Prospective Clinical, Virologic, and Immunologic Assessment of COVID-19 in Transplant Recipients

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Background. Several studies have described the clinical features of COVID-19 in solid-organ transplant recipients. However, many have been retrospective or limited to more severe cases (hospitalized) and have not routinely included serial virological sampling (especially in outpatients) and immunologic assessment. **Methods.** Transplant patients diagnosed with COVID-19 based on a respiratory sample PCR were prospectively followed up to 90 d. Patients provided consent for convalescent serum samples and serial nasopharyngeal swabs for SARS-CoV-2 antibody (antinucleoprotein and anti-RBD) and viral load, respectively. **Results.** In the 161 SOT recipients diagnosed with COVID-19, the spectrum of disease ranged from asymptomatic infection (4.3%) to hospitalization (60.6%), supplemental oxygen requirement (43.1%), mechanical ventilation (22.7%), and death (15.6%). Increasing age (OR, 1.031; 95% CI, 1.001-1.062; $P = 0.046$) and ≥2 comorbid conditions (OR, 3.690; 95% CI, 1.418-9.615; $P = 0.007$) were associated with the need for supplemental oxygen. Allograft rejection was uncommon (3.7%) despite immunosuppression modification. Antibody response at ≥14 d postsymptoms onset was present in 90% (anti-RBD) and 76.7% (anti-NP) with waning of anti-NP titers and stability of anti-RBD over time. Median duration of nasopharyngeal positivity was 10.0 d (IQR, 5.5–18.0) and shedding beyond 30 d was observed in 6.7% of patients. The development of antibody did not have an impact on viral shedding. **Conclusions.** This study demonstrates the spectrum of COVID-19 illness in transplant patients. Risk factors for severe disease are identified. The majority form antibody by 2 wk with differential stability over time. Prolonged viral shedding was observed in a minority of patients. Reduction of immunosuppression was a safe strategy.

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INTRODUCTION

More than 1 y into the pandemic, our understanding of how SARS-CoV-2 infection affects transplant recipients has increased, but many unanswered questions remain. Hospitalization is reported in 66%–87% and mortality varies widely from 3.0% to 18.7%,[1-11] depending on the population studied, increasing up to 40% in those with severe disease.[12,13] Much of the literature remains focused toward hospitalized patients rather than the full spectrum of COVID-19 in transplant recipients and is limited by relatively short duration of follow-up for a disease, which we now understand can have a protracted clinical course. Transplant-specific complications, including allograft rejection, have not been comprehensively assessed but are of importance given that immunosuppression is frequently modified during the treatment of COVID-19.

Following SARS-CoV-2 infection, most individuals, including transplant recipients, will develop neutralizing IgG antibodies,[3,14] and in nonimmunocompromised hosts, the presence of such antibodies is associated with a reduction of SARS-CoV-2 reinfection at 6 mo.[15,16] An understanding of humoral response to natural SARS-CoV-2 infection in transplant recipients will be useful when interpreting vaccine response; however, factors determining the

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magnitude of antibody response in transplant recipients remain unclear.

In nonimmunocompromised persons, viral load (VL) kinetics and duration of viral shedding display significant heterogeneity and may correlate with disease severity and infectivity,[17,18] but not necessarily the magnitude of immune response.[19] Prolonged shedding of SARS-CoV-2 has been documented in transplant recipients; however, an association with severity of the disease has not been established.[14] Quantitative PCR is considered the optimal method for assessing viral burden but is often substituted for the less than ideal surrogate, cycle threshold.[20]

We herein present a comprehensive overview of our center's experience of COVID-19 in SOT recipients over a 12-mo period and the lessons learned regarding the clinical course, outcomes, complications, and in a subset of patients antibody response and viral shedding in the prevaccination era.

MATERIALS AND METHODS

Recruitment/Cohort/Ethics

This prospective, single-center study encompassed all SOT recipients who were diagnosed with COVID-19 over a 12-mo period starting from the date our first patient was diagnosed (March 19, 2020). The University Health Network Transplant Centre performs approximately 700 solid-organ transplants annually and has comprehensive follow-up of over 6000 transplant recipients. All patients with a positive SARS-CoV-2 diagnosis are captured by the program. Inpatients and outpatients were included if they had a positive clinical nucleic acid test for SARS-CoV-2 infection on a respiratory specimen. All patients were assessed in real-time by a transplant infectious diseases physician and followed for outcomes at 30, 60, and 90 d using a standardized form with a modified version of the WHO clinical progression scale applied.[20] A subset of consenting patients provided serum at ≥14 d after COVID-19 symptom onset. Nasopharyngeal (NP) swabs were collected from consenting patients at serial timepoints post-diagnosis by nursing staff (inpatient) or were midnasal swabs that were self-collected following verbal and written instructions (outpatient). Outpatient swabs were rapidly delivered to the laboratory by courier. The study was approved by the institutional research ethics board. All patients or their delegates provided informed consent. A study overview is found in Figure S1, SDC, http://links.lww.com/TP/C254.

Antibody Testing

Serological testing for anti-SARS-CoV-2 IgG antibody was performed using 2 different assays according to manufacturer's instructions: an anti-NP chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories, USA)[21] and an antispike RBD electrochemiluminescent immunoassay (ECLIA) (Roche, Switzerland). Index measurements of ≥1.4 and ≥0.8 U/mL were considered positive, respectively. The sensitivity and specificity of the anti-NP assay are 100% and 99.6% and the anti-RBD assay are 98.8% and 99.98% at >14 d postsymptom onset.[22,23]

Viral Load Measurement

Quantitative VL measurements were performed on viral RNA extracted from nasopharyngeal swabs using fluorogenic probe-based real-time RT-qPCR (Norgen Biotek, Canada). Two hundred eighty microliters of swab solution were processed using the QIAamp Viral RNA kit (Qiagen, Germany), following the manufacturer's instructions. The PCR assay was based on the World Health Organization SARS-CoV-2 assay, which targets the RNA-dependent RNA polymerase (RdRP) and envelope (E) genes of SARS-CoV-2.[24] Quantification was performed using the RdRP assay and an 8-point standard-curve using the kit's positive control (starting concentration: 500 000 copies/μL). The limit of quantification (LOQ) was defined as the lowest standard point consistently detected within 40 cycles during standardization. The LOQ was 50 copies/mL. Values below the LOQ, including undetectable VLs, were set to half the LOQ, 25 copies/mL. All samples with undetectable VLs were validated for technical errors using an RNaseP endogenous internal amplification control (Norgen Biotek). The duration of viral shedding was calculated as the middle of the interval between the last positive swab (including the diagnostic swab) and the first negative swab, calculated from the time of symptoms. When the last study swab was positive, we used this time as the duration of shedding as a conservative estimate. To assess factors influencing the duration of viral shedding, we compared those who shed virus for longer and shorter than the median duration of viral shedding in our cohort.

Statistics

Demographics were analyzed using descriptive statistics. Categorical variables were compared using χ^2 or Fisher's exact test. Continuous variables were compared using Mann-Whitney *U* test or Spearman's correlation. The multivariate model was performed using binary logistic regression including variables that had a *P* value of <0.1 on univariate analysis. The number of comorbidities rather than individual comorbidities was used in the multivariate model. Statistical significance was defined at the level of $P < 0.05$. All statistical analyses were performed using IBM SPSS (version 25) or Prism (version 9, GraphPad Software, USA). Figures were prepared using Microsoft Excel (2016) and Prism.

RESULTS

Clinical Characteristics

During the study period, 161 SOT recipients of the ~6000 patients followed at our center were diagnosed with COVID-19. All patients were followed up for a minimum of 30 d, and 136 (84.5%) were followed for the full 90 d or until death. The demographics and baseline characteristics of transplant recipients with COVID-19 are shown in Table 1. Symptoms at presentation are shown in Table S1, SDC, http://links.lww.com/TP/C254. Most (65.6%) were male with a median age of 58.0 y. Kidney transplant recipients comprised 50.6% of the cohort. A variant of concern was identified in 6 patients (based on mutation screening for N501Y).[25] Immunosuppression was reduced in 66.9% of cases; most commonly, the antiproliferative was reduced with a mean dose reduction of 80.3%.

TABLE 1.

Demographics of transplant recipients with COVID-19

	All patients (n = 160)	No oxygen requirement (WHO outcome class 1–4) (n = 91)	Oxygen requirement (WHO outcome class 5–10) (n = 69)	*P*
Baseline characteristics				
Age, median (IQR), y	58.0 (48.0–67.0)	55.0 (42.0–64.0)	62.0 (52.0–69.5)	0.006
Male, n (%)	105 (65.6)	60 (65.9)	45 (65.2)	0.925
Race, n (%)				
				0.438
Asian	20 (12.5)	10 (11.0)	10 (14.5)	
Black	16 (10.0)	12 (13.2)	4 (5.8)	
Hispanic	9 (5.6)	4 (4.4)	5 (7.2)	
Indian/subcontinent	34 (21.3)	21 (23.1)	13 (8.8)	
White	81 (50.6)	44 (48.4)	37 (53.6)	
Comorbid conditions, n (%)				
Hypertension	119 (74.4)	60 (65.9)	59 (85.5)	**0.005**
ACEi/ARB use	52 (32.5)	28 (30.8)	24 (34.8)	0.591
Diabetes mellitus	71 (47.3)	34 (37.4)	37 (53.6)	**0.040**
BMI > 30	28 (17.5)	12 (13.2)	16 (23.2)	0.099
Coronary artery disease	29 (18.1)	16 (17.6)	13 (18.8)	**0.038**
Congestive cardiac failure	4 (2.5)	1 (1.1)	3 (4.3)	0.190
Chronic lung disease (including CLAD)	21 (13.1)	6 (6.6)	15 (21.7)	**0.005**
Current smoker	4 (2.5)	3 (3.3)	1 (1.4)	0.445
Chronic kidney disease (grade 3–5)	82 (51.9)	41 (45.1)	42 (60.9)	**0.047**
Active malignancy	7 (4.4)	2 (2.2)	5 (7.2)	0.122
Other immunosuppressive condition	3 (1.9)	2 (2.2)	0 (0.0)	0.064
Number of comorbidities, n (%)				
				<0.001
0–1	48 (30.0)	39 (42.8)	9 (13.0)	
≥2	112 (70.0)	52 (57.1)	60 (86.9)	
Transplant				
Time from transplant, median (IQR), d	2075 (647–3833)	1777 (488–3906)	2091 (894–3762)	0.189
Retransplant, n (%)	15 (9.4)	8 (8.8)	7 (10.1)	0.771
Organ, n (%)				
				<0.001
Heart	9 (5.6)	6 (6.6)	3 (4.3)	
Kidney, liver-kidney	82 (51.3)	44 (48.3)	38 (55.1)	
Kidney-pancreas	8 (5.0)	3 (3.3)	5 (7.2)	
Liver	37 (23.1)	31 (34.1)	6 (8.7)	
Lung, heart-lung	24 (15.0)	7 (7.7)	17 (24.6)	
Baseline immunosuppression, n (%)				
Prednisone	124 (77.5)	59 (64.8)	65 (94.2)	**<0.001**
Tacrolimus	120 (75.0)	70 (76.9)	50 (72.5)	0.519
Level ≥10 ng/mL	15 (9.4)	7 (7.7)	8 (11.6)	0.292
Cyclosporine	37 (23.1)	20 (22.0)	17 (24.6)	0.693
Mycophenolic acid	113 (70.6)	57 (62.6)	56 (81.1)	**0.011**
TDD ≥720 mg	83 (51.9)	42 (46.2)	41(59.4)	0.786
Azathioprine	12 (7.5)	10 (10.9)	4 (5.8)	0.453
mTORi	6 (3.8)	4 (4.4)	2(2.8)	0.170
Rejection and enhanced immunosuppression <6 mo before COVID-19 diagnosis, n (%)				
Biopsy-proven rejection	6 (3.8)	4(4.4)	2 (2.9)	0.382
Basiliximab	4 (2.5)	3 (3.3)	1 (1.4)	0.324
Antithymocyte globulin	6 (3.8)	5 (5.5)	1 (1.4)	0.163
Rituximab	1 (0.6)	0 (0.0)	1 (1.4)	0.183
Alemtuzumab	1 (0.6)	0 (0.0)	0 (0.0)	0.194

Bolding indicates variables with $P < 0.05$.

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CLAD, chronic lung allograft dysfunction; COVID-19, coronavirus disease 2019; IQR, interquartile range; mTORi, mammalian target of rapamycin inhibitor; TDD, total daily dose; WHO, World Health Organization.

Hospitalization for COVID-19 occurred in 97 (60.6%) patients and 69 (43.1%) required oxygen. In the hospital, therapies used were remdesivir (n = 32), dexamethasone (n = 55), tocilizumab (n = 2), and convalescent plasma (n = 1). A potential source of COVID-19 transmission was identified in 60.2% (n = 97) of cases, including household (n = 69, 42.9%), social (n = 11, 6.8%), occupational (n = 7, 4.3%), nosocomial (n = 8, 8.2%), and long-term care facility (LTC) (n = 1, 0.6%). Five (3.1%) patients had a history of recent travel without a specific, known, COVID-19 positive contact. There was 1 case of donor-derived SARS-CoV-2 transmission via a lung transplant recipient, which has been previously described and is henceforth excluded from analysis due to the idiosyncratic nature of this case.[26] The epidemic curve of SARS-CoV-2 infection in transplant patients mirrored the epidemic curve in Ontario, Canada (data not shown).[27]

Clinical Outcomes and Complications

The spectrum of COVID-19 severity is shown in Figure 1 (based on WHO severity score). Notably, 7 (4.4%) patients were asymptomatic of SARS-CoV-2 infection, including the only patient included in this cohort who had received the COVID-19 vaccination before infection (2 doses of mRNA-1273, Moderna). Outcomes by transplanted organ are shown in Table 2; 97/160 (60.6%) required hospitalization, and 69/160 (43.1%) needed supplemental oxygen. ICU admission occurred in 27/160 (16.9%). Two patients required extracorporeal membrane oxygenation (ECMO), only 1 of whom survived to hospital discharge, 83 d post-diagnosis. Forty-five patients (28.1%) had an acute kidney injury. Pulmonary imaging demonstrated ground-glass opacities or infiltrates in 85 of 103 (82.7%). One-third (n = 27, 31.8%) of patients with evidence of pneumonitis required ICU admission ($P < 0.001$) and all patients admitted to ICU had evidence of pneumonitis on imaging.

Twenty-five patients (15.6%) died, including 12 kidney, 4 liver, 8 lung, and 1 heart transplant recipient. All deaths were attributed to COVID-19. In 2 patients, gastrointestinal catastrophes (small bowel obstruction and perforated diverticulum) contributed to death. Death occurred at a median of 15 d (IQR, 8–27) post-COVID-19 diagnosis, and all patients were hospitalized at the time of death. Six deaths occurred 30 or more d post-COVID-19 diagnosis.

Factors Associated With Disease Severity

Factors significantly associated with oxygen requirement (WHO score ≥5) (on univariate analysis) included older age ($P = 0.006$), 2 or more baseline comorbidities ($P < 0.001$), hypertension ($P = 0.005$), diabetes mellitus (0.040), chronic kidney disease Grade 3-5 ($P = 0.047$); lung transplant recipient ($P = 0.005$); prednisone use ($P < 0.001$); and mycophenolic acid ($P = 0.011$) at the time of SARS-CoV-2 diagnosis. On multivariate analysis, factors that were associated with more severe disease were increasing age (OR, 1.031; 95% CI, 1.001-1.062; $P = 0.046$) and having ≥2 comorbid conditions (OR, 3.690; 95% CI, 1.418-9.615; $P = 0.007$). Risk factors for death included increasing age (median 66 versus 56 y; $P < 0.001$) and 2 or more comorbidities ($P = 0.002$). Although not statistically significant, lung transplant recipients had a higher incidence of death following SARS-CoV-2 infection and all, but 1 had a preexisting chronic lung allograft dysfunction.

Infectious Complications

Treatment for concomitant bacterial pneumonia was common (n = 74, 46.3%), but an etiologic agent was proven

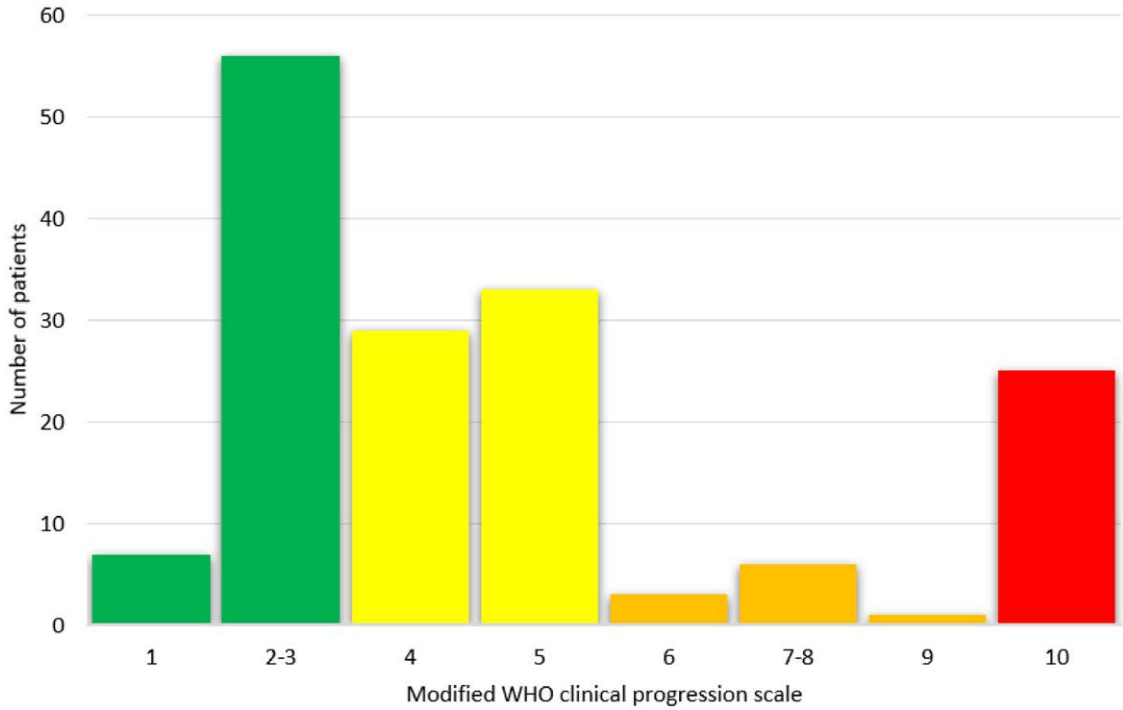

FIGURE 1. Modified WHO progression scale of transplant recipients with coronavirus disease 2019. Number of solid-organ transplant recipients with modified WHO severity scores. 1–3 (green bars): ambulatory mild disease, 4–5 (yellow bars): hospitalized moderate disease, 6–9 (orange bars): hospitalized severe disease, and 10 (red bars): death. WHO, World Health Organization.

TABLE 2.

Outcomes from coronavirus disease 2019 by type of transplant

	WHO score	Heart (n = 9), n (%)	Kidney, liver-kidney (n = 82), n (%)	Kidney-pancreas (n = 8), n (%)	Liver (n = 37), n (%)	Lung, heart-lung (n = 24), n (%)	Total (n = 160), n (%)	*P*
Hospitalization	4	5 (55.6)	53 (64.6)	8 (100.0)	12 (32.4)	19 (79.1)	97 (60.6)	0.001
Oxygen requirement	5	3 (33.3)	38 (46.3)	5 (62.5)	6 (16.2)	11 (45.8)	69 (43.1)	0.001
ICU	6	0 (0.0)	17 (20.7)	0 (0.0)	2 (5.4)	8 (33.3)	27 (16.9)	0.010
Mechanical ventilation	7–8	0 (0.0)	15 (18.3)	0 (0.0)	2 (5.4)	5 (20.8)	22 (13.8)	0.093
ECMO	9	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)	1 (4.1)	2 (1.25)	2.5
Death	10	1 (11.1)	12 (14.6)	0 (0.0)	4 (10.8)	8 (33.3)	25 (15.6)	0.153

Bolding indicates variables with $P < 0.05$.
ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; WHO, World Health Organization.

in just 4 cases (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Stenotrophomonas maltophilia*). During acute COVID-19, 8 (5.0%) patients were bacteremic (coagulase-negative staphylococcus, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus mirabilis*, *Clostridium perfringens*, and *Pseudomonas aeruginosa*). In lung transplant recipients, there was 1 case of probable *Aspergillus fumigatus* tracheobronchitis and 1 case of pulmonary *Candida albicans* isolation in the setting of bronchial anastomosis dehiscence.

Noninfectious Complications Including Rejection

Several noninfectious complications were observed during acute COVID-19. Gastrointestinal complications included 1 episode each of pancreatitis in a heart transplant recipient, small bowel obstruction, and perforated diverticulitis. Coagulopathic and thrombotic complications included a case, each of renal thrombosis leading to infarction, pulmonary embolus, and retroperitoneal bleed in the setting of therapeutic anticoagulation. There were 6 episodes of allograft rejection diagnosed during the follow-up period, 2 each within 30, 60, and 90 d of COVID-19

TABLE 3.

Demographics and comparisons of antibody response to severe acute respiratory syndrome coronavirus 2 infection in transplant recipients

		Anti-RBD		Anti-NP	
	Total antibody cohort, N (%)	Antibody level, median (IQR)	*P*	Antibody level, median (IQR)	*P*
	N = 60	66.2 (5.7–185.0)		3.5 (1.1–5.7)	
Age, median (IQR), y	53.1 (14.5)				
≤50	21 (35.0)	45.9 (4.7–141.4)	0.249	3.3 (1.3–4.5)	0.310
>50	39 (65.0)	84.8 (9.7–220.3)		3.5 (1.1–6.4)	
Time from transplant, median (IQR), d	1947.5 (598.5–3330.7)				
≤6 mo posttransplant	5 (8.3)	3.87 (2.2–242.2)	0.53	1.0 (0.3–3.2)	0.052
≥6 mo posttransplant	55 (91.7)	73.5 (9.1–185.1)		3.9 (1.6–5.8)	
Organ					
Heart	3 (5.0)[a]	138.7 (126.8–150.6)		7.1 (5.6–8.2)	
Kidney	32 (53.3)	39.1 (3.3–155.7)		3.2 (0.9–4.8)	
Kidney-pancreas	3 (5.0)	143.2 (14.6–185.9)		5.0 (4.8–5.1)	
Liver	14 (23.3)	202.8 (36.1–690.6)		5.6 (2.6–7.2)	
Lung	8 (13.3)	53.8 (1.6–75.8)	0.153	1.5 (0.3–3.4)	**0.031**
Non-lung	52 (86.7)	87.5 (6.9–190.1)		3.9 (1.7–5.9)	
Immunosuppression at time of COVID-19 diagnosis					
Calcineurin inhibitor	59 (98.3)				
Cyclosporine	16 (26.7)	68.7 (4.0–205.9)	0.689	3.2 (1.3–6.0)	0.946
Tacrolimus	43 (71.7)	76.9 (9.1–185.1)		3.8 (1.3–5.7)	
Antimetabolite	49 (81.7)				
Azathioprine	6 (10.0)	35.2 (8.1–95.8)		5.4 (2.9–6.3)	
Mycophenolic acid	43 (71.7)	60.9 (4.4–158.9)		3.2 (1.0–5.3)	
MPA TDD ≤720 mg	26 (43.3)	74.6 (8.8–186.4)	0.91	4.4 (1.8–6.0)	**0.011**
MPA TDD >720 mg	17 (28.3)	17.0 (0.68–113.8)		2.0 (0.4–3.3)	
Prednisone	47 (78.3)	57.7 (3.8–143.4)	0.003	3.3 (0.9–5.0)	**0.010**
No steroid	13 (21.7)	202.8 (74.1–837.6)		5.6 (3.4–7.2)	

Bolding indicates variables with $P < 0.05$.
[a]Anti-RBD data is missing for 1 heart transplant recipient.
COVID-19, coronavirus disease 2019; IQR, interquartile range; MPA, mycophenolic acid; NP, nucleoprotein; RBD, receptor-binding domain; TDD, total daily dose.

diagnosis. Five cases were biopsy proven, and 1 was diagnosed clinically. Of the 107 SOT recipients whose immunosuppression regimen was altered during COVID-19, 4 patients (3.7%) experienced allograft rejection within 90 d of COVID-19 diagnosis.

Antibody Responses

To assess the humoral response to SARS-CoV-2, we measured anti-RBD and anti-NP antibodies in the serum of 60 (37.5%) patients from this cohort at ≥14 d postsymptom onset (Table 3). The median time from COVID-19 symptom onset to serum collection was 40.5 d (IQR, 34.0–50.8 d). Anti-RBD was positive in 90% (n = 54) and anti-NP in 76.7% (n = 46) with median antibody levels of 66.3 U/mL (IQR, 5.7–185.0) and 3.5 U/mL (IQR, 1.1–5.7), respectively. For anti-RBD, the percent of transplant recipients who tested positive at 14–29 d, 30–59 d, and >60 d post–symptom onset were 83.3%, 87.5%, and 100%, respectively (Figure 2A). For anti-NP, the corresponding proportions were 83.3%, 73.2%, and 57.1%, respectively (Figure 2B). These data suggest anti-RBD antibody levels are relatively stable in SOT recipients over the first 60 d, whereas anti-NP responses fluctuate and wane over time. Anti-NP and -RBD antibody levels were directly correlated with each other ($P < 0.001$) (Figure S2, SDC, http://links.lww.com/TP/C254).

Prednisone use at baseline was associated with both lower anti-RBD ($P = 0.003$) and anti-NP levels ($P = 0.011$) at convalescence. MPA total daily dose of >720 mg at baseline was associated with lower levels of anti-NP ($P = 0.011$) (Table 2). There was no significant difference in antibody levels based on disease severity or treatment received.

Viral Loads and Duration of Viral Shedding

Of the 60 patients with serology, a subset of 30 patients submitted serial nasopharyngeal swabs for VL testing (total swabs N = 159; median of 6 swabs [range 1–7]). Of these, 13 of 30 remained outpatients. All patients had a positive diagnostic swab; in follow-up study swabs, SARS-CoV-2 VL was detected in 18/30 (60.0%) patients. The range of detectable and peak VLs are shown in Figure 3A and C, respectively. The median duration of viral shedding from symptom onset was 10.0 d (IQR, 5.5–18.0) (Figure 3B and D). Viral load decreased over time, and only 2 patients had a calculated duration of shedding ≥30 d (6.7%) (Figure 3B and D). Those who shed SARS-CoV-2 for <10.0 d were kidney (42.9%), liver (28.6%), lung (21.4%), and heart (7.1%) recipients. Those who shed virus for ≥10 d from symptom onset were kidney (43.8%), liver (25.0%), kidney-pancreas (12.5%), lung (12.5%), and heart (6.3%) transplant recipients. No baseline factors (sex, age [>50 y old], transplanted organ, hospitalization status, or baseline immunosuppression) were statistically associated with prolonged shedding (defined as ≥10.0 d from symptom onset; $P > 0.05$ for all comparisons). Furthermore, there were no differences in anti-RBD ($P > 0.99$) or anti-NP ($P = 0.38$) antibody positivity among those with or without prolonged SARS-CoV-2 shedding.

DISCUSSION

We present a large, prospective single-center study of COVID-19 with in-depth assessment and follow-up of every SOT recipient from our center over a 12-mo period and have identified several key lessons. Unlike many previous studies, which included mainly hospitalized patients, sourced data from a central registry, or retrospectively analyzed cases, we included both inpatients and outpatients who were assessed prospectively. With 90-d follow-up for most patients, we were able to accurately assess outcomes, acute and subacute complications of COVID-19, which are not fully appreciated within a shorter follow-up period. COVID-19 causes a wide spectrum of disease in transplant recipients from asymptomatic infection (4.3%) to hospitalization (60.6%), supplemental oxygen (43.1%) requirement, mechanical ventilation (22.7%), and death (15.6%). As previously demonstrated, we found the presence of comorbid conditions to be associated with poorer outcomes[1,2,6] and the highest mortality in lung transplant recipients. Household transmission is the most common source of SARS-CoV-2 acquisition in SOT recipients and

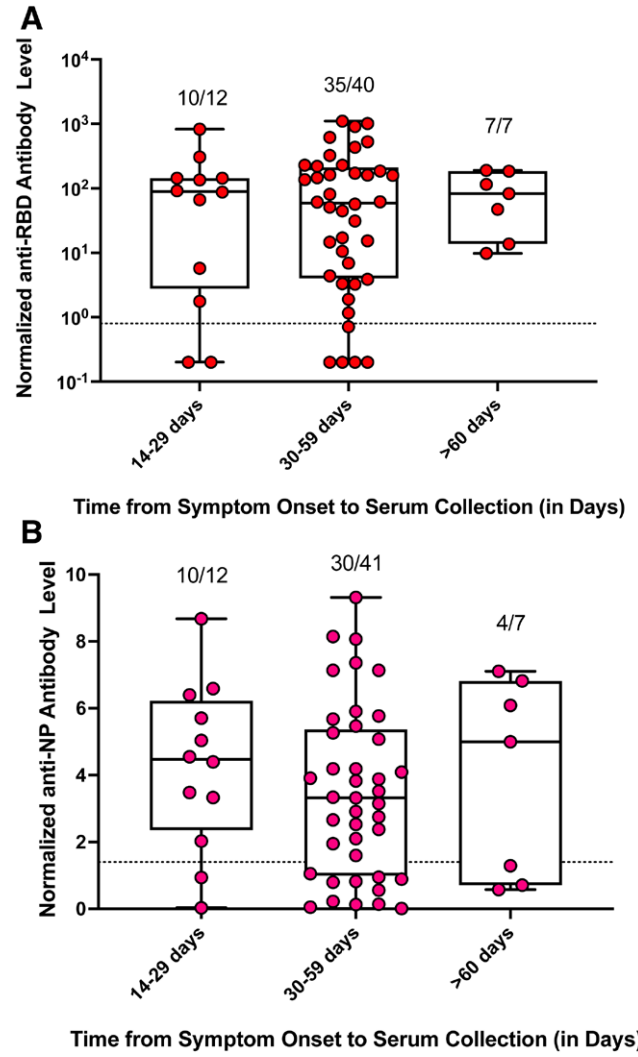

FIGURE 2. Antibody responses in solid-organ transplant recipients. Antispike RBD (A) and anti-NP (B) antibody responses in n = 60 solid-organ transplant recipients. Dashed horizontal lines indicate cutoff for positivity of each test. The fraction of individuals positive at each time period is also denoted above each box plot. All serum samples were ≥14 d postsymptom onset. Plots indicate median and interquartile range. NP, nucleoprotein; RBD, spike receptor-binding domain.

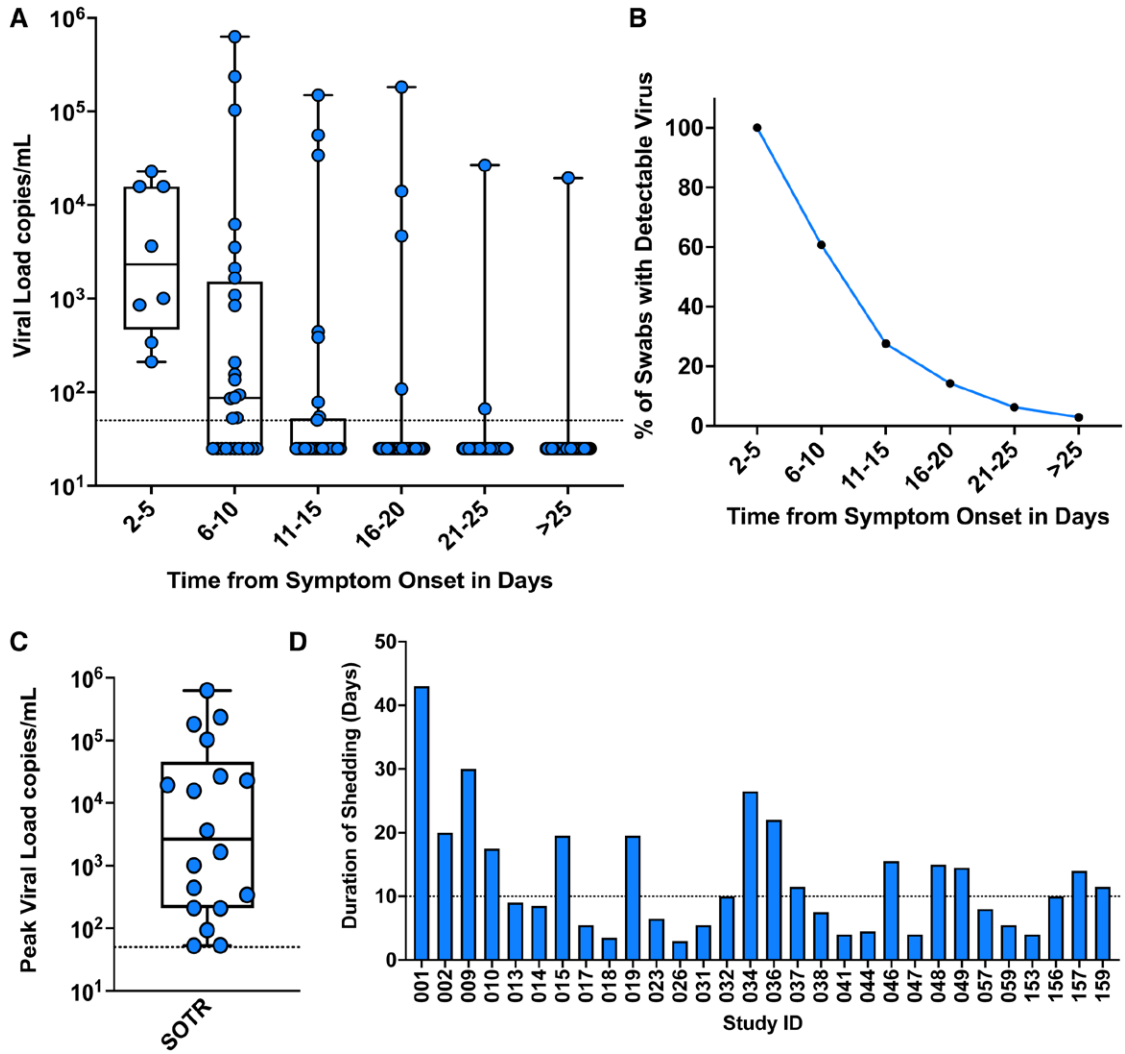

FIGURE 3. Viral loads in SOTRs. A, Viral loads measured in n = 30 SOTRs. Each dot corresponds to a nasopharyngeal swab result collected during the range of days from symptom onset shown on the x-axis. Box and whisker plots show the median plus the interquartile range for each date interval. B, Percent of swabs tested with a detectable viral load in the days following symptom onset. C, Peak viral load for each patient with at least 1 detectable viral load. Bars show median and interquartile range. Dashed horizontal line indicated the limit of quantification. D, Duration of detectable viral load for individual patients in the study. SOTR, solid-organ transplant recipient.

the epidemic curve mirrors that of the wider community, indicating that preventative measures must focus on the wider environment beyond the individual. Rates of allograft rejection post-COVID-19 have been reported rarely. We have demonstrated allograft rejection is uncommon post-COVID-19, even in the setting of immunosuppression modification, and most episodes occur >30 postinfection. Interestingly, proven coinfections including bacteria and fungi were also uncommon.

Our study and others show that transplant recipients can mount SARS-CoV-2–specific antibodies within 2 wk of COVID-19 symptoms, and these antibodies may be durable for 2–6 mo. In a study of 18 kidney transplants, Hartzell et al demonstrated that most patients develop SARS-CoV-2–specific antibodies from day 10 postsymptom onset.[28] Boyarsky et al found that, in a cohort of 18 organ transplant recipients, at a median of 98 d pot-COVID-19 diagnosis, 78% had a reactive screening immunoassay, of which 83% had detectable anti-S1 antibodies. All patients who did not mount an antibody response received convalescent plasma or intravenous immunoglobulin for management of COVID-19.[29] We found a high rate of antibody response to both RBD and NP. Also, anti-RBD and anti-NP were correlated with each other ($P < 0.0001$), but anti-RBD was more durable than the latter in the months following SARS-CoV-2 infection. Seropositivity for anti-NP in our study ranged from 57.1% to 73.2% >30 d from symptom onset, which is compatible with another study finding seroprevalence of 51% at 4–8 wk postdiagnosis.[30] Consistent with the findings of Burack et al, we identified convalescent and baseline immunosuppression as factors that appear to modify the magnitude of the humoral response.[30]

A major strength of our study is the analysis of viral shedding especially in outpatients. Viral loads in outpatients are challenging to capture since patients are in home isolation. However, we used a method for self-swabbing, which is has been validated to detect SARS-CoV-2 and also used in COVID-19 vaccine studies.[31,32] Most previous analyses of viral shedding have focused on transplant recipients with

severe disease requiring hospitalization.[33-35] Benotmane et al performed virological assessment on 40 kidney transplant recipients,[14,36] where almost 3-quarters (74.4%) had peak VL occur at diagnosis, and 25% of patients showed persistent viral shedding after 30 d. In another study, 10 of 47 (21%) of SOT recipients undergoing PCR testing at least 20 d after symptom onset tested positive for SARS-CoV-2.[37] Again, the potential bias of disease severity exists as individuals with severe COVID-19 are over-represented in many studies. We observed a relatively low rate of prolonged shedding, which may be related to the inclusion of many patients with mild disease severity. Our data suggest that the current quarantine period (in Canada) of up to 20 d for a COVID-positive transplant patient suggested by public health guidelines is adequate for those with mild to moderate disease. Importantly, most of these studies, including ours, only report VL and do not confirm whether the amount of shed virus is of an infectious nature. Of note, some studies, like ours, show no relationship between NP VLs and COVID-19 disease severity.[14]

Strengths of our study include a comprehensive prospective capture of all COVID-19 positive patients with low ascertainment bias, both inpatients and outpatients, as well as long-term follow-up of patients. The study was limited in that not all patients provided serum and nasopharyngeal swabs. We may not have captured all asymptomatic patients. A unique strength of our study is the determination of viral shedding in outpatients. In summary, our study provides key insights into the full spectrum of disease severity of COVID-19 in SOT recipients based on a prospective assessment of both mild and hospitalized cases couple with supporting serological and virological data. These data may also provide a sound base for comparison when assessing the effectiveness of SARS-CoV-2 vaccinations and the differential impact of SARS-CoV-2 variants.

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REFERENCES

1. Pereira MR, Mohan S, Cohen DJ, et al. COVID-19 in solid organ transplant recipients: initial report from the US epicenter. *Am J Transplant*. 2020;20:1800–1808.
2. Kates OS, Haydel BM, Florman SS, et al. Coronavirus disease 2019 in solid organ transplant: a multicenter cohort study. *Clin Infect Dis*. [Epub ahead of print. August 7, 2020]. doi:10.1093/cid/ciaa1097
3. Søfteland JM, Friman G, von Zur-Mühlen B, et al. COVID-19 in solid organ transplant recipients: a national cohort study from Sweden. *Am J Transplant*. 2021;21:2762–2773.
4. Roberts MB, Izzy S, Tahir Z, et al. COVID-19 in solid organ transplant recipients: dynamics of disease progression and inflammatory markers in ICU and non-ICU admitted patients. *Transpl Infect Dis*. 2020;22:e13407.
5. Chaudhry ZS, Williams JD, Vahia A, et al. Clinical characteristics and outcomes of COVID-19 in solid organ transplant recipients: a cohort study. *Am J Transplant*. 2020;20:3051–3060.
6. Sharma P, Chen V, Fung CM, et al. COVID-19 outcomes among solid organ transplant recipients: a case-control study. *Transplantation*. 2021;105:128–137.
7. Yi SG, Rogers AW, Saharia A, et al. Early experience with COVID-19 and solid organ transplantation at a US high-volume transplant center. *Transplantation*. 2020;104:2208–2214.
8. Felldin M, Søfteland JM, Magnusson J, et al. Initial report from a Swedish high-volume transplant center after the first wave of the COVID-19 pandemic. *Transplantation*. 2021;105:108–114.
9. Adamson CS. Antiviral agents: discovery to resistance. *Viruses*. 2020;12:E406.
10. Ali T, Al-Ali A, Fajji L, et al. Coronavirus disease-19: disease severity and outcomes of solid organ transplant recipients: different spectrums of disease in different populations? *Tranplantation*. 2021;105:121–127.
11. Ali Malekhosseini S, Nikoupour H, Gholami S, et al. A report of 85 cases of COVID-19 and abdominal transplantation from a single center: what are the associated factors with death among organ transplantation patients. *Transplantation*. 2021;105:90–99.
12. Molnar MZ, Bhalla A, Azhar A, et al; STOP-COVID Investigators. Outcomes of critically ill solid organ transplant patients with COVID-19 in the United States. *Am J Transplant*. 2020;20:3061–3071.
13. Miarons M, Larrosa-García M, García-García S, et al; Vall d'Hebron COVID-19 Working Group. COVID-19 in solid organ transplantation: a matched retrospective cohort study and evaluation of immunosuppression management. *Transplantation*. 2021;105:138–150.
14. Benotmane I, Gautier-Vargas G, Wendling MJ, et al. In-depth virological assessment of kidney transplant recipients with COVID-19. *Am J Transplant*. 2020;20:3162–3172.
15. Lumley SF, O'Donnell D, Stoesser NE, et al; Oxford University Hospitals Staff Testing Group. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N Engl J Med*. 2021;384:533–540.
16. Azzi Y, Parides M, Alani O, et al. COVID-19 infection in kidney transplant recipients at the epicenter of pandemics. *Kidney Int*. 2020;98:1559–1567.
17. Pujadas E, Chaudhry F, McBride R, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir Med*. 2020;8:e70.
18. Fajnzylber J, Regan J, Coxen K, et al; Massachusetts Consortium for Pathogen Readiness. SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun*. 2020;11:5493.
19. Thieme CJ, Anft M, Paniskaki K, et al. Robust T cell response toward spike, membrane, and nucleocapsid SARS-CoV-2 proteins is not associated with recovery in critical COVID-19 patients. *Cell Rep Med*. 2020;1:100092.
20. WHO Working Group on the Clinical Characterisation and Management of COVID-19 Infection. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis*. 2020;20:e192–e197.
21. Bryan A, Pepper G, Wener MH, et al. Performance characteristics of the Abbott architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. *J Clin Microbiol*. 2020;58:e00941–e00920.
22. Theel ES, Harring J, Hilgart H, et al. Performance characteristics of four high-throughput immunoassays for detection of IgG antibodies against SARS-CoV-2. *J Clin Microbiol*. 2020;58:e01243–e01220.
23. Elecsys Anti-SARS-CoV-2 S. *Package Insert 2020-09, V1.0; material numbers 09289267190 and 09289275190e*. Elecsys; 2020.
24. World Health Organization. Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR. 2020. Available at https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf?sfvrsn=d381fc88_2. Accessed May 20, 2021.
25. Public Health Ontario. SARS-CoV-2 (COVID-19 virus) variant of concern (VoC) surveillance. 2021. Available at https://www.publichealthontario.ca/en/laboratory-services/test-information-index/covid-19-voc. Accessed April 20, 2021.
26. Kumar D, Humar A, Keshavjee S, et al. A call to routinely test lower respiratory tract samples for SARS-CoV-2 in lung donors. *Am J Transplant*. 2021;21:2623–2624.
27. Government of Canada. Coronavirus disease 2019 (COVID-19): epidemiology update. 2021. Available at https://www.canada.ca/content/dam/phac-aspc/documents/services/diseases-maladies/2019-novel-coronavirus-infection/COVID19-epi-update-eng-2020-04-02.pdf. Accessed April 20, 2021.
28. Hartzell S, Bin S, Benedetti C, et al. Evidence of potent humoral immune activity in COVID-19-infected kidney transplant recipients. *Am J Transplant*. 2020;20:3149–3161.
29. Boyarsky BJ, Ou MT, Werbel WA, et al. Early development and durability of SARS-CoV-2 antibodies among solid organ transplant recipients: a pilot study. *Transplantation*. 2021;105:e52–e53.
30. Burack D, Pereira MR, Tsapepas DS, et al. Prevalence and predictors of SARS-CoV-2 antibodies among solid organ transplant recipients with confirmed infection. *Am J Transplant*. 2021;21:2254–2261.
31. McCulloch DJ, Kim AE, Wilcox NC, et al. Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection. *JAMA Netw Open*. 2020;3:e2016382.

32. Voysey M, Clemens SAC, Madhi SA, et al; Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet*. 2021;397:99–111.
33. Zhu L, Gong N, Liu B, et al. Coronavirus disease 2019 pneumonia in immunosuppressed renal transplant recipients: a summary of 10 confirmed cases in Wuhan, China. *Eur Urol*. 2020;77:748–754.
34. Italiano J, Bush R, Acharya R, et al. Persistent viral shedding despite seroconversion in a kidney transplant recipient with severe extrapulmonary COVID-19. *BMJ Case Rep*. 2020;13:e239612.
35. Roedl K, Heidenreich S, Pfefferle S, et al. Viral dynamics of SARS-CoV-2 in critically ill allogeneic hematopoietic stem cell transplant recipients and immunocompetent patients with COVID-19. *Am J Respir Crit Care Med*. 2021;203:242–245.
36. Gaston DC, Malinis M, Osborn R, et al. Clinical implications of SARS-CoV-2 cycle threshold values in solid organ transplant recipients. *Am J Transplant*. 2021;21:1304–1311.
37. Theodore DA, Greendyke WG, Miko B, et al. Cycle thresholds among solid organ transplant recipients testing positive for SARS-CoV-2. *Transplantation*. 2021;105:1445–1448.