COVID-19 in heart transplant patients: Case reports from Brazil

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Abstract
Introduction: The COVID-19 pandemic continues, with a late hyperinflammatory phase. The immunosuppressive therapy used in heart transplant patients, in theory, could reduce inflammation, thus benefitting patients with COVID-19. So far, however, there is still very little literature on this subject.

Methods: This is a single-center retrospective study. We described laboratory parameters and clinical outcomes from 11 heart transplant patients with COVID-19 assisted at Dante Pazzanese Institute of Cardiology between March and July 2020.

Results: Patients with ages of between 35 and 79 years were enrolled, and heart transplantation ranged from 3 to 264 months. The main comorbidities were diabetes mellitus (9/11; 81.8%), hypertension (10/11; 90.9%), and chronic renal disease (6/11; 54.5%). Cyclosporine A was used in 10 (90.9%) patients, mycophenolate mofetil in 9 (81.8%) patients, and mTOR inhibitor in 5 (45.5%) patients. Fever and cough were observed in 8 (72.7%) patients, and dyspnea and gastrointestinal symptoms in 5 (45.5%) patients. Lymphopenia was observed in 10 (90.9%) patients and thrombocytopenia in 5 (45.5%) patients. The higher level of troponin associated with chest tomography above 50% of bilateral pulmonary infiltrates with ground-glass opacity (GGO) was observed in those with the worst outcomes. Nine patients needed intensive care, and hospital stay ranged from 4 to 21 days, with 2 (18.2%) patients requiring vasopressor drugs and mechanical ventilation, and three (27.3%) patients dying due to COVID-19 complications.

Conclusion: Heart transplant patients had similar symptoms and outcomes as the general population; immunosuppressive therapy seems not to have protected them. Patients who presented higher levels of troponin and D-dimer, associated with greater GGO pulmonary infiltrates, had worse outcomes. More studies with larger cohorts may clarify immunosuppressive effects on COVID-19 outcomes.

KEYWORDS
COVID-19, D-dimer, heart transplantation, SARS-CoV-2, troponin
The SARS-CoV-2 is responsible for the coronavirus disease 2019 (COVID-19); the SARS-CoV-2 infection continues to spread globally with devastating results, and it is considered by the World Health Organization (WHO) as a pandemic disease. COVID-19 has also been responsible for many deaths around the world, including Brazil. According to the Brazilian Health Ministry, 358,425 individuals died up to April 14, 2021, and the lethality was 2.6%.

In theory, one might anticipate a higher attack rate of pneumonia, acute respiratory syndrome, and septic shock for organ transplant recipients. However, immunosuppression can inhibit the secondary hyperinflammation caused by a cytokine storm, responsible for the majority of deaths from COVID-19. Similarly, it can lead to atypical clinical presentations or increasing the risk of adverse events. Herein, we report a single-center retrospective study from heart transplant patients with COVID-19, as a preliminary observational context to inform treatment and clinical outcomes.

2 | METHODS

2.1 | Ethical statement

This study was approved by the institutional review board of Dante Pazzanese Institute of Cardiology (protocol 4.433.303), which was conducted in accordance with Resolution 466 by the Brazilian Health Council/National Health Surveillance Agency and ICH-GCP for good clinical practices.

2.2 | Patients and demographic data

Of the 145 adult heart transplant recipients routinely followed in our institution between March and July 2020, 11 of them presented to our institution for COVID-19 care and were included in this study. After heart transplantation, all patients received a standard immunosuppressive regimen with cyclosporine at a 4 mg/kg/day dose, mycophenolate mofetil at a 1.5 g/day dose, and prednisone at a 0.4 mg/kg daily dose. Alternative immunosuppressive therapy was prescribed whenever necessary. All data, such as clinical history, laboratory results, inflammatory and radiological issues, and specific drugs used, were retrospectively collected.

The primary outcome was death caused by COVID-19; a secondary outcome was the need for intensive care unit (ICU), mechanical ventilation, along with acute renal dysfunction.

2.3 | RT-PCR for COVID-19 diagnosis

To extract SARS-CoV-2 nucleic acids from nasopharyngeal swabs, the QIAamp® Viral RNA (Cat. #52906; Qiagen, GmbH, Hilden, GY) was applied. The RT-PCR was carried out in aRotor-Gene thermal cycler by using QuantiTect Probe RT-PCR Master Mix (Cat. # 204 443; Qiagen GmbH, Hilden, GY).

The primers used for RT-PCR analysis were recommended by the US Centers for Disease Control and Prevention, commercially designed (2019-nCoV RUO Kit) by Integrated DNA Technologies, Coralville, IA, USA (www.idtdna.com), containing primers N1 and N2 specific for SARS-CoV-2 and human RNase P as positive controls. The samples were considered positive when all measured parameters (N1, N2, and RNase P) showed lower than 35 Ct (cycle threshold).

2.4 | Serology for IgG detection

Enzyme-linked immunosorbent assay (ELISA) was applied to detect IgG anti-SARS-CoV-2 protein N (GenBank: QIG56001.1) in serum of patients. The 96-well plates were firstly adsorbed with 1µg/ml of N protein, incubated overnight at 4°C, blocked with 1% bovine serum albumin diluted in PBS buffer for 1h at 37°C, and then incubated with the serum (dilution 1/100) for 2 h at 37°C. The HRP anti-human IgG secondary antibody (Sigma, USA) at 1:30 000 of dilution was added and incubated at 37°C for 1 h. The TMB substrate (Thermo Scientific, USA) was added to each well and stopped with 1N HCl after 3 min of incubation. The absorbance was read at 450 nm. The cutoff value assumed was 0.450 AU.

3 | RESULTS

The age of patients enrolled was between 35 and 79 years. The main comorbidities were diabetes mellitus (9/11; 80%), hypertension (10/11; 90%), and chronic renal disease (6/11; 54%). Heart transplantation ranged from 3 to 264 months. Cyclosporine A was used in 10 (90%) patients, mTOR inhibitor in 45% (5/11) patients, and mycophenolate in 80% (9/11) patients. Immunosuppression was discontinued in two patients: One had septic shock and the other had severe leukopenia (270/mm³), and both died. Common symptoms at onset of illness were fever and cough in 72% of cases, and dyspnea and gastrointestinal symptoms in 45% of cases (Table 1).

Regarding laboratory parameters at admission or routine follow-up, lymphopenia (<1.5/1000 mm³) was observed in 90% (10/11) of cases and thrombocytopenia (<150/1000 mm³) in almost 50% (5/11) of cases. Troponin was higher in two out of three patients who died. Increased inflammatory markers were common and higher in those requiring intensive care (Table 2).

Chest tomography was performed in 82% (9/11) of patients, six presented less than 50% of bilateral pulmonary infiltrates with ground-glass opacity (GGO), and three had more than 50% of bilateral pulmonary infiltrates with ground-glass opacity associated with worse prognosis (Table 3).

Only one patient received hydroxychloroquine as an alternative therapy for COVID-19. None of the patients received remdesivir, because it was not approved for clinical use in our country until the
time of this study. Hospital stay was 4–21 days, with death occurring in 3 patients (27.3%). Vasopressors and mechanical ventilation were used in 20% of patients. None patient received extracorporeal membrane oxygenation (Table 3).

### DISCUSSION

In this study, we described eleven COVID-19 cases from a heart transplant patient cohort routinely followed up in our hospital, including nine cases of severe or critical COVID-19, and 2 cases of mild-to-moderate ambulatory COVID-19. The nine severe or critical COVID-19 patients sought emergency care due to difficulty breathing, and all of them were hospitalized. The two mild-to-moderate cases were minimally asymptomatic, suspected of SARS-CoV-2 infection during a routine visit, confirmed by serology around two months after COVID-19 symptoms, and therefore, they did not go through thoracic tomography as well.

Notably, at the beginning of pandemic outbreak, all nasopharyngeal swabs were tested at government-designated referral laboratory, and samples were missed by logistic services (2 cases with RT-PCR results lost and other 2 cases no longer in the acute phase of infection during routine follow-up).

We observed a 27% (3/11) mortality rate, slightly higher than in another study, which observed 15% (2/13) mortality rate. However, our result was close to that found by Latif et al (32%; 7/22) and Bottio et al (29.7%; 14/47), the largest cases in this population to date, in which the authors observed a double fatality rate in heart transplant recipients than in general population. The most common symptoms observed in our study group were similar to the report described in Spain, as well as in a multicenter study from Italy.

Four patients (36%) in this study required intensive care, all with higher than 1000 ng/ml D-dimer, and three with above 50% of bilateral pulmonary infiltrates and worse clinical outcomes, progressing to death. Although our hospital offers venous-arterial extracorporeal membrane oxygenation (VA-ECMO), one elder patient opted for palliative treatment and the family refused this treatment. Two others patients did not fulfill the ELSO guidelines, and both died of septic shock.

Some authors observed an elevated D-dimer in individuals with COVID-19, suggesting possible disseminated intravascular coagulation. Furthermore, the group that received low molecular weight heparin had a lower mortality rate. Interestingly, like Singhvi et al, no episodes of thromboembolic event were observed in our study. Half of our patients had higher than 1000 ng/ml D-dimer and none of them treated with anticoagulant therapy, because it was not recommended by the institutional guideline at that time. It is worth to mention that D-dimer is not routinely assessed in our service, and then, most of our patients have no baseline values assessed.

In this study, patients who had elevated circulating troponin, often present hemodynamic instability, vasoactive drugs were required and had worst outcomes, as the literature points out. Furthermore, patients suffering from viral sepsis usually present myocardial injury and elevate circulating troponin. The cardiac troponin concentration is associated with early mortality, as well as postdischarge cardiovascular morbidity.

The number of patients with lymphopenia (<1.5/1000 mm³) and thrombocytopenia (<150/1000 mm³) was higher in this study, 90% (10/11) and 45% (5/11), respectively, versus previous reports in

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**TABLE 1** Characteristics and COVID-19 symptoms in heart transplant patients

| Inpatients/ outpatients | Age (years) | Gender | Time from transplant (months) | Comorbidities | Immunosuppression | Symptoms |
|-------------------------|------------|--------|-------------------------------|---------------|-------------------|----------|
| 1                       | 79         | Male   | 264                           | HTN, DM, CRD  | CyA, MMF, CSs     | Fever, cough, dyspnea |
| 2                       | 67         | Male   | 264                           | HTN, DM, CRD  | mTOR, MMF, CSs    | Cough, dyspnea |
| 3                       | 52         | Female | 192                           | HTN, DM, obesity | CyA, MMF, CSs     | Fever, cough, dyspnea |
| 4                       | 50         | Male   | 84                            | HTN, DM       | CyA, mTOR, MMF, CSs | Fever, cough, GID |
| 5                       | 35         | Female | 3                             | DM            | CyA, MMF, CSs     | Fever, cough |
| 6                       | 69         | Male   | 42                            | HTN, DM, CRD, obesity | CyA, mTOR, CSs     | Fever, cough, dyspnea, GID |
| 7                       | 51         | Male   | 72                            | HTN, DM, CRD  | CyA, mTOR, MMF, CSs | Fever, cough, dyspnea |
| 8                       | 74         | Male   | 124                           | HTN, DM, CRD  | CyA, mTOR, CSs    | GID     |
| 9                       | 37         | Male   | 11                            | HTN            | CyA, MMF, CSs     | Fever   |
| 10                      | 73         | Male   | 223                           | HTN, DM, CRD  | CyA, MMF, CSs     | No symptoms |
| 11                      | 44         | Male   | 36                            | HTN            | CyA, MMF, CSs     | Fever, cough, GID |

Note: Abbreviations: CRD, chronic renal disease; CSs, corticosteroids; CyA, cyclosporine A; DM, diabetes mellitus; GID, gastrointestinal disorder; HTN, hypertension; MMF, mycophenolate mofetil; mTOR, mTOR inhibitor. *Indicate which is outpatients and death individual.
| Inpatients/ Outpatients | Total leukocytes (1000 mm³) | Lymphocytes (1000 mm³) | Platelets (1000 mm³) |
|-------------------------|----------------------------|------------------------|---------------------|
|                         | Baseline                   | Admission/ Outpatient\* | Discharge/ Death\*  |
|                         | Baseline                   | Admission/ Outpatient\* | Discharge/ Death\*  |
|                         | Baseline                   | Admission/ Outpatient\* | Discharge/ Death\*  |
| 1                       | 4.9                        | 6.1                    | 13.7\*              |
| 2                       | 6.8                        | 9.8                    | 14.7\*              |
| 3                       | 6.6                        | 3.7                    | 3.4                 |
| 4                       | 6.4                        | 7.7                    | 3.4                 |
| 5                       | 6.1                        | 8.3                    | 5.2                 |
| 6                       | 6.1                        | 2.8                    | 0.3\*               |
| 7                       | 5.7                        | 10.5                   | 7.7                 |
| 8                       | 5.2                        | 5.1                    | 5.7                 |
| 9\*                     | 9.4                        | 9.6                    | 9.7                 |
| 10\*                    | 4.6                        | 6.1\*                  | 6.1                 |
| 11                      | 8.3                        | 5.4                    | 8.0                 |

| Inpatients/ Outpatients | Troponin I (ng/L) | D-dimer (ng/ml) | C-reactive protein (mg/dL) | B-type natriuretic peptide (pg/ml) |
|-------------------------|-------------------|----------------|---------------------------|-----------------------------------|
|                         | Baseline          | Admission/ Outpatient\* | Discharge/ Death\* | Baseline          | Admission/ Outpatient\* | Discharge/ Death\* | Baseline          | Admission/ Outpatient\* | Discharge/ Death\* |
| 1                       | 0.01              | NA\*             | NA\*          | 1836              | NA\*              | 231              | NA\*              | 21               | 25\*               |
| 2                       | NA\*             | 0.41             | NA\*          | 1397              | NA\*              | 4200             | NA\*              | 40\*             | 8.8\*              |
| 3                       | 0.02             | 0.02             | NA            | 287               | 235               | 1010             | 1230             | 910\*           |
| 4                       | 0.04             | 0.01             | NA            | NA\*             | 0.5               | 2823             | NA\*             | 0.5              | 0.5              |
| 5                       | 0.08             | 0.03             | 0.07          | NA\*             | 675               | 3810             | 2800             | 6250            |
| 6                       | 0.02             | 0.12             | 1.42\*        | NA\*             | 4061              | 2300             | 7350\*           |
| 7                       | 0.01             | 0.07             | 0.02          | 2642              | 6933              | 219               | 1074             | 174\*           |
| 8                       | 0.01             | 0.01             | 0.01          | 1839              | 1300              | 82               | 490\*            |
| 9\*                     | 0.01             | 0.01             | 0.01          | NA\*             | 147               | 89               | 84               | 105\*           |
| 10\*                    | 0.01             | 0.01             | 0.01          | NA\*             | NA\*             | 394               | 694              | 777\*           |
| 11                      | 0.01             | 0.01             | 0.01          | 205               | 341               | 397               | 2               | 1.3\*            |

Note: Abbreviations: NA, not accomplished. *Indicate death patients.
nontransplanted and transplanted populations.\textsuperscript{20,21} This was likely due to the use of immunosuppressant or an additional symptom of COVID-19.

Although in vitro studies suggest that mycophenolate mofetil is inhibitor of coronaviruses,\textsuperscript{22} the interferon alpha combined with cyclosporine therapy was effective in reducing MERS-CoV replication.\textsuperscript{23} On the other hand, immunosuppressive therapy can increase susceptibility to the infection, decreasing an effective response to the treatment. The high mortality rate in this study does not suggest beneficial protection of immunosuppression; further randomized studies are necessary to assess each immunosuppressant individually.

The small cohort from a sole transplantation center is one major limitation of this study. However, it seems to be a common limitation in studies involving heart transplant patients infected by SARS-CoV-2. One must acknowledge that we did not routinely test all patients; therefore, we might have underestimated the prevalence of COVID-19 in patients with heart transplantation. Unfortunately, given the limited national resources, it was possible only to test symptomatic individuals.

It is not possible to draw conclusions as to specific therapies for COVID-19, or in managing immunosuppression, from a small observational, noninterventional study such as this. However, it does provide insight into the scope and magnitude of the burden of the disease. We hope that data the described herein could help to clarify the pathogeneses of COVID-19 into the heart transplant context.

5 | CONCLUSION

Heart transplant patients had comparable symptoms and outcomes as the general population; the immunosuppressive therapy background did not appear to protect them. Patients who presented higher levels of troponin and D-dimer with higher GGO pulmonary infiltrates had worse outcomes. Studies with larger cohorts may contribute to clarifying the immunosuppressive effect on COVID-19 outcomes.

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CONFLICT OF INTEREST
The authors have no conflicts of interest regarding the content of this manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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