cough (38%), was the most common presenting symptom (1).

In our cohort of heart transplant recipients, we observed a two-fold prevalence of infection, approximately 17 cases per 1000 individuals. Although solid organ transplant recipients are typically more aware of infection prevention and use of personal protective equipment, the prevalence of COVID-19 in this population was higher than in the general population. This higher prevalence is likely the result of the higher susceptibility of heart transplant recipients to infections due to their chronic immunosuppressed state. In Northern Italy, the case-fatality rate of the overall population is 15.4%, whereas that of our heart transplant patients was 29.7%. The Italian population older than 70 years accounts for 85% of all COVID-19 related deaths with a case fatality rate greater than 26% (1). The case fatality rate of heart transplant recipients with COVID-19 are similar to the 25% mortality reported by Latif et al. (3) in their cohort. A recent meta-analysis of COVID-19 cases showed a greater risk of developing severe and lethal disease in males, especially if they are older than 65 years and active smokers. Comorbidities such as hypertension, diabetes and cardiovascular and respiratory disease can also influence prognosis (6). Our findings confirm these observations. According to our univariable analysis, age, higher NYHA class, diabetes, chronic kidney disease, peripheral vascular disease, CAV and lower oxygen saturation at admission (related to severe pulmonary involvement and delay in hospital referral), may account for the observed higher case-fatality rate. In the non-surviving population, diabetes-related cardiovascular effects are exemplified by the higher rate of extracardiac arteriopathy and previous PCI as well as a lower mean eGFR. As reported in a meta-analysis by Li et al., diabetes mellitus was two-fold more frequent in ICU/severe cases than in non-diabetic individuals (7). Diabetes mellitus itself can also be with an abnormal immune response characterized by increased cytokine production and inhibition of macrophages and T-cell activation, which leads to higher susceptibility to infection (8). Impaired glycemic control is also associated with an increased risk of bacterial co-infection and pneumonia (9).

Differences in age, eGFR and prednisone use were observed between hospitalized and non-hospitalized patients, but the relevance of these findings remains unknown due to the small sample size of this study.

The majority (81%) of patients in this case series was admitted to the hospital because of fever and hypoxemic respiratory failure. Of the individuals with severe primary pulmonary involvement, 32% only needed non-invasive ventilation whereas only 4% required mechanical ventilation. Nevertheless mortality rate was much higher (30%) for patients with primarily pulmonary presentation and, in fact, respiratory failure was their leading cause of death. These poor outcomes may be related to the rapidity with which the pandemic spread in Northern Italy, leading to a critical shortage of ICU beds over a period of only a few days. In these circumstances, heroic efforts were made to manage very ill patients in non-acute care settings. It is plausible that lack of healthcare resources in the midst of an overwhelming emergency may have contributed to the high mortality rate of our patients' cohort.

Elimination of immunosuppressive therapy has been reported in few cases of solid organ transplant patients infected with the SARS-CoV-2 virus (10). However, we hypothesized that suspension could lead to allograft failure due to rebound activation of immunological memory, potentially exacerbated by the hyperinflammatory state. Instead, the center participating in this prospective observational study elected to reduce immunosuppressive therapy in markedly lymphopenic and febrile patients so that the components of acquired immune could be recruited against the viral infection. While we are aware of the ongoing controversies surrounding the use of hydroxychloroquine, this drug was used in most patients based on its anti-inflammatory activity (11). Despite the lack of agreement in the scientific community and awareness of drug interactions with calcineurin and mTOR inhibitors (12) antiretroviral therapy with ritonavir/lopinavir was used in nearly 50% of the patients. In only one case we used tocilizumab, a monoclonal antibody against IL-6. Tocilizumab is currently used in some forms of cytokine-release-syndromes and is currently under investigation for treatment of COVID-19, though clear benefits have not yet been demonstrated (13).

We observed no COVID-19 related myocarditis. Although endomyocardial biopsies have not been performed due to the enormously strained healthcare resources, persistently normal LVEF, only slight changes in myocardial biomarkers and lack of requirement for mechanical circulatory support, are consistent with the belief that COVID-19 myocarditis did not occur in our heart transplant recipients.

Broad spectrum prophylactic antibiotics were used to avoid superimposed bacterial infection, the risk of which was heightened by lymphocytopenia and protracted hospitalization. Beta-lactamases

and macrolides were predominantly used and a second line of targeted antibiotic therapy was used when a specific pathogen was identified. The fact that of five patients with confirmed superimposed bacterial infections four developed sepsis and two died, confirms the wisdom of our approach which may have averted more deaths related to superimposed bacterial infections.

In patients with severe disease, we used therapeutic doses of low molecular weight heparin to prevent venous thromboembolic risk. Coagulopathy with microcirculation thrombophilia in severe forms is related to hyperinflammatory state due to the infection itself and correlates with the poorest outcomes (14). Tests of aggregometry and thromboelastography were not performed due to the urgency of the situation and the scarcity of resources.

During the follow-up, unfortunately, we observed the occurrence of two new deaths and a second wave of infections involving 6 new patients.

Regarding the two deaths, the first patient was self-quarantined and his immunosuppressive regimen was left unchanged. He died due to CMV related pulmonary infection. It can be hypothesized that an alteration in the immunosuppressive state has favored the CMV replication. The second patient, in whom immunosuppressive therapy was previously reduced, was discharged home with standard therapy. Probably, this was enough to trigger a rejection that proved itself later, with cardiogenic shock. The death then occurred from complications following hospitalization. Immunosuppressant plasma levels should be strictly monitored after therapy modifications during the disease and follow-up.

As for the new infections we registered, in 5 cases, being asymptomatic, as in the previous cases described, we did not modify the immunosuppressive therapy, we did not add antiviral drugs and we treated the patients with careful home monitoring. In one case, where pulmonary involvement was more important, the patient was hospitalized and treated as discussed above by reducing immunosuppressive therapy and adding antibiotics to avoid opportunistic infections. The clinical characteristics of the patients self-quarantined were comparable regardless the cohort considered.

The data presented here highlights that heart transplant recipients are extremely vulnerable to the unfavorable consequences of the COVID-19 infection. The fact that some patients have been successfully treated during self-quarantine at home does not warrant lowering the attention of care, and indeed underlines the importance of early referral of infected heart transplant recipients to their

respective programs. In fact, there is a risk, even if the cases observed are few in our experience, that on the one hand we go towards a state of excessive immunosuppression with the evidence of opportunistic infections, on the other towards a state of under-immunosuppression with the possibility of rejection.

The high mortality we observed seems to be unrelated to allograft dysfunction or rejection. The reduction of immunosuppression in severe forms of disease is crucial for two fundamental aspects: first, promoting the immune response against viral infection, and secondly, reducing the risk of superinfections related to hospitalization. For future hospitalized heart transplant recipients with COVID-19, our strategy will consist in reduction of immunosuppression and antibiotic prophylaxis, along with the best supportive care available. We believe that in the asymptomatic forms, immunosuppressive therapy may remain unchanged until the first symptoms emerge. One of the main findings, in the light of this experience, is that there is always a careful monitoring of the patient, because at any moment he can take different paths. Furthermore, targeted SARS-CoV-2 antiviral drugs seem to be closer, hoping that they will soon be available for the physician.

Study limitation

We acknowledge the several limitations of this study. Firstly, in the absence of a control group, despite enrolling the largest series to date of heart transplant recipients with COVID-19, the study is observational in nature. The cohort presented may not represent the heart transplant population at large, because of its geographic restriction to Northern Italy and their diagnosis early in the course of the pandemic. The prevalence of SARS-CoV-2 infection may have been underestimated because asymptomatic heart transplant recipients were not tested. As mounting evidences are emerging about the absence of benefits of using anti-inflammatory or antiviral therapies (11), some of the treatments proposed for our patients may seem outdated. These limitations should be viewed in the context of a rapidly spreading infection that overwhelmed the Northern Italian Health System, despite the heroic efforts of its healthcare providers.

Conclusion

Heart transplant recipients are especially vulnerable to infection with SARS-CoV-2 infection and experienced a twofold higher mortality compared to the general population. Specific risk factors have been identified for severe disease and higher mortality. The wisdom of reducing the intensity of immunosuppression and of using anti-inflammatory and antibiotic prophylaxis requires study in

larger populations. The severity of COVID-19 disease in heart transplant recipients underscores the critical importance of early and timely referral to specialized heart transplant centers. Despite the acknowledged limitations, this study evaluated the largest population of heart transplant recipients infected with SARS-CoV-2 to date.

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Clinical perspectives

Data on heart transplanted patients in the COVID-19 era are still very limited. This is a retrospective, observational, and multicentric study, which have included the leading heart transplant Centers of Northern Italy, where the SARS-CoV-2 pandemia has been more devasting. We included all heart transplanted patients positive to SARS-CoV-2 at these Centers. Thus, this article is the study with largest comprehensive data, among those already published, on the SARS-CoV-2 infection prevalence and clinical presentation.

The risk of SARS-CoV-2 infection in patients treated by immunosuppressive agents is double in comparison with general population. Even the mortality resulted doubled in the heart transplant population. The reduction of immunosuppressive therapy, associated to hydroxychloroquine and broad-spectrum antibiotics administration, has been the common strategy, and it accounted for a mortality of 30%.

Translational outlook

The dataset created for the pandemic emergency is prospective, and we are collecting data relating to new cases, as well as the clinical evolution of those already recognized as infected.
Future studies will concern the effects of the reduction of immunosuppressive therapy, and the systemic effects of the Covid-19 virus by controlling the immune response elicited by the virus itself in each patient.

References

1. Istituto Superiore di Sanità. Sorveglianza Integrata COVID-19 in Italia, aggiornamento del 30 Giugno 2020. https://www.epicentro.iss.it/coronavirus/sars-cov-2-dashboard (30 June 2020).

2. Fei Li, Jie Cai , Nianguo Dong. s.l. First Cases of COVID-19 in Heart Transplantation From China. Journal of Heart and Lung Transplantation, 2020. doi:10.1016/j.healun.2020.03.006

3. Latif F, Farr MA, Clerkin KJ, Habal MV, Takeda K, Naka Y et al. Characteristics and Outcomes of Recipients of Heart Transplant With Coronavirus Disease 2019. JAMA Cardiol. 2020 May 13:e202159.

4. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ and HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet, 2020 Mar, Vols. 395(10229):1033-1034.

5. Pereira MR, Mohan S, Cohen DJ, Husain SA, Dube GK, Ratner LE, Arcasoy S, Aversa MM, Benvenuto LJ, Dadhani D, Kapur S, Dove LM, Brown RS, Rosenblatt RE, Samstein B, Uriel N, Farr MA, Satlin M, Small CB, Walsh T, Kodiyanplakkal RP, Miko BA, Aaron JG, Tsapepas DS, Emond JC, Verna EC COVID-19 in Solid Organ Transplant Recipients: Initial Report from the US Epicenter. Am J Transplant. 2020 Apr 24 doi:10.1111/ajt.15941

6. Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, Li Q, Jiang C, Zhou Y, Liu S, Ye C, Zhang P, Xing Y, Guo H, Tang W.. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. J Infect. 2020 Apr 23. pii: S0163-4453(20)30234-6. doi:10.1016/j.jinf.2020.04.021

7. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clin Res Cardiol. 2020 May;109(5):531-538

8. Critchley JA, Carey IM, Harris T, DeWilde S, Hosking FJ, Cook DG. Glycemic Control and Risk of Infections Among People With Type 1 or Type 2 Diabetes in a Large Primary Care Cohort Study. Diabetes Care. 2018 Oct;41(10):2127-2135

9. Ferlita S, Yegiazaryan A, Noori N, Lal G, Nguyen T, To K, Venketaraman V. Type 2 Diabetes Mellitus and Altered Immune System Leading to Susceptibility to Pathogens, Especially Mycobacterium tuberculosis. J Clin Med. 2019 Dec 16;8(12)

10. Zhu L, Xu X, Ma K, Yang J, Guan H, Chen S, Chen Z, Chen G.. Successful recovery of COVID-19 pneumonia in a renal transplant recipient with long-term immunosuppression. Am J Transplant, 2020 Mar 17. doi:10.1111/ajt.15869

11. FDA Letter Revoking of Emergency Use Authorization for Emergency Use of Chloroquine Phosphate and Hydroxychloroquine Sulfate, June 15, 2020. U.S. Food & Drug Administration. https://www.fda.gov/media/138945/download. June 15, 2020.

12. Elens L, Langman LJ, Hesselink DA, Bergan S, Moes DJAR, Molinaro M, Venkataramanan R, Lemaitre F.. Pharmacologic treatment of transplant recipients infected with SARS-CoV-2: considerations regarding therapeutic drug monitoring and drug-drug interactions [published online ahead of print, 2020 Apr 15]. Ther Drug Monit. 2020; doi:10.1097/FTD.0000000000000761

13. Alzghari SK, Acuña VS. Supportive Treatment with Tocilizumab for COVID-19: A Systematic Review. J Clin Virol. 2020 Apr 21;127:104380. Doi: 10.1016/j.jcv.2020.104380

14. Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, Navalesi P, Simioni P. COVID-19-Related Severe Hypercoagulability in Patients Admitted to Intensive Care Unit for Acute Respiratory Failure. Thromb Haemost. 2020 Apr 21. doi:10.1055/s-0040-1710018

Central Illustration. SARS-CoV-2 in Heart Transplant Recipients: A multicenter analysis in the Northern Italy

Table 1

	All (n=47)	Survivors (n = 33)	Non Survivors (n = 14)	p	Home self-quarantined patients (n=9)	Hospitalized patients (n=38)	p
Demographics							
Age (years)	61,84±14,51	57,67±14,87	71,67±7,24	0,002	48,64±17,34	64,97±12,02	0,002
Sex (male)	37(79%)	25(76%)	12(86%)	0,700	6(67%)	31(82%)	0,377
Time from Htx (years)	10,46±8,70	9,46±8,61	12,75±8,75	0,242	6,55±9,81	11,29±8,35	0,164
Risk Factors							
BMI (kg/m2)	25,27±4,80	25,32±4,21	25,15±6,13	0,915	25,82±4,10	25,13±4,99	0,703
Obesity	8(17%)	5 (15%)	3 (21%)	0,679	1(11%)	7(18%)	0,600
Arterial Hypertension	30(64%)	22(67%)	8(57%)	0,741	5(56%)	25(66%)	0,704
Dyslipidemia	22(47%)	14(42%)	8(57%)	0,524	4(44%)	18(47%)	0,874
Diabetes Mellitus	8(17%)	3(9%)	5(36%)	0,040	1(11%)	7(18%)	0,600
Former smoker	11(23%)	8(24%)	3(21%)	0,835	3(33%)	8(21%)	0,419
Peripheral vascular disease	8(17%)	3(9%)	5(36%)	0,040	0(0%)	8(21%)	0,323
COPD	3(6%)	2(6%)	1(7%)	0,890	0(0%)	3(8%)	0,384
Stroke	1(2%)	0(0%)	1(7%)	0,298	0(0%)	1(2%)	0,623
Malignancy	5(11%)	3(9%)	2(14%)	0,627	2(22%)	3(8%)	0,240
Dialysis	6(13%)	4(12%)	2(14%)	0,839	0(0%)	6(16%)	0,579

GFR (ml/min)	48,17±32,14	55,00±33,93	30,82±18,72	0,004	82,90±35,30	39,72±25,29	<0,001
Previous PCI	11(23%)	5(15%)	6(36%)	0,040	0(0%)	11(29%)	0,092
CMR	7(15%)	4(12%)	3(21%)	0,404	1(11%)	6(16%)	0,771
AMR	1(2%)	0(0%)	1(7%)	0,295	0(0%)	1(3%)	0,633
CAV Score	0,41±0,89	0,17±0,59	1,00±1,21	0,039	0,14±0,38	0,46±0,95	0,398
NYHA class				0,023			0,218
I	35(74%)	28(85%)	7(50%)		8(100%)	27(71%)	
II	8(17%)	3(9%)	5(36%)		0(0%)	8(21%)	
III	3(6%)	1(3%)	2(14%)		0(0%)	3(8%)	
IV	0(0%)	0(0%)	0(0%)		0(0%)	0(0%)	
LVEF (%)	54,48±10,38	56,72±9,04	48,71±12,05	0,083	56,40±9,48	54,00±10,77	0,654
Immunosoppressive Therapy							
Cyclosporine	34(81%)	21(64%)	13(93%)	0,073	6(67%)	28(74%)	0,939
Tacrolimus	12(26%)	11(33%)	1(7%)	0,073	2(22%)	10(26%)	0,939
Prednisone	19(40%)	14(42%)	5(36%)	0,749	6(67%)	13(34%)	0,033
Mycophenolate	26(55%)	18(55%)	8(57%)	0,955	5(56%)	22(58%)	0,707
Everolimus	12(26%)	8(24%)	4(29%)	0,800	2(22%)	10(26%)	0,939
Azathioprine	3(6%)	1(3%)	2(14%)	0,216	0(0%)	3(8%)	0,411
Anticoagulant Therapy	4(9%)	3(9%)	1(7%)	0,782	0(0%)	4(11%)	0,330
Laboratory Results at last FU							
WBC count	6,91±2,75	6,67±2,82	7,59±2,63	0,46	5,56±2,54	7,30±2,74	0,176

	(cells per 10^9/L)					1					
	Lymphocyte (cells per 10^9/L)		1,71±1,05		1,57±0,97	2,13±1,22	0,234		1,24±0,69	1,85±1,10	0,217	

Baseline characteristics of heart transplant population with COVID-19. Abbreviations: BMI, body mass index; COPD chronic obstructive pulmonary disease; GFR, glomerular filtration rate; PCI, percutaneous coronary intervention; NYHA, New York Heart Association; FU, follow up; LVEF, left ventricular ejection fraction; WBC, white blood cells.

Table 2

	All (n=47)	Survivors (n = 33)	Non Survivors (n = 14)	p
COVID-19 onset				
Presenting symptoms	44(93%)	30(91%)	14(100%)	0,544
Fever	41(87%)	27(82%)	14(100%)	0,159
Cough	33(70%)	21(64%)	12(86%)	0,175
Shortness of breath	33(70%)	19(58%)	14(100%)	0,004
Myalgia	21(45%)	15(45%)	6(43%)	0,870
Headache	9(19%)	5(15%)	4(29%)	0,419
Anosmia	7(15%)	7(21%)	0(0%)	0,166
Sinusitis	3(6%)	3(9%)	0(0%)	0,548
Gastrointestinal	10(21%)	9(27%)	1(7%)	0,242
Nasopharyngeal swab test positive	47(100%)	33(100%)	14(100%)	1,000
X-ray Pneumonia	34(72%)	20(61%)	14(100%)	0,005
Fever peak (°C)	38,16±0,56	38,02±0,55	38,40±0,53	0,041
Duration of fever (days)	7,46±3,79	6,58±2,90	9,08±4,75	0,141
Duration of symptoms (days)	9,80±7,31	10,05±7,82	9,00±5,93	0,766
Hospitalization	38(81%)	24(73%)	14(100%)	0,030
SpO2 at admission (%)	91,90±5,92	93,93±5,04	86,55±4,68	<0,001
Worst SpO2 during hositalization (%)	87,81±8,95	91,30±7,49	79,27±6,13	<0,001
Acute Respiratory Distress Syndrome	13(28%)	2(6%)	11(79%)	<0,001
Laboratory Results at Admission				
WBC count (cells per 10^9/L)	5,57±3,03	5,06±2,70	7,47±3,63	0,061
Hb (g/dl)	11,00±2,27	11,17±2,42	10,57±1,85	0,489
Platelets (cells per 10^9/L)	180,82±69,79	184,71±72,52	155,50±60,10	0,600
Lymphocyte (cells per 10^9/L)	1,61±2,17	1,73±2,28	0,70±0,53	0,544
Eosinophilis (cells per 10^9/L)	0,15±0,35	0,16±0,36	0,18±0,41	0,933
CRP (mg/dl)	31,58±42,45	26,22±32,53	50,70±67,33	0,383
PCT (ng/ml)	1,14±2,53	0,51±1,12	4,56±6,07	0,519
Serum creatinine (mg/dl)	3,28±3,98	3,26±4,54	3,34±2,26	0,149
Serum Creatinine in hospital peak (mg/dl)	3,12±2,36	2,70±2,31	4,14±2,27	0,034
AST (U/L)	25,69±15,85	21,01±11,19	46,00±19,29	0,008

	ALT (U/L)		20,88±15,57		17,08±9,21	32,25±25,91	0,092	
	TnI in hospital peak		41,85±88,18		13,07±6,67	99,40±144,58	0,253	

COVID-19 onset characteristics among heart transplant population. Abbreviations: SpO2, oxygen saturation; WBC, white blood cells; Hb, haemoglobin; CRP, c-reactive protein; PCT, procalcitonin; AST, aspartate aminotransferase; ALT, alanine transaminase; TnI, troponin I.

Table 3

	All (n=47)	Survivors (n = 33)	Non Survivors (n = 14)	p
Treatment and outcome				
Hydroxychloroquine	38(81%)	24(73%)	14(100%)	0,155
Lopinavir/Ritonavir	21(45%)	13(39%)	8(57%)	0,526
Tocilizumab	1(2%)	1(3%)	0(0%)	0,482
Iperimmune Plasma	0(0%)	0(0%)	0(0%)	1,000
Corticosteroid bolus therapy	10(21%)	8(24%)	2(14%)	0,361
Management of Immunosoppressive Therapy [a]				
Reduction Cyclosporine dose (n=34)	6(18%)	3(14%)	3(30%)	0,653
Reduction Tacrolimus dose (n=12)	5(42%)	5(45%)	0(0%)	0,377
Reduction Mycophenolate dose (n=30)	17(57%)	13(62%)	4(44%)	0,623
Reduction Everolimus dose (n=12)	3(25%)	2(25%)	1(25%)	1,000
Reduction Azathioprine dose (n=3)	3(100%)	1(100%)	2(100%)	1,000
Antibiotics prophylaxis	39(83%)	26(79%)	13(93%)	0,735
Anticoagulant Therapy	13(28%)	11(33%)	2(14%)	0,282
ICU stay	4(9%)	2(6%)	2(14%)	0,616
NIV	15(32%)	4(12%)	11(79%)	<0,001
Invasive Ventilation	2(4%)	0(0%)	2(14%)	0,129
Pronation	3(6%)	2(6%)	1(7%)	0,896
CVVH	0(0%)	0(0%)	1(7%)	0,368
ECMO support	0(0%)	0(0%)	0(0%)	1,000
Inotropic Support	3(6%)	0(0%)	3(21%)	0,043
Nitric Oxide	1(2%)	0(0%)	1(7%)	0,310
Complications				
Neurological	1(2%)	0(0%)	1(7%)	0,333
Gastrointestinal	1(2%)	1(3%)	0(0%)	0,464
Infective				
Bacterial co-infection	5(11%)	2(%6)	3(21%)	0,307
Fungal co-infection	0(0%)	0(0%)	0(0%)	1,000
Viral co-infection	0(0%)	0(0%)	0(0%)	1,000
Sepsis	4(9%)	2(6%)	2(14%)	0,307
In Hospital length of stay (days)	17,79±10,70	23,21±8,91	8,50±6,17	<0,001

Treatment and outcome of heart transplant population with COVID-19. Abbreviations: ICU, intensive care unit; NIV, non-invasive ventilation; CVVH, continuous veno-venous hemofiltration; ECMO, Extracorporeal membrane oxygenation.

[a] percentage of patients with a reduction in immunosuppressant dosage.

Table 4

	All (n=6)
Demographics	
Age (years)	59,0(48,3-73,5)
Sex (male)	4(67%)
Time from Htx (years)	7,8(3,7 – 18,3)
Risk Factors	
BMI (kg/m2)	26,9(22,7-29,9)
Obesity	2(33%)
Arterial Hypertension	4(67%)
Dyslipidemia	2(33%)
Diabetes Mellitus	2(33%)
Former smoker	0(0%)
Peripheral vascular disease	0(0%)
COPD	1(17%)
Stroke	0(0%)
Malignancy	2(33%)
Dialysis	0(0%)
GFR (ml/min)	43,5(39,0-73,5)
Previous PCI	1(17%)
CMR	1(17%)
AMR	0(0%)

	CAV Score	0(0-3)	
	NYHA class		
	I	4(66%)	
	II	2(33%)	
	III	0	
	IV	0	
	LVEF (%)	58,5(55,8-62,8)	
	Immunosoppressive Therapy		
	Cyclosporine	3(50%)	
	Tacrolimus	1(17%)	
	Prednisone	3(50%)	
	Mycophenolate	4(67%)	
	Everolimus	1(17%)	
	Azathioprine	0(0%)	
	Anticoagulant Therapy	0(0%)	
	Laboratory Results at last FU		
	WBC count (cells per 10^9/L)	5,48(5,11-6,66)	
	Lymphocyte (cells per 10^9/L)	1,22(0,92-1,77)	
	COVID-19 onset		
	Presenting symptoms	4(66%)	
	Fever	4(66%)	
	Cough	2(33%)	
	Shortness of breath	2(33%)	

	Myalgia	2(33%)	
	Headache	1(17%)	
	Anosmia	0(0%)	
	Sinusitis	0(0%)	
	Gastrointestinal	0(0%)	
	Nasopharyngeal swab test positive	6(100%)	
	X-ray Pneumonia	1(17%)	
	Fever peak (°C)	38,1(37,7-38,4)	
	Duration of fever (days)	4,8(4,5-5,0)	
	Duration of symptoms (days)	6,0(4,8-7,0)	
	Hospitalization	1(17%)	
	Modification of Immunosoppressive Therapy	1(17%)	
	Antibiotics prophylaxis	4(67%)	
	Death	0(0%)	

Characteristics of Cohort 2 SARS-CoV-2 positive heart transplant population. Abbreviations: BMI, body mass index; COPD chronic obstructive pulmonary disease; GFR, glomerular filtration rate; PCI, percutaneous coronary intervention; NYHA, New York Heart Association; FU, follow up; LVEF, left ventricular ejection fraction; WBC, white blood cells.