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Sequential IgG antibody monitoring for virus-inactivated and adenovirus-vectored COVID-19 vaccine in Brazilian healthcare workers

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Abstract

Vaccination certainly is the best way to fight against the COVID-19 pandemic. In this study, the seroconversion effectiveness of two vaccines against severe acute respiratory syndrome coronavirus 2 was assessed in healthcare workers: virus-inactivated CoronaVac (CV, $n = 303$), and adenovirus-vectored Oxford–AstraZeneca (AZ, $n = 447$). The immunoglobulin G (IgG) antibodies anti-spike glycoprotein and anti-nucleocapsid protein were assessed by enzyme-linked immunosorbent assay at the time before vaccination (T1), before the second dose (T2), and 30 days after the second dose (T3). Of all individuals vaccinated with AZ, 100% ($n = 447$) exhibited seroconversion, compared to 91% ($n = 276$) that were given CV vaccine. Among individuals who did not respond to the CV, only three individuals showed a significant increase in the antibody level 4 months later the booster dose. A lower seroconversion rate was observed in elders immunized with the CV vaccine probably due to the natural immune senescence, or peculiarity of this vaccine. The AZ vaccine induced a higher humoral response; however, more common side effects were also observed. Nonvaccinated convalescent individuals revealed a similar rate of anti-spike IgG to individuals that were given two doses of CV vaccine, which suggests that only a one-shot COVID-19 vaccine could produce an effective immune response in convalescents.

KEYWORDS

COVID-19 serological testing, immunoglobulin G, nucleocapsid protein, SARS-CoV-2, spike glycoprotein

1 │ INTRODUCTION

COVID-19 is a highly transmissible viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is considered by the World Health Organization a pandemic disease.[1] The clinical spectrum of COVID-19 is wide, encompassing asymptomatic,[2–4] mild respiratory illnesses, and severe viral pneumonia followed by respiratory failure and death.[5,6] The symptoms of COVID-19 are also very heterogeneous, including fever, dry cough, dyspnea, headache, dizziness, generalized weakness, vomiting, and diarrhea,[6,7] as well as olfactory and taste dysfunction.[8] Furthermore, the increase of inflammatory cytokines,[9] disseminated intravascular coagulation, high D-dimer and fibrinogen levels with prolonged thrombin time,[10] and presence of ground-glass opacity in chest computed tomography are also often observed.[3,11]

Many efforts have been made to discover drugs to treat COVID-19, as well as vaccines.[12,13] Two vaccines against

SARS-CoV-2 were assessed in this study. The first one is CoronaVac (CV), which was developed by Sinovac Life Sciences in partnership with the Butantan Institute (São Paulo, Brazil), using a classical inactivated virus approach.[14] Data showed that after two consecutive doses, the humoral response induced by CV was detected in around 99.0% of individuals,[15,16] and the efficacy to avoid severe symptoms and death was 50.38%.[17] Other vaccine, ChAdOx1 nCoV-19 (AZD1222), was developed by the University of Oxford and the AstraZeneca company (AZ) in collaboration with Fiocruz (Rio de Janeiro, Brazil). This is a vector-based vaccine using a recombinant nonreplicating chimpanzee adenovirus as a vector,[14] the efficacy observed was 90.0%.[18]

All vaccines follow the same final pathway, basically, a major histocompatibility complex (MHC) Class I or Class II on the professional antigen-presenting cells display the virus fragment (peptides) to antigen-specific CD8+ cytotoxic T-cell or CD4+ T-helper cell, respectively, and trigger B cells for the antibody production.[13] MHC I and MHC II molecules are extremely polymorphic; above 10 000 different alleles of MHC I molecules have been identified,[19] suggesting that the same vaccine could induce a different immune response due to genetic diversity.

This prospective longitudinal study aimed to assess the seroconversion effectiveness of two vaccines used in Brazil at the beginning of the COVID-19 pandemic and contribute to a better understanding of anti-SARS-CoV-2 protective immunity.

2 | METHODS

2.1 | Ethical statement

This study was submitted to the Ministry of Health Scientific Research (Brazil Platform, CAAE number: 42309321.1.0000.5462) and approved by the Dante Pazzanese Institute of Cardiology institutional ethics committee (number: 4.507.032, January 22, 2021). All participants provided their written consent, and the study was conducted following Resolution 466 of the Brazilian Health Council/National Health Surveillance Agency and ICH-GCP for good clinical practices.

2.2 | Participants in the study and vaccination protocol

All healthcare workers, administrative and support staff working at our hospital, and those who agreed to receive anti-SARS-CoV-2 vaccines were eligible for this study. Vaccination was performed with a dose of 0.5 ml, respecting the recommended interval between the first and second doses, 28 days for CV and 90 days for AZ vaccine.

A total of 1362 individuals were vaccinated between January and February of 2021, 470 (34.5%) immunized with CV, and 892 (65.5%) with AZ vaccine. However, only 303 in the CV group and 447 in the AZ group completed the planned blood collection scheme for this

study. Those without three samples collected were withdrawn from the study.

2.3 | Blood collections and serum storage

Three sequential blood samples were obtained via antecubital venipuncture by using BD Vacutainer serum collection tubes on the vaccination day, before the first dose (T1), before the second dose (T2), and 30 days after the second dose (T3). All samples were collected between January to July 2021. All serum samples were stored at −30°C in single-use aliquots.

2.4 | Enzyme-linked immunosorbent assay for immunoglobulin G antibody detection

The protocol to assess the anti-SARS-CoV-2 antibody was based on our previous publication.[20,21]

Ten COVID-19 negative serums and another 10 positive serums were pooled and rerun on every assay plate as interassay control. Results higher than the cutoff value of 0.300 optical density (OD) were reported as positive for the anti-SARS-CoV-2 antibody.

2.5 | Data analysis

All clinical, laboratory, and demographic variables were stored on the Research Electronic Data Capture (REDCap) database, supported by Brazilian REDCap Consortium.

Statistical analysis was performed using the SPSS Statistics V22.0 software (IBM) and Graph Pad Prism® Version 6 for Windows (GraphPad Software).

Numeric variables with a normal distribution were presented as mean ± standard deviation and compared by the t-test, whereas those without a normal distribution were presented as median and interquartile range and compared by using the Mann–Whitney U test or Kruskal–Wallis test. Categorical variables were presented in percentage and were compared by using the Pearson χ^2 test or Fisher's exact test, when appropriate. Statistical significance $p < 0.05$.

3 | RESULTS

3.1 | Characteristics of participants in the study

The research subjects were categorized into three groups, according to the vaccine-induced immune response: no responder, responder, and previous sensitized (Table 1). Although there was no significance in gender between the groups, the massive participation of women in this study was observed ($n = 543$; 72.4%). The group of nonresponders immunized with CV was significantly older (57.8 vs. 48.6 and 45 years; $p < 0.05$). There were no differences in the race or ethnicity

TABLE 1 Demographic data of participants categorized by seroresponse to the CoronaVac and AstraZeneca vaccine

Vaccines	CoronaVac (n = 303)				Astra Zeneca (n = 447)			
Seroresponse	Nonresponders (n = 27)	Responders (n = 201)	Previous sensitized (n = 75)	p	Nonresponders (n = 0)	Responders (n = 271)	Previous sensitized (n = 176)	p
Gender, n (%)								
Female	14 (51.9)	135 (67.2)	54 (72.0)	0.166	0	198 (73.1)	142 (80.7)	0.07
Male	13 (41.8)	66 (32.8)	21 (28.0)		0	73 (26.9)	34 (19.3)	
Age (year)								
Mean (standard deviation)	**57.8 (13.9)**	48.6 (14.4)	45.0 (15.6)	**<0.05**	0	43.7 (10.2)	44.5 (11.4)	0.440
Race/ethnicity, n (%)								
White/Caucasian	19 (70.4)	134 (66.7)	43 (57.3)		0	132 (48.7)	86 (48.9)	
Black/African–Brazilian	2 (7.4)	23 (11.4)	10 (13.3)		0	39 (14.4)	29 (16.5)	
White and Black mixed	3 (11.1)	33 (16.4)	16 (21.3)	0.400	0	84 (31.0)	53 (30.1)	0.671
Asian	2 (7.4)	6 (3.0)	1 (1.3)		0	8 (3.0)	2 (1.1)	
Brazilian–Indian	0	1 (0.5)	0		0	2 (0.7)	0	
Not declared	1 (3.7)	4 (2.0)	5 (6.7)		0	6 (2.2)	6 (3.4)	
Staff group, n (%)								
Doctor	9 (33.3)	66 (32.8)	14 (18.9)	**<0.05**	0	9 (3.3)	4 (2.3)	**0.029**
A nurse or nursing assistant	8 (29.6)	83 (41.3)	**47 (63.5)**		0	66 (24.4)	**57 (32.8)**	
Healthcare worker[a]	4 (14.8)	25 (12.4)	4 (5.4)		0	72 (26.6)	34 (19.5)	
Administrative or executive	1 (3.7)	13 (6.5)	7 (9.5)		0	79 (29.2)	41 (23.6)	
Security and cleaning staff	0	4 (2.0)	0		0	19 (7.0)	25 (14.4)	
Other	5 (18.5)	10 (5.0)	2 (2.7)		0	26 (9.6)	13 (7.5)	
Exposure to SARS-CoV-2 at work								
Exposure	11 (40.7)	106 (52.7)	**51 (68.9)**	0.014	0	68 (25.2)	**62 (35.2)**	0.025
Nonexposure	16 (59.3)	95 (47.3)	23 (31.1)		0	202 (74.8)	114 (64.8)	
Comorbidities, n (%)								
Hypertension	**11 (40.7)**	40 (19.9)	12 (16.0)	**0.032**	0	34 (12.5)	**42 (23.9)**	**0.002**
Diabetes	**10 (37.0)**	14 (7.0)	10 (13.3)	**<0.05**	0	16 (5.9)	17 (9.7)	0.143
Asthma	2 (7.4)	13 (6.5)	1 (1.3)	0.142	0	12 (4.4)	11 (6.3)	0.512

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Dentist, pharmacist, psychologist, social assistant, biologist, physiotherapist, and physical professional.

variable. The number of individuals previously infected by SARS-CoV-2 was higher between nurses or nursing assistants than in other staff groups in both vaccines, CV ($p < 0.05$) and AZ ($p = 0.029$). Similar results were also observed in professionals who were exposed to SARS-CoV-2 at work, CV ($p = 0.014$), and AZ ($p = 0.025$). The percentage of individuals with hypertension and diabetes is significantly higher in the nonresponders group immunized with CV, this is probably associated with the elderly people in this group.

3.2 | Comparison of side effects of two vaccines

At the first dose of the vaccines, 54 (18.3%) of individuals immunized by CV and 245 (55.8%) by AZ experienced at least one side effect ($p < 0.05$), and a similar rate was observed at the second dose, 56 (18.9%), and 178 (42.2%) in CV and AZ, respectively ($p < 0.05$). The common side effects of vaccines were (CV vs. AZ): injection site pain: 29 (9.6%) versus 160 (35.8%), headache: 23 (7.6%) versus 128

(28.6%), myalgia: 13 (4.3%) versus 130 (29.1%), fatigue: 6 (2%) versus 86 (19.2%), and fever: 1 (0.3%) versus 54 (12.1%) (Figure 1).

Among 1362 healthcare workers immunized by COVID-19 vaccines in our hospital, only a 39-year-old woman developed Guillain–Barré syndrome (GBS) on the 42nd day after taking the first dose of the AZ vaccine, and the diagnosis was confirmed by cerebrospinal fluid analyses and electromyography; therefore, the second dose was not recommended. Several studies have shown the association between COVID-19 and GBS development,[22,23] and this issue should be better explored mainly for vaccines using new technologies, such as adenovirus vectors or messenger RNA (mRNA).

3.3 | Adenovirus-vectored vaccinated individuals exhibited higher anti-spike immunoglobulin G

All individuals vaccinated with the AZ vaccine exhibited seroconversion, compared to 91% of seroconverted individuals that were given the CV vaccine (Table 1). It was interesting to note that

non-vaccinated convalescent individuals, revealed a similar rate of anti-spike immunoglobulin G (IgG) (mean: 0.7502 OD) to individuals that were given two doses of CV vaccine (mean: 0.7065 OD). However, individuals that were immunized with the AZ vaccine exhibited higher rates of anti-spike IgG, which were even potentiated when individuals were previously exposed to SARS-CoV-2 (Figure 2).

## 3.4 | Immune response delay in elderly individuals who received a virus-inactivated vaccine

Individuals who did not respond to the CV vaccine at the endpoint (T3) were invited to collect a new blood sample (T4) around 4 months after the second dose. Seven individuals remained without detectable IgG anti-S-protein (OD < 0.300), the other seven individuals had a slight increase in the antibody level, with OD between 0.400 and 0.700, and three showed a significant increase in the antibody level, with OD > 1.200 (Figure 3). No one in the group reported symptoms related to COVID-19.
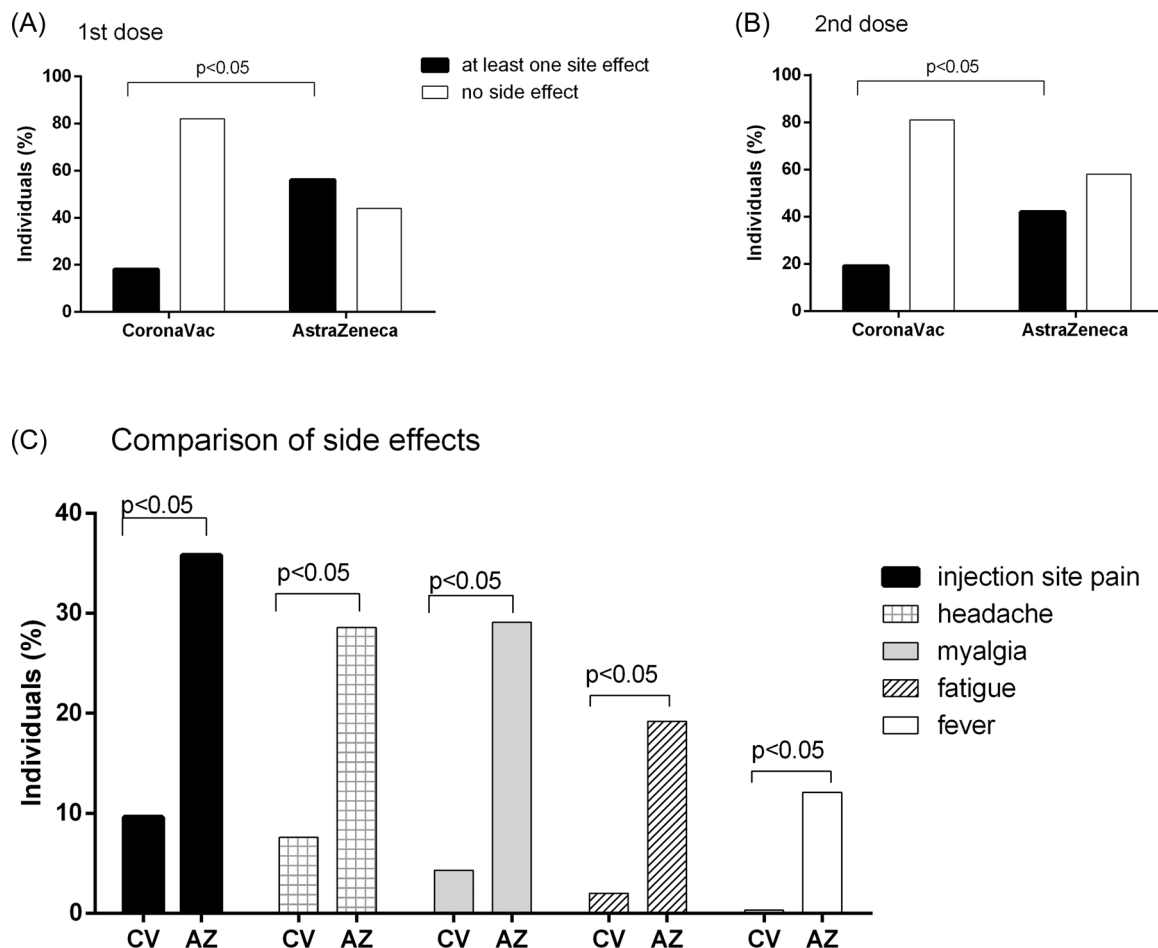
FIGURE 1 Frequency comparison of side effects between CoronaVac (CV) and AstraZeneca (AZ) vaccines. At the first dose, 54 (18.3%) of individuals immunized by CV and 245 (55.8%) by AZ experienced at least one side effect (A), and a similar rate was observed at the second dose, 56 (18.9%) and 178 (42.2%) in CV and AZ, respectively (B). (C) The common side effects of vaccines were injection site pain, headache, myalgia, fatigue, and fever. Statistical analysis was performed by Fisher's exact test ($p < 0.05$).
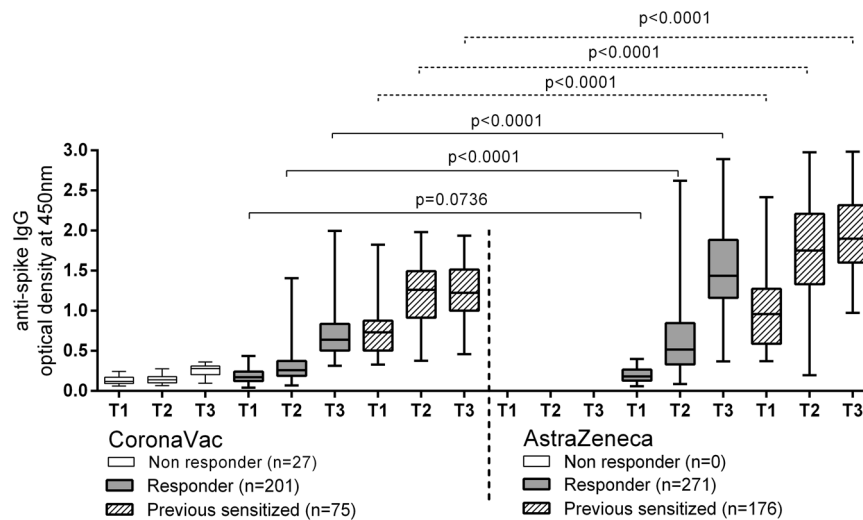
FIGURE 2 Comparison of immune response between CoronaVac and AstraZeneca vaccines. Serum IgG antibody against SARS-CoV-2 spike protein was assessed by enzyme-linked immunosorbent assay. The results were obtained at 450 nm absorbance and the cutoff point set was 0.300 optical density. The blood was drawn sequentially on the vaccination day before the first dose (T1), before the second dose (T2), and 30 days after the second dose (T3). IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
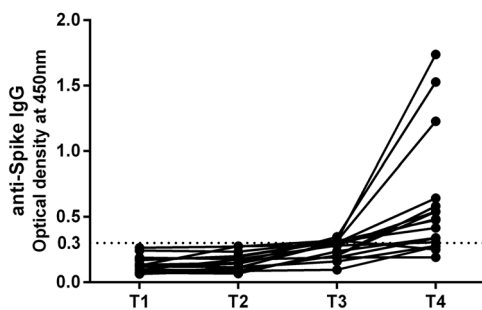
FIGURE 3 Later immune response of CoronaVac vaccine. Out of 27 individuals who did not respond to CoronaVac, 17 returned 3 months after time T3 to collect a new sample (T4, 4 months after the second dose). The enzyme-linked immunosorbent assay was applied to assess the anti-SARS-CoV-2 S protein antibody. The cutoff point set was 0.300 OD. Seven individuals remained without a detectable antibody against S-protein in T4 (<0.300), the other seven had a slight increase in antibody level (0.400–0.700), and three showed a significant increase in the level of antibody (>1.200). IgG, immunoglobulin G; OD, optical density; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

3.5 | Induction of anti-spike and anti-nucleocapsid antibodies by CV

The CV vaccine is produced with the whole virus-inactivated particles, then antibody anti-nucleocapsid was assessed to confirm the specificity of the enzyme-linked immunosorbent assay (ELISA). The statistics analyzed by the Pearson test showed a good correlation between the level of antibodies against spike proteins and nucleocapsid protein (Figure 4).

3.6 | Frequency of SARS-Cov-2 infection in vaccinated individuals

With only one dose of vaccine, 2 (0.7%) in the group immunized by CV and 16 (3.6%) in the group immunized by AZ had COVID-19 diagnosed by reverse transcriptase-polymerase chain reaction, and after the second dose of the vaccine, 11 (3.6%) and 4 (0.9%) of individuals had COVID-19 in the CV and AZ vaccine groups, respectively. It is noteworthy that none of them needed hospital care.

4 | DISCUSSION

SARS-CoV-2 was quickly disseminated in Brazil; in many states, persons infected had severe symptoms, exceeding the capacity of the hospitals. At the peak of the disease, persons even lost their lives due to the lack of oxygen. The high human-to-human contagious rate forced our country to adopt severe measures of restrictive social life, including a complete lockdown in more critical cities, except for essential services. This situation led to a financial crisis, people losing their jobs, and most of them going hungry. The solution may lie in mass vaccination, but at the beginning of the pandemic, there were not sufficient vaccines, and it was necessary to set priority groups for vaccination. In general, healthcare workers who were on the front lines in combating COVID-19 had priority to receive the vaccine.

The CV was the first vaccine applied in our hospital, and the older workers had priority for immunization, therefore the CV group in this study had a higher average age. Twenty-seven CV-immunized individuals (8.9%) had undetectable antibodies. Our data corroborate the Karameses and Tutuncu report,[24] of which among 235 participants over 65 years with comorbidities, 27 (11.4%) had

(A)   T2 (r=0.8515 p<0.0001)   (B)   T3 (r=0.7628 p<0.0001)
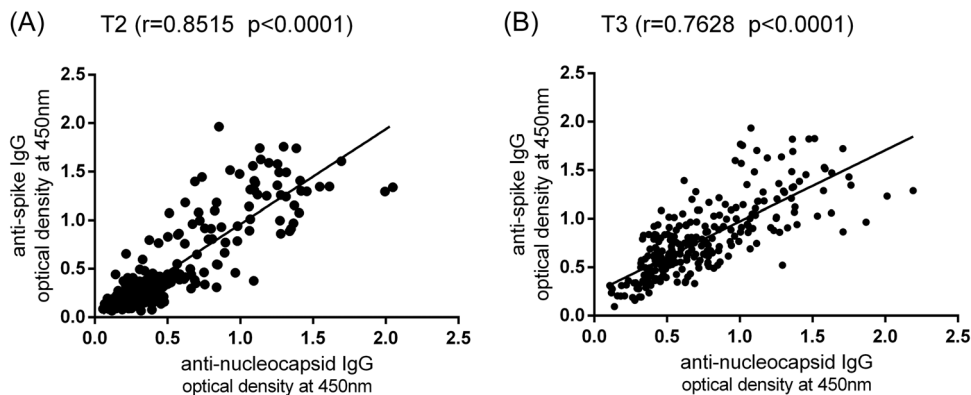
FIGURE 4 CoronaVac vaccine-induced antibody production against SARS-CoV-2 spike and nucleoprotein at the same level. The serum from 303 individuals immunized by CoronaVac on the day of the (A) second dose (T2) and 30 days after the (B) second dose (T3) was assessed by enzyme-linked immunosorbent assay to detect antibodies anti-spike protein and nucleoprotein. The results showed absorbance (optical density). Statistical analysis was performed by Pearson's two-tailed test ($α = 0.05$). IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

negative antibody detection. The immune senescence, which is often observed in older people probably harmed the effectiveness of the vaccine. Interestingly, three nonresponders from the CV group showed intense antibody levels similar to an infection 4 months after the second dose, but completely asymptomatic. Our data showed that the CV induced the production of antibodies against S-protein, as well as N-protein, probably also against other SARS-CoV-2 proteins not tested in this study. Further studies are needed to clarify the protective function of these antibodies since the antibodies detected in this study by ELISA were binding antibodies rather than neutralizing antibodies.

In this study, the number of participants who reported any side effects, such as headache, myalgia, fatigue, and fiver was significantly higher in the AZ group than in the CV. Additionally, the percentage of individuals who reported any side effects following the first and second dose of the AZ vaccine was 55.8% and 42.2%, respectively, which is in line with 50.15% and 52.6% of other studies.[18,25] The data in the literature suggests that adenovirus-vectored vaccines usually induce more side effects.[24–26]

The serological analysis of samples harvested before the vaccination allowed to discriminate those who had been previously exposed to SARS-CoV-2, and allowed to observe the profile of immune response to the vaccine. Before vaccination, 251 out of 750 (33.5%) of our hospital staff enrolled in this study already had antibodies against SARS-CoV-2 spike protein. Our result is slightly higher than the research reported by University Hospitals Birmingham NHS Foundation Trust, the UK in April 2020, which revealed an overall seroprevalence of 24.4% ($n = 126/516$).[27] However, these data are noticeably higher than another Brazilian study reported by Oliveira et al.,[28] which observed 5.5% of seroprevalence among individuals enrolled between March and July 2020. These conflicting results are probably because the study was performed more than 1 year before ours, and also before the worst COVID-19 pandemic peak in our country.

In both vaccines assessed in this study, the previous sensitized group presented a similar level of antibody anti-spike protein in T2 (before the second dose) and T3 (30 days after the second dose). These results suggest that only a single dose may produce efficient antiviral immune responses in COVID-19 convalescents.

Some studies are exploring the association between vaccination and autoimmune disease, including GBS.[29,30] The attributable risk was estimated at one additional case of GBS for every 100 000 doses of influenza vaccine administered.[31] Although the individual risk for autoimmune complications is likely to be small, it raises concerns to better explore adverse effects associated with the use of new technologies, such as adenovirus-vectored or mRNA for mass vaccination. In Brazil, the COVID-19 vaccination has been well accepted, and it is associated with a daily decrease in the death rate caused by COVID-19.[32] However, SARS-CoV-2 mutants that emerged recently (omicron) contain more than 30 changes to spike proteins[33] which may result in loss of antibody neutralization to SARS-CoV-2 in the present vaccinated population and an additional booster shot is now recommended by health authorities.

5 | CONCLUSION

The seroconversion rate was lower among elders immunized with the virus-inactivated vaccine, probably due to the natural immune senescence or peculiarity of this vaccine. The adenovirus-vectored vaccine induced a higher humoral response; however, more common side effects were also observed. The individuals previously exposed to SARS-CoV-2 could receive only one dose of the vaccine, which will be enough to boost an effective immune response.

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