SARS-CoV-2 antibody dynamics among kidney transplant recipients 3 months after BNT162b2 vaccination: a prospective cohort study

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Abbreviations: ANOVA - analysis of variance; ATG - anti-thymocyte globulin; CNI - calcineurin inhibitor; GFR - glomerular filtration rate; GMT - Geometric mean titers; IQR - interquartile range; mTOR – mammalian target of rapamycin; OR – odds ratio; RMC - Rabin medical center; RBD – receptor binding domain
ABSTRACT

Data regarding immunogenicity of mRNA SARS-CoV-2 vaccines among kidney transplant recipients in the months following vaccination are lacking. We aimed to investigate humoral immune response at 3-4 months post vaccination among a cohort of kidney transplant recipients, compared to control group of dialysis patients. Anti-spike antibodies were tested at 1 and 3-4 months after vaccination. Of 259 kidney transplant recipients tested at median time of 110 days from second vaccine dose, 99 (38%) were seropositive, compared with 83% (101/122) control patients. Younger age, better renal function, and lower immunosuppression levels were associated with seropositivity. 14% (13/94) of participants seropositive at 1 month became seronegative at follow up, and 12% (18/165) became seropositive. The latter were mainly individuals with higher antibody levels at 1 month. Antibody levels at 3-4 months were significantly reduced in both study groups, though the decline was more pronounced in the control group. Kidney transplant recipients present poor antibody response to mRNA SARS-CoV-2 vaccination, with only 38% seropositive at 3-4 months. Nevertheless, the decay in antibody response over time is modest, and some patients may present delayed response, reaching adequate antibody levels at 3-4 months. Low seropositivity rates in this group call for investigating other immunization strategies.

Keywords: antibodies, COVID-19, immune response, kidney transplant, mRNA vaccine
INTRODUCTION

Inadequate antibody response to SARS-CoV-2 mRNA vaccination has been increasingly reported among solid organ transplant (SOT) recipients. Several studies in kidney transplant recipients have shown poor humoral responses up to one month following the second mRNA vaccine dose, ranging between 29 to 54% seropositivity\(^1,2\). Heart transplant recipients have shown similar response rates\(^3\), while lung transplant recipients have shown even worse responses (18% seropositivity at 14-21 days following the second vaccine dose)\(^4\).

Factors associated with reduced humoral response in these studies included increased age, lower glomerular filtration rate (GFR), and type and dose of immunosuppressive medication. Treatment with belatacept or anti-thymocyte globulin (ATG); higher doses of mycophenolic acid; or higher blood levels of calcineurin inhibitors were all associated with lower seropositivity rates\(^1,3,5\).

Among transplant recipients following natural COVID-19 infection, few small studies demonstrated similar immediate humoral and cellular response in comparison with healthy controls, soon after the infection\(^6,7\). A study following liver transplant recipients over 6 months after natural infection showed lower seropositivity rate at 3 and 6 months when compared to healthy controls, but with a similar decline in seropositivity rates over time (77% seropositivity at 3 months to 63% at 6 months)\(^8\).

No data are currently available regarding the durability of antibody response following vaccination in transplant recipients. Among heathy subjects, Widge et al. reported elevated antibodies persisting at 90 days, and more recently, 209 days after the second dose of mRNA-1273 (Moderna) vaccine in all 34 participants, despite a slight decrease in levels\(^9,10\). Geometric mean titers (GMTs) exceeded those of
convalescing non-vaccinated controls. In comparison, Naaber et al demonstrated a significant decrease in antibody levels among 122 healthy volunteers at 6 and 12 weeks following BNT162b2 vaccine (Pfizer)\(^1\).

In this second stage of a prospective cohort study following kidney transplant recipients after BNT162b2 vaccination, we assessed anti-spike (anti-S) antibody levels at 3 months after the second vaccine dose.

**MATERIALS AND METHODS**

**Study procedures and data collection**

This was a prospective, single center, cohort study, evaluating kidney transplant recipients with functioning graft that had been vaccinated with 2 doses BNT162b2 vaccine, 21 days apart. We included adult recipients (>18) receiving first vaccine dose at least one month following the transplantation surgery. Participants who had documented infection with COVID-19 at any time prior to vaccination were excluded from the study. The study was conducted according to the declarations of Helsinki and Istanbul and was approved by the ethic committee of Rabin medical center (RMC). The settings, data collection, immunosuppression regimen data, and laboratory methods have been previously described [Supplement 1], as well as outcomes at 2-4 weeks following the second vaccine dose\(^1\). In this phase of the study, we further tested consenting participants for SARS-CoV-2 antibody levels at ~3 months after second vaccine dose. The SARS-CoV-2 IgG II Quant (Abbott\(^\circ\)) assay, testing antibodies against the receptor binding domain (RBD) of the spike protein, was used, with level > 50 AU/mL considered positive\(^12\). A control group consisted of dialysis patients, evaluated in the same manner. The rationale for this control group was data from two studies, showing robust antibody response to mRNA vaccines.
among dialysis patients, similar to healthy population\textsuperscript{13,14}. Dialysis patients that were treated with immunosuppressant medications were excluded from the control group.

**Statistical analysis**

For comparison of continuous variables between groups we used Student-t test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. The main outcome of the study was seropositivity for SARS-CoV-2 at three months (defined as antibody titer > 50 AU/ml)\textsuperscript{12}. Secondary outcomes included log transformed antibody titer (1 was added to the antibody levels and the product was transformed to decimal logarithmic scale), and the change in antibody levels between the two evaluation timepoints.

Univariate and multivariate logistic regression analyses were performed with seropositivity as outcome. Multivariate analysis was done using forward regression with a p value of 0.05 for inclusion. Variables introduced into the model included: age, gender, BMI, donor type, time from transplantation, diabetes status, time from immunization to evaluation, eGFR, high calcineurin inhibitor (CNI) blood level, mycophenolic acid dose, treatment with ATG (within 6 month before the first vaccine dose), treatment with high dose corticosteroids (at least 250 mg methyl prednisolone for at least 3 days, within 6 month before the first vaccine dose), treatment with inhibitors of mammalian target of rapamycin (mTOR) and treatment with cyclosporine. For evaluation of the log transformed antibody titer, we used simple and multiple linear regression analysis. Multivariate analysis was done using forward regression with a p value of 0.05 for inclusion.

To evaluate antibody dynamics between the first and the second evaluation we used repeated measure analysis of variance (ANOVA) with the antibody levels at one
month and 3-4 months as the repeated measures. We evaluated for interactions between the control and the study groups as well as patients' variables in the study group, and the change in antibody levels. All variables with significant interaction (p<=0.05) were introduced into multivariate model. For this analysis age was divided into two groups (>=50 years vs. <50 years) as well as mycophenolic acid dose (no treatment or <=360 mg vs. >360 mg).

We also evaluated the difference between the log transformed antibody titer at one month and 3-4 months. The difference was calculated as log \((1+ \text{Ab}_{\text{one month}})/(1+\text{Ab}_{3-4 \text{ months}})\). We used simple and multiple linear regression analysis. Multivariate analysis was done using forward regression with p value of 0.05 for inclusion with all variables mentioned above. All analyses were done using SPSS version 26 (IBM INC, Armonk, NY).

**RESULTS**

**Seropositivity rates**

Of the original 308 consenting kidney transplant recipients five had COVID-19 infection during the interval and one patient died. Forty-four patients were unavailable for a repeat blood sample during the study period. Two hundred fifty-nine patients (84%) had available measurements of anti-S antibodies at 3-4 months. The control group included 122 dialysis patients from the RMC dialysis units\(^{13}\). For baseline characteristics of the study and control groups see Table 1 and Table S1.

For the study group, antibody levels were collected at a median time of 110 days (interquartile range [IQR] 98-122 days) from the second vaccine dose. Of the 94 patients who were seropositive at one month, 13 (13.8%) turned seronegative; and of the 165 patients seronegative at one month, 18 (11.7%) turned seropositive. (See supplemental Figure 2 for visual description of antibody dynamics among these 13
seronegative and 18 seropositive patients). Overall, of the 259 patients included, 99 (38.2%) were seropositive for anti-S antibodies at 3-4 months, compared to 112/308 (36.4%) at one month. Median antibody titer was 17.7 AU/mL (IQR 2.7-156) compared to 15.5 AU/mL (IQR 3.5–163.6) at one month. Of the 122 patients in the control group, 101 (82.8%) were seropositive (p<0.001 vs study group) at 3-4 months, compared to 114/122 (93.4%) at one month. The values for antibody levels and log transformed antibody levels at one and 3-4 months for the study and control groups are presented in Table 2 and Figure 1. The distribution of log transformed difference between anti-S antibody levels at one and 3-4 months was normal and is depicted in Figure 2.

Factors associated with seropositivity at 3-4 months

On univariate and multivariate analyses, the variables that were associated with anti-S positivity in the study group included younger age (odds ratio [OR] 1.042 per year, 95% confidence interval [CI] 1.02 - 1.065, p<0.001), eGFR above 60 ml/min/1.73 m² (OR 3.788, 95% CI 2.113 - 6.789, p<0.001), lower mycophenolic acid dose (OR 1.468 per 360 mg decline, 95% CI1.135 - 1.898, p=0.003), and low CNI blood level (OR 1.806, 95% CI 1.027 - 3.177, p=0.04). For the full analyses see Table 3. This was also, shown in a second analysis, using the log transformed anti-S antibody levels as a continuous variable (See Supplementary Table S3).

Similar results were also found on additional analyses including only the 239 patients (92.3%) treated with tacrolimus, performed using tacrolimus level as a continuous variable. The results of this analysis are detailed in supplementary Tables S4 and S5.
Transformation from seronegative at one month to seropositive at 3-4 months

Of the 165 seronegative patients at one month, 18 (11.7%) became seropositive. Median antibody levels at 1 month were 29 AU/ml (IQR 14-42 AU/ml) and increased to a median of 80 AU/ml (IQR 57-139 IU/ml) at 3 months. In univariate logistic regression analysis, higher antibody levels at one month (OR 1.107 per AU/ml, 95% CI 1.065 - 1.152, p<0.001) and eGFR above 60 ml/min/1.73m² (OR 4.105, 95% CI 1.389 - 12.13, p=0.011) were associated with seroconversion at three months. These findings remained significant on multivariate analysis, as well as younger age, that was also significantly associated with seroconversion (OR 1.058, 95% CI 1.008 - 1.109, p=0.022). The results of the analysis are presented in supplementary Table S6.

Transformation from seropositive at one month to seronegative at 3-4 months

Of the 94 seropositive patients at one month, 13 (13.8%) became seronegative. Median antibody levels at 1 month were 92 AU/ml (IQR 67-184 AU/ml) decreased to a median of 26 AU/ml (IQR 18-36 IU/ml) at 3 months. In univariate logistic regression analysis these 13 patients were at longer duration from transplantation, lower BMI, less likely to be treated with tacrolimus and had lower antibody levels at 1 month. This sample size was too small to perform multivariate analysis (13 patients). (See Supplemental Table 2)

Dynamics of anti-S antibody levels between 1 month and 3-4 months.

Anti-S antibody levels at one and three months for the study and control groups are presented in Table 2 and Figure 1. The anti-S antibody levels at 3-4 months were significantly reduced in the study groups (p=0.007) and the control group (p<0.001) compared to one month. Similar findings were demonstrated in an analysis including
only patients seropositive at 1 month (Figure S1 and Table S7). The reduction in levels was significantly lower in the study vs control groups (p<0.001).

**Association between anti-S antibodies dynamics and patients' variables**

Antibody level decline over time was associated with GFR below 60 ml/min/1.73m² (p=0.028), diabetes mellitus (p=0.026), no treatment or low dose of mycophenolic acid (p<0.001), and positive antibody response at one month (p=0.006) on univariate and multivariate analysis. This was shown using repeated measures ANOVA and validated on evaluation of factors associated with the log transformed difference in antibody levels between one and 3-4 months (Table 4 and supplementary Table S8 respectively).

Treatment with mTOR inhibitors, which had no interaction with antibody level change by univariate analysis, significantly interacted with antibody decline when introduced into the multivariate model (p=0.014). The results of the repeated measure ANOVA are detailed in Table 4.

**DISCUSSION**

In this cohort of 259 kidney transplant recipients 3-4 months following second dose mRNA vaccination, we demonstrated that antibody response remained low (38%) at similar rates to those observed at 1 month. Among seropositive patients at 1 month, ~14% had declining antibodies to a level of seronegativity at 3 months. At the same time, ~12% of patients seronegative at 1 month, seroconverted to positive. This conversion to seropositivity was more likely in younger patients, those with increased eGFR, and those who had higher antibody levels at 1 month. For the entire cohort,
antibody levels declined over time, but at a slower rate in comparison to the control group of dialysis patients.

The findings of similar seropositivity rates with a modest decline at 3 months are in accordance to current knowledge on the immune response to natural SARS-CoV-2 infection. In patients after natural COVID-19 infection, studies demonstrate either sustained levels\textsuperscript{15,16} or progressive modest decline of IgG levels to spike protein (25% decrease by day 105, and 46% by day 115 for anti-RBD antibodies in one study)\textsuperscript{17}. Seropositivity rates remain high months after natural infection, reported to be 88-90% after 6-8 months\textsuperscript{17-19}. Neutralization antibody dynamics is reported to be similar to that of anti-RBD antibodies\textsuperscript{17,18}.

A decline in antibody titer is expected in the weeks following vaccination in general. Such decline has also been demonstrated among immunocompetent individuals after mRNA COVID-19 vaccines. Among healthcare workers, a reduction in IgG levels has been observed each month following the second BNT162b2 dose, as well as a decline in neutralizing antibody titers, decreasing by a factor of 3.9 at 3 months\textsuperscript{20}. It has also been demonstrated that anti-RBD antibody titers decline faster than anti-spike antibody titers, which were more stable over time\textsuperscript{9,21,22}. Naaber et al. found a decline in anti-RBD from peak mean levels of 26928 AU/mL at 1 week after the second mRNA vaccine dose to 13943 AU/mL at six weeks (45% decrease), and to 5702 AU/mL at 12 weeks after the second dose (77% decrease) among 90 healthy individuals. These levels however remained significantly higher compared to patients recovered from natural infection. Older age was associated with a decreased antibody titer in each time point tested in this study\textsuperscript{11}. Similarly, Mcda et al. reported a drop of 50% in anti-RBD IgG levels at three months after vaccination compared to peak levels measured after the second vaccine dose\textsuperscript{23}. In a group of 142 hemodialysis
patients, Berar-Yanay et al. demonstrated response rate of 94% 1 month after second
dose declining to 78% at 3 months after the second dose of BNT162b2\textsuperscript{24}.

Among kidney transplant recipients, Boyarsky et al. recently reported antibody
response at 3 months after 2 dose mRNA vaccination. In this study, among 75 patients
with low-positive titers at 1 month, 35 (47\%) became high positive at 3m, indicating
that a delayed response may be possible, as suggested by our results. In this study,
overall 43\% of patients had an increase in titer from 1 to 3 months, 35\% had a
decrease, and 21\% remained stable. Four percent of seropositive patients at 1 month
became seronegative at 3 months, lower than the 14\% in our study\textsuperscript{25}. An additional
recent study including 312 SOT recipients demonstrated consistent results of
relatively stable antibodies over time. At ~1 month following a second dose of mRNA
vaccine, 63\% were seropositive; at ~3 months, 72\% were seropositive; and at 6
months, 72\% as well. Seven percent of seropositive patients at 1 month became
seronegative at 6 months\textsuperscript{26}.

Regarding patients who were seronegative and turned seropositive, we cannot rule out
a delayed antibody response for SOT recipients, as also suggested by the two studies
described above\textsuperscript{25,26}. Peak antibody response has been previously demonstrated at
between day 15 to 45 after natural infection\textsuperscript{17,22}. We were unable to explain why this
was more likely in younger patients and those with higher eGFR, however it seems
reasonable that patients with some antibody response (negative according to test
interpretation, but levels above zero) were more likely to mount antibody response at
a later stage. Nevertheless, it is still possible that some of the patients became
seropositive due to test limitations and margin of error.
To some extent, serum antibodies are a surrogate marker for vaccine effectiveness in immunocompromised patients. Annual anti-HBs titers monitoring is recommended for kidney transplant recipients for decisions on re-vaccination\textsuperscript{27}. Monitoring antibody response to SARS-CoV-2 vaccine may also serve as a potential tool to identify the need and timing of potential revaccination. Using this surrogate, we assume that the vast majority of kidney transplant recipients seropositive at one month are still protected at 3 months. As the strategy of administrating a third mRNA dose is evolving\textsuperscript{28}, these findings support one month as a reasonable time point for serological assessment for decision making.

This study has several limitations. The importance of waning immunity, specifically antibody levels, in protecting against disease is still uncertain. Moreover, in our study we did not test neutralizing antibodies. However, high correlation between anti-RBD antibodies and neutralizing antibodies has been documented\textsuperscript{22,29}, and even modest levels of neutralizing antibodies have been demonstrated to provide protection against COVID-19\textsuperscript{18} for the long term. In addition, detectable antibodies months after acute COVID-19 have been shown to be associated with protection against re-infection\textsuperscript{30}. Thus, we assume that seropositivity at 1-3 months represents, at least to some extent, protection against infection. Another limitation in this regard is that no tests for T cell response to vaccine were conducted in this study.

In summary, we document similarly low rates of antibody response at 3- and 1-month following mRNA vaccine in kidney transplant recipients, with 14% of seropositive patients at 1 month presenting antibody decay to seronegative test levels. Additional studies are needed to determine the correlation between antibody response months after vaccination and protection against disease. The low seropositivity rates of 38%
among kidney transplant recipients deserves attention and consideration of vaccination strategies aiming to improve the immune response.

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CONFLICT OF INTEREST STATEMENT

The authors of this manuscript have no conflicts of interest to disclose. The results presented in this paper have not been published previously in whole or part.

DATA AVAILABILITY STATEMENT

The original data from this study may be available upon reasonable request to the corresponding author.

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Figure 1 - log transformed anti S-protein antibody levels at one and 3-4 months for the study and the control groups
Figure 2 - distribution of log transformed difference between anti-S antibody levels at one and 3-4 months for entire cohort.
| Variable                                                                 | All (N=259)     | Seropositive (N=99) | Seronegative (N=160) | p-value  |
|--------------------------------------------------------------------------|-----------------|---------------------|----------------------|----------|
| **Age (years), mean±SD**                                                 | 58.22±13.37     | 54.1±13.3           | 60.77±12.8           | <0.001   |
| **Female gender, N (%)**                                                 | 88 (34%)        | 34 (34.3%)          | 54 (33.8%)           | 0.922    |
| **Time from transplantation (years), mean±SD**                          | 7.1±7.51        | 6.17±5.91           | 7.67±8.31            | 0.119    |
| **First three months, N (%)**                                           | 10 (3.9%)       | 3 (3%)              | 7 (4.4%)             | 0.585    |
| **Living donor, N (%)**                                                 | 198 (76.4%)     | 82 (82.8%)          | 116 (72.5%)          | 0.052    |
| **eGFR (per ml/min/1.73m2), mean±SD**                                   | 62.15±22.3      | 70.54±22.43         | 56.96±20.64          | <0.001   |
| **eGFR below 60 ml/min/1.73m2, N (%)**                                  | 126 (48.6%)     | 29 (29.3%)          | 97 (60.6%)           | <0.001   |
| **Diabetes mellitus, N (%)**                                            | 45 (17.4%)      | 14 (14.1%)          | 31 (19.4%)           | 0.28     |
| **Time from second vaccine dose, mean±SD**                              | 110.21±16.65    | 108.47±19.06        | 111.28±14.93         | 0.188    |
| **BMI (kg/m2), mean±SD**                                                | 27.12±4.56      | 27.11±4.19          | 27.12±4.79           | 0.983    |
| **No mycophenolic acid, N (%)**                                         | 70 (27%)        | 33 (33.3%)          | 37 (23.1%)           |          |
| **Low dose mycophenolic acid, N (%)**                                   | 20 (7.7%)       | 9 (9.1%)            | 11 (6.9%)            | 0.38     |
| **Medium dose mycophenolic acid, N (%)**                                | 99 (38.2%)      | 35 (35.4%)          | 64 (40%)             |          |
| **High dose mycophenolic acid, N (%)**                                  | 70 (27%)        | 22 (22.2%)          | 48 (30%)             |          |
| **Tacrolimus, N (%)**                                                   | 239 (92.3%)     | 95 (96%)            | 144 (90%)            | 0.81     |
| **Cyclosporine, N (%)**                                                 | 20 (7.7%)       | 4 (4%)              | 16 (10%)             |          |
| **Tacrolimus level (ng/ml), mean±SD**                                   | 7.74±2.14       | 7.1±1.7             | 8.17±2.29            | <0.001   |
| **Cyclosporine level**                                                  | 129.8±58.14     | 118.5±42.9          | 132.6±62.22          | 0.676    |
| **mTOR inhibitor, N (%)**                                               | 22 (8.5%)       | 11 (11.1%)          | 11 (6.9%)            | 0.235    |
| **High CNI level, N (%)**                                               | 150 (57.9%)     | 48 (48.5%)          | 102 (63.8%)          | 0.016    |
| **High dose CS*, N (%)**                                                | 22 (8.5%)       | 5 (5.1%)            | 17 (10.6%)           | 0.118    |
| **Treatment with Rituximab, N (%)**                                     | 4 (1.5%)        | 0 (0%)              | 4 (2.5%)             | 0.113    |
| **Treatment with ATG, N (%)**                                           | 12 (4.6%)       | 3 (3%)              | 9 (5.6%)             | 0.334    |

*All patients were on corticosteroid treatment, this indicates percent with high dose

Abbreviations: ATG – antithymocyte globulin; BMI – body mass index; CNI – calcineurin; CS – corticosteroids; eGFR – estimated Glomerular filtration rate; mTOR -mechanistic target of rapamycin; SD – standard deviation;
Table 2. Antibody levels and log transformed antibody levels at one and 3-4 months for the study and control groups

|               | Control group | Study group | p-value |
|---------------|---------------|-------------|---------|
| **One month** |               |             |         |
| Antibody level (AU/ml)* | 1533.05 (321-2903.2) | 16.10 (3.8-157.2) | <0.001  |
| log antibody level (log AU/ml) # | 2.98±1.48 | 1.48±0.97 | <0.001  |
| **3-4 months** |               |             |         |
| Antibody level (AU/ml)* | 364.40 (90.7-859.6) | 17.70 (2.7-156) | <0.001  |
| Log antibody level (log AU/ml) # | 2.46±1.36 | 1.36±0.97 | <0.001  |

* median (interquartile range)  # mean ± standard deviation
Table 3. Factors associated with seropositivity at 3-4 months – univariate and multivariate analysis

| Variable                                | Univariate          |         |         |         |         |         |         |
|-----------------------------------------|---------------------|---------|---------|---------|---------|---------|---------|
|                                         | OR                  | 95% CI  | p       | OR      | 95% CI  | p       |
| Younger age (per year decrease)         | 1.039               | 1.019   | 1.060   | 0.000   | 1.042   | 1.020   | 1.065   | 0.000   |
| Female gender                           | 1.027               | 0.605   | 1.742   | 0.922   | -       | -       | -       | -       |
| Time from transplantation (per year)    | 0.972               | 0.938   | 1.008   | 0.122   | 0.956   | 0.917   | 0.997   | 0.036   |
| Living donor                            | 1.830               | 0.977   | 3.425   | 0.059   | -       | -       | -       | -       |
| eGFR (per ml/min/1.73m^2)               | 1.030               | 1.017   | 1.043   | 0.000   | -       | -       | -       | -       |
| eGFR above 60 ml/min/1.73m^2            | 3.716               | 2.173   | 6.356   | 0.000   | 3.788   | 2.113   | 6.789   | 0.000   |
| Diabetes mellitus                       | 0.685               | 0.344   | 1.364   | 0.282   | -       | -       | -       | -       |
| Time from second vaccine dose (per day) | 0.986               | 0.959   | 1.014   | 0.327   | -       | -       | -       | -       |
| BMI (per kg/m^2)                        | 0.999               | 0.946   | 1.056   | 0.983   | -       | -       | -       | -       |
| Mycophenolic acid dose (per 360 mg)    | 1.261               | 1.012   | 1.572   | 0.039   | 1.468   | 1.135   | 1.898   | 0.003   |
| Cyclosporine                            | 0.379               | 0.123   | 1.168   | 0.091   | -       | -       | -       | -       |
| mTOR inhibitor                          | 1.693               | 0.705   | 4.067   | 0.239   | -       | -       | -       | -       |
| Low CNI level                           | 1.869               | 1.123   | 3.109   | 0.016   | 1.806   | 1.027   | 3.177   | 0.040   |
| High dose CS                            | 0.447               | 0.160   | 1.254   | 0.126   | -       | -       | -       | -       |
| Treatment with ATG                      | 0.524               | 0.138   | 1.985   | 0.342   | -       | -       | -       | -       |

Abbreviations: ATG – antithymocyte globulin; BMI – body mass index; CI – confidence interval; CNI – calcineurin; CS – corticosteroids; eGFR – estimated Glomerular filtration rate; mTOR -mechanistic target of rapamycin; OR – odds ration; SD – standard deviation;
Table 4. Factors that interact with antibody level change between one and 3-4 month by repeated measures ANOVA

| Variable                                           | N     | Log Ab level 1 month | Log Ab level 3 months | p interaction UV | p interaction MV |
|----------------------------------------------------|-------|-----------------------|------------------------|-------------------|------------------|
| All                                                | 259   | 1.48±0.97             | 1.36±0.97              | <0.001            | 0.013            |
| No antibody response at one month                  | 165   | 0.86±0.44             | 0.81±0.64              |                   |                  |
| Positive antibody response at one month            | 94    | 2.58±0.6              | 2.33±0.61              | 0.001             | 0.006            |
| Age >50                                            | 187   | 1.37±0.92             | 1.24±0.9               | 0.460             | -                |
| Age<=50                                            | 72    | 1.77±1.06             | 1.68±1.06              |                   |                  |
| Male gender                                        | 171   | 1.53±0.98             | 1.4±0.95               | 0.859             | -                |
| Female gender                                      | 88    | 1.4±0.97              | 1.29±0.99              |                   |                  |
| eGFR >=60                                          | 133   | 1.75±0.98             | 1.69±0.98              | 0.032             | 0.028            |
| eGFR<60                                            | 126   | 1.2±0.89              | 1.02±0.82              |                   |                  |
| Living donor                                       | 61    | 1.34±0.93             | 1.22±0.94              | 0.956             | -                |
| Deceased donor                                     | 198   | 1.53±0.98             | 1.41±0.97              |                   |                  |
| No DM                                              | 214   | 1.5±0.99              | 1.41±0.97              | 0.026             | 0.065            |
| DM                                                 | 45    | 1.41±0.92             | 1.15±0.93              |                   |                  |
| No/low dose mycophenolic acid                      | 90    | 1.85±1.06             | 1.51±0.98              | <0.001            | <0.001           |
| Medium/high dose mycophenolic acid                 | 169   | 1.29±0.87             | 1.28±0.95              |                   |                  |
| BMI <30                                            | 192   | 1.49±0.94             | 1.38±0.95              | 0.621             | -                |
| BMI >=30                                           | 67    | 1.46±1.07             | 1.32±1.02              |                   |                  |
| CNI level<7 ng/ml                                  | 109   | 1.68±1.02             | 1.53±1.01              | 0.363             | -                |
| CNI level>=7 ng/ml                                 | 150   | 1.34±0.92             | 1.24±0.91              |                   |                  |
| No treatment with ATG                              | 247   | 1.5±0.96              | 1.39±0.96              | 0.133             | -                |
| Treatment with ATG                                 | 12    | 1.17±1.32             | 0.86±1.06              |                   |                  |
| No mtor inhibitor                                  | 237   | 1.47±0.99             | 1.35±0.98              | 0.449             | 0.014            |
| mTOR inhibitors                                    | 22    | 1.59±0.75             | 1.55±0.77              |                   |                  |
| no high dose CS                                    | 237   | 1.53±0.97             | 1.42±0.96              | 0.995             | -                |
| high dose CS                                       | 22    | 0.91±0.78             | 0.79±0.83              |                   |                  |

Abbreviations: Ab – antibodies; ATG – antithymocyte globulin; BMI – body mass index; CNI – calcineurin; CS – corticosteroids; DM – diabetes mellitus; eGFR – estimated Glomerular filtration rate; mTOR – mechanistic target of rapamycin; SD – standard deviation;