Antibody and T-cell responses 6 months after COVID-19 mRNA-1273 vaccination in patients with chronic kidney disease, on dialysis, or living with a kidney transplant

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Short title: COVID-19 vaccination in kidney patients

Abstract

Background The immune response to COVID-19 vaccination is inferior in kidney transplant recipients (KTR), and to a lesser extent in patients on dialysis or with chronic kidney disease (CKD). We assessed the immune response 6 months after mRNA-1273 vaccination in kidney patients and compared this to controls.

Methods 152 participants with CKD stages G4/5 (eGFR <30 mL/min/1.73m^2), 145 participants on dialysis, 267 KTR, and 181 controls were included. SARS-CoV-2 Spike S1-specific IgG antibodies were measured by fluorescent bead-based multiplex-immunoassay, neutralizing antibodies to ancestral, Delta and Omicron (BA.1) variants by plaque reduction, and T-cell responses by IFN-γ release assay.

Results At 6 months after vaccination S1-specific antibodies were detected in 100% of controls, 98.7% of CKD G4/5 patients, 95.1% of dialysis patients, and 56.6% of KTR. These figures were comparable to the response rates at 28 days, but antibody levels waned significantly. Neutralization of the ancestral and Delta variant was detected in most participants, whereas neutralization of Omicron was mostly absent. S-specific T-cell responses were detected 6 months in 75.0% of controls, 69.4% of CKD G4/5 patients, 52.6% of dialysis patients, and 12.9% of KTR. T-cell responses at 6 months were significantly lower than responses at 28 days.

Conclusions Although seropositivity rates at 6 months were comparable to that at 28 days after vaccination, significantly decreased antibody levels and T-cell responses were observed. The combination of low antibody levels, reduced T-cell responses, and absent neutralization of the newly-emerging variants indicates the need for additional boosts or alternative vaccination strategies in KTR.

Trial registration number

NCT04741386 (ClinicalTrials.gov)

Key words

COVID-19, mRNA-1273 vaccine, chronic kidney disease, kidney transplantation, dialysis,

Introduction

Coronavirus disease 2019 (COVID-19)-associated mortality risk is 3- to 4-fold higher in patients with severely impaired kidney function, patients on dialysis and kidney transplant recipients (KTR), compared to the general population[1]. Therefore, sustained effectiveness of COVID-19 vaccination in the face of novel emerging variants is of great importance for these patients.

We recently performed a clinical trial in approximately 800 participants to assess the immunogenicity, tolerability and safety of the mRNA-1273 COVID-19 vaccine in kidney patients[2]. In particular KTR showed a combination of low antibody and non-detectable T-cell responses 28 days following the second vaccination. Notably, almost all dialysis patients and patients with chronic kidney disease (CKD G4/5) showed seroconversion, but antibody levels were significantly lower compared to controls[3]. This is in accordance with other smaller studies that described lower seroconversion rates in patients on dialysis, and in transplant recipients 28 days after two doses of mRNA vaccines[4,5].

mRNA vaccines have recently been shown to induce durable immunological memory, with protection against newly-emerging SARS-CoV-2 variants in healthy individuals[6,7]. However, especially among older individuals and patients on immunosuppression, antibody levels rapidly wane over a period of 6 months[8]. These data underline the importance of long-term follow-up of high-risk patients with kidney disease to assess the need for additional boosts or alternative vaccination strategies.

We studied the concentration of spike S1 binding antibodies, the level of neutralizing antibodies to the ancestral, Delta and Omicron (BA.1) variant, and T-cell responses at 6 months after mRNA-1273 COVID-19 vaccination in patients with severely impaired kidney function, patients on dialysis, KTR, and control subjects without known kidney disease.

Methods

The design of the Dutch Renal patients COVID-19 VACcination (RECOVAC) Immune Response study was published previously[2]. Ethical approval was obtained from the Dutch Central Committee on Research Involving Human Subjects (CCMO, NL76215.042.21) and the local ethics committees of the participating centers.

Study participants and COVID-19 vaccination

Four different cohorts were included in the study. Cohort A (controls), consisted of subjects without kidney disease (eGFR >45 mL/min/1.73m^2), cohort B of patients with severely impaired kidney function (eGFR <30 mL/min/1.73m^2 or CKD stages G4/5); cohort C of patients on hemodialysis or peritoneal dialysis; and cohort D of KTR. The control cohort included partners, siblings or household members of participants in cohorts B, C, and D. All participants received two mRNA-1273 COVID-19 vaccinations (®Moderna Biotech Spain, S.L.) with an interval of 28 days. Blood samples were collected at baseline, prior to second vaccination, and 28 days and 6 months after the second vaccination. Patients who experienced COVID-19 before or during the study were excluded.

SARS-CoV-2 Spike S1-specific IgG antibody response and virus neutralizing antibodies

SARS-CoV-2 Spike S1-specific IgG antibodies were measured in serum samples by a validated fluorescent bead-based multiplex-immunoassay, as previously described and expressed as international Binding Antibody Units per mL (BAU/mL) [9,10]. Participants were classified as seropositive or seronegative, cut-off was set at S1-specific IgG antibody concentration ≥10 BAU/mL[10,11]. Nucleocapsid antibodies were measured at all time points by multiplex immunoassay, as previously described, and classified as positive or negative[12].

Plaque reduction neutralization tests (PRNT) against the ancestral SARS-CoV-2 and the Delta and Omicron variant were performed as described previously[7,11]. For feasibility, it was a priori decided to measure neutralizing antibodies only in a random sample of 20 patients per group with measurable S1 specific IgG antibodies at 6 months included in one of the participating centers (Erasmus MC Rotterdam).

SARS-CoV-2 specific T-cell response

SARS-CoV-2-specific T-cell responses were measured in all subjects participating at the Erasmus MC Rotterdam. This concerned 40 control subjects, 36 CKD G4/5 patients, 38 dialysis patients and 62 kidney transplant recipients. Measurement was performed by a commercially available Interferon-gamma (IFN-ɣ) release assay (IGRA) according to the manufacturer's instructions (QuantiFERON, QIAGEN)[13]. Results from the Ag2 (peptides covering the entire S protein) assay were expressed in IU/ml after subtraction of the negative control values as interpolated from a standard calibration curve.

Antibody decay

Previous studies have shown that decay of SARS-CoV-2-specific antibodies most likely follow an exponential pattern over time[14]. We therefore calculated decay in antibodies over time using an exponential formula, to estimate antibody half-life and time to reach certain antibody levels:

- $y= a*b^X$, where y is the S1 IgG antibody level at 6 months, a is the S1 IgG antibody level at 28 days, b is the slope and X is time.
- The slope b was calculated as: Log10(b)=(Log10(y)-Log10(a))/(X(y) – X(a)).
- Half-life was subsequently calculated as: Half-life=Log10(0.5)/Log10(b).
- Time to reaching a certain S1 IgG antibody level (c) was calculated as: Time until c=X(a)+(Log10(c)-Log10(a))/Log10(b)

Statistical analysis

Continuous data are presented as mean with standard deviation (SD) or as median and interquartile interval (IQI) in case of non-normal distribution. Categorical data are presented as percentages. Differences between patient groups and the control group were tested using independent *t* test, Mann Whitney-U test or Pearson Chi-2 test depending on data distribution, with Bonferroni correction for multiple testing. Differences within study cohorts over time were tested using paired sample *t* test, Wilcoxon singed rank test or Pearson Chi-2 test depending on data distribution. The correlation between the S1 IgG antibody levels measured at 28 days and 6 months after the second vaccination was tested by performing Pearson correlation. All analyses were performed with the statistical software IBM SPSS statistics version 23.0 (SPSS Inc., Chicago, IL). Figures were created with the software GraphPad Prism version 5.00 (GraphPad Software, San Diego, California)**.** A two-sided P-value <0.05 was adopted to denote statistical significance, and corrected in case of multiple testing using Bonferroni correction unless stated otherwise.

Results

Baseline characteristics

A flow chart of study enrollment is depicted in Figure 1.. In total, 181 controls, 152 patients with CKD G4/5, 145 dialysis patients, and 267 KTR were included for the analysis of binding antibody

levels 6 months after vaccination. Baseline characteristics of these participants are shown in Table 1.

SARS-CoV-2 Spike S1-specific IgG antibody response at 6 months

All controls retained seropositivity 6 months after vaccination. The seropositivity rates in patients with CKD G4/5 or patients on dialysis were 98.7% or 95.1%, respectively (compared to 100% and 99.3% at day 28). In KTR, the seropositivity rate was 57.7% at day 28, and of these patients 14.9% (n = 23) became seronegative at 6 months (P < 0.001). Remarkably, 17.7% (n = 20) of seronegative KTR at day 28 became seropositive at 6 months (Figure 2A). Overall, 56.6% of KTR was seropositive at month 6.

In all four groups the S1-specific IgG antibody levels declined significantly from day 28 to month 6, but with good correlation between the 2 timepoints (R=0.88, P<0.001) (Figure S1). In controls, levels decreased 7.7-fold from 3009 to 380 BAU/mL. In CKD G4/5 patients levels decreased 7.5-fold from 2380 to 309 BAU/mL, in dialysis patients 9-fold from 1585 to 165 BAU/mL, and in KTR 2.3-fold from 25 to 16 BAU/mL (all P<0.001) (Figure 2B). At 6 months after vaccination, S1-specific IgG antibody levels in dialysis patients and KTR were significantly lower when compared to controls (both P<0.001). Whereas all CKD G4/5 patients, dialysis patients and controls had a decrease in S1-specific IgG antibody levels, 32 KTR (18%) had an increase in S1-specific IgG antibody levels. None of these patients reported having contracted COVID-19, or had detectable nucleocapsid antibodies.

Decay in S1-specific IgG antibodies and predictors of decay

Since significant waning of S1-specific IgG antibody levels was detected at 6 months after vaccination in all groups, we calculated the antibody half-life assuming an exponential decay. The overall half-life in the entire cohort was 52 days (41-69), and this was comparable between the four groups (Table 2, Figure 3). With an exponential decay model, we calculated the time until seronegativity. This time was 451 days after the second vaccination in controls and 442 days in CKD G4/5 patients, whereas it was significantly shorter at 381 days in dialysis patients and 308 days in KTR (both P<0.001 compared to control group). Several characteristics differed significantly between participants in whom S1-specific IgG antibody levels declined faster (half-

life below 52 days) or slower (half-life above 52 days) (Supplemental Table 1), but these characteristics were not consistent between the different groups.

Neutralizing antibodies targeting SARS-CoV-2 variants

In a selection of 20 participants per group with S1-specific IgG antibodies, neutralization against the ancestral SARS-CoV-2, the Delta and Omicron (BA.1) variants was assessed (Figure 4). Neutralizing antibodies to the ancestral and Delta SARS-CoV-2 strains were detected in all controls, CKD G4/5 patients, and patients on dialysis at 28 days (Figure 4). At 6 months, in several participants in the control, CKD G4/5 and dialysis groups, levels of Delta virus neutralizing antibodies had dropped below the detection levels. Levels of neutralizing antibodies were significantly lower at 6 months as compared to 28 days after second vaccination (Figure 4). In KTR, not all participants showed neutralizing antibodies against the ancestral and Delta variant. Notably, waning of neutralizing antibodies was not apparent in KTR at 6 months, compared to 28 days post vaccination. Levels of neutralizing antibodies against the ancestral strain and Delta strain correlated with the levels of S1-specific IgG antibodies at 28 days and 6 months after second vaccination (ancestral/Wild type R=0.88 and R=0.85 resp., both P<0.001; Delta R=0.83 and R=0.85 resp., both P<0.001) (Figure S2A and B)). Notably, neutralization of the newly-emerged Omicron variant was hardly detected in any of the groups, both at 28 days and 6 months after vaccination. Correlation plots of neutralizing antibodies against Omicron and S1-specific IgG antibodies showed that Omicron was only neutralized at high titers of S1-specific IgG, both at 28 days and at 6 months after second vaccination (R=0.51, and R=0.55, both P<0.001) (Figure S2C).

SARS-CoV-2 specific T-cell responses at 6 months

A detectable SARS-CoV-2-specific T-cell response (defined as IFN-ɣ concentration of ≥0.15 IU/mL after specific stimulation) was observed in 87.5% and 75.0% of controls, 77.8% and 69.4% of CKD G4/5 patients, and 73.3% and 52.6% of dialysis patients at 28 days and 6 months, respectively (P<0.001, P=0.002 and P<0.001 resp.) (Figure 5A). A detectable T-cell response was observed in 17.7% of KTR at day 28 and of these patients 45.5% (n = 5) had a non-detectable T-cell response at 6 months (P<0.001)..

T-cell responses correlated to the levels of S1-specific binding antibodies, both at 28 days and at 6 months after second vaccination (R=0.64 and R=0.59 resp., both P<0.001) (Figure S3). Median IFN-ɣ level at 6 months tended to be lower in CKD G4/5 patients (0.28 IU/mL) and were significantly lower in dialysis patients (0.21 IU/mL) as compared to controls (0.76 IU/mL) (P=0.06 and P=0.04 resp.). Median IFN-ɣ levels were also significantly lower in KTR at 0.02 IU/ml when compared to controls (P<0.001). Nevertheless, in the KTR, median IFN-ɣ levels at 6 months were not significantly lower compared to 28 days, opposed to an observed significant decline in controls, CKD G4/5, and dialysis patients (Figure 5B).

Out of 62 KTR, 3 (4.8%) showed an increase in IGRA-response, defined as >0.15 IU/mL at both timepoints with doubling between day 28 and month 6. An increase in SARS-CoV-2-specific T-cell response between day 28 and month 6 was observed in 2 controls (5.0%), 4 CKD G4/5 (11.1%) and none of the dialysis patients. There was no relation between a rise in S1 antibody titers and an increase in T-cell response in the KTR.

Safety

Overall, 25 safety events were reported between day 28 and month 6. Significantly more safety events occurred in dialysis patients and KTRs compared to controls (6.2% and 4.5% versus 0%, P=0.003 and P=0.01 resp.). None were classified as related to COVID-19 vaccination (Table 3). In total, 10 patients died due to variable causes, in none of these cases this was classified as related to COVID-19 vaccination. Finally, in the KTR group one participant experienced allograft rejection, which was not related to COVID-19 vaccination and recovered after treatment with methylprednisolone and subsequently thymoglobulin.

Discussion

In this study, we demonstrate waning of binding antibodies, neutralizing antibodies, and T-cell responses in different groups of kidney patients at 6 months after vaccination with the mRNA-1273 COVID-19 vaccine. The slope of these decreases in vaccine induced immunity were similar among patient groups and controls. Consequently, SARS-CoV-2-specific antibodies and T-cells became undetectable in a substantial proportion of dialysis patients and especially KTR, when compared to controls. At 6 months after vaccination, neutralizing antibodies to the circulating Omicron variant were hardly detected in any of the groups.

Until now, data on durability of the response to vaccination were not available from adequately powered and controlled vaccination studies in kidney patients, although stronger waning of antibodies in dialysis patients was previously observed[15]. Additionally, a study in 312 solid organ transplant recipients showed that seropositivity rates after mRNA vaccination remained relatively stable until 6 months[16]. Interestingly, similar to our observations, this study also detected an increase in seropositivity in 43 solid organ transplant recipients (14.7%), but the authors could not exclude asymptomatic infections as a cause of this increase. This phenomenon of increasing antibody concentrations was also observed by Hall *et al* in a small subgroup of transplant recipients who received placebo vaccination but nevertheless had an increase in anti-RBD antibody levels[17]. We detected an increase in antibody concentration in 18% of the KTR between 28 days and 6 months after vaccination, while these participants had not reported SARS-CoV-2 infection and we did not detect nucleocapsid-specific antibodies. This late increase in antibody levels could be explained by ongoing delayed mRNA vaccine-induced B cell stimulation and/or delayed plasma cell differentiation in KTR[18].

Neutralizing antibodies are regarded as an important correlate of protection against developing severe COVID-19[19,20]. We show that the majority of kidney patients with measurable binding antibodies can still neutralize both the ancestral SARS-CoV-2, as well as the Delta variant. Neutralizing antibodies were significantly lower in KTR, although waning was less pronounced in this group. In accordance with current literature, cross-neutralization of the emerging Omicron variant (BA.1) was strongly reduced, and almost none of the sera obtained 6 months after vaccination neutralized this variant[7,21–23].

The absence of cross-neutralization explains that re-infections and breakthrough infections with the Omicron variant are now frequently seen. The Omicron (BA.1) variant is the first variant that formed a novel antigenic cluster[24], explaining the reduced vaccine efficacy against this variant. Fortunately, a lower risk of severe disease after infection with this variant was described[25]. This is potentially due to inherent differences in viral properties between the Omicron and previously circulating variants. The absence of cross-neutralization of Omicron suggests that other immunological mechanisms than virus neutralization are involved in cross-protection against severe disease. These might include effector functions mediated by non-neutralizing antibodies and virus-specific T-cells. We therefore speculate that the combination of low binding antibody levels and reduced T-cell responses, in conjunction with lack of neutralization of the Omicron variant, as observed in KTR, could predispose to more severe disease upon infection with the Omicron variant.

Thus far, limited data on virus-specific T-cell responses in high-risk groups of kidney patients have been reported, especially during standardized follow-up. In healthy individuals, cell-mediated immune responses are detectable up to at least 8 months after vaccination[6,7]. We observed a trend to waning T-cell responses in this study, however compared to the antibody concentrations, T-cell responses were relatively stable. T-cell responses were undetectable in the peripheral blood obtained from the majority of KTR 6 months after vaccination. However, the fact that we could not measure SARS-CoV-2 specific T-cells in the circulation does not exclude that functional T-cells could have been present in the lymphoid organs to play a role in B-cell activation and antibody production. An in-depth analysis of virus-specific T-cells is required to better understand the differences in phenotype and functionality of T-cells between groups.

We report the immunogenicity of mRNA-1273 vaccination 6 months after completion of the two-shot regimen, but a third vaccination is already implemented in clinical practice in high-risk groups. Our data show that 6 months after the second vaccination, especially in KTR, the levels of neutralizing antibodies against the ancestral and emerging variants as well as T cell responses are low or not detectable. This underlines the importance of the third vaccination as standard part of a complete COVID-19 vaccination schedule in KTR. Moreover, when an increased COVID-19 risk exists, this vulnerable patient group should be protected by masks and social distancing as advised by the CDC (https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html). Finally, AZD7442 (Evusheld) could be considered as pre-exposure prophylaxis in severely immunocompromised patients. Additionally, our data show a similar half-life of antibodies in all groups. Although seroconversion rates were high in CKD G4/5 and dialysis patients, antibody levels in these patient groups were lower than in controls. This suggests that the interval between the second and third vaccination should be shorter in these patients than in the general population.

The main strength of our study is the prospective design with the inclusion of different kidney patients as well as a control cohort. The study assessed both (functional) antibody responses, as well as T-cell responses at predefined fixed time points using standardized assays. Study limitations include: (1) all patients received the mRNA1273 (Moderna) COVID-19 vaccine, which precludes conclusions about the response to other vaccines; (2) patients using immunosuppressive therapy were excluded at baseline from the CKD G4/5 as well as dialysis cohorts, which may have skewed the seroconversion rate and waning of antibodies in these patients. On the other hand, this enabled specific evaluation of the role of impaired kidney function and kidney function replacement treatment.

In conclusion, although seropositivity rates at 6 months after vaccination were comparable to response rates 28 days after vaccination, significantly decreased antibody levels and T-cell responses were observed in all groups. Especially KTR displayed a combination of low antibody levels, few detectable T-cell responses, and lack of neutralization of circulating variants 6 months after vaccination. Alternative strategies to improve immunogenicity of COVID-19 vaccines, including additional boosts with (variant-adapted) COVID-19 vaccines should be considered to reduce the risk of severe disease in these vulnerable patients.

NOTES

Authors' Contributions

RG and JS designed the study protocol. FB, MK, MR, CB, DvB, RvdM, ER, RdV and LH contributed to the protocol design. DD, MK, AM and PV provided intellectual content of critical importance to the study. AM, CB, RvB, DD, DG, GdH, CG and RdV were involved with data acquisition. NR provided the COVID-19 vaccine. MK, MR, CB, DvB, FB, DD, RG, AM, RvdM, CG, RdV, LH and JS participated in the study, analysis of the data, preparation of the manuscript.

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Conflict of interest statement

CCB reports a Dutch Kidney Foundation grant and support from Procare II study, unrelated to this work; a role as Executive Editor / Social Media Editor for Transplantation Journal. MMLK reports participation in an advisory board on Maribavir for Takeda. None other declared.

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Table 1. Baseline characteristics per study group.

	Control (n=181)	**CKD G4/5** (n=152)	**Dialysis** (n=145)	**KTR** (n=267)
Female, n (%)	107 (59.1)	53 (34.9)	48 (33.1)	123 (46.1)
Caucasian, n (%)	167 (92.3)	135 (88.8)	121 (83.4)	243 (91.0)
Age (years)	58.4 ± 12.9	60.6 ± 13.4	60.0 ± 13.8	55.9 ± 14.1
BMI (kg/m^2)	27.5 ± 5.3	27.8 ± 5.2	26.7 ± 5.7	27.0 ± 4.6
SBP (mmHg)	146.3 ± 22.7	151.1 ± 24.1	139.2 ± 25.8	146.4 ± 21.3
DBP (mmHg)	84.8 ± 11.6	84.1 ± 11.9	78.2 ± 16.5	84.5 ± 10.9
Current smoking, n (%)	31 (17.1)	23 (15.1)	33 (22.8)	28 (10.5)
Current alcohol consumption, n (%)	108 (59.7)	60 (39.5)	32 (22.1)	105 (39.3)
Number of comorbidities	0 (0-1)	1 (1-2)	1 (1-2)	1 (1-2)
Comorbidities, n (%)				
- Hypertension	49 (27.1)	126 (82.9)	96 (66.2)	219 (82.0)
- Diabetes Mellitus	17 (9.4)	40 (26.3)	35 (24.1)	56 (21.0)
- History of coronary artery disease	9 (5.0)	33 (21.7)	33 (22.8)	35 (13.1)
- Heart failure	2 (1.1)	12 (7.9)	10 (6.9)	13 (4.8)
- Chronic lung disease	14 (7.7)	16 (10.5)	14 (9.7)	12 (4.5)
- History of malignancy[1]	9 (5.0)	20 (13.2)	34 (23.4)	40 (15.0)
- Auto-immune disease	4 (2.2)	3 (2.0)	5 (3.4)	14 (5.2)
Lymphocytes (10^9/L)	2.0 (1.6-2.5)	1.6 (1.2-2.0)	1.2 (0.9-1.6)	1.3 (0.9- 1.9)
eGFR (ml/min/1.73m^2)	82.3 ± 18.5	17.7 ± 6.1	-	49.5 ± 18.9
Primary renal diagnosis, n (%)				
- Primary glomerulonephritis	-	18 (11.8)	14 (9.7)	53 (19.9)
- Pyelonephritis	-	1 (0.7)	1 (0.7)	54(1.5)
- Interstitial nephritis	-	7 (4.6)	4 (2.8)	9 (3.4)
- Familial/hereditary renal diseases	-	25 (16.4)	19 (13.1)	51 (19.1)
- Congenital diseases	-	6 (3.9)	5 (3.4)	18 (6.7)
- Vascular diseases	-	31 (20.4)	27 (18.6)	26 (9.7)
- Secondary glomerular/systemic disease	-	4 (2.6)	7 (4.8)	12 (4.5)
- Diabetic Kidney Disease	-	9 (5.9)	21 (14.5)	10 (3.7)
- Other	-	29 (19.1)	24 (16.6)	39 (14.6)
- Unknown	-	22 (14.4)	23 (15.9)	45 (16.8)
Dialysis characteristics, n (%)				
- Hemodialysis	-	-	110 (75.9)	-
- Peritoneal dialysis	-	-	35 (24.1)	-
- Time on dialysis (months)	-	-	30.0 (14.0-69.8)	-
Transplant characteristics				

- First kidney transplant, n (%)	-	-	-	208 (77.9)
- Time after last transplantation (years)	-	-	-	6.0 (2.0-13.0)
- Last transplant				
○ Living, n (%)	-	-	-	183 (68.5)
○ Pre-emptive, n (%)	-	-	-	98 (36.7)
Number of immunosuppressive agents	-	-	-	2 (2-3)
Immunosuppressive treatment at baseline, n (%)				
- Steroids	-	-	-	203 (76.0)
- Azathioprine	-	-	-	32 (12.0)
- Mycophenolate mofetil	-	-	-	183 (68.5)
- Calcineurin inhibitor	-	-	-	221 (82.8)
- mTor inhibitor	-	-	-	17 (6.4)
- Other	-	-	-	5 (1.9)
- Induction with rituximab last year, n (%)	-	-	-	2 (0.7)
Received kidney transplant after baseline, n (%)	-	13 (8.6)	13 (9.0)	1 (0.4)
Start dialysis after baseline, n(%)	-	9 (5.9)	-	1 (0.4)
Immunosuppressive treatment at month 6, n (%)				
- Steroids	-	13 (8.6)	11 (7.6)	196 (73.4)
- Azathioprine	-	-	1 (0.7)	31 (11.6)
- Mycophenolate mofetil	-	11 (7.2)	9 (6.2)	181 (67.8)
- Calcineurin inhibitor	-	13 (8.6)	11 (7.6)	214 (80.1)
- mTor inhibitor	-	2 (1.3)	2 (1.4)	17 (6.4)

Variables are presented as mean ± SD, or as median (IQ interval) in case of non-normal distribution.
Abbreviations are: CKD, chronic kidney disease; KTR, kidney transplant recipient; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate.
[1] Including melanomas, excluding all other skin malignancies

Table 2. Decay in S1-specific IgG antibody level per study group assuming an exponential decay.

	Control	CKD G4/5	Dialysis	KTR
Half-Life (days)	52.6 (43.2-65.5)	52.4 (43.4-72.6)	49.6 (40.4-63.5)	52.9 (32.7-81.3)
Seroresponse defined as ≥10 BAU/mL at day 28, n (%)	181 (100)	152 (100)	144 (100)	154 (57.7)
S1 IgG level (BAU/mL) in seropositive subjects	3009 (1812-4797)	2380 (1267-4569)	1587 (702-3121)**	310 (57.1-1041)**
Time to 10 BAU/mL (days) in seropositive subjects	451 (378-569)	442 (368-610)	381 (313-494)**	308 (119-473)**

Variables are presented as mean ± SD, as median (IQ interval) in case of non-normal distribution or as number (percentage) in case of categorical data.
P-values were calculated using Mann-Whitney U test with the control group as reference group. Bonferroni correction was applied for multiple testing.
Abbreviations are: CKD, chronic kidney disease; KTR, kidney transplant recipient
* P<0.05
** P<0.001

Table 3. List of serious adverse events between 28 days and 6 months after second vaccination.

	Control (N=181)	**CKD G4/5** (N=152)	**Dialysis** (N=145)	**KTR** (N=267)
Serious adverse events				
Any serious adverse event, n (%)	0	4 (2.6)	9 (6.2)	12 (4.5)
Related to vaccination, n (%)	-	0	0	0
Not related to vaccination, n (%)				
- Urinary tract infection	-	2 (1.3)	1 (0.7)	7 (2.6)
- Malaise	-	2 (1.3)	-	1 (0.4)
- Colitis	-	-	1 (0.7)	-
- Fever	-	-	2 (1.4)	2 (0.7)
- Fluid overload	-	-	1 (0.7)	-
- Syncope	-	-	1 (0.7)	-
- Choledocholithiasis	-		1 (0.7)	-
- Infected haematoma	-	1 (6.6)	1 (0.7)	-
- Varicella zoster infection	-	-	-	1 (0.4)
- Acute renal insufficiency	-	-		1 (0.4)
- Femoropopliteal artery byspass thrombosis	-	-	-	1 (0.4)
- Abdominal pain	-	-	1 (0.7)	-
- Rectal blood loss	-	-	1 (0.7)	-
- Amputation			1 (0.7)	
- Hypocalcemia	-	-	-	1 (0.4)
- Acute renal rejection	-	-	-	1 (0.4)
- PTLD	-	-	1[1] (0.7)	-
- CMV infection	-	-	1 (0.7)	-

Variables are given as number and percentage. P-values were calculated using Chi-squared test.
Abbreviations are: CKD, chronic kidney disease; KTR, kidney transplant recipient; PTLD, post transplantation lymphoproliferative disorder; CMV, Cytomegalovirus; P-val, P-value.
[1]Subject received a kidney transplant after baseline visit

FIGURE LEGENDS

Figure 1. Subject enrollment and outcomes 6 months after second vaccination.

Figure 2. A) Seroconversion rate and B) S1-specific IgG antibody levels at day 28 and month 6 after second SARS-CoV-2 vaccination per cohort; Depicted are scatter dot plots with a line indicating the median level. P-values were calculated using Wilcoxon signed rank test.

Figure 3. Decay in antibodies according to exponential decay model. (A) Decay per group in subjects with antibody value at day 28 ≥10 BAU/mL. (B) Estimated time until 10 BAU/mL.

Figure 4. Levels of neutralizing antibodies against the ancestral SARS-CoV-2 (Wild type), and the recently emerged Delta and Omicron variants per subgroup and compared to level of neutralizing antibodies at 6 months. The dotted horizontal line indicates the lower limit of detection (LLoD) of neutralization (titer of 20). P-values were calculated using Wilcoxon signed rank test.

Figure 5. SARS-CoV-2-specific T-cell response in all subjects in one of the participating centers. A)Percentage of T-cell responders per group 6 months after vaccination (defined as Ag2 ≥0.15 IU/mL). B) Individual IFNγ levels per group, with the horizontal line representing the median value. Dotted horizontal line indicates the threshold of detectable T-cell response (≥0.15 IU/mL). P-values were calculated using Wilcoxon signed rank test.

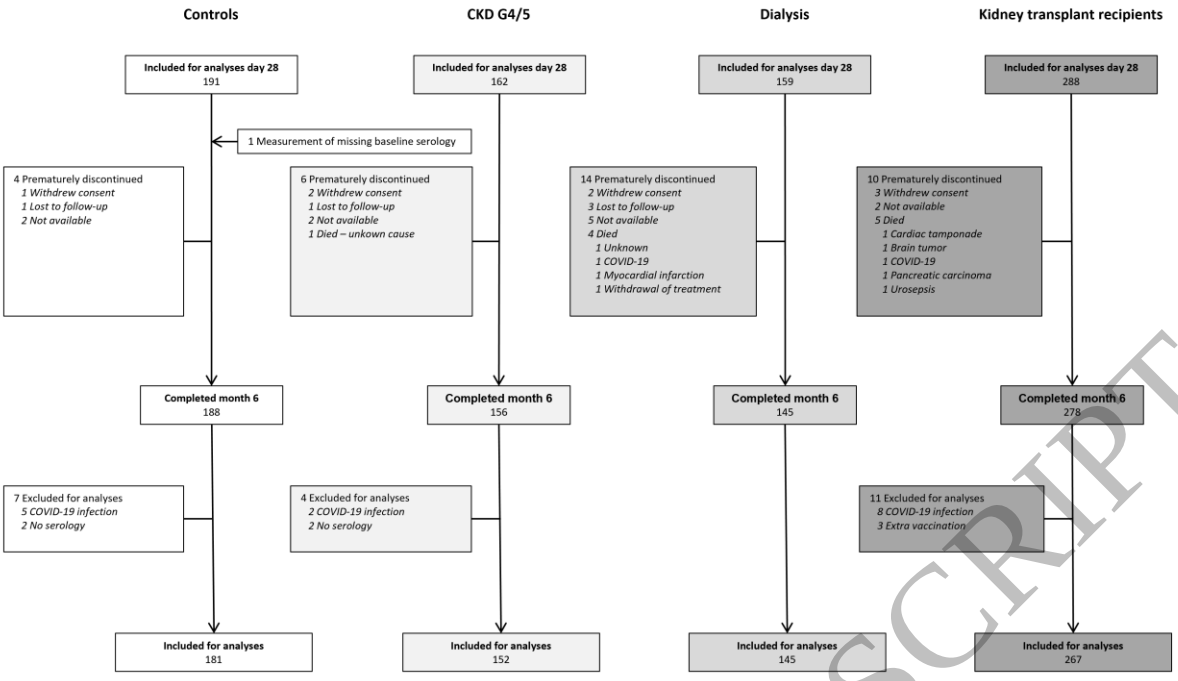

Figure 1
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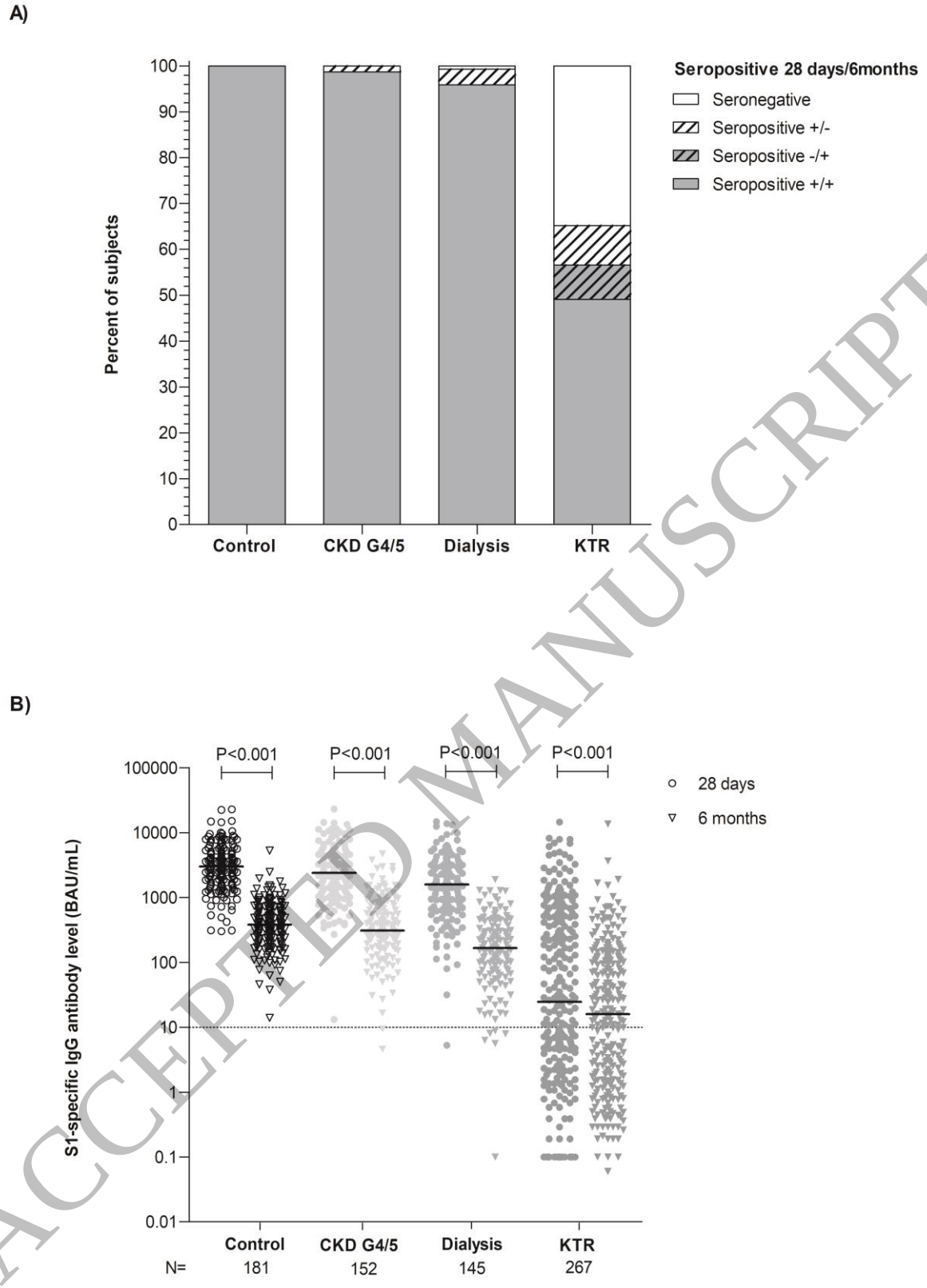

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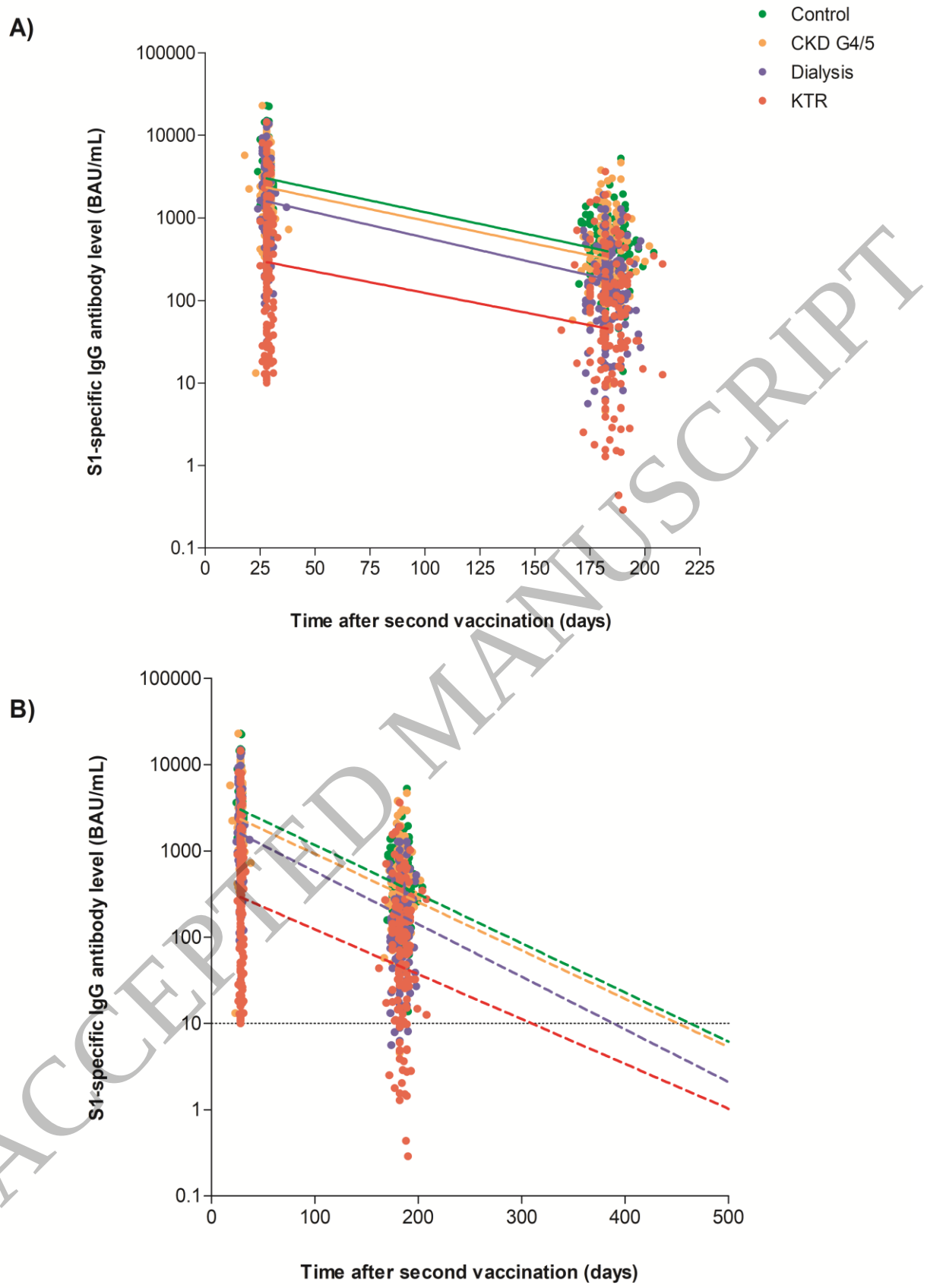

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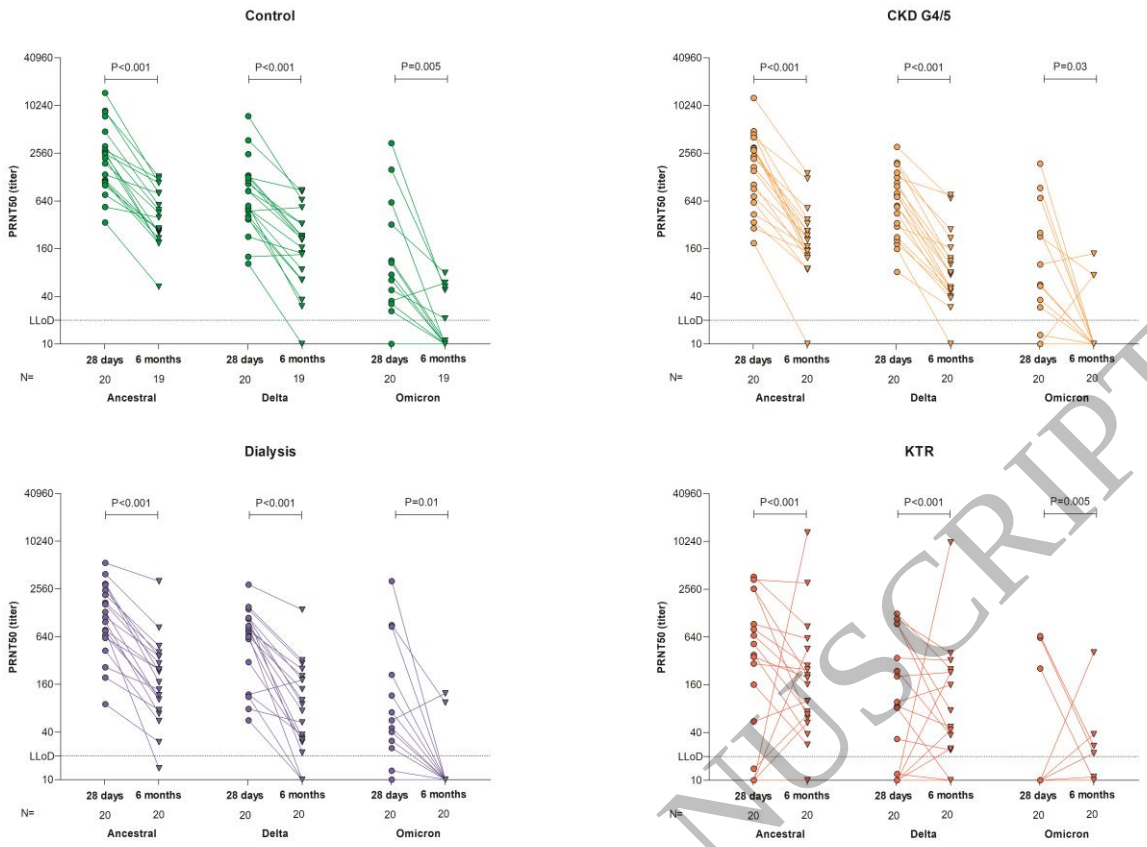

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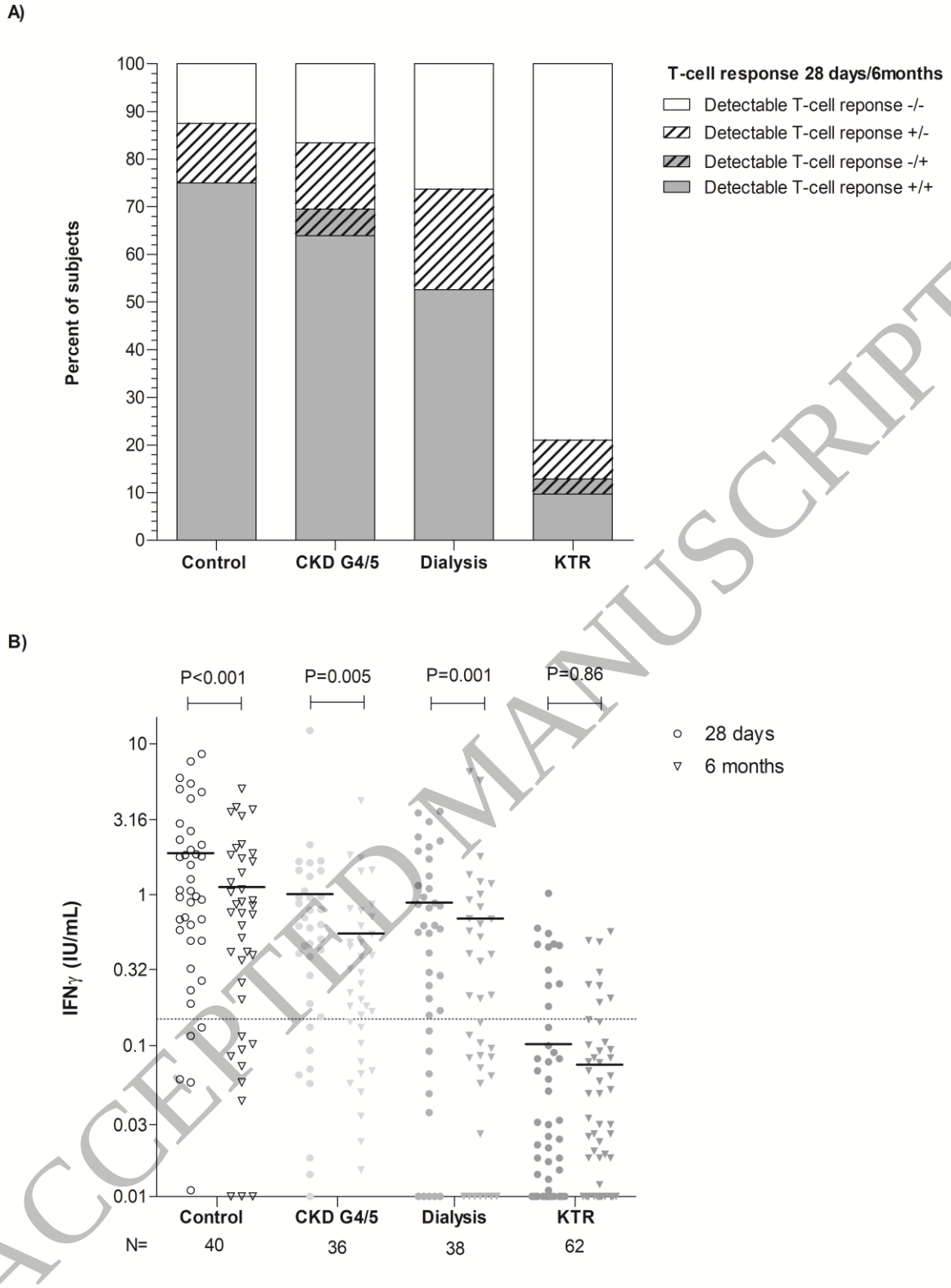

Figure 5
160x211 mm (x DPI)