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Analysis of the humoral and cellular response after the third COVID-19 vaccination in patients with autoimmune hepatitis

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Abstract

Background & Aims: To explore the humoral and T-cell response to the third COVID-19 vaccination in autoimmune hepatitis (AIH).

Methods: Anti-SARS-CoV-2 antibody titers were prospectively determined in 81 AIH patients and 53 healthy age- and sex-matched controls > 7 days (median 35) after the first COVID-19 booster vaccination. The spike-specific T-cell response was assessed using an activation-induced marker assay (AIM) in a subset of patients.

Results: Median antibody levels were significantly lower in AIH compared to controls (10908 vs. 25000 AU/mL, p<0.001), especially in AIH patients treated with MMF (N=14, 4542 AU/mL, p=0.004) or steroids (N=27, 7326 AU/mL, p=0.020). Also, 48% of AIH patients had antibody titers below the 10% percentile of the healthy controls (9194 AU/mL, p<0.001). AIH patients had a high risk of failing to develop a spike-specific T-cell response (15/34 (44%) vs. 2/16 (12%), p=0.05) and showed overall lower frequencies of spike-specific CD4+T cells (median: 0.074% vs 0.283;p=0.01) after the booster vaccination compared to healthy individuals. In 34/81 patients, antibody titers before and after booster vaccination were available. In this subgroup, all patients but especially those without detectable/low antibodies titers (<100 AU/mL) after the second vaccination (N=11/34) showed a strong, 148-fold increase.

Conclusion: A third COVID-19 vaccination efficiently boosts antibody levels and T-cell responses in AIH patients and even seroconversion in patients with absent immune response after two vaccinations, but to a lower level compared to controls. Therefore, we suggest routinely assessing antibody levels in AIH patients and offering additional booster vaccinations to those with suboptimal response.

Lay summary:

Recently, we demonstrated that the immune response after the second COVID-19 vaccination in patients with autoimmune hepatitis is reduced. Here, we show that a third dose efficiently increases antibody levels but the response remains weaker than in controls. Therefore, assessing antibody levels and offering a second booster vaccination for all AIH patients with a suboptimal response is proposed.

Highlights:

- Antibody levels and the spike-specific T-cell response were prospectively assessed in patients with AIH and a control group of healthy individuals after first COVID-19 booster vaccination.
- A third COVID-19 vaccination efficiently boosts the humoral immune response in AIH patients and a seroconversion can be achieved in all patients.
- However, antibody levels remain significantly lower than in controls and the magnitude of the spike-specific T-cell response does not increase compared to the response after two vaccinations.
- Multivariate logistic regression analysis reveals immunosuppression with MMF or steroids as risk factors for lower antibody levels.
- Therefore, it is proposed to assess antibody levels in all patients and to offer early booster vaccination for patients with low response after the third vaccination.

Introduction

Various reports show a reduced immune response to COVID-19 vaccinations in patients on immunosuppression (1), including liver transplant recipients (2). Moreover, we have reported recently, that AIH patients show an impaired B- and T-cell response after the 2nd COVID-19 vaccination, even in the absence of or under mild immunosuppressive treatment (3). Also, AIH patients seemed to have an increased risk to acquire SARS-CoV-2 infection (3). At the same time, immunosuppressive drugs (4, 5) and advanced stages of chronic liver diseases (6) are associated with a more severe course of COVID-19. Additionally, various SARS-CoV-2 variants have been identified as being partly resistant to antibody-mediated neutralization requiring induction of higher antibody titers for protection (5-7). Indeed, a third dose of a COVID-19 vaccine demonstrated effectiveness for boosting the vaccination response and preventing severe outcomes in the general population and patients under immunosuppression like solid organ transplant recipients or patients with rheumatic disease (8-10)

In this prospective observational study, we aimed to explore the humoral and T-cell response after the third COVID-19 vaccination in patients with AIH compared to healthy controls in a real-world setting.

Patients and Methods

Study population and data collection

Consecutive non-pregnant patients ≥ 18 years with diagnosed AIH presenting at the YAEL outpatient clinic of the University Medical Center Hamburg-Eppendorf (UKE) for routine who were SARS-CoV-2 vaccinated with a three-dose regimen with either an mRNA (BNT162b2; BioNTech SE / Pfizer or mRNA-1273; Moderna Biotech) or vector-based (AZD1222; AstraZeneca) basic immunization and an mRNA-based booster dose visits between January and February 2022, were enrolled in this prospective observational cohort study. To assess a previous COVID-19 infection, all AIH patients were tested for antibodies against SARS-CoV-2 nucleocapsid. In addition, data from 53 nucleocapsid-negative control subjects (being part of the cohorts described in (2) and (11)) matched by age, sex, and time since the third vaccination were included. In all participants, the immune response was determined > 7 days after the third 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), and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki. All participants signed written informed consent.

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

The vaccine-specific humoral immune response was quantitatively determined by the anti-SARS-CoV-2 spike receptor-binding domain (RBD) assay (Roche Elecsys anti-SARS-CoV-2 S Ig ElectroChemiLuminescent ImmunoAssay (ECLIA)) with a cut-off at 0.8 U/mL (sensitivity 93.9%, specificity 99.6%) (12). To detect silent infections, the

existence of anti-nucleocapsid antibodies was qualitatively assessed by the Roche Elecsys anti-SARS-CoV-2 N Ig ECLIA (sensitivity 93.6%, specificity 99.8%) (13).

The spike-specific T-cell response measured by an activation-induced marker assay (AIM)

Peripheral blood mononuclear cells (PBMC) 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), as previously described (3). The stimulation index (SI) was calculated by dividing the percentages of $CD154^+CD4^+$ T cells in the stimulated sample by the respective unstimulated value. Responders were defined as patients with a stimulation index (SI) of > 2. 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. All continuous variables are given in median levels, if not stated otherwise. A binary logistic regression model was constructed based on rational assumptions to predict a low immune response. All parameters with p-values < 0.1 were included in a multivariate analysis in order to identify independent risk factors for reduced vaccination response.

Significance was expected for p-values smaller than 0.05. SPSS Statistics Version 26 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 8.0.0 (GraphPad Software. San Diego California, USA) were used for statistical analyses and to create figures, respectively.

Results

Study Cohort

A total of 95 consecutive patients with AIH anti-spike antibodies and anti-nucleocapsid antibodies were prospectively assessed. Patients in whom the exact information on the date of the third vaccination (N=3) was not available, the diagnosis of AIH was not confirmed (N=2), who reported previous SARS-CoV-2-infection (N=7) or anti-nucleocapsid antibodies were detectable as a sign of previous SARS-CoV-2 infection (N=2), were excluded from further analysis. Hence, 81 AIH patients and 53 controls who had received a third COVID-19 vaccination were included in the final analysis. The vaccination response in AIH patients and healthy controls were tested after a median of 35 (IQR 26-55) and 33 days (IQR 23-42, p=0.156), respectively. Additionally, antibody levels after the second vaccination were available in a subgroup of 34 patients. Characteristics of patients and controls included in the main analysis are given in **Table 1.**

Reduced humoral immune response in patients with AIH even after booster vaccination

After the third vaccination, antibody levels of AIH patients remained significantly lower compared to controls (10908 vs. 25000 AU/mL, p<0.001; **Fig. 1B**). Along this line, 48% and 72% of AIH patients had lower antibody titers than 9194 AU/mL and 19047 AU/mL, which corresponds to the 10% and 25% percentile of antibody levels from healthy controls (**Table 1**). In the subgroup of 34 patients, who were tested for antibody levels also after the second vaccination, all patients apart from one, who was under an immunosuppressive regimen containing anti-TNF-therapy, experienced a considerable, on average 30-fold increase in antibody levels after the third vaccination.

Hence, the median antibody levels increased from 260 AU/ml prior booster vaccination to 8666 AU/mL ($p<0.001$) after a third COVID19 vaccination (**Fig. 1A**). Also, all patients without seroconversion after two vaccinations (N=5/34), demonstrated antibody levels >1000 AU/ml after a third vaccination."

Risk factors for a reduced humoral vaccination response in patients with autoimmune hepatitis

Seventy-three of the 81 AIH patients analyzed were under immunosuppressive medication (N=73) which was associated with lower antibody titers after a third vaccination compared to AIH patients without immunosuppression (8859 vs. 25000 AU/mL (median)). This was especially true for treatment with mycophenolate mofetil (MMF) or prednisolone (**Table 2**), and both treatments were identified as independent risk factors for markedly reduced antibody levels (< 10% percentile of controls) in a multivariate logistic regression analysis with odds-ratios of 4.75 (95%-CI: 1.01-22.36) and 7.30 (95%-CI: 1.29-41.23). In contrast, immunosuppression with azathioprine was not identified as independent risk factor for a reduced humoral vaccination response in patients with AIH (OR: 0.84, 95%-CI: 0.27-2.55). Nevertheless, antibody titers of patients with azathioprine monotherapy (N=36) were significantly lower than in healthy individuals (13496 vs. 25000 AU/mL (median), $p<0.001$) or AIH patients without immunosuppression (25000 AU/mL (median), $p<0.001$), but still significantly higher than in patients treated with MMF (4305 AU/mL (median), $p=0.047$) or prednisolone only (4882 AU/mL (median), $p=0.011$), and notably higher than in patients under treatment with a combination of two immunosuppressants (7588 AU/mL (median), $p=0.096$) (**Table 2**). In the subgroup of patients solely treated with azathioprine, the dosage of azathioprine (range 25 – 200mg) had no detectable impact on the humoral vaccination response ($p=0.669$). Likewise, when looking at patients with higher dosage

of immunosuppression (≥7.5 mg prednisolone, >100 mg azathioprine or > 1000mg MMF per day), no dose-dependent effects could be observed in this small real-world cohort. Of note, the lowest antibody levels were detected in the small subgroup of patients (n=3) with a combined immunosuppression consisting of prednisolone and MMF (4305 vs. 12341 AU/mL, p=0.041, **Table 2**).

In the subgroup of patients, in whom antibody titers prior a third vaccination were available, 11/34 had antibody titers below the cut-off for a "borderline response" (≤ 100 AU/mL) after the second vaccination (median 29 AU/mL). Interestingly, these patients experienced an especially efficient, 148-fold boost in their antibody levels after the first booster vaccination. Nevertheless, antibody levels remained significantly lower than in the rest of the AIH patients (4511 vs. 13496 AU/mL (median), p=0.002) (**Fig. 1C**).

No other parameter including liver cirrhosis or older age could be identified to be associated with reduced antibody titers of AIH patients (**Table 2**). Although the time interval between third vaccination and determination of antibody levels varied between patients, the range (IQR: 26-55 days) was small and a relevant impact on antibody levels could be ruled out (p=0.495, **Table 3**).

Spike-specific T-cell response

The spike-specific T-cell response was assessed by the upregulation of the activation-induced markers (AIM) CD154 and CD137 in 34 AIH patients and 16 health individuals. While all 34 AIH patients demonstrated antibody levels >1000 AU/mL, 44% (N=15) of the AIH patients showed no spike-specific T-cell response after the third vaccination. In contrast, this was the case in only 12% (N=2) of controls (**Fig. 2A**, **Table 1**). In comparison to our results of AIH patients after two vaccinations (3) a spike-specific T-cell response was not more frequently detectable after booster vaccination (55% vs.

56%). Moreover, frequencies of spike-specific $CD4^+$ T cells were significantly lower in AIH patients as compared to healthy controls (**Fig. 2B**).

Of note, patients without a robust T-cell response had no reduced antibody titers (median 15777 AU/mL, range 1384-22058) and vice versa, high antibody levels were not associated with a strong T-cell response **(Fig. 2C**). In contrast, most healthy controls with antibody levels > 10000 AU/mL, had also a robust T-cell response (**Fig. 2C**). No particular risk factor for impaired T-cell response could be identified.

Discussion

In this observational study, we assessed the humoral and cellular vaccination response after the third COVID-19 vaccination in a cohort of 81 patients with AIH compared to healthy controls. Our data demonstrate a strong boost of antibody levels in all patients, especially in those with low antibodies after the second vaccination. Nevertheless, antibody levels remained significantly lower than in healthy controls, and a spike-specific T-cell response could only be detected in 54% of the AIH patients.

However, it is important to highlight the strong 148-fold boost of antibody levels in low responders (<100AU/mL). In comparison, a cohort of health care workers with a predominantly strong response after the second vaccination demonstrated only a 7-fold increase in antibody levels (11). Hence, all patients achieved seroconversion as well as antibody levels above the cut-off for borderline/low response (<100 AU/mL), also those without seroconversion after two vaccinations. Therefore, these findings demonstrate an efficient increase of antibody levels by booster vaccinations in low responders. Still, almost half of the AIH patients had antibody titers below the 10% percentile from healthy controls and in many no measurable T cell response was induced.

We have herein refrained from defining a cut-off for optimal antibody titers after three vaccinations, given the wide range of clinically used antibody assays, and foremost, because the required level of antibodies to be fully protected against infection and their relative neutralizing activity are still ill-defined. Nevertheless, higher antibody levels at least decrease the risk of infection (15). Hence, we believe that patients with low or absent antibody responses should be offered a second booster vaccination.

Overall the results of this study are in line with the results observed in a large cohort of patients with autoimmune rheumatic diseases, in which patients who remained seronegative after two vaccinations achieved seroconversion as well as a strong

increase in antibody levels after booster vaccination, but nevertheless maintained lower antibody levels than the rest of the cohort (10).

In addition, we could demonstrate that immunosuppressive therapy, especially with MMF or steroids, was linked with a lower humoral response. These effects have also been described for patients with autoimmune rheumatic diseases, whose antibody levels have been additionally negatively affected by biologicals and older age (10). Of note, immunosuppression with azathioprine which represents the standard, long-term treatment in AIH was not identified as independent risk factor for reduced humoral vaccination response and was associated with notably higher antibody levels than immunosuppression with steroids or MMF, although patients tended to have lower antibody levels than healthy controls. If this is due to the medication or a spontaneous immunosuppression as pathogenic mechanism of the disease itself, like postulated elsewhere (14), has to be further investigated.

Furthermore, we previously found that in liver transplant recipients from the same area various comorbidities were linked with reduced vaccination response (2). However, due to the low prevalence of comorbidities in our cohort of AIH patients, we could not perform a comprehensive analysis of their impact on the vaccination response.

Even after a third vaccination, only 54% of the patients showed a detectable immune-response after stimulation with a peptide pool derived of the SARS-CoV-2 spike glycoprotein. This frequency is not higher than that observed after the second vaccination (3). Moreover, in contrast to healthy controls, high antibody levels were not predictive of a robust T cell response. These findings are somewhat unexpected, given the fact that most AIH patient were only under mild immunosuppression with low dose azathioprine and/or steroids. However, further, more comprehensive analysis of the T cell response in larger prospective cohorts and agreement on standardized tests are

required to understand the clinical significance of these results and the general assessment of the T cell response in routine practice.

Moreover, a limitation of this study is that the vaccination response was not measured at a fixed time point after booster vaccination, although in most patients, antibodies were determined within a relatively short time interval. Therefore, it seems unlikely this has induced a major bias.

This study demonstrates the efficacy of a third vaccination against COVID-19 to boost the humoral response in most patients with AIH. Nevertheless, antibody levels remained significantly lower in AIH compared to controls. Therefore, we propose assessment of antibody titers after third vaccination for all AIH patients and offering a second booster vaccination to patients with suboptimal serological response, especially in the presence of additional risk factors. If feasible, adaptation of immunosuppression prior to booster immunization should be considered in patients on MMF and / or steroid therapy.

List of Abbreviations

AIH	Autoimmune hepatitis
AIM	Activation-induced marker assay
AU	Arbitrary unit
COVID-19	Coronavirus disease 2019
EASL	European Association for the Study of the Liver
ECLIA	ElectroChemiLuminescent ImmunoAssay
MMF	Mycophenolate mofetil
PBMC	Peripheral blood mononuclear cells
RBD	Receptor-binding domain
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
SI	Stimulation index
UKE	University Medical Center Hamburg-Eppendorf

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Table 1: Baseline characteristics and immune response to COVID-19 booster vaccination

1A: Total cohort (N=134)	AIH (N=81)	Controls (N=53)	p
Age (median years, IQR)	60 (52-68)	57 (55-60)	0.051
Females (n, %)	67 (83)	42 (79)	0.614
Time 3rd vacc. – follow-up (median days, IQR)	35 (26-55)	33 (23-42)	0.156
Abs levels RBD (median AU/mL, IQR)	10908 (4748-20042)	25000 (19047-25000)	**<0.001**
Seroconversion (n, %)	81 (100)	53 (100)	1.000
≥100 AU/mL (n, %)	79 (98)	53 (100)	0.518
≥1000 AU/mL (n, %)	77 (95)	53 (100)	0.152
≥9194 AU/mL (n, %)	42 (52)	48 (91)	**<0.001**
≥19047 AU/mL (n, %)	23 (28)	40 (75)	**<0.001**
T-cell response with SI > 2 (n/N, %)	19/34 (56)	14/16 (88)	0.053
1B: AIH patients (N=81)			
BMI (median kg/m^2, IQR)	25.7 (22.1-30.5)		
Creatinine (median mg/dL, IQR)	0.8 (0.7-0.9)		
GFR (median mL/min, IQR)	86 (74-96)		
HbA1c (median %, IQR)	5.6 (5.2-6.1)		
Diabetes (n, %)	12 (15)		
Arterial hypertension (n, %)	27 (33)		
Cirrhosis (n, %)	19 (23)		
IgG (median g/L, IQR)	12.8 (10.1-16.9)		
GOT (median U/L, IQR)	35 (27-49)		
GPT (median U/L, IQR)	27 (20-47)		
Lymphocytes (median 10^9/L, IQR)	1.37 (0.98-1.73)		
T-Lymphocytes (median /µL, IQR)	992 (739-1273)		
B-Lymphocytes (median /µL, IQR)	64 (43-113)		
Immunosuppression (n, %)	73 (90)		
Steroids (n, %)	27 (31)		
If yes, dosage (median mg, range)	5 (5-20)		
Azathioprine (n, %)	50 (62)		
If yes, dosage (median mg, range)	75 (25-175)		
MMF (n, %)	14 (17)		
If yes, dosage (median mg, range)	1500 (1000-2000)		

Abs: antibodies, AIH: autoimmune hepatitis, AU: arbitrary units, BMI: body mass index, GFR: glomerular filtration rate, GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, IQR: interquartile range, MMF: mycophenolate mofetil, RBD: receptor binding domain, SI: Stimulation index, UDCA: ursodeoxycholic acid
Statistical analysis was performed by Mann-Whitney test and Pearson Chi^2-test.

Table 2: Antibody levels in dependence of the presence of potential risk factors

	Yes, AU/mL	No, AU/mL	p
Age > 65 years (n=25/81)	8859 (4181-19243)	11933 (6381-20126)	0.549
Female (n=67/81)	9187 (4717-19285)	14702 (4808-25000)	0.323
Cirrhosis (n=19/81)	8859 (2607-20210)	11525 (5574-20137)	0.526
Diabetes (n=12/81)	8692 (3391-22090)	11525 (4748-20042)	0.800
Arterial hypertension (n=27/81)	14792 (7595-22588)	7985 (4173-17619)	**0.031**
Immunosuppression (n=73/81)	8859 (4652-17693)	25000 (19377-25000)	**0.005**
Steroids (n=27/81)	7326 (4305-12341)	14017 (4763-22573)	**0.020**
Prednisolone (n=13/81)	4882 (2377-8093)	12706 (6381-21523)	**0.014**
Prednisolone mono (n=3/13)	3246 (2607-X)	5734 (1913-7709)	1.000
≥7.5 mg/d prednisolone (n=5/13)	2607 (1120-13472)	5734 (3511-7174)	0.622
Azathioprine (n=50/81)	12437 (6240-19957)	8122 (3246-22058)	0.559
Azathioprine mono (n=36/50)	13496 (6052-20352)	7588 (5314-12641)	0.096
>100mg/d (n=7/36)	13359 (10908-16045)	13633 (4642-21149)	0.815
MMF (n=14/81)	4542 (1224-8144)	12936 (6624-21978)	**0.004**
MMF mono (n=9/14)	4305 (1299-8204)	4778 (941-13715)	0.898
>1000mg/d (n=5/9)	4778 (1898-16161)	4401 (401-16500)	0.730
Combined immunosuppression (n=28/81)	10356 (5254-24763)	10908 (4652-19209)	0.746
Prednisolone + MMF (n=3/28)	4305 (1214-X)	12341 (6653-25000)	**0.041**
UDCA (n=25/81)	7322 (4800-16673)	12341 (4586-22568)	0.391

AIH: autoimmune hepatitis, AU: arbitrary units, IQR: interquartile range, MMF: mycophenolate mofetil, RBD: receptor binding domain, UDCA: ursodeoxycholic acid. Statistical analysis was performed by Mann-Whitney test.

Table 3: Risk of AIH patients of antibody levels < 10% percentile of controls (9194 AU/mL) after a third SARS-CoV-2 vaccination based on the RBD immunoassay

	Univariate OR (95%-CI)	p	Multivariate OR (95%-CI)	p
Total cohort				
Age, years	1.00 (0.97-1.03)	0.994		
Sex, female (n=109)	0.95 (0.38-2.42)	0.921		
Days after 3rd vaccination	1.01 (0.99-1.03)	0.172		
Immunosuppression (IS)	7.60 (0.89-64.92)	0.064		
Patients only				
Age, years	0.99 (0.96-1.02)	0.627		
Sex, female (n=67)	0.54 (0.16-1.78)	0.310		
Days after 3rd vaccination	1.01 (0.99-1.03)	0.495		
Cirrhosis (n=19)	1.31 (0.47-3.68)	0.608		
BMI, kg/m^2	0.97 (0.88-1.07)	0.572		
Diabetes (n=12)	1.09 (0.32-3.72)	0.889		
Hypertension (n=27)	0.40 (0.15-1.05)	0.062		
IS (n=73)	7.60 (0.89-64.92)	0.064		
Steroids (n=27)	**4.04 (1.50-10.91)**	**0.006**		
Prednisolone (n=13)	**7.86 (1.62-38.23)**	**0.011**	**7.30 (1.29-41.23)**	**0.024**
Predni mono (n=3)	3.42 (0.34-34.32)	0.297		
Predni ≥7.5 mg/d (n=5)	4.69 (0.50-43.89)	0.176		
Budesonide (n=14)	1.40 (0.35-5.63)	0.638		
MMF (n=14)	**5.11 (1.30-20.01)**	**0.019**	**4.75 (1.01-22.36)**	**0.049**
MMF mono (n=9)	4.38 (0.85-22.53)	0.078		
MMF >1g/d (n=5)	3.64 (0.69-19.23)	0.129		
Azathioprine (n=50)	0.52 (0.21-1.30)	0.162		
Aza mono (n=36)	**0.33 (0.13-0.83)**	**0.019**	0.84 (0.27-2.55)	0.836
Aza >100mg/d (n=7)	0.27 (0.05-1.39)	0.117		
Combined IS (n=28)	1.12 (0.45-2.80)	0.808		
Predni + MMF (n=3)	n.a.			
UDCA (n=25)	1.64 (0.63-4.26)	0.306		
HbA1c, %	1.71 (0.73-4.01)	0.215		
eGFR, mL/min	0.99 (0.97-1.02)	0.578		
IgG, g/L	0.93 (0.85-1.01)	0.085		
Lymphocytes, 10^9/L	0.79 (0.35-1.79)	0.575		

BMI: body mass index, CI: confidence interval, eGFR: estimated glomerular filtration rate, IS: Immunosuppression, MMF: mycophenolate mofetil, OR: odds ratio, UDCA: ursodeoxycholic acid

Figure legends:

1A: Anti-SARS-CoV-2 antibody levels after second and third COVID-19 vaccination in patients with AIH. Statistical analysis was performed by Wilcoxon matched-pairs signed rank test.

1B: Anti-SARS-CoV-2 antibody levels after third COVID-19 vaccination in patents with AIH compared to healthy controls. Statistical analysis was performed by Mann-Whitney test.

1C: Anti-SARS-CoV-2 antibody levels after third COVID-19 vaccination in AIH patients with anti-SARS-CoV-2 Abs levels <100 AU/ml after the 2nd vaccination (low) compared to AIH patients with SARS-CoV-2 Abs levels >100 AU/ml after the 2nd vaccination (high).

P-values: *<0.05;**<0.01;***<0.001;****<0.0001.

2A: Percentages of AIH patients with a detectable T-cell response defined as SI>2 compared to healthy controls.

2B: Percentages of CD154^{+} CD4^{+} T cells after third COVID-19 vaccination in AIH patients compared to healthy controls.

2C: Cross-correlation of SARS-CoV-2 antibody levels and T-cell response as assessed by the stimulation index (SI) in patients with AIH and healthy controls. A SI>2 was defined as detectable T-cell response (dashed line).

P-values: *<0.05;**<0.01;***<0.001;****<0.0001.

Figure 1A

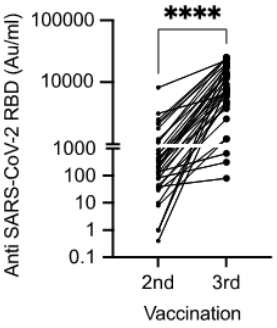

Figure 1B

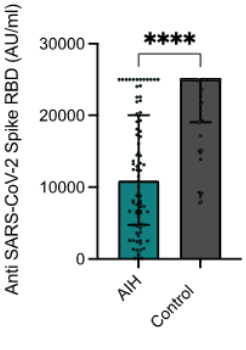

Figure 1C

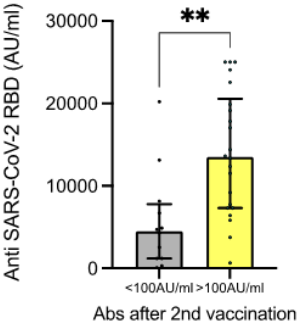

Figure 2A

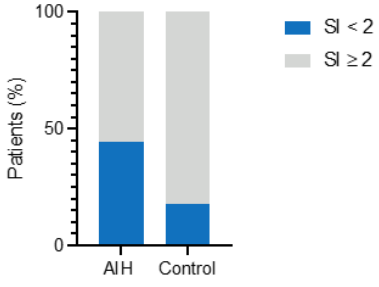

Figure 2B

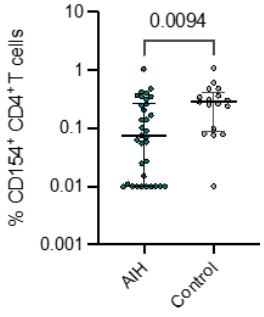

Figure 2C