ORIGINAL ARTICLE

SARS-CoV-2 vaccination response in patients with autoimmune hepatitis and autoimmune cholestatic liver disease

Paul Duengelhoef[1,2] | Johannes Hartl[1,3] | Darius Rüther[1,3] | Silja Steinmann[1,3] | Thomas T. Brehm[1,4] | Jan Philipp Weltzsch[1,3] | Fabian Glaser[1,3] | G. M. Schaub[1,4] | Martina Sterneck[1] | Marcial Sebode[1,3] | Christina Weiler-Normann[1,3] | Marylyn M. Addo[1,4,5] | Marc Lütgehetmann[4,6] | Friedrich Haag[2] | Christoph Schramm[1,3,7,8] | Julian Schulze zur Wiesch[1,4] | Ansgar W. Lohse[1,3,4,8]

[1]Department of Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[2]Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[3]European Reference Network on Hepatological Diseases (ERN RARE-LIVER), Hamburg, Germany

[4]Partner Site Hamburg-Lübeck-Borstel-Riems, German Center for Infection Research (DZIF), Hamburg, Germany

[5]Department for Clinical Immunology of Infectious Diseases, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany

[6]Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[7]Martin-Zeitz Center for Rare Diseases, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[8]Hamburg Center for Translational Immunology (HCTI), Hamburg, Germany

Correspondence

Ansgar W. Lohse and Johannes Hartl, Department of Internal Medicine, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20249 Hamburg, Germany.
Email: alohse@uke.de and j.hartl@uke.de

Abstract

Background/Aims: In this observational study, we explored the humoral and cellular immune response to SARS-CoV-2 vaccination in patients with autoimmune hepatitis (AIH) and patients with cholestatic autoimmune liver disease (primary sclerosing cholangitis [PSC] and primary biliary cholangitis [PBC]).

Methods: Anti-SARS-CoV-2 antibody titers were determined using the DiaSorin LIAISON and Roche immunoassays in 103 AIH, 64 PSC, and 61 PBC patients and 95 healthy controls >14 days after the second COVID-19 vaccination. The spike-specific T-cell response was assessed using an activation-induced marker assay (AIM) in a subset of individuals.

Results: Previous SARS-CoV-2 infection was frequently detected in AIH but not in PBC/PSC (10/112 (9%), versus 4/144 (2.7%), $p = 0.03$). In the remaining patients, seroconversion was measurable in 97% of AIH and 99% of PBC/PSC patients, respectively. However, in 13/94 AIH patients antibody levels were lower than in any healthy control, which contributed to lower antibody levels of the total AIH cohort when compared to PBC/PSC or controls (641 vs. 1020 vs. 1200 BAU/ml, respectively). Notably, antibody levels were comparably low in AIH patients with ($n = 85$) and without immunosuppression ($n = 9$). Also, antibody titers significantly declined within 7 months after the second vaccination. In the AIM assay of 20 AIH patients, a spike-specific T-cell response was undetectable in 45% despite a positive serology, while 87% (13/15) of the PBC/PSC demonstrated a spike-specific T-cell response.

Conclusion: Patients with AIH show an increased SARS-CoV-2 infection rate as well as an impaired B- and T-cell response to SARS-CoV-2 vaccine compared to PBC and PSC patients, even in the absence of immunosuppression. Thus, antibody responses to vaccination in AIH patients need to be monitored and early booster immunizations considered in low responders.

KEYWORDS
autoimmune hepatitis, chronic liver disease, immunosuppression, primary biliary cholangitis, primary sclerosing cholangitis, SARS-CoV-2 vaccination

INTRODUCTION

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) vaccine-induced immune response of different cohorts with liver disease including cirrhotic patients and liver transplant recipients has recently been published.[1] It demonstrated lower spike-specific antibody levels after the second SARS-CoV-2 vaccination in transplant patients but equally high antibody levels in cirrhotic patients compared to HC. In contrast, the spike-specific T-cell response was reduced in both patient groups. Another study revealed that patients with various autoimmune diseases and immunosuppression like rheumatic arthritis were also less likely to reach seroconversion after SARS-CoV-2 vaccination.[2] However, the vaccination response in patients with autoimmune liver diseases has not been studied comprehensively so far, nor have risk factors for an inadequate vaccination response been explored in this vulnerable cohort.

This prospective observational study compared the humoral and T-cellular immune response to SARS-CoV-2 vaccination in a large cohort of patients with AIH and cholestatic autoimmune liver disease (PBC and PSC). Also, predictors of low response to vaccination were explored in this cohort.

PATIENTS AND METHODS

Study population and data collection

Consecutive non-pregnant patients ≥18 years with diagnosed AIH, PSC, or PBC presenting at the YAEL outpatient clinic of the University Medical Center Hamburg-Eppendorf (UKE) for routine visits between July and September 2021 were enrolled in this prospective observational cohort study in case of SARS-CoV-2 vaccination with a two-dose regimen, consisting of an mRNA (BNT162b2; BioNTech SE/Pfizer or mRNA-1273; Moderna Biotech) or vector-based vaccine (AZD1222; AstraZeneca). Clinical data were obtained from the patients' electronic medical records. In addition, previous data from 95 control subjects matched by age, vaccination regimen, and time since second vaccination were included.[3] In all participants, the immune response was determined >2 weeks after the second vaccination. The study was approved by the local Ethics Committee of Hamburg, Germany (Reg. numbers PV7103, PV7298, EV5332) and the Paul Ehrlich Institute, the German Federal Institute for Vaccines and Biomedicines (Reg. number NIS508). All participants signed written informed consent.

Key summary

- This is the first report that comprehensively assessed the immune reponse after the second SARS-CoV-2 vaccination in patients with autoimmune liver diseases.
- 103 patients with autoimmune hepatitis (AIH) as well as 125 patients with primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) were included.
- The humoral and cellular vaccination was compared to a control group of heathy individuals that was matched by age, sex, and time from second vaccination.
- In almost all patients a humoral vaccination response could be detected.
- Nevertheless, AIH patients demonstrated a reduced humoral response compared to healthy controls (HC) or patients with cholestatic liver disease.
- Despite a positive serology, no spike-specific T cell response was detected in half of the patients with AIH, but in almost all patients with PBC or PSC.
- The impaired vaccination response in AIH was also observed in patients in remission without receiving immunosuppressive drugs.

Investigation of the COVID-19 vaccine-specific humoral and T-cell response

The vaccine-specific humoral immune response was quantitatively determined by the DiaSorin LIAISON XL anti-SARS-CoV-2 TrimericS IgG ChemiLuminescent ImmunoAssay (sensitivity 99.4%, specificity 99.8%, cut-off 33.8 BAU/mL[4]), further denoted as anti-S Trimer, and the Roche Elecsys anti-SARS-CoV-2 S Ig ElectroChemiLuminescent ImmunoAssay (sensitivity 93.9%, specificity 99.6%, cut-off 0.8 U/mL[5]), further denoted as anti-S RBD. To detect silent infections, the existence of anti-nucleocapsid antibodies was qualitatively assessed by

TABLE 1 Baseline characteristics and humoral response to SARS-CoV-2 vaccination

	AIH (n = 94)	PBC/PSC (n = 123)	Controls (n = 95)	p
Age (mean years, SD)	53 (17)	52 (15)	51 (8)	0.108
Females (n, %)	74 (79)	80 (65)	72 (75)	0.056
Vaccine regimen				**0.025**
mRNA/mRNA (n, %)	83 (88)	105 (85)	90 (95)	
BNT162b2 (n, %)	77 (82)	91 (74)	87 (92)	
mRNA-1273 (n, %)	6 (6)	14 (11)	3 (3)	
AZD1222/AZD1222 (n, %)	6 (6)	5 (4)	2 (2)	
AZD1222/mRNA (n, %)	5 (5)	13 (11)	3 (3)	
Time 2nd vaccination - follow-up (mean days, SD)	85 (36)	79 (29)	85 (28)	0.062
Transient elastography (median kPa, IQR)	6.5 (5.3–10.6)	6.0 (4.8–8.2)		0.331
Cirrhosis (n, %)	33 (35)	21 (17)		**0.002**
Diabetes (n, %)	9 (10)	13 (11)		0.810
BMI (median kg/m^2, IQR)	27.0 (23.3–31.0)	25.0 (22.6–28.0)		**0.048**
Arterial hypertension (n, %)	31 (33)	31 (25)		0.209
Creatinine (median mg/dl, IQR)	0.82 (0.72–0.92)	0.80 (0.74–0.90)		0.946
GFR (median mL/min, IQR)	88 (76–103)	81 (74–92)		0.729
HbA1c (median %, IQR), n = 43	5.4 (4.9–5.9)	5.8 (5.3–6.4)		0.238
Lymphocytes (median 10^9/L, IQR)	1.2 (0.7–1.6)	1.8 (1.3–2.3)		**<0.001**
T-lymphocytes (median/μL, IQR), n = 50	717 (404–1008)	899 (633–1482)		0.151
B-lymphocytes (median/μL, IQR), n = 50	54 (37–146)	187 (138–323)		**0.001**
Immunosuppression (n, %)	85 (90)	31 (25)		**<0.001**
Steroids (n, %)	31 (33)	11 (9)		**<0.001**
If yes: Dosage (median mg, range)	5 (2–40)	5 (2–15)		0.871
Azathioprine (n, %)	59 (63)	6 (5)		**<0.001**
If yes: Dosage (median mg, range)	75 (10–200)	75 (50–150)		0.884
MMF (n, %)	7 (7)	0		**0.003**
Other (n, %)	14 (15)	25 (20)		0.302
≥2 Immunosuppressants (n, %)	27 (29)	9 (7)		**<0.001**
≥3 Immunosuppressants (n, %)	1 (1)	0		0.433
Antibody titer trimer (median BAU/mL, IQR)	641 (227–1440)	1020 (432–1770)	1100 (552–1780)	**0.002**
Seroconversion (n, %)	91 (97)	122 (99)	95 (100)	0.125
≥100 BAU/mL (n, %)	84 (89)	119 (97)	95 (100)	**0.001**
≥552 BAU/mL (n, %)	51 (54)	86 (70)	72 (76)	**0.005**
Antibody titer RBD (median AU/mL, IQR)	980 (348–2304)	1743 (810–3450)	1262 (646–2370)	**0.008**
Seroconversion (n, %)	93 (99)	123 (100)	95 (100)	0.309
≥100 AU/mL (n, %)	82 (87)	121 (98)	95 (100)	**<0.001**
≥646 AU/mL (n, %)	56 (60)	95 (77)	71 (76)	**0.014**

Note: p-values < 0.05 were highlighted in bold.

Abbreviations: AIH, autoimmune hepatitis; BAU, binding antibody units; BMI, body mass index; GFR, glomerular filtration rate; IQR, interquartile range; MMF, mycophenolate mofetil; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; RBD, receptor binding domain; SD, standard deviation; UDCA, ursodeoxycholic acid.

the Roche Elecsys anti-SARS-CoV-2 N Ig.ElectroChemiLuminescent ImmunoAssay (sensitivity 93.6%, specificity 99.8%).[6]

The spike-specific T-cell response measured by an AIM assay

For assessment of the spike-specific T-cell response, peripheral blood mononuclear cells were isolated from EDTA-blood by Ficoll-PaqueT™ density gradient centrifugation and frozen at −80°C until analysis. After thawing, a minimum of 1×10^6 cells were stimulated with an overlapping 15-mer peptide pool derived from the full sequence of the SARS-CoV-2 spike glycoprotein (PepMix™ SARS-CoV-2 Spike Glycoprotein, JPT Peptide Technologies) or left unstimulated for 18 h at 37°C after adding 1 μL Ultra-LEAF™ purified anti-human CD40 antibody (BioLegend). Cells were stained with an antibody cocktail (Table S1) for the detection of surface molecules. All samples were analyzed on a BD FACS Canto II, and FlowJo version 10.8.0 (BD Biosciences) was used for the flow cytometric analysis.

Statistical analysis

Pearson Chi^2 test and Fisher's exact test were used to test the difference in dichotomous variables between two or more groups. Normally and abnormally distributed continuous variables were compared by t-test and Mann-Whitney test when comparing two groups or Kruskal-Wallis test when comparing more than two groups, respectively. A binary logistic regression model was constructed based on rational assumptions to predict a low immune response. Significance was expected for p-values smaller than 0.05. SPSS Statistics Version 26 (IBM Corp.) and GraphPad Prism version 8.0.0 (GraphPad Software) were used for statistical analyses and to create figures, respectively.

RESULTS

Patient characteristics

In a total of 112 consecutive patients with AIH and 144 consecutive patients with cholestatic liver disease (70 PBC, 74 PSC), anti-spike antibodies and anti-nucleocapsid antibodies were prospectively assessed >14 days following the second SARS-CoV-2 vaccination. Patients in whom the exact information on the date of the second vaccination or the vaccination regime was not available were only included in the analysis on seroprevalence of SARS-CoV-2 infection but not in the analysis on vaccination response. Convalescent patients were analyzed separately (flow chart, Figure.S1), Ultimately, 94 AIH patients and 123 patients (59 PBC, and 64 PSC) with autoimmune cholestatic liver disease were included in the final analysis of vaccination response and were compared to a control group of 95 healthy individuals who were matched by age, sex, and time since second vaccination. Characteristics of patients and controls included in the main analysis are given in Table 1.

Seroprevalence of SARS-CoV-2 infection in vaccinated individuals with autoimmune liver disease

In total, 11 patients (AIH: 8/112 (8%), PBC: 1/70 (1%), PSC: 2/74 (3%)) were tested positive for nucleocapsid antibodies indicating previous SARS-CoV-2 infection. In addition, two AIH patients and one PSC patient reported a previous SARS-CoV-2 infection despite
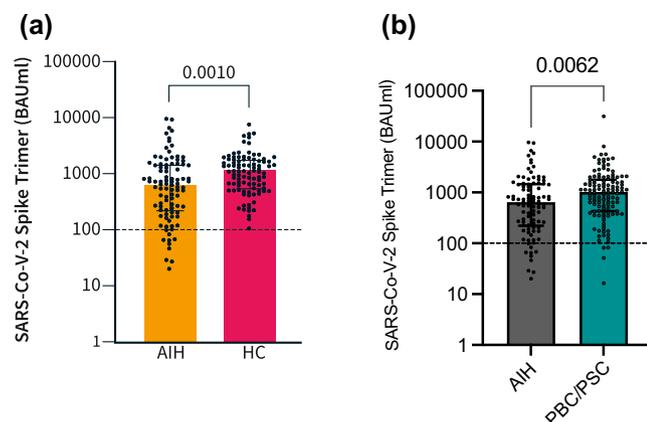

FIGURE 1 Serological response after second Covid-19 vaccination according to anti-S Trimer (a) Patients with autoimmune hepatitis (AIH) compared to healthy controls (HC) (b) AIH compared to patients with cholestatic autoimmune liver disease (primary biliary cholangitis/primary sclerosing cholangitis). Statistical analysis was performed by Mann-Whitney test; dotted horizontal lines indicate cut-off value for borderline response (<100 BAU/mL); bars and solid horizontal lines indicate medians and interquartile ranges
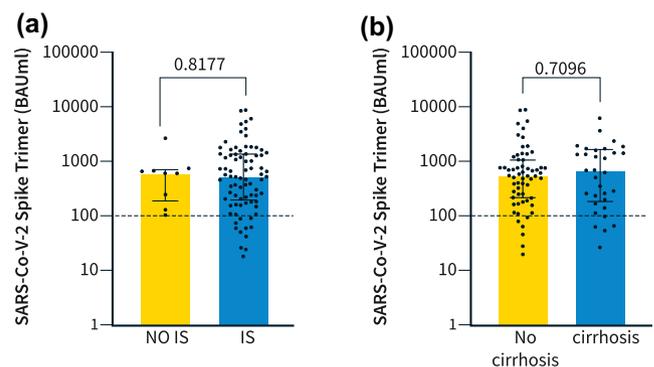

FIGURE 2 Comparison of humoral response according to the anti-S Trimer in autoimmune hepatitis (AIH) patients (AIH) under various conditions (a) AIH with liver cirrhosis compared to AIH without liver cirrhosis (b) Patients in biochemical remission (No IS) compared to patients undergoing immunosuppressive therapy (IS). Statistical analysis was performed by Mann-Whitney test; dotted horizontal lines indicate cut-off value for "borderline response" (<100 BAU/mL); bars indicate medians; solid horizontal lines indicate interquartile ranges

the absence of nucleocapsid antibodies. Thus, in our cohort SARS-CoV-2 infections had occurred more frequently in patients with AIH than in patients with cholestatic liver diseases (10/112%, 9% vs. 4/144%, 2.7%, $p = 0.03$).

Of note, only eight patients were aware of previous SARS-CoV-2 infection and reported mildly symptomatic COVID-19 (AIH: 6/112 (5%), PBC: 1/70 (1%), PSC: 1/74 (1%)), while in six patients the infection had taken a silent course. No patient had been hospitalized because of COVID-19.

Reduced humoral immune response in patients with AIH

In AIH, almost all patients (91/94%, 97%) achieved seroconversion. Nevertheless, antibody titers were significantly lower in comparison to HC when measured by the Trimer assay ($p = 0.001$; Figure 1a) and tended to be lower when the RBD assay was used ($p = 0.08$; Figure S2A). Although the difference in median antibody levels between the total cohort of AIH patients and controls was moderately pronounced, it is noteworthy that in a subgroup of 13 (14%) AIH patients, antibody levels were lower than the lowest antibody titer measured in any healthy control (Figure 1a, Figure S2A). The characteristics of this subgroup of AIH patients are shown in Table S2. No individual risk factor for impaired antibody response could be detected in this subgroup.

Irrespective of the assay used, antibody titers in AIH were significantly lower when compared to patients with cholestatic liver diseases (Figure 1b, Figure S2B). Therefore, we next explored whether this difference might be related to the distribution of potential risk factors for an impaired vaccination response. Patients with AIH did not differ with regard to age, sex, time since second vaccination, diabetes,

TABLE 2 Comparison of autoimmune hepatitis (AIH) patients with and without immunosuppression

	IS (n = 85)	No IS (n = 9)	p
Age (mean years, SD)	52 (17)	60 (17)	0.138
Females (n, %)	68 (80)	6 (67)	0.395
Time 2nd vaccination - follow-up (mean days, SD)	84 (35)	104 (42)	0.117
Transient elastography (median kPa, IQR)	6.7 (5.3–12.0)	5.7 (4.2–8.1)	0.243
Cirrhosis (n, %)	30 (35)	3 (33)	1.0
BMI (median kg/m^2, IQR)	27.4 (23.3–31.0)	24.7 (22.3–29.8)	0.435
Diabetes (n, %)	9 (10)	0	0.593
Arterial hypertension (n, %)	29 (34)	2 (22)	0.713
Creatinine (median mg/dL, IQR)	0.81 (0.72–0.93)	0.82 (0.72–0.88)	0.888
GFR (median mL/min, IQR)	88 (76–102)	86 (76–104)	1.0
HbA1c (median %, IQR)	5.4 (4.8–5.8)	-	-
IgG (median g/L, IQR)	13.9 (10.4–16.9)	12.0 (11.2–14.4)	0.459
GOT (median U/L, IQR)	27 (20–43)	30 (25–35)	0.654
GPT (median U/L, IQR)	25 (15–42)	29 (24–34)	0.710
Lymphocytes (median 10^9/L, IQR)	1.13 (0.64–1.56)	1.76 (1.23–2.22)	**0.023**
T-lymphocytes (median/µL, IQR)	717 (404–1008)	988 (401-X)	0.771
B-lymphocytes (median/µL, IQR)	49 (33–141)	252 (240-X)	**0.038**
Antibody titer trimer (median BAU/mL, IQR)	580 (217–1490)	669 (208–780)	0.812
Seroconversion (n, %)	82 (96)	9 (100)	1.0
≥100 BAU/mL (n, %)	75 (88)	9 (100)	0.590
≥552 BAU/mL (n, %)	45 (53)	6 (67)	0.501
Antibody titer RBD (median AU/mL, IQR)	926 (313–2579)	1363 (337–2110)	0.959
Seroconversion (n, %)	84 (99)	9 (100)	1.0
≥100 AU/mL (n, %)	74 (87)	8 (89)	1.0
≥646 AU/mL (n, %)	50 (59)	6 (67)	1.0

Note: p-values < 0.05 were highlighted in bold.

Abbreviations: BAU, binding antibody units; BMI, body mass index; GFR, glomerular filtration rate; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; IQR, interquartile range; IS, immunosuppression; RBD, receptor binding domain; SD, standard deviation; UDCA, ursodeoxycholic acid.

arterial hypertension, or renal impairment when compared to patients with cholestatic liver disease (Table 1). However, AIH patients had more frequently liver cirrhosis (35% vs. 17%, p = 0.002) and immunosuppressive treatment (90% vs. 25%, p < 0.001) along with reduced B-cell counts (54/μL vs. 187/μL, p = 0.001). Also, AIH patients tended to have less frequently received a heterologous vaccination (5% vs. 11%, p = 0.16). Hence, the potential impact of these factors on antibody levels was further explored.

Albeit most AIH patients require long-term immunosuppressive treatment,[7] nine (10%) AIH patients were in biochemical remission and had been off immunosuppressive therapy for at least 6 months. Interestingly, AIH patients without immunosuppression had comparably low antibody levels as those under immunosuppression (Figure 2a, Figure S3A), as well as lower antibody titers than HC (p = 0.050). Of note, no difference between AIH patients with and without immunosuppression was observed concerning age, time since second vaccination, or any other baseline characteristic (Table 2). Moreover, there was no difference in antibody levels between AIH patients with and without liver cirrhosis (Figure 2b, Figure S3B). As heterologous vaccination was more frequent in patients with cholestatic liver disease, the results of the different patient groups (AIH vs. Primary biliary cholangitis/PSC) were compared separately after the exclusion of patients who were vaccinated with a heterologous vaccine regime. Here, the antibody levels of AIH patients were still significantly lower compared to PSC/PBC patients (anti-S Trimer: 669 BAU/ml (IQR 227 vs. 1480) versus 1020 BAU/ml (480–1610), p = 0.027; anti-S RBD: 1142 AU/ml(301–2670) versus 1705 AU/ml (875–2823), p = 0.023) (Table S3).

Hence, no extrinsic factor which might have contributed to the lower antibody levels among AIH patients could be identified.

Humoral immune response in patients with PBC and PSC

When patients with cholestatic liver disease (PBC or PSC) were analyzed separately, PBC patients had lower antibody titers than patients with PSC (Figure S4) despite less frequent immunosuppressive treatment (12 vs. 38%). However, it must be noted that PBC patients had a longer time interval after the second vaccination (85 vs. 73 days (mean)) and were significantly older (61 vs. 44 years (mean)) with consecutively lower glomerular filtration rate (81 vs. 100 ml/min (median)) than patients with PSC (Table S2).

Risk factors for reduced humoral vaccination response in patients with autoimmune liver disease

The predictive degree of immunity to SARS-CoV-2 based on antibody levels has not been determined yet. Herein, we defined the cut-off for a "borderline response" at 100 BAU/mL (and 100 AU/mL) in line with previous reports and randomized trials.[1,8–10] Also, the cut-off level of 100 BAU/ml represents the lowest antibody level measured in any

healthy control in this study. Furthermore, an additional cut-off for a "low" positive response was set at the 25% percentile of antibody levels from HC in the respective assay (i.e. 552 BAU/mL (anti-S Trimer) and 646 AU/mL (anti-RBD)).

Both endpoints, "borderline" and "low" response occurred more frequently in patients with AIH than in patients with autoimmune cholestatic liver disease ("borderline response": 11% vs. 3%, p = 0.028; "low response": 46% vs. 30%, p = 0.18; Table 1; Figure 3).

In the total cohort of patients with autoimmune liver diseases, an independent effect on both endpoints, "borderline response" as well as "low response", was demonstrated for only two parameters that is the use of steroids and time after second vaccination (Table 3, Table S3 and S4).

Thus, the impact of time after second vaccination was further investigated. The time interval varied between 2 weeks and 7 months and was not different between study groups (Table 1). During this period, a decrease of antibody titers was observed in all study groups independently of the antibody assay applied (Figure 4, Figure S5). Moreover, there was no difference between the patient groups in how steep the decline of antibody levels occurred. Overall, in patients with >3 months after the second vaccination, antibody levels were only half as high as in the group <3 months after second vaccination (anti-S Trimer: 1310 vs. 636 BAU/mL, p < 0.001; anti-RBD: 1977 vs. 988 AU/mL, p < 0.001).

Although no other factor was independently linked with borderline response in the small subset of patients with antibody levels <100 BAU/ml, age, sex, diabetes, and hypertension indicated some impact on "low response" consistently in both assays but this failed to reach significance in the multivariate analysis (Table 3, Table S4).

Heterologous vaccination was associated with increased antibody levels, but only in the Trimer assay (Table 3) and only in patients with cholestatic liver disease (1770 vs. 1010 BAU/ml, p = 0.019) as
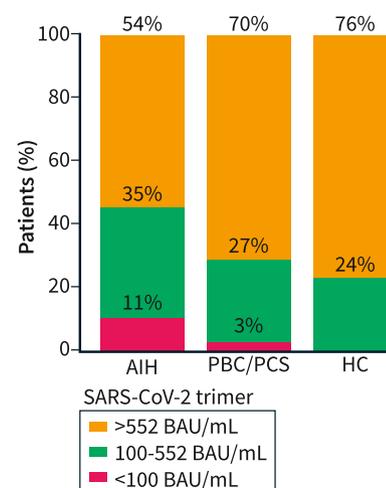

FIGURE 3 Serological response after second Covid-19 vaccination according to anti-S Trimer. Distribution of antibody levels in the respective study groups based on the cut-off for "borderline response" (<100 BAU/ml) and "low response" (<552 BAU/ml)

compared to patients with AIH (546 vs. 728 BAU/ml, $p = 0.798$). Of note, even in the separate analysis of patients who had received a homologous vaccination regime, the antibody levels of AIH patients were still significantly lower compared to PSC/PBC patients.

Spike-specific T-cell response

In addition to antibody-titers, the spike-specific T-cell response was assessed by the upregulation of the activation-induced markers (activation-induced marker assay (AIM)) CD154 and CD137 in 20 AIH patients, 15 patients with cholestatic liver disease (13 PBC, 2 PSC), and 7 controls to examine the spike specific T-cell response.

Almost every second AIH patient (9/20, 45%) did not show a specific CD4+ T-cell response upon stimulation, while this was the case in only 2/15 (13.3%) patients with cholestatic liver disease (both PBC) and none of the controls (Figure 5a). Moreover, frequencies of spike-specific CD4+ and CD8+ T cells tended to be lower in AIH patients as compared to HC ($p = 0.14$, and $p = 0.05$, respectively), whereby in line with previous results, frequencies of spike specific CD8+ T cells were lower than respective frequencies of CD4+ T cells (Figure S6).[11,12]

In the total cohort of patients with autoimmune liver diseases, a low humoral response (<552 BAU/ml) was linked with lower frequencies of spike-specific CD4+ T-cells and lower T-cell response upon stimulation (Figure S7). However, the overall correlation

TABLE 3 Binary logistic regression analysis for risk of antibody titers <552 BAU/mL (anti-S Trimer)

	Univariate OR (95%-CI)	p	Multivariate OR (95%-CI)	p
Total cohort				
Age (per 10 years)	**1.25 (1.08-1.45)**	**0.003**	**1.23 (1.05-1.47)**	**0.009**
Sex	0.62 (0.35–1.07)	0.087		
Heterologous vaccination	**1.85 (1.11-3.07)**	**0.018**	**2.08 (1.18-3.66)**	**0.012**
Time after 2nd vaccination (per 30 days)	**1.59 (1.25-2.03)**	**<0.001**	**1.67 (1.28-2.17)**	**<0.001**
Immunosuppression	**1.92 (1.19-3.12)**	**0.008**	**2.10 (1.24-3.54)**	**0.006**
Patients only				
Age (per 10 years)	**1.28 (1.09-1.49)**	**0.003**	1.14 (0.94–1.39)	0.174
Sex	**0.48 (0.25-0.92)**	**0.027**	0.87 (0.41–1.84)	0.705
Heterologous vaccination	**2.02 (1.16-3.53)**	**0.013**	**2.29 (1.21-4.33)**	**0.011**
Time after 2nd vaccination (per 30 days)	**1.46 (1.12-1.89)**	**0.005**	**1.53 (1.14-2.05)**	**0.005**
PBC/PSC	**0.51 (0.29-0.89)**	**0.018**	0.73 (0.38–1.41)	0.351
Immunosuppression	1.65 (0.94–2.89)	0.080		
Steroids	**2.51 (1.26-4.97)**	**0.009**	**2.71 (1.22-6.02)**	**0.014**
MMF	2.35 (0.51–10.78)	0.271		
Azathioprine	1.60 (0.88–2.89)	0.123		
UDCA	0.61 (0.34–1.12)	0.111		
Diabetes	**3.42 (1.37-8.57)**	**0.009**	2.48 (0.89–6.91)	0.083
Hypertension	**1.97 (1.08-3.59)**	**0.027**	1.32 (0.64–2.72)	0.450
Transient elastography	0.99 (0.96–1.02)	0.386		
Cirrhosis	0.82 (0.43–1.56)	0.535		
BMI	1.05 (0.99–1.11)	0.129		
HbA1c	1.19 (0.79–1.79)	0.399		
Creatinine	1.93 (0.67–5.55)	0.221		
IgG	0.97 (0.91–1.03)	0.258		
Lymphocytes	1.05 (0.93–1.19)	0.431		
T-lymphocytes	1.00 (1.00–1.00)	0.709		
B-lymphocytes	1.00 (0.99–1.00)	0.308		

Note: p-values < 0.05 were highlighted in bold.

Abbreviations: BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; OR, odds ratio; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; UDCA, ursodeoxycholic acid.
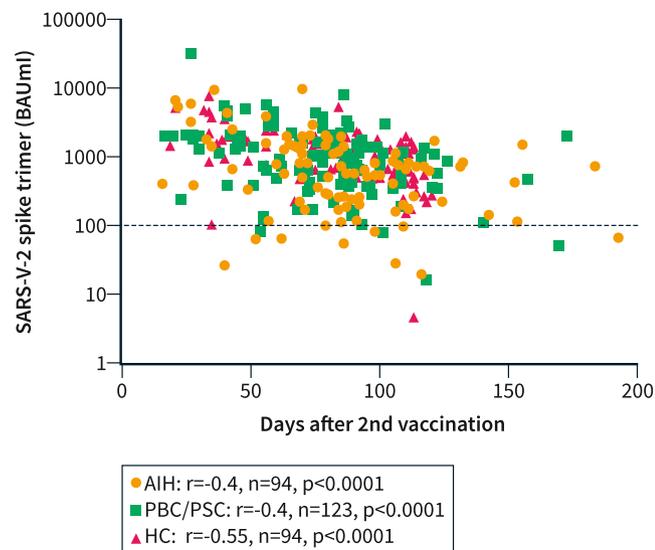
FIGURE 4 Humoral response after second vaccination by the anti-S Trimer over time. Comparison of the antibody titer according to the time point after second dose in patients with autoimmune hepatitis (AIH, *black dots*), patients with cholestatic liver disease (primary biliary cholangitis/primary sclerosing cholangitis, *turquoise squares*) and healthy controls (HC, *purple ascending triangles*). Dotted horizontal line indicates cut-off value for "borderline response" (<100 BAU/mL)

between the humoral and cellular immune response was of only minor stringency in all study groups. In patients with AIH, the antibody levels demonstrated some correlation with frequencies of spike-specific CD4$^+$ T-cells (Figure S8) but did not correlate with the stimulation index of spike specific CD4+ T cells ($r = = 0.18$, $p = 0.44$). Along this line, 6/13 (46%) AIH patients showed no T-cell response despite high antibody titers ≥552 BAU/ml (anti-S Trimer). In contrast, this was only observed in 1/12 (8%) of the patients with PBC/PSC (Figure 5b). *Nevertheless, also in patients with PBC/PSC, the correlation between humoral and cellular response was not significant ($r = 0.12$, $p = 0.24$), but tended to be significant in HC ($r = 0.26$, $p = 0.08$).*

Immune response in convalescents

Of the nine convalescent AIH patients, eight received an mRNA-based vaccination and all developed a humoral immune response with high antibody levels ranging from 418 BAU/mL to 16,700 BAU/mL (median 3680 BAU/ml). Cellular immune response was only assessed in two convalescent AIH patients after receiving SARS-CoV-2 vaccination with a two-dose regimen (BNT162b2; respectively), but none showed a spike-specific T-cell response despite high anti-SARS-CoV-2 titers.

DISCUSSION

This observational study is the first report that comprehensively and prospectively assessed the humoral and cellular immune response in patients with AIH following the second anti-SARS-CoV-2 vaccination.

Moreover, the vaccination response was compared to age- and sex-matched study groups of patients with cholestatic autoimmune liver disease (PBC/PSC) and HC.[3]

Almost all AIH patients (97%) achieved a seroconversion. Nevertheless, a subgroup of about 15% of patients with AIH showed a considerably reduced humoral immune response, which resulted in moderately reduced antibody levels of the total AIH cohort when compared to healthy individuals or patients with cholestatic liver diseases. Also, in contrast to some previous reports[11] but in accordance with more recent publications,[12] our data indicate a significant decrease of antibody levels in all study groups within a relatively short period of 7 months. In only every second AIH patient a spike-specific T-cell response was detectable despite high antibody levels. For instance, 36% showed no spike-specific T-cell response despite antibody titers >552 BAU/ml. Collectively, these data demonstrate a moderately reduced humoral SARS-CoV-2 vaccination response in AIH as well as an impaired T cell response compared to PBC/PSC and HC.

These results are somewhat unexpected, given the fact that most AIH patients in our cohort were only under mild immunosuppressive therapy receiving low dose azathioprine w/o low dose prednisolone. Notably, 10% of the AIH patients were in biochemical remission and had been off immunosuppressive therapy for at least 6 months, and even these patients demonstrated comparably low antibody titers as AIH patients under immunosuppression. These findings are in line with a previous study that reported that untreated AIH patients did not respond to tetanus toxoid booster immunization.[13] Moreover, this previous report demonstrated an active suppression of T-cell autoreactivity by peripheral blood cells collected in phases of disease remission. Thus, based on these observations a general immunocompromised state may exist in AIH which might also have contributed to the reduced vaccination response and the diminished effects of heterologous vaccination in our AIH cohort. Along this line, potential risk factors of impaired vaccination were equally distributed between study groups, and therefore, no extrinsic factor that might have contributed to the reduced vaccination response in AIH could be identified.

A recent report demonstrated an inferior survival rate after liver transplantation in patients with AIH compared to PBC or PSC caused by an increased susceptibility to infection.[14] Accordingly, AIH patients had an increased risk of SARS-CoV-2-infection compared to patients with PBC/PSC, as a previous SARS-CoV-2-infection was detected in 9% of AIH patients but only in 2.7% of the patients with PSC/PBC. These rates are higher/lower than an observed convalescent rate of 4.7% in a healthcare worker cohort of the same region.[3] Although these data were collected 2–4 months earlier in May 2021, a relevant increase in the convalescent rate since then seems unlikely, given the low SARS-CoV-2 incidence rate in Germany from May to September 2021. While immunosuppression due to AIH-intrinsic mechanisms or/and medication may have contributed to the increased risk of infection in AIH, the mechanisms that might have protected patients with PBC/PSC from SARS-CoV-2 infection are unknown.
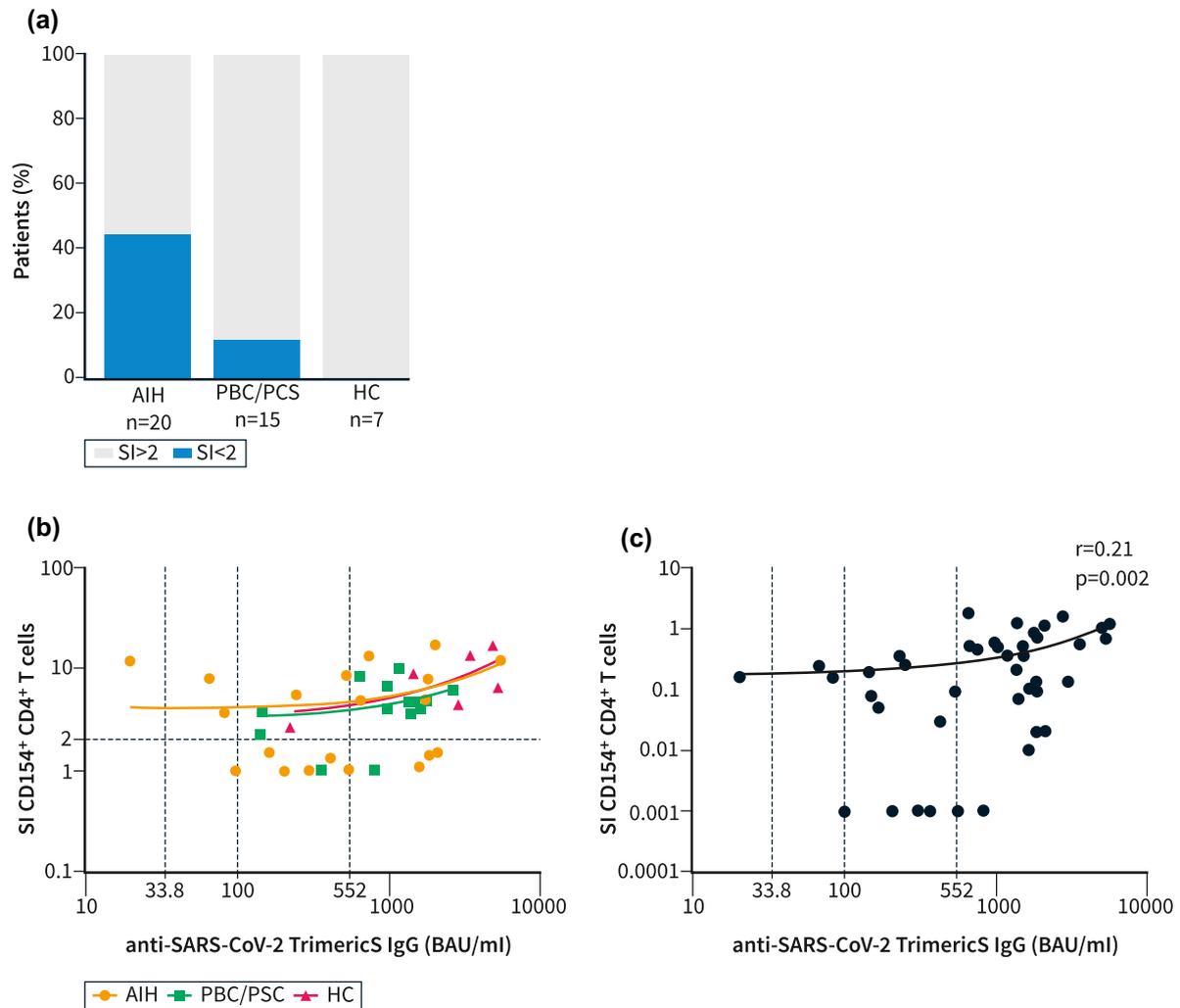
FIGURE 5 T cell response after second SARs-CoV-2 vaccination in patients with autoimmune hepatitis (AIH), patients with cholestatic autoimmune liver disease (primary biliary cholangitis/primary sclerosing cholangitis) and healthy controls (HC) (a) Responder rates within patient groups according to CD4⁺ (left) and CD8⁺ (right) T cell. Stimulation index (SI) was calculated by dividing the percentages of CD154⁺ CD4⁺ T cells or rather either CD154⁺ or CD137⁺ CD8⁺ Tcells in the stimulated sample by the respective unstimulated value. A SI of >2 was interpreted as a positive reaction. SI below 1 were set to 1 (b) Stimulation indices (SI) compared to the humoral response (c) Correlation of percentages of CD154⁺ CD4⁺ T cells to antibody levels (anti-S Trimer). Dotted horizontal line indicates cut-off value for an adequate T cell response; dotted vertical lines indicate cut-off values for no, "borderline", or "low response" (<33,8 BAU/mL, <100 BAU/mL, <552 BAU/mL)

As we only assessed anti-SARS-CoV-2 antibodies in vaccinated patients and in those with a history of COVID-19 disease, we might have missed silent SARS-CoV-2 infections in unvaccinated patients. For this reason, we cannot estimate the overall infection rate. However, the study started at end of July 2021, at a time at which the vast majority had received a vaccination, hence, it seems unlikely that the overall infection rate in patients with autoimmune liver disease was considerably higher. Also, only 5 patients reported mild COVID-19 symptom and none of the patients had been hospitalized because of COVID-19. Hence, in line with previous reports on patients with AIH[15] and other autoimmune diseases,[16,17] patients with autoimmune liver diseases were not at high risk of a severe disease course.

We and others have reported previously[1,18] that in a group of patients with chronic liver disease of mixed etiology, liver cirrhosis

was not associated with lower antibody levels. Accordingly, we did not observe any effect of liver cirrhosis in the overall cohort of patients with autoimmune liver disease, nor in AIH, which is of clinical relevance since about one-third of AIH patients have already liver cirrhosis at the time of diagnosis.

Previously identified risk factors of impaired SASR-CoV-2 vaccination response in liver transplant patients[1,19] and patients with inflammatory bowel disease[20] like the strength of immunosuppression, use of azathioprine, diabetes and arterial hypertension were associated with low antibody levels in the univariate analysis and thus seemed to also have some impact on patients with autoimmune liver diseases. Nevertheless, in the multivariate analysis, only immunosuppression with steroids had an independent effect consistently in both antibody-assays, emphasizing its importance for reduced vaccination response as shown for various patient groups before.[19,21,22] However,

the sample size may have been too small, to detect more subtle pronounced effects, which is clearly a limitation of this study. Especially the small sample size in the subgroups including AIH patients without immunosuppression (n = 9), patients with the lowest antibodies (n = 13) and infection rates among AIH patients and PBC/PSC (n = 11) limits the significance of the results. Furthermore, not standardizing timepoints of blood collection after second vaccination is a main methodological limitation of this study. Although time since second vaccination was equally distributed between study groups, the time interval varied considerably, and therefore, might have introduced bias. In addition, this study is limited by the restricted number of patients in whom the T-cell response was explored. In addition, we did not look for the functionality of the T cells (e.g. via cytokine staining), hence the full T cell response and level of protection in our cohort is still unknown. Finally, it has to be pointed out that the level of circulating SARS-CoV-2 antibodies that renders safe protection against symptomatic breakthrough infection has not been established yet. So, the chosen cut-offs of a borderline and low humoral response should only be regarded as an estimate based on the range of antibody levels in controls and the current literature.[23]

In conclusion, almost all patients with autoimmune liver diseases showed a detectable humoral response to SARS-CoV-2 vaccine. However, patients with AIH demonstrated an impaired spike-specific T-cell response and reduced antibody levels when compared to HC or patients with PBC/PSC. Notably, this was also observed in AIH patients without immunosuppressive medication, suggesting that a state of generalized immunosuppression may exist in AIH. Along this line, AIH patients seem to have an increased risk to acquire a SARS-CoV-2-infection, while – for unknown reasons - patients with PBC/PSC might be protected from infection. Thus, antibody responses to vaccination in AIH patients need to be monitored and early booster immunizations considered in low responders.

CONFLICT OF INTEREST
All authors declare that they have no known competing financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT
Individual participant data will not be shared.

ORCID
Johannes Hartl https://orcid.org/0000-0002-4037-9938
Darius Rüther https://orcid.org/0000-0003-3783-380X

REFERENCES
1. Ruether DF, Schaub GM, Duengelhoef PM, Haag F, Brehm TT, Fathi A, et al. SARS-CoV2-specific humoral and T-cell immune response after second vaccination in liver cirrhosis and transplant patients. Clin Gastroenterol Hepatol. 2022;20:162–172.
2. Kearns P, Siebert S, Willicombe M, Gaskell C, Kirkham A, Pirrie S, et al. Examining the immunological effects of COVID-19 vaccination in patients with conditions potentially leading to diminished immune response capacity – the OCTAVE trial; 2021. https://ssrn.com/abstract=3910058
3. Brehm TT, Thompson M, Ullrich F, Schwinge D, Addo MM, Spier A, et al. Low SARS-CoV-2 infection rates and high vaccine-induced immunity among German healthcare workers at the end of the third wave of the COVID-19 pandemic. Int J Hyg Environ Health. 2021;238:113851.
4. Bonelli F, Blocki FA, Bunnell T, Chu E, De La OA, Grenache DG, et al. Evaluation of the automated LIAISON((R)) SARS-CoV-2 TrimericS IgG assay for the detection of circulating antibodies. Clin Chem Lab Med. 2021;59(8):1463–7.
5. Patel EU, Bloch EM, Clarke W, Hsieh YH, Boon D, Eby Y, et al. Comparative performance of five commercially available serologic assays to detect antibodies to SARS-CoV-2 and identify individuals with high neutralizing titers. J Clin Microbiol. 2021;59(2).
6. Riester E, Majchrzak M, Mühlbacher A, Tinguely C, Findeisen P, Hegel JK, et al. Multicentre performance evaluation of the Elecsys anti-SARS-CoV-2 immunoassay as an aid in determining previous exposure to SARS-CoV-2. Infect Dis Ther. 2021;10(4): 2381–97.
7. Hartl J, Ehlken H, Weiler-Normann C, Sebode M, Kreuels B, Pannicke N, et al. Patient selection based on treatment duration and liver biochemistry increases success rates after treatment withdrawal in autoimmune hepatitis. J Hepatol. 2015;62(3): 642–6.
8. Resman Rus K, Korva M, Knap N, Avšič Županc T, Poljak M. Performance of the rapid high-throughput automated electrochemiluminescence immunoassay targeting total antibodies to the SARS-CoV-2 spike protein receptor binding domain in comparison to the neutralization assay. J Clin Virol. 2021;139:104820.
9. Hall VG, Ferreira VH, Ku T, Ierullo M, Majchrzak-Kita B, Chaparro C, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. N Engl J Med. 2021;385:1244–1246.
10. Ragone C, Meola S, Fiorillo PC, Penta R, Auriemma L, Tornesello ML, et al. HLA does not impact on short-medium-term antibody response to preventive anti-SARS-cov-2 vaccine. Front Immunol. 2021;12(2999).
11. Doria-Rose N, Suthar MS, Makowski M, O'Connell S, McDermott AB, Flach B, et al. Antibody persistence through 6 Months after the second dose of mRNA-1273 vaccine for covid-19. N Engl J Med. 2021;384(23):2259–61.
12. Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 covid-19 vaccine over 6 months. N Engl J Med. 2021;385(24):e84.
13. Lohse AW, Kögel M, Meyer zum Büschenfelde KH. Evidence for spontaneous immunosuppression in autoimmune hepatitis. Hepatology. 1995;22(2):381–8.
14. Heinemann M, Adam R, Berenguer M, Mirza D, Malek-Hosseini SA, O'Grady JG, et al. Longterm survival after liver transplantation for autoimmune hepatitis: results from the European liver transplant registry. Liver Transplant. 2020;26(7):866–77.
15. Marjot T, Buescher G, Sebode M, Barnes E, Barritt AS, Armstrong MJ, et al. SARS-CoV-2 infection in patients with autoimmune hepatitis. J Hepatol. 2021;74(6):1335–43.
16. Picchianti Diamanti A, Rosado MM, Nicastri E, Sesti G, Pioli C, Laganà B. Severe acute respiratory syndrome coronavirus-2 infection and autoimmunity 1 year later: the era of vaccines. Front Immunol. 2021;12:708848.
17. Kastritis E, Kitas GD, Vassilopoulos D, Giannopoulos G, Dimopoulos MA, Sfikakis PP. Systemic autoimmune diseases, anti-rheumatic

therapies, COVID-19 infection risk and patient outcomes. Rheumatol Int. 2020;40(9):1353–60.

18. Thuluvath, PJ, Robarts, P, Chauhan, M. Analysis of antibody responses after COVID-19 vaccination in liver transplant recipients and those with chronic liver diseases. J Hepatol.

19. Rabinowich L, Grupper A, Baruch R, Ben-Yehoyada M, Halperin T, Turner D, et al. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. J Hepatol. 2021.

20. Kennedy NA, Lin S, Goodhand JR, Chanchlani N, Hamilton B, Bewshea C, et al. Infliximab is associated with attenuated immunogenicity to BNT162b2 and ChAdOx1 nCoV-19 SARS-CoV-2 vaccines in patients with IBD. Gut. 2021;70(10):1884–93.

21. Stumpf J, Siepmann T, Lindner T, Karger C, Schwöbel J, Anders L, et al. Humoral and cellular immunity to SARS-CoV-2 vaccination in renal transplant versus dialysis patients: a prospective, multicenter observational study using mRNA-1273 or BNT162b2 mRNA vaccine. The Lancet Regional Health – Europe. 2021;9.

22. Deepak P, Kim W, Paley MA, Yang M, Carvidi AB, Demissie EG, et al. Effect of immunosuppression on the immunogenicity of mRNA vaccines to SARS-CoV-2 : a prospective cohort study. Ann Intern Med. 2021;174(11):1572–85.

23. Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nat Med. 2021;27:2032–2040.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.