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A Multicenter Cohort Study From India of ABO-Incompatible Kidney Transplantation in Post—COVID-19 Patients

Vivek Kute, Deepak Shankar Ray, Sonal Dalal, Umapati Hegde, Suraj Godara, Vivek Pathak, M.M. Bahadur, Dinesh Khullar, Sandeep Guleria, S Vishwanath, Ashay Singhare, Dinesh Yadav, Shyam Bihari Bansal, Sanshriti Chauhan, and Hari Shankar Meshram

ABSTRACT

Background. There is a dearth of data regarding the consequences of ABO-incompatible kidney transplant (ABOiKTx) among post—COVID-19 candidates.

Methods. The study was designed as a retrospective, multicentric cohort study across 11 sites in India, from August 2020 to December 2021. The data for ABOiKTx conducted for post—COVID-19 candidates were investigated. The primary outcome of biopsy-proven acute rejection was compared with the ABO protocol implemented through Kaplan-Meier analysis. The secondary outcomes were graft loss, patient survival, and infections.

Results. A total of 38 ABOiKTx with candidates of median (interquartile range) age of 38.5 (31.25-47.5) years were performed. Nineteen cases had mild COVID-19 severity, while 9 cases (23.6%) had an oxygen requirement. Six (15.7%) donors also were post—COVID-19. The most common ABO incompatibility reported was A to O in 14 (36.8%) pairs followed by B to O in 10 (26.3%) pairs. The maximum isoagglutinin titer cutoff was 1:2048 and 1:64 for baseline and pre-transplant levels, respectively. The median time from COVID-19 infection to surgery was 130 (63.2-183) days. Biopsy-proven acute rejection, graft loss, and mortality were 13.1%, 2.6%, and 2.6%, respectively. The Breslow-Wilcoxon’s P value in Kaplan-Meier plots were 0.57 and 0.93 for thymoglobulin-based induction and high dose rituximab-based regimen, respectively. The incidence of reinfection was 2.6%. Two (5.2%) urinary tract infections were reported. No cytomegalovirus or BK polyomavirus infection was reported. The median serum creatinine at 1 year of follow-up was 1.1 (0.8-1.3) mg/dL.

Conclusions. Our report implies that ABOiKTx in post—COVID-19 candidates can be successfully performed with no major deviation from standard ABO protocol.

COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection, has radically impacted the world, and transplantation communities were one of the most vulnerable groups for the associated morbidity and mortality [1–5]. Transplant activities around the world were significantly curtailed during the COVID-19 pandemic, leading to significant concerns about the impact on transplantation outcomes. ABOiKTx has been considered a high-risk procedure due to the high risk of acute rejection, graft loss, and mortality [1-5]. The availability of ABO compatible kidneys for transplantation has been limited during the pandemic period, leading to an increase in ABOiKTx. The impact of this on long-term outcomes is not well understood. Our report provides valuable insights into the outcomes of ABOiKTx in post—COVID-19 candidates in India.
ABO TRANSPLANT IN POST—COVID-19

world showed marked decline during fluctuating phases of the pandemic and various geographic regions [6,7]. As the COVID-19 toll expanded, there was also a build-up of post—COVID-19 candidates awaiting transplantation. With the emerging concerns of post—COVID-19 reports globally, [8,9], emergent research work in the context of transplantation is required. Organ transplantation deals with various immunosuppression (IS) and there exists a practical dilemma on optimal strategy of IS regimen in this pandemic due to the threat of provoking SARS-CoV-2 infection. ABO-incompatible kidney transplant (ABOiKTx) endorses an added initial bombardment of IS, and conducting such transplantation in a post—COVID-19 candidate has theoretically aggravated risk. The time to start IS, dosing of IS, wait time from COVID-19 infection to surgery, and the outcome in such ABOiKTx are unspecified.

India has one of the leading worldwide living donation programs but lacks a national kidney paired donation registry. Thus, ABOiKTx is commonly done in many centers. In this report, we have aimed to explore the IS protocol, wait time from infection to surgery, outcomes, and longitudinal follow-up of the ABOiKTx conducted in post—COVID-19 candidates. To the best of our understanding, this is the largest cohort study of ABOiKTx reported so far in post—COVID-19 candidates. The report would be a resource material for living donation practices in the pandemic and will help in the decisiveness of the optimal waiting time and IS regimen in such complicated scenarios.

MATERIALS AND METHODS

Ethical Statement

The study protocol received ethical approval from the institutional review board (registration number: ECR143/Inst/GJ/20 13/RR-19). The study also complied with the retrospective design of the STROBE statement. All the transplantation procedures were performed in accordance with the principles of the Declaration of Helsinki, the Declaration of Istanbul, and the Transplantation of Human Organ and Tissue Act, and good clinical practice guidelines. Informed written consent was taken from the donor and recipients before surgeries, after explaining all pros and cons of ABO protocol and potential consequences of flaring of SARS-CoV-2 sequelae.

Study Design, Settings, and Population

We did a retrospective longitudinal cohort study of post—COVID-19 wailtsted individuals who underwent renal transplantation across 11 transplant centers (Institute of Kidney Diseases and Research Center, Dr HL Trivedi Institute of Transplantation Sciences, Ahmedabad, Gujarat, India; Rabindranath Tagore International Institute of Cardiac Sciences, Kolkata, West Bengal, India; Gujarat Kidney Foundation, Ahmedabad, Gujarat; Mulijibhai Patel Urological Hospital, Nadiad, Gujarat, India; Mahatma Gandhi Medical College & Hospital, Jaipur, Rajasthan, India; Kovai Medical Center and Hospital, Coimbatore, Tamil Nadu; Jaslok Hospital and Research Center, Mumbai, Maharashtra, India; Max Saket Complex, Max Super Specialty Hospital, Saket, Delhi, India; Indraprastha Apollo Hospital, New Delhi, India; Manipal Hospital, Bangalore, India; Medanta, The Medicity, Gurugram, Har- yana, India) where the index case was transplanted in September 2020, and the last case was transplanted in October 2021. The index case acquired COVID-19 in August 2020 and the last case in September 2021. The last date of follow-up was December 1, 2021. Patients were considered for inclusion if they were aged 18 years or older and had fit the criteria for confirmed SARS-CoV-2 infection by the World Health Organization [10]. Patients with probable and suspected SARS-CoV-2 were not included. Patients were followed up until death or until their last follow-up visit (incongruent/in-person).

Laboratory Tests

SARS-CoV-2 was detected in nasal and throat swabs by use of a real-time polymerase chain reaction test (PCR). We documented routine blood tests done as part of the usual donor-recipient evaluation of each pair (blood group, inactive parathormone anti-ABO titer, donor-specific antibody human leukocyte match for A, B, DR, crossmatch, flow cytometry, and cytomegalovirus [CMV] serology). We also included laboratory tests done during COVID-19 admission, when available, and included hemoglobin, total leukocyte count, neutrophil-lymphocyte ratio, platelet counts, C-reactive protein, ferritin, D-dimer, and lactate dehydrogenase. The follow-up blood reports collected for analysis included renal functions, urine routine, CMV serology, and BKV blood and urine. At all the sites, all available patient data were collected until death or last follow-up. The follow-up of individual centers adhered to the standard practices of transplantation, with added telemedicine monitoring was done on a case-to-case basis for clinical screening and epide miologic screening to ensure safety in the pandemic.

Clinical Data and Definitions

The demographic data (age, sex, height, weight, and body mass index) were recorded along with comorbidities (hypertension, diabetes, heart disease, and cerebrovascular accidents) were recorded from patient medical records. COVID-19 vaccination history was also elicited. Respiratory support required during COVID-19 was classified as follows: supplemental oxygen when delivered by low flow oxygen devices, like nasal cannula or face mask; high flow oxygen, high-frequency nasal cannula, non-rebreathing mask, high flow oxygen devices; noninvasive ventilation like bilevel positive pressure ventilation; and mechanical ventilation. Mild COVID-19 illness was defined as cases without any form of oxygen need, while moderate had low flow oxygen devices requirement and severe needed support higher than low flow oxygen devices [11].

Data Management

Demographic, clinical, laboratory, anti—COVID-19 treatment, and outcome data were extracted from electronic medical records and case files using individual transplant centers’ policies and practices. Data were entered by members of the research/clinical care teams of respective transplant centers using a proforma sheet developed and distributed by the authors VK and HSM. All data from different centers were assembled as a single file by VK and crosschecked by their VK, HSM, and SC for any errors. Outliers (implausible values) for variables and dates were identified and clarified by communication with the data entry teams of individual centers. Anonymized data were downloaded for statistical analysis. The data were locked for analysis on December 1, 2021.

SARS-CoV-2 Free Pathway for ABO-Incompatible Transplant

Per the national guidelines [12], all recipients and donors practiced hand hygiene, face mask, and cough etiquette. To limit the nosocomial spread, a nominated hemodialysis subunit was used for pretransplant patients,
and health care workers were reallocated for the care of transplant. Clinical and epidemiologic screening of health care workers was done on daily basis, and PCR was done only in symptomatic cases. In unfortunate cases, where health care workers became positive, all the contacts were isolated and quarantined for PCR screening. The prerequisites for transplantation were 2 consecutive PCR negative reports, no SARS-CoV-2 symptoms for at least 28 days for both donors and recipients, and normal or decreasing radiological features. Anti-SARS-CoV-2 antibodies were not done in most of the centers and was not a criterion to proceed for transplant. All procedures and decisions were performed in accordance with the national guidelines for COVID-19 recovered candidates [13].

### Outcome Measures

The primary outcome was measured in terms of biopsy-proven acute rejection (BPAR) concerning the ABO protocol. The secondary outcomes measured were graft loss (defined as the need for a permanent return to dialysis after transplant at any point of time); all-cause mortality; delayed graft function (defined as a requirement of hemodialysis within the first week of transplant surgery); infections (bacterial, CMV, BKV, and urinary tract infections); clinical rejection; and early SARS-CoV-2 infection defined as infection within 1 month of the transplant.

### Statistical Procedures

No high-evidence data about the research question was available at the time of study design, so no statistical tool was used for computing the sample size of the study. Continuous data were expressed as median and interquartile range, range and mean (standard deviation) as justified. All categorical variables were reported as frequencies and percentages. The data were rounded to 2 decimal points. There was no loss to follow-up, and the data on the primary outcomes was available for all the cases. Missing data for variables related to secondary outcomes were handled with list deletion during analysis. As the frequency of events was low, so multivariable and logistic regression analysis was not done. Treatment regimen used was considered statistically significant for reporting this study.

### RESULTS

From April 2020 to December 31, 2020 a total of 38 ABOiKTx were done from post—COVID-19 candidates, 6 of which also had post—COVID-19 donors. Table 1 shows the COVID-19 course of the recipient. The most common incompatibility observed in the cohort was A to O in 14 pairs followed by B to O in 10 pairs. A to B, B to A, and AB to A donation was done in 4 (5.2%) pairs each. In 2 (2.6%) cases, AB to B was done. The data for HLA was available for 32 ABOiKTx, which showed the mean HLA mismatch for A, B, and DR locus as 1.24 (0.57), 1.24 (0.57), and 1.3 (0.47), respectively. The median age of the recipient was 38.5 (31.25-47.5) years. The youngest candidate was 7 years old while the oldest was 67 years old. All donors were females and female to male donation corresponded to only 6 (15.7%) cases. The most frequent native kidney disease diagnosed in the cohort was unknown followed by diabetes and hypertension in 11 (28.9%), 7 (18.4%), and 4 (10.5%) cases, respectively. The frequency of events was low, so multivariable and logistic regression analysis was not done.

### Table 1. COVID-19 Admission in Transplant Candidate

| Cumulative symptoms during COVID-19 course | n = 38 |
|-------------------------------------------|-------|
| Subjective fever                          | 31 (81.5) |
| Cough/expectoration                       | 26 (68.4) |
| Difficulty in breathing                   | 7 (18.4) |
| Anosmia/ageusia                           | 9 (23.6) |
| Nausea/vomiting                           | 5 (13.1) |
| Fatigue/malaise                           | 14 (36.8) |
| Diarrhea                                  | 1 (2.6) |

| Oxygen support required                   |       |
|-------------------------------------------|-------|
| Never required                            | 19 () |
| Low flow oxygen by face mask or nasal prong | 8 (21) |
| High flow oxygen by NRB or HFNC          | 0 (0) |
| BiPAP/NIV                                | 1 (2.6) |
| Mechanical ventilation                    | 0 (0) |

| Peak laboratory reports of COVID-19 course |       |
|-------------------------------------------|-------|
| Hb g/dL                                   | 9 (8-9.9) |
| TLC, $10^9$ cell counts mm$^3$             | 5.6 (4.25-7.1) |
| Neutrophil percentage                     | 72 (68.35-80.5) |
| Lymphocyte percentage                     | 21 (12.05-28) |
| CRP, mg/L                                 | 34 (11.65-65.775) |
| D dimer, ng/mL                            | 490.5 (357.75-846.5) |
| IL-6, pg/mL                               | 16.5 (16.25-16.75) |
| Ferritin, ng/mL                           | 682 (454-800) |
| LDH, IU/L                                 | 384 (313-451) |
| X-ray abnormalities detected              | 38 (100) |

Treatment regimen used

| Remdesivir                                 | 14 (36.8) |
| Anticoagulation                            | 20 (52.6) |
| Steroids                                   | 20 (52.6) |
| Ivermectin                                 | 1 (2.6) |
| Doxycycline                                | 5 (13.1) |
| Azithromycin                               | 2 (5.3) |

BiPAP, bi-level positive pressure ventilation; CRP, C-reactive protein; HFNC, high-frequency nasal cannula; IL-6, interleukin-6; LDH, lactate dehydrogenase; NIV, noninvasive ventilation; NRB or HFNC, non-rebreather mask; TLC, total leukocyte count.
(2.6%) were partially vaccinated before surgery. Twenty-three cases had positive CMV serology for both donors and recipients, while only 6 cases both were negative. The data for the other 9 cases were not captured. The pretransplant laboratory tests revealed serum albumin; inactive parathormone; hemoglobin; WBC; neutrophil-lymphocyte ratio; platelet counts and CRP values of 3.6 (3.2-4) g/dL; 136 (84-290) pg/mL; 10.4 (9.3-11.8) g/dL; 7 (6.3-8.3) × 10^9 cells mm^3; 3.4 (4.8-2.9) lacs and 6 (5-10.5) g/L. All recipients had chest radiology. Table 2 shows the outcome of the post−COVID-19 candidate. The waiting time from PCR negative to surgery and PCR positive to surgery was 118 (55-168); 18-350 days and 130 (63.2-183); 37-360 days, respectively. Preconditioning protocol consisted of Rituimab given 14 days before transplant. Tacrolimus (0.08 mg/kg) and mycophenolate (360 mg TDS or QDS depending on weight) were started at least 7 days before transplant. The median dose of RTX was 200 (200-500) mg with a minimum dose of 100 and a maximum of 500 mg. Thirteen cases had RTX with 500 mg dose, while 26 had below 375 mg/kg m^2.
One case was on non-RTX based induction protocol. The median plasmapheresis/ immunoadsorption (PEX/IA) performed in preconditioning was 4 (2-5). In 3 cases, no PEX/IA was done and a maximum of 8 cycles were done in 1 case. The median baseline ABO titer was 1:128 (1:64-1:128). The lowest cut-off iso-agglutinin titer was 1:8 and the maximum titer before transplant recorded was 1:2048. The median tire after PEX/IA was achieved was 1:8 (1:4-1:8). The least titer reached was 1:2 and the maximum cutoff titer before surgery was 1:64. 18 (73.6%) cases received interleukin-2 blockers as induction while 11 received ATG (3, 1.5, and 1 mg/kg was given for 7, 2, and 2 cases). We have also surveyed the transplant centers regarding any changes in the ABOiKT protocol, where all centers responded with no change in the IS regimen in post−COVID-19 cases, irrespective of the initial COVID-19 severity. In total, 5 (13.1%) BPAR were confirmed, of which 3 had antibody-mediated rejection and 2 were acute cellular rejection. The treatment received for antibody-mediated rejection was 4 to 5 cycles of plasma exchange and 3 doses of methylprednisolone in acute cellular rejection. There was no increase in ABO titer in any of the cohorts during follow-up. In the Kaplan-Meier analysis for the high-dose vs low-dose RTX, we found there was no statistical difference (P value = 0.93) in BPAR (Fig 1). No delayed graft function was reported in the whole cohort. One death at 6 months of transplant life was reported with fungal pyelonephritis which suffered graft loss during treatment.

| Characteristics          | n = 38 |
|--------------------------|--------|
| Days from the onset of COVID-19 symptoms to first PCR positive | 3 (3-4); 2-6 |
| Days from first PCR positive to the first negative | 12 (8-15); 5-66 |
| Days from first PCR negative to surgery | 118 (55-168); 18-350 |
| Positive PCR to surgery | 130 (63.2-183); 37-360 |
| Total negative PCR reports before transplant | 3 (2-4); 1-5 |
| Days of hospital stay after surgery | 9 (9-10) |
| Days of nadir serum creatinine | 4 (4-7) |
| Nadir serum creatinine | 1.1 (0.8-1.3) |
| Discharge | 1.1 (0.8-1.3) |
| 1 mo | 1 (0.8-1.2) |
| 3 mo | 1.1 (0.8-1.2) |
| 6 mo | 1.1 (0.8-1.5) |
| 1 y | 1.1 (0.8-1.3) |
| BPAR | 5 (13.1) |
| Graft loss | 1 (2.6) |
| Fungal pyelonephritis | 1 (2.6) |
| UTI | 2 (5.2) |
| Death | 1 (2.6) |

Serum creatinine is reported in mg/dL. Out of 5 BPAR, 3 had antibody-mediated rejection and 2 had acute cellular rejection. BPAR, biopsy-proven acute rejection; PCR, polymerase chain reaction; UTI, urinary tract infection.

Figure 1. Incidence of biopsy proven acute rejection (BPAR) 1A: Kaplan Meier plot between rituximab high dose and low dose. 1B: Kaplan Meier plot between thymoglobulin and other induction.
DISCUSSION

We hereby report the protocol and outcome of ABO-incompatible kidney transplantation performed in post-COVID-19 candidates, which is the largest cohort study reported so far in this context. We aimed to pursue the research question of optimal IS regimen, outcomes, and follow-up in ABOiKTx in this subgroup of transplant candidates. The rationale behind organizing the study is the abnormal follow-up in discharged/recovered COVID-19 cases in the general population, which has taken the limelight in current research [14–16].

In this pandemic, many transplant centers have opted in for a lesser potent induction/IS regimen as a general measure, the future implications of which are yet to be disclosed [17]. ABOiKTx is traditionally feared for infection flare due to bombing of heavy IS in the preparative period of transplant. A recent meta-analysis [18] comprising of largest ABO transplants concluded that ABO has increased mortality compared to ABO compatible in 1, 3, and 5 years, strikingly with infectious complications. The optimal IS regimen for conducting an ABOiKTx among post-COVID-19 candidates poses a special situation that should be dealt with extreme caution and vigilance. The authors have previously reported [19] 12 ABOiKTx in post-COVID-19 candidates, but due to small sample size, lack of detailed IS protocol, and short follow-up, no valuable conclusion was made for ABOiKTx.

The majority of our centers used a single dose of RTX 2 weeks before the transplant, with a dosage ranging from 100 to 375 mg/m². Total plasma exchange with 5% albumin or fresh-frozen plasma was conducted in most centers, but double-filtration plasmapheresis was used in some patients. The frequency of antibody removal was decided according to the baseline isoaagglutinin titer. In standard practices for ABO protocol, rituximab and Immunoadsorption are the best methods reported in the previous metaanalysis, although the quality of evidence was not high. In a previous report, low doses of RTX have shown to be effective in preventing rejection in 213 ABO transplants, but with no added benefit in terms of infectious complications [20]. However, a few recently published metaanalysis [21,22] have found dose low dose RTX to be of the same efficacy and with a lower chance of infection compared to 350 mg/m² dose. Organ transplantation has been reported with the suboptimal response with COVID-19 vaccine in high-level data [23] and more recent reports are favoring a booster dose [24]. Additionally, the risk of blunted response is linked with the rituximab-based regimen [25], belatacept [26], or mycophenolate-based regimen [27].

Echoing all the above shreds of evidence and risk of flaring of COVID-19 with the low immune response to COVID-19 vaccine, low dose RTX should be preferred. Our report had a similar incidence of BPAR in low dose compared to high dose RTX, which further bolsters the low dose approach. The continuation of high potent induction drugs and IS regimen in ABO protocol might decrease the immunogenicity of COVID-19 vaccines, but the decision to significantly alter the IS regimen is a matter of debate and is currently under research.

The study is entitled to provide helpful data for transplant centers across the world about the safety of high-risk transplants like ABOiKTx in post-COVID-19 candidates with no change from the standard regimen. We gathered data from 11 high-volume transplant centers of India, so experiences of all different centers were clubbed together to reach a consensus for conducting ABO transplants, hence practical replication of our model is not an issue, and is a major strength of our report. A serial follow-up for an additional year further strengthens our report. Still, the study holds some limitations. First, the obvious retrospective design, which has its innate limitations. Second, cases with higher COVID-19 severity constituted less in numbers. Third, the exact real-world burden of COVID-19 reactivation/reinfection along with their severity in post-COVID-19 ABOiKTx patients was not possible as the COVID-19 cases declined considerably after August in India. So, a firm conclusion cannot be interpolated for the risk of acquiring COVID-19 in the study population. Fourth, we failed to add a control group of ABOiKT without COVID-19 in the pandemic, due to logistics involved in data collection. However, the satisfactory outcomes in our cohort counteract the lack of this control group.

CONCLUSIONS

We conclude that ABOI transplants in post-COVID-19 candidates can be safely performed with no tailoring of immunosuppression by appropriate COVID-19 safety measures. In our study, ABOiKTx transplants were done with no modification from the standard immunosuppression of the ABOiKTx protocol. We hope, the study may encourage continuing difficult transplantation like ABOiKTx in post-COVID-19 candidates, as managing immunosuppression in the context of COVID-19 is still less known.

DATA AVAILABILITY

Data will be made available on request.

DISCLOSURE

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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