Past COVID-19 and immunosuppressive regimens affect the long-term response to anti-SARS-CoV-2 vaccination in liver transplant recipients

Pierluigi Toniutto, Edmondo Falleti, Sara Cmet, Annarosa Cussigh, Laura Veneto, Davide Bitetto, Ezio Fornasiere, Elisa Fumolo, Carlo Fabris, Assunta Sartor, Roberto Peressutti, Francesco Curcio, Laura Regattin, Lucrezia Grillone

Table of contents

Table S1. Association between prevaccination demographic and clinical characteristics of COVID-19 naïve liver transplanted patients (N=131) with regards to the development of a positive (≥0.8 U/ml) or negative (<0.8 U/ml) anti-SARS-CoV-2 s-RBD antibody response, as assessed one month (31±2 days) after the second dose of the Pfizer® BTN162b2 vaccine. Categorical parameters are presented as frequencies (%), and the Pearson chi-squared test was used for statistical comparisons. Continuous variables are presented as medians (interquartile range), for serum immunosuppressive drug serum levels, they are presented as the means (±SE), and the rank-sum test (Mann–Whitney) was used for statistical comparisons. Stepwise regression with a forward approach was used to discriminate independent predictive variables to achieve a positive antibody response after vaccination in a multivariate logistic model analysis.

	Univariate analysis			Multivariate analysis		
	Anti-s RBD IgG negative (N = 44)	Anti-s RBD IgG positive (N = 87)	p	O.R.	95% C.I.	p
Age at LT (years)	60.5 (56.9-65.7)	57.1 (50.4-61.5)	0.005			
Male gender	28 (63.6)	64 (73.6)	0.241			
BMI (kg/m^2)	26.3 (24.2-29.3)	25.5 (23.2-28.5)	0.485			
Months between LT and vaccination	73.3 (19.6-145)	126 (59.1-195)	0.017			
Etiology (HCV, HBV, AH, AI, other)	9, 8, 19, 4, 4 (20.5, 18.2, 43.2, 9.1, 9.1)	19, 13, 39, 9, 7 (21.8, 14.9, 44.8, 10.3, 8.0)	0.989			
HCC	17 (38.6)	30 (4.5)	0.640			
DM	17 (38.6)	29 (33.3)	0.548			
Dyslipidemia	13 (29.6)	16 (18.4)	0.146			

Alcohol consumption >40 gr/day	5 (11.4)	4 (4.6)	0.148			
HTN	22 (50.0)	36 (41.4)	0.348			
Presence of esophageal varices	2 (4.6)	4 (4.6)	0.989			
Presence of ascites	3 (6.8)	1 (1.6)	0.075			
IS single drug frequency						
Tacrolimus	26 (59.1)	59 (67.8)	0.323			
Cyclosporine	10 (22.7)	21 (24.1)	0.858			
MMF	40 (90.9)	18 (21.7)	<0.001			
Everolimus	2 (4.6)	10 (11.5)	0.193			
Prednisone	6 (13.6)	7 (8.1)	0.312			
Double-triple IS including MMF	34 (77.3)	17 (19.5)	<0.001			
(MMF+T; +C; +E; +P; +C+P)	22, 8, 1, 1, 2	8, 8, 1, 0, 0				
	(50, 18.2, 2.3, 2.3, 4.5)	(9.2, 9.2, 1.1, 0, 0)				
Double-triple IS excluding MMF	3 (6.8)	11 (12.6)	0.308			
(T+E, T+A, T+P, C+P, T+E+P)	0, 0, 2, 0, 1	3, 1, 5, 1, 1				
	(0, 0, 4.5, 0, 2.3)	(3.4, 1.1, 5.7, 1.1, 1.1)				
Any double IS therapy	34 (77.3)	27 (31.0)	<0.001			
Any triple IS therapy	3 (3.9)	1 (0.0)	0.075	<0.01	<0.01-0.073	<0.001
Serum IS drug levels or daily dose [#]						
Tacrolimus (ng/ml)	3.36 ±3.54	4.50 ±8.38	0.635			
Cyclosporine (ng/ml)	8.25 ±16.6	13.6 ±28.7	0.618			
MMF (g/day)	1.59 ±0.68	0.30 ±0.62	<0.001	0.091	0.036-0.228	<0.001
Everolimus (ng/ml)	0.23 ±1.05	0.53 ±1.78	0.312			

Prednisone (mg/day)	0.62 ±1.71	0.46 ±1.73)	0.421			
IS levels with respect to reference [#]						
Below	20 (45.5)	43 (49.4)	0.667			
Above	1 (2.3)	5 (5.8)	0.369			
Hemoglobin (g/dl)	13.0 (11.7-14.3)	13.6 (12.6-15.0)	0.017			
Leukocytes (n/µl)	4630 (4055-6105)	5980 (4860-7120)	<0.001	1.001	1.000-1.001	0.005
Neutrophils (n/µl)	3070 (2430-3820)	3540 (2860-4310)	0.016			
Albumin (g/dl)	4.30 (3.95-4.60)	4.24 (4.10-4.42)	0.607			
Bilirubin (mg/dl)	0.57 (0.39-0.77)	0.66 (0.43-1.00)	0.165			
eGFR (ml/min/1.73m^2)	53.1 (45.6-70.3)	65.1 (46.5-79.6)	0.123			
AST (IU/l)	15.5 (13-22)	20 (16-25)	0.005			
ALT (IU/l)	14 (9-22)	17 (12-24)	0.053			
INR	1.02 (0.96-1.12)	1.05 (1.00-1.16)	0.093			
25-OH-Vitamin D (ng/ml)	31.2 (27.7-34.0)	30.9 (25.0-35.0)	0.642			

LT: liver transplantation; BMI: body mass index; HBV: hepatitis B virus; HCV: hepatitis C virus, AH: alcoholic; AI: autoimmune; HCC: hepatocellular carcinoma; DM: diabetes mellitus; HTN: arterial hypertension; MMF: mycophenolate mofetil; IS: immunosuppressive; T: tacrolimus, C: cyclosporine, E: everolimus, A: azathioprine, P: prednisone; [#]reference blood levels evaluated within 1 month before vaccination for each IS drug were calculated in accordance with Cillo et al.[35] ; eGFR: estimated glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR international normalized ratio. Logistic model estimation parameters: pseudo $R^2 = 0.525$; area under the ROC curve = 0.935; correct classification = 88.6%.

Table S2. Association between prevaccination demographic and clinical characteristics of COVID-19 naïve liver transplanted patients (N=126) with regards to the development of a positive (≥0.8 U/ml) or negative (<0.8 U/ml) anti-SARS-CoV-2 s-RBD antibody response, as assessed 4 months (125±5 days) after the second dose of the Pfizer® BTN162b2 vaccine. Categorical parameters are presented as frequencies (%), and the Pearson chi-squared test was used for statistical comparisons. Continuous variables are presented as medians (interquartile range), for serum immunosuppressive drug levels, they are presented as the means (±SE), and the rank-sum test (Mann–Whitney) was used for statistical comparisons. Stepwise regression with a forward approach was used to discriminate independent predictive variables to achieve a positive antibody response after vaccination in a multivariate logistic model analysis.

	Univariate analysis			Multivariate analysis		
	Anti-s RBD IgG negative (N = 29)	Anti-s RBD IgG positive (N = 97)	p	O.R.	95% C.I.	p
Age at LT (years)	60.8 (58.2-65.8)	57.4 (51.1-61.8)	0.003	0.923	0.865-0.992	0.030
Male gender	19 (65.5)	69 (71.1)	0.563			
BMI (kg/m^2)	26.4 (24.5-28.7)	25.5 (23.4-28.7)	0.551			
Months between LT and vaccination	60.0 (19.1-98.4)	117 (59.1-189)	0.011			
Etiology (HCV, HBV, AH, AI, other)	6, 6, 11, 3, 3 (20.7, 20.7, 37.9, 10.3, 10.3)	21, 15, 45, 10, 6 (21.6, 15.5, 46.4, 10.3, 6.2)	0.865			
HCC	10 (34.5)	37 (38.1)	0.721			
DM	9 (31.0)	33 (34.0)	0.765			
Dyslipidemia	8 (27.6)	20 (20.6)	0.428			

Alcohol consumption >40 gr/day	3 (10.3)	7 (7.2)	0.584			
HTN	13 (44.8)	41 (42.3)	0.807			
Presence of esophageal varices	2 (6.9)	4 (4.1)	0.538			
Presence of ascites	2 (6.9)	2 (2.1)	0.193			
Immunosuppressive treatment						
Tacrolimus	20 (69.0)	62 (63.9)	0.617			
Cyclosporine	5 (17.1)	24 (24.7)	0.400			
MMF	25 (86.2)	30 (30.9)	<0.001			
Everolimus	2 (6.9)	9 (9.3)	0.690			
Prednisone	5 (17.2)	7 (7.2)	0.249			
Double-triple IS including MMF	23 (79.3)	25 (25.8)	<0.001			
(MMF+T; +C; +E; +P; +C+P)	16, 4, 1, 1, 1	13, 10, 1, 0, 1				
	(55.2, 13.8, 3.4, 3.4, 3.4)	(13.4, 10.3, 1, 0, 1)				
Double-triple IS excluding MMF	3 (10.3)	9 (9.3)	0.864			
(T+E, T+A, T+P, C+P, T+E+P)	0, 0, 2, 0, 1	2, 1, 4, 1, 1				
	(0.0, 0.0, 6.9, 0.0, 3.4)	(2.1, 1.0, 4.1, 1.0, 1.0)				
Any double IS therapy	24 (82.8)	32 (33.0)	<0.001			
Any triple IS therapy	2 (6.9)	2 (2.1)	0.193			
Serum IS drug levels or daily dose						
Tacrolimus (ng/ml)	3.86 ±0.61	3.58 ±0.36	0.451			
Cyclosporine (ng/ml)	4.75 ±2.11	13.8 ±2.89	0.240			
MMF (g/d)	1.57 ±0.14	0.49 ±0.08	<0.001	0.265	0.134-0.525	<0.001
Everolimus (ng/ml)	0.34 ±0.24	0.43 ±0.17	0.922			

Prednisone (mg/d)	0.80 ±0.35	0.36 ±0.14	0.115			
IS levels with respect to reference[#]						
below	15 (51.7)	43 (44.3)	0.483			
above	1 (3.5)	4 (4.1)	0.870			
Hemoglobin (g/dl)	12.8 (11.6-13.6)	13.6 (12.8-15.0)	0.005			
Leukocytes (n/µl)	4520 (4010-5550)	5980 (4700-6750)	<0.001	1.001	1.000-1.001	0.012
Neutrophils (n/µl)	3900 (2350-3770)	3480 (2840-4310)	0.029			
Albumin (g/dl)	4.50 (4.07-4.60)	4.24 (4.10-4.41)	0.204			
Bilirubin (mg/dl)	0.53 (0.37-0.63)	0.64 (0.47-0.97)	0.022			
eGFR (ml/min/1.73 m^2)	57.9 (45.9-70.0)	65.1 (48.1-80.1)	0.112			
AST (IU/l)	15 (13-21)	19 (16-25)	0.003			
ALT (IU/l)	12 (9-17)	18 (12-27)	0.001			
INR	1.03 (0.96-1.14)	1.21 (0.98-2.05)	0.548			
25-OH-Vitamin D (ng/ml)	32.3 (28.6-34.0)	30.9 (25.0-35.0)	0.268			

LT: liver transplantation; BMI: body mass index; HBV: hepatitis B virus; HCV: hepatitis C virus; AH: alcoholic hepatitis; AI: autoimmune; HCC: hepatocellular carcinoma; DM: diabetes mellitus; HTN: arterial hypertension; MMF: mycophenolate mofetil; IS: immunosuppressive; T: tacrolimus; C: cyclosporine; E: everolimus; A: azathioprine; P: prednisone; [#]reference blood levels evaluated within 1 month before vaccination for each IS drug were calculated in accordance with Cillo et al.[35]; eGFR: estimated glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR international normalized ratio. Logistic model estimation parameters: pseudo $R^2 = 0.434$; area under the ROC curve = 0.910; correct classification = 88.9%.

Table S3. Impact of each single or in combination immunosuppressive drug in influencing the anti-SARS-CoV-2 s-RBD antibody response, assessed at every time point after Pfizer® BTN162b2 vaccine in COVID-19 naïve liver transplanted patients. At the top are presented single and in combination immunosuppressive drugs. In the left column are presented, starting from the top and from each time point after vaccine dose, the mean of anti-SARS-CoV-2 s-RBD antibody titer. For every time point after vaccine dose, the number of patients (N.) treated with each single or in combination immunosuppressive drug, the mean and the percentage deviation (%Dev) of anti-SARS-CoV-2 s-RBD antibody titers are displayed in the rows.

	T	**C**	**E**	**T+A**	**T+E**	**C+P**	**T+P**	**T+E+P**	**M**	**M+T**	**M+C**	**M+E**	**M+P**	**M+C+P**
Three weeks (19 days) after the 1st vaccine dose (mean antiSARS-CoV-2 s-RBD antibody titer: 2.03 U/ml; N=131)														
N.	42	12	5	1	3	1	7	2	7	30	16	2	1	2
Mean	5,3	2,8	0,6	0,5	0,0	0,0	0,5	0,0	0,0	0,7	0,03	0,0	0,0	0,0
%Dev	162	39	*-71*	*-75*	*-100*	*-100*	*-75*	*-100*	*-100*	*-68*	*-98*	*-100*	*-100*	*-100*
One month (31±2 days) after the 2nd vaccine dose (mean antiSARS-CoV-2 s-RBD antibody titer: 340 U/ml; N=131)														
N.	42	12	5	1	3	1	7	2	7	30	16	2	1	2
Mean	614	1087	253	228	91,5	168	347	2,5	0,2	31	27	0,7	0,0	0,0
%Dev	81	220	*-25*	-33	*-73*	*-51*	2	*-99*	*-100*	*-91*	*-92*	*-100*	*-100*	*-100*
Four months (125±5 days) after the 2nd vaccine dose (mean antiSARS-CoV-2 s-RBD antibody titer: 185 U/ml, N=126)														
N.	42	12	5	1	2	1	6	2	7	29	14	2	1	2
Mean	319	414	284	274	64	72	88	3,9	27	50	66	12,	0,0	5,7
%Dev	72	124	54	48	*-65*	*-61*	*-52*	*-98*	*-85*	*-73*	*-65*	*-93*	*-100*	*-97*
Six months (165±4 days) after the 2nd vaccine dose (mean antiSARS-CoV-2 s-RBD antibody titer: 122 U/ml, N=123)														

N.	42	11	5	1	3	1	6	2	6	27	14	2	1	2
Mean	209	228	182	306	37	61	70	3,2	38	42	40	37	0,5	3,7
%Dev	71	87	49	151	*-70*	*-50*	*-42*	*-97*	*-69*	*-66*	*-67*	*-70*	*-100*	*-97*

T: tacrolimus; C: cyclosporine; M: mycophenolate mofetil; E: everolimus; A: azathioprine; P: prednisone.

Table S4. Linear regression analysis evaluating the demographic and clinical contributors to the development of anti-SARS-CoV-2 s-RBD antibody response to Pfizer® BTN162b2 vaccine in COVID-19-naïve liver transplanted patients. Antibody titer values are considered as continuous variable, and they were assessed at each time point after vaccination. Stepwise regression with a forward approach was used to discriminate best fitting variables to predict antibody response after vaccination in a multivariate linear model analysis. All variables presented in table 2, that were significantly associated at the univariate regression test, have been selected to run in the multivariate linear model. At three weeks after the first and at 1 month after the second vaccine dose, data were available in all patients (N=131), while at 4 and 6 months after the second vaccine dose data were available in 126 and 123 patients respectively.

Anti-SARS-CoV-2 s-RBD antibody titer values	t	Coef.	95% C.I.	p
Three weeks (19 days) after the first vaccine dose				
Age at transplantation (years)	-2.63	-0.017	-0.029 -0.004	0.009
MMF daily dose (g/day)	-6.10	-0.322	-0.427 -0.218	<0.001
eGFR (ml/min/1.73m^2)	2.34	0.007	0.001 0.012	0.021
(Model $R^2 = 0.315$)				
One month (31±2 days) after the second vaccine dose				
Age at transplantation (years)	-3.84	-0.028	-0.042 -0.014	<0.001
MMF daily dose (g/day)	-11.36	-1.163	-1.365 -0.960	<0.001

Alcohol consumption >40gr/day	-2.52	-0.646	-1.154 -0.138	0.013
Serum hemoglobin levels (gr/dl)	2.52	0.146	0.031 0.260	0.013
(Model $R^2 = 0.568$)				
Four months (125±5 days) after the second vaccine dose				
Age at liver transplantation (years)	-4.51	-0.033	-0.047 -0.018	<0.001
MMF daily dose (g/day)	-7.07	-0.799	-1.023 -0.575	<0.001
(Model $R^2 = 0.385$)				
Six months (165±4 days) after the second vaccine dose				
Age at transplantation (years)	-4.00	-0.028	-0.043 -0.014	<0.001
MMF daily dose (g/day)	-5.69	-0.688	-0.928 -0.449	<0.001
(Model $R^2 = 0.328$)				

MMF: mycophenolate mofetil; eGFR: estimated glomerular filtration rate.

Table S5. Association between prevaccination demographic and clinical characteristics of COVID-19-naïve liver transplanted patients (N=131) with regards to the development of a strong positive (≥100 U/ml) or negative (<100 U/ml) anti-SARS-CoV-2 s-RBD antibody response, as assessed one month (31±2 days) after the second dose of the Pfizer® BTN162b2 vaccine. Categorical parameters are presented as frequencies (%), and the Pearson chi-squared test was used for statistical comparisons. Continuous variables are presented as medians (interquartile range), for serum immunosuppressive drug levels, they are presented as the means (±SE), and the rank-sum test (Mann–Whitney) was used for statistical comparisons. Stepwise regression with a forward approach was used to discriminate independent predictive variables to achieve a strong antibody response after vaccination in a multivariate logistic model analysis.

	Univariate analysis			Multivariate analysis		
	Anti-s RBD IgG <100 U/ml (N = 80)	Anti-s RBD IgG ≥100 U/ml (N = 51)	p	O.R.	95% C.I.	p
Age at LT (years)	59.8 (54.4-64.7)	55.7 (48.3-60.5)	0.002	0.941	0.898-0.987	0.013
Male gender	58 (72.5)	34 (66.7)	0.476			
BMI (kg/m^2)	25.5 (23.6-29.1)	26.2 (23.4-28.5)	0.878			
Months between LT and vaccination	77.3 (25.9-169.9)	153.9 (72.4-200.8)	0.001			
Etiology (HCV, HBV, AH, AI, other)	16, 15, 40, 5, 4 (20.0, 18.8, 50.0, 6.3, 5.0)	12, 6, 18, 8, 7 (23.5, 11.8, 35.3, 15.7, 13.7)	0.082			
HCC	32 (40.0)	15 (29.4)	0.218			
DM	32 (40.0)	14 (27.5)	0.142			
Dyslipidemia	20 (25.0)	9 (17.7)	0.323			
Alcohol consumption >40gr/day	9 (11.3)	0 (0.0)	<0.001	<0.01	<0.01-<0.01	<0.001

HTN	35 (43.8)	23 (45.1)	0.880			
Presence of esophageal varices	4 (5.0)	2 (3.9)	0.773			
Presence of ascites	3 (3.8)	1 (2.0)	0.562			
IS treatment						
Tacrolimus	50 (62.5)	35 (68.3)	0.474			
Cyclosporine	19 (23.8)	12 (23.5)	0.977			
MMF	53 (66.3)	5 (9.8)	<0.001			
Everolimus	6 (7.5)	6 (11.8)	0.409			
Prednisone	8 (10.0)	5 (9.8)	0.971			
Double-triple IS including MMF	46 (57.5)	5 (9.8)	<0.001			
(MMF+T; +C; +E; +P; +C+P)	27, 14, 2, 1, 2	3, 2, 0, 0, 0				
	(33.8, 17.5, 2.5, 1.3, 2.5)	(5.9, 3.9, 0, 0, 0)				
Double-triple IS excluding MMF	6 (7.5)	8 (15.7)	0.139			
(T+E, T+A, T+P, C+P, T+E+P)	1, 0, 3, 0, 2	2, 1, 4, 1, 0				
	(1.3, 0, 3.8, 0, 2.5)	(3.9, 2, 7.8, 2, 0)				
Any double IS therapy	48 (60.0)	13 (25.5)	<0.001			
Any triple IS therapy	4 (5.0)	0 (0.0)	0.105			
Serum IS drug levels or daily dose						
Tacrolimus (ng/ml)	4.62 ±0.99	3.33 ±0.38	0.728			
Cyclosporine (ng/ml)	10.4 ±2.7	13.9 ±3.8	0.701			
MMF (g/day)	1.11 ±0.10	0.13 ±0.06	<0.001	0.162	0.072-0.365	<0.001
Everolimus (ng/ml)	0.34 ±0.15	0.57 ±0.27	0.478			
Prednisone (mg/day)	0.50 ±0.19	0.54 ±0.25	0.951			
IS levels with respect to reference[#]						
Below	36 (45.0)	27 (52.9)	0.375			

Above	5 (6.3)	1 (2.0)	0.252
Hemoglobin (g/dl)	13.2 (12.1-14.7)	13.6 (12.6-15.0)	0.089
White blood cells (n/µl)	5510 (4480-6495)	5790 (4680-7350)	0.160
Neutrophils (n/µl)	3280 (2780-3980)	3580 (2700-4490)	0.343
Albumin (g/dl)	4.24 (4.02-4.59)	4.23 (4.10-4.44)	0.479
Bilirubin (mg/dl)	0.60 (0.44-0.84)	0.66 (0.40-1.01)	0.908
eGFR (ml/min/1.73 m^2)	57.9 (46.3-70.7)	66.9 (44.8-82.4)	0.093
AST (IU/l)	17.5 (14-23)	21 (16-26)	0.029
ALT (IU/l)	14 (10.5-22.5)	18 (14-29)	0.021
INR	1.04 (0.98-1.13)	1.06 (1.00-1.16)	0.094
25-OH-Vitamin D (ng/ml)	31.0 (225.5-34.0)	31.0 (26.7-37.2)	0.410

LT: liver transplantation; BMI: body mass index; HBV: hepatitis B virus; HCV: hepatitis C virus; AH: alcoholic; AI: autoimmune; HCC: hepatocellular carcinoma; DM: diabetes mellitus; HTN: arterial hypertension; MMF: mycophenolate mofetil; IS: immunosuppressive; T: tacrolimus; C: cyclosporine; E: everolimus; A: azathioprine; P: prednisone; [#]reference blood levels evaluated within 1 month before vaccination for each IS drug were calculated in accordance with Cillo et al.[35]; eGFR: estimated glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR international normalized ratio. Logistic model estimation parameters: pseudo $R^2 = 0.363$; area under the ROC curve = 0.873; correct classification = 78.6%.

Table S6. Association between prevaccination demographic and clinical characteristics of COVID-19-naïve liver transplanted patients (N=126) with regards to the development of a strong positive (≥100 U/ml) or negative (<100 U/ml) anti-SARS-CoV-2 s-RBD antibody titer, as assessed 4 months (125±5 days) after the second dose of the Pfizer® BTN162b2 vaccine. Categorical parameters are presented as frequencies (%), and the Pearson chi-squared test was used for statistical comparisons. Continuous variables are presented as medians (interquartile range), for serum immunosuppressive drug levels, they are presented as the means (±SE), and the rank-sum test (Mann–Whitney) was used for statistical comparisons. Stepwise regression with a forward approach was used to discriminate independent predictive variables to achieve a strong antibody response after vaccination in a multivariate logistic model analysis.

	Univariate analysis			Multivariate analysis		
	Anti-s RBD IgG <100 U/ml (N = 81)	Anti-s RBD IgG ≥100 U/ml (N = 45)	p	O.R.	95% C.I.	p
Age at LT (years)	59.8 (54.4-64.6)	56.9 (49.2-60.9)	0.010	0.946	0.907-0.985	0.008
Male gender	57 (70.4)	31 (68.9)	0.862			
BMI (kg/m^2)	25.6 (23.4-28.7)	26.2 (23.7-28.5)	0.878			
Months between LT and vaccination	76.1 (25.9-179)	149 (66.7-196)	0.015			
Etiology (HCV, HBV, AH, AI, other)	16, 14, 41, 6, 4 (19.8, 17.3, 50.6, 7.4, 4.9)	11, 7, 15, 7, 5 (24.4, 15.6, 33.3, 15.6, 11.1)	0.223			
HCC	29 (35.8)	18 (40.0)	0.641			
DM	28 (34.6)	14 (31.1)	0.693			
Dyslipidemia	20 (24.7)	8 (17.8)	0.371			

Alcohol consumption >40gr/day	9 (11.1)	1 (2.2)	0.077			
HTN	38 (46.9)	16 (35.6)	0.217			
Presence of esophageal varices	4 (4.9)	2 (4.4)	0.901			
Presence of ascites	3 (3.7)	1 (2.2)	0.649			
Immunosuppressive treatment						
Tacrolimus	50 (61.7)	32 (71.1)	0.290			
Cyclosporine	20 (24.7)	9 (20.0)	0.549			
MMF	46 (56.8)	9 (20.0)	<0.001			
Everolimus	8 (9.8)	3 (6.7)	0.541			
Prednisone	9 (11.1)	3 (6.7)	0.415			
Double-triple IS including MMF	40 (49.4)	8 (17.8)	<0.001			
(MMF+T; +C; +E; +P; +C+P)	25, 10, 2, 1, 2	4, 4, 0, 0, 0				
	(30.9, 12.3, 2.5, 1.2, 2.5)	(8.9, 8.9, 0, 0, 0)				
Double-triple IS excluding MMF	8 (9.9)	4 (8.9)	0.856			
(T+E, T+A, T+P, C+P, T+E+P)	2, 0, 3, 1, 2	0, 1, 3, 0, 0				
	(2.5, 0, 3.7, 1.2, 2.5)	(0, 2.2, 6.7, 0, 0)				
Any double IS therapy	44 (54.3)	12 (26.7)	0.003			
Any triple IS therapy	4 (4.9)	0 (0.0)	0.130			
Serum IS drug levels or daily dose[#]						
Tacrolimus (ng/ml)	3.69 ±0.40	3.66 ±0.49	0.967			
Cyclosporine (ng/ml)	10.68 ±2.33	13.3 ±4.85	0.743			
MMF (g/d)	0.99 ±0.10	0.27 ±0.08	<0.001	0.363	0.214-0.616	<0.001
Everolimus (ng/ml)	0.51 ±0.20	0.22 ±0.17	0.374			

Prednisone (mg/d)	0.49 ±0.17	0.39 ±0.24	0.760
IS levels with respect to reference[#]			
Below	34 (42.0)	24 (53.3)	0.220
Above	4 (4.9)	1 (2.2)	0.454
Hemoglobin (g/dl)	13.0 (12.1-14.4)	14.1 (13.1-15.0)	0.011
White blood cells (n/µl)	5600 (4470-6450)	5980 (4700-7170)	0.151
Neutrophils (n/µl)	3320 (2540-4090)	3590 (2860-4490)	0.248
Albumin (g/dl)	4.24 (4.03-4.56)	4.24 (4.10-4.50)	0.479
Bilirubin (mg/dl)	0.59 (0.43-0.81)	0.66 (0.43-0.97)	0.908
eGFR (ml/min/1.73m^2)	58.6 (45.9-71.8)	70.2 (50.7-81.0)	0.041
AST (IU/l)	18 (14-24)	18 (16-23)	0.454
ALT (IU/l)	16 (11-25)	18 (12-21)	0.366
INR	1.02 (0.98-1.08)	1.07 (1.01-1.18)	0.006
25-OH-Vitamin D (ng/ml)	30.9 (27.2-34.0)	32.0 (25.0-36.2)	0.688

LT: liver transplantation; BMI: body mass index; HBV: hepatitis B virus; HCV: hepatitis C virus; AH: alcoholic; AI: autoimmune; HCC: hepatocellular carcinoma; DM: diabetes mellitus; HTN: arterial hypertension; MMF: mycophenolate mofetil; IS: immunosuppressive; T: tacrolimus; C: cyclosporine; E: everolimus; A: azathioprine; P: prednisone; [#]reference blood levels evaluated within 1 month before vaccination for each IS drug were calculated in accordance with Cillo et al.[35]; eGFR: estimated glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR international normalized ratio. Logistic model estimation parameters: pseudo $R^2 = 0.170$; area under the ROC curve = 0.758; correct classification = 69.8%.

Table S7. Association between prevaccination demographic and clinical characteristics of COVID-19 naïve liver transplanted patients (N=123) with regards to the development of a strong positive (≥100 U/ml) or negative (<100 U/ml) anti-SARS-CoV-2 s-RBD antibody response assessed 6 months (165±4 days) after the second dose of the Pfizer® BTN162b2 vaccine. Categorical parameters are presented as frequencies (%), and the Pearson chi-squared test was used for statistical comparisons. Continuous variables are presented as medians (interquartile range), and for serum immunosuppressive drug levels, they are presented as the means (±SE), and the rank-sum test (Mann–Whitney) was used for statistical comparisons. Stepwise regression with a forward approach was used to discriminate independent predictive variables to achieve a strong antibody response after vaccination in a multivariate logistic model analysis.

	Univariate analysis			Multivariate analysis		
	Anti-s RBD IgG <100 U/ml (N = 87)	Anti-s RBD IgG ≥100 U/ml (N = 36)	p	O.R.	95% C.I.	p
Age at LT (years)	59.1 (54.0-63.0)	55.5 (46.0-61.1)	0.023	0.950	0.912-0.989	0.012
Male gender	63 (72.4)	25 (69.4)	0.740			
BMI (kg/m^2)	25.6 (23.2-28.7)	25.4 (23.7-28.8)	0.936			
Months between LT and vaccination	76.1 (30.9-188.3)	153 (76.2-197)	0.007			
Etiology (HCV, HBV, AH, AI, other)	19, 15, 41, 6, 6 (21.8, 17.2, 47.1, 6.9, 6.9)	7, 6, 13, 5, 5 (19.4, 16.7, 36.1, 13.9, 13.9)	0.469			
HCC	34 (39.1)	13 (36.9)	0.758			

DM	30 (34.5)	12 (33.3)	0.903			
Dyslipidemia	19 (21.8)	20 (22.2)	0.963			
Alcohol consumption >40 gr/day	10 (11.5)	0 (0.0)	0.034	<0.01	<0.01-<0.01	<0.001
HTN	45 (51.7)	11(30.6)	0.032			
Presence of esophageal varices	4 (4.6)	2 (5.6)	0.822			
Presence of ascites	3 (3.5)	1 (2.8)	0.849			
Immunosuppressive treatment						
Tacrolimus	57 (65.5)	24 (66.7)	0.903			
Cyclosporine	21 (24.1)	7 (19.4)	0.572			
MMF	46 (52.9)	6 (16.7)	<0.001			
Everolimus	8 (9.2)	4 (11.1)	0.745			
Prednisone	10 (11.5)	2 (5.6)	0.313			
Double-triple IS including MMF	41 (47.1)	5 (13.9)	<0.001			
(MMF+T; +C; +E; +P; +C+P)	24, 12, 2, 1, 2	3, 2, 0, 0, 0				
	(27.6, 13.8, 2.3, 1.1, 2.3)	(8.3, 5.6, 0, 0, 0)				
Double-triple IS excluding MMF	10 (11.5)	3 (8.3)	0.604			
(T+E, T+A, T+P, C+P, T+E+P)	3, 0, 4, 1, 2	0, 1, 2, 0, 0				
	(3.4, 0, 4.6, 1.1, 2.3)	(0, 2.8, 5.6, 0, 0)				
Any double IS therapy	47 (54.0)	8 (22.2)	0.001			
Any triple IS therapy	4 (4.6)	0 (0.0)	0.191			
Serum IS drug levels or daily dose[#]						
Tacrolimus (ng/ml)	4.61 ±0.90	3.48 ±0.57	0.516			
Cyclosporine (ng/ml)	10.5 ±2.65	10.2 ±4.08	0.877			

MMF (g/d)	0.89 ±0.10	0.26 ±0.10	<0.001	0.421	0228-0.776	0.006
Everolimus (ng/ml)	0.53 ±0.19	0.37 ±0.19	0.838			
Prednisone (mg/d)	0.55 ±0.18	0.42 ±0.29	0.353			
IS levels with respect to reference[#]						
Below	38 (43.7)	21 (58.3)	0.139			
Above	4 (4.6)	1(2.8)	0.642			
Hemoglobin (g/dl)	13.2 (12.1-14.7)	14.1 (13.5-15.0)	0.024	1.300	1.003-1.678	0.047
Leukocytes (n/µl)	5730 (4500-6590)	5950 (4690-7060)	0.450			
PMN neutrophils (n/µl)	3360 (2780-4180)	3500 (2700-4540)	0.689			
Albumin (g/dl)	4.30 (4.10-4.53)	4.24 (4.10-4.57)	0.574			
Bilirubin (mg/dl)	0.59 (0.40-0.89)	0.68 (0.45-0.99)	0.329			
eGFR (ml/min/1.73 m^2)	58.6 (45.1-73.1)	69.8 (53.8-83.2)	0.022			
AST (IU/l)	18 (14-24)	19.5 (17-25)	0.089			
ALT (IU/l)	15 (11-22)	19 (13-27)	0.019			
INR	1.02 (0.97-1.12)	1.07 (1.03-1.17)	0.021			
25-OH-Vitamin D (ng/ml)	31.0 (27.2-35.0)	31.5 (25.0-36.7)	0.893			

LT: liver transplantation; BMI: body mass index; HBV: hepatitis B virus; HCV: hepatitis C virus; AH: alcoholic; AI: autoimmune; HCC: hepatocellular carcinoma; DM: diabetes mellitus; HTN: arterial hypertension; MMF: mycophenolate mofetil; IS: immunosuppressive; T: tacrolimus; C: cyclosporine; E: everolimus; A: azathioprine; P: prednisone; [#]reference blood levels evaluated within 1 month before vaccination for each IS drug were calculated in accordance with Cillo et al.[35]; eGFR: estimated glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR international normalized ratio. Logistic model estimation parameters: pseudo $R^2 = 0.212$; area under the ROC curve = 0.787; correct classification = 73.2%.