Immune response after mRNA COVID-19 vaccination in heart transplant recipients: long-term follow-up and evaluation of a third vaccination

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Solid organ transplant recipients (SOTRs) are considered to be at high risk for severe complications and even death when infected with COVID-19.[1] In heart transplant recipients (HTRs), COVID-19 is associated with a mortality rate of up to 25%.[2] Yet, some recently published data indicate a similar safety profile but reduced immune responses to COVID-19 mRNA vaccine in SOTRs compared to data from immunocompetent persons.[3]

We compared the humoral and cellular responses after the administration of two doses of COVID-19 mRNA vaccines in heart transplant recipients (HTRs) against health controls (health care workers of the Medical university of Vienna). Moreover, six months after primary vaccinations, SARS-CoV-2 antibody kinetics and the efficacy of the third dose of COVID-19 vaccine were evaluated. Antibodies against the SARS-CoV-2 receptor-binding domain and the nucleocapsid protein (RBD) were determined up to two weeks before vaccination, 3-6 weeks after first immunization (median 26 days), 3-10 weeks (median 45 days) and 5-6 months after the second vaccination (median 167 days). The time point was chosen to identify peak levels after the second vaccination and to have the first data on antibody kinetics. The Elecsys® Anti-SARS-CoV-2 S immunoassay was used to quantitatively determine antibodies to the RBD of the viral spike (S) protein and nucleocapsid-specific antibodies.[4] T cell responses were measured using *ex vivo* ELISpot assays, and results were considered positive when mean spot counts were at least three times higher than those of three unstimulated wells.[5]

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The baseline characteristics, treatments of HTRs and administered vaccines are listed in **Table 1.**

The seroconversion rates and median SARS-CoV-2 antibody levels following mRNA vaccination were significantly lower in HTRs than in HCs: 15% vs. 94%, $p < 0.001$; 0.2 (0.2–0.2) vs. 51.7 (12.03–154.25), $p < 0.001$ after the first immunisation, and 60% vs. 100%, $p < 0.001$; 2.80 (0.2–92.40) vs. 1488 (616–2500), $p < 0.001$ after the second immunisation. Antibody levels significantly increased in both HTRs ($p < 0.001$) and HCs ($p < 0.001$) after administering the second vaccine dose. **(Figure 1A).** Five to six months after giving the second dose, SARS-CoV-2 S median antibody titers in HCs decreased significantly (658 [280.5–864], $p < 0.001$), whereas in HTRs, no significant change in the median antibody levels was detected (34.95 [0.7–182.25], $p = 0.248$). After administering the third dose of COVID-19 vaccine, seroconversion was detected in 36 out of 43 (84 %) HTRs, including 13 of 20 HTRs (65 %), with no initial antibody response. SARS-CoV-2 antibody levels increased significantly in HTRs who received a third vaccine (2500 [IQR 159–2500], $p < 0.001$), as well as in HCs (2500 [2500–2500], $p < 0.001$). However, antibody levels were still significantly lower in HTRs compared with HCs ($p < 0.001$) **(Figure 1B).**

Logistic regression analysis was performed to evaluate the potential association of variables such as age, sex, vaccine, years since heart transplantation, and immunosuppressive therapy after receiving COVID-19 vaccines. In univariate analysis, therapy with ciclosporin was associated with significantly higher odds for seroconversion (OR = 4.25 [1.32–16.62], p = 0.22). However, possible negative effects on seroconversion were observed for therapy with tacrolimus (OR = 0.38 [0.13–1.06]) and with mycophenolate mofetil (MMF) (OR = 0.26 [0.05-0.93]), but they failed to reach statistical significance. After adjusting for age and sex, therapy with tacrolimus (OR = 0.21 [0.06–0.067], p = 0.011) was determined to be associated with significantly lower odds for seroconversion **(Supplemental Table 1).**

Within six months after administering the second vaccine dose, antibody levels increased in HTRs who were treated with MMF therapy (21 out of 37 (57%), 26.50 [0.20–113], p = 0.027). In HTRs without MMF therapy, no significant change in antibody levels was detected after six months (79.20 [25.68–569.50], p = 0.477) **(Supplemental Figure 2A).** In HTRs with (2484 [165.50–2500], p < 0.001) and without (2500 [1200.25–2500], p = 0.009) MMF therapy, antibody levels increased significantly after receiving the third vaccine dose **(Supplemental Figure 2B).**

T cell responses were induced in 77% (13/17) HTRs and 100% (16/16) HCs. No difference was observed in the magnitude of T cell responses in HTRs compared with HCs (310 [60–342.5] SFCs/106 PBMC vs. 337.5 [164.25–494] SFCs/106 PBMC, p = 0.377) **(Figure 1C).** Of the 13 HTRs with detectable T cell responses, 8 (62%) developed humoral responses, whereas in 5 (38 %) seroconversions did not occur after the second vaccination.

The use of triple immunosuppressive therapy during the first three years after solid organ transplantation, especially the inclusion of antimetabolite and calcineurin inhibitor tacrolimus, seems to be associated with a reduced immune response to the COVID-19 vaccines. Consistent with previous reports by those of SOTRs, we showed a lower seroconversion rate in HTRs treated with tacrolimus and MMF.[6-8]

As expected, antibody levels declined after six months in most vaccine recipients. HTRs, however, displayed striking divergent antibody kinetics as HTRs, who received MMF, showed even an increase of antibody titers after six months. We propose that treatment with MMF might delay efficient B cell responses and therefore early seroconversion, which also suggests the determination of SARS-CoV-2 antibody levels later than four weeks after vaccination in this group of patients.

Our data on cellular responses are comparable to those published by Hererra *et al.,* but the level of T cell responses was higher than that observed in lung or kidney organ recipients. [6,9] In contrast, Schramm *et al*. showed inadequate cellular responses in heart and lung transplant recipients using interferon-γ

release assay (IGRA) for whole blood samples.[10] To our knowledge, comparative studies of the sensitivity of the two test methods for determining cellular responsiveness to SARS-CoV-2 peptides have nor be published yet.

The limitation of our study is the small sample size of T cell analyses, due to which the impact of immunosuppressive therapies on cellular responses could not be investigated in detail. Nevertheless, we confirm that HRTs can mount cellular responses even in the absence of seroconversion.

In summary, our results showed reduced humoral responses in HTRs and highlighted the complexity and unpredictability of immune responses in immunocompromised patients. Results from our study and other studies in solid organ transplant recipients suggest the urgent need for an improved prophylaxis strategy. Cellular responses appear to be less affected by immunosuppression and remain preserved even in some patients in which seroconversion did not occur. However, it is still unclear the role of cellular response in protection against SARS-COV-2. Most initial non- responders and almost all responders benefited from a third vaccine either through seroconversion or an increase in antibody levels. Further studies are needed to confirm the clinical importance of COVID-19 vaccination in this population.

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Author contributions

All authors contributed to manuscript preparation. S.T and S.W. contributed to the study design. L.S. contributed to data analysis. K.U-Ü and F.W. contributed to patient recruitment. T.P. and H.H. performed antibody measurements. J.A. and M.K. contributed to cellular assays.

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Competing interests

No competing interests are declared.

Data availability

The data that support the findings of this study are available from the corresponding author (ST) upon reasonable request.

Ethics approval

Ethical approval for this study was granted by the local ethics committee of the Medical University of Vienna, Austria. Patients gave written informed consent to participate in the study.

Authorship:

ST, SB and SW contributed to the conception or design of the work. LS, ST and SW contributed to the acquisition, analysis, or interpretation of data for the work. TP and HH performed antibody measurements. MK and JA, contributed to cellular assays. FW and KU contributed to patient recruitment. ST and SW drafted the manuscript. EF critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Table 1: Characteristics of heart transplant recipients (HTRs) and healthy controls (HCs) at baseline

Figure 1. Humoral and cellular response in HTRs and in HCs after COVID-19 mRNA immuniSation (the horizontal line indicates the cutoff for seroconversion, and circles represent individual antibody titers): (A) SARS-CoV-2 S antibody levels (BAU/ml); (B) Change in SARS-CoV-2 S antibody levels (BAU/ml) over time in HCs and HTRs – the circles represent individual antibody titers; (C) T cell response to SARS-CoV-2 mRNA vaccination. T cell response rates and magnitudes in HTRs and HCs – the bars indicate the proportion of patients with a T cell response against SARS-CoV-2 peptide pools at 2–4 weeks after second dose vaccination.

Table 1: Heart transplant recipients (HTRs) and healthy controls (HCs) characteristics at baseline

	HTR n = 65	HC n = 65	P value
Age (median)	64 (55 – 69)	50 (42 – 57)	<0.001
Sex (n, %)			1
Female	20 (30.8)	21 (32.3)	
Male	45 (69.2)	44 (67.7)	
Years between heart organ transplantation and vaccination, median (IQR)	8 (5 – 15)	NA	
Therapy (n, %)		NA	
Ciclosporin	21 (32.3)		
Tacrolimus	36 (55.4)		
mTOR inhibitor	19 (29.2)		
Everolimus	13 (20)		
Sirolimus	6 (9.2)		
MMF	49 (75.4)		
Prednisone	18 (27.7)		
Dual therapy (n, %)	52 (80)		
Triple therapy (n, %)	13 (20)		
Therapy combinations (n, %)		NA	
Ciclosporin, prednisone	3 (4.6)		
Ciclosporin, MMF	11 (16.9)		
mTOR inhibitor, ciclosporin	3 (4.6)		
mTOR inhibitor, MMF	7 (10.8)		
mTOR inhibitor, tacrolimus	5 (7.7)		
Tacrolimus, prednisone	3 (4.6)		
Tacrolimus, MMF	20 (30.8)		
Ciclosporin, prednisone, MMF			
mTOR inhibitor, ciclosporin, prednisone	3 (4.6)		
mTOR inhibitor, MMF, prednisone	1 (1.5)		
mTOR inhibitor, tacrolimus, prednisone	1 (1.5)		
mTOR inhibitor, tacrolimus, MMF	1 (1.5)		
Tacrolimus, MMF, prednisone	1 (1.5)		
	6 (9.2)		
Vaccine first dose (n, %)			
mRNA-1273	5 (7.8)	0	
BNT162b2	61 (92)	66 (100)	
Vaccine second dose (n, %)			
mRNA-1273	5 (7.8)	0	
BNT162b2	61 (92)	66 (100)	
Vaccine third dose (n, %)			
AZD1222	2 (5)	0	
mRNA-1273	8 (22)	3 (6)	
BNT162b2	33 (77)	50 (94)	
Covid-19 diagnosed before vaccination	2 (3.1)	2 (3.1)	1

IQR (the interquartile range); HTR (heart transplant recipients), HC (healthy control); MMF (Mycophenolate mofetil)