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 (10 908 vs. 25 000 AU/ml, p < .001), especially in AIH patients treated with MMF (N = 14, 4542 AU/ml, p = .004) or steroids (N = 27, 7326 AU/ml, p = .020). Also, 48% of AIH patients had antibody titers below the 10% percentile of the healthy controls (9194 AU/ml, p < .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 = .05) and showed overall lower frequencies of spike-specific CD4+ T cells (median: 0.074% vs 0.283; p = .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 the 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 responses.

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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1 | INTRODUCTION

Various reports show a reduced immune response to COVID-19 vaccinations in patients on immunosuppression, including liver transplant recipients. Moreover, we have reported recently that AIH patients show an impaired B- and T-cell response after the second COVID-19 vaccination, even in the absence of or under mild immunosuppressive treatment. Also, AIH patients seemed to have an increased risk to acquire SARS-CoV-2 infection. At the same time, immunosuppressive drugs and advanced stages of chronic liver diseases 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. Indeed, the 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 a rheumatic disease.

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.

2 | PATIENTS AND METHODS

2.1 | Study population and data collection

Consecutive non-pregnant patients ≥18 years with diagnosed AIH presenting at the YAEI 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 Refs. [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.

2.2 | 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%). 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%).

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.

Key points

- Antibody levels and the spike-specific T-cell response were prospectively assessed in patients with AIH and a control group of healthy individuals after the 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 the low response after the third vaccination.
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 18h at 37°C after adding 1 μl Ultra-LEAF™ purified antihuman CD40 antibody (BioLegend), as previously described. 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 analysed 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-squared 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 < .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 .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 vaccine response in AIH patients and healthy controls were tested after a median of 35 (IQR 26–55) and 33 days (IQR 23–42, p = .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 (10 908 vs. 25 000 AU/ml, p < .001; Figure 1B). Along this line, 48% and 72% of AIH patients had lower antibody titers than 9194 AU/ml and 19 047 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 an 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 < .001) after a third COVID19 vaccination (Figure 1). 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 analysed 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. 25 000 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 an 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 (13 496 vs. 25 000 AU/ml [median], p < .001) or AIH patients without immunosuppression (25 000 AU/ml [median], p < .001), but still significantly higher than in patients treated with MMF (4305 AU/ml [median], p = .047) or prednisolone only (4882 AU/ml [median], p = .011), and notably higher than in patients under treatment with a combination of two immunosuppressants (7588 AU/ml [median], p = .096) (Table 2). In the subgroup of patients solely treated with azathioprine, the dosage of azathioprine (range 25–200 mg) had no detectable impact on the humoral vaccination response (p = .669). Likewise, when looking at patients with a higher dosage of immunosuppression (>7.5 mg prednisolone, >100 mg azathioprine or >1000 mg 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 combined immunosuppression consisting of prednisolone and MMF (4305 vs. 12 341 AU/ml, p = .041, Table 2).
In the subgroup of patients, in whom antibody titers prior to 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. 13,496 AU/ml [median], p = .002) (Figure 1C).

Table 2 provides a more comprehensive overview of the baseline characteristics and immune response to COVID-19 booster vaccination in both AIH and control groups.

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

|                      | AIH (N = 81) | Controls (N = 53) | p      |
|----------------------|--------------|-------------------|--------|
| 1A: Total cohort (N = 134) |              |                   |        |
| Age (median years, IQR) | 60 (52–68)   | 57 (55–60)        | .051   |
| Females (n, %)         | 67 (83)      | 42 (79)           | .614   |
| Time third vacc.—follow-up (median days, IQR) | 35 (26–55) | 33 (23–42) | .156   |
| Abs levels RBD (median AU/ml, IQR) | 10,908 (4,748–20,042) | 25,000 (19,047–25,000) | <.001 |
| Seroconversion (n, %)  | 81 (100)     | 53 (100)          | 1.000  |
| ≥100AU/ml (n, %)       | 79 (98)      | 53 (100)          | .518   |
| ≥1000AU/ml (n, %)      | 77 (95)      | 53 (100)          | .152   |
| ≥914AU/ml (n, %)       | 42 (52)      | 48 (91)           | <.001  |
| ≥19,047AU/ml (n, %)    | 23 (28)      | 40 (75)           | <.001  |

1B: AIH patients (N = 81)

|                      |              |                   |        |
| BMI (median kg/m², 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 10⁹/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) |                   |        |

Note: Abbreviations: 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 Chi²-test.

In the subgroup of patients, in whom antibody titers prior to 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. 13,496 AU/ml [median], p = .002) (Figure 1C).

No other parameter including liver cirrhosis or older age could be identified to be associated with reduced antibody titers in AIH patients (Table 2). Although the time interval between the 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 = .495, Table 3).

3.4 " 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 healthy 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 (Figure 2; Table 1). In comparison to our results of AIH patients after two vaccinations, 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 compared to healthy controls (Figure 2B).

Of note, patients without a robust T-cell response had no reduced antibody titers (median 15 777 AU/ml, range 1384–22 058) and vice versa, high antibody levels were not associated with a strong T-cell response (Figure 2C). In contrast, most healthy controls with antibody levels >10 000 AU/ml had also a robust T-cell response (Figure 2C). No particular risk factor for impaired T-cell response could be identified.

TABLE 2 Antibody levels in dependence on the presence of potential risk factors

| Risk Factor          | Yes, AU/ml | No, AU/ml | p     |
|----------------------|------------|-----------|-------|
| Age > 65 years (n = 25/81) | 8859 (4181–19 243) | 11 933 (6381–20 126) | .549 |
| Female (n = 67/81)    | 9187 (4717–19 285) | 14 702 (4808–25 000) | .323 |
| Cirrhosis (n = 19/81) | 8859 (2607–20 210) | 11 525 (5574–20 137) | .526 |
| Diabetes (n = 12/81)  | 8692 (3391–22 090) | 11 525 (4748–20 042) | .800 |
| Arterial hypertension (n = 27/81) | 14 792 (7595–22 588) | 7985 (4173–17 619) | .031 |
| Immunosuppression (n = 73/81) | 8859 (4652–17 693) | 25 000 (19377–25 000) | .005 |
| Steroids (n = 27/81)  | 7326 (4305–12 341) | 14 017 (4763–22 573) | .020 |
| Prednisolone (n = 13/81) | 4882 (2377–80 93) | 12 706 (6381–21 523) | .014 |
| Prednisolone mono (n = 3/13) | 3246 (2607-X) | 5 734 (1913–7709) | 1.000 |
| ≥7.5 mg/d prednisolone (n = 5/13) | 2607 (1120–13 472) | 5 734 (3511–7174) | .622 |
| Azathioprine (n = 50/81) | 12 437 (6240–19 957) | 8122 (3246–22 058) | .559 |
| Azathioprine mono (n = 36/50) | 13 496 (6052–20 352) | 7 588 (5314–12 641) | .096 |
| >100mg/days (n = 7/36) | 13 359 (10 908–16 045) | 13 633 (4642–21 149) | .815 |
| MMF (n = 14/81)       | 4542 (1224–81 44) | 12 936 (6624–21 978) | .004 |
| MMF mono (n = 9/14)   | 4305 (1299–82 04) | 4 778 (941–13 715) | .898 |
| >1000 mg/days (n = 5/9) | 4778 (1898–16 161) | 4 401 (401–16 500) | .730 |
| Combined immunosuppression (n = 28/81) | 10 356 (5254–24 763) | 10 908 (4652–19 209) | .746 |
| Prednisolone + MMF (n = 3/28) | 4305 (1214-X) | 12 341 (6653–25 000) | .041 |
| UDCA (n = 25/81)      | 7 322 (4800–16 673) | 12 341 (4586–22 568) | .391 |

Note: Statistical analysis was performed by Mann–Whitney test.

Abbreviations: AIH, autoimmune hepatitis, AU, arbitrary units, IQR, interquartile range, MMF, mycophenolate mofetil, RBD, receptor-binding domain, UDCA, ursodeoxycholic acid.
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 in 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 (<100 AU/ml). In comparison, a cohort of healthcare workers with a predominantly strong response after the second vaccination demonstrated only a seven-fold increase in antibody levels. Hence, all patients achieved seroconversion as well as antibody levels above the cut-off for borderline/low

| Abbreviations: AIH, autoimmune hepatitis; AU, arbitrary units; BMI: body mass index, CI: confidence interval, eGFR: estimated glomerular filtration rate, IS, Immunosuppression, MMF, mycophenolate mofetil, OR, odds ratio, RBD, receptor-binding domain; UDCA: ursodeoxycholic acid. |

### TABLE 3 Risk of AIH patients of antibody levels <10% 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)       | .994 |                         |      |
| Sex, female (n = 109) | 0.95 (0.38–2.42)    | .921 |                         |      |
| Days after third vaccination | 1.01 (0.99–1.03) | .172 |                         |      |
| IS                  | 7.60 (0.89–64.92)     | .064 |                         |      |
| Patients only       |                        |      |                          |      |
| Age, years          | 0.99 (0.96–1.02)       | .627 |                         |      |
| Sex, female (n = 67) | 0.54 (0.16–1.78)     | .310 |                         |      |
| Days after third vaccination | 1.01 (0.99–1.03) | .495 |                         |      |
| Cirrhosis (n = 19)  | 1.31 (0.47–3.68)       | .608 |                         |      |
| BMI, kg/m²          | 0.97 (0.88–1.07)       | .572 |                         |      |
| Diabetes (n = 12)   | 1.09 (0.32–3.72)       | .889 |                         |      |
| Hypertension (n = 27) | 0.40 (0.15–1.05)   | .062 |                         |      |
| IS (n = 73)         | 7.60 (0.89–64.92)     | .064 |                         |      |
| Steroids (n = 27)   | 4.04 (1.50–10.91)      | .006 |                         |      |
| Prednisolone (n = 13) | 7.86 (1.62–38.23) | .011 | 7.30 (1.29–41.23)       | .024 |
| Predni mono (n = 3) | 3.42 (0.34–34.32)     | .297 |                         |      |
| Predni ≥7.5 mg/days (n = 5) | 4.69 (0.50–43.89) | .176 |                         |      |
| Budesonide (n = 14) | 1.40 (0.35–5.63)      | .638 |                         |      |
| MMF (n = 14)        | 5.11 (1.30–20.01)     | .019 | 4.75 (1.01–22.36)       | .049 |
| MMF mono (n = 9)    | 4.38 (0.85–22.53)     | .078 |                         |      |
| MMF >1 g/day (n = 5) | 3.64 (0.69–19.23)  | .129 |                         |      |
| Azathioprine (n = 50) | 0.52 (0.21–1.30) | .162 |                         |      |
| Aza mono (n = 36)   | 0.33 (0.13–0.83)      | .019 | 0.84 (0.27–2.55)       | .836 |
| Aza >100 mg/day (n = 7) | 0.27 (0.05–1.39) | .117 |                         |      |
| Combined IS (n = 28) | 1.12 (0.45–2.80) | .808 |                         |      |
| Predni+MMF (n = 3)  | n.a.                   |      |                         |      |
| UDCA (n = 25)       | 1.64 (0.63–4.26)      | .306 |                         |      |
| HbA1c, %            | 1.71 (0.73–4.01)      | .215 |                         |      |
| eGFR, ml/min        | 0.99 (0.97–1.02)      | .578 |                         |      |
| IgG, g/L            | 0.93 (0.85–1.01)      | .085 |                         |      |
| Lymphocytes, 10⁹/L  | 0.79 (0.35–1.79)      | .575 |                         |      |
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 of 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. 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.

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. Of note, immunosuppression with azathioprine which represents the standard, long-term treatment in AIH was not identified as an 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 owing to the medication or spontaneous immunosuppression as a pathogenic mechanism of the disease itself, like postulated elsewhere, 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. However, owing 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 from the SARS-CoV-2 spike glycoprotein. This frequency is not higher than that observed after the second vaccination. 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 patients 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 that 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 the 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.

**AUTHOR CONTRIBUTIONS**

Conceptualization: DFR, AWL, JH; Data curation: PMD, TTB, SS, JPW, FG, ML, GMS, JH; Formal analysis: DFR, PMD, JH; Investigation: DFR, PMD, JH; Methodology: DFR, PMD, FH, JSzW,
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CONFLICTS 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

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

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