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Systemic oxidative stress may be associated with reduced IgG antibody titers against SARS-CoV-2 in vaccinated kidney transplant recipients: A post-hoc analysis of the RECOVAC-IR observational study

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) poses an increased risk for severe illness and suboptimal vaccination responses in patients with kidney disease, in which oxidative stress may be involved. Oxidative stress can be reliably measured by determining circulating free thiols (R–SH, sulfhydryl groups), since R–SH are rapidly oxidized by reactive species. In this study, we aimed to examine the association between serum free thiols and the ability to mount a humoral immune response to SARS-CoV-2 vaccination in kidney patients.

Methods: Serum free thiol concentrations were measured in patients with chronic kidney disease stages 4/5 (CKD G4/5) ($n = 46$), on dialysis ($n = 43$), kidney transplant recipients (KTR) ($n = 73$), and controls ($n = 50$). Baseline serum free thiol and interferon-γ-induced protein-10 (IP-10) – a biomarker of the interferon response – were analyzed for associations with seroconversion rates and SARS-CoV-2 spike (S1)-specific IgG concentrations after two doses of the mRNA-1273 vaccine.

Results: Albumin-adjusted serum free thiol concentrations were significantly lower in patients with CKD G4/5 ($P < 0.001$), on dialysis ($P < 0.001$), and KTR ($P < 0.001$), as compared to controls. Seroconversion rates after full vaccination were markedly reduced in KTR (52.1%) and were significantly associated with albumin-adjusted free thiols (OR = 1.76, $P = 0.033$). After adjustment for MMF use, hemoglobin, and eGFR, this significance was not sustained (OR = 1.49, $P = 0.241$).

Conclusions: KTR show suboptimal serological responses to SARS-CoV-2 vaccination, which is inversely associated with serum R–SH, reflecting systemic oxidative stress. Albeit this association was not robust to relevant confounding factors, it may at least partially be involved in the inability of KTR to generate a positive serological response after SARS-CoV-2 vaccination.

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[1] This study was performed using the RECOVAC immune response (IR) cohort (ClinicalTrials.gov NCT04741386; The Immune-response and Safety of COVID-19 Vaccination in Patients With Chronic Kidney Disease, on Dialysis, or Living With a Kidney Transplant - A Prospective, Controlled, Multicenter Cohort Study by the RECOVAC Consortium). A list of RECOVAC Collaborators is added in the Acknowledgments.

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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a global health crisis with devastating impact on human society. While there has been great progress in the fight against the virus and COVID-19 restrictions are gradually disappearing, individuals with a compromised immune system remain at increased risk for severe illness. They frequently show a blunted antibody response to SARS-CoV-2 vaccination as is especially the case for organ transplant recipients [1]. Long-term immunosuppression negatively impacts immunogenicity of SARS-CoV-2 vaccinations, in which several underlying mechanisms are likely to be involved.

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage [2]. Reactive species - including reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) - are chemically reactive molecules with essential physiological functions, albeit an overproduction under pathological circumstances may result in oxidative stress. The systemic redox status is reliably reflected by extracellular free thiols, which are organosulfur compounds carrying a free sulfhydryl (R–SH) group that are rapidly oxidized by ROS and other reactive species, resulting in reduced levels of free thiols in circumstances of oxidative stress [3–5]. Aside from ROS, sulfhydryl groups are at least equally receptive to oxidative modification by RSS and RNS, including hydrogen sulfide (H_2S) and nitric oxide (NO)-related metabolites. A few years ago, an integrative conceptual framework was created that aims to describe the interactions among different types of reactive species, including ROS, RNS, RSS, and reactive carbonyl species (RCS), as well as their interactions with downstream biological targets [6–8]. In particular, an important role in the RSI is fulfilled by RSS, and cysteine-based redox switches (consisting of extracellular free thiols) are considered to play a central role in the RSI since they serve as the main transducing components of redox regulation [6,9]. Extracellular free thiols, consisting of both protein-bound free thiols and low-molecular-weight (LMW) free thiols like glutathione and cysteine, not only present potent antioxidant buffering capacity, they also direct a variety of transducing proteins such as membrane transporters, ion channels, enzymes, and transcription factors, and enable both short-term and longer-term biological adaptations [6]. Thus, quantification of serum free thiol concentrations is often considered an easy, minimally invasive, reproducible, and robust method to determine the degree of systemic oxidative stress.

Recently, direct evidence has been provided with regard to the presence of oxidative stress in COVID-19, even in non-hospitalized individuals with mild COVID-19 [10–12]. At a functional level, this may result in modulation of the SARS-CoV-2-targeted immune response, since reactive species have previously been shown to modulate many cellular defense pathways, thereby allowing for adaptation to changes in the oxidative micro-environment [13]. For example, ROS have been demonstrated to block the activation of the stimulator of interferon genes (STING), thereby suppressing the type I interferon (IFN) response [14]. This finding is especially relevant to COVID-19, in which a dysregulated IFN response is considered to be involved in the progression to severe illness [15]. Similar mechanisms could also operate in the immunological response to SARS-CoV-2 vaccination.

Patients with kidney diseases are known to have higher levels of oxidative stress, but they are also at increased risk of developing severe COVID-19, which may be attributable to poorly functioning physiological defense mechanisms [4,16,17]. In this study, we therefore hypothesized that the serological antibody response to SARS-CoV-2 vaccination may be related to serum free thiol concentrations in patients with an impaired kidney function or on kidney replacement therapy. Furthermore, we aimed to examine a potential relationship between a disrupted whole-body redox balance and the efficiency of the innate immune response in patients with an impaired kidney function or on kidney replacement therapy. To this end, we determined serum concentrations of the chemokine IFN-γ-induced protein 10 (IP-10), as a surrogate biomarker for the IFN response.

Abbreviations

BMI	Body mass index
CKD G4/5	Chronic kidney disease stages 4/5
CNI	Calcineurin inhibitors
COVID-19	Coronavirus disease 2019
CRP	*C*-reactive protein
eGFR	Estimated glomerular filtration rate
FT	Free thiols
Hb	Hemoglobin
IFN	Interferon
IP-10	Interferon-γ-induced protein 10
KEAP1	Kelch-like ECH-associated protein 1
KTR	Kidney transplant recipients
MCP-1	Monocyte chemoattractant protein 1
MMF	Mycophenolate mofetil
mTOR	Mammalian target of rapamycin
MxA	Myxovirus-resistance protein A
NF-κB	Nuclear factor κB
NRF2	Nuclear factor erythroid 2-related factor 2
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSS	Reactive sulfur species
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
STING	Stimulator of interferon genes
WBC	White blood cell count

2. Materials and methods

2.1. Study population and study design

This study was carried out as a post-hoc analysis by using data from the RECOVAC immune response (IR) study, which is a prospective, controlled multicenter study aimed at examining the immunogenicity and safety of SARS-CoV-2 vaccination in patients with kidney disease and KTR [18]. Written informed consent was obtained from all study participants. In total 162 patients and 50 control subjects participating in the University Medical Center Groningen (UMCG) were included in the current study, including patients with chronic kidney disease stages 4–5 (CKD G4/5) ($n = 46$), patients on dialysis ($n = 43$), KTR ($n = 73$), and control subjects ($n = 50$). The CKD G4/5 group included patients that were not on dialysis nor had a history of kidney transplantation. Patients on dialysis, including hemodialysis and peritoneal dialysis, had no history of kidney transplantation nor used immunosuppressive therapy. In a subset of KTR ($n = 28$), kidney transplantations were preemptive, meaning transplantation was performed prior to the initiation of dialysis. The control subjects were spouses, siblings or household members of participants in the other study groups. All participants received two doses of mRNA-1273 SARS-CoV-2 vaccination (Moderna Biotech Spain, S.L.) with an interval of 28 days in accordance with the manufacturer's guidelines. The study was approved by the Institutional

Review Board (IRB) of the UMCG (IRB no. 2020/662) and carried out according to the principles of the Declaration of Helsinki (2013).

2.2. Data collection

As part of the RECOVAC-IR study, information on demographics, body mass index (BMI), smoking history, medication use, and medical history was collected for all included subjects. Blood samples were collected at baseline (i.e., prior to vaccination), one month after the first vaccination, 28 days after the second vaccination, and at 6 months follow-up. In all participants, standard laboratory measurements were performed, including hemoglobin (Hb), white blood cell count (WBC), platelet count, *C*-reactive protein (CRP), creatinine, and albumin. Serum albumin and CRP were measured by turbidimetry using an automated analyzer (Roche Modular, Roche Diagnostics, Mannheim, Germany), whereas serum creatinine was measured by photometry (Roche Modular, Roche Diagnostics, Mannheim, Germany). An automated hematology analyzer (Sysmex XE-2100, Sysmex Corporation, Kobe, Japan) was used to measure Hb, WBC, and platelet count. The estimated glomerular filtration rate (eGFR) was calculated by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Serum levels of *anti*-S1 SARS-CoV-2 IgG antibodies were measured with a validated fluorescent bead-based multiplex-immunoassay with a specificity of 99.7% and sensitivity of 91.6%, as previously described [19,20]. Levels were expressed as international binding antibody units per mL (BAU/mL). Measurements were performed on all samples collected at the different time points. Baseline *anti*-S1 SARS-CoV-2 IgG antibodies were quantified to identify and exclude subjects who had a SARS-CoV-2 infection prior to vaccination, whereas measurements at the second and third time points were performed to assess the humoral immune response after the first and second vaccination, respectively. Participants were classified as responder or non-responder based on seroconversion, with a threshold for seropositivity set at a level of *anti*-S1 SARS-CoV-2 IgG antibodies $\geq$10 BAU/mL [21]. This threshold was determined based on previously performed receiver operating characteristics analysis [20].

2.3. Measurements of serum free thiols and IP-10

As for the current study, concentrations of free thiols and IP-10 were measured in the serum samples reused from the RECOVAC-IR study. Serum concentrations of free thiols were measured at two different time points: at baseline (prior to vaccination) and immediately before second vaccination. Measurements were performed as previously described, but with minor modifications [22,23]. Serum samples were stored at −80 °C until further analysis to avoid unintended thiol oxidation. After thawing, samples were four-fold diluted using 0.1 M Tris buffer (pH 8.2). Background absorbance was measured using the CLARIOstar Plus microplate reader (BMG Labtech, Ortenberg, Germany) at 412 nm, alongside a reference measurement at 630 nm. Subsequently, samples were incubated with 20 μL 1.9 mM 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent, CAS no. 69-78-3, Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M phosphate buffer (pH 7.0) for 20 min at room temperature. Subsequently, sample absorbance was measured again. Final concentrations of serum free thiols were determined by parallel measurement of an L-cysteine (CAS no. 51-90-4, Fluka Biochemika, Buchs, Switzerland)-based calibration curve (range: 15.6–1000 μM) in 0.1 M Tris/10 mM EDTA (pH 8.2). Intra- and inter-day coefficients of variation (CV) were all <10%. Serum free thiol concentrations were also adjusted to serum albumin concentrations by calculating the free thiol/albumin ratio (expressed as μmol/g of albumin). This procedure was additionally performed to rule out the potential effect of albumin on the amount of potentially detectable free thiols, since serum albumin harbors the largest amount of free thiols in physiological conditions [24]. Concentrations of serum IP-10 were measured by enzyme-linked immunosorbent assay (ELISA) (Duoset DY266, R&D Systems, Minneapolis, Canada), as previously described [25].

2.4. Statistical analysis

Continuous data were presented as mean ± standard deviation (SD) in case of normal distributions, and as medians with interquartile ranges (IQR) for skewed data. Categorical data were presented as proportions *n* with corresponding percentages (%). Assessment of normality was performed both visually, using histograms and normal probability (Q-Q) plots, and statistically using Shapiro-Wilk normality tests. Differences between two study groups were assessed using independent sample *t*-tests or Mann-Whitney *U*-tests, depending on data distribution. Comparisons between more than two study groups for continuous variables were tested using one-way analysis of variance (ANOVA) or Kruskal-Wallis tests, depending on data distribution. To better specify the observed differences between groups, post-hoc analyses were performed - Games-Howell post-hoc analysis in case of ANOVA and Dunn-Bonferroni post-hoc analysis secondary to Kruskal-Wallis tests. As for categorical variables, comparison between groups were performed using chi-square tests or Fisher's exact tests, as appropriate. Pairwise Z-tests with Bonferroni corrections were performed following chi-square as post-hoc testing of categorical data. Within-group comparisons for continuous skewed variables were performed using Wilcoxon signed-rank tests (2 time points) or Friedman tests ($\geq$3 time points).

To identify subject parameters that were associated with both *anti*-S1 SARS-CoV-2 IgG antibody titers and seroconversion rates, linear regression analyses and logistic regression analyses were performed, respectively. Standardized values (Z-scores) of continuous variables were used for analysis, whereas *anti*-S1 SARS-CoV-2 IgG antibody titers - as outcome variable - were log-transformed. Multivariable linear and logistic regression analyses using backward selection (P_{OUT}>0.05) was performed to identify factors significantly associated with seroconversion rates and antibody titers, which were subsequently adjusted for when studying relationships between the biomarkers of interest (FT and IP-10) and seroconversion rates or *anti*-S1 SARS-CoV-2 IgG antibody titers. To identify potential interaction effects of albumin-adjusted free thiols with relevant clinical variables on seroconversion rates, logistic regression analyses were performed across various relevant clinical subgroups and through testing for potential effect modification by including terms in the models ($P_{interaction} < 0.05$). Furthermore, additional mediation analyses were conducted to analyze whether the associations between MMF use and seroconversion rates and/or *anti*-S1 SARS-CoV-2 IgG titers were mediated by albumin-adjusted free thiols. This was done by performing distinct regression models to analyze every association separately, followed by an estimation of the indirect effect of MMF use on the outcomes seroconversion rates and *anti*-S1 SARS-CoV-2 IgG titers using the SPSS PROCESS macro v4.2 extension. Regarding associations with seroconversion rates, odds ratios, corresponding 95% confidence intervals (CI), and *P*-values were reported. As for associations with *anti*-S1 SARS-CoV-2 IgG antibody titers, standardized β-coefficients and *P*-values were reported to indicate strength, direction and significance of observed associations. St. β-coefficients represented the difference in albumin-adjusted serum free thiol concentrations per 1-SD increment for continuous variables and the difference in free thiol concentrations compared to the implied reference group for categorical variables. Two-sided *P*-values <0.05 were considered to be statistically significant. The IBM SPSS Statistics 28.0 software package (SPSS Inc., Chicago, IL, USA) and Python programming language (v.3.9.0, Python Software Foundation) were used for data analysis and data visualization, using the *pandas* (v.1.2.3), *numpy* (v.1.20.0), *matplotlib* (v.3.4.1), *seaborn* (v.0.11.1), and *zepid* (v.0.9.0) packages.

3. Results

3.1. Cohort characteristics

Baseline characteristics per study group are presented in Table 1. No significant differences were observed in age ($P = 0.194$), gender ($P = 0.460$), or BMI ($P = 0.681$) between the groups. Immunosuppression was only used by KTR, in the following order of frequency: steroids (98.6%), calcineurin inhibitors (CNI) (90.4%), mycophenolate mofetil (MMF) (86.1%), azathioprine (4.1%), and mammalian target of rapamycin (mTOR) inhibitors (2.7%). Regarding the dialysis group, 72.1% of cases ($n = 31$) received hemodialysis and 27.9% of cases ($n = 12$) peritoneal dialysis. One individual in the control group was seropositive for *anti*-S1 SARS-CoV-2 IgG antibodies at baseline and was therefore excluded from further analysis.

3.2. Serum free thiol and IP-10 concentrations in kidney patients

Serum free thiol concentrations were significantly lower in patients with CKD G4/5 (mean ± SD 124.0 ± 50.4 μM, $P < 0.001$), on dialysis (137.5 ± 62.2 μM, $P < 0.001$), and KTR (150.5 ± 41.3 μM, $P < 0.001$), as compared to controls (190.6 ± 36.5 μM) (Fig. 1A). Similar results were observed after adjustment for albumin, with significantly reduced albumin-adjusted serum concentrations of free thiols in patients with CKD G4/5 (median [IQR] 2.8 [2.1–3.5] μmol/g of albumin, $P < 0.001$), on dialysis (3.1 [2.3–4.6] μmol/g, $P < 0.001$), and KTR (3.5 [2.9–4.1] μmol/g, $P < 0.001$), as compared to controls (4.4 [3.7–4.9] μmol/g). Additional differences in albumin-adjusted serum free thiols were observed between CKD G4/5 patients and KTR ($P = 0.036$). Among dialysis patients, albumin-adjusted serum free thiols did not differ significantly between patients on hemodialysis (3.2 [2.4–4.6] μmol/g) and peritoneal dialysis (3.0 [1.7–4.5] μmol/g, $P = 0.498$). Furthermore, among KTR, no significant differences were observed in albumin-adjusted serum free thiols between MMF users (3.5 [2.9–4.1] μmol/g) and non-MMF users (3.6 [2.5–4.0] μmol/g, $P = 0.712$). Serum free thiol levels were stable over time and demonstrated no significant differences between measurements at baseline and after vaccination in patients with CKD G4/5 ($P = 0.481$), on dialysis ($P = 0.913$), KTR ($P = 0.228$), and controls ($P = 0.348$).

Baseline IP-10 concentrations were significantly different between the groups ($P = 0.011$), with significantly higher concentrations in dialysis patients (28.8 [10.7–74.5] pg/mL) than in KTR (9.0 [0.1–52.3] pg/mL, $P = 0.007$) and controls (9.8 [0.1–32.2] pg/mL, $P = 0.004$) (Fig. 1B). IP-10 concentrations significantly increased over time in both KTR ($P = 0.040$) and controls ($P = 0.001$) (Supplementary Table S1). Finally, in KTR no significant associations were observed between baseline albumin-adjusted serum free thiol concentrations and IP-10 concentrations at baseline (St.β = 0.030, $P = 0.799$), after one dose of vaccination (St.β = −0.041, $P = 0.730$), or after two doses of vaccination (St.β = 0.017, $P = 0.891$).

3.3. Anti-S1 SARS-CoV-2 IgG antibody response

Seroconversion rates after two doses of vaccination were reduced in KTR when compared to controls (52.1% vs. 100%, $P < 0.001$), whereas seroconversion rates were high in both CKD G4/5 (100%) and dialysis (100%) patients (Table 2, Fig. 2). Thus, non-responders in this study were only present among KTR, with 52.1% of KTR ($n = 38$) being responder and 47.9% of KTR ($n = 35$) being non-responder. After one dose of vaccination, seroconversion rates were significantly lower in KTR (13.7%) as compared to patients with CKD G4/5 (95.7%, $P < 0.001$), on dialysis (81.4%, $P < 0.001$) and controls (98.0%, $P < 0.001$). Furthermore, significant differences in seroconversion rates after the first dose of vaccination were observed between patients on dialysis and controls (81.4% vs. 98.0%, $P = 0.042$).

The results of reduced seroconversion rates in KTR are supported by the lower *anti*-S1 SARS-CoV-2 IgG antibody levels, with levels after two doses of vaccination being significantly lower in KTR (11.3 [3.7–200.9] BAU/mL), as compared to controls (2967.7 [1883.6–4286.4] BAU/mL, $P < 0.001$). Although seroconversion rates after two doses of vaccination

Table 1
Baseline demographic, clinical, and biochemical characteristics per study group.

	Control ($n = 50$)	KTR ($n = 73$)	Dialysis ($n = 43$)	CKD G4/5 ($n = 46$)	*P*-value
Age (years)	62.0 [50.0–68.8]	58.0 [47.5–67.0]	61.0 [51.0–71.0]	61.5 [50.8–68.0]	0.194
Female, n (%)	29 (58.0)	36 (49.3)	19 (44.2)	20 (43.5)	0.460
BMI (kg/m^2)	27.4 [25.0–30.5]	27.7 [24.8–30.7]	26.8 [24.1–31.0]	28.1 [25.0–33.0]	0.681
Current smoking, n (%)	6 (12.0)	2 (2.7)	11 (25.6)	4 (8.7)	**0.002**
Current immunosuppression	–	73 (100)	–	–	–
Steroids		72 (98.6)			
Azathioprine		3 (4.1)			
MMF		63 (86.1)			
CNIs		66 (90.4)			
mTOR inhibitor		2 (2.7)			
Comorbidities					
Hypertension, *n* (%)	19 (38.0)	54 (74.0)	25 (58.1)	32 (69.6)	**<0.001**
Diabetes mellitus, *n* (%)	6 (12.0)	12 (16.4)	12 (27.9)	9 (19.6)	0.242
Coronary artery disease, *n* (%)	5 (10.0)	8 (11.0)	9 (20.9)	7 (15.2)	0.386
Heart failure, *n* (%)	1 (2.0)	4 (5.5)	4 (9.3)	5 (10.9)	0.258
Chronic lung disease, *n* (%)	7 (14.0)	4 (5.5)	1 (2.3)	6 (13.0)	0.102
Past malignancy, *n* (%)	4 (8.0)	8 (11.0)	11 (25.6)	6 (13.0)	0.072
Auto-immune disease, *n* (%)	1 (2.0)	4 (5.5)	0 (0)	2 (4.3)	0.486
Laboratory measurements					
Hb (g/dL)	8.7 [8.1–9.2]	7.9 [7.3–8.7]	6.9 [6.1–7.5]	7.4 [6.7–8.3]	**<0.001**
CRP (mg/L)	1.8 [1.0–3.3]	1.7 [1.1–4.7]	3.7 [1.8–7.0]	2.3 [1.0–4.8]	**0.012**
WBC (× 10^9/L)	6.0 [5.0–7.7]	8.2 [6.6–10.4]	6.9 [5.6–8.8]	6.6 [5.6–7.8]	**<0.001**
Neutrophils (× 10^9/L)	3.5 [2.7–4.5]	6.5 [5.0–8.7]	4.5 [3.5–5.9]	4.3 [3.6–5.1]	**<0.001**
Platelets (× 10^9/L)	244.0 [216.0–292.5]	239.0 [205.0–290.0]	250.0 [199.0–288.0]	220.0 [190.0–251.0]	**0.029**
Albumin (g/L)	45.0 [43.0–46.0]	43.0 [41.5–45.0]	41.0 [38.0–45.0]	43.0 [41.0–45.5]	**0.001**
Creatinine (μmol/L)	74.0 [63.5–87.5]	122.0 [96.5–158.5]	669.0 [450.0–818.0]	282.0 [225.0–365.0]	**<0.001**
eGFR (mL/min/1.73m [2])	83.5 [70.4–95.5]	50.5 [38.4–62.0]	N/A	17.6 [13.1–23.7]	**<0.001**

Data are presented as mean ± SD, median [IQR] or proportion *n* with corresponding percentages (%). *P*-values <0.05 were considered statistically significant (indicated in bold). Abbreviations: BMI, body mass index; CKD G4/5, chronic kidney disease stages 4/5; CNI, calcineurin inhibitors; CRP, *C*-reactive protein; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; IP-10, interferon-γ-induced protein 10; KTR, kidney transplant recipients; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; N/A, not applicable; WBC, white blood cell count.

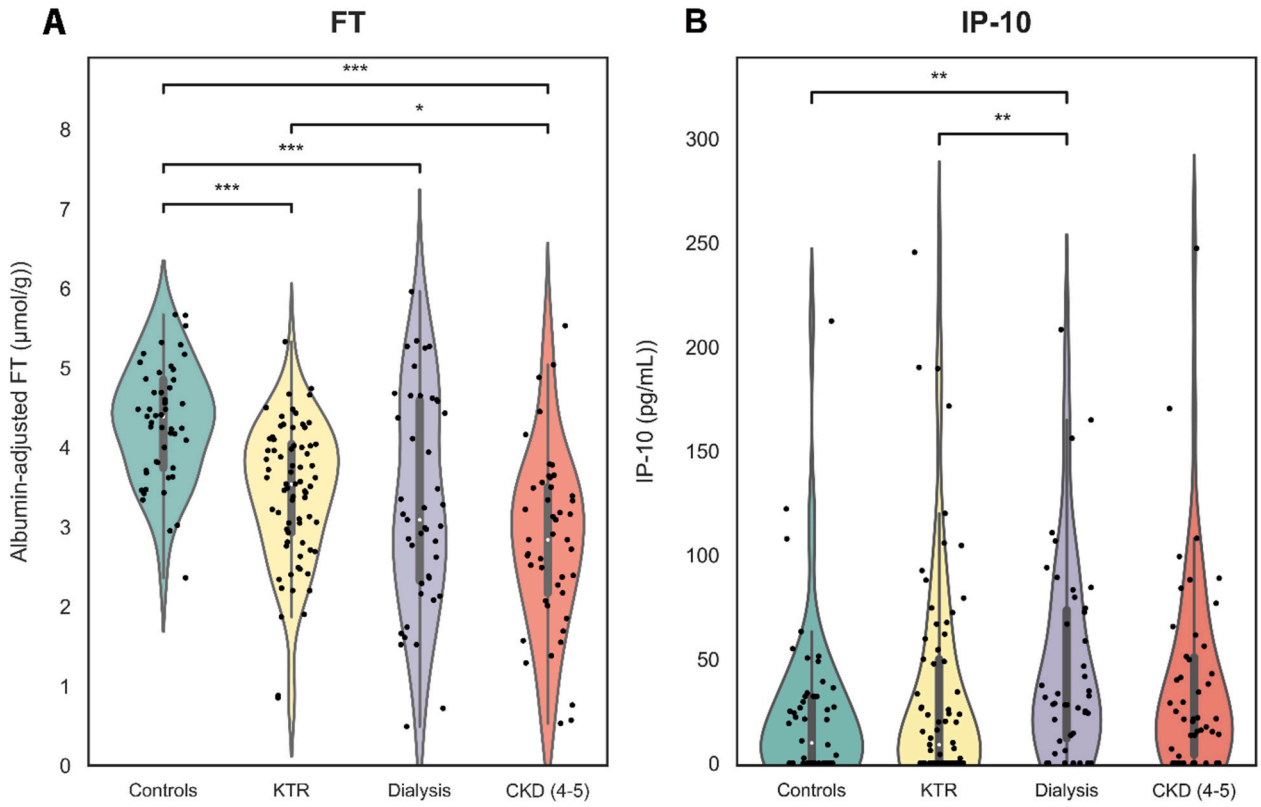

Fig. 1. Serum FT and IP-10 levels in patients with kidney disease, kidney transplant recipients, and controls. (**A**) Serum albumin-adjusted FT concentrations were significantly reduced in all patient groups compared with controls and significantly lower in patients with CKD G4/5 than in KTR. (**B**) Serum IP-10 levels were significantly higher in patients on dialysis than in KTR and controls. Statistical analyses were performed using Kruskal-Wallis test with post-hoc analysis in all study groups. $^{**}P < 0.01$, and $^{***}P < 0.001$. Abbreviations: CKD (4–5), chronic kidney disease stages 4/5; FT, free thiols; IP-10, interferon-γ-induced protein 10; KTR, kidney transplant recipients.

Table 2
Anti-S1 SARS-CoV-2 IgG antibody titers and seroconversion rates after first (upper) and second (lower) dose of vaccination, and at 6 months follow-up.

	Control ($n = 49$)	KTR ($n = 73$)	*P*-value	Dialysis ($n = 43$)	*P*-value	CKD G4/5 ($n = 46$)	*P*-value
First dose of vaccination							
Responder, *n* (%)	48 (98.0)	10 (13.7)	<0.001	35 (81.4)	0.042	44 (95.7)	NS
S1 IgG antibody titers (BAU/mL)	353.4 [153.2–676.5]	0.8 [0.6–4.1]	**<0.001**	67.6 [11.4–175.4]	**<0.001**	195.8 [87.9–320.0]	0.459
Second dose of vaccination							
Responder, *n* (%)	49 (100)	38 (52.1)	**<0.001**	43 (100.0)	NS	46 (100.0)	NS
S1 IgG antibody titers (BAU/mL)	2967.7 [1883.6–4286.4]	11.3 [3.7–200.9]	**<0.001**	1549.4 [675.6–2489.6]	**0.016**	2614.4 [1610.4–4383.9]	1.000
6 months follow-up*							
Responder, *n* (%)	48 (100)	42 (58.3)	**<0.001**	39 (100)	NS	43 (100)	NS
S1 IgG antibody titers (BAU/mL)	477.3 [266.1–760.9]	15.4 [2.0–67.7]	**<0.001**	131.8 [63.6–430.4]	**0.001**	541.0 [228.2–1171.0]	0.865

Data are presented as median [IQR] or proportion *n* with corresponding percentages (%). *P*-values were calculated using Kruskal-Wallis test with post-hoc analysis in case of continuous skewed data and chi-square test with post-hoc analysis in case of categorical data. *P*-values <0.05 were considered statistically significant (indicated in bold). *Control ($n = 1$) and patients with CKD G4/5 ($n = 3$), on dialysis ($n = 4$), and KTR ($n = 1$) were removed from analysis as they received a COVID-19 diagnosis during the study (after second vaccination) or data at 6 months follow-up was not available. Abbreviations: CKD G4/5, chronic kidney disease stages 4/5; KTR, kidney transplant recipients; IgG, immunoglobulin G; NS, non-significant; S1, Spike 1.

were 100% in patients on dialysis, their antibody levels at the same time point were significantly lower than in controls ($P = 0.016$). Among dialysis patients, antibody levels did not differ significantly between patients on hemodialysis (1580.2 [843.1–2888.0] BAU/mL) and peritoneal dialysis (1172.8 [477.3–2014.5] BAU/mL, $P = 0.522$). No significant differences were observed between CKD G4/5 patients and controls after the first dose of vaccination (195.8 [87.9–320.0] vs. 353.4 [153.2–676.5], $P = 0.459$) and second dose of vaccination (2614.4 [1610.4–4383.9] vs. 2967.7 [1883.6–4286.4], $P = 1.000$). At 6 months follow-up, *anti*-S1 SARS-CoV-2 antibody levels significantly decreased as compared to those measured after two doses of vaccination in seropositive individuals in all study groups ($P < 0.001$) (Supplementary Table S1).

3.4. Associations between serum FT and IP-10 levels with seroconversion rates and anti-S1 SARS-CoV-2 IgG antibody titers in kidney transplant recipients

As a large proportion of the KTR group were non-responders after two vaccinations, baseline predictors of seroconversion rates were investigated in these patients. Multiple baseline characteristics differed significantly between responders and non-responders (Supplementary Table S2). Logistic regression analysis was performed to examine their associations with responder status (Fig. 3). Univariable analysis demonstrated that free thiol concentrations (OR = 1.67 per SD, $P = 0.049$), albumin-adjusted free thiol concentrations (OR = 1.76 per SD, $P = 0.033$), eGFR (OR = 2.28, $P = 0.004$), and Hb (OR = 2.41, $P = 0.003$)

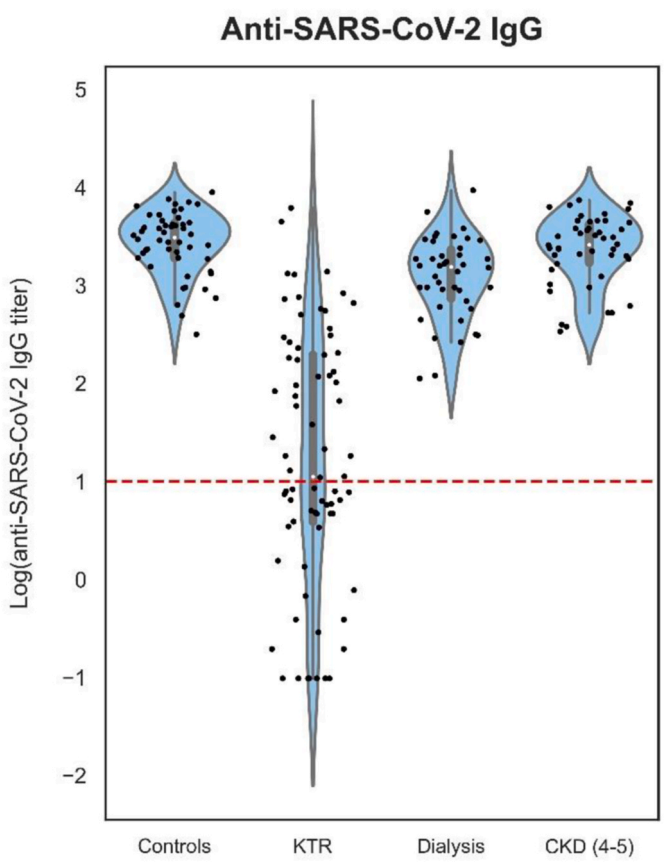

Fig. 2. Log-transformed *anti*-S1 SARS-CoV-2 IgG antibody titers in patients with CKD G4/5, on dialysis, KTR, and controls. Antibody titers were lowest in KTR compared with all other groups ($P < 0.001$). Non-responders to two doses of vaccination were only present among KTR, whereas seroconversion rates were 100% for the other groups. Statistical analyses were performed using Kruskal-Wallis test with post-hoc analysis in all study groups. Abbreviations: CKD (4–5), chronic kidney disease stages 4/5; IgG, immunoglobulin G; KTR, kidney transplant recipients; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

were significantly positively associated with being a responder, whereas the current use of MMF demonstrated an inverse association with responder status (OR = 0.10, $P = 0.030$). After adjustment for MMF use, Hb, and eGFR, however, the significance of the association with albumin-adjusted serum free thiol concentrations (OR = 1.49, $P = 0.241$) vanished. Additional univariable linear regression analysis demonstrated that albumin-adjusted free thiols significantly co-correlated with eGFR (St.β = 0.373, $P = 0.001$), but not with Hb (St.β = 0.203, $P = 0.085$) or MMF use (St.β = 0.136, $P = 0.250$). No significant interactions were observed of albumin-adjusted free thiols with Hb ($P = 0.648$), eGFR ($P = 0.808$), and MMF use ($P = 0.293$) and associations with responder status (Supplementary Table S3). Finally, mediation analysis did not support albumin-adjusted free thiols as mediator in the association between MMF use and seroconversion rates, as the bootstrap 95% CIindicated that the indirect effect was not significant (B = 0.214, 95% CI = −0.460, 1.015).

Serum IP-10 levels showed no association with seroconversion rates (OR = 1.16 per SD, $P = 0.546$), neither after adjustment for MMF use, Hb, and eGFR (OR = 0.79 per SD, $P = 0.446$). This is in line with our findings that IP-10 levels did not significantly differ between responders and non-responders (Supplementary Table S2). When comparing logistic regression models, we observed that a model including albumin-adjusted free thiols and IP-10 – in addition to the clinical variables Hb, eGFR, and MMF use – performed significantly better than a model with clinical variables only (likelihood ratio [LR] 1.87, Nagelkerke R^2 [R^2_N] 0.424, $P < 0.001$) (Supplementary Table S6).

As for *anti*-S1 SARS-CoV-2 IgG antibody levels, albumin-adjusted free thiol concentrations (St.β = 0.240, $P = 0.040$), Hb concentrations (St.β = 0.290, $P = 0.013$), and eGFR (St.β = 0.334, $P = 0.004$) showed a significant positive association with log-transformed antibody titers, whereas MMF use was inversely correlated to log-transformed antibody titers (St.β = −0.346, $P = 0.003$) in univariable linear regression analyses (Fig. 4, Supplementary Table S7). In multivariable linear regression analysis with backward selection, only MMF use (St.β = −0.364, $P < 0.001$) and eGFR (St.β = 0.352, $P = 0.001$) appeared to be independently associated with antibody titers. Albumin-adjusted free thiol concentrations did not reach statistical significance in multivariable regression analysis (St.β = 0.138, $P = 0.229$), nor was there a significant association between IP-10 concentrations and log-transformed *anti*-S1 SARS-CoV-2 IgG antibody titers (St.β = 0.010, $P = 0.927$). Mediation analysis suggested that the association between MMF use and *anti*-S1 SARS-CoV-2 IgG titers was not mediated by albumin-adjusted FT, as the indirect effect was not significant, as demonstrated by the bootstrap 95% confidence intervals (B = 0.103, 95% CI -0.218, 0.439).

In linear regression analysis, no significant associations of albumin-adjusted free thiols and IP-10 concentrations with *anti*-S1 SARS-CoV-2 IgG antibody titers were present in other study groups (Fig. 4, Supplementary Table S7).

4. Discussion

In this study, we investigated the association between serum concentrations of free thiols - as surrogate biomarker for systemic oxidative stress - and the humoral immune response to SARS-CoV-2 vaccination in patients with impaired kidney function or on kidney replacement therapy. Most importantly, we demonstrated that KTR show markedly reduced seroconversion rates after two doses of the mRNA-1273 vaccine (Moderna Biotech Spain, S.L.), which was significantly associated with baseline serum concentrations of free thiols in univariable analysis. This association was not sustained, however, when adjusting for relevant clinical confounding factors. Our findings may contribute the understanding of suboptimal immune responses to vaccination in KTR. Indeed, decreased seroconversion rates in response to SARS-CoV-2 vaccination in KTR have previously been demonstrated by others, which appeared to be associated with the use of immunosuppression, older age, and reduced kidney function, among others [21,26] Our results indicate that a disrupted whole-body redox balance may play a role in the observed blunted humoral immune response.

Our results show a significant positive association between albumin-adjusted serum free thiols – as a marker of systemic oxidative stress – with achieving antibody titers above the serological protection threshold c.q. being a responder to *anti*-SARS-CoV-2 vaccination in KTR. Interestingly, systemic oxidative stress was particularly increased in patients with CKD G4/5, whereas an association with responder status was only found in KTR. This observation implies that, regarding a positive serological response to SARS-CoV-2 vaccination, an unfavorable redox status is primarily of relevance in circumstances of a suppressed immune system. Of note, no such association was observed when adjusting for factors that were also significantly associated with seroconversion rates. Considering this, the observed positive association between albumin-adjusted serum free thiols and the probability of being a responder is likely to (at least partially) be explained by their use of MMF and/or their significantly lower eGFR and Hb concentrations. In literature, eGFR and Hb levels are known to be positively associated with serum free thiols [23]. Indeed, our results demonstrated a significant correlation between serum free thiols and eGFR in KTR, which may have influenced our results. Mycophenolate mofetil (MMF, CellCept®) is an immune-suppressing antimetabolite that selectively inhibits T-cell and B-cell proliferation by inhibiting the synthesis of purine [27]. The association between MMF use and oxidative stress is currently uncertain with existing literature reporting conflicting results [28–31]. In the

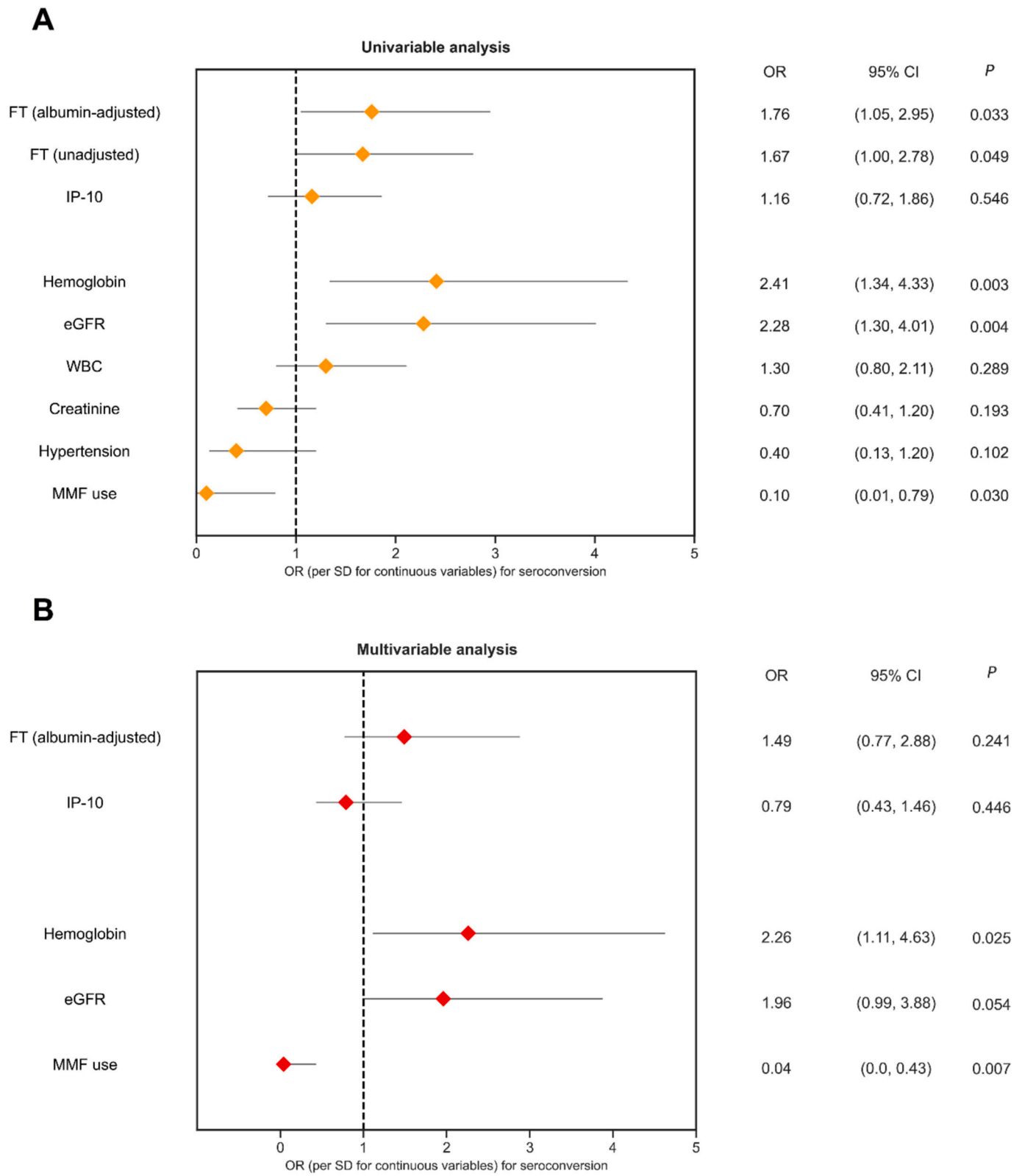

Fig. 3. Forest plots demonstrating odds ratios (OR) with corresponding 95% confidence intervals (CI) for associations between patient characteristics and serum FT and IP-10 levels and the odds of achieving seroconversion after two doses of vaccination. Results are presented from univariable (panel A) and multivariable (B) logistic regression analyses. ORs for continuous variables correspond with 1 SD increment. *P*-values <0.05 were considered statistically significant. Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; FT, free thiols; IP-10, interferon-γ-induced protein 10; MMF, mycophenolate mofetil; OR, odds ratio; SD, standard deviation; WBC, white blood cell count.

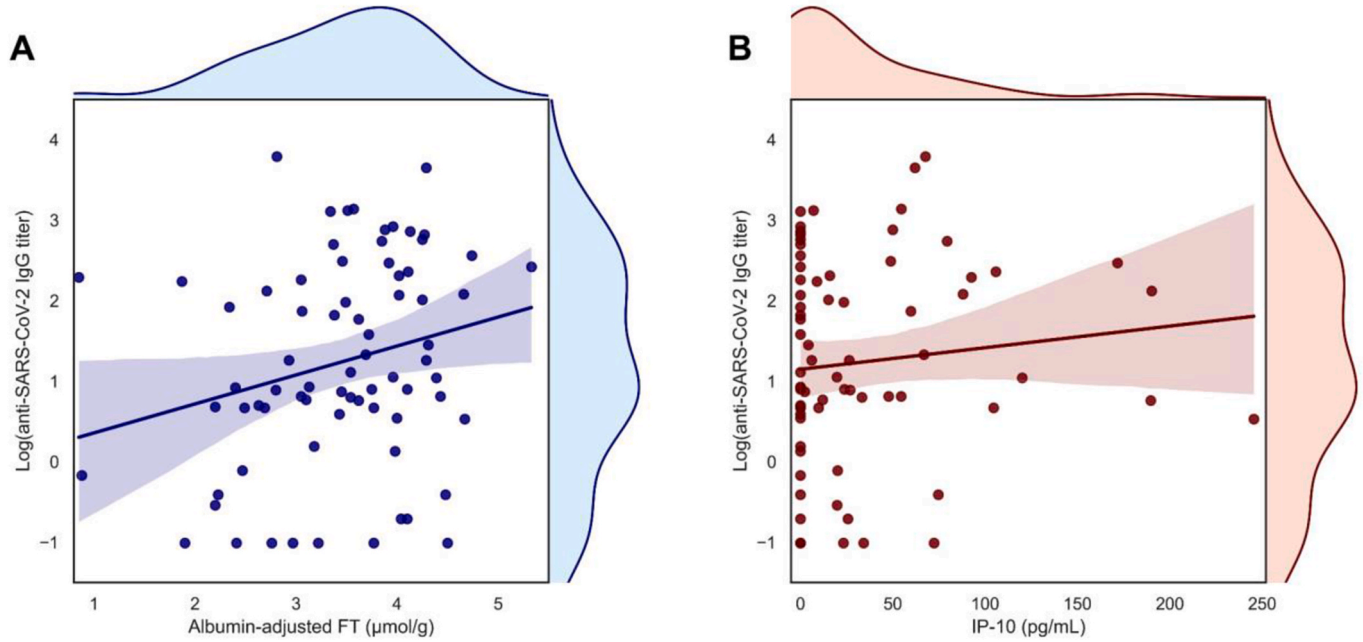

Fig. 4. Pre-vaccination serum levels of albumin-adjusted FT correlate with log-transformed *anti*-S1 SARS-CoV-2 IgG antibody titers after two doses of vaccination, whereas baseline IP-10 levels do not. In linear regression analyses, albumin-adjusted FT levels were indeed significantly associated with log-transformed *anti*-S1 SARS-CoV-2 IgG antibody titers, albeit significance vanished after adjustment for confounding variables. Abbreviations: FT, free thiols; IgG, immunoglobulin G; IP-10, interferon-γ-induced protein 10; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

current study, serum free thiols were similar between MMF users and non-MMF users among KTR and no significant association was present between free thiols and MMF use. Multivariable analysis, however, demonstrated MMF to be independently inversely associated with being a responder and with *anti*-S1 SARS-CoV-2 IgG antibody titers, which is in line with previous studies, in which the use of MMF was identified as an important factor influencing the immunogenicity of vaccination in KTR and other immunocompromised individuals [21,32,33]. Our observations raises questions on whether and how the use of immunosuppression in KTR may alter the systemic redox status, which should be elucidated in future studies.

Reactive species are able to affect the host immune response by modulation of various cellular defense pathways [13]. For example, ROS may modify the activity of transcription factors, including the nuclear factor κB (NF-κB), involved in the activation of pro-inflammatory cytokines and chemokines, as well as in inflammasome regulation, all of which are important players in COVID-19 [13,34,35]. One way in which ROS may modulate immune responses is by blocking STING, thereby suppressing the IFNresponse [14]. Indeed, IFNs – upon induction in the innate immune response – have enhancing effects on adaptive immune cells (e.g., T-cells and B-cells), thereby mediating a crosstalk between innate and adaptive immunity during viral infection [36]. This is particularly relevant to COVID-19, where dysregulation of IFN-signaling is thought to be an important factor in the development of severe disease [15]. However, our results do not support our initial hypothesis that oxidative stress leads to a decrease in STING-induced IFNproduction, thereby negatively affecting the SARS-CoV-2 vaccination response. No significant association between baseline albumin-adjusted free thiols and IP-10 levels were found, nor did IP-10 levels significantly correlate with seroconversion rates or *anti*-S1 SARS-CoV-2 IgG antibody titers after two doses of vaccination in KTR. To this matter, several considerations need to be considered. First, we solely quantified IP-10 levels – an IFN-γ-induced chemokine – as surrogate biomarker for the IFN response. In line with our results in KTR and controls, increased IP-10 levels have previously been observed following SARS-CoV-2 infection and vaccination [37,38] and positive correlations between IP-10 levels and post-vaccination antibody responses exist for other infectious diseases [39,40]. However, other IFN-related proteins, such as monocyte chemoattractant protein 1 (MCP-1) and myxovirus-resistance protein A (MxA), are known markers of IFN expression and quantification thereof could possibly provide a more granular insight into the IFN signature [25]. Furthermore, as explained above, there are multiple mechanisms – in addition to IFN-signaling – in which reactive species may interfere with the humoral immune response after SARS-CoV-2 vaccination that could play a role in patients with impaired kidney function or those on kidney replacement therapy. Finally, the relation between oxidative stress and the antibody response after SARS-CoV-2 vaccination is likely to be influenced by other factors (vide supra).

Strengths of this study include its embedding in a prospectively conducted cohort study, which facilitated the analysis of associations between free thiols and IP-10 levels and *anti*-S1 SARS-CoV-2 IgG antibody titers, while taking into account a number of relevant clinical parameters from a comprehensively characterized dataset. Furthermore, we not only evaluated associations between baseline free thiols and IP-10 and *anti*-S1 SARS-CoV-2 IgG antibody titers after two doses of vaccination, but also at 6 months, allowing us to make observations at longer follow-up. In addition, we leveraged a highly-sensitive and validated fluorescence bead-based microparticle immunoassay, of which test characteristics have been associated with neutralization assays that are commonly considered to be biologically more accurate [41]. Several limitations of this study warrant recognition. First, we solely measured the immune response after two doses of vaccination, whereas the administration of additional vaccine doses has been recommended for kidney transplant recipients based upon multiple studies showing improved immune responses [42–44]. Second, we only quantified antibodies against the spike S1 protein of SARS-CoV-2 and thereby assessed humoral immunity, whereas we did not take into account cellular or T-cell-mediated immunity. Independent of antibody formation, however, T-cell-mediated immunity against SARS-CoV-2 is equally important for achieving adequate immunological protection against COVID-19. Indeed, post-vaccination T-cell responses are expected to be suppressed considering the inhibiting effect of immunosuppressive agents on T-cell function, and previous studies have provided direct evidence for a reduced T-cell response following SARS-CoV-2

vaccination in KTR [45–47]. Nevertheless, some studies have demonstrated that anti-spike S1-SARS-CoV-2 antibody levels correlated with the degree of protection against disease severity of COVID-19 [48]. Furthermore, the current study design was not appropriate for proper causality analyses, and cautious interpretation of our results on the correlation between systemic oxidative stress and reduced antibody responses to COVID-19 vaccination in KTR is warranted. Although an effort was made to address potential biases (including confounding, interaction, and mediation) to gain a mechanistic view on our results, no convincing evidence of causality could be provided. Since our results on the correlation between oxidative stress and post-vaccination *anti*-S1 SARS-CoV-2 IgG antibody responses pertain to KTR only, generalizability to other patient groups is limited. As such, future studies with causal study designs and with different study populations are necessary to clarify these concepts in more detail.

Finally, we solely quantified a single biomarker, represented by serum free thiols, of systemic oxidative stress. Although this biomarker has repeatedly been shown to provide a robust and powerful read-out of the *in vivo* systemic redox status [6], a more integrative approach incorporating multiple redox-regulated biomarkers would surely do better in characterizing alterations in the human redox signaling network and, thus, in that of systemically measurable levels of redox perturbations, especially considering the dynamic nature of oxidative stress as pathophysiological phenomenon. Combining stable biomarkers of different types of reactive species and of multiple redox-regulated metabolic pathways would be an ideal approach to establish integrative redox biomarker signatures. More recently, efforts to realize these combined approaches are on their way, but are lagging behind when compared to other biological areas since (consensus-based) criteria that candidate biomarkers should fulfill for reliable reflections of distinct reactive species as well as distinct redox-regulated metabolic pathways in clinical settings are currently lacking [7,8,49]. A few years ago, a mass spectrometry-based analysis of the systemic thiol redox metabolome, consisting of 12 specific thiol measurements, including total free thiols, was described, which could theoretically be utilized in different human biofluids [50]. Such biomarker platforms should be further optimized and be investigated for their potential use in clinical stratification and monitoring effects of redox-modulating therapeutics. In this context, pure "redox metabolomics" approaches are also being developed, albeit this builds another layer of complexity with several technological and methodological challenges that may appear [51]. However, a 'multi-omics' characterization of the critical elements of the RSI, consisting of 1) nutritional components (e.g. amino acids, H_2S-donors, and vitamins), 2) transducing elements, consisting of cysteine-based redox switches e.g. systemic free thiols, and 3) stable end products of the RSI, encompassing *S*-, *N*- and O-derived metabolites, could be employed to better understand the redox signaling network [6]. Also, such multi-omics approached would need to be installed within well-characterized patient cohorts, preferably complemented by routine measurements of blood parameters and accurate clinical metadata. These strategies could help to expand the granularity of the human redox architecture in clinical settings, while also providing clues to the key hubs of interactions underlying redox biology.

5. Conclusions

To conclude, we demonstrated that the suboptimal humoral immune response after 2 doses of the mRNA-1273 SARS-CoV-2 vaccine in KTR is associated with lower levels of serum free thiols indicating increased systemic oxidative stress. Although this association was not sustained after adjustment for relevant clinical confounding factors, it may nevertheless shed light on the potential involvement – albeit limited in effect size – of oxidative stress in the suboptimal antibody response to SARS-CoV-2 vaccination in KTR. Future studies are warranted to further elucidate the relationship between systemic oxidative stress and vaccination responses and to externally assess the value of serum free thiols as potential predictive biomarker for seroconversion after SARS-CoV-2 vaccination in KTR.

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CRediT authorship contribution statement

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradbiomed.2024.02.018.

References

[1] A. Lee, S.Y. Wong, L.Y.A. Chai, et al., Efficacy of covid-19 vaccines in immunocompromised patients: systematic review and meta-analysis, Br. Med. J. 376 (2022) e068632.
[2] H. Sies, Oxidative stress: a concept in redox biology and medicine, Redox Biol. 4 (2015) 180–183.
[3] A.R. Bourgonje, A.E. Abdulle, M.F. Bourgonje, et al., Serum free sulfhydryl status associates with new-onset chronic kidney disease in the general population, Redox Biol. 48 (2021) 102211.
[4] L. Boekhoud, J. Koeze, E.C. van der Slikke, et al., Acute kidney Injury is associated with lowered plasma-free thiol levels, Antioxidants 9 (11) (2020) 1135.
[5] N.A. Spraakman, A.M. Coester, A.R. Bourgonje, et al., Systemic and renal dynamics of free sulfhydryl groups during living donor kidney transplantation, Int. J. Mol. Sci. 23 (17) (2022) 9789.
[6] M.M. Cortese-Krott, A. Koning, G.G.C. Kuhnle, et al., The reactive species interactome: evolutionary emergence, biological significance, and opportunities for redox metabolomics and personalized medicine, Antioxidants Redox Signal. 27 (10) (2017) 684–712.
[7] E. Malard, S. Valable, M. Bernaudin, E. Pérès, L. Chatre, The reactive species interactome in the brain, Antioxidants Redox Signal. 35 (14) (2021) 1176–1206.
[8] A.R. Bourgonje, M. Feelisch, K.N. Faber, A. Pasch, G. Dijkstra, H. van Goor, Oxidative stress and redox-modulating therapeutics in inflammatory bowel disease, Trends Mol. Med. 26 (11) (2020) 1034–1046.
[9] K. Ono, T. Akaike, T. Sawa, et al., Redox chemistry and chemical biology of H2S, hydropersulfides, and derived species: implications of their possible biological activity and utility, Free Radic. Biol. Med. 77 (2014) 82–94.
[10] L.E. van Eijk, A. Tami, J.L. Hillebrands, et al., Mild coronavirus disease 2019 (COVID-19) is marked by systemic oxidative stress: a pilot study, Antioxidants 10 (12) (2021) 2022.
[11] A.K. Kalem, B. Kayaaslan, S. Neselioglu, et al., A useful and sensitive marker in the prediction of COVID-19 and disease severity: thiol, Free Radic. Biol. Med. 166 (2021) 11–17.
[12] B.F. Dagcioglu, A. Keskin, R. Guner, et al., Thiol levels in mild or moderate COVID-19 patients: a comparison of variant and classic COVID-19 cases, Int. J. Clin. Pract. 75 (11) (2021) e14753.
[13] M. Schieber, N.S. Chandel, ROS function in redox signaling and oxidative stress, Curr. Biol. 24 (10) (2014) R453–R462.
[14] L. Tao, A. Lemoff, G. Wang, et al., Reactive oxygen species oxidize STING and suppress interferon production, Elife 9 (2020) e57837.
[15] Y.M. Kim, E.C. Shin, Type I and III interferon responses in SARS-CoV-2 infection, Exp. Mol. Med. 53 (5) (2021) 750–760.
[16] M.P. Kao, D.S. Ang, A. Pall, A.D. Struthers, Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options, J. Hum. Hypertens. 24 (1) (2010) 1–8.
[17] V. Mahalingasivam, G. Su, M. Iwagami, M.R. Davids, J.B. Wetmore, D. Nitsch, COVID-19 and kidney disease: insights from epidemiology to inform clinical practice, Nat. Rev. Nephrol. 18 (8) (2022) 485–498.
[18] M.M.L. Kho, M.E.J. Reinders, C.C. Baan, et al., The RECOVAC IR study: the immune response and safety of the mRNA-1273 COVID-19 vaccine in patients with chronic kidney disease, on dialysis or living with a kidney transplant, Nephrol. Dial. Transplant. 36 (9) (2021) 1761–1764.
[19] G. den Hartog, R.M. Schepp, M. Kuijer, et al., SARS-CoV-2-Specific antibody detection for seroepidemiology: a multiplex analysis approach accounting for accurate seroprevalence, J. Infect. Dis. 222 (9) (2020) 1452–1461.
[20] G. den Hartog, E.R.A. Vos, L.L. van den Hoogen, et al., Persistence of antibodies to severe acute respiratory syndrome coronavirus 2 in relation to symptoms in a nationwide prospective study, Clin. Infect. Dis. 73 (12) (2021) 2155–2162.
[21] J.F. Sanders, F.J. Bemelman, A.L. Messchendorp, et al., The RECOVAC immune-response study: the immunogenicity, tolerability, and safety of COVID-19 vaccination in patients with chronic kidney disease, on dialysis, or living with a kidney transplant, Transplantation 106 (4) (2022) 821–834.
[22] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1) (1959) 70–77.
[23] M.F. Bourgonje, A.R. Bourgonje, A.E. Abdulle, et al., Systemic oxidative stress, aging and the risk of cardiovascular events in the general female population, Front Cardiovasc Med 8 (2021) 630543.
[24] L. Turell, R. Radi, B. Alvarez, The thiol pool in human plasma: the central contribution of albumin to redox processes, Free Radic. Biol. Med. 65 (2013) 244–253.
[25] W.M. Lambers, K. de Leeuw, B. Doornbos-van der Meer, G.F.H. Diercks, H. Bootsma, J. Westra, Interferon score is increased in incomplete systemic lupus erythematosus and correlates with myxovirus-resistance protein A in blood and skin, Arthritis Res. Ther. 21 (1) (2019) 260.
[26] B. Rozen-Zvi, D. Yahav, T. Agur, et al., Antibody response to SARS-CoV-2 mRNA vaccine among kidney transplant recipients: a prospective cohort study, Clin. Microbiol. Infect. 27 (8) (2021) 1173, e1-.e4.
[27] T.M. Sievers, S.J. Rossi, R.M. Ghobrial, et al., Mycophenolate mofetil, Pharmacotherapy 17 (6) (1997) 1178–1197.
[28] H. Ferjani, A. Achour, H. Bacha, S. Abid, Tacrolimus and mycophenolate mofetil associations: induction of oxidative stress or antioxidant effect? Hum. Exp. Toxicol. 34 (11) (2015) 1119–1132.
[29] J. Park, H. Ha, J. Seo, et al., Mycophenolic acid inhibits platelet-derived growth factor-induced reactive oxygen species and mitogen-activated protein kinase activation in rat vascular smooth muscle cells, Am. J. Transplant. 4 (12) (2004) 1982–1990.
[30] K.H. Huh, H.J. Ahn, J. Park, et al., Mycophenolic acid inhibits oleic acid-induced mesangial cell activation through both cellular reactive oxygen species and inosine monophosphate dehydrogenase 2 pathways, Pediatr. Nephrol. 24 (4) (2009) 737–745.
[31] C. Van den Branden, B. Ceyssens, M. Pauwels, et al., Effect of mycophenolate mofetil on glomerulosclerosis and renal oxidative stress in rats, Nephron Exp. Nephrol. 95 (3) (2003) e93–e99.
[32] W.R. Mulley, K. Visvanathan, A.C. Hurt, et al., Mycophenolate and lower graft function reduce the seroresponse of kidney transplant recipients to pandemic H1N1 vaccination, Kidney Int. 82 (2) (2012) 212–219.
[33] A. Grupper, L. Rabinowich, D. Schwartz, et al., Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus, Am. J. Transplant. 21 (8) (2021) 2719–2726.
[34] J. Tschopp, K. Schroder, NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nat. Rev. Immunol. 10 (3) (2010) 210–215.
[35] L.E. van Eijk, M. Binkhorst, A.R. Bourgonje, et al., COVID-19: immunopathology, pathophysiological mechanisms, and treatment options, J. Pathol. 254 (4) (2021) 307–331.
[36] F. McNab, K. Mayer-Barber, A. Sher, A. Wack, A. O'Garra, Type I interferons in infectious disease, Nat. Rev. Immunol. 15 (2) (2015) 87–103.
[37] C. Bergamaschi, E. Terpos, M. Rosati, et al., Systemic IL-15, IFN-γ, and IP-10/CXCL10 signature associated with effective immune response to SARS-CoV-2 in BNT162b2 mRNA vaccine recipients, Cell Rep. 36 (6) (2021) 109504.
[38] G. Tut, T. Lancaster, P. Sylla, et al., Antibody and cellular immune responses following dual COVID-19 vaccination within infection-naive residents of long-term care facilities: an observational cohort study, Lancet Healthy Longev 3 (7) (2022) e461–e469.
[39] T.D. Querec, R.S. Akondy, E.K. Lee, et al., Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans, Nat. Immunol. 10 (1) (2009) 116–125.
[40] H.I. Nakaya, J. Wrammert, E.K. Lee, et al., Systems biology of vaccination for seasonal influenza in humans, Nat. Immunol. 12 (8) (2011) 786–795.
[41] V.V. Edara, W.H. Hudson, X. Xie, R. Ahmed, M.S. Suthar, Neutralizing antibodies against SARS-CoV-2 variants after infection and vaccination, JAMA 325 (18) (2021) 1896–1898.
[42] N. Kamar, F. Abravanel, O. Marion, C. Couat, J. Izopet, A. Del Bello, Three doses of an mRNA covid-19 vaccine in solid-organ transplant recipients, N. Engl. J. Med. 385 (7) (2021) 661–662.
[43] V.G. Hall, V.H. Ferreira, T. Ku, et al., Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients, N. Engl. J. Med. 385 (13) (2021) 1244–1246.
[44] I. Benotmane, G. Gautier, P. Perrin, et al., Antibody response after a third dose of the mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients with minimal serologic response to 2 doses, JAMA 326 (11) (2021) 1063–1065.
[45] N. Chavarot, A. Ouedrani, O. Marion, et al., Poor anti-SARS-CoV-2 humoral and T-cell responses after 2 injections of mRNA vaccine in kidney transplant recipients treated with belatacept, Transplantation 105 (9) (2021) e94–e95.
[46] J.S.F. Sanders, A. Lianne Messchendorp, R.D. de Vries, et al., 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, Clin. Infect. Dis. 76 (3) (2022) e188–e199.
[47] J. Stumpf, J. Schwöbel, T. Lindner, et al., Risk of strong antibody decline in dialysis and transplant patients after SARS-CoV-2mRNA vaccination: six months data from the observational Dia-Vacc study, Lancet Reg Health Eur 17 (2022) 100371.
[48] D.S. Khoury, D. Cromer, A. Reynaldi, et al., Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection, Nat. Med. 27 (7) (2021) 1205–1211.
[49] J. Santolini, S.A. Wootton, A.A. Jackson, M. Feelisch, The Redox architecture of physiological function, Curr Opin Physiol 9 (2019) 34–47.
[50] M. Feelisch, M.M. Cortese-Krott, J. Santolini, S.A. Wootton, A.A. Jackson, Systems redox biology in health and disease, EXCLI J 21 (2022) 623–646.
[51] A.R. Bourgonje, D. Kloska, A. Grochot-Przęczek, M. Feelisch, A. Cuadrado, H. van Goor, Personalized redox medicine in inflammatory bowel diseases: an emerging role for HIF-1α and NRF2 as therapeutic targets, Redox Biol. 60 (2023) 102603.