SUPPLEMENTARY MATERIALS

METHODS

Used Serologic Assays

Abbott SARS-CoV-2 IgG II Quant-test (Abbott S IgG)

- Chemiluminescence microparticle immunoassay (CMIA).
- Quantifies IgG-type antibodies against the RBD of the viral S-protein
- Threshold for positivity: ≥ 50 AU/mL
- Maximum value: > 80.000 AU/mL

Roche Elecsys anti-SARS-CoV-2 S (Roche S tAb)

- Electrochemiluminescence sandwich immunoassay (ECLIA)
- Quantifies total antibodies directed against the receptor-binding domain (RBD) of the viral spike (S) protein
- Threshold for positivity: ≥ 0.8 U/ml
- Maximum value: > 250 U/mL

DiaSorin Liaison SARS-CoV-2 TrimericS IgG (DiaSorin TriS IgG)

- Chemiluminescence immunoassay (CLIA)
- Quantifies IgG antibodies against a trimeric S-protein antigen
- Threshold for positivity: ≥ 13 AU/ml
- Maximum value: > 800 AU/ml

Siemens SARS-CoV-2 IgG (Siemens sCOVG)

- Electrochemiluminescence sandwich immunoassay (ECLIA)
- Quantifies IgG antibodies directed against the receptor-binding domain (RBD) of the viral spike (S1) protein
- Threshold for positivity: ≥ 1.0 U/ml

- Maximum value: > 150 AU/ml

Wantai SARS-CoV-2 IgG ELISA (Wantai S IgG)

- Enzyme-Linked ImmunoSorbent Assay (ELISA)
- Quantifies IgG antibodies directed against the receptor-binding domain (RBD) of the viral spike (S1) protein
- Threshold for positivity: ≥ 0.75 AU/ml
- Maximum value: 14.500 AU/ml

All antibody levels were expressed in binding antibody units per milliliter (BAU/mL) which are traceable to the WHO international standard for anti-SARS-CoV-2 immunoglobulin, by multiplying antibodies titers by the conversion factors suggested by the manufacturers:

- Roche S tAb (U/ml): 1.0
- Abbott S IgG (AU/ml): 0.143
- DiaSorin TriS (AU/mL): 2.6
- Siemens sCOVG (U/mL): 21.8
- Wantai S IgG (AU/mL): 1.0

Detectable antibody responses were classified as “Weak” or “Good” with a threshold of 250 BAU/mL which has been associated to an estimate close to 90% of mRNA-1273 vaccine-induced protection against symptomatic COVID-19 in immunocompetent patients for anti-Spike antibodies in the COVE trial and close to 80% of ChAdOx1 nCoV-19 efficacy in the AZD12222 trial.

Table S1 – Interpretation of serological assays

	WHO international standard	Abbott S IgG	Roche S tAb	DiaSorin TriS	Siemens sCOVG	Wantai S IgG
Unit	BAU/mL	AU/mL	U/mL	AU/mL	U/mL	AU/mL
Positivity	-	≥ 50	≥ 0.8	≥ 13	≥ 1.0	≥ 0.75
Weak response	< 250	< 1748	< 250	< 96	< 11.5	< 250
Good response	≥ 250	≥ 1748	≥ 250	≥ 96	≥ 11.5	≥ 250

Table S2 - Used serologic assay and vaccine types

	Overall
	n = 687
Serologic assay	
Abbott S IgG	373 (54.3)
Roche S tAb	185 (26.9)
DiaSorin TriS IgG	42 (6.1)
Siemens sCOVG	55 (8.0)
Wantai S IgG	32 (4.7)
mRNA vaccines	
BNT162b2	657 (95.6)
mRNA-1273	30 (4.4)

All results are n (%).

Statistics

Proportions were assessed with an exact binomial confidence interval; Chi-square, Mann-Whitney test, MacNemar test and Mann-Whitney U test were used as appropriate. Significant variables in univariate analysis were used to build a logistic regression model to assess factors independently associated with the absence of antibody response after two vaccine doses. All tests were 2-sided with $\alpha = .05$. Analyses were performed using R Statistical Software (version 4.0.3).

RESULTS

Serologic response after 2 doses of vaccine

Table S3 – Univariate analysis of factors associated with the absence of serologic response after 2 doses of mRNA vaccine against SARS-CoV-2

	Overall	No detectable serologic response	Detectable serologic response	Serologic response rate	p-value*
	n = 687	n = 149	n = 538		
Age					0.690
≥ 50 years old	463 (67.4)	111 (74.5)	352 (65.4)	76%	
< 50 years old	224 (32.6)	38 (25.5)	186 (34.6)	83%	
Sex †					1.0
Male	399 (59.0)	91 (63.6)	308 (57.8)	77%	
Female	277 (41.0)	52 (36.4)	225 (42.2)	81%	
Disease Type †					0.765
Myeloid malignancy	439 (69.0)	97 (77.0)	342 (67.1)	78%	
Lymphoid malignancy	168 (26.4)	27 (21.4)	141 (27.6)	84%	
Nonmalignant	29 (4.6)	2 (1.6)	27 (5.3)	93%	
Conditioning†					0.726
RIC	430 (68.7)	99 (76.2)	331 (66.7)	77%	
MAC	196 (31.3)	31 (23.8)	165 (33.3)	84%	
Donor Type †					0.180
Matched unrelated	333 (50.8)	71 (54.2)	262 (49.9)	79%	
HLA-identical sibling	194 (29.6)	26 (19.8)	168 (32.0)	87%	
Haplo-identical	129 (19.7)	34 (26.0)	95 (18.1)	74%	
Pretransplant CMV Serostatus†					0.945
Recipient seronegative	282 (48.0)	44 (39.6)	238 (50.0)	84%	
Recipient seropositive	305 (52.0)	67 (60.4)	238 (50.0)	78%	
History of GVHD requiring systemic treatment†	239 (38.4)	51 (39.8)	188 (38.0)	78%	1.0
Time since transplantation					< 0.001
< 12 months	144 (21.0)	77 (51.7)	67 (12.5)	47%	
≥ 12 months	543 (79.0)	72 (48.3)	471 (87.5)	87%	
Disease relapse after HSCT†	61 (10.7)	17 (15.3)	44 (9.6)	72%	1.0
Ongoing antineoplastic treatments	74 (11.2)	26 (19.5)	48 (9.1)	65%	0.015
Ongoing immunosuppressive treatments (< 3 months) †	203 (30.0)	89 (60.5)	114 (21.6)	56%	< 0.001
Rituximab (< 6 months) †	29 (4.4)	24 (16.3)	5 (1.0)	17%	< 0.001
Absolute lymphocyte count (G/L) †					< 0.001
≥ 1G/L	421 (70.2)	51 (37.5)	370 (79.7)	88%	
< 1G/L	179 (29.8)	85 (62.5)	94 (20.3)	52%	
T-CD4+ cell count †					0.001
≥ 500/mm^3	174 (35.4)	14 (13.2)	160 (41.6)	92%	
< 500/mm^3	317 (64.6)	92 (86.8)	225 (58.4)	71%	
B-CD19+ cell count †					< 0.001
≥ 100/mm^3	274 (72.7)	24 (28.6)	250 (85.3)	91%	
< 100/mm^3	103 (27.3)	60 (71.4)	43 (14.7)	41%	

Gammaglobulinemia †					< 0.001
≥ 6 g/L	395 (71.3)	62 (50.4)	333 (77.3)	84%	
< 6 g/L	159 (28.7)	61 (49.6)	98 (22.7)	62%	

All results are n (%). *after Bonferroni Correction for multiple (16) tests. † Missing data: 11 for sex, 51 for disease type, 61 for conditioning, 31 for donor type, 100 for CMV serostatus, 64 for history of GVHD, 117 for disease relapse, 29 for anti-neoplastic treatment, 11 for immunosuppressive treatments, 35 for Rituximab, 87 for lymphocytes count, 196 for T-CD4+ cell count, 310 for B-CD19+ cell count and 133 for gammaglobulinemia. Vaccine efficacy rate is the proportion of patients with a detectable antibody response. RIC for reduced intensity conditioning, MAC for myeloablative conditioning, HSCT for hematopoietic stem cell transplantation, GVHD for graft versus host disease, CMV for cytomegalovirus.

Table S4 – Multivariate analysis of factors associated with the absence of antibody response after 2 doses of mRNA vaccine against SARS-CoV-2

	Multivariate model 1 n = 578		**Multivariate model 2** n = 352	
	Odds-ratio (95%CI)	**p-value***	**Odds-ratio (95%CI)**	**p-value***
Time since transplantation < 1 year	2.73 (1.63 to 4.59)	< 0.001	4.67 (2.41 to 9.07)	< 0.001
Antineoplastic treatments (< 3 months)	1.74 (0.93 to 3.23)	0.403	1.47 (0.62 to 3.5)	1.0
Immunosuppressive treatments (< 3 months)	3.41 (2.09 to 5.55)	< 0.001	2.75 (1.37 to 5.54)	0.032
Rituximab (< 6 months)	13.61 (4.1 to 45.19)	< 0.001	3.87 (0.72 to 20.8)	0.809
Absolute lymphocyte count < 1 G/L	3.09 (1.88 to 5.09)	< 0.001	-	-
T-CD4+ cell count < 500/mm^3	-	-	1.94 (0.88 to 4.29)	0.718
B-CD19+ cell count < 100/mm^3	-	-	5.72 (2.75 to 11.87)	< 0.001
Gammaglobulinemia < 6 g/L	-	-	1.09 (0.53 to 2.26)	1.0

Multivariate model 1 is adjusted in the full sample with available lymphocyte count (n=578, including 453 patients with an antibody response and 125 without), while multivariate model 2 is adjusted in a subset of patients with available gammaglobulinemia and T-CD4+ and B-CD19+ cells count (n=352, including 275 patients with a detectable antibody response and 77 without). * After Bonferroni Correction for multiple tests (5 and 7 tests). All variables significantly associated with antibody response in univariate analysis were included in the multivariate analysis.

Focus on patients vaccinated within the first year from HSCT

Table S5 –Univariate analysis of factors associated with the absence of serologic response after 2 doses of mRNA vaccine against SARS-CoV-2 in patients vaccinated within the first year from HSCT

	Overall	No detectable serologic response	Detectable serologic response	Serologic response rate	p-value*
	n = 144	n = 77	n = 67		
Age					0.068
≥ 50 years old	100 (69.4)	59 (76.6)	41 (61.2)	41%	
< 50 years old	44 (30.6)	18 (23.4)	26 (38.8)	59%	
Sex †					0.888
Male	89 (64.0)	47 (65.3)	42 (62.7)	47%	
Female	50 (36.0)	25 (34.7)	25 (37.3)	50%	
Disease Type †					0.246
Myeloid malignancy	93 (75.6)	50 (82.0)	43 (69.4)	46%	
Lymphoid malignancy	28 (22.8)	10 (16.4)	18 (29.0)	64%	
Nonmalignant	2 (1.6)	1 (1.6)	1 (1.6)	50%	
Conditioning†					0.448
RIC	90 (73.2)	47 (77.0)	43 (69.4)	48%	
MAC	33 (26.8)	14 (23.0)	19 (30.6)	58%	
Donor Type †					0.256
Matched unrelated	56 (44.8)	32 (51.6)	24 (38.1)	43%	
HLA-identical sibling	26 (20.8)	10 (16.1)	16 (25.4)	62%	
Haplo-identical	43 (34.4)	20 (32.3)	23 (36.5)	53%	
Pretransplant CMV Serostatus†					0.086
Recipient seronegative	43 (45.3)	17 (35.4)	26 (55.3)	40%	
Recipient seropositive	52 (54.7)	31 (64.6)	21 (44.7)	60%	
History of GVHD requiring systemic treatment†	34 (28.8)	23 (37.7)	11 (19.3)	32%	0.045
Time since transplantation					0.136
< 6 months	28 (19.4)	19 (24.7)	9 (13.4)	32%	
6-12 months	116 (80.6)	58 (75.3)	58 (86.6)	50%	
Disease relapse after HSCT†	12 (11.5)	7 (13.7)	5 (9.4)	42%	0.706
Ongoing antineoplastic treatments	18 (14.4)	9 (14.3)	9 (14.5)	50%	1.0
Ongoing immunosuppressive treatments (< 3 months) †	83 (58.0)	49 (64.5)	34 (50.7)	41%	0.136
Rituximab (< 6 months) †	19 (13.3)	16 (21.1)	3 (4.5)	16%	0.008
Absolute lymphocyte count (G/L) †					0.023
≥ 1G/L	54 (39.4)	21 (29.6)	33 (50.0)	61%	
< 1G/L	83 (60.6)	50 (70.4)	33 (50.0)	40%	
T-CD4+ cell count †					0.014
≥ 500/mm^3	26 (22.8)	7 (12.3)	19 (33.3)	73%	
< 500/mm^3	88 (77.2)	50 (87.7)	38 (66.7)	43%	
B-CD19+ cell count †					< 0.001
≥ 100/mm^3	46 (50.5)	13 (28.3)	33 (73.3)	72%	
< 100/mm^3	45 (49.5)	33 (71.7)	12 (26.7)	27%	

Gammaglobulinemia †					0.054
≥ 6 g/L	85 (64.4)	38 (55.9)	47 (73.4)	55%	
< 6 g/L	47 (35.6)	30 (44.1)	17 (26.6)	36%	

All results are n (%).† Missing data: 5 for sex, 21 for disease type, 21 for conditioning, 19 for donor type, 49 for CMV serostatus, 26 for history of GVHD, 40 for disease relapse, 19 for anti-neoplastic treatment, 1 for immunosuppressive treatments, 1 for Rituximab, 7 for lymphocytes count, 30 for T-CD4+ cell count, 53 for B-CD19+ cell count and 12 for gammaglobulinemia. Vaccine efficacy rate is the proportion of patients with a detectable antibody response. RIC for reduced intensity conditioning, MAC for myeloablative conditioning, HSCT for hematopoietic stem cell transplantation, GVHD for graft versus host disease, CMV for cytomegalovirus.

Table S6 – Multivariate analysis of factors associated with the absence of antibody response after 2 doses of mRNA vaccine against SARS-CoV-2 in patients vaccinated within the first year from HSCT

	Multivariate model 1 n = 115		**Multivariate model 2** n = 70	
	Odds-ratio (95%CI)	**p-value**	**Odds-ratio (95%CI)**	**p-value**
Time since transplantation < 6 months	1.74 (0.59 to 5.1)	0.311	1.57 (0.35 to 7.07)	0.557
History of GVHD requiring systemic treatment	2.71 (1.07 to 6.87)	0.036	1.96 (0.57 to 6.7)	0.283
Rituximab (< 6 months)	5.47 (1.39 to 21.48)	0.015	2.54 (0.42 to 15.52)	0.311
Absolute lymphocyte count < 1 G/L	3.01 (1.28 to 7.07)	0.011	-	-
T-CD4+ cell count < 500/mm^3	-	-	2.19 (0.63 to 7.64)	0.218
B-CD19+ cell count < 100/mm^3	-	-	4.06 (1.19 to 13.88)	0.026

Multivariate model 1 is adjusted in the full sample with available lymphocyte count and gammaglobulinemia (n=115, including 56 patients with an antibody response and 59 without), while multivariate model 2 is adjusted in a subset of patients with available T-CD4+ and B-CD19+ cells count (n=70, including 35 patients with a detectable antibody response and 35 without). All variables significantly associated with antibody response in univariate analysis were included in the multivariate analysis except for the time since transplantation which was forced into the model.

Third dose analysis

Table S7 – Characteristics at baseline of patients included in the third dose analysis

	Overall	**No or minimal response after dose 2** (< 250 BAU/mL)	**Good response after dose 2** (≥ 250 BAU/mL)
	n=181	n=116	n=65
Age (years) : median [IQR]	60.5 [49.5, 66.9]	61.3 [50.0, 66.4]	58.7 [49.5, 68.0]
Sex (female): n (%)	71 (39.4)	43 (37.4)	28 (43.1)
Disease type: n (%)			
Myeloid malignancy	131 (73.2)	88 (77.2)	43 (66.2)
Lymphoid malignancy	41 (22.9)	25 (21.9)	16 (24.6)
Nonmalignant	7 (3.9)	1 (0.9)	6 (9.2)
Myeloablative conditioning: n (%)	44 (25.3)	29 (26.1)	15 (23.8)
Donor Type: n (%)			
Matched unrelated	101 (56.7)	64 (56.1)	37 (57.8)
HLA-identical sibling	43 (24.2)	28 (24.6)	15 (23.4)
Haplo-identical	34 (19.1)	22 (19.3)	12 (18.8)
History of GVHD requiring systemic treatment: n (%)	65 (44.8)	41 (44.1)	24 (46.2)
Time since transplantation (months): median [IQR]	18.5 [10.1, 42.6]	12.2 [7.7, 24.4]	37.0 [19.9, 51.2]
< 12 months	65 (35.9)	58 (50.0)	7 (10.8)
≥ 12 months	116 (64.1)	58 (50.0)	58 (89.2)
Disease relapse after HSCT: n (%)	16 (11.1)	14 (15.4)	2 (3.8)
Ongoing antineoplastic treatments: n (%)	25 (14.2)	19 (16.8)	6 (9.5)
Ongoing immunosuppressive treatments (< 3 months from the first dose): n (%)	78 (43.3)	67 (58.3)	11 (16.9)
Rituximab (< 6 months from the first dose): n (%)	16 (8.9)	16 (13.9)	0 (0.0)
Absolute lymphocyte count (/mm^3): median [IQR]	1170.0 [600.0, 2200.0]	831.0 [500.0, 1787.0]	1640.0 [1120.0, 2440.0]
T-CD4+ cell count (/mm^3): median [IQR]	303.0 [178.5, 620.0]	253.0 [139.5, 712.5]	379.0 [239.5, 562.0]
B-CD19+ cell count (/mm^3): median [IQR]	159.0 [60.0, 378.0]	86.5 [1.0, 200.0]	361.0 [230.0, 547.5]
Gammaglobulinemia (g/L): median [IQR]	7.2 [4.8, 9.6]	6.3 [4.3, 9.1]	7.6 [6.1, 10.2]
mRNA vaccines dose 1 and 2: n (%)			
BNT162b2	179 (98.9)	114 (98.3)	65 (100.0)
mRNA-1273	2 (1.1)	2 (1.7)	0
mRNA vaccines dose 3: n (%)			
BNT162b2	178 (98.3)	113 (97.4)	65 (100.0)
mRNA-1273	3 (1.7)	3 (2.6)	0
Time interval between second and third vaccine dose (days): median [IQR]	54.0 [34.0, 73.8]	60.0 [42.0, 75.0]	35.0 [31.0, 69.0]
Time interval between third vaccine dose and serology (days): median [IQR]	30.0 [27.0, 35.0]	29.0 [26.0, 35.0]	31.0 [29.0, 35.0]

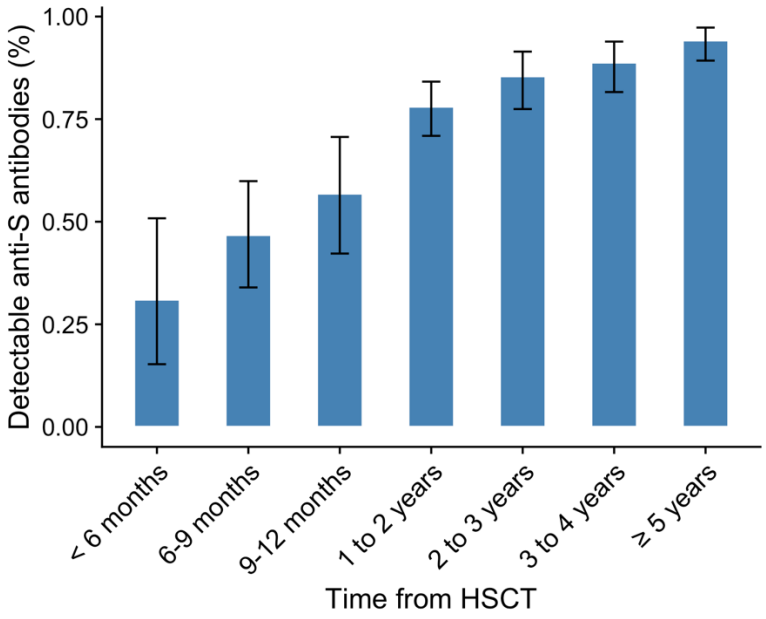
Characteristics of all patients in the cohort with a third dose and an available subsequent serologic testing.

Table S8 – Univariate analysis of factors associated with the emergence of detectable serologic response after a third vaccine dose

	No detectable serologic response after dose 3	**Detectable serologic response after dose 3**	**p-value***
	n=41	n=29	
Age (years): median [IQR]	61.8 [52.1, 66.4]	58.0 [49.7, 66.6]	0.547
Sex (female): n (%)	13 (32.5)	13 (44.8)	0.429
Disease type: n (%)			0.394
Myeloid malignancy	34 (85.0)	22 (75.9)	
Lymphoid malignancy	6 (15.0)	6 (20.7)	
Nonmalignant	0 (0.0)	1 (3.4)	
Myeloablative conditioning: n (%)	7 (17.1)	11 (39.3)	0.074
Donor Type: n (%)			0.167
Matched unrelated	26 (65.0)	15 (51.7)	
HLA-identical sibling	9 (22.5)	5 (17.2)	
Haplo-identical	5 (12.5)	9 (31.0)	
History of GVHD requiring systemic treatment: n (%)	15 (48.4)	11 (40.7)	0.749
Time since transplantation (months): median [IQR]			1.0
< 12 months	25 (51.0)	17 (58.6)	
≥ 12 months	16 (39.0)	12 (41.4)	
Pretransplant CMV Serostatus			0.273
recipient seronegative	13 (43.3.0)	7 (25.9)	
recipient seropositive	17 (56.7)	20 (74.1)	
Disease relapse after HSCT: n (%)	6 (19.4)	3 (12.0)	0.705
Ongoing antineoplastic treatments: n (%)	8 (20.0)	3 (10.3)	0.454
Ongoing immunosuppressive treatments (< 3 months from the first dose): n (%)	28 (70.0)	18 (62.1)	0.666
Rituximab (< 6 months from the first dose): n (%)	11 (26.8)	4 (13.8)	0.311
Absolute lymphocyte count (/mm^3): median [IQR]	580.0 [400.0, 918.5]	823.0 [500.0, 1550.0]	0.048
T-CD4+ cell count (/mm^3): median [IQR]	176.0 [113.5, 471.5]	226.0 [132.5, 450.0]	0.448
B-CD19+ cell count (/mm^3): median [IQR]	0.0 [0.0, 66.7]	85.0 [49.0, 156.0]	0.016
Gammaglobulinemia (g/L): median [IQR]	5.7 [3.6, 8.0]	6.3 [4.3, 8.8]	0.535
mRNA vaccines dose 1 and 2: n (%)			1.0
BNT162b2	40 (97.6)	28 (96.6)	
mRNA-1273	1 (2.4)	1 (3.4)	
Vaccine switch for the third dose: n (%)	0	2 (6.9)	0.328
Time interval between second and third vaccine dose (days): median [IQR]	57.0 [47.0, 73.0]	60.0 [42.0, 76.2]	0.449
Time interval between third vaccine dose and serology (days): median [IQR]	30.0 [22.5, 32.0]	28.5 [26.8, 34.2]	0.754

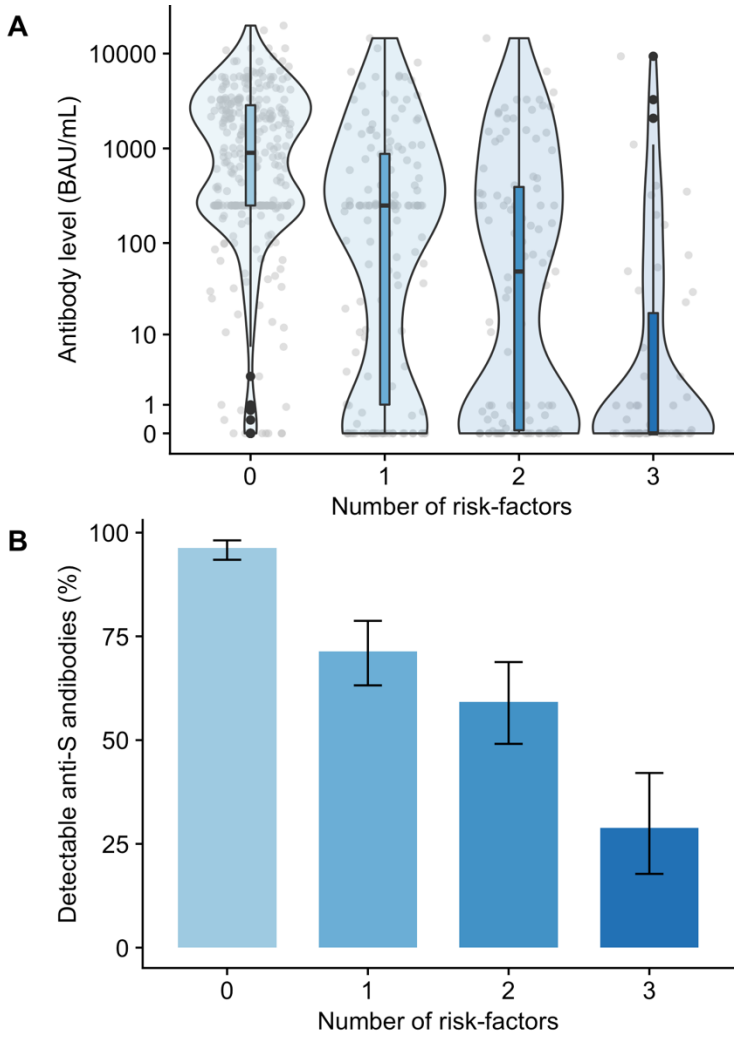
All patients with a negative serologic response after two doses are included in this table.

Figure S1 - Anti-Spike response after 2 vaccine doses by time from allogeneic HSCT



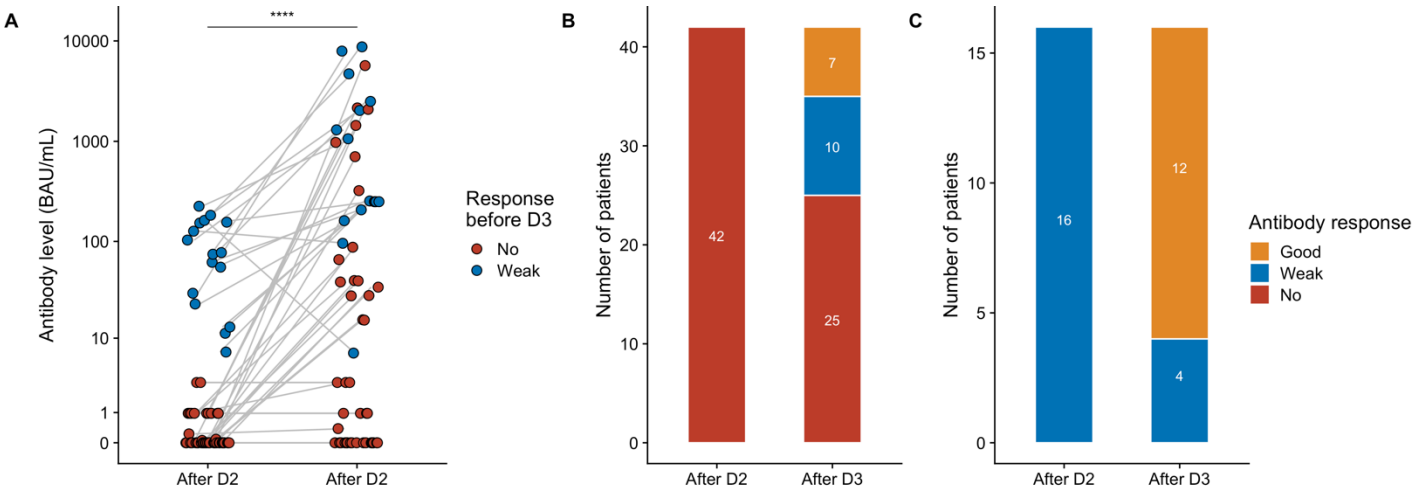
Serologic response to a two-dose vaccination according to the time from allogeneic hematopoietic stem cell transplantation (HSCT). The positivity threshold was given by the manufacturer for each used serological assay.

Figure S2 - Anti-Spike response by number of risk-factors associated with immunization after 2 vaccine doses



Serologic response to a two-dose vaccination according to the number of risk factors associated with immunization after dose 2 (see table 2 and figure 1). Panel A: Anti-spike antibody level. The violin plots contain interior box plots with upper and lower horizontal edges the 25th and 75th percentiles of antibody level and middle line the 50th percentile. Panel B: proportion of detectable anti-spike antibodies with 95% confidence interval. Risk-factors for absence of humoral response were significant variables in multivariate model 1: time-interval from transplantation < 12 months, absolute lymphocyte count <1G/L, systemic immunosuppressive treatments within 3 months of vaccination and use of rituximab within 6 months.

Figure S3 – Antibody response after a third dose of SARS-CoV-2 vaccine among patients within the first year from HSCT



Antibody response before and after the third dose (D3) among patients vaccinated within the first year after transplantation. Panel A: antibody levels (in BAU/mL) after the second and a third dose of vaccine. Dots represent individual values and are filled according to the response after dose 2 (red for no response, blue for response < 250 BAU/mL). Antibody level significantly increased after dose 3 ($p < 0.001$, Mann-Whitney U test). Panel B-C: antibody qualitative response to the third dose classified according antibody levels among patients with no- (B) or weak- (V) prior detectable response. “No” for undetectable response, “Weak” for response < 250 BAU/mL and “Good” for response ≥ 250 BAU/mL. D2 is for the dose 2 and D3 for dose 3.