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Lower seroprevalence for SARS-CoV-2-specific antibodies among kidney transplant recipients compared to the general population in the city of Sao Paulo, Brazil

Marina P. Cristelli[1] [ORCID] | **Laila A. Viana[1]** | **Carlos M. Fortaleza[2]** | **Celso Granato[3]** |
Monica R. Nakamura[1] | **Daniel W.C.L. Santos[1]** | **Renato Demarchi Foresto[1]** |
Helio Tedesco-Silva[1] | **Jose Medina-Pestana[1]**

[1] Transplant Division, Hospital do Rim, Universidade Federal de São Paulo (UNIFESP), São Paulo, Sao Paulo, Brazil

[2] Infectious Diseases Division, Universidade Estadual Paulista (UNESP), Botucatu, Sao Paulo, Brazil

[3] Infectious Diseases Division, Universidade Federal de São Paulo (UNIFESP), São Paulo, Sao Paulo, Brazil

Correspondence
Marina Pontello Cristelli, Transplant Division, Hospital do Rim, Universidade Federal de São Paulo (UNIFESP), Borges Lagoa, 960, São Paulo, Brazil 04038-002.
Email: ninacristelli@yahoo.com.br

Abstract

Background: Kidney transplant recipients have higher COVID-19 associated mortality compared to the general population. However, as only symptomatic patients seek medical attention, the current level of exposure, the main sources of acquisition, and the behavior of humoral immunity over time are poorly understood.

Methods: This cross-sectional prospective single-center study recruited kidney transplant recipients of any age living in Sao Paulo. A sample size of 401 patients was calculated considering the 17.2% seroprevalence in the municipality population from a published survey, a 95% confidence interval and an absolute error of 2%.

Results: Of the 2636 eligible patients, 416 were included. The seroprevalence for IgG anti-SARS-CoV-2 was 8.2%. Seroconversion rate decreased with increasing age, from 15.7% (18–35 years) to 8.3% (36–60 years) and 4.2% (>60 years, $p = 0.042$). Seropositivity among previously confirmed COVID-19 patients was 68.4%, followed by 9.4% in those with flu-like symptoms and only 4.6% among asymptomatic patients ($p < 0.0001$). Seroprevalence was significantly higher among patients reporting household contact ($p = 0.018$). Twenty-seven from the 34 IgG+ patients had a second test after 59 (IQR 50–63) days, and, in 33%, the IgG index became below the positivity threshold.

Conclusions: In this cohort of kidney transplant recipients, the seroprevalence for IgG anti-SARS-CoV-2 was lower than that of the general population, decreased with ageing, and was associated with household contacts. In a considerable proportion of the patients, there was a significant decay in the IgG levels in a short period of time. Therefore, preventive strategies, such as prioritization for vaccination, should be urgently considered.

KEYWORDS
COVID-19, kidney transplant, seroprevalence

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1 | INTRODUCTION

Population-based data on the seroprevalence of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are now available worldwide, showing wide variability depending on age, ethnicity, socio-economic level, geographic region, and timing of the survey during the pandemic.[1,2] In Brazil, a country with one of the highest number of cases,[3] a large cross-sectional study including more than 30,000 households from 133 sentinel cities in all states, showed an increase in seroprevalence from 1.9% in May to 3.1% in June, 2020.[4] Infection rates are higher among health professionals[2,5,6] and younger people due to higher environmental and mobility exposure, respectively.[1,4]

Transplant recipients have a higher COVID-19-associated mortality compared to the general population.[7–9] Yet, because only symptomatic patients seek health-care assistance, the actual level of exposure is not known. Whether routine and unscheduled medical visits to transplant centers increase the risk of infection, compared to the general population, is still unknown.

In Sao Paulo, a city with more than 12 million inhabitants (8,407,202 inhabitants aged 18 or over) and a population density of 8,054/kmš, a serosurvey with six phases was established to estimate the percentage of adults infected by SARS-CoV-2. Seroprevalence increased from 11.5% in June (15th to 24th) to 17.9% in July (20th to 29th), and to 26.2% in October (1st to 10th) 2020.[10] Therefore, we sought to determine and compare the seroprevalence for SARS-CoV-2 among kidney transplant recipients living in the city taking advantage of this survey in the general population. We also explored the risk factors associated with the presence of a positive IgG serology and the antibodies titers in a follow up samples two months apart.

2 | METHODS

2.1 | Study design and population

This was a cross-sectional prospective single-center study. The study was approved by the Ethics Committee (#35321020.9.0000.8098). Eligible patients were all kidney transplant recipients of any age currently living in Sao Paulo, with at least one outpatient visit within the last year ($n = 2,636$). Due to mobility restrictions, only those patients with a scheduled face-to-face appointment were screened. Only patients providing the written informed consent were enrolled in the study. No one of the patients included in the present analysis were enrolled in COVID-19 vaccine clinical trials or have undergone vaccination prior to antibody measurement.

2.2 | Sampling

The sample size calculation was performed using the OpenEpi software, version 3.01, considering the total population of eligible patients ($n = 2,636$) and the 17.2% prevalence of the event of interest (seropositivity) in the general population according to the municipal epidemiological survey.[10] With a prespecified confidence interval of 95.0% and an absolute error of 2%, the expected total sample size was 401 people.

2.3 | Regional stratification

Of the 2636 kidney transplant recipients eligible for the survey, 19% live in the North, 32% in the South, 3% in the Center, 19% in the East, and 27% in the West region of the city. Since regional differences may influence the degree of exposure to the infection, the study population was constituted to represent the regional distribution of patients. Therefore, all the patients with a scheduled in-person outpatient visit beginning of September 1st, 2020 were screened until a representative sample of each city region was completed, which occurred on 25th of September 2020.

2.4 | Clinical evaluation

All enrolled patients answered a questionnaire containing the following questions: (1) Do you work as a health professional?; (2) have you had prior SARS-CoV-2 infection confirmed by either viral detection by RT-PCR testing or serologic testing?; (3) Have you had flu-like symptoms (that included fever, chills, headache, muscle or body aches, cough, sore throat, runny nose, fatigue, nausea, vomiting, and diarrhea) in the last six months?; (4) Have you have close contact (for at least 15 minutes) with a suspicious or confirmed case of SARS-CoV-2 infection in the past six months? Socio-demographic and clinical characteristics of interest were obtained from the electronic institutional records and included age, gender, ethnicity, blood type, past history of diabetes, donor source (deceased or living), class I and class II panel reactive antibody (PRA), time from transplantation, and region of residence. Mixed ethnicity refers to people of mixed race between Caucasians and Africans.

2.5 | Anti-SARS-CoV-2 antibody testing

The presence of anti-SARS-CoV-2 antibodies (IgG) was determined in a serum sample obtained at the scheduled in-person outpatient visit. Patients with a positive test were invited to provide a follow-up sample two months later to analyze antibody titers decay. Anti-SARS-CoV-2 antibody (IgG) was determined using the Abbott SARS-CoV-2 IgG assay on the Abbott Architect system. The assay is a chemiluminescent microparticle immunoassay for qualitative detection of IgG against the SARS-CoV-2 nucleoprotein. The results were interpreted as positive according to the manufacturer's instructions with a cut-off antibody index of 1.68 arbitrary units.

2.6 | Statistical analysis

Continuous variables were presented as median and interquartile ranges (IQR). Comparisons were made using the non-parametric

Mann–Whitney U test for independent samples and the Wilcoxon signed-rank test for dependent samples. Categorical variables were reported as frequencies and percentages, and comparisons were made using the Pearson chi-square test. Differences or effects were considered significant with a $p < 0.05$. Attack rate was defined as the percentage of the persons at risk that had a confirmed COVID-19 diagnosis either by RT-PCR or antigen testing. Patients who had a positive serologic test performed for the purpose of serosurvey studies were not included in the numerator; therefore, seroprevalence rates are expected to be higher than attack rates. Statistical analysis was performed using the SPSS program v. 22.0 (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Study cohort

Of the 2636 eligible patients, 552 subjects with a scheduled in-person appointment were screened to construct the representative regional cohorts. Of them, 64 were no longer living in Sao Paulo, and 50 patients did not consent to participate in the study. Finally, 22 patients had no serologic test result at the time of this analysis, resulting in 416 patients included for analysis (Supplementary Material 4). There were no differences comparing the demographic characteristics of the study cohort with the eligible population living in Sao Paulo, except for a longer median time after transplantation. Importantly, the study cohort well represented the regional distribution of residence of the eligible population (Supplementary Material 1).

3.2 | Seroprevalence for SARS-CoV-2 infection

In this cohort ($n = 416$), the seroprevalence for SARS-CoV-2 infection was 8.2% (n = 34). Seroconversion rate decreased with increasing age strata, from 15.7% (18-35 years) to 8.3% (36-60 years) and 4.2% (older than 60 years). Seropositivity was associated with previous confirmed diagnosis of COVID-19 in 68.4% of the patients, with a median time between diagnosis and serology testing of 103 (IQR 98–134) days. Seropositivity among patients with flu-like symptoms was 9.4% and only 4.6% among asymptomatic patients. Among five (15%) patients with flu-like syndrome in the six months prior to the investigation, three had contact with a confirmed or suspected person with COVID-19. Seroprevalence was significantly higher among patients reporting household contact (Supplementary Material 2).

3.3 | Comparison between IgG+ and IgG− patients

Patients showing seroconversion were younger, primarily due to a lower representation of the older than 60 years stratum compared to IgG- patients. There were no differences in gender, ethnicity, and blood type, history of diabetes mellitus, donor type, and time after transplantation. There were also no differences in environmental conditions such as regions of residence, health care profession, and contact tracing. Notably, compared to IgG− patients, a higher proportion of IgG+ patients had previous confirmed SARS-CoV-2 infection (38% vs. 2%, $p < 0.0001$, Table 1).

3.4 | Anti-SARS-CoV-2 antibody testing and follow-up

The median IgG anti-SARS-CoV-2 index of the 34 patients with positive serology was 3.1 arbitrary units (IQR 2.4–5.4). There were no differences in median IgG index according to clinical status (Supplementary Material 3). Twenty-seven patients had a second test after 59 (IQR 50–63) days, showing a median index of 2.6 arbitrary units (IQR 1.6–4.2). There were no differences in median IgG index comparing the first and second measurements (Supplementary Material 3) but, importantly, in nine patients (33%) the IgG index became below the positivity threshold, indicating the phenomena of seroreversion (Supplementary Material 5).
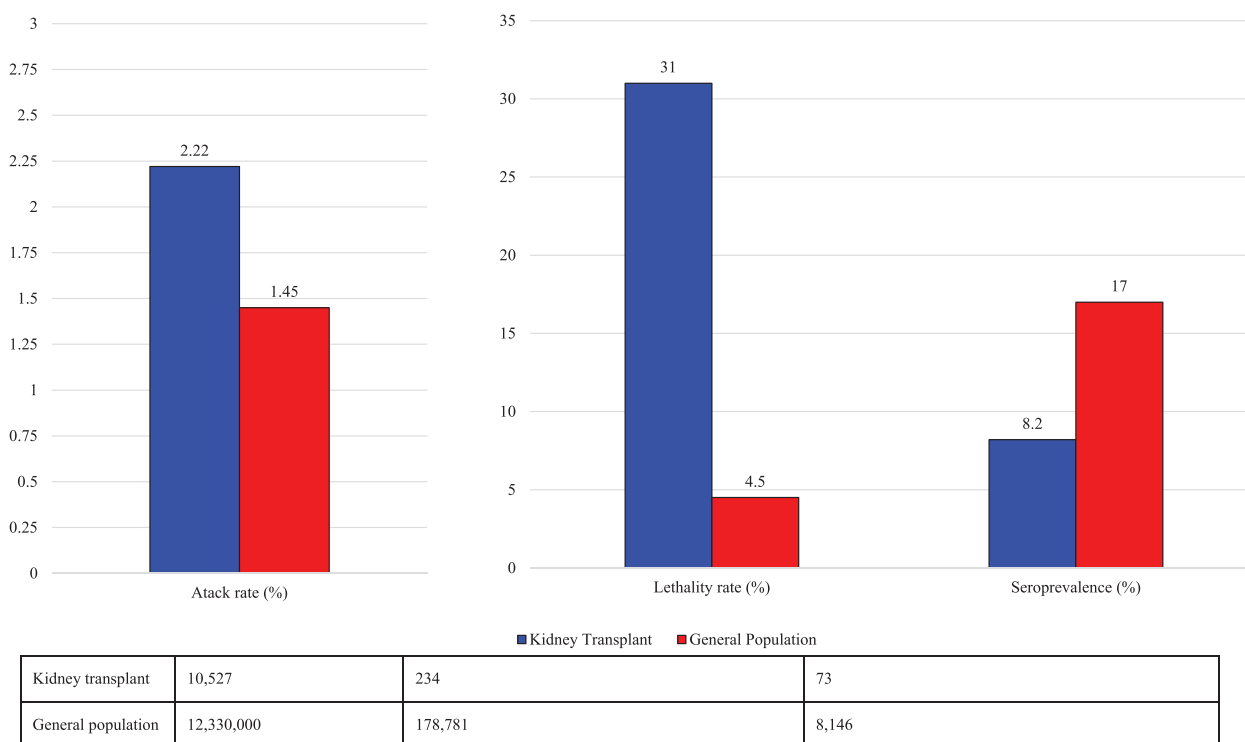
4 | DISCUSSION

This is the first Latin American analysis of the seroprevalence of IgG antibodies for SARS-CoV-2 among kidney transplant recipients. This survey was conducted in São Paulo, one of the epicenters of the COVID-19 pandemic in Brazil. The reported 8.2% seroprevalence is lower than that observed in the general population, 17.9% in July 2020, and 26.2% in October 2020.[10] The reasons behind the seroprevalence difference observed are not completely understood, involving, but not limited to, differences in the attack rate, lethality rate, antibody response, and adherence to non-pharmacological measures. The attack rate was higher among transplant recipients than that in general population reported by the municipality,[11] as illustrated in Figure 1. This may have been possibly due to an observation bias, since these individuals have broader access to SARS-CoV-2 testing, thanks to coordinated and sequential measures implemented by the transplant center since the beginning of the pandemic.[12] Conversely, the COVID-19 lethality rate was 10-times higher among the kidney transplant recipients compared to the general population (30% vs. 4%)[11] (Figure 1), potentially reducing the number of IgG+ recipients during the survey. Finally, pharmacological immunosuppression may impair the antibody response, such as what is observed for seroconversion after other viral diseases and vaccines.[13]

Some evidence suggests that certain blood groups would influence susceptibility to SARS-CoV-2 infection (group A patients would be more susceptible, for example), for reasons related to the interaction between the virus and target cells, but not yet completely established.[14] In the present analysis, however, this association was not observed. There was a clear association between household contact and seroprevalence, as already demonstrated in previous surveys in the general population.[4,15] Since prolonged and unprotected household contact represents the greatest risk of spreading the disease,

TABLE 1 Comparison of demographic characteristics of the patients with a positive (IgG+) and negative (IgG-) serology for SARS-CoV-2

Parameters	Study cohort (n = 416)	IgG+ (n = 34)	IgG- (n = 382)	p value
Median age, years (IQR)	52 (41–61)	47 (34–55)	53 (42–61)	0.034
18–35 years	59 (14.2)	9 (26.5)	50 (13.1)	0.042
36–60 years	240 (57.7)	20 (58.8)	220 (57.6)	
Older than 60 years	117 (28.1)	5 (14.7)	112 (29.3)	
Male gender, n (%)	246 (59)	19 (56)	226 (59)	0.734
Ethnicity, n (%)				0.229
Caucasian	187 (45)	10 (29)	177 (46)	
Mixed	126 (30)	14 (41)	112 (29)	
Black	80 (19)	9 (26)	71 (19)	
Other	23 (6)	2 (6)	22 (6)	
Blood type, n (%)				0.479
A	146 (35)	12 (35)	134 (35)	
B	57 (13)	7 (21)	50 (13)	
O	188 (45)	13 (38)	175 (46)	
AB	13 (3)	2 (6)	11 (3)	
Missing	12 (3)	0	12 (3)	
Diabetes, n (%)	59 (14)	4 (12)	55 (14)	0.746
Deceased donor transplant, n (%)	285 (69)	24 (71)	261 (68)	0.719
Median time after transplantation, years (IQR)	8 (4–13)	6 (4–10)	8 (4–13)	0.373
Region of residence, n (%)				0.755
North	83 (20)	7 (20)	76 (20)	
South	138 (33)	9 (27)	129 (34)	
Center	7 (2)	1 (3)	6 (1)	
West	70 (17)	8 (23)	62 (16)	
East	118 (28)	9 (27)	109 (29)	
Health care professional, n (%)	6 (1)	0	6 (1)	0.142
Contact with a confirmed or suspicious person, n (%)	95 (23)	11 (34)	84 (22)	0.125
Household	39 (9)	8 (24)	31 (10)	
Work colleagues	69 (17)	5 (15)	64 (17)	
Both	13 (3)	2 (6)	11 (3)	
Clinical status at the serosurvey, n (%)				<0.0001
Previously confirmed COVID-19	19 (5)	13 (38)	6 (2)	
18–35 years	3	1	2	
36–60 years	15	11	4	
Older than 60 years	1	1	0	
Flu-like syndrome within the last 6 months	53 (13)	5 (15)	48 (13)	
18–35 years	10	3	7	
36–60 years	31	2	29	
Older than 60 years	12	0	12	
Asymptomatic	344 (82)	16 (47)	328 (85)	
18–35 years	46	5	41	
36–60 years	194	7	187	
Older than 60 years	104	4	100	

| Kidney transplant | 10,527 | 234 | 73 |
| General population | 12,330,000 | 178,781 | 8,146 |

FIGURE 1 Attack and lethality rates from COVID-19 and seroprevalence for IgG anti-SARS-CoV-2 among the studied population of kidney transplant recipients and the general population of the city of Sao Paulo. Data regarding the general population was obtained from references number 10 and 11

any prevention strategy should also focus on the patient's behavior at home. In addition, the social vulnerability of patients, added to the accumulation of comorbidities and the state of immunosuppression, results in a high risk of death, suggesting the need to prioritize this population in government vaccination strategies.

In this cohort of patients, the seroprevalence decreased with increasing age, tendency not observed in the serosurvey conducted in the city of Sao Paulo (seroprevalence randomly ranging from 15.8% to 22% across the age strata).[10] Possible explanations include a selection bias, because older people have higher COVID-19-associated mortality and the relationship between immunosenescence and lower vaccine immune responses.[16] On the contrary, increased exposure to the virus among younger people, as they represent the workforce and are more socially active, less severe clinical presentation and more robust humoral response may be involved in the higher percentage of seroconversion among the younger kidney transplant recipients.

Within 60 days, a third of the patients presented antibody decay to non-reagent levels. This phenomenon has already been observed for SARS-CoV-2[15,17,18] and other viruses[19] in the general population, and other viruses although its clinical significance is still uncertain. Furthermore, up to now, the role of cellular immunity, the most important component for virus infection control, has not been thoroughly evaluated. A North American study that evaluated 87 patients between 1.3 and 6.2 months after infection with SARS-CoV-2 observed a preservation of the magnitude of the memory B-cell compartment, despite a significant decay of specific IgM and IgG antibody against the

SARS-CoV-2 receptor binding domain and of the neutralizing activity in plasma.[20]

The limitations of the present analysis include the fact that it was transversal and included a population from a specific region of the country, which does not allow extrapolation to other periods of time and space. Moreover, it is not possible to infer about population- or person-level protective immunity, since the presence of antibodies does not imply these antibodies protect the person. Third, the absence of a plaque-reduction neutralization test for confirming the IgG ELISA positive results prevents any conclusion about quantitative information on antibody titers that inhibit viral infection. Finally, in an ongoing pandemic, a large proportion of persons will be recently infected and therefore will be negative by serologic testing.[21]

In conclusion, in this cohort of kidney transplant recipients living in the São Paulo city, the the seroprevalence for IgG anti-SARS-CoV-2 was lower than that of the general population and decreased with ageing. The main source of exposition was household contact and there was seroreversion in a considerable proportion of these patients in a short period of time suggesting the need prioritization of preventive strategies for these individuals.

AUTHOR CONTRIBUTIONS

Marina P. Cristelli, Laila A. Viana, Carlos M. Fortaleza, Helio Tedesco-Silva, Jose Medina-Pestana, Daniel W.C.L. Santos, and C.Gdesigned the study. Marina P. Cristelli, Laila A. Viana, Monica R. Nakamura, C.Z., and Renato Demarchi Foresto obtained the data. Marina P. Cristelli,

Laila A. Viana, Carlos M. Fortaleza, Celso Granato, and Helio Tedesco-Silva analyzed the data. Marina P. Cristelli, Laila A. Viana, Carlos M. Fortaleza, and Helio Tedesco-Silva drafted and revised the paper. All authors approved the final version of the manuscript. Marina P. Cristelli, Laila A. Viana, and Carlos M. Fortaleza equally contributed to the study.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ORCID

Marina P. Cristelli https://orcid.org/0000-0002-2813-0400

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SUPPORTING INFORMATION

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