

**This item is the archived peer-reviewed author-version of:**

SARS-CoV-2 mRNA vaccination is not associated with the induction of anti-HLA or non-HLA antibodies

**Reference:**

Wijtvliet Veerle, Verheyden Sonja, Depreter Barbara, Heylen Christine, Coeman Elke, Abrams Steven, De Winter Benedicte, Massart Annick, Hellemans Rachel, Pipeleers Lissa, ....- SARS-CoV-2 mRNA vaccination is not associated with the induction of anti-HLA or non-HLA antibodies  
Transplant immunology - ISSN 1878-5492 - 74(2022), 101670  
Full text (Publisher's DOI): <https://doi.org/10.1016/J.TRIM.2022.101670>  
To cite this reference: <https://hdl.handle.net/10067/1893990151162165141>

# **SARS-CoV-2 mRNA vaccination is not associated with the induction of anti-HLA or non-HLA antibodies**

Veerle P W M Wijtvliet<sup>1,2</sup>, Sonja Verheyden<sup>3</sup>, Barbara Depreter<sup>3</sup>, Christine Heylen<sup>4</sup>, Elke Coeman<sup>4</sup>, Steven Abrams<sup>5,6</sup>, Benedicte Y De Winter<sup>1</sup>, Annick Massart<sup>1,2</sup>, Rachel Hellemans<sup>1,2</sup>, Lissa Pipeleers<sup>7</sup>, Frans H J Claas<sup>1,8</sup>, Kevin K Ariën<sup>9,10</sup>, Karl Martin Wissing<sup>7</sup>, Daniel Abramowicz<sup>1,2</sup>, Kristien J Ledeganck<sup>1</sup>

<sup>1</sup>Laboratory of Experimental Medicine and Pediatrics and member of the Infla-Med Centre of Excellence, University of Antwerp, Edegem, Belgium.

<sup>2</sup>Department of Nephrology and Hypertension, Antwerp University Hospital, Edegem, Belgium.

<sup>3</sup>Department of Hematology, HLA and Molecular Hematology Laboratory, Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium.

<sup>4</sup>Immucor Inc., Norcross, GA, United States.

<sup>5</sup>Department of Family Medicine and Population Health, Global Health Institute, University of Antwerp, Antwerp, Belgium.

<sup>6</sup>I-BioStat, Data Science Institute, Hasselt University, Diepenbeek, Belgium

<sup>7</sup>Department of Nephrology, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium.

<sup>8</sup>Department of Immunology, Leiden University Medical Center, Leiden, Netherlands.

<sup>9</sup>Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium.

<sup>10</sup>Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.

Corresponding author: Veerle Wijtvliet, MD. Antwerp University Hospital, Department of Nephrology and Hypertension. Drie Eikenstraat 655, 2650 Edegem, Belgium.

Electronic address: [veerle.wijtvliet@uantwerpen.be](mailto:veerle.wijtvliet@uantwerpen.be)

Phone number: +32 3 265 2591.

Abbreviations: Ab = antibodies; AD-BCR = Antigen Density corrected BCR; ARHGDIB = Rho GDP dissociation inhibitor beta; BCM = Background Corrected MFI; BCR = Background Corrected Ratio; BNT162b2 = Pfizer–BioNTech COVID-19 vaccine; COVID-19 = coronavirus disease 2019; CTR = controls; EC = ethics committee; HD = patients on haemodialysis; HLA = human leukocyte antigen; IgG = immunoglobulin G; IQR = interquartile range; KTR = kidney transplant recipients; LMX kit = LIFECODES LifeScreen Deluxe kit; LSA kit = LIFECODES single-antigen bead kit; MFI = median fluorescence intensity; MMF = mycophenolate mofetil; mRNA = messenger RNA; mRNA-1273 = Moderna COVID-19 vaccine; n = number; NA = not applicable; PE = phycoerythrin; PCR = polymerase chain reaction; PD = patients on peritoneal dialysis; RBD = receptor-binding domain; SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2; SD = standard deviation

## **Abstract**

**Background:** SARS-CoV-2 vaccination is strongly recommended in kidney transplant recipients (KTR) and dialysis patients. Whether these vaccinations may trigger alloantibodies, is still debated.

**Methods:** In the current study we evaluated the effect of SARS-CoV-2 mRNA vaccines on anti-Human Leukocyte Antigen (HLA) and 60 anti-non-HLA antibody profiles in clinically stable KTR and dialysis patients. In total, we included 28 KTR, 30 patients on haemodialysis, 25 patients on peritoneal dialysis and 31 controls with a positive seroresponse 16-21 days after the first dose of either the SARS-CoV-2 mRNA BNT162b2 or mRNA-1273 vaccine. Both anti-HLA and anti-non-HLA antibodies were determined prior to vaccination and 21 to 35 days after the second vaccine dose.

**Results:** Overall, the proportion of patients with detectable anti-HLA antibodies was similar before and after vaccination (class I 14% vs. 16%,  $p = 0.48$ ; class II 25% before and after vaccination). After vaccination, there was no pattern in 1) additionally detected anti-HLA antibodies, or 2) the levels of pre-existing ones. Additional anti-non-HLA antibodies were detected in 30% of the patients, ranging from 1 to 5 new anti-non-HLA antibodies per patient. However, the clinical significance of anti-non-HLA antibodies is still a matter of debate. To date, only a significant association has been found for anti-non-HLA ARHGDIB antibodies and long-term kidney graft loss. No additionally developed anti-ARHGDIB antibodies or elevated level of existing anti-ARHGDIB antibodies was observed.

**Conclusion:** The current data indicate that SARS-CoV-2 mRNA vaccination does not induce anti-HLA or anti-non-HLA antibodies, corroborating the importance of vaccinating KTR and dialysis patients.

## **Keywords**

Antibodies, human leukocyte antigen (HLA), minor antigen, SARS-CoV-2, vaccination

## 1. Introduction

The ongoing coronavirus disease 2019 (COVID-19) pandemic mainly affects dialysis patients and kidney transplant recipients since they are at higher risk of severe COVID-19 and mortality [1-5]. As a consequence, these vulnerable populations are prioritized for immunization with SARS-CoV-2 mRNA vaccines (BNT162b2 or mRNA-1273) in numerous countries [6]. However, phase 3 trials of SARS-CoV-2 vaccines have excluded these patients so far, resulting in limited data on the safety of these vaccines. As already observed in other vaccine studies, repeated injections of adjuvanted influenza vaccines may trigger the development of anti-human leukocyte antigen (HLA) antibodies, donor-specific or not, in kidney transplant recipients [7, 8]. At the start of our investigation, no studies had investigated a potential association between SARS-CoV-2 vaccination and subsequent development of anti-non-HLA antibodies. This could be important, because a significant association has been described for presence of anti-non-HLA ARHGDI B antibodies and the development of long-term kidney graft loss [9, 10]. Therefore, studies are needed to evaluate the potential of SARS-CoV-2 mRNA vaccines to elicit anti-HLA or non-HLA antibodies. Indeed, it is crucial to investigate whether SARS-CoV-2 mRNA vaccines may induce anti-HLA or non-HLA antibodies in dialysis patients on the waiting list for a kidney transplantation, as these could negatively affect their future transplant eligibility and transplant outcomes. Furthermore, a small number of reports have shown that SARS-CoV-2 mRNA vaccines may trigger the development of subsequent kidney transplant rejection [11, 12] that was associated [11] or not [12] with the appearance of donor-specific anti-HLA antibodies. Therefore, the aim of the current study was to investigate in kidney transplant recipients, dialysis patients, and healthy controls if 1) additional anti-HLA-antibodies, ARHGDI B or other non-HLA antibodies did

develop after SARS-CoV-2 mRNA vaccination, and if 2) the median fluorescence intensity (MFI) values of pre-existing anti-HLA, ARHGDIB and other non-HLA antibodies did increase after vaccination with either two-dose BNT162b2 (Pfizer) or mRNA-1273 (Moderna) vaccination.

## **2. Objective**

The goal of the present study was to evaluate the possible association between two-dose SARS-CoV-2 mRNA vaccination and subsequent development of anti-non-HLA and/or anti-HLA antibodies in kidney transplant recipients, dialysis patients and healthy controls.



### 3. Material and Methods

#### 3.1 Patients

##### *3.1.1 Anti-Human Leukocyte Antigen (HLA) Antibody testing*

*Study group:* The study group consisted of a random sample of kidney transplant recipients (KTR) and dialysis patients, who were under active follow-up at the Antwerp University Hospital or University Hospital Brussels [13]. They all received a first dose of SARS-CoV-2 mRNA vaccine in March-April 2021. Patients who seroconverted 16-21 days after the first dose of SARS-CoV-2 mRNA vaccine, were included. Seroconversion was defined as anti-receptor-binding domain (RBD) immunoglobulin G (IgG) positivity (signal-to-noise ratio > 1), measured using an in-house Luminex assay, as earlier described [13, 14]. Exclusion criteria were patients < 18 years, transplantation within 1 month, plasmapheresis within 1 month, ongoing allograft rejection during study sampling, leukopenia (<2500/ $\mu$ l), intravenous immunoglobulins injection within 3 months, and refusal of the patient to participate in the study.

*Immunocompetent control group:* To compare anti-HLA antibody responses between immunocompromised and immunocompetent subjects, healthy control adults were recruited through a digital invitation within the Antwerp University Hospital and University of Antwerp. Those who showed a seroconversion for SARS-CoV-2 following primary SARS-CoV-2 mRNA vaccination between March and May 2021 were included.

The study was performed in accordance with the Declaration of Helsinki and approved by the ethical committee of the Antwerp University Hospital/University Hospital Brussels and

University of Antwerp/the Free University of Brussels (no. EC 21/05/076). Samples were collected after written informed consent.

### ***3.1.2 Anti-Non-HLA Antibody Testing***

Within the study group subjected to anti-HLA antibody testing, a subgroup of kidney transplant recipients and dialysis patients younger than 70 years was selected for anti-non-HLA antibody testing. Dialysis patients older than 70 years were not included, as they are generally not eligible for kidney transplantation. Control patients were not included for logistic reasons.

## **3.2 Study design**

This was a prospective, interventional, longitudinal, multicentre study, in which serum samples were collected from each patient at day 1 (before vaccination), 16 to 21 days after the first dose of the vaccine, and 21 to 35 days after the second vaccine dose, as previously described in detail [13]. Samples were processed by and stored at the “Biobank Antwerpen” (Antwerp, Belgium; ID: BE71030031000) [15].

## **3.3 Detection of anti-HLA antibodies**

All samples taken pre-vaccination (day 1) and post-vaccination (21 to 35 days after the second vaccine dose) were tested for the presence of anti-HLA antibodies. First, all sera were screened using a LIFECODES LifeScreen Deluxe (LMX) kit (lot numbers 3011324 3011049-LMX and 3011429 3011286-LMX) according to manufacturer’s protocol (Immucor®, USA). Subsequently, the sera with a positive result on LMX testing, were subjected to a second test using LIFECODES single-antigen bead (LSA) kits (lot numbers 3011654 3011426-SA1 and 3011545 3011301-SA2) (Immucor®, USA) to determine the specificity of class I anti-HLA-A, -B

and -C and class II anti-HLA-DRB1, -DQA1, -DQB1, -DPA1 and -DPB1 IgG antibodies. Quality control was built in the LSA kit by the inclusion of one positive and one negative control serum sample. These controls were included in each test to determine if a technical error or reagent failure had occurred. Furthermore, both a positive and negative control bead were included to monitor sample's performance. Results were expressed as raw median fluorescence intensity (MFI) values. The background MFI value, provided by the manufacturer per single antigen bead, was subtracted from the raw MFI value to calculate the *Background Corrected MFI* (BCM). By dividing the BCM by the raw MFI value of the lowest ranked antigen bead for that locus (also named the calculated control (CalcCON)), the *Background Corrected Ratio* (BCR) was determined. Subsequently, *Antigen Density corrected BCR values* (AD-BCR) were generated by normalization of the BCR to the amount of antigen on each bead, as found in the lot-specific recording sheet. These values were calculated with the MATCHIT! antibody software version 1.3.0 (Immucor®, USA), as provided by the manufacturer. The MatchIT software assigned a LSA bead as positive when 2 of the 3 above calculated values were fulfilled (BCM > 2000, BCR > 5, AD-BCR > 5)

### **3.4 Detection of anti-non-HLA antibodies**

The non-HLA multiplex bead panel (Immucor®, USA) included 60 non-HLA antigens conjugated to polystyrene beads (supplemental table 1). Testing was performed by Immucor according to manufacturer's protocol. Forty  $\mu$ L of capture beads were incubated with 10  $\mu$ L of serum for 30 minutes. After washing, IgG bound to capture beads were detected using phycoerythrin (PE) conjugated goat anti-human IgG for 30 minutes. Results were expressed as raw MFI values. An antigen bound bead was considered positive if the MFI value exceeded the corresponding MFI threshold. This MFI threshold was determined using sera from 100

never-transfused males, by calculating mean MFI + 3 standard deviations. The background MFI value, provided by the manufacturer per single antigen bead, was subtracted from the raw MFI value to calculate the *Background Corrected MFI* (BCM).

### **3.5 Statistical analysis**

The study was exploratory by design. IBM SPSS statistics version 28.0 and statistical software package R were used for statistical analysis. Continuous variables were presented as mean  $\pm$  standard deviation (SD) for variables having a symmetrical empirical distribution or normally distributed variables or median (interquartile range) for skewed variables. Proportions estimated from paired observations, were compared using the McNemar's test with continuity correction. All statistical tests were performed two-sided with p-values  $< 0.05$  considered to be statistically significant.

## **4. Results**

### **4.1 Patient characteristics**

#### ***4.1.1 Anti-Human Leukocyte Antigen (HLA) Antibody testing***

In total, 28 kidney transplant recipients, 30 patients on haemodialysis, 25 patients on peritoneal dialysis and 31 controls were included. The patient characteristics and the flow of patient inclusion are depicted in Table 1 and Figure 1, respectively.

#### ***4.1.2 Anti-Non-HLA Antibody Testing***

A subgroup of 28 kidney transplant recipients, 28 patients on haemodialysis, and 14 patients on peritoneal dialysis were selected for anti-non-HLA antibody testing. The patient characteristics and the flow of patient inclusion are shown in Table 1 and Figure 1, respectively.

### **4.2 Anti-HLA antibodies**

Among the patients who were negative prior to vaccination by LMX-LSA testing, only two patients (2/114; 2%) converted from negative anti-HLA class I antibodies prior to vaccination to positive antibodies 1 month after full-dose vaccination. In these two cases, the observed MFIs after vaccination were very low (one KTR developed anti-HLA B8 antibodies with a *Background Corrected MFI* (BCM) of 431, and one patient on peritoneal dialysis developed anti-HLA B45 antibodies with a BCM of 206. No relevant rise in MFI values was observed in these two patients, as shown in Table 2A. Regarding class II anti-HLA antibodies, none of the patients converted from negative to positive. Overall, the percentage of patients with positive anti-HLA antibody responses pre-vaccination vs. 1 month after 2-dose vaccination, remained

also unaffected (class I 14% vs. 16%,  $p = 0.48$ ; class II 25% before and after vaccination; Figure 2).

Sixteen patients had positive test results on both pre- and post-vaccination samples for anti-HLA class I antibodies, and 28 patients for class II antibodies. No pattern was seen in additionally detected anti-HLA antibodies post-vaccination. In total, 9 subjects developed 16 new anti-HLA antibodies with a non-relevant median increase in MFI of 195. Along the same line, no rise in MFI of pre-existing anti-HLA-antibodies (Supplemental Table 2) was observed.

### **4.3 Anti-Non-HLA antibodies**

Screening of 70 sera pre- versus post-vaccination, revealed additional induction of anti-non-HLA antibody specificities after two-dose SARS-CoV-2 vaccination in 21 patients (30%). An overview of the identified anti-non-HLA antibodies and corresponding MFI values is depicted in Supplemental Table 3. In particular, no development of additional anti-ARHGDIB antibodies was observed. No pattern was noted in the development of other specific anti-non-HLA antibodies after SARS-CoV-2 mRNA vaccination. In patients with pre-existing anti-non-HLA-antibodies before vaccination, MFI values were considered increased if the median MFI, measured in at least 3 samples, increased  $> 1000$  post-vaccination vs. pre-vaccination. Only anti-non-HLA antibody specificity 'IL8' met these criteria. Furthermore, only one patient exhibited pre-existing anti-ARHGDIB antibodies, but its MFI values were decreased after vaccination. Finally, none of the included kidney transplant recipients developed a rejection after 2-dose SARS-CoV-2 mRNA vaccination.

## 5. Discussion

In this study, we examined the effect of two-dose SARS-CoV-2 mRNA vaccines on anti-HLA- and non-HLA antibody profiles in clinically stable kidney transplant recipients and dialysis patients. No clear pattern in either anti-HLA antibody or non-HLA antibody development was observed one month after receiving the second vaccine dose, suggesting that SARS-CoV-2 mRNA vaccination does not induce allosensitization. Future longitudinal studies are needed to confirm these findings and examine whether there is a long-term effect of SARS-CoV-2 mRNA vaccines on anti-HLA or non-HLA antibody development or an effect of SARS-CoV-2 mRNA vaccines on anti-HLA or non-HLA antibody development in patients who did not seroconvert after vaccination.

Several studies investigated the influence of various vaccines on anti-HLA antibody profiles, with conflicting results [7, 16, 17]. Cordero *et al.* studied a cohort of 490 solid organ transplant recipients (SOTR) vaccinated with an adjuvanted influenza H1N1 vaccine, and reported no association between vaccination and production of *de novo* donor-specific anti-HLA antibodies or rejection [16]. Katerinis *et al.* followed two independent cohorts of kidney transplant recipients until 6 months after H1N1 vaccination [7]. Sixteen of 92 (17.3%) and 7 of 59 (11.9%) patients developed both donor-specific and non-donor-specific anti-HLA antibodies, which were generally of low levels. Only two patients developed clinical events possibly associated to *de novo* development of anti-HLA antibodies: one patient was diagnosed with a thrombotic microangiopathy two weeks after the second H1N1 dose, and the other patient developed an acute humoral rejection two months after the second H1N1 dose. Nevertheless, other factors than influenza vaccination could have triggered these events. Lindemann *et al.* examined the development of anti-HLA antibodies following vaccination with Prevenar 13® (Pfizer, New

York, NY, USA) against *Streptococcus pneumoniae* in 47 clinically stable kidney transplant recipients [17]. They reported that anti-HLA class I and II antibodies increased significantly at month 1 and 12 after vaccination ( $p < 0.05$ ) in female KTR. No *de novo* donor-specific antibodies were detected [17]. Nevertheless, these vaccines are different vaccine types than the SARS-CoV-2 mRNA vaccines, which form a new class of vaccines on the market.

To date, two case reports describe the development of anti-HLA antibodies after SARS-CoV-2 vaccination. Xu et al. describe a patient waiting for a second kidney transplantation, who developed anti-HLA-DR7 antibodies 37 days after the second dose of BNT162b2 was administered [18]. HLA DR7 was the mismatched antigen with the failing first kidney allograft, which makes the authors speculate that the BNT162b2 vaccine may have activated a memory B cell response to HLA DR7. Abu-Khader et al. reported another patient on the kidney transplant recipient list without sensitization history (e.g., no previous transplants or transfusions) before vaccination, who developed anti-HLA-antibodies against B57 and B58 eighteen days after receiving the first dose of BNT162b2 vaccine [19].

With respect to kidney allograft rejection after SARS-CoV-2 mRNA vaccination, two case reports documented the development of T-cell mediated acute rejection after vaccination. One case report described a 53-year-old kidney transplant recipient, who developed a T-cell mediated acute rejection after receiving his second mRNA-1273 vaccine. Anti-HLA antibody testing did not show any *de novo* developed donor-specific antibodies [12]. One other case of T-cell mediated rejection was reported eight days after the administration of a second BNT162b2 vaccine dose, but in that instance novel donor-specific class II anti-HLA antibodies had been detected. No further anti-HLA antibody specification was mentioned [11]. In addition, two large studies showed contradictory results regarding anti-HLA antibody



development [20]. First, Al Jurdi et al. followed 58 kidney transplant recipients until two months after their second SARS-CoV-2 mRNA vaccination (BNT162b2 or mRNA-1273). At a median of 85 days after initial vaccination, none of the kidney transplant recipients had developed *de novo* anti-HLA antibodies. Only one patient developed a T-cell mediated rejection 40 days after initial vaccination, following conversion from Tacrolimus to Belatacept two days before vaccination [21]. Second, Sattler et al. detected no *de novo* anti-HLA antibodies, no increase of existing anti-HLA antibodies and no cases of acute rejection after the second BNT162b2 vaccine dose [22].

Besides the well-known effect of anti-HLA antibodies on kidney allograft survival, the clinical significance of non-HLA antibodies is still a matter of debate. To date, only a significant association has been found for the presence of anti-non-HLA ARHGDIB antibodies and the development of long-term kidney graft loss [9, 10]. In our study, no development of additional anti-ARHGDIB or MFI rise of existing anti-ARHGDIB antibodies was observed. Examining the other 59 anti-non-HLA antibodies, only a relevant rise in pre-existing anti-IL8-antibodies was noted, with unknown clinical significance.

The main limitation of our study is its relatively limited sample size possibly restricting the detection of small differences in anti-HLA and non-HLA antibody levels. Furthermore, we could not exclude natural fluctuation of non-HLA antibody measurements, as no non-vaccinated control group was included in this study. However, our results are consistent with the expected incidence of the observed development of anti-HLA antibodies along time. Indeed, it is estimated that 5% to 9% of kidney transplant recipients develop *de novo* donor-specific anti-HLA antibodies each year [23, 24]. As around 5 million kidney transplant recipients have been vaccinated for SARS-CoV-2 infection worldwide, we believe that the

small number of case reports on kidney transplant recipients with development of additional anti-HLA-antibodies after SARS-CoV-2 mRNA vaccination, does not necessarily imply a causal relationship between SARS-CoV-2 mRNA vaccination and development of anti-HLA antibodies. Based on our data and those of others, it seems that the risk of developing anti-HLA antibodies due to SARS-CoV-2 vaccination is extremely low and that development of additional anti-HLA antibodies is rather an activation of previously induced memory B cells. Therefore, we believe the benefit of getting vaccinated outweighs the risk of developing anti-HLA or non-HLA antibodies.

## **Author contributions**

DA and KL designed and coordinated the study. VW, SV, BD, CH, EC, LP, KA, and KMW collected the data. VW, SV, CH, and SA interpreted and analyzed the data. VW, DA and KL wrote the report. VW, SV, BD, CH, SA, BDW, AM, RH, LP, FHJ, KMW, DA and KL critically reviewed the paper. All authors read and approved the final version.

## **Acknowledgements**

The authors would like to thank the study nurses E. Meersman, S. Verhofstede, N. Marmitte and T. Bogaerts for their excellent and efficient help. We also thank E. Snelders and J. Michiels for their logistical support, the lab technicians of the Laboratory of Experimental Medicine and Pediatrics and L. Heyndrickx of the Institute of Tropical Medicine for the sample processing and P. Moons from Biobank Antwerp for his technical support. Special thanks to all the kidney transplant recipients, dialysis patients and control patients who made this study possible.

## **Declarations of interest**

None.

## **Funding**

The authors received no specific funding for this work.

## **Data availability statement**

Requests for original and additional data should be directed to the corresponding author.

## References

- [1] S. Anand, M. Montez-Rath, J. Han, J. Bozeman, R. Kerschmann, P. Beyer, J. Parsonnet, G.M. Chertow, Prevalence of SARS-CoV-2 antibodies in a large nationwide sample of patients on dialysis in the USA: a cross-sectional study, *The Lancet* 396(10259) (2020) 1335-1344.
- [2] Y. Cheng, R. Luo, K. Wang, M. Zhang, Z. Wang, L. Dong, J. Li, Y. Yao, S. Ge, G. Xu, Kidney disease is associated with in-hospital death of patients with COVID-19, *Kidney international* 97(5) (2020) 829-838.
- [3] K.J. Jager, A. Kramer, N.C. Chesnaye, C. Couchoud, J.E. Sánchez-Álvarez, L. Garneata, F. Collart, M.H. Hemmeler, P. Ambühl, J. Kerschbaum, Results from the ERA-EDTA Registry indicate a high mortality due to COVID-19 in dialysis patients and kidney transplant recipients across Europe, *Kidney international* 98(6) (2020) 1540-1548.
- [4] C.M. Petrilli, S.A. Jones, J. Yang, H. Rajagopalan, L. O'Donnell, Y. Chernyak, K.A. Tobin, R.J. Cerfolio, F. Francois, L.I. Horwitz, Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study, *bmj* 369 (2020).
- [5] E.J. Williamson, A.J. Walker, K. Bhaskaran, S. Bacon, C. Bates, C.E. Morton, H.J. Curtis, A. Mehrkar, D. Evans, P. Inglesby, J. Cockburn, H.I. McDonald, B. MacKenna, L. Tomlinson, I.J. Douglas, C.T. Rentsch, R. Mathur, A.Y.S. Wong, R. Grieve, D. Harrison, H. Forbes, A. Schultze, R. Croker, J. Parry, F. Hester, S. Harper, R. Perera, S.J.W. Evans, L. Smeeth, B. Goldacre, Factors associated with COVID-19-related death using OpenSAFELY, *Nature* 584(7821) (2020) 430-436.
- [6] S. Caillard, O. Thauvat, COVID-19 vaccination in kidney transplant recipients, *Nature Reviews Nephrology* 17(12) (2021) 785-787.
- [7] I. Katerinis, K. Hadaya, R. Duquesnoy, S. Ferrari-Lacraz, S. Meier, C. van Delden, P.-Y. Martin, C.-A. Siegrist, J. Villard, De Novo Anti-HLA Antibody After Pandemic H1N1 and Seasonal Influenza Immunization in Kidney Transplant Recipients, *American Journal of Transplantation* 11(8) (2011) 1727-1733.
- [8] T. Fairhead, E. Hendren, K. Tinckam, C. Rose, C.H. Sherlock, L. Shi, N.S. Crowcroft, J.B. Gubbay, D. Landsberg, G. Knoll, J. Gill, D. Kumar, Poor seroprotection but allosensitization after adjuvanted pandemic influenza H1N1 vaccine in kidney transplant recipients, *Transpl Infect Dis* 14(6) (2012) 575-83.
- [9] E.G. Kamburova, M.L. Gruijters, T. Kardol-Hoefnagel, B.W. Wisse, I. Joosten, W.A. Allebes, A. van der Meer, L.B. Hilbrands, M.C. Baas, E. Spierings, C.E. Hack, F.E. van Reekum, A.D. van Zuilen, M.C. Verhaar, M.L. Bots, A. Drop, L. Plaisier, R.C.A. Melchers, M.A.J. Seelen, J.S. Sanders, B.G. Hepkema, A.J.A. Lambeck, L.B. Bungener, C. Roozendaal, M.G.J. Tilanus, C.E. Voorter, L. Wieten, E.M. van Duijnhoven, M. Gelens, M.H.L. Christiaans, F.J. van Ittersum, S.A. Nurmohamed, N.M. Lardy, W. Swelsen, K. van der Pant, N.C. van der Weerd, I.J.M. Ten Berge, A. Hoitsma, P.J.M. van der Boog, J.W. de Fijter, M.G.H. Betjes, S. Heidt, D.L. Roelen, F.H. Claas, F.J. Bemelman, H.G. Otten, Antibodies against ARHGDI B are associated with long-term kidney graft loss, *Am J Transplant* 19(12) (2019) 3335-3344.
- [10] A. Senev, H.G. Otten, E.G. Kamburova, J. Callemeyn, E. Lerut, V. Van Sandt, D. Kuypers, M.P. Emonds, M. Naesens, Antibodies Against ARHGDI B and ARHGDI B Gene Expression Associate With Kidney Allograft Outcome, *Transplantation* 104(7) (2020) 1462-1471.
- [11] A. Del Bello, O. Marion, A. Delas, N. Congy-Jolivet, M. Colombat, N. Kamar, Acute rejection after anti-SARS-CoV-2 mRNA vaccination in a patient who underwent a kidney transplant, *Kidney Int* 100(1) (2021) 238-239.
- [12] J.T. Bau, L. Churchill, M. Pandher, H. Benediktsson, L.A. Tibbles, S. Gill, Acute Kidney Allograft Rejection Following Coronavirus mRNA Vaccination: A Case Report, *Transplant Direct* 8(2) (2022) e1274.
- [13] V. Wijtvliet, K.K. Ariën, S. Abrams, M.M. Couttenye, F. Mestrez, J. Mariën, B.Y. De Winter, P. Van Damme, L. Pipeleers, K.M. Wissing, D. Abramowicz, K.J. Ledeganck, mRNA-1273 vaccine (Moderna): a better option than BNT162b2 (Pfizer) in kidney transplant recipients and dialysis patients?, *Nephrol Dial Transplant* (2021).

- [14] J. Mariën, A. Ceulemans, J. Michiels, L. Heyndrickx, K. Kerkhof, N. Foque, M.A. Widdowson, L. Mortgat, E. Duysburgh, I. Desombere, H. Jansens, M. Van Esbroeck, K.K. Ariën, Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay, *J Virol Methods* 288 (2021) 114025.
- [15] BE 71030031000; Biobank Antwerp [BB190007], BBMR-ERIC, Belgian [BIORESOURCE]. .
- [16] E. Cordero, A. Bulnes-Ramos, M. Aguilar-Guisado, F. González Escribano, I. Olivas, J. Torre-Cisneros, J. Gavaldá, T. Aydillo, A. Moreno, M. Montejo, M.C. Fariñas, J. Carratalá, P. Muñoz, M. Blanes, J. Fortún, A. Suárez-Benjumea, F. López-Medrano, C. Roca, R. Lara, P. Pérez-Romero, Effect of Influenza Vaccination Inducing Antibody Mediated Rejection in Solid Organ Transplant Recipients, *Front Immunol* 11 (2020) 1917-1917.
- [17] M. Lindemann, S. Oesterreich, B. Wilde, U. Eisenberger, N. Muelling, P.A. Horn, F.M. Heinemann, O. Witzke, Sex-Specific Differences in HLA Antibodies after Pneumococcal Vaccination in Kidney Transplant Recipients, *Vaccines (Basel)* 7(3) (2019) 84.
- [18] Q. Xu, P. Sood, D. Helmick, J.S. Lomago, A.D. Tevar, A. Zeevi, Positive flow cytometry crossmatch with discrepant antibody testing results following COVID-19 vaccination, *Am J Transplant* 21(11) (2021) 3785-3789.
- [19] A. Abu-Khader, W. Wang, M. Berka, I. Galaszkiwicz, F. Khan, N. Berka, SARS Cov-2 vaccination induces de novo donor-specific HLA antibodies in a renal transplant patient on waiting list: A case report, *HLA* 99(1) (2022) 25-30.
- [20] D. Kumar, P. Kimball, G. Gupta, COVID-19 vaccine does not alter panel reactive antibody or flow cytometric cross match in kidney transplant candidates, *Transpl Immunol* 69 (2021) 101469.
- [21] A. Al Jurdi, R.B. Gassen, T.J. Borges, Z. Solhjoui, F.E. Hullekes, I.T. Lape, O. Efe, A. Alghamdi, P. Patel, J.Y. Choi, M.T. Mohammed, B. Bohan, V. Pattanayak, I. Rosales, P. Cravedi, C.N. Kotton, J.R. Azzi, L.V. Riella, Non-Invasive Monitoring for Rejection in Kidney Transplant Recipients After SARS-CoV-2 mRNA Vaccination, *Front Immunol* 13 (2022) 838985.
- [22] A. Sattler, E. Schrezenmeier, U.A. Weber, A. Potekhin, F. Bachmann, H. Straub-Hohenbleicher, K. Budde, E. Storz, V. Proß, Y. Bergmann, L.M. Thole, C. Tizian, O. Hölsken, A. Diefenbach, H. Schrezenmeier, B. Jahrsdörfer, T. Zemojtel, K. Jechow, C. Conrad, S. Lukassen, D. Stauch, N. Lachmann, M. Choi, F. Halleck, K. Kotsch, Impaired humoral and cellular immunity after SARS-CoV-2 BNT162b2 (tozinameran) prime-boost vaccination in kidney transplant recipients, *J Clin Invest* 131(14) (2021).
- [23] R.L. Heilman, A. Nijim, Y.M. Desmarteau, H. Khamash, M.J. Pando, M.L. Smith, H.A. Chakkera, J. Huskey, R. Valdez, K.S. Reddy, De novo donor-specific human leukocyte antigen antibodies early after kidney transplantation, *Transplantation* 98(12) (2014) 1310-5.
- [24] C. Wiebe, I.W. Gibson, T.D. Blydt-Hansen, M. Karpinski, J. Ho, L.J. Storsley, A. Goldberg, P.E. Birk, D.N. Rush, P.W. Nickerson, Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant, *Am J Transplant* 12(5) (2012) 1157-67.

**Table 1: Patient characteristics of subjects in the anti-HLA antibody study group & non-HLA antibody study group**

**Legend Table 1: Patient characteristics.** In the first four columns, patient characteristics of subjects disposed to anti-Human Leukocyte Antigen (HLA) antibody testing, are depicted. Within the study group subjected to anti-HLA antibody testing, a subgroup of kidney transplant recipients and dialysis patients younger than 70 years was selected for anti-non-HLA antibody testing. The characteristics of these subjects disposed to anti-non-HLA antibody testing are represented in the last three columns.

	Anti-HLA antibody study group				Anti-Non-HLA antibody study group		
	Kidney transplant recipients	Patients on haemodialysis	Patients on peritoneal dialysis	Controls	Kidney transplant recipients	Patients on haemodialysis	Patients on peritoneal dialysis
Number of subjects	28	30	25	31	28	28	14
Male (n (%))	17 (60.7)	19 (63.3)	16 (64.0)	9 (29.0)	17 (60.7)	18 (64.3)	7 (50)
Median age, years (IQR)	55 (43-62)	60 (48-70)	66 (52-74)	65 (57-69)	55 (43-62)	60 (48-68)	55 (43-61)
Median interval last transplantation-2 <sup>nd</sup> vaccination, years (IQR)	6 (2-12)	NA	NA	NA	6 (2-12)	NA	NA
Number of kidney transplantations (n (%))							
1	24 (85.7)	NA	NA	NA	24 (85.7)	NA	NA
2	2 (7.1)				2 (7.1)		
3	2 (7.1)				2 (7.1)		
Number of immunosuppressive drugs (n (%))							
1	17 (60.7)	NA	NA	NA	17 (60.7)	NA	NA
2	10 (35.7)				10 (35.7)		
3	1 (3.6)				1 (3.6)		
Number of patients on MMF/MPA (n (%))	11 (39.9)	NA	NA	NA	11 (39.9)	NA	NA
Multi-organ transplantation (n (%))	0 (0)	NA	NA	NA	0 (0)	NA	NA
Median interval start dialysis-2 <sup>nd</sup> vaccination, years (IQR)	NA	2 (1-4)	1 (1-2)	NA	NA	2 (1-4)	1 (0-2)
Diabetes mellitus (n (%))	4 (14.3)	13 (43.3)	9 (36)	NA	4 (14.3)	12 (42.9)	4 (28.6)
PCR-proven history of SARS-CoV-2 infection	6 (21.4)	5 (16.7)	1 (4.0)	Unknown	6 (21.4)	4 (14.3)	1 (7.1)
Vaccine type (n (%))							
BNT162b2 (Pfizer)	14 (50)	15 (50)	5 (20)	29 (94.0)	14 (50)	15 (53.6)	4 (28.6)
mRNA-1273 (Moderna)	14 (50)	15 (50)	20 (80)	2 (6.0)	14 (50)	13 (46.4)	10 (71.4)

*Abbreviations:* HLA = Human Leukocyte Antigen; IQR = interquartile range; MMF = mycophenolate mofetil; MPA mycophenolic acid; n = number; NA = not applicable; PCR = polymerase chain reaction

**Table 2: Detection of additional anti-HLA antibody specificities post-vaccination vs. pre-vaccination**

**Legend Table 2: Overview of additionally detected anti-Human Leukocyte Antigens (HLA) antibodies class I and anti-HLA class II.** Results are expressed as median difference (increased versus decreased) in background corrected median fluorescence intensity (MFI) values for each additionally detected anti-HLA antibody post-vaccination. Results were interpreted relevant if the median MFI increased > 1000. Row A. shows the MFI pre-vaccination versus post-vaccination in subjects who tested generally negative for anti- HLA class I antibodies before vaccination, but positive 21-35 days after two-dose vaccination. Row B. shows the MFI of additionally detected anti-HLA antibodies pre-vaccination versus post-vaccination, in subjects who tested generally positive for either anti-HLA class I or II antibodies both before and after vaccination. In total, 9 subjects developed 16 new anti- HLA antibodies with an increased MFI (non-relevant median increase in MFI of 195).

A. Negative pre-vaccination; positive post-vaccination						
Anti-HLA Ab		Increased MFI* post- vs. pre-vaccination			Decreased MFI post- vs. pre-vaccination	
Class	I	B8	n = 1	+193	n = 0	NA
		B45	n = 0	NA	n = 1	-199
B. Positive pre-vaccination; positive post-vaccination						
Anti-HLA Ab		Increased MFI* post- vs. pre-vaccination			Decreased MFI post- vs. pre-vaccination	
Class	I	A23	n = 1	+475	n = 0	NA
		A25	n = 1	+ 390	n = 0	NA
		A32	n = 1	+ 358	n = 0	NA
		A80	n = 1	+144	n = 0	NA
		B27	n = 1	+191	n = 0	NA
		B42	n = 1	+264	n = 0	NA
		B55	n = 1	+152	n = 0	NA
		B56	n = 1	+195	n = 0	NA
		B67	n = 1	+888	n = 0	NA
		B82	n = 1	+186	n = 0	NA
	Cw10	n = 1	+534	n = 0	NA	
	II	DR1	n = 1	+46	n = 0	NA
		DR14	n = 1	+74	n = 0	NA
		DR17	n = 0	NA	n = 1	-42
		DR18	n = 0	NA	n = 1	-99
		DR52	n = 1	+523	n = 0	NA
DR53		n = 2	+284	n = 0	NA	

\* Expressed as median difference in background corrected MFI values. Interpreted as relevant increased MFI if median MFI increased > 1000.



*Abbreviations: Ab = antibodies; HLA = human leukocyte antigens; MFI = median fluorescence intensity; n = number; NA = not applicable*

## Legends to figure

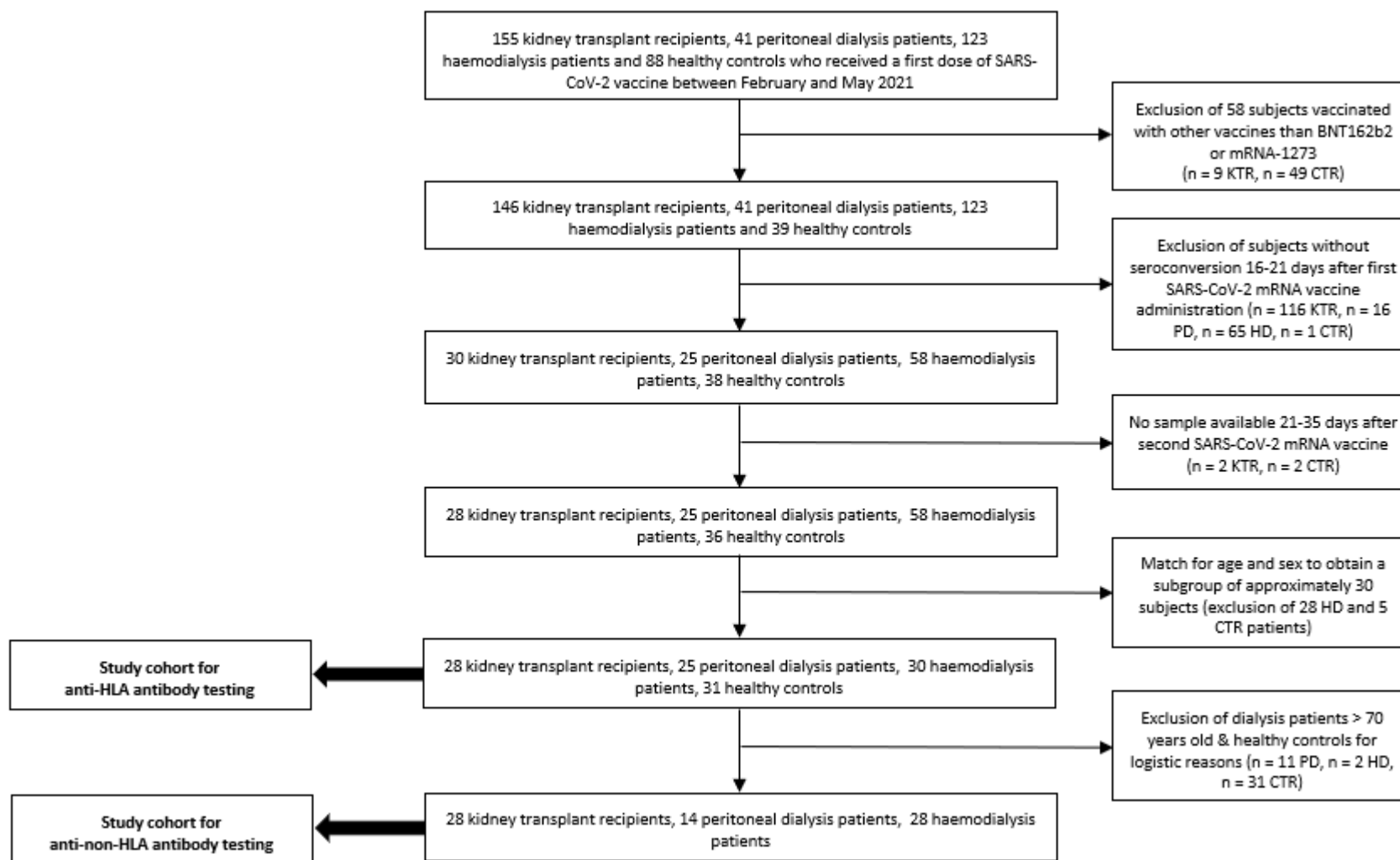
### Figure 1: Flow-chart of patient selection

*Abbreviations: CTR = controls; HD = patients on haemodialysis; HLA = human leukocyte antigens; KTR = kidney transplant recipients; PD = patients on peritoneal dialysis*

**Figure 2: Percentage of patients with positive antibody responses (to at least one bead) pre-vaccination (*white*) and 1 month after 2-dose SARS-CoV-2 mRNA vaccination (*black*). P-values were calculated using the McNemar's test with continuity correction.** The frequency of anti-HLA antibodies remained overall unchanged post-vaccination in the study group of 114 subjects (28 kidney transplant recipients, 30 patients on haemodialysis, 25 patients on peritoneal dialysis and 31 controls).

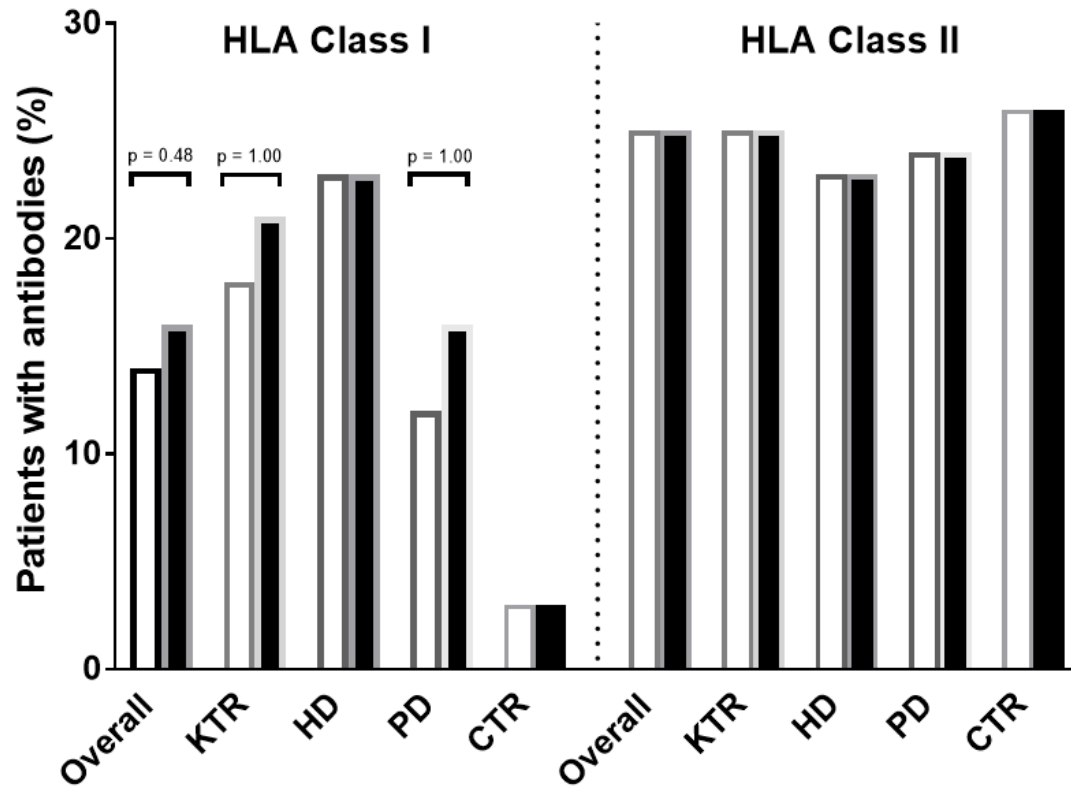
*Abbreviations: CTR = controls; HD = patients on haemodialysis; HLA = human leukocyte antigens; KTR = kidney transplant recipients; PD = patients on peritoneal dialysis*

**Figure 1: Flow-chart of patient selection**



Abbreviations: CTR = controls; HD = patients on haemodialysis; HLA = human leukocyte antigens; KTR = kidney transplant recipients; PD = patients on peritoneal dialysis

**Figure 2: Percentage of patients with positive antibody responses (to at least one bead) pre-vaccination (*white*) and 1 month after 2-dose SARS-CoV-2 mRNA vaccination (*black*). P-values were calculated using the McNemar’s test with continuity correction. The frequency of anti-HLA antibodies remained overall unchanged post-vaccination in the study group of 114 subjects (28 kidney transplant recipients, 30 patients on haemodialysis, 25 patients on peritoneal dialysis and 31 controls).**



Abbreviations: CTR = controls; HD = patients on haemodialysis; HLA = human leukocyte antigens; KTR = kidney transplant recipients; PD = patients on peritoneal dialysis