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SARS-CoV-2 vaccine booster in solid organ transplant recipients previously immunised with inactivated versus mRNA vaccines: A prospective cohort study

Martín Dib,[a,b,1] Nicole Le Corre,[c,d,1] Catalina Ortiz,[b] Daniel García,[b,e] Marcela Ferrés,[c,d] Constanza Martinez-Valdebenito,[c,d] Cinthya Ruiz-Tagle,[f] María José Ojeda,[f] Manuel A. Espinoza,[g] Aquiles Jara,[h] Juan Pablo Arab,[i] Ricardo Rabagliati,[f] Cecilia Vizcaya,[d] María Elena Ceballos,[f] Mauricio Sarmiento,[j] Sebastián Mondaca,[j] Macarena Viñuela,[k] Antonia Pastore,[k] Vania Szwarcfiter,[k] Elizabeth Galdames,[a] Aldo Barrera,[c,d] Pablo Castro,[l] Nicolás MS Gálvez,[m] Jorge A. Soto,[m] Susan M. Bueno,[m] Alexis M. Kalergis,[m,n] Bruno Nervi,[j] and M. Elvira Balcells[f]*

[a]Instituto de Trasplantes, Red de Salud UC CHRISTUS, Chile
[b]Departamento de Cirugía Digestiva, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[c]Laboratorio de Infectología y Virología Molecular, Red de Salud UC CHRISTUS, Chile
[d]Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[e]Equipo de Cirugía Digestiva, Servicio de Cirugía, Hospital Dr Eduardo Pereira, Valparaíso, Chile
[f]Departamento de Enfermedades Infecciosas del Adulto, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[g]Departamento de Salud Pública, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[h]Departamento de Nefrología, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[i]Departamento de Gastroenterología, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[j]Departamento de Hematología y Oncología, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[k]Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[l]División de Enfermedades Cardiovasculares, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[m]Millennium Institute on Immunology and Immunotherapy, Santiago, Chile. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile
[n]Departamento de Endocrinología, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

Summary

Background Solid-organ transplant (SOT) recipients have worse COVID-19 outcomes than general population and effective immunisation in these patients is essential but more difficult to reach. We aimed to determine the immunogenicity of an mRNA SARS-CoV-2 vaccine booster in SOT recipients previously immunised with either inactivated or homologous SARS-CoV-2 mRNA vaccine.

Methods Prospective cohort study of SOT recipients under medical care at Red de Salud UC-CHRISTUS, Chile, previously vaccinated with either CoronaVac or BNT162b2. All participants received a BNT162b2 vaccine booster. The primary study end point was anti-SARS-CoV-2 total IgG antibodies (TAb) seropositivity at 8-12 weeks (56-84 days) post booster. Secondary end points included neutralising antibodies (NAb) and specific T-cell responses.

Findings A total of 140 (50% kidney, 38% liver, 6% heart) SOT recipients (mean age 54 [13.6] years; 64 [46%] women) were included. Of them, 62 had homologous (three doses of BNT162b2) and 78 heterologous vaccine schedules (two doses of CoronaVac followed by BNT162b2 booster). Boosters were received at a median of 21.3 weeks after primary vaccination. The proportion achieving TAb seropositivity (82.3% vs 65.4%, $P = 0.035$) and NAb positivity (77.4% vs 55.1%, $P = 0.007$) were higher for the homologous versus the heterologous group. On the other hand, the number of IFN-γ and IL-2 secreting SARS-CoV-2-specific T-cells did not differ significantly between groups.

Interpretation This cohort study shows that homologous mRNA vaccine priming plus boosting in SOT recipients, reaches a significantly higher humoral immune response than inactivated SARS-CoV-2 vaccine priming followed by heterologous mRNA booster.

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*Corresponding author at: María Elvira Balcells; Departamento de Enfermedades Infecciosas, Facultad de Medicina, Pontificia Universidad Católica de Chile; Diagonal Paraguay 362, 6° piso; Santiago, Chile.
E-mail address: ebalcells@uc.cl (M.E. Balcells).

[1] These authors contributed equally to this manuscript.

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Research in context

Evidence before this study

SARS-CoV-2 vaccination is safe and highly effective at attenuating disease severity and preventing death. Inactivated vaccines are well established and relatively simple to manufacture and have been widely implemented during the COVID-19 pandemic in several Regions, particularly in Asia, Central and South America after emergency use approval. In Chile, inactivated virus vaccine CoronaVac was used in over 70% of the cases for primary vaccination in the general population. However, inactivated vaccines are less immunogenic and studies are showing that seropositivity provided declines earlier than with newer SARS-CoV-2 mRNA vaccines. Immunocompromised patients, such as solid organ transplant (SOT) recipients, are at high risk of severe COVID-19 outcomes and therefore ensuring effective immunisation in this group is key. The WHO has recommended that primary vaccine series should be extended to include an additional dose in moderately to severely immunocompromised patients. The optimal timing for the additional doses and the best vaccine combination (heterologous versus homologous) for boosters are currently unknown. We searched PubMed for articles published between database inception and February 15th, 2022, using search terms describing "SARS-CoV-2" or "COVID-19", "vaccine" and "solid organ transplant". The pooled proportion of antibody response rate after a third dose of homologous SARS-CoV-2 mRNA vaccine was 50.3% (95% CI: 37.1-63.5) in SOT recipients, as described in a recent systematic review by Efros et al. Only very few studies have reported so far on the immunogenicity of heterologous additional doses in SOT recipients, and most of these have not found significant differences between different booster schemes, although inactivated vaccines have not been included.

Added value of this study

This is the first study to compare the response to an homologous versus heterologous vaccine schedule in SOT recipients primarily immunised with either inactivated or mRNA SARS-CoV-2 vaccines. We found that homologous mRNA vaccine priming and boosting reaches a higher specific humoral immune response than inactivated SARS-CoV-2 vaccine priming followed by an mRNA vaccine booster. Recipients at higher risk for low response were kidney transplant recipients, triple immunosuppression users and fewer years from transplant.

Implications of all the available evidence

Previous and our findings suggest that in highly immunocompromised patients, such as SOT recipients, primary immunisation with an inactivated SARS-CoV-2 vaccine should be considered as an alternative only if mRNA vaccines are not available. Offering an mRNA vaccine booster after receiving a primary immunisation with inactivated SARS-CoV-2 vaccine does not induce the level of specific humoral response provided by an mRNA booster after homologous primary mRNA immunisation. Given the high rate of non-responders even after booster doses for both vaccine schemes, additional strategies such as extended primary series or combination of vaccine types need urgent evaluation in transplant recipients.

Introduction

To date, SARS-CoV-2 vaccines have become the mainstay of the pandemic response globally after showing to be a safe and highly effective strategy for preventing COVID-19 and its more severe outcomes. However, evidence gathered shows that vaccine effectiveness wanes a few months after primary immunisation, compromising mainly the vaccine protection against infection but also against severe disease.[1] As of February 3rd 2022, over 92% of Chilean general population has received two doses of SARS-CoV-2 vaccine, and the inactivated virus vaccine CoronaVac has been used in over 70% of the cases.[2] Large local observational studies have also shown that IgG seropositivity is lower and declines earlier with CoronaVac than with mRNA (BNT162b2) vaccination.[3] This fact is the basis for recommending booster doses, particularly in immunocompromised and older individuals.

In accordance with World Health Organization (WHO)'s Strategy to Achieve Global COVID-19 Vaccination by mid-2022, the first priority of a vaccination program is to reduce mortality and severe disease and to

protect health systems. Among the most important measures to achieve this goal is to maximize coverage among those most likely to become seriously ill.[4]

Immunocompromised individuals, such as solid organ transplant (SOT) recipients, are at higher risk of suffering from more severe COVID-19 outcomes.[5] Additionally, the immune response to vaccines in transplant recipients is less robust than in the general population, such as humoral response is induced in only 34% of vaccinees after two doses of mRNA-based SARS-CoV-2 vaccine, and in only 21% after two doses of inactivated vaccine.[6, 7] Furthermore, despite detectable T-cell immunity after completing mRNA vaccination, recent studies describe higher rates of COVID-19 breakthrough infections and worse outcomes compared with general population; with up to 27% of vaccinated SOT recipients requiring hospitalization, >10% requiring admission to the intensive care unit, and >5% dying.[8]

Inadequate responses to current COVID-19 vaccines can be addressed through different strategies, such as extended primary series for those at-risk, and vaccine boosting after primary series completion to tackle fading immunity. The WHO (for all COVID-19 vaccines having received WHO emergency-use listing) has recently recommended that the primary vaccine series should be extended to include an additional dose in moderately to severely immunocompromised persons, including transplant recipients.[9] Optimal timing of additional doses in an extended primary series, and the benefits of heterologous versus homologous additional doses in these patients is currently unknown.

Here we report the results of a prospective study assessing the humoral and cellular immune responses induced by an mRNA (BNT162b2) vaccine booster in SOT recipients having previously completed a primary immunisation schedule with either inactivated (CoronaVac) or mRNA (BNT162b2) SARS-CoV-2 vaccines.

Methods

Study population and design

All adult immunocompromised patients in Chile, including SOT recipients, were prioritised in the national immunisation plan and offered a primary SARS-CoV-2 vaccine series starting in early February 2021, with either two doses of BNT162b2, Pfizer-BioNTech (3 weeks apart each) or two doses of CoronaVac, Sinovac Biotech (4 weeks apart). The vaccine regimen selection depended on local availability at the time. In addition, a booster with BNT162b2 vaccine was recommended from August 2021 onwards for all vaccinated patients.

All SOT recipients under post-transplant medical care at the Transplant Institute, Red de Salud UC-CHRISTUS (Santiago, Chile) older than 17 years, having received a graft in the previous 10 years and planning to receive this vaccine booster were invited to participate. Patients transplanted earlier than 3 months before enrolment and those reporting a previous SARS-CoV-2 infection were excluded. Baseline demographics and clinical data were collected by questionnaire in an electronic case report form at enrolment. Immunosuppression regimen was recorded. Target trough levels of tacrolimus and cyclosporine were maintained according to each organ transplant protocol and the post-transplant period. After the first 3 months post-transplant, most tacrolimus trough goal levels ranged between 6-8 ng/mL and cyclosporine trough levels between 100-200 ng/mL. When necessary, previous vaccine schedule was confirmed consulting the national vaccine registry. The BNT162b2 booster was provided at the national vaccination centres. Patients were re-contacted 8–12 weeks (56-84 days) after the BNT162b2 booster, and a blood sample was collected (at a home visit or in the transplant clinic).

For analysis, participants receiving a primary vaccine series with two doses of CoronaVac followed by the BNT162b2 booster were classified as the "heterologous vaccine group", and participants receiving a primary vaccine series with two doses of BNT162b2 followed by the BNT162b2 booster were classified as the "homologous vaccine group".

Outcomes

The primary outcome was the difference in seropositivity (detectable SARS-CoV-2 spike protein total IgG antibodies (TAb) at a level ≥11 RU/ml) between the homologous and heterologous vaccination groups at 8-12 weeks following the third dose (BNT162b2 booster). Secondary immunogenicity outcomes were the proportion of participants with positive SARS-CoV-2 neutralising antibodies (NAb); the percentage of neutralising activity (expressed as inhibition percentage of NAb); geometric mean concentration (GMC) of anti-S1 IgG; and specific T-cell response to SARS-CoV-2 antigens. We also explored patient's characteristics at baseline and any potential associations between clinical characteristics and vaccine responses.

Determination of anti-SARS-CoV-2 IgG antibodies

A commercial ELISA (SARS-CoV-2 QuantiVac, Euroimmun, Lübeck, Germany) was used for quantitative *in vitro* determination of human IgG antibodies against the S1 domain of SARS-CoV-2. Data were expressed in Relative Units per ml (RU/ml), and values ≥11 RU/ml were interpreted as positive according to the manufacturer's instructions. All assays were performed in duplicate.

Determination of neutralising antibodies against SARS-CoV-2

The presence of NAb against the S1 receptor binding domain (RBD) of SARS-CoV-2 from Wuhan was

determined using the SARS-CoV-2 Surrogate Virus Neutralisation Test (sVNT) Kit (Cat. L00847, GenScript, New Jersey, USA), according to the manufacturer's instructions. This method uses the cPass^{TM} technology allowing a rapid detection of total neutralising antibodies mimicking the interaction between the virus and ACE2 at the host cells, and is developed as an ELISA test. Serum samples are diluted to a ratio of 1:10; the test assesses the presence/absence of NAb and allows the interpretation of the inhibition rate as = [1 −(OD450$_{nm}$ value of Sample/OD450$_{nm}$ value of Negative Control)] × 100%. A percentage of neutralisation ≥30 at a 1:10 sample dilution was considered positive. All assays were performed in duplicate.

T-cell immune response

A subgroup of 60 enroled patients was evaluated for SARS-CoV-2-specific T-cell responses. The subgroup was selected as follows: (i) equal proportion of homologous and heterologous schedule (ii) similar age median between groups (iii) >7 months from transplant (iv) no ongoing organ rejection (v) a balance between the type of transplant between groups.

Peripheral blood mononuclear cells (PBMCs) were isolated with the SepMate PBMC isolation system (SepMateTM) and cryopreserved. Specific anti-SARS-CoV-2 T-cell response was assessed by a commercial human IFN-γ/IL-2 double-colour enzymatic ELISPOT assay (#hIFNgIL2, Cellular Technology Limited, OH, USA). This technique captures information on polyfunctional T cells responses by detecting simultaneously two cytokines secretion by specific anti-SARS-CoV-2 T cells. Briefly, T cells were stimulated with Mega Pools (MPs) of peptides derived from the SARS-CoV-2 proteome (peptides donated by La Jolla Institute).[10] These included two sets of peptides derived from the Spike protein (MP-S) and the remaining proteins (MP-R), and two sets of peptides derived from the whole SARS-CoV-2 proteome (CD8A and CD8B), both determined *in silico* to optimally stimulate $CD4^+$ T and $CD8^+$ T-cell as previously described.[10] On the same day of thawing the cells, ELISPOT plates (PVDF membrane) were activated with 70% ethanol and coated with human IFN-γ and IL-2 capture antibodies and incubated at 4 °C overnight. ELISPOT plates were washed with PBS 1X, and the MPs were used as the specific stimulus. The MPs were prepared individually in CTL medium 1% L-glutamine (#25030149, ThermoFisher Scientific) at a final concentration of 2 ug/mL and 100uL were added to each well. A total of 3×10^5 cell in 100uL of CTL medium 1% L-glutamine were added, obtaining a final concentration of peptides MPs of 1 ug/mL. As positive control of T-cell response, phytohemagglutinine (PHA; #10576-015, Gibco) at final concentration of 50 mg/mL was used in an independent stimulation, and a negative control without stimulation was included. Cells were incubated 48 hours at 37°C and 5% CO2 in a humid incubator. After incubation, the IFN-γ and IL-2 spots were developed following the manufacturer's instructions. Background value was subtracted from measured results. ELISPOT results were expressed as the sum of SFC stimulated with MP-S and MP-R, expressed as MP S-R, and stimulated with MP-CD8A and MP-CD8B, expressed as MP-CD8. The Spot Forming Cells (SFCs) were counted on an ImmunoSpot S6 Micro Analyzer. As we studied an immunocompromised population, participants without a response to the PHA (positive control) were also included in the analysis.

Statistical analyses

For sample size calculation, we considered that (i) a large SOT series reported that 41% of SOT recipients vaccinated with BNT162b2 reached TAb seropositivity, and an homologous BNT162b2 booster led to a 27% increase (68% TAb)[11], (ii) we found that after primary CoronaVac vaccination (2 doses) in SOT recipients, TAb seropositivity was reached in only 20% of patients.[7] We assumed that a 25% higher proportion of TAb seropositivity would be reached with the homologous boosting (mRNA vaccines only) versus the heterologous boosting (inactivated followed by mRNA vaccine). Thus, we estimated a sample size of 63 patients per arm considering a significance level of 5% and a statistical power of 80%.

Dichotomous variables were compared with the Chi-squared test or Fisher's exact test, and continuous variables with Student's t-test or Mann-Whitney test. The quantitative measurement of anti-SARS-CoV-2 IgG antibodies was expressed in geometric mean concentration (GMC). Binary outcomes such as seropositivity in NAb or TAb were analysed with multivariable logistic regression adjusting for potential confounding covariates. We chose as confounding variables all those covariates which showed statistical significance ($P < 0.05$) in the regression model. We confirmed this selection using the stepwise technique. Analyses and graphs were performed using RStudio version 1.4.1717 and GraphPad Prism 9.3.1. A *P*-value <0.05 was considered statistically significant.

Ethics

This study was approved by the institutional review board of the Pontificia Universidad Católica de Chile. Informed consent was obtained from all participants.

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any aspect of the study design, analysis of results or decision to submit for publication.

Results

Study participants

A total of 339 adult SOT recipients were transplanted between 10 years and 3 months prior to the study initiation and were being followed in our Transplant Institute. Of these, 254 patients fulfilled inclusion criteria, and 147 participants accepted and were enrolled between October 6th and December 13th, 2021. Five participants were excluded from the analysis after enrolment as they were found to have exclusion criteria (previous unreported COVID-19 in two cases, transplant date >10 years or <3 months in two cases, a different SARS-CoV-2 vaccine administered in one case) and two participants were excluded as blood sample was taken outside the 8-12 week post-vaccine booster range, therefore, a total of 140 participants were included in the analysis (Figure 1). Participants' mean age was 54 years old, and 64 (46%) were women. In total, 70 participants (50%) had a kidney transplant, 53 (37.9%) a liver transplant, 8 (5.7%) a heart transplant and 9 (6.4%) a double organ transplant (kidney plus other organ), with a median time of 3.4 years since transplant. Demographic and clinical characteristics of participants are shown in Table 1.

Of all enrolled participants, 78 (55.7%) had received a primary immunisation schedule with CoronaVac and 62 (44.3%) with BNT162b2. The BNT162b2 vaccine booster was administered at a mean of 21.3 weeks (Interquartile range (IQR) 20.8-22.2) after completing the primary immunisation schedule, and participants were sampled at mean of 10.9 weeks (IQR 9.8-11.3) after the booster. Participants from the homologous vaccine group were younger (mean age 48 vs 59 years, $P < 0.001$), had a lower proportion of diabetes (12.9 vs 26.9%, $P = 0.042$), included a higher proportion of kidney transplant (62.9 vs 39.7%, $P = 0.006$) and lower proportion of liver transplant (27.4% vs 46.2%, $P = 0.023$), than the heterologous group. Also, the time from primary vaccine completed to the booster vaccine was shorter for the homologous vaccine group (median of 21.0 vs 21.8 weeks, $P = 0.022$). Triple immunosuppression was being used in the majority of patients (79/140, 56.4%), including 50% of the heterologous and 64.5% of the homologous vaccine schedule patients, a non-significant difference. Of these 79 patients, the vast majority (81%) were on corticosteroids, mycophenolate mofetil and calcineurin inhibitors.

No severe adverse events were reported after vaccine boosters.

Humoral response

The proportion of participants presenting positive total IgG antibodies for homologous and heterologous vaccine group was 82.3% and 65.4% ($P = 0.035$), and GMC titers were 58.7 and 30.9 RU/ml ($P = 0.060$), respectively (Figure 2 a, b).

The proportion of participants who reached NAb positivity for the homologous and heterologous group was 77.4% and 55.1% ($P = 0.007$), respectively. When neutralising capacity was analysed, the homologous group reached a median of 96.6% inhibition versus 51.8% in the heterologous group ($P = 0.038$) (Figure 2 c, d).

Predictors associated with humoral response to vaccine

Both TAb and NAb development were associated with having received the homologous vaccine schedule (OR 2.45, 95% CI 1.10-5.47; and OR 2.79, 95% CI 1.33-5.87, respectively), longer time from transplant to primary vaccine (OR 1.23, 95% CI 1.05-1.44; and OR 1.23, 95% CI 1.07-1.42, per doubling of years, respectively) and type of transplant (liver vs others) (OR 2.96, 95% CI 1.24-7.08; and OR 2.53, 95% CI 1.17-5.47, respectively). Also, triple immunosuppression use was negatively associated with TAb development (OR 0.42, 95% CI 0.19-0.94) (Suppl. Figure 1). In multivariable analysis, the homologous vaccine schedule was still significantly associated with TAb and NAb seropositivity after adjusting for age, time from transplant to primary vaccine, type of organ graft, diabetes, and triple immunosuppression use (Table 2).

For all study participants, a strong correlation between TAb concentration and NAb neutralising activity expressed as inhibition percentage was found (r=0.929, $P < 0.0001$) (Suppl. Figure 2).

T-cell response

A subgroup of 60 enrolled patients was evaluated for SARS-CoV-2-specific T-cell responses. The IFN-γ response tended to be higher in the homologous than in the heterologous vaccine group although not statistically different, with a median of 19 versus 4 SCF/3×10^5 cells for MP S-R ($P = 0.25$) and 4 versus 1.5 SCF/3×10^5 cells ($P = 0.08$) for MP-CD8, respectively (Figure 3a). Similarly, the homologous vaccine group showed a higher IL-2 response upon stimulation with a median of 46 versus 12 SCF/3×10^5 cells for MP S-R ($P = 0.07$) and 4 versus 0.5 SCF/3×10^5 cells for MP-CD8 ($P = 0.06$), all non-statistically significant differences (Figure 3b).

Discussion

In the present study, we found that homologous mRNA vaccine priming and boosting in SOT recipients reaches a higher specific humoral immune response than inactivated SARS-CoV-2 vaccine priming followed by an mRNA vaccine booster. This finding is important given

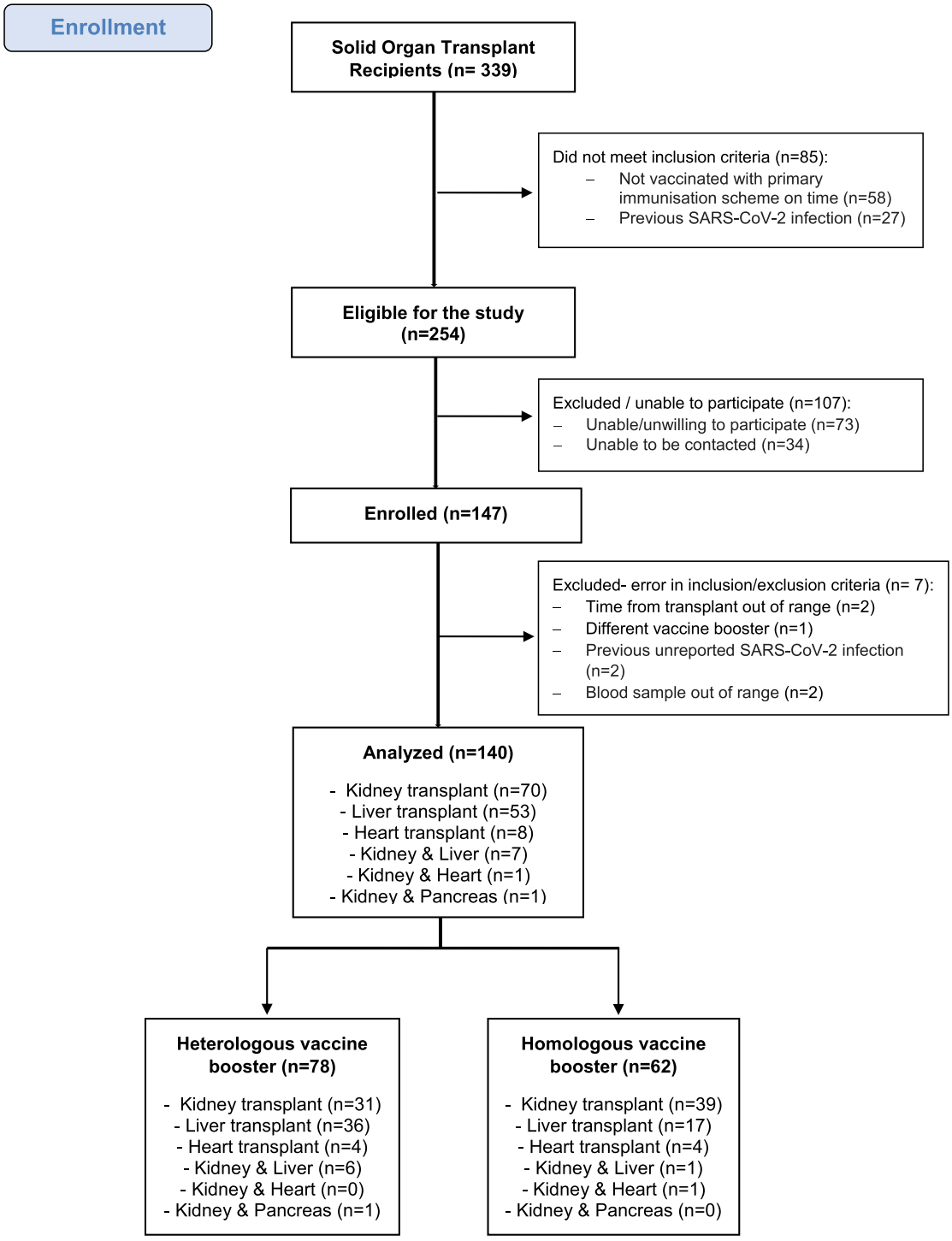

Figure 1. Study flow-chart. Adult solid organ recipients transplanted within 10 years prior to study initiation, under medical care at the Transplant Institute, Red de Salud UC CHRISTUS.

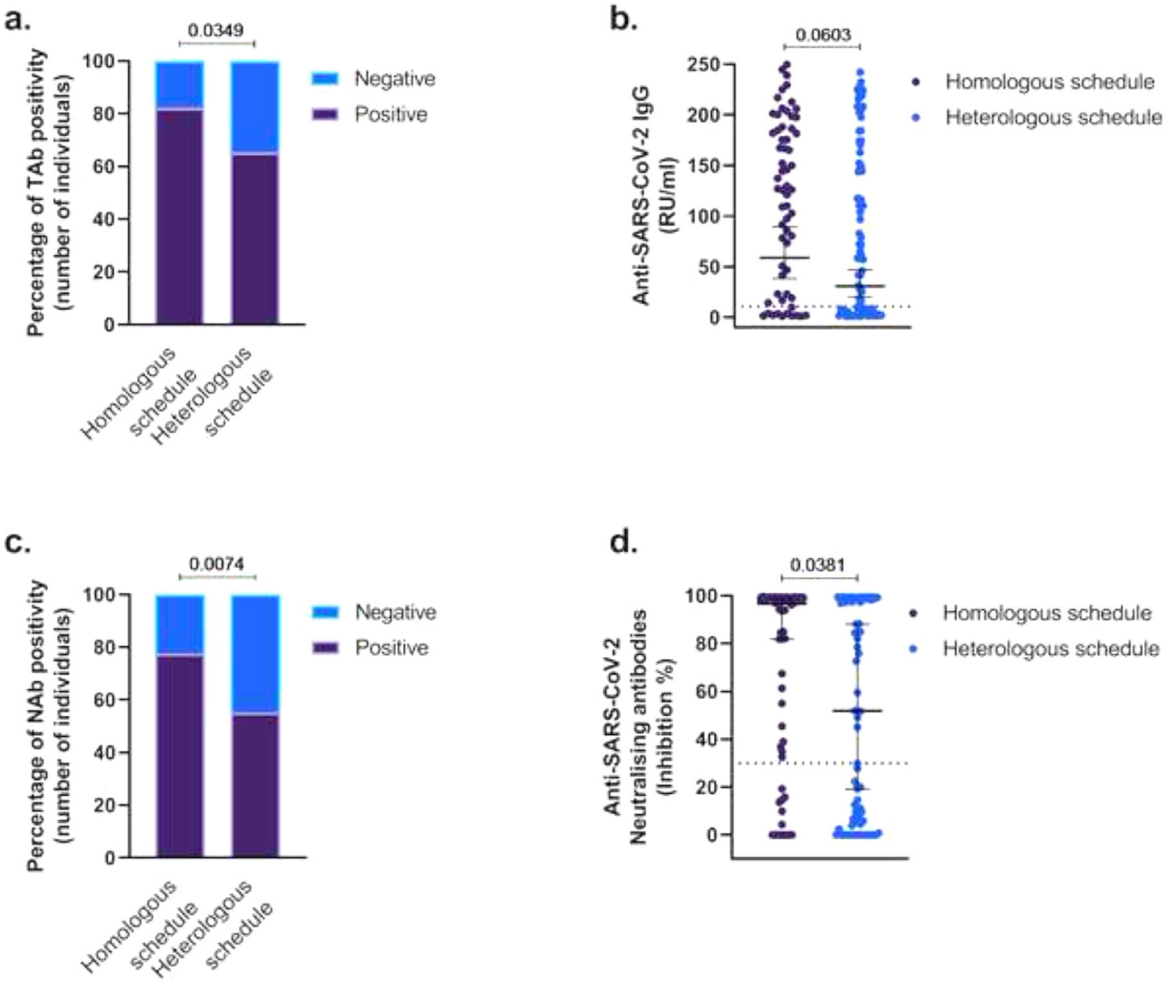

Figure 2. Humoral response against SARS-CoV-2 in SOT recipients with an homologous or heterologous vaccination schedule. Serum was obtained between 8-12 weeks after the mRNA vaccine in the homologous group (n = 62) and the heterologous group (n = 78). Distribution for **(a)** frequency of total IgG (TAb) anti-S1 positivity (≥11 relative units per ml, RU/ml), **(b)** total IgG anti-S1 geometric mean concentration (GMC) (95%CI), RU/ml), **(c)** neutralising antibodies (NAb) positivity (≥30% of inhibition rate) and **(d)** neutralising activity (median (IQR) of percentage of inhibition). Dotted line in (b) and (d) show seropositivity cut-off. Statistical significance was calculated with Fisher test (a,c), Mann-Whitney (b,d); two-tailed P values are shown.

that traditional inactivated SARS-CoV-2 vaccines such as CoronaVac (Sinovac), BBIBP-CorV (Sinopharm Beijing), or BBV152 (Bharat Biotech) have been widely implemented, mainly in low- and middle-income countries after emergency use approval.[12]

SOT patients require life-long immunosuppression regimens that commonly include some combination of a calcineurin inhibitor, steroids, and/or an anti-metabolite that non-specifically inhibit T- and B-cells. Weak humoral and T-cell immune responses have been described in transplant recipients after full primary immunisation with SARS-CoV-2 mRNA vaccines, with TAb seropositivity reached in only one third of patients.[6] We recently described that SOT recipients also had an impaired humoral response to inactivated vaccines, with only 20% of vaccinees attaining TAb seropositivity and positive neutralising response 8-12 week after primary immunisation with CoronaVac.[7] Furthermore, in a prospective, phase 4 clinical trial that included over 3000 adult kidney transplant recipients vaccinated with CoronaVac in Brazil, the proportion of patients with positive TAb antibodies was 43% four weeks after the second dose, and although the incidence of COVID-19 was reduced (6.4% versus 4.2%; P < 0.0001), the 28-day lethality rate remained unchanged (25% vs 22%; P = 0.534).[13]

Multiple vaccine doses can boost the primary immune response to viral infections by providing supplementary innate immune activation signals, promoting further expansion of previously activated T- and B-cell clones, and promoting germinal centres responses.[14] A recent systemic review and meta-analysis

	Total (*n* = 140)	Heterologous Vaccine Group[a] (*n* = 78)	Homologous Vaccine Group[b] (*n* = 62)	*P* Value
Demographics				
Age, mean (SD), years	54.4 (13.6)	59.5 (13.2)	48.1 (11.2)	**<0.001**
Female (No, %)	64 (45.7)	36 (46.2)	28 (45.2)	0.91
BMI, mean (SD)	26.7 (4.6)	27.1 (4.3)	26.2 (4.9)	0.24
Comorbidities				
Hypertension (No, %)	83 (59.3)	48 (61.5)	35 (56.5)	0.54
Diabetes (No, %)	29 (20.7)	21 (26.9)	8 (12.9)	**0.042**
Asthma or COPD (No, %)	5 (3.6)	2 (2.6)	3 (4.8)	0.47
Chronic renal disease (No, %)	3 (2.1)	2 (2.6)	1 (1.6)	0.99
Chronic liver disease (No, %)	8 (5.7)	6 (7.7)	2 (3.2)	0.30
Current immunosuppressive therapy				
Prednisone (No, %)	95 (67.9)	50 (64.1)	45 (72.6)	0.29
Prednisone dose >15 mg/d (No, %)	2 (1.4)	2 (2.6)	0 (0.0)	0.50
Mycophenolate mofetil (No, %)	97 (69.3)	52 (66.7)	45 (72.6)	0.45
Tacrolimus (No, %)	104 (74.3)	58 (74.4)	46 (74.2)	0.98
Cyclosporine (No, %)	27 (19.3)	16 (20.5)	11 (17.7)	0.68
Rapamicine (No, %)	16 (11.4)	8 (10.3)	8 (12.9)	0.62
Triple immunosupressor	79 (56.4)	39 (50.0)	40 (64.5)	0.08
Years since transplant				
Median (IQR)	3.4 (1.4–6.0)	3.0 (1.2–6.2)	3.7 (2.0–5.9)	0.34
≤1	22 (15.7)	16 (20.5)	6 (9.7)	0.08
1 - ≤3	46 (32.9)	23 (29.5)	23 (37.1)	0.34
>3 - ≤5	25 (17.9)	14 (17.9)	11 (17.7)	0.97
>5	47 (33.6)	25 (32.1)	22 (35.5)	0.67
Type of transplant				
Kidney (No, %)	70 (50.0)	31 (39.7)	39 (62.9)	**0.006**
Liver (No, %)	53 (37.9)	36 (46.2)	17 (27.4)	**0.023**
Heart (No, %)	8 (5.7)	4 (5.1)	4 (6.5)	0.73
Kidney-liver (No, %)	7 (5.0)	6 (7.7)	1 (1.6)	0.13
Kidney-pancreas (No, %)	1 (0.7)	1 (1.3)	0 (0.0)	0.99
Kidney-heart (No, %)	1 (0.7)	0 (0.0)	1 (1.6)	0.44
Time between 2nd and booster vaccine dose, median (IQR), weeks	21.3 (20.8–22.2)	21.8 (20.6–22.7)	21.0 (20.9–21.7)	**0.022**
Time between booster and blood sampling, median (IQR), weeks	10.9 (9.8–11.3)	10.4 (9.6–11.3)	11.0 (10.3–11.6)	0.10

***Table 1*: Baseline characteristics of solid organ transplant recipients participants.**
[a] Inactivated (CoronaVac) primary vaccine followed by mRNA (BNT162b2) booster.
[b] mRNA (BNT162b2) primary vaccine schedule followed by an homologous (BNT162b2) booster. Abbreviations: BMI: body mass index; COPD: chronic obstructive pulmonary disease; SD: standard deviation; IQR: interquartile range.

	TAb Positivity[a]			NAb Positivity[b]		
Vaccine group	**No. (%)**	**OR (95% CI), *P value***	**adOR (95% CI), *P value***	**N (%)**	**OR (95% CI), *P value***	**adOR (95% CI), *P value***
Heterologous group[c] (*n* = 78)	51 (65.38)	1 *(reference)*	1 *(reference)*	43 (55.13)	1 *(reference)*	1 *(reference)*
Homologous group [d] (*n* = 62)	51 (82.26)	2.45 (1.10–5.47), *P* = 0.028	2.66 (1.04–6.85), *P* = 0.042	48 (77.42)	2.79 (1.33–5.87), *P* = 0.007	2.94 (1.20–7.23), *P* = 0.019

***Table 2*: Frequency of total anti-SARS-CoV-2 IgG antibodies (TAb) and neutralising antibodies (NAb) in solid-organ transplant recipients receiving the heterologous (inactivated vaccine followed by mRNA vaccine booster) versus the homologous (mRNA vaccine and booster) immunisation schedule.**
[a] Number of participants reaching the total anti-SARS-CoV-2 S1 IgG antibodies (TAb) cut-off (≥11 RU/ml).
[b] Number of participants reaching the cut-off (≥30%) in SARS-CoV-2 neutralising antibodies (NAb) for test positivity.
[c] CoronaVac primary schedule followed by BNT162b2 vaccine booster.
[d] BNT162b2 primary schedule followed by homologous BNT162b2 booster. Abbreviations: OR: odds ratio; CI: confidence interval; adOR: adjusted odds ratio. Adjusted ORs covariates: age, time from transplant to primary vaccine schedule, type of transplant (liver versus others), diabetes, and triple immunosuppression.

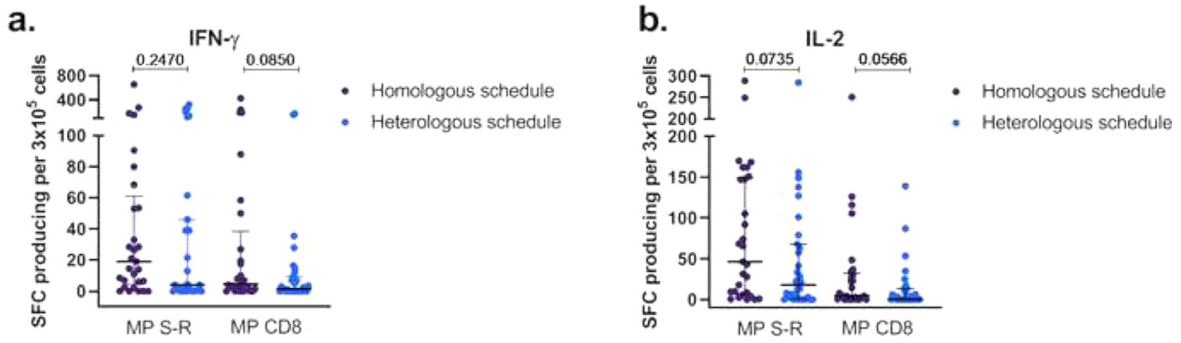

Figure 3. Evaluation of IFN-γ and IL-2 secreting Spot Forming T cells in SOT recipients with homologous and heterologous vaccination schedules. PBMCs (3×10^5 cells) obtained between 8-12 weeks after mRNA vaccine in the homologous group (n = 29) and the heterologous group (n = 31) were stimulated with megapool of peptides (MP S-R), or megapool of peptides (MP CD8) from SARS-CoV-2 proteins. **(a)** IFN-γ-secreting spot forming T cells (SFC) and **(b)** IL-2-secreting SFC were quantified by ELISPOT. Medians and interquartile ranges are shown. Statistical significance was calculated with Mann-Whitney test.

of seven studies including a total of 853 SOT recipients having received a third dose an mRNA vaccine showed that antibody response occurred in 6.4-69.2% of patients, and the pooled proportion of antibody response rate was 50.3% (95%CI 37.1-63.5, I2 = 90%).[15] In a small SOT recipients case series, nor even a fourth dose of an mRNA SARS-CoV-2 vaccine succeeded in improving immunity among the non-responders.[16]

Combining - heterologous - vaccine schedules, either as vaccine priming or as boosting with adenoviral or mRNA vaccines, is being explored as a strategy to improve response to SARS-CoV-2 vaccines in observational studies. In a large study in healthcare workers (n = 13,121), priming vaccination with the heterologous adenoviral (ChAdOx1-S) and mRNA (BNT162b2) combination conferred better immunogenicity than the homologous BNT162b2, with ChAdOx1-S vaccine inducing a weaker IgG response but a stronger T-cell response than BNT162b2 after the priming dose.[17] Thus, both the mRNA-1273 and BNT162b2 vaccines have already received emergency-use authorization by the FDA after completion of primary vaccination with any FDA-authorized or approved COVID-19 vaccine.[18] Less is known regarding the role of inactivated vaccines in these heterologous schedules, although a recent study showed that a BNT162b2 booster was superior to an homologous inactivated (CoronaVac) booster in healthy participants non-responders to a primary inactivated vaccine series.[19]

In SOT recipients, heterologous priming vaccination also led to an improved response in one study, with a higher humoral response obtained after mRNA priming, and a more robust T-cell response after vector priming.[20] Regarding the effectiveness of additional vaccine doses for transplant recipients being non-responders to a primary schedule, there is still unclear superiority of an homologous versus heterologous combination. In a recent clinical trial including 197 kidney transplant recipients, a third dose induced an antibody response in 35% of the homologous (mRNA only) group versus 42% of the heterologous (adenoviral) group, with no statistically significant difference.[21] Another large cohort study of kidney transplant recipients showed that TAb seroconversion was observed in 75% of patients with an heterologous schedule (ChAdOx1-S priming followed by mRNA vaccine, n = 28) and in 67.8% of patients with an mRNA exclusive schedule (3 doses, n = 345), a non-significant difference.[22]

A strength of the present study is the assessment of the cellular immune response that included both $CD4^+$ and $CD8^+$ T cells, as current knowledge on vaccine-induced cellular immunity in transplant recipients is limited to mRNA and adenoviral vaccines. SOT recipients generate robust T-cell responses following natural SARS-CoV-2 infection that correlate with disease severity, but comparatively lower T-cell responses following mRNA vaccination.[23] In a cohort of either kidney (n = 133) or kidney-pancreas (n = 15) recipients, only 35% developed SARS-CoV-2 S-ELISpot positivity after two doses of the mRNA-1273 vaccine.[24] On the other hand, in liver (n = 58) and heart (n = 46) recipients, the median SFC response after two mRNA-1273 doses was 24.5 and 10 per 2×10^5 PBMCs, respectively (79% of SARS-CoV-2 S-ELISpot positivity).[25] Even if laboratory techniques are not entirely comparable, SOT recipients in our study presented a similar amount of IFN-γ-producing T-cells after homologous mRNA booster, but lower for the heterologous vaccine group (median IFN-γ SCF 19 and 4 per 3×10^5 PBMCs, respectively). Besides, these highly immunosuppressed subjects developed a polyfunctional memory T-cells, producing IFN-γ and IL-2, an important cytokine for maintaining immune memory and T-cell proliferation.[26] Attaining a specific T-cell response in these patients may be needed to prevent severe outcomes with Omicron variant, as recently reported.[27]

Several factors can undermine the immune response to SARS-CoV-2 vaccines in transplant recipients. Older

age, more intense immunosuppressive drug regimens including depleting antibodies and anti-metabolites, corticosteroid use, triple immunosuppression, lymphopenia, and shorter time since transplant are well-described risk factors.[28] In this cohort, we found that kidney transplant, triple immunosuppression and fewer years from transplant associated with lower responses. The modulation of immunosuppressors, such as reducing mycophenolate dose or withdrawing belatacept costimulation blockade, has been proposed as another strategy to improve vaccine effectiveness in SOT recipients.[29]

The present study has some limitations. Importantly, the allocation of different primary vaccination regimens was not randomised. Due to vaccines local availability at the time, the group allocated to primary mRNA vaccines was younger and included more kidney transplant recipients than the group that received the inactivated vaccines. However, adjusting for these variables in the multivariate analysis did not change the study main findings. Additionally, this study did not specifically compare immune response before and after the mRNA booster, as it was aimed at comparing immunogenicity induced by the three-dose schedules. Finally, our study does not provide information on vaccine effectiveness regarding the protection against actual infection or disease. Also, prior SARS-CoV-2 infection before blood sampling was not systematically assessed, and asymptomatic infections despite immunosuppression have been described.[30]

In conclusion, our study is the first to compare the response to an homologous versus heterologous vaccine booster that included inactivated vaccines in SOT recipients. Our findings show that offering a BNT162b2 vaccine booster after receiving a primary immunisation with CoronaVac does not induce the level of immune response provided by a BNT162b2 booster after homologous primary BNT162b2 immunisation. Therefore, in highly immunocompromised patients, primary immunisation with an inactivated SARS-CoV-2 vaccine should be considered as an alternative only if homologous primary BNT162b2 immunisation is not available. Moreover, given the high rate of non-responders even after booster doses for both vaccine schedules, other strategies such as extended primary series with additional doses, different timing between doses, and combination of vaccine types, need urgent evaluation in SOT recipients.

Contributors

B.N., N.L.C., M.D. and M.E.B. conceived the study. M. F., C.O., D.G., C.V., M.S., R.R., M.E.C. and S.M. made substantial contributions to the design and methodology of the study. C.M.V., C.R.T., A.B., N.M.S.G. and J. A.S. performed laboratory investigation and assisted the analysis of data. C.O., D.G., A.J., J.P.A., M.V., A.P., V.S., E.G. and P.C. led the participants recruitment, data collection and clinical data curation. M.E.B., N.L.C., M. D. performed the main analysis of results and wrote the original draft. M.J.O. and M.A.E. did the statistical analysis and contributed to interpretation of data. B.N., M.E. B., N.L.C., S.M.B. and A.M.K. were responsible for funding of the study. All authors revised and approved the final version of the manuscript.

Data sharing statement

Anonymised participant data can be made available upon requests directed to the corresponding author. Proposals will be reviewed on the basis of scientific merit and previous ethics approval.

Declaration of interests

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lana.2022.100371.

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