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ORIGINAL PAPER

Anti-SARS-CoV-2 Seroconversion in COVID-19 Convalescent Kidney Transplant Recipients Compared with Non-transplanted Patients

Ricardo Martins Garcia[1], Vinicius Lafico Teixeira Gomes[1], Renato Demarchi Foresto[1,*], Mônica Rika Nakamura[1], Marcia Araújo Toffoli de Jesus[1], Elisabeth França Lucena[1], Renata Pimentel Rissoni[2], Marina Pontello Cristelli[1], Helio Tedesco Silva Junior[1], Lúcio Requião-Moura[1], José Medina Pestana[1]

1.Hospital do Rim e Hipertensão – Fundação Oswaldo Ramos – São Paulo/SP – Brazil. 2.Prefeitura de Ipaussu – Secretaria Municipal de Saúde e Higiene – Ipaussu/SP – Brazil.

*Corresponding author: rdforesto@gmail.com

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ABSTRACT

Objective: Due to immunosuppression, kidney transplant recipients (KTRs) might have lower seroconversion after COVID-19 than non-KTRs. Thus, we aimed to evaluate the seroconversion rate after COVID-19 between KTRs and non-KTRs. **Methods:** This cohort study enrolled three non-paired groups of patients with COVID-19: 601 KTRs, 211 healthcare workers (HCWs), and 170 non-transplanted inhabitants (INHs) in a countryside city in Brazil. The anti-severe acute respiratory syndrome coronavirus 2 nucleocapsid antibody was assessed 14 days after diagnosis. The primary outcome was seroconversion. **Results:** The KTRs were older, had more comorbidities and severe COVID-19. Compared to HCWs and INHs, admission to the intensive care unit (ICU; 44.9% vs. 0% vs. 1.8%, p<0.001), mechanical ventilation requirement (32.3% vs. 0% vs. 1.8%, p<0.001), and death (28.8% vs. 0% vs. 1.2%, p<0.001) were significantly higher in KTRs. Seroconversion did not differ between the groups: 76.2% in KTRs, 74.9% in HCWs, and 82.2% in INHs (p=0.35). In a group-adjusted multivariable logistic regression, while a short period between infection and blood sample collection reduced the probability of seroconversion (adjusted odds ratio [aOR]=0.986), the presence of fever (aOR=1.737, p=0.017), cough (aOR=1.785, p=0.005), and requirement for ventilatory support (OR=1.981, p=0.017) increased the risk. **Conclusions:** Clinical severity, mechanical ventilation requirement and death by COVID-19 were significantly higher among the KTRs. However, among the survivors, KTRs had a similar seroconversion prevalence associated with clinical severity parameters and a shorter time of blood sample collection.

Descriptors: COVID-19; SARS-CoV-2; Kidney Transplant Recipients; Seroconversion.

Soroconversão de Anticorpos Anti-SARS-CoV-2 em Receptores de Transplante Renal Convalescentes com COVID-19 em Comparação com Pacientes Não Transplantados

RESUMO

Objetivo: Devido à imunossupressão, receptores de transplante renal (RTRs) podem ter menor soroconversão após COVID-19 do que indivíduos não-transplantados. Assim, nosso objetivo foi avaliar a taxa de soroconversão após COVID-19 entre RTRs e não-RTRs. **Métodos:** Este estudo de coorte envolveu três grupos não pareados de pacientes com COVID-19: 601 RTRs, 211 profissionais de saúde (PSs) e 170 habitantes não transplantados (HNTs) em uma cidade do interior do Brasil. O anticorpo anti-SARS-CoV-2 foi avaliado 14 dias após o diagnóstico. O desfecho primário foi a taxa de soroconversão. **Resultados:** Os RTRs eram mais idosos, com mais comorbidades e COVID-19 grave. Em comparação com

profissionais de saúde e HNTs, admissão na unidade de terapia intensiva (UTI; 44,9% vs. 0% vs. 1,8%, p<0,001), necessidade de ventilação mecânica (32,3% vs. 0% vs. 1,8%, p<0,001), e óbito (28,8% vs. 0% vs. 1,2%, p<0,001) foram significativamente maiores em RTRs. A soroconversão não diferiu entre os grupos: 76,2% em RTRs, 74,9% em PSs e 82,2% em HNTs (p=0,35). Em uma regressão logística multivariada ajustada ao grupo, enquanto um curto período entre a infecção e a coleta da amostra de sangue reduziu a probabilidade de soroconversão (odds ratio [aOR] = 0,986), a presença de febre (aOR = 1,737, p = 0,017), tosse (aOR=1,785, p=0,005) e necessidade de suporte ventilatório (OR=1,981, p=0,017) aumentaram o risco. **Conclusões:** A gravidade clínica, a necessidade de ventilação mecânica e a morte por COVID-19 foram significativamente maiores entre os RTRs. No entanto, entre os sobreviventes, os RTRs tiveram prevalência de soroconversão semelhante associada aos parâmetros de gravidade clínica e menor tempo de coleta de amostra de sangue.

Descritores: COVID-19; SARS-CoV-2; Receptores de Transplante Renal; Soroconversão.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic poses varied and unprecedented challenges to the scientific community and public health system authorities. Initial studies aimed at describing the full spectrum of the clinical syndrome and establishing the best strategies for diagnosis and clinical management.[1–3] Regarding the specific immunological response to the infection, serology has played a central role in diagnosis, epidemiological inquiry and vaccine immunogenicity since the first tests were available.[4,5] Furthermore, the seroconversion and length of time of circulating antibodies in convalescent patients may be associated with clinical severity.[6] Due to adaptive immune response impairment caused by immunosuppressive agents, COVID-19 convalescent kidney transplant recipients (KTRs) seem to have lower seroconversion rates after the disease, and they are more susceptible to severe infection.[7]

Subsequently, the approved severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines demonstrated high immune reactogenicity, followed by an initial and significant reduction infections and deaths in countries with high vaccine coverage rates.[8,9] Unfortunately, the first reports showed low immunogenicity and effectiveness of vaccines for transplanted patients, regardless of the vaccine platform, leading to a negligible impact on the incidence of the disease with a modest reduction in the risk of death.[10–14]

More recently, the world has been impacted by the emergence of the more contagious new variant Omicron, associated with an increased number of new cases.[15] Compared with the previous variants, Omicron is more likely to reinfect, suggesting a possible immune escape mechanism.[16] Even in fully vaccinated patients, the neutralization antibody activity seems lower for the Omicron variant than for previous variants; however, compared to the Delta variant, the neutralization for Omicron is less impacted in convalescent and vaccinated patients.[16] Thus, investigating the immune response to a previous infection in convalescent patients — before vaccine availability — may be helpful in patients at a higher risk of COVID-19 severity.

Therefore, the present study aimed to evaluate the seroconversion rate after COVID-19 among KTRs compared to non-transplanted patients before the first case of the Delta variant in Brazil, before the vaccination campaign in the country. When this study was conducted, Brazil was one of the most affected countries in the world, with the second-largest absolute number of cases and the third highest number of deaths due to COVID-19.

METHODS

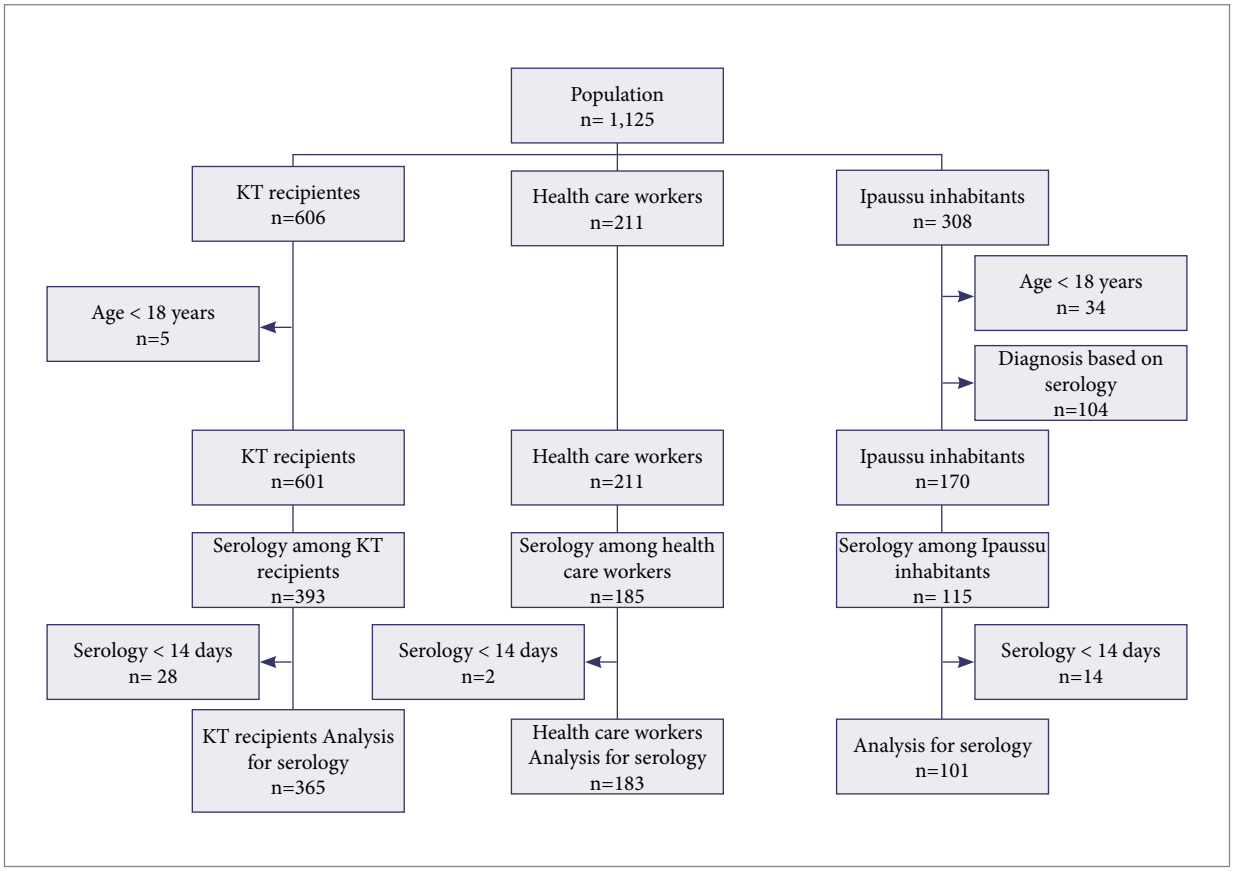
Study design and population

This retrospective cohort study included patients diagnosed with COVID-19 from three independent groups: KTRs, health care workers (HCWs) and inhabitants (INHs) of a city in the countryside of the state of São Paulo, Brazil (Ipaussu). All KTRs were followed in the Hospital do Rim, located in the capital of São Paulo, and all HCWs worked at this hospital. The city of Ipaussu is 224 miles from the capital, and it was chosen for this analysis because the Hospital do Rim assumed the matrix support strategies for COVID-19 diagnosis. The local ethics committee approved the study (identification number CAAE 35321020.9.0000.8098, approval number 4.417.135). Informed consent was obtained or exempted following the guidelines of the Declaration of Helsinki, specific national legislation and local institutional review board recommendations.

The eligible participants were adults older than 18 years who had symptomatic COVID-19 between March 20 and October 29, 2020, diagnosed using a real-time polymerase chain reaction (RT-PCR) test. Screening diagnoses in asymptomatic patients were not considered, and those diagnosed through serology or viral antigen detection were excluded. The final follow-up date was March 31, 2021, or the date of death. Serological data were collected between May 2020 and February 15,

2021. For the seroconversion analysis, patients with the serological data collected in the first 14 days following the COVID-19 diagnosis were excluded.

The at-risk population comprised all transplanted patients treated at the Hospital do Rim (11,875 patients), 1,032 HCWs, and all inhabitants living in Ipaussu in 2021 (estimated at 14,506 people, according to the official numbers of the Brazilian government). Between March and October 2020, 1,125 patients had COVID-19 in three groups: 606 among KTRs, 211 among HCWs, and 308 INHs (Fig. 1). Thirty-nine patients were excluded because they were younger than 18: 5 among KTRs and 34 among INHs. Additionally, among INHs, 104 were excluded because the diagnosis was not based on RT-PCR. Therefore, 982 patients were included: 601 (61.2%) KTRs, 211 (21.5%) HCWs, and 170 (17.3%) INHs.



Source: Elaborated by the authors.

Figure 1. Population disposition.

Data and definitions

The data source for the KTRs was electronic medical records. For HCWs, a self-based survey was applied at diagnosis (in-person or telehealth) and when they returned to their regular activities. For the INHs, a self-based survey was also conducted at the time of diagnosis and completed when the blood sample for serology assessment was collected.

Variables of interest were grouped into demographic data and symptoms/signs at COVID-19 diagnosis. The first group included age, sex, ethnicity, weight, height, smoking status, hypertension, diabetes, chronic obstructive pulmonary disease (COPD), previous heart disease and cancer. The following symptoms were obligatorily questioned: cough, coryza, nasal congestion, sore throat, dyspnea, headache, ageusia, anosmia, myalgia, and diarrhea. Fever was considered when the patient presented an axillary temperature higher than 37.8 °C. Dyspnea was defined as any degree of shortness of breath or difficulty in breathing subjectively self-reported by the patient, both reported at any time during the infection. Hypoxemia was considered when the patient presented peripheral oxygenation lower than 94%.

Clinical management was conducted according to the local practice of the healthcare service where the patients were referenced and categorized as domiciliary or in-hospital (ward or intensive care unit [ICU]).

Outcomes

The primary outcome was seroconversion after COVID-19 infection. The time of serological data collection is detailed in the following subsections. The secondary outcomes were hospitalization (ward or ICU), requirement for ventilatory support

(noninvasive or mechanical ventilation), and death attributable to COVID-19. Indications for hospitalization, inward allocation or ICU admission, and indication for noninvasive or mechanical ventilation were based on local practices in each center to which patients were referenced.

Serology assessment

Only patients with serologies collected at least 14 days post-infection were considered for seroconversion analysis. Patients who died or survived but did not have serology performed at any time or had serological data collected before day 14 of infection were excluded (Fig. 1).

The anti-SARS-CoV-2 nucleocapsid antibody was assessed by chemiluminescent microparticle immunoassay using the SARS-CoV-2 IgG Reagent Kit 6S60 (ARCHITECT Systems, Abbott®). This assay is an automated two-step immunoassay for the qualitative and quantitative detection of IgG antibodies against SARS-CoV-2 in human serum and plasma. First, the patient sample, SARS-CoV-2 antigen-coated paramagnetic microparticles and assay diluent were combined and incubated. If present, IgG antibodies bind to the SARS-CoV-2 antigen-coated microparticles. Next, the mixture was washed and anti-human IgG acridinium-labeled conjugates were added to create a reaction mixture and incubated. Following a wash cycle, the pre-trigger and trigger solutions were added. The resulting chemiluminescent reaction was measured as a relative light unit (RLU) and expressed as an index calculated by the ratio between the sample result and the producer's cut-off.

Statistical analysis

Continuous variables are summarized as median and interquartile intervals and compared among the three groups using the Kruskal–Wallis test. In contrast, the categorical variables and outcomes were summarized and compared using the X2 test.

All patients were stratified into two groups according to seroconversion status. The Mann-Whitney U test was performed among these groups to compare continuous variables, whereas categorical variables were compared using the X2 test. Multivariable analysis to identify independent predictors of seroconversion was performed using generalized linear mixed models with binary logistic regression adjusted for the groups (as random intercept): KTRs, HCWs, or INHs. Variables poorly associated with seroconversion in the univariate analysis ($p>0.20$), collinear variables and those with more than 5% missing values were not considered in the multivariate model. Imputation of missing data was unnecessary because any selected variable presented a missing value. The discrimination performance of the multivariable model was tested using the area under the receiver operating characteristic curve (AU-ROC). Statistical analyses were performed using SPSS version 26 (IBM, Armonk, NY, USA), and statistical significance was defined as a p-value<0.05, with a 95% confidence interval (95% CI).

RESULTS

Demographic data and clinical outcomes stratified by groups

Initially, 982 patients were enrolled: 601 KTRs, 211 HCWs, and 170 INHs. Demographic data are shown in Table 1. As expected, there were several differences between the groups. KTRs were older and had a higher incidence of smoking, hypertension, diabetes, previous heart disease and cancer. In contrast, INHs were more frequently white and had a higher body mass index (BMI). In addition, reports of COPD were more frequent among HCWs. Missing data were observed only for weight (n = 196, 19.9%), height (n = 192, 19.5%), BMI (n = 201, 20.5%), and ethnicity (n = 7, 0.7%).

Several differences were observed in the COVID-19 symptoms and signs (Table 2). Among the KTRs, diarrhea, dyspnea, and hypoxemia were the most frequent. HCWs more frequently presented upper respiratory symptoms such as coryza, nasal congestion, sore throat, fever and headache. Finally, cough and ageusia were more common among INHs. No differences were observed in the presence of anosmia.

As expected, the need for advanced health care was significantly more frequent among KTRs: 71.5% were hospitalized (vs. 3.8% among HCWs and 57.6% among INHs; $p<0.001$), and 44.9% required ICU (vs. 0% among HCWs and 1.8% among INHs; $p<0.001$). Similarly, the requirement for non-invasive ventilation (51.7% vs. 0% vs. 15.3%; $p<0.001$) and mechanical ventilation (32.3% vs. 0% vs. 1.8%; $p<0.001$) were more frequent in the KTRs (vs. HCW and INHs, respectively). Finally, the fatality rate among KTRs recipients was 28.8%, whereas only 1.2% of INHs died ($p<0.001$). No deaths were observed among HCWs. The outcomes are summarized in Table 3.

Table 1. Demographic data.

Variables	Non-missing values n=982	Kidney transplant recipients n=601	Healthcare workers n=211	Inhabitants of Ipaussu n=170	p-value
Age (years)	982	54.0 (44.0; 62.0)	37.0 (29.0; 44.0)	42.0 (31.0; 52.5)	<0.001
Male – n (%)	982	365 (60.7)	81 (38.4)	73 (42.9)	<0.001
Ethnicity – n (%)	975				<0.001
White		*391 (65.1)*	*129 (63.2)*	*147 (86.5)*	
Mixed		*131 (21.8)*	*44 (21.6)*	*13 (7.6)*	
Afro-Brazilian		*70 (11.6)*	*20 (9.8)*	*10 (5.9)*	
Other		*9 (1.5)*	*11 (5.4)*	-	
Weight (kg)	786	73.5 (63.0; 84.2)	75.0 (63.7; 86.0)	83.7 (70.2; 92.1)	<0.001
Height (m)	790	1.67 (1.60; 1.73)	1.67 (1.60; 1.75)	1.63 (1.57; 1.70)	0.006
BMI (kg/m^2)	781	26.6 (23.4; 29.7)	26.3 (23.9; 29.4)	30.2 (26.3; 33.8)	<0.001
Overweight – n (%)		*218 (39.4)*	*56 (42.1)*	*33 (35.1)*	*0.57*
Obesity – n (%)		*132 (23.8)*	*29 (21.8)*	*49 (52.1)*	*<0.001*
Smokers – n (%)	982	131 (21.8)	18 (8.5)	2 (1.2)	<0.001
Hypertension – n (%)	982	421 (70.0)	14 (6.6)	12 (7.1)	<0.001
Diabetes – n (%)	982	188 (31.3)	5 (2.4)	15 (8.8)	<0.001
COPD – n (%)	982	11 (1.8)	12 (5.7)	2 (1.2)	0.004
Heart disease – n (%)	982	59 (9.8)	6 (2.8)	5 (2.9)	<0.001
Cancer – n (%)	982	35 (5.8)	0 (0.0)	0 (0.0)	<0.001

BMI: Body Mass Index; COPD: Chronic Obstructive Pulmonary Disease. Source: Elaborated by the authors.

Table 2. Symptoms and signs at COVID-19 diagnosis.

Symptoms or signs	Kidney transplant recipients n=601	Health care workers n=211	Inhabitants of Ipaussu n=170	p-value
Fever – n (%)	356 (40.8)	95 (45.0)	74 (43.5)	<0.001
Cough – n (%)	349 (58.1)	115 (54.5)	118 (69.4)	0.008
Coryza – n (%)	122 (20.3)	123 (58.3)	39 (22.9)	<0.001
Nasal congestion – n (%)	101 (16.8)	122 (57.8)	10 (5.9)	<0.001
Sore throat – n (%)	53 (8.8)	87 (41.2)	60 (35.3)	<0.001
Myalgia – n (%)	292 (48.6)	133 (63.0)	88 (51.8)	0.001
Diarrhea – n (%)	199 (33.1)	30 (14.2)	42 (24.7)	<0.001
Headache – n (%)	150 (25.0)	144 (68.2)	73 (42.9)	<0.001
Ageusia – n (%)	3 (0.5)	59 (28.0)	51 (30.0)	<0.001
Anosmia – n (%)	192 (32.3)	68 (32.2)	53 (31.2)	0.96
Dyspnea – n (%)	304 (50.6)	36 (17.1)	42 (28.6)	<0.001
Hypoxemia – n (%)*	187 (31.1)	2 (0.9)	0 (0.0)	0.004

*Missing values: 23; for all other variables, there was no single value. Source: Elaborated by the authors.

Table 3. Clinical outcomes stratified by the three non-paired groups.

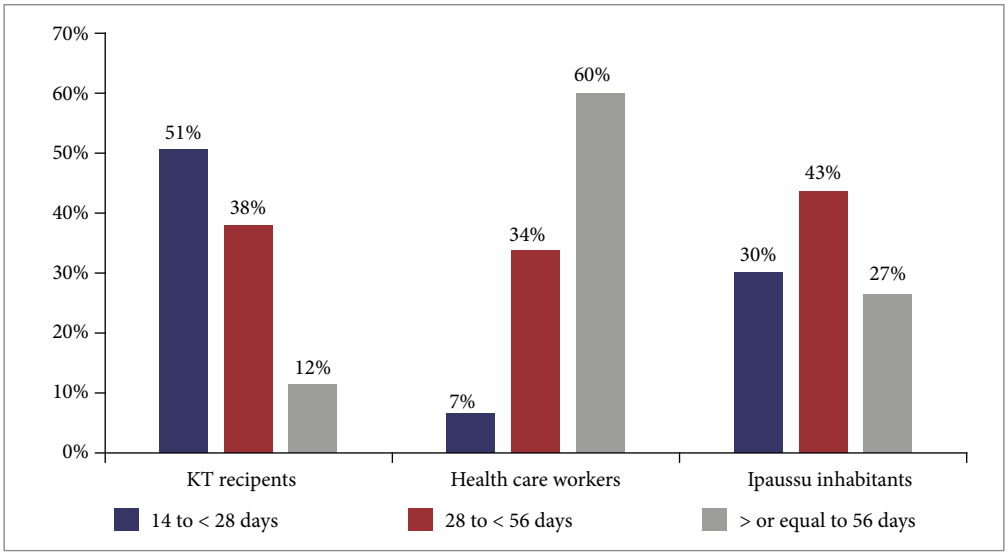
Outcomes*	Kidney transplant recipients n=601	Health care workers n=211	Inhabitants of Ipaussu n=170	p-value
Home care – n (%)	167 (27.8)	203 (96.2)	72 (42.4)	<0.001
In-hospital ward – n (%)	430 (71.5)	8 (3.8)	98 (57.6)	<0.001
Intensive care unit – n (%)	270 (44.9)	0 (0.0)	3 (1.8)	<0.001
Non-invasive ventilation – n (%)	311 (51.7)	0 (0.0)	26 (15.3)	<0.001
Mechanical ventilation – n (%)	194 (32.3)	0 (0.0)	3 (1.8)	<0.001
Death – n (%)	173 (28.8)	0 (0.0)	2 (1.2)	<0.001

*There are no missing values. Source: Elaborated by the authors.

Assessing serological data of COVID-19 in convalescent patients

Serum for serology was collected from 649 (66.1%) patients at least 14 days after the infection: 365 (60.7%) KTRs, 183 (86.7%) HCWs, and 101 (59.4%) INHs. The median time for blood sample collection was different in the three populations, being significantly earlier among KTRs: 28.0 (22.0; 42.0) days versus 45.0 (24.0; 63.0) days among INHs versus 67.0 (42.0; 108.0) days among HCW, p<0.001. Figure 2 shows the frequency of sample collection at three different periods stratified by population (14 to < 28 days,

28 days < 56 days, and ≥ 56 days). The serological data of most KTRs (51%) were collected in the first period, whereas 60% of HCWs were collected in the third time. For INHs, the collection distribution was more homogenous in all three time periods.



Source: Elaborated by the authors.

Figure 2. Frequency of the sample collection in three different periods.

There were no differences in the seroconversion rates between the groups: 76.2% (n=278) in KTRs, 74.9% (n=137) in HCWs, and 82.2% (n=83) in INHs, p=0.35. According to the groups, a small but statistically significant difference was observed in the anti-SARS-CoV-2 IgG index. The highest IgG anti-SARS-CoV-2 index was observed among INHs, 5.8 (4.1; 7.5) RLU, compared to 5.4 (3.8; 6.7) in KTRs and 4.4 (3.4; 6.6) in HCW (Table 4).

Table 4. Seroconversion prevalence and titer.

Results	Kidney transplant recipients n=601	Health care workers n=211	Inhabitants of Ipaussu n=170	p-value
Serology* – n (%)	365 (60.7)	183 (86.7)	101 (59.4)	<0.001
Positive – n (%)	278 (76.2)	137 (74.9)	83 (82.2)	0.35
Time from infection to serology collection (days)	28.0 (22.0; 42.0)	67.0 (42.0; 108.0)	45.0 (24.0; 63.0)	<0.001
Titer (relative light unit)	5.4 (3.8; 6.7)	4.4 (3.4; 6.6)	5.8 (4.1; 7.5)	0.009

*Including only serologies assessed 14 days after diagnosis. Source: Elaborated by the authors.

Probability of seroconversion after COVID-19 infection

Among the survivors, 498 (76.7%) were positive for anti-SARS-CoV-2 IgG after infection recovery. The univariate analysis compared patients with positive and negative serology (Table 5). The BMI (27.2 vs. 22.9 kg/m2, p=0.006), frequency of fever (60.0 vs. 45.0%, p=0.001), cough (63.3 vs. 48.3%, p=0.001), and requirement for non-invasive ventilation (25.9 vs. 14.6%, p=0.004) were higher in patients with positive serology. In contrast, the time for serology collection was shorter (35.0 vs. 51.0 days, p<0.001).

Variables with a p-value <0.20 in the previous analysis were considered for multivariable modeling: age, sex, smoking, fever, cough, sore throat, headache, hypoxemia, severity and time between the infection diagnosis and serology. Severity was defined as ventilatory assistance, considering both invasive and non-invasive strategies. BMI was excluded from the modeling because of missing values higher than 5% (n=101; 15.6%). Despite the low number of missing patients (n=23; 3.5%), hypoxemia was excluded owing to collinearity with severity.

The multivariate analysis adjusted for groups is presented in Table 6. The probability of seroconversion was increased by 63% if fever (adjusted odds ratio [aOR]=1.637; 95% CI=1.093–2.452; p=0.017) was present and by 78% if cough (aOR=1.785; 95% CI=1.192–2.673; p=0.005) was present during the infection. While the requirement for ventilatory assistance was associated with a 98% higher likelihood of seroconversion (aOR=1.981; 95% CI=1.131–3.469; p=0.017), a longer time between infection and serology collection was associated with a lower probability: each day reduced the odds by 1.4% (aOR= 0.986; 95% CI=0.982–0.990; p<0.001). Finally, the multivariable model achieved an AU-ROC of 0.739 (95% CI=0.684–0.776).

Table 5. Univariable analysis for seroconversion stratified by seropositive and seronegative patients.

Variables	Non-missing values n=649	Positive n=498 76.7%	Negative n=151 23.3%	p-value
Demographic				
Age (years)	649	45.0 (37.0; 56.0)	44.0 (34.9; 54.0)	0.12
Male – n (%)	649	261 (52.4)	66 (43.7)	0.06
Ethnicity – n (%)	643	-	-	0.80
White		*326 (66.1)*	*105 (70.0)*	
Mixed		*102 (20.7)*	*29 (19.3)*	
Afro-Brazilian		*55 (11.2)*	*14 (9.3)*	
Other		*10 (2.0)*	*2 (1.3)*	
Weight (kg)	553	75.1 (65.0; 88.0)	71.3 (60.0; 84.0)	0.004
Height (m)	553	1.66 (1.60; 1.73)	1.65 (1.58; 1.72)	0.135
BMI (kg/m^2)	548	27.2 (24.2; 30.8)	25.7 (22.9; 29.6)	0.006
Overweight		*167 (39.7)*	*47 (37.0)*	*0.59*
Obesity		*125 (29.7)*	*27 (21.3)*	*0.06*
Smokers – n (%)	649	59 (11.8)	25 (16.6)	0.13
Hypertension – n (%)	649	216 (43.4)	57 (37.7)	0.22
Diabetes – n (%)	649	98 (19.7)	26 (17.2)	0.50
COPD – n (%)	649	12 (2.4)	5 (3.3)	0.54
Heart disease – n (%)	649	28 (5.6)	7 (4.6)	0.64
Cancer – n (%)	649	14 (2.8)	3 (2.0)	0.58
Symptoms and signs				
Fever – n (%)	649	299 (60.0)	68 (45.0)	0.001
Cough – n (%)	649	315 (63.3)	73 (48.3)	0.001
Coryza – n (%)	649	154 (30.9)	57 (37.7)	0.12
Nasal congestion – n (%)	649	126 (25.3)	48 (31.8)	0.11
Sore throat – n (%)	649	104 (20.9)	40 (26.5)	0.15
Myalgia – n (%)	649	278 (55.8)	89 (58.9)	0.50
Diarrhea – n (%)	649	143 (28.7)	41 (27.2)	0.71
Headache – n (%)	649	200 (40.2)	71 (47.0)	0.13
Ageusia – n (%)	649	65 (13.1)	18 (11.9)	0.71
Anosmia – n (%)	649	177 (35.5)	60 (39.7)	0.35
Dyspnea – n (%)	626	155 (32.4)	45 (30.6)	0.69
Hypoxemia – n (%)	626	72 (15.0)	14 (9.5)	0.09
Outcomes				
Home care – n (%)	649	264 (53.0)	94 (62.3)	0.05
Ward – n (%)	649	233 (46.8)	57 (37.7)	0.05
Intensive care unit – n (%)	649	66 (13.3)	14 (9.3)	0.19
Non-invasive ventilation – n (%)	649	129 (25.9)	22 (14.6)	0.004
Mechanical ventilation – n (%)	649	21 (4.2)	2 (1.3)	0.07
Serology				
Time from infection to serology collection (days)	649	35.0 (24.0; 59.0)	51.0 (25.0; 112.0)	<0.001

Patients for whom serology was collected within 14 days after the COVID-19 diagnosis were excluded. BMI: Body Mass Index; COPD: Chronic Obstructive Pulmonary Disease. Source: Elaborated by the authors.

Table 6. Univariable and multivariable group-adjusted model for seroconversion probability.

Variables	Univariable			Adjusted Multivariable		
	OR	95% CI	P	aOR	95% CI	p-value
Age (each year old)	1.012	0.998; 1.026	0.108	1.005	0.988; 1.023	0.552
Male sex	1.418	0.983; 2.046	0.062	1.331	0.885; 2.001	0.169
Smoking	0.667	0.408; 1.126	0.133	0.602	0.341; 1.062	0.080
Headache	0.756	0.524; 1.091	0.135	0.821	0.532; 1.267	0.372
Sore throat	0.732	0.481; 1.116	0.147	0.790	0.474; 1.317	0.366
Fever	1.834	1.270; 2.648	0.001	1.637	1.093; 2.452	0.017
Cough	1.839	1.274; 2.656	0.001	1.785	1.192; 2.673	0.005
Ventilatory support	1.966	1.208; 3.199	0.007	1.981	1.131; 3.469	0.017
Time from infection to serology collection (each day)	0.989	0.985; 0.992	<0.001	0.986	0.982; 0.990	<0.001

Adjusted for a group of patients: kidney transplant recipients, health care workers or Ipaussu inhabitants. The area under the receiver operating characteristic curve for predicting positive serology was 0.730 (95% CI 0.684–0.776). OR: Odds Ratio; CI: Confidence Interval; aOR: adjusted Odds Ratio. Source: Elaborated by the authors.

DISCUSSION

Our study observed similar seroconversion rates after recovery from SARS-CoV-2 infection among transplanted and non-transplanted patients. Therefore, we expected the seroconversion rate to be lower among KTRs because of the adaptive immune impairment caused by the chronic use of immunosuppressive agents. To test the primary hypothesis, we compared the seroconversion rate among KTRs with two independent and non-paired populations: HCWs who worked at the same hospital where the KTRs were treated and the general population living in a small city in our state. As expected, the groups differed in demographic data and COVID-19-attributable symptoms and signs. Notably, dyspnea and hypoxemia were significantly more frequent among KTRs, and consequently, they had worse clinical outcomes. Furthermore, until the last longitudinal observation, none of the HCWs had died. Finally, we observed that the seroconversion probability seemed related to COVID-19 severity.

In the first studies, the incidence of IgG against SARS-CoV-2 nucleocapsid protein varied between 80–100% within two or three weeks after infection.[17–20] However, for solid organ transplant recipients, reports based on nucleocapsid protein response have found a seroconversion rate of 50% at a median of seven weeks after COVID-19 confirmed diagnosis.[21] Some factors were attributed to playing a role in these seroconversion discrepancies. For instance, the short period between transplantation and COVID-19 diagnosis is less associated with the probability of seroconversion.[21] However, patients with a recent report of acute rejection treatment or under an immunosuppressive maintenance regimen based on more than two agents are also less likely to produce a specific humoral response against SARS-CoV-2.[21] Other traditional factors related to a limited humoral response to viral infections include immunological induction with T cell-depleting drugs, a cumulative number of comorbidities and poor baseline kidney function.[22] These data indirectly suggest an impact of the state of immunodepression on COVID-19 adaptive response among those patients.

In contrast, the seroconversion rate in KTRs is closer to that in non-KTRs when the virus-specific serological response is assessed early during the acute phase of infection. In a study enrolling patients hospitalized due to COVID-19, SARS-CoV-2 IgG response was observed in 77% of the KTRs 14 days after the diagnosis, and the titers were comparable with the immunocompetent controls.[7] The same has been observed for T cell-specific responses.[7] In a retrospective study comprising 3,192 COVID-19 patients carried out in three centers in Wuhan, critical illness was a risk factor for longer viral positivity, varying from 24 to 18 days among critically and non-critically ill patients, respectively, and a consequent increased rate of seroconversion from the first (44.6%) to the fourth week (93.3% vs. 81.5%) in non-critically ill patients.[23] In another study, IgM, IgG, and IgA titers (receptor binding domain spike protein, RDB) were higher in hospitalized patients than in those who did not require hospitalization, for whom a more rapid decline in titers was observed.[6] In our study, we observed a similar seroconversion rate among the groups, but the KTRs had more criteria for severity. We found a 2-fold higher probability of seroconversion if ventilatory support was required, a clinical marker of severe acute respiratory syndrome. Thus, COVID-19 severity seems to be associated with the likelihood of humoral and cellular responses, even in immunosuppressed patients.

As expected, we found discrepancies between the groups regarding COVID-19 clinical presentation and outcomes. Respiratory symptoms were predominant, followed by fever and other systemic symptoms in the three groups, but there was a higher frequency of dyspnea and hypoxemia in KTRs. Unlike other diseases caused by respiratory viruses, one peculiar characteristic of COVID-19 is the spectrum of clinical presentations with several systemic symptoms, despite the predominance of the respiratory syndrome.[1,24] The same is observed in KTRs, with 40–70% presenting with cough, dyspnea and fever,[25–27] and severe acute respiratory syndrome is the leading cause of hospitalization.[28] For KTRs, gastrointestinal symptoms are persistent, mainly diarrhea, as confirmed in our cohort.[25–27] While the respiratory syndrome is a predictor of severity, we previously demonstrated that diarrhea is a predictor of hospitalization among KTRs.[27] The presence of replicant viruses in the feces of patients with COVID-19 has been reported, although the direct effect of the virus in the gut tissue has not been well defined.[29,30] The use of immunosuppressive drugs, such as mycophenolate acid, is commonly associated with diarrhea, and coinfection by intestinal parasites or cytomegalovirus may explain the high frequency of diarrhea among KTRs.[31–33]

Our study was conducted before the immunization campaign in Brazil. While a substantial reduction in cases and deaths followed vaccination in the overall population, it was modest among the KTRs. Recently, data from the national UK register enrolling 39,260 KTRs demonstrated that vaccination did not reduce SARS-CoV-2 infection in these patients and that the risk of death was reduced by 31% four weeks after the second dose of ChAdOx1-S, but it was not reduced after two doses of BNT162b2.[11] In a phase IV clinical trial, we evaluated the effectiveness of an inactivated viral vaccine in 3,371 KTRs.[14] Despite decreasing the number of cases from 64/1,000 persons at risk (before vaccination) to 42/1,000 persons at risk (after vaccination), we did not observe any impact on case-fatality rates. In contrast, recent studies found that non-KTRs with pre-existing immunity derived from infection have an antibody titer and memory B cells significantly boosted in the short term after one dose of vaccine.[34,35] Data regarding the vaccine response in KTRs recovered from COVID-19 are unknown; however, evidence from the overall population highlights the possibility of a better immunological response for recovered patients.

Lastly, our study has several limitations, some related to the retrospective and observational design; therefore, potential selection bias should be carefully considered. In addition, we compared three unpaired groups. While the decision not to pair the individuals imposed several differences regarding demographic characteristics, we aimed to describe the main differences between these groups, highlighting the differences between KTRs and non-KTRs. Furthermore, we adjusted the multivariable model for the groups to reduce the impact of these imbalances on the primary outcome among the populations. Moreover, the study was carried out early in the pandemic and before the vaccination of KTRs in our country. Finally, although serology was performed in the same laboratory for all patients, the standard method for serology was based on antibodies against the nucleocapsid proteins, which are less sensitive than the RBD spike protein.

In conclusion, we observed that KTRs presented a different clinical spectrum from the two non-paired populations of KTRs during the pandemic before immunization. In contrast to the primary hypothesis, the seroconversion rate was similar among KTRs and non-KTRs, and variables related to more severe infection were associated with the likelihood of seroconversion, independent of transplantation.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Substantive scientific and intellectual contributions to the study: Garcia RM, Gomes VLT, Foresto RD, Nakamura MR, Jesus MAT, Lucena EF, Rissoni RP, Cristelli MP, Silva Junior HT, Requião-Moura L, Pestana JM; **Conception and design:** Foresto RD, Nakamura MR, Jesus MAT, Lucena EF, Rissoni RP, Cristelli MP, Silva Junior HT, Requião-Moura L, Pestana JM; **Data analysis and interpretation:** Foresto RD, Cristelli MP, Silva Junior HT, Requião-Moura L, Pestana JM; **Article writing:** Garcia RM, Gomes VLT, Foresto RD; **Critical revision:** Foresto RD, Requião-Moura L; **Final approval:** Garcia RM, Gomes VLT, Foresto RD, Nakamura MR, Jesus MAT, Lucena EF, Rissoni RP, Cristelli MP, Silva Junior HT, Requião-Moura L, Pestana JM.

AVAILABILITY OF RESEARCH DATA

Data will be provided upon request.

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