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Original Article

Humoral and cellular immune correlates of protection against COVID-19 in kidney transplant recipients

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ABSTRACT

As solid organ transplant recipients are at high risk of severe COVID-19 and respond poorly to primary SARS-CoV-2 mRNA vaccination, they have been prioritized for booster vaccination. However, an immunological correlate of protection has not been identified in this vulnerable population. We conducted a prospective monocentric cohort study of 65 kidney transplant recipients who received 3 doses of BNT162b2 mRNA vaccine. Associations among breakthrough infection (BTI), vaccine responses, and patient characteristics were explored in 54 patients. Symptomatic COVID-19 was diagnosed in 32% of kidney transplant recipients during a period of 6 months after booster vaccination. During this period, SARS-CoV-2 delta and omicron were the dominant variants in the general population. Univariate Analyses identified the avidity of SARS-CoV-2 receptor binding domain binding IgG, neutralizing antibodies, and SARS-CoV-2 S2-specific interferon gamma responses as correlates of protection against BTI. No demographic or clinical parameter correlated with the risk of BTI. In multivariate analysis, the risk of BTI was best predicted by neutralizing antibody and S2-specific interferon gamma responses. In conclusion, T cell responses may help compensate for the suboptimal antibody response to booster vaccination in kidney transplant recipients. Further studies are needed to confirm these findings.

1. Introduction

Solid organ transplant (SOT) recipients are at high risk of severe COVID-19 and death.[1–4] This high risk has been attributed to immunosuppressive therapies and to common comorbidities, including cardiovascular disease, diabetes, and obesity, that have also been associated with severe COVID-19 and death in the general population.[5,6] Although they had not been included in COVID-19 vaccine trials, SOT recipients were offered COVID-19 vaccination early in vaccination campaigns. Unfortunately, their responses to COVID-19 vaccines were weaker

Abbreviations: ADCP, antibody-dependent cellular phagocytosis; BTI, breakthrough infection; LLOD, lower limit of detection; RBD, receptor binding domain; SOT, solid organ transplant; VIF, variance inflation factor; VOC, variant of concern; Wuhan NT_{50}, Wuhan neutralizing antibody titer.

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than those of healthy adults: neutralizing antibodies were not detectable in most patients, even after 2 doses of mRNA vaccine[7]. These low vaccine responses were associated with a high incidence of breakthrough infections (BTIs), including severe cases.[8–12] SOT recipients were therefore prioritized for a booster with a third dose of COVID-19 vaccine. Higher levels of SARS-CoV-2 neutralizing antibodies were induced by booster as than by primary vaccination in these patients, in line with what is observed in the general population.[13–15] Yet, the level of immunity against BTI achieved by booster vaccination of SOT recipients, especially in the context of emerging SARS-CoV-2 variants, remains poorly defined.[16].

Serum levels of SARS-CoV-2 neutralizing antibodies are correlated with protection against COVID-19 in the general population.[17–20] In addition, booster mRNA vaccination is associated with improved vaccine effectiveness in healthy adults.[21] An immune correlate of protection against COVID-19 in SOT patients has not been identified. Despite their lower levels in SOT recipients, neutralizing antibodies are likely to play a key role given their importance in other populations. On the other hand, other immune effectors, in particular T lymphocytes, could contribute to protection in patients with limited vaccine-induced humoral immunity.[22]

Sociodemographic and clinical factors could also contribute to the risk of BTI in SOT patients. Previous studies identified age, female gender, and comorbidities, such as metabolic syndrome and kidney disease, as risk factors for SARS-CoV-2 BTI.[23,24] It is unclear whether these factors increase the risk of BTI directly, or by reducing the immune response to COVID-19 vaccination. Identifying risk factors for BTI in SOT recipients and their potential impact on COVID-19 vaccine immunogenicity would help identify patients who are at high risk and guide COVID-19 booster immunization strategies.

This prospective cohort study was undertaken to identify risk factors of BTI in kidney transplant recipients who had received 3 doses of BNT162b2 COVID-19 mRNA vaccine. Associations with vaccine-induced immune responses, sociodemographic and clinical factors were explored.

2. Materials and methods

2.1. Study design and population

This single-center prospective phase IV investigator-initiated study of the immunogenicity of the BNT162b2 vaccine (Pfizer-BioNTech) in kidney transplant recipients was approved by the ethics committee of the Erasme Hospital (P2020/284 and A2021/131) and by the Belgian Federal Agency for Medicines and Health Products (EudraCT 2021-000-412-28). Kidney transplant recipients aged at least 18 years were recruited in the Department of Nephrology, Dialysis and Transplantation of the Erasme Hospital, Belgium. They were informed and gave informed consent before the administration of first dose of BNT162b2 vaccine. Patients transplanted with multiple organs were excluded from the study. Patients infected with SARS-CoV-2 before BNT162b2 vaccination were not included in this report. Previous SARS-CoV-2 infection was defined as a history of a positive PCR on nasopharyngeal swab before study recruitment and/or a positive serology (Wantai SARS-CoV-2 IgG Elisa, Supplementary Materials) at recruitment, before administration of the first dose of BNT162b2 vaccine. Patients were seen by a study clinician at enrolment before the first dose, one month after the second dose, and the day of the third dose of the vaccine. Demographic and clinical data were recorded, including age, gender, body mass index, comorbidities (hypertension, diabetes, cardiovascular disease, chronic kidney insufficiency defined with eGFR <30mL/min in CKD-EPi and evolutive or treated cancer), characteristics of transplantation history (duration, rank, graft function, induction treatment, and immunosuppressive drugs), and absolute lymphocyte count.

All patients received the BNT162b2 vaccine on day 0 and day 21, according to the Belgian national vaccination program. The first vaccine dose was administered between March 2, 2021, and March 18, 2021, and the second dose between March 23, 2021, and April 8, 2021. The third dose of BNT162b2 vaccine was administered between August 17, 2021, and 20, 2021, following an amendment of the study protocol approved by the Belgian Federal Agency for Medicines and Health Products. BNT162b2 is a lipid nanoparticle–formulated, nucleoside-modified mRNA vaccine encoding a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein.[25] The 30 μg/0.3 mL dose was administered by intramuscular injection into the upper arm. Vaccines were administered by a trained study nurse at Erasme Hospital. Participants were observed for a minimum of 15 minutes after vaccination. No serious adverse events were observed.

2.2. Breakthrough infection

After recruitment, symptomatic SARS-CoV-2 BTI, defined as the occurrence of fever, chills, cough, dyspnea, rhinitis, anosmia, dysgeusia, abdominal pain or diarrhea, associated with a positive SARS-CoV-2 PCR on nasopharyngeal swab, were recorded. Patients were informed of symptoms compatible with COVID-19 and were encouraged to be tested by PCR of a nasopharyngeal swab in case of such symptoms. Patients were regularly contacted by phone and/or by email to limit unreported BTI. The following data were recorded when a BTI was diagnosed: symptoms, date of symptom onset, oxygen requirement, organ failure and treatment, including curative monoclonal antibodies. The date of BTI was defined as the date of symptom onset. Only the first symptomatic BTI was included in the data analysis.

2.3. Immune response to BNT162b2 vaccination

Humoral and cellular immune responses to BNT162b2 vaccination were measured one month after the second vaccine dose (D2D28) and one month after the third vaccine dose (D3D28). Prevaccination immune responses to SARS-CoV-2 were analyzed as baseline. Humoral immune responses measured included SARS-CoV-2 receptor binding domain (RBD)-specific binding IgG and avidity, SARS-CoV-2 Wuhan, B.1.617.2 Delta variant (83DJ-1), BA.1 Omicron variant and BA.2 Omicron variant neutralizing antibodies, and SARS-CoV-2 S1 and S2 domain binding and phagocytosis inducing IgG. Cellular immune responses measured included S1 and S2 domain-specific Interferon gamma-producing cells. For detailed laboratory methods, see Supplementary Material.

2.4. Statistical analyzes

The occurrence of SARS-CoV-2 BTI during the study period (from March 2, 2021, to March 18, 2021, to February 1, 2022) was plotted as a Kaplan–Meier survival curve. The incidence of SARS-CoV-2 infection diagnosed in Belgium during the study period and the proportion of infections with variants of concern (VOC) circulating in Belgium was included in the graph to provide the epidemiological context of study.[26] The incidence rate (person-day rate) of BTI was defined as the ratio of BTI divided by the time each patient was observed, totaled for all the patients. It was calculated for 2 different periods: (1) from the first day of vaccination to D3D28, and (2) from D3D28 to February 1, 2022 when the fourth dose of COVID-19 vaccine was administered. Descriptive data were evaluated with geometric means (95% CI) for continuous variables, and *n* (%, 95% CI) for binary values. Descriptive data were compared by *t*-test or Wilcoxon—Mann–Whitney rank test for parametric and nonparametric values, respectively, according to the D'Agostino normality test. Fisher test was performed to compare the risk of BTI according to the stratification of the immune parameters. The Spearman test was performed to assess the correlation between immune parameters. A Wilcoxon signed-rank test was used to test paired sampled, when comparing two-time points. The proportion of participants with antibody levels higher than the lower limit of detection (LLOD) at D2D28 or D3D28 were calculated for SARS-CoV-2 RBD binding IgG (LLOD: 5.4 IU/mL according to WHO standard) and neutralizing antibodies (LLOD: 77 IU/mL according to WHO standard). For RBD binding IgG and neutralizing antibodies, antibody concentrations below the limit of detection were assigned a value equal to half of the LLOD before the

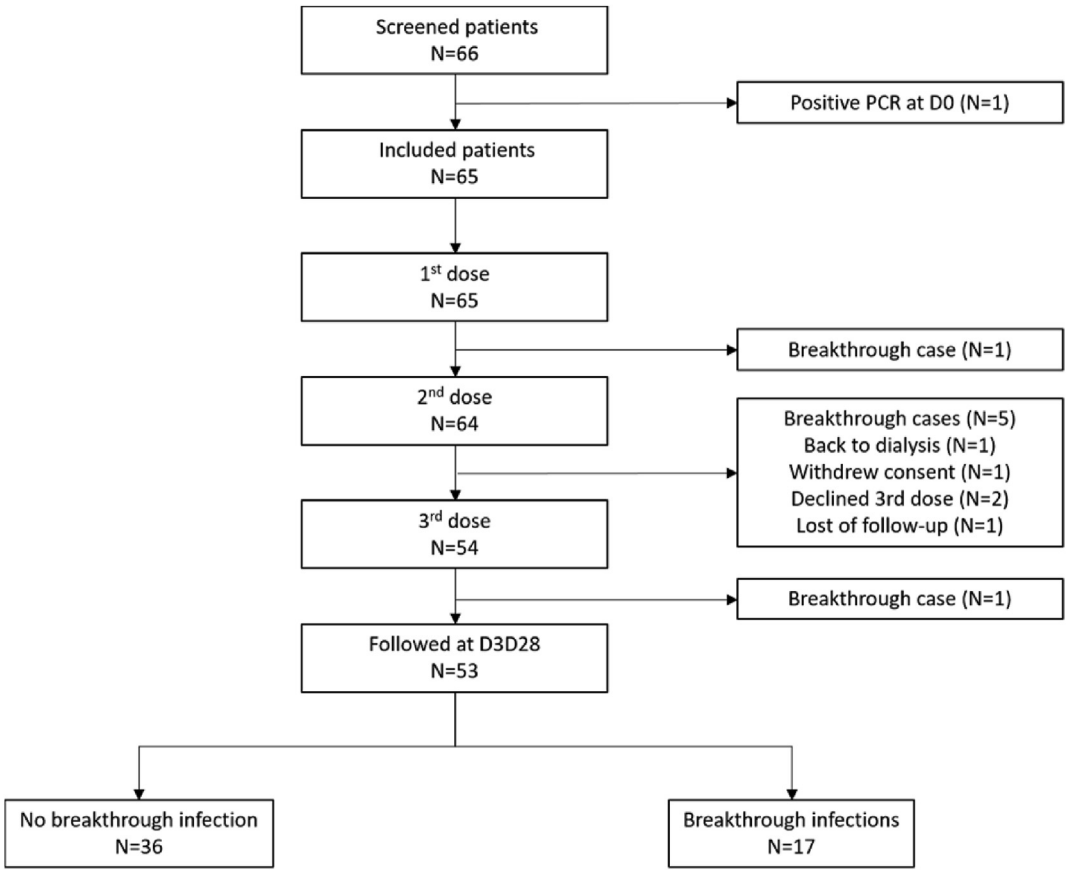

Figure 1. Study flow chart.

transformation. RBD IgG avidity was measured for samples with detectable binding RBD IgG only. For Interferon gamma ELISpot, data equal to 0 or less than 0 after subtraction of background were assigned a value of 1 before the transformation. Values higher than 300 spots forming cells/250 000 PBMC were censured at 1.200/ 10^6 PBMC. The association between immune variables and demographic and clinical variables, were explored by linear regressions. Univariate logistic regression was used to explore the factors associated with the occurrence of BTI for the period between D3D28 and February 1, 2022. Variables with a *P* value of $< .1$ in univariate Analyses were included in a variance inflation factor (VIF) analysis. To avoid multicollinearity, only variables with VIF <10 were included in multivariate analysis. A multivariate logistic regression model was built to analyze risk factors of BTI, adjusting for age, kidney graft function, and mycophenolate mofetil use, considered as important variables influencing immune responses to SARS-CoV-2 vaccination, based on our linear regression data and on published data.[27,28] Missing data were excluded from the final analysis. The final model was selected based on backward selection of variables. All statistical analyses were done using R version 4.2.0.[29]

3. Results

3.1. Study population

Sixty-six kidney transplant recipients were screened for the study. One patient was excluded because of a positive PCR test on the day of the first COVID-19 vaccination. Characteristics of the 65 patients included in the study are shown in Supplementary Table S1. The mean age was 58.2 years. The mean duration of transplantation was 7.4 years. Immunosuppressive treatment included steroids (78%), mycophenolate mofetil (52%), azathioprine (25%), tacrolimus (68%), cyclosporine (14%), and everolimus (23%). During follow-up, one patient required chronic dialysis, one patient withdrew consent, one patient was lost to follow-up between the second and the third vaccination, and 2 patients declined the third dose of vaccine (Fig. 1). No patients died during the study period.

3.2. Incidence of SARS-CoV-2 BTI

Six cases of symptomatic SARS-CoV-2 BTI were diagnosed between the first and the third COVID-19 vaccination. Thus, 54 patients were followed-up and received the third dose of the vaccine, on average 161 days after having received the first vaccine dose (Fig. 1). One case of symptomatic BTI was diagnosed within 28 days of the third dose of vaccine. At D3D28, 53 patients remained free of symptomatic BTI. Seventeen cases were diagnosed between 28 days after the third vaccination and the fourth vaccination, when the study follow-up ended (Fig. 1). Symptoms related to BTI are shown in table S2. One patient experienced moderate COVID-19 requiring oxygen supply, and the other 16 patients had mild COVID-19. Curative monoclonal antibodies were administered to 14 patients (83%). At the end of the study period, 36 patients remained free of symptomatic BTI.

Figure 2 depicts the cumulative number of symptomatic BTI in the study population over time and in relation to the incidence of SARS-CoV-2 infection with individual VOC in the Belgian population. During the first period (before D3D28), the incidence rate of SARS-CoV-2 BTI in the study population was 0.63 (0.25; 1.30) cases per 1000 person-days. During this period, the daily incidence of COVID-19 was estimated at less than 1 case per 1000 inhabitants in Belgium, and the alpha and delta VOC were dominant. During the second period (after D3D28), the incidence rate of BTI in the study population was about 4-

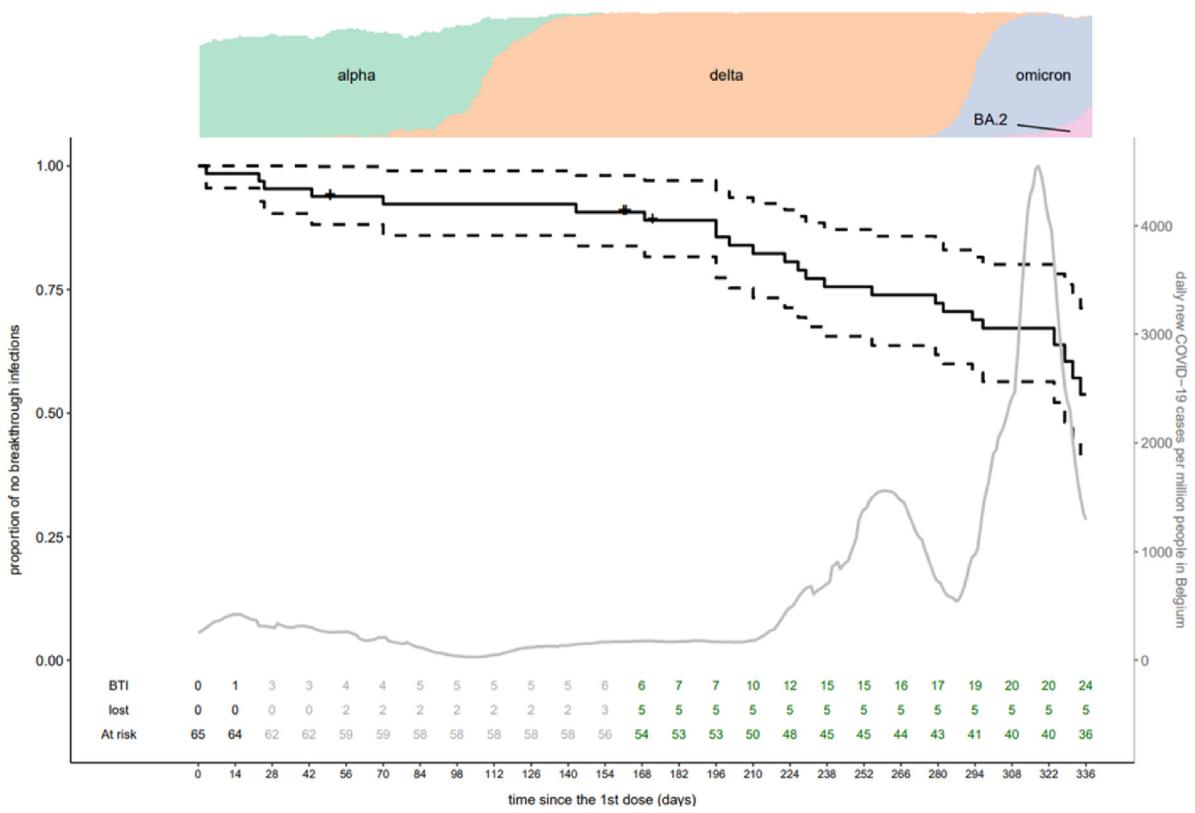

Figure 2. Occurrence of symptomatic BTI in relation to incidence of COVID-19 in the Belgian population. The black line represents the proportion of kidney transplant recipients who remained free of SARS-CoV-2 BTI during the study period. The dotted lines are 95% confidence intervals. Day 0 is the date of the first vaccine dose for each patient. Patients were followed-up until the first day of administration of the fourth vaccine dose (February 1, 2022). Each cross represents a patient lost to follow-up. Numbers of patients with BTI, lost to follow-up, and at risk of BTI are indicated in the lower part of the graph (black numbers: before the second vaccine dose; gray: between the second and third dose of vaccine; green: after the third vaccine dose). The gray line represents daily confirmed COVID-19 cases per million people 7 days rolling average in Belgium. Day 0 for the gray line is the median date of the first vaccination of the study population (March 10, 2021). Proportions of infections with VOC are presented in the upper part of the figure (alpha in green, delta in orange, omicron BA.1 in gray and omicron BA.2 in pink, and others in white). BTI, breakthrough infection

fold higher: 2.74 (1.60; 4.38) cases per 1000 persons-days (comparison of incidence rates between the 2 periods: $P < .001$). During this second period, the estimated incidence of COVID-19 also increased in the Belgian population and the delta and BA.1 omicron VOC were dominant (Fig. 2).

3.3. Immune response to COVID-19 vaccination

Administration of a third dose of COVID-19 vaccine significantly increased the levels and quality of SARS-CoV-2 spike-specific antibodies (Fig. 3A and Supplementary Table S3). The geometric mean serum concentration of RBD binding IgG increased from 8 IU/mL after 2 doses of vaccine to 35 IU/mL after 3 vaccine doses. The proportion of patients with detectable RBD binding IgG was 51% after 3 vaccinations (27/53). Avidity of RBD binding IgG also increased in patients with detectable RBD IgG with a five-fold slower dissociation rate between the second and third dose of vaccine (from geometric mean of k_{off} : 0.0016 s^{-1} after the second dose to geometric mean of k_{off} : 0.0003 s^{-1} after the third dose of vaccine). Neutralizing antibody levels increased similarly, with only 2% of patients having detectable SARS-CoV-2 neutralizing antibodies after 2 doses and 45% of patients after 3 vaccine doses. In contrast, Interferon gamma responses to SARS-CoV-2 S1 and S2 were not significantly different after the second and third vaccine doses (Fig. 3A and Supplementary Table S3). These similar responses overall involved inter-patient heterogeneity, with increased responses in some patients and decreased responses in others. As a result, Interferon gamma responses after the second and third vaccine doses were only moderately correlated (Fig. 3B).

3.4. Risk factors of SARS-CoV-2 BTI after booster COVID-19 vaccination

Potential risk factors for SARS-CoV-2 BTI after the third dose of COVID-19 vaccine were first explored by univariate analysis using logistic regression (Fig. 4 and Supplementary Table S4). An increased risk of BTI in kidney transplant recipients was associated with low RBD IgG avidity (odds ratio (95% CI): 0.07 (0.003; 0.61), $P = .04$), low titer of neutralizing antibodies (0.15 (0.02; 0.64), $P = .027$), and low frequency of S2-specific, and not S1-specific, Interferon gamma-producing cells (0.30 (0.11; 0.69), $P = .009$). No significant association was detected between the risk of BTI after booster COVID-19 vaccination and the demographic or clinical variables that were analyzed (Fig. 4 and Supplementary Table S4). Of note, no significant association was detected between the risk of BTI after D3D28 and the humoral or cellular immune responses measured 28 days after the second dose of vaccine (Supplementary Fig. S1)

The absence of significant association between demographic and clinical variables and the risk of BTI led us to explore their potential association with the immune response to COVID-19 vaccination. As shown in Supplementary Table S5, older age was associated with lower humoral immune responses to booster vaccination. Lower kidney graft function and mycophenolate mofetil use were associated with lower humoral and cellular immune responses to booster vaccination, whereas the use of azathioprine was associated with higher vaccine responses. Together, these results indicate that demographic and clinical variables are associated with differences in immune responses to booster COVID-19 vaccination in kidney transplant recipients even if they were not significantly associated with the risk of BTI in this population.

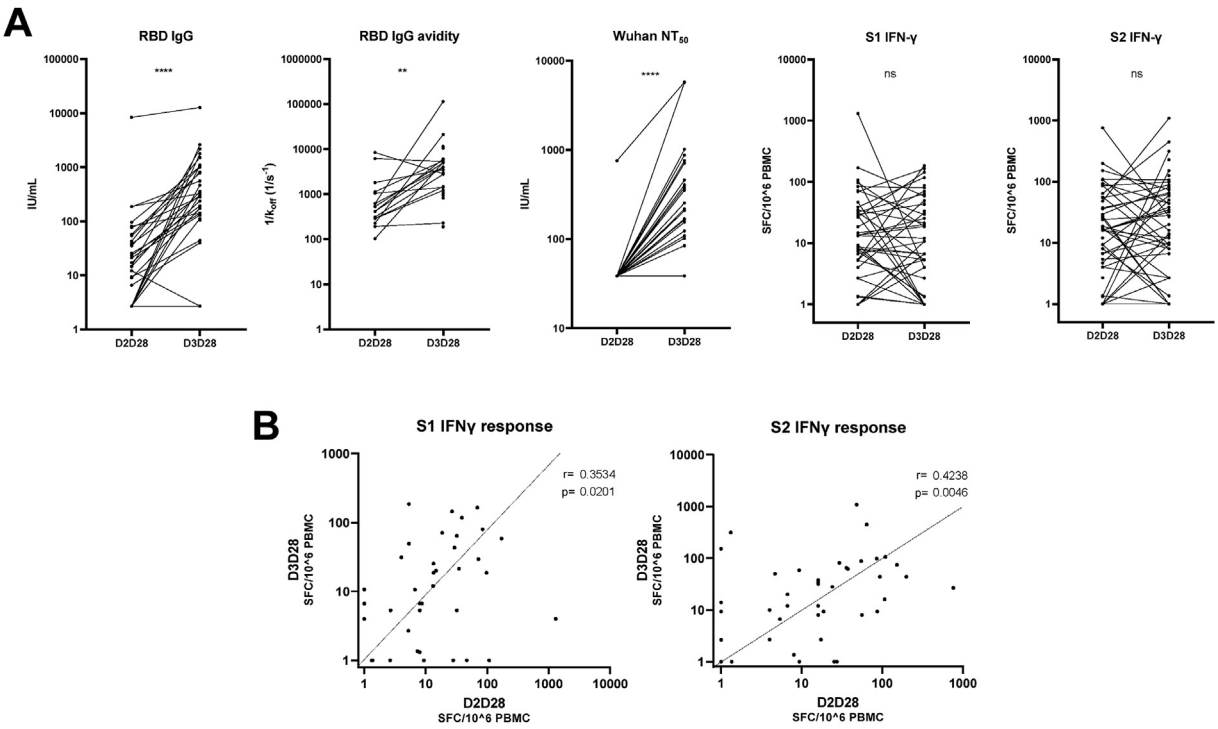

Figure 3. Immune response to second and third COVID-19 mRNA vaccinations in kidney transplant recipients. (A) Immune responses, including SARS-CoV-2 receptor binding domain (RBD)-specific binding IgG titer (RBD IgG) and avidity (RBD IgG avidity), SARS-CoV-2 Wuhan neutralizing antibody titer (Wuhan NT_{50}) and S1 (S1 Interferon gamma) and S2 (S2 Interferon gamma) domain-specific Interferon gamma-producing cells, were measured one month after the 2nd dose (D2D28) and one month after the 3rd dose (D3D28) of vaccine among kidney transplant recipients naive of symptomatic BTI at D3D28 ($n = 53$). Each data point represents a sample. Samples were available for 51 patients at D2D28 and 53 patients at D3D28. The lower limit of detection (LLOD) is 5.4 IU/mL for RBD binding IgG and 77 IU/mL for Wuhan NT_{50}. Twenty-one patients had detectable RBD binding IgG at D2D28 and 27 at D3D28. One patient had detectable neutralizing antibodies at D2D28 and 24 at D3D28. RBD IgG avidity was only measured for samples with RBD binding IgG levels > 5.4 IU/mL. Statistical comparisons were made using Wilcoxon signed-rank test. RBD: receptor binding domain. Wuhan NT_{50}: 50% neutralizing antibody titer against Wuhan strain. S1 or S2 Interferon gamma: S1 or S2 specific PMBC responses. ** $P < .01$; and **** $P < .0001$. (B) Spearman correlation between S1 and S2 IFNγ response measured at D2D28 and D3D28. The dotted line indicates the function f(X)=Y.

3.5. Multi-parametric analysis of immune response to booster COVID vaccination in patients with or without symptomatic BTI

Additional parameters of the humoral immune response to COVID-19 vaccination were measured to further explore the association between the immune response to booster vaccination and the risk of BTI in kidney transplant recipients. Patients with BTI had lower levels of S1-specific, but not S2-specific, binding IgG as compared with patients who did not develop symptomatic BTI (Fig. 5A). In parallel, patients with BTI also had lower levels of S1-specific, but not S2-specific, IgG activating cellular phagocytosis (antibody-dependent cellular phagocytosis, [ADCP]). In line with the univariate analysis, patients who developed SARS-CoV-2 BTI after booster vaccination had significantly lower RBD binding IgG titer and lower levels of neutralizing antibodies as compared with patients who remained free of symptomatic BTI (Fig. 5B). A similar trend was observed with RBD IgG avidity. After booster vaccination, only a few patients had detectable neutralizing antibodies against delta ($n = 4$), omicron BA.1 ($n = 1$), and omicron BA.2 ($n = 4$) (Supplementary Table S6). None of them developed symptomatic BTI. Analysis of cellular immune responses confirmed the results of the univariate analysis: patients with BTI had significantly lower frequencies of S2-specific, and not S1-specific, Interferon gamma producing cells, as compared with patients with no BTI (Fig. 5C). Humoral immune response parameters (RBD IgG, RBD IgG avidity, Wuhan NT_{50} (Wuhan neutralizing antibody titer), S1 IgG, S2 IgG, S1 IgG ADCP and S2 IgG ADCP) were highly correlated (Fig. 5D). In contrast, significant but lower correlations were observed between humoral and cellular immune response (S1 Interferon gamma and S2 Interferon gamma) parameters.

3.6. Multivariate analysis of risk factors of BTI

Multivariate analysis was performed to identify the immunological factors that best predicted the risk of BTI after booster vaccination. Variables with a P value of <.1in the univariate analysis were included in the analysis of VIF. Among them, RBD IgG avidity and S1 ADCP had a VIF above 10 and were excluded from the analysis to avoid collinearity. Based on the associations observed between clinical variables and COVID-19 vaccine responses (Supplementary Table S5), this analysis was adjusted for age, kidney graft function (eGFR), and mycophenolate use. Wuhan neutralizing antibody titer and S2 Interferon gamma response were the best predictors of BTI after booster vaccination, with odds ratios of 0.04 ([<0.001; 0.50], $P = .028$) and 0.15 [(0.02; 0.67], $P = .037$), respectively (Table 1). Based on this analysis, we estimated that patients with a 10-fold increase in Wuhan NT_{50} had on average a 28-fold lower risk of BTI, and patients with a 10-fold increase in S2 Interferon gamma responses had on average a 7-fold lower risk of BTI.

To further explore the relative contribution of neutralizing antibody and of S2 Interferon gamma responses in protection against BTI, patients were categorized on the basis of median immune responses (Fig. 6). Among the 18 patients with low humoral and cellular responses, 10 developed symptomatic BTI (Fig. 6A). The lowest risk of BTI was observed in patients with high humoral and cellular responses (1 BTI among 16 patients) (Fig. 6A, B). An intermediate and similar risk was observed in patients with either high humoral or high cellular responses (4 BTI among 15 patients) (Fig. 6A, B, C).

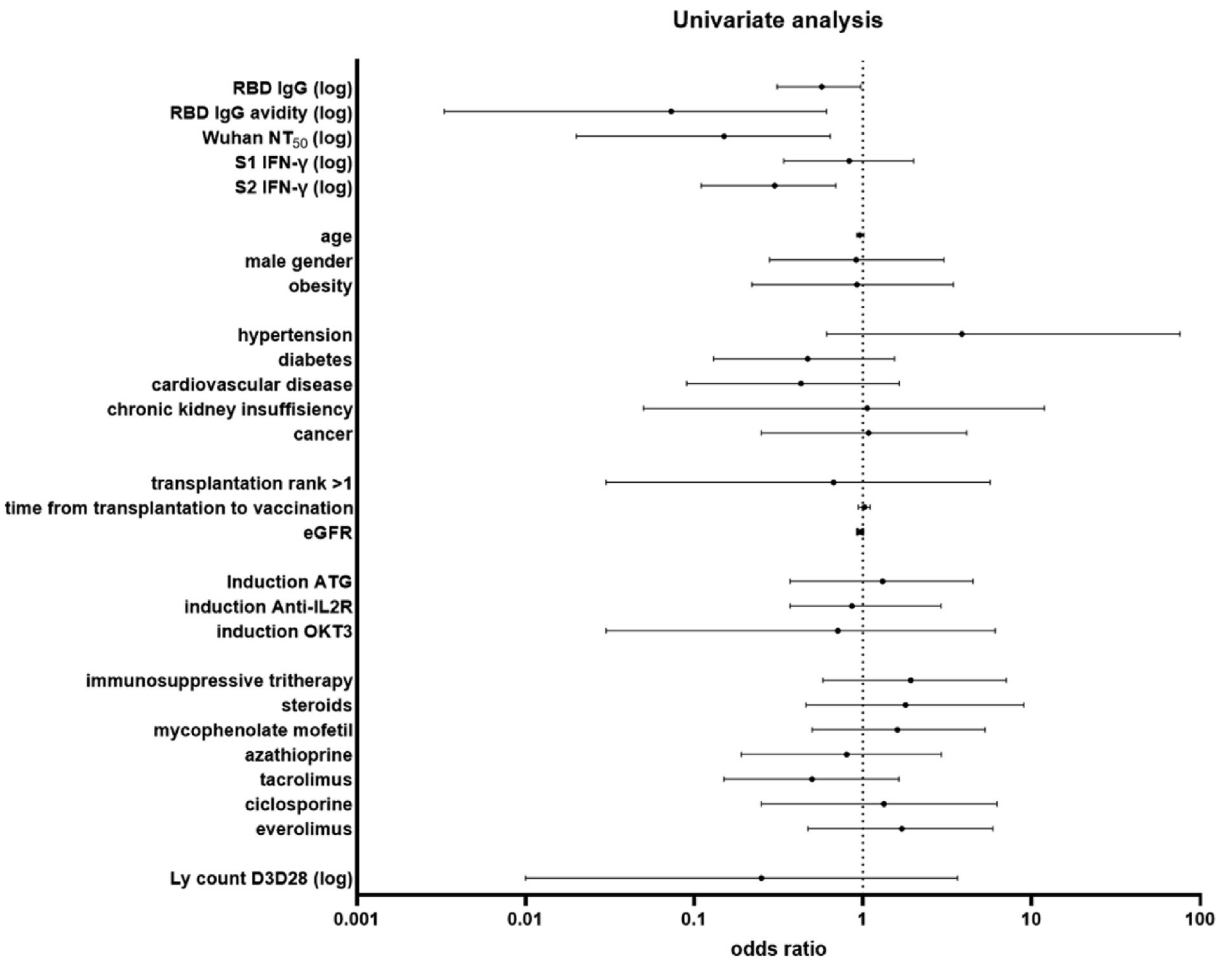

Figure 4. Univariate analysis of risk factors of symptomatic breakthrough infection in kidney transplant recipients. Univariate logistic regression was used to assess the relationship between immunological, demographic, and clinical parameters and the occurrence of symptomatic BTI. Obesity was defined as BMI $>$ 30 kg/m^2. Transplantation rank was divided into first transplantation and second or more transplantation. Immunological parameters and absolute lymphocyte counts were log10 transformed before analysis. RBD: receptor binding domain. Wuhan NT_{50}: 50% neutralizing antibody titer against Wuhan strain. S1 or S2 Interferon gamma: S1 or S2-specific PMBC responses. eGFR: estimated glomerular function rate; OKT3: muromumab; ATG: anti-thymoglobulin. Ly count: absolute lymphocyte count.

4. Discussion

In this prospective cohort study, we observed that both humoral and cellular immune responses to booster COVID-19 vaccination predicted protection against symptomatic BTI in kidney transplant recipients. Although age, kidney graft function, and the use of mycophenolate mofetil were associated with altered vaccine responses, no significant association was observed between these parameters, or other demographic and clinical parameters, and the occurrence of BTI. These data emphasize the importance of inducing adequate immunity through vaccination to protect this vulnerable population from COVID-19.

Our analysis was focused on kidney transplant recipients who had not been diagnosed with COVID-19 either before vaccination or before the response to booster vaccination was measured. Patients had low humoral immune responses after 2 doses of mRNA vaccine, confirming previous studies in SOT recipients.[13–15] Humoral immunity was significantly enhanced by booster vaccination, as observed in the general population.[21] In contrast, cellular immune responses were not significantly different following 2 doses of vaccination and after booster vaccination. This observation is in line with recent studies conducted on healthy adults.[30] This overall stability of cellular responses involved inter-patient heterogeneity in the response dynamics, suggesting heterogeneity in the establishment of T cell memory after vaccination. Despite enhanced immunity after booster vaccination, patients remained at high risk of SARS-CoV-2 BTI. Thirty-two percent of our study population developed symptomatic BTI in the first 6 months after booster vaccination. Most patients received therapeutic monoclonal antibodies to prevent severe COVID-19 and only one patient required oxygen supplementation. The incidence of BTI after the third vaccine dose was significantly higher than the one observed before booster vaccination, most likely because of the higher virus circulation of delta and omicron VOC during this period. This observation further emphasizes the importance of booster vaccination to prevent COVID-19 in SOT recipients.[8,9] Follow-up analyses of patients who remained free of BTI indicated that antibody levels decayed after the third vaccine dose and were further boosted by a fourth dose of vaccine (Supplementary Fig. S2).

To our knowledge, this is the first report on correlate of protection against COVID-19 in SOT recipients. As observed in the general population, the antibody response to COVID-19 vaccination was associated with the protection of kidney transplant recipients against BTI with VOC.[20] As reported in the general population, the strongest association with protection against BTI in SOT was observed with Wuhan neutralizing antibody titer.[18,20] In addition to SARS-CoV-2 neutralization, antibodies mediate viral control via Fc-dependent effector functions, including ADCP. We observed high correlations between the titers of binding, neutralizing, and ADCP-promoting antibodies against SARS-CoV-2 RBD and S1 subunit, indicating a strong coordination between the diverse effector components of the antibody response. S1-specific ADCP-promoting IgG was higher in patients who remained free of BTI after booster vaccination, supporting the notion that they may

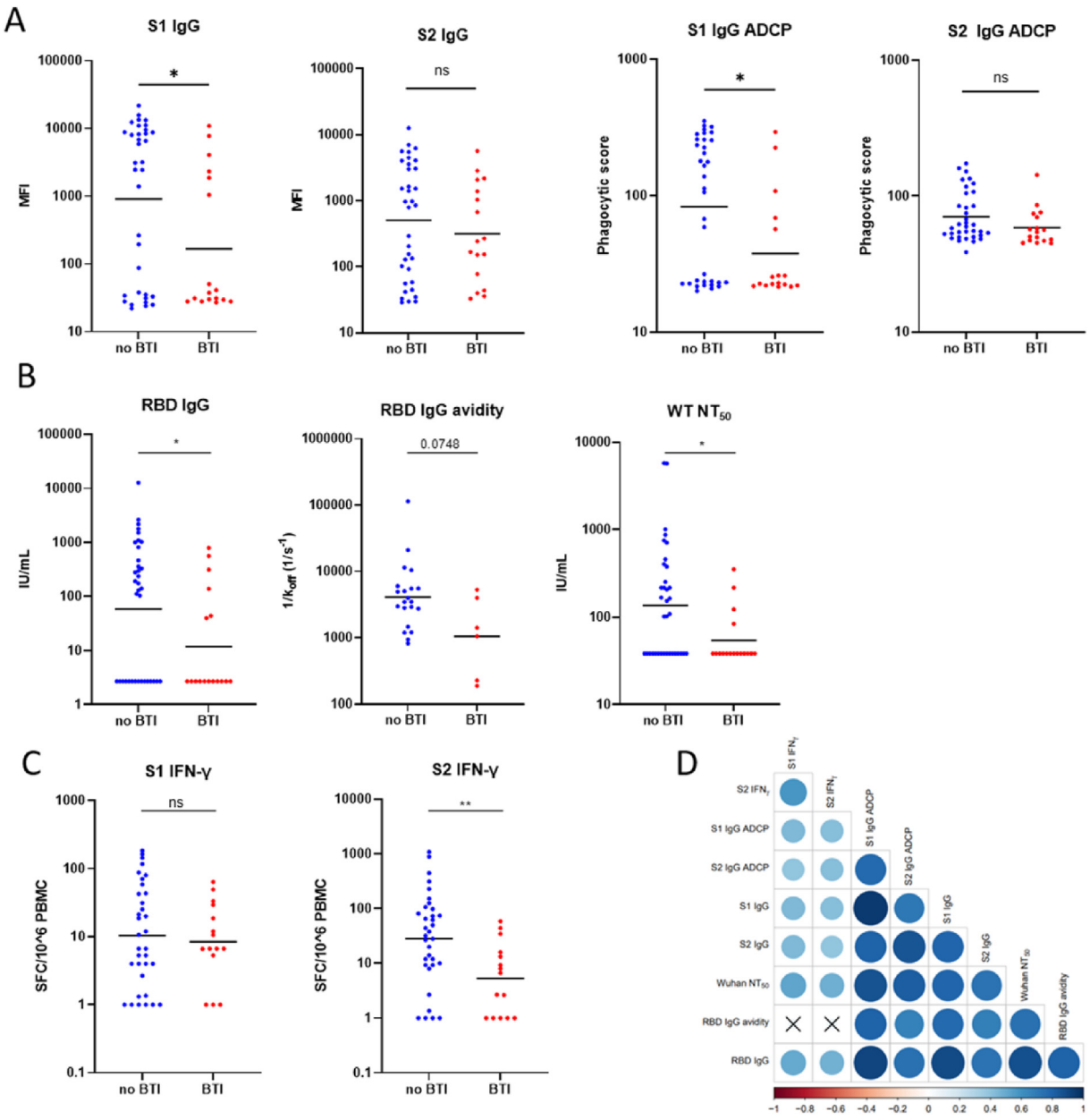

Figure 5. Immune response to booster COVID-19 vaccination in kidney transplant recipients with and without symptomatic BTI. Humoral and cellular immune responses were measured 28 days after a third dose of BNT162b2 COVID-19 vaccine in 53 kidney transplant recipients who developed (red symbols) or did not develop (blue symbols) symptomatic SARS-CoV-2 BTI after vaccination. Black bars indicate geometric means. Variables were compared with Wilcoxon—Mann–Whitney test. * $P < .05$; ** $P < .01$. (A) S1-specific binding IgG (S1 IgG), S2-specific binding IgG (S2 IgG), S1-specific binding IgG antibody–dependant cellular phagocytosis (S1 IgG ADCP), and S2-specific binding IgG antibody –dependant cellular phagocytosis (S1 IgG ADCP). (B) SARS-CoV-2 receptor binding domain (RBD)-specific binding IgG titer (RBD IgG) and avidity (RBD IgG avidity), SARS-CoV-2 Wuhan neutralizing antibody titer (Wuhan NT_{50}). (C) S1-specific cells producing Interferon gamma (S1 Interferon gamma) and S2-specific cells producing Interferon gamma (S2 Interferon gamma). (D) Pearson correlation matrix of immune response parameters. Correlation coefficients are color coded. Circle size indicates the absolute value of corresponding correlation coefficients. All correlations were statistically significant, except those marked by a cross instead of a circle.

also contribute to vaccine-induced protection. In contrast, S2-specific binding and ADCP-promoting IgG were similar in patients who developed or did not develop BTI. This observation suggests a dominant role of S1-specific antibodies in mediating protection against BTI in this population.

The S2-specific Interferon gamma response to booster vaccination was the second most important predictor of BTI. As no significant association was observed with S1-specific Interferon gamma responses, our data suggest a differential role of SARS-CoV-2 subunits in mediating protection after mRNA vaccination. As the S2 subunit is more conserved than the S1 subunit across SARS-CoV-2 VOC, it may be an important target for cell-mediated immunity against the delta and omicron variants to which our study population was exposed.

As suggested previously, T cell immunity may be particularly important in patients with defective humoral responses to COVID-19 vaccination.[22,31] Supporting this notion, we observed that high cellular responses were associated with a reduced risk of BTI both in patients with undetectable humoral responses and in patients with high humoral responses to booster vaccination. However, as S2 Interferon gamma responses were correlated with neutralizing antibody responses, the 2 parameters provide overlapping information about the immune response to booster vaccination. The role of cellular responses may therefore

Table 1
Multivariate analysis of risk factors of symptomatic BTI in kidney transplant recipients, adjusted for age, mycophenolate mofetil, and estimated glomerular function rate.

Variables	Complete initial model		Complete final model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* -value
RBD IgG	1.21 (0.19; 16.1)	0.848	-	-
Wuhan NT_{50}	0.03 (0.00; 1.10)	0.091	0.04 (0.00; 0.50)	0.028
S1 IgG	0.98 (0.14; 5.94)	0.980	-	-
S2 Interferon gamma	0.14 (0.02; 0.69)	0.041	0.15 (0.02; 0.67)	0.037
age	0.83 (0.71; 0.93)	0.006	0.83 (0.71; 0.93)	0.005
Mycophenolate mofetil use	0.97 (0.10; 9.17)	0.979	0.96 (0.12; 7.80)	0.965
eGFR	0.96 (0.88; 1.04)	0.340	0.96 (0.89; 1.03)	0.295

Only significant variables in univariate analysis and with VIF <10 were included in the initial multivariate model. The final model was obtained using backward selection of immunological features.

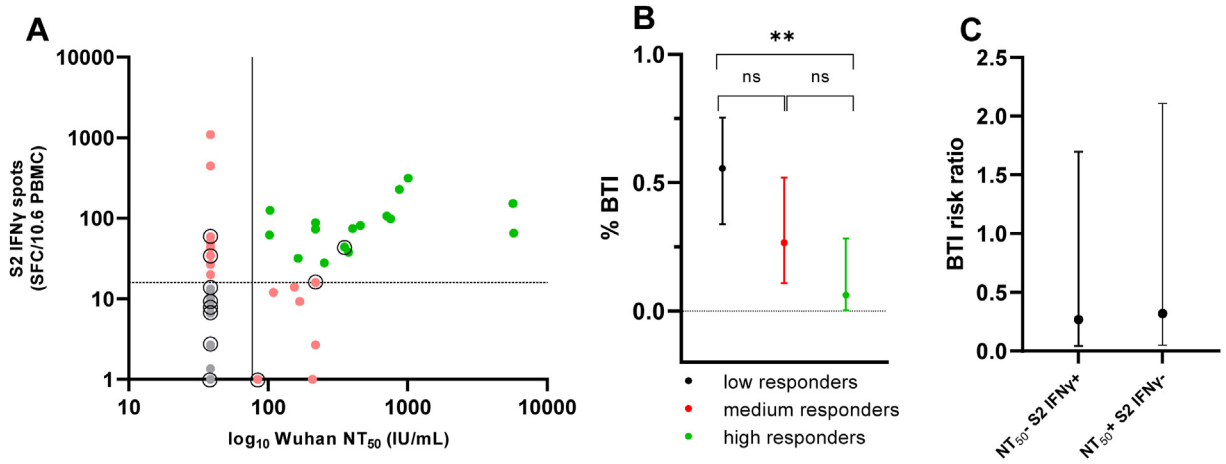

Figure 6. Risk BTI according to neutralizing antibody against Wuhan and S2 T cell response. (A) Patients were categorized on the basis of median neutralizing antibody titer and S2 Interferon gamma spot frequency in low responders (gray symbols; low neutralizing antibody titer and low S2 IFNγ spot frequency), medium responders (red symbols; high neutralizing antibody titer or high S2 IFNγ spot frequency) and high responders (green symbols; high neutralizing antibody titer and high S2 IFNγ spot frequency). Circled symbols indicate patients with BTI. Dotted line indicates the median S2 IFNγ response (16.1 SFC/10^6 PBMC). Black line indicates the median neutralizing antibody titer (77 IU/mL). (B) Geometric mean percentage and 95% CI of patients with BTI in the 3 patient categories. **: $P < .01$; ns: not significant. Fisher test. (C) Odds ratio and 95% CI of risk of BTI in patients with low neutralizing antibody titer with high or low S2 IFNγ spot frequency (NT_{50}- S2 IFNγ+) and in patients with low S2 IFNγ spot frequency with high or low neutralizing antibody titer (NT_{50}+ S2 IFNγ-).

include an improvement in the prediction of the risk of BTI and a direct contribution to immunity against BTI.

This study has several limitations. First, its sample size is relatively low because it was a single-center study. This limitation was decreased by the high incidence of symptomatic BTI during a period when delta and omicron VOC were dominant, allowing us to detect statistically significant associations with immunological parameters. A larger sample size may have allowed us to detect significant associations with demographic and clinical parameters. Further studies of correlates of protection in SOT recipients are needed to validate our observations. Second, the study did not include systematic screening of SARS-CoV-2 by PCR and therefore did not allow us to diagnose asymptomatic SARS-CoV-2 BTI. Such systematic screening was considered too challenging by the patients and by the study team. Instead, priority was placed on diagnosing symptomatic BTI, which we consider to be diagnosed with high sensitivity. Third, the study does not provide information regarding immunity against severe COVID-19, as most patients experiencing a BTI received therapeutic monoclonal antibodies and consequently presented mostly mild symptoms. Finally, while the very strong dominance of delta and omicron VOC in the Belgian population during the study period indicates that these were likely the cause of BTI in our study population, sequencing of SARS-CoV-2 was not performed systematically after the diagnosis of BTI. Differences in correlates of protection against delta and omicron variants have been suggested and may have influenced our results.[32]

In conclusion, this study shows that humoral and cellular immune responses induced by booster vaccination correlate with protection against SARS-CoV-2 BTI in kidney transplant recipients. These data emphasize the importance of reaching and maintaining a high level of immunity through vaccination in this vulnerable population and suggest that vaccines inducing potent cellular immune responses, such as mRNA vaccines, may be particularly useful in populations with suboptimal humoral immune responses to vaccination. Further studies are needed to confirm these findings.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request, following materials transfer agreements.

Author contributions

D.K., N.G., A.L.M., and A.M., conceptualized the study. D.K. and P.P. wrote the clinical study protocol. D.K. obtained permission from the ethics committee and A.F.M.P.S., M.E.G., A.L.M., and A.M. secured the funding of the study. D.K., N.G., and A.L. conducted the clinical study. A.L.M. overviewed the clinical study. D.K., N.G., V.O., D.G., M.V., A.W., J.M., and L.H. conducted the laboratory analyzes. A.M., P.P., I.D., M.E.A., A.Mat, and K.K.A. overviewed the laboratory analyzes. D.K., N.G., S.D., D.G. and L.H. analyzed the data. D.K., N.G., S.D., M.E.A., and A.M. interpreted the data. D.K. and A.M. drafted the manuscript. All co-authors reviewed and approved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2023.02.015.

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References

1. Requião-Moura LR, Sandes-Freitas TV de, Viana LA, et al. High mortality among kidney transplant recipients diagnosed with coronavirus disease 2019: Results from the Brazilian multicenter cohort study. *PLoS One*. 2021;16(7), e0254822. https://doi.org/10.1371/journal.pone.0254822.
2. Villanego F, Mazuecos A, Pérez-Flores IM, et al. Predictors of severe COVID-19 in kidney transplant recipients in the different epidemic waves: Analysis of the Spanish Registry. *Am J Transplant*. 2021;21(7):2573–2582. https://doi.org/10.1111/ajt.16579.
3. Hilbrands LB, Duivenvoorden R, Vart P, et al. COVID-19-related mortality in kidney transplant and dialysis patients: results of the ERACODA collaboration. *Nephrol Dial Transplant*. 2020;35(11):1973–1983. https://doi.org/10.1093/ndt/gfaa261.
4. Jager KJ, Kramer A, Chesnaye NC, et al. Results from the ERA-EDTA Registry indicate a high mortality due to COVID-19 in dialysis patients and kidney transplant recipients across Europe. *Kidney Int*. 2020;98(6):1540–1548. https://doi.org/10.1016/j.kint.2020.09.006.
5. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature*. 2020;584(7821):430–436. https://doi.org/10.1038/s41586-020-2521-4.
6. Udomkarnjananun S, Kerr SJ, Townamchai N, et al. Mortality risk factors of COVID-19 infection in kidney transplantation recipients: a systematic review and meta-analysis of cohorts and clinical registries. *Sci Rep*. 2021;11(1), 20073. https://doi.org/10.1038/s41598-021-99713-y.
7. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *JAMA*. 2021;325(21):2204. https://doi.org/10.1001/jama.2021.7489.
8. Caillard S, Chavarot N, Bertrand D, et al. Occurrence of severe COVID-19 in vaccinated transplant patients. *Kidney Int*. 2021. https://doi.org/10.1016/j.kint.2021.05.011, 0(0.
9. Qin CX, Moore LW, Anjan S, et al. Risk of breakthrough SARS-CoV-2 infections in adult transplant recipients. *Transplantation*. 2021;105(11):e265. https://doi.org/10.1097/TP.0000000000003907.
[10]. Yetmar ZA, Bhaimia E, Bierle DM, Ganesh R, Razonable RR. Breakthrough COVID-19 after SARS-CoV-2 vaccination in solid organ transplant recipients: An analysis of symptomatic cases and monoclonal antibody therapy. *Transplant Infect Dis*. 2022;24(2):e13779. https://doi.org/10.1111/tid.13779.
11. Anjan S, Natori Y, Fernandez Betances AA, et al. Breakthrough COVID-19 infections after mRNA vaccination in solid organ transplant recipients in Miami, Florida. *Transplantation*. 2021;105(10):e139–e141. https://doi.org/10.1097/TP.0000000000003902.
12. Sun J, Zheng Q, Madhira V, et al. Association between immune dysfunction and COVID-19 breakthrough infection after SARS-CoV-2 vaccination in the US. *JAMA Intern Med*. 2022;182(2):153–162. https://doi.org/10.1001/jamainternmed.2021.7024.
13. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients. *New Eng J Med*. 2021;385:661–662. https://doi.org/10.1056/NEJMc2108861.
14. Benotmane I, Gautier G, Perrin P, et al. Antibody response after a third dose of the mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients with minimal serologic response to 2 doses. *JAMA*. 2021;326(11):1063–1065. https://doi.org/10.1001/jama.2021.12339.
15. Karaba AH, Zhu X, Liang T, et al. A third dose of SARS-CoV-2 vaccine increases neutralizing antibodies against variants of concern in solid organ transplant recipients. *Am J Transplant*. 2022;22(4):1253–1260. https://doi.org/10.1111/ajt.16933.
16. Hoffman TW, Meek B, Rijkers GT, van Kessel DA. Serologic response to a third dose of an mRNA-based SARS-CoV-2 vaccine in lung transplant recipients. *Transpl Immunol*. 2022;72, 101599. https://doi.org/10.1016/j.trim.2022.101599.
17. Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27(11):2032–2040. https://doi.org/10.1038/s41591-021-01540-1.
18. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27(7):1205–1211. https://doi.org/10.1038/s41591-021-01377-8.
19. Bergwerk M, Gonen T, Lustig Y, et al. Covid-19 breakthrough infections in vaccinated health care workers. *New Eng J Med*. 2021;385:1474–1484. https://doi.org/10.1056/NEJMoa2109072.
20. Cromer D, Steain M, Reynaldi A, et al. Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe*. 2022;3(1):e52–e61. https://doi.org/10.1016/S2666-5247(21)00267-6.
21. Lustig Y, Gonen T, Meltzer L, et al. Superior immunogenicity and effectiveness of the third compared to the second BNT162b2 vaccine dose. *Nat Immunol*. 2022;23(6):940–946. https://doi.org/10.1038/s41590-022-01212-3.
22. Kent SJ, Khoury DS, Reynaldi A, et al. Disentangling the relative importance of T cell responses in COVID-19: leading actors or supporting cast? *Nat Rev Immunol*. 2022;22(6):387–397. https://doi.org/10.1038/s41577-022-00716-1.
23. Yamal JM, Appana S, Wang M, et al. Trends and correlates of breakthrough infections with SARS-CoV-2. *Front Public Health*; 2022:10. https://www.frontiersin.org/article/10.3389/fpubh.2022.856532. Accessed June 20, 2022.
24. MD DL, Barkley E, Kibitel J, PharmD NC, McNitt J. Which comorbidities increase the risk of a COVID-19 breakthrough infection? epic research. https://epicresearch.org/articles/which-comorbidities-increase-the-risk-of-a-covid-19-breakthrough-infection. Accessed April 1, 2022.
25. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;10, NEJMoa2034577. https://doi.org/10.1056/NEJMoa2034577. Published online December.
26. Belgium COVID-19 Dashboard - Sciensano. Google Data Studio. Accessed August 16, 2022. =http://datastudio.google.com/reporting/c14a5cfc-cab7-4812-848c-0369173148ab/page/urrUC?feature=opengraph.
27. Hamaya T, Hatakeyama S, Yoneyama T, et al. Seroprevalence of SARS-CoV-2 spike IgG antibodies after the second BNT162b2 mRNA vaccine in Japanese kidney transplant recipients. *Sci Rep*. 2022;12(1):5876. https://doi.org/10.1038/s41598-022-09897-0.

28. Manothummetha K, Chuleerarux N, Sanguankeo A, et al. Immunogenicity and risk factors associated with poor humoral immune response of SARS-CoV-2 vaccines in recipients of solid organ transplant: A systematic review and meta-analysis. *JAMA Network Open.* 2022;5(4), e226822. https://doi.org/10.1001/jamanetworkopen.2022.6822.
29. R: The R project for statistical computing. https://www.r-project.org/. Accessed August 15, 2022.
30. Maringer Y, Nelde A, Schroeder SM, et al. Durable spike-specific T cell responses after different COVID-19 vaccination regimens are not further enhanced by booster vaccination. *Sci Immunol.* 2022;7(78), eadd3899. https://doi.org/10.1126/sciimmunol.add3899.
31. Wherry EJ, Barouch DH. T cell immunity to COVID-19 vaccines. *Science.* 2022;377(6608):821–822. https://doi.org/10.1126/science.add2897.
32. Stærke NB, Reekie J, Nielsen H, et al. Levels of SARS-CoV-2 antibodies among fully vaccinated individuals with Delta or Omicron variant breakthrough infections. *Nat Commun.* 2022;13(1):4466. https://doi.org/10.1038/s41467-022-32254-8.