



SARS-CoV-2 mRNA vaccines do not worsen autoimmunity in patients with autoimmune liver diseases

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ARTICLE INFO

Handling Editor: C Selmi

Keywords:

Liver autoantibodies

mRNA SARS-CoV-2 vaccines

Autoimmune liver diseases

ABSTRACT

Introduction and aims: mRNA vaccines against Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) infection have been associated with immune-related adverse reactions. We aimed at investigating whether SARS-CoV-2 vaccines may worsen autoimmune reactions in patients with autoimmune liver diseases.

Methods: We centrally tested a large panel of liver- and non-liver-related autoantibodies in patients with primary biliary cholangitis (PBC), autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), and in healthcare workers (HW) before and after SARS-CoV-2 mRNA vaccines.

Results: 49 PBC, 35 AIH, 9 PSC and 38 HW were included. The proportion of subjects with at least one autoantibody positivization after vaccination was 11 % for HW, 37 % for AIH, 35 % for PBC and 56 % for PSC patients, patients having a significantly higher frequency of positivization as compared to HW. The proportion of seropositive subjects before vaccination who had at least one autoantibody negativization was 25 % for HW, 57 % for AIH, 40 % for PBC and 50 % for PSC, AIH patients having a significantly higher frequency of negativization as compared to HW. In the AIH group, the number of autoantibody negativizations was higher than the number of positivizations. The BNT162b2 vaccine was associated with a higher risk of developing new autoantibodies as compared to the mRNA-1273 vaccine. No new-onset autoimmune disease was observed after one year. One AIH patient had a relapse after vaccination.

Conclusion: mRNA SARS-CoV-2 vaccines do not induce short-term worsening of autoimmunity in patients with autoimmune liver diseases.

1. Introduction

The recent pandemic caused by Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2), a highly transmissible and pathogenic virus causing coronavirus disease 2019 (COVID-19), had a devastating global impact, leading to an unprecedented fast development of anti-COVID-19

vaccines. Two mRNA vaccines, BNTb262 and mRNA-1273, have been approved in Switzerland, and a massive vaccination campaign has started at the end of 2020. mRNA SARS-CoV-2 vaccines have proved to be highly effective in preventing severe and fatal COVID-19, even after the advent of highly transmissible variants and sub-variants [1–3]. Since the vaccines are stimulating type I interferon (IFN) responses, the

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<https://doi.org/10.1016/j.jaut.2024.103325>

Received 14 July 2024; Received in revised form 27 September 2024; Accepted 5 October 2024

Available online 15 October 2024

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promotion of autoimmune diseases was an important safety concern [4]. While mild local and systemic adverse reactions associated with the brisk immune response elicited by mRNA vaccines are common, rare severe adverse events have been reported, including hepatitis with autoimmune features and flares of pre-existing autoimmune diseases [5–7].

Circulating autoantibodies are a key diagnostic marker in several autoimmune diseases, and their presence reflects an immune activation against self-antigens, which can predate clinical disease onset [8].

Circulating autoantibodies have been reported after COVID-19 vaccination in case reports [9,10], case series of patients developing overt autoimmune diseases [7] and in healthy individuals [11], raising concerns about safety of mRNA SARS-CoV-2 vaccines in patients predisposed to autoimmunity. However, these results have not been confirmed by a recent study which included patients with non-hepatic autoimmune diseases and tested for autoantibodies before and after mRNA SARS-CoV-2 vaccines [12]. Whether COVID-19 vaccines may worsen autoimmune reactions in patients with autoimmune liver diseases is unknown.

2. Aims

In this study, we aimed to investigate if autoantibodies appear de novo or disappear, and/or if the titer of pre-existing autoantibodies increases or decreases by \geq than two dilutions, as measured by indirect immunofluorescence (IIF), in the serum of patients affected by autoimmune liver diseases (AILD) after mRNA COVID-19 vaccination.

Additionally, we aimed to investigate.

- factors that may influence autoantibody appearance and disappearance after mRNA SARS-CoV-2 vaccination, including sex, age at diagnosis, type of mRNA vaccine, type of AILD and immunosuppressive treatment at vaccination
- onset of hepatic and extrahepatic autoimmune diseases after COVID-19 mRNA vaccination
- association of AILD course with autoantibodies appearance or increasing titers

3. Methods

3.1. Patients and controls

The Swiss Autoimmune Liver Disease (AILD) Cohort Studies (<https://sasf.unibas.ch/7studies.php>), initiated in 2017, are a nationwide prospective registry with biobank including patients diagnosed at each participating center with autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) or variant syndromes, the diagnosis being based on established criteria [13–17]. The Swiss PBC cohort study also includes patients with isolated positive PBC serology (isPBC), defined as positive anti-mitochondrial antibody (AMA) and/or positive PBC-specific anti-nuclear antibody (ANA) with normal serum alkaline phosphatase (ALP) level, i.e. patients without cholestasis and normal or non-available liver histology [18]. Serum samples are collected at enrollment and subsequently once a year. For the purpose of the present study, additional serum samples were collected before and after mRNA SARS-CoV-2 vaccines (see below).

Sera and clinical data from patients collected within the frame of the Swiss AILD Cohort Studies were used for the present project. The inclusion criteria are as follows.

- Age \geq 18 years
- A diagnosis of AIH, PBC, PSC, isPBC or of variant syndrome, the latter being defined as abnormal cholangiogram in AIH patients for the AIH/PSC variant syndrome and as severe interface hepatitis at liver biopsy and positive ANA and/or anti-smooth muscle antibody (SMA) in PBC patients for the AIH/PBC variant syndrome

- Patients having received any mRNA vaccine against COVID-19 according to the manufacturer label
- Availability of at least one serum sample collected before vaccination
- Availability of at least one serum sample collected after vaccination

Active disease for AIH is defined as abnormal serum transaminase levels and for PBC as abnormal serum ALP level.

Sera from age and sex matched health care workers (HW) of nursing homes, collected in the context of the COV-RISK study, carried out by the Institute for Research in Biomedicine and the Università della Svizzera Italiana, were used as healthy controls.

Clinical data collected within the frame of the Swiss AILD cohort studies were re-analysed in order to evaluate associations between serological and clinical features. These include (according to the Swiss AIH/PBC/PSC cohort study protocols): year of birth; sex; immunosuppressive treatment at the time of COVID-19 vaccination; concomitant extrahepatic autoimmune diseases at the time of vaccination and after 6 and 12 months; date of COVID-19 vaccination(s) and name of the vaccine(s); AIH relapses; annual liver biochemical tests; liver transplant status.

Serum samples from patients with autoimmune diseases collected at the following timepoints have been analysed.

- Pre-pandemic sample, if available (= collected before September 1, 2019)
- Pre-vaccination sample, as close as possible to the vaccination date
- Post-vaccination sample 1 = 1–3 months post-vaccination
- Post-vaccination sample 2 = 6 months post-vaccination

Serum samples from healthy controls collected at the following timepoints were analysed.

- Pre-vaccination sample, taken within 3 months before vaccination
- Post-vaccination sample 1 = 1–2 months post-vaccination
- Post-vaccination sample 2 = 6 months post-vaccination

Biosamples and clinical data were re-analysed under code. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki and was approved by the local Ethics Committee (2021-00365, Rtf CETI 3822). Written informed consent was obtained from each patient at enrolment in the Swiss AILD Cohort Studies; in addition, patients gave their consent and signed the relevant addendum in order to collect additional serum samples.

Enrolled health care workers have signed the additional informed consent form called “Dichiarazione di consenso per il riutilizzo di dati (genetici) e materiale biologico in forma codificata” (Consent declaration for the use of data (genetic) and biological material in anonymous fashion).

The project was approved by the Scientific Committee of the Swiss AILD Cohort Studies.

3.2. Autoantibody testing

The laboratory tests were performed at EUROIMMUN, Lübeck, Germany. The starting dilution for IIF on all substrates was 1:10. Positivity cut-off was 1:100 on all substrates except fixed human neutrophils, where it was 1:10. Samples were tested by IIF on HEp-20-10 cells for detection of anti-nuclear antibody (ANA) (patterns reported according to guidelines [19]), by IIF on rat kidney, liver and stomach tissue for detection of the following liver-specific autoantibodies: SMA, anti-liver kidney microsomal antibody type 1 (LKM1), anti-liver cytosol type 1 antibody (LC1), and anti-mitochondrial antibody (AMA). SMA was tested also by IIF on vascular smooth muscle (VSM) 47 cells. IIF on human neutrophils was used for the detection of anti-neutrophil cytoplasmic antibodies (ANCA). Anti-myeloperoxidase (MPO) and anti-proteinase 3 (PR3) antibodies were tested by EUROLINE

immunoassay system (anti-MPO, -PR3). Atypical pANCA is defined as positive perinuclear staining pattern on both ethanol and formaldehyde-fixed neutrophils [20]. In addition, all sera were also assessed by IIF on primate liver substrate. All sera were analysed by immunoblot (EUROLINE Autoimmune Liver Diseases 9 Ag plus F-Actin, IgG) for antibodies targeting soluble liver antigen (SLA), M2, M2-3E, Sp100, pro-myelocytic leukemia (PML), gp210, LKM-1, LC-1, Ro-52, and F-Actin. The same immunoassay was used to detect antibodies targeting 15-hydroxyprostaglandin dehydrogenase (PGDH).

Antibodies against specific ANA autoantigens were also tested by the EUROLINE immunoassay system ANA profile 23 (EUROIMMUN), which allows detection of 23 autoantibodies targeting nuclear autoantigens: anti-ds-DNA, anti-nucleosome, anti-histone, anti-SS-A, anti-Ro-52, anti-SS-B, anti-Smith, anti-ribonucleoprotein, anti-Mi2 α , anti-Mi2 β , anti-Ku, anti-CENP A, anti-CENP B, anti-sp100, anti-PML, anti-Scl 70, anti-PM-Scl100, anti-PM-Scl75, anti-RP11, anti-RP155, anti-gp210, anti-PCNA, anti-DFS70. Anti-cyclic citrullinated peptides (CCP) antibody, anti-transglutaminase IgA, anti-thyroid peroxidase (TPO) antibodies were tested by ELISA (EUROIMMUN).

In addition, anti-SARS-CoV-2 antibodies elicited by natural infection or by anti-COVID-19 vaccinations were measured by ELISA (EUROIMMUN).

To avoid issues related to sequential testing of the same sample, tests were performed in parallel using different aliquots, with only minor differences in storage time between them.

4. Statistical analysis

Categorical variables are described by counts and percentages; continuous variables are described by median and interquartile range. Statistical analysis was performed with Prism 10 for macOS, Excel and R. The Mann-Whitney *U* test was used to compare quantitative data between two groups. The Kruskal-Wallis rank sum test was used to compare quantitative data between more than two groups and, if significant, Wilcoxon rank sum test was used for pairwise comparisons.

The association between the appearance or the increasing titer of at least one autoantibody and different covariates was tested by means of bivariate analysis and appropriate tests (Fisher's exact or Chi-squared). *P* values below 0.05 (two-tailed) were considered significant in all analyses.

Different multivariate logistic regression models were fitted for a set of sociodemographic and clinical variables using positivization of at least one autoantibody as dependent variable. We evaluate the significance of parameters using Wald's test and 95 % CI for odds ratios (ORs).

5. Results

Ninety-three AILD patients, including 35 (38 %) AIH (34 type 1 and one type 2), 37 (40 %) PBC, 12 (13 %) isPBC, nine (10 %) PSC, and 38 (41 %) HW were enrolled. Demographic and clinical features are shown in Table 1. All AIH patients were on immunosuppressive treatment at the time of vaccination: 19 on thiopurines, coupled with prednisone in nine of them, 12 on mycophenolate mofetil, coupled with prednisone in 10 of them, and the remaining four patients were on prednisone monotherapy. Five AIH patients had the PBC variant syndrome, and six had the PSC variant syndrome. All PBC patients but one were on ursodeoxycholic acid (UDCA) at time of vaccination; five had elevated ALP levels at time of vaccination, including the untreated patient. The vast majority (95 %) of the HWs, who were prioritized in the vaccination campaign being healthcare workers, received the BNT162b2 vaccine, that was approved before the mRNA-1273 vaccine in Switzerland. The rate of anti-spike antibody positivization after vaccination was high and not statistically different in all groups. Autoantibody results before and after vaccination are shown in Fig. 1. Autoantibodies that changed after vaccination were highly heterogeneous in all groups, without the changes involving some autoantibodies more than others.

Table 1

Demographic and clinical features of the study population.

Group	PBC n = 49	AIH n = 35	PSC n = 9	Healthy Controls n = 38
Female sex, n (%)	44 (90)	26 (74)	5 (56)	30 (79)
Age at vaccination, years	65 (55–72)	68 (55–72.5)	59 (55–64)	54 (47–61)
BNT162b2 vaccine (%)	17 (35) ^a	21 (60)	6 (67)	36 (95)
mRNA-1273 vaccine (%)	31 (65) ^a	14 (40)	3 (33)	2 (5)
Patients on immunosuppression at vaccination, n (%)	3 (6)	35 (100)	2 (22)	0 (0)
Patients with inactive disease at time of vaccination	44 (90)	32 (91)	NA	NA
Seronegative subjects before vaccination, n (%)	1 (2)	2 (6)	3 (33)	14 (37)
Time from pre-pandemic sample to 1st vaccine dose (median, days)	866 (772–966)	785 (646–896)	866 (844–898)	- ^b
Time from pre-vaccination sample to 1st vaccine dose (median, days)	219 (113–371)	236 (88–325)	66 (37–130)	38 (35–41)
Time from 2nd vaccine dose to 1st post-vaccine sample (median, days)	60 (35–97)	43 (37–62)	47 (32–73)	33 (33–35)
Time from 2nd vaccine dose to 2nd post-vaccine dose (median, days)	178 (146–205)	194 (181–204)	183 (178–196)	264 (264–266)
Anti-spike antibodies before vaccination	3 (6)	0 (0)	1 (11)	7 (18)
Anti-spike antibodies after vaccination	47 (98)	30 (86)	8 (89)	37 (97)
Follow-up^c (median, months)	30 (29–31)	31 (31–32)	31 (30–31)	35 (35–35)
Disease duration^d (median, months)	51 (30–129)	61 (33–111)	65 (15–76)	–
Number of patients with concomitant extrahepatic autoimmune diseases, n (%)				
Rheumatoid arthritis	3	0	0	–
Thyroiditis	3	4	0	–
Sjogren syndrome	7	0	0	–
Systemic sclerosis	3	0	0	–
Skin disorders	2 ^e	1 ^f	1 ^g	–
Celiac disease	0	2	0	–
Inflammatory bowel disease	0	1	6	–

PBC, primary biliary cholangitis; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis.

^a Type of vaccine for one patient unknown.

^b No pre-pandemic sample available.

^c Time from second vaccination to the end of 2023.

^d Time from diagnosis to vaccination.

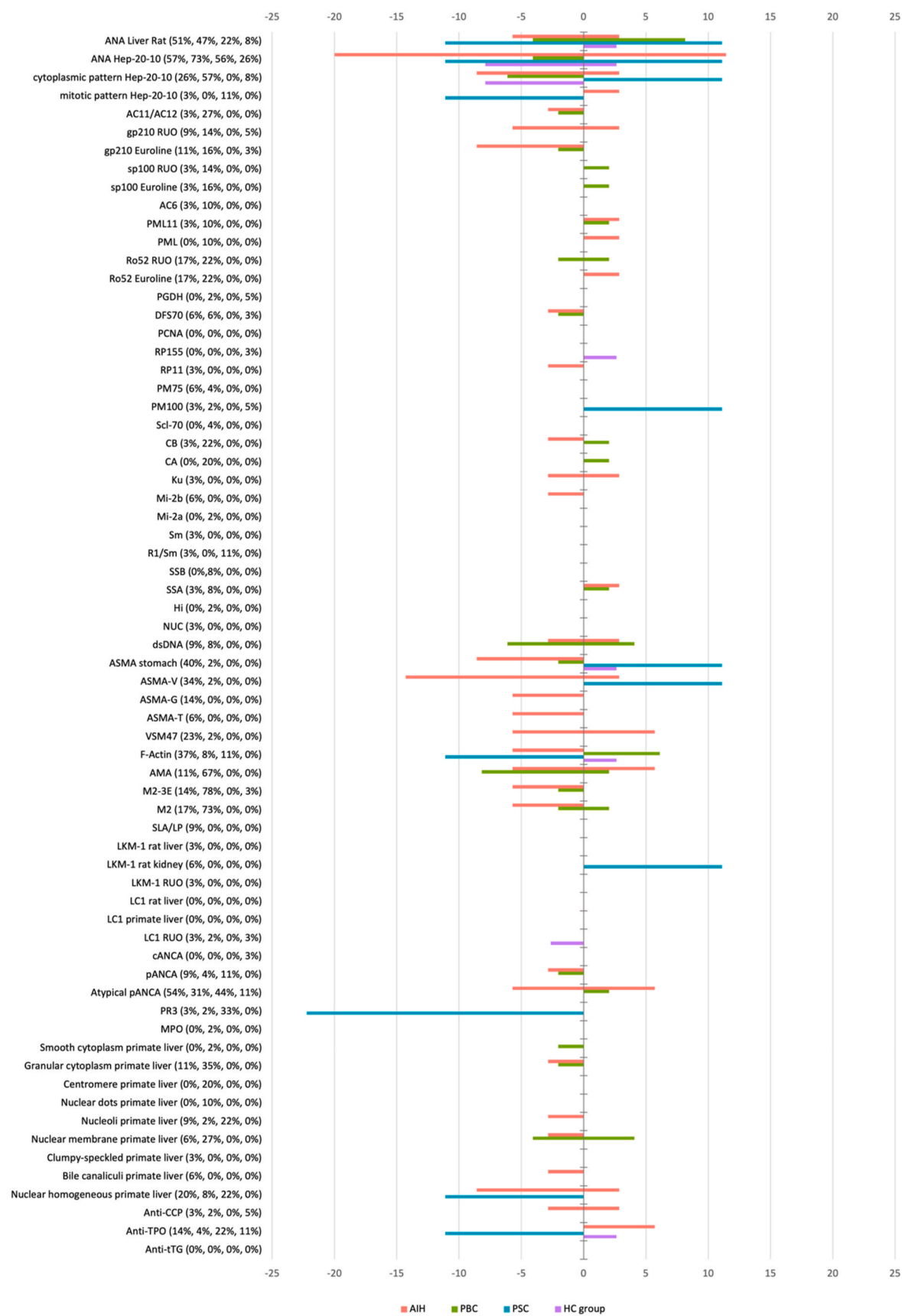
^e one lichen, one cutaneous lupus erythematosus.

^f cutaneous lupus erythematosus.

^g pemphigus vulgaris.

The proportion of subjects who had at least one autoantibody positivization after vaccination was 11 % for HW, 37 % for AIH, 35 % for PBC and 56 % for PSC patients. AILD patients had a significantly higher frequency of positivization as compared to HW (Kruskal-Wallis test: $p = 0.013$; Wilcoxon rank sum test: HW-AIH $p = 0.020$, HW-PBC $p = 0.027$, HW-PSC $p = 0.017$); the frequency of positivization was similar in the AIH, PBC and PSC (Wilcoxon rank sum test AIH-PBC = 0.511, PBC-PSC = 0.285, AIH-PSC = 0.511).

To further evaluate this aspect, we performed a multivariate logistic regression considering HW and AILD patients. PSC patients were the most likely to develop new specificities or having increasing titres of



(caption on next page)

Fig. 1. Summary of autoantibody changes after vaccination.

The horizontal axis shows percentages of negativization with negative numbers, and of positivizations with positive numbers.

Percentages after autoantibody name refer to the proportion of positive subjects before vaccination, the first percentage referring to autoimmune hepatitis, the second to primary biliary cholangitis, the third to primary sclerosing cholangitis and the fourth to healthy controls.

ANA, anti-nuclear antibody; PML, promyelocytic leukemia; PGDH, phosphoglycerate dehydrogenase; DFS, dense fine speckled; PCNA, proliferating cell nuclear antigen; RP, ribosomal protein; PM, Polymyositis; Scl, Scleroderma; CB, centromere B; CA, centromere A; Sm, Smith; R1/Sm, R1 component of Sm; SSB, Sjögren Syndrome antigen B; SSA, Sjögren Syndrome antigen A; Hi, histone; NUC, nucleobindin 1; dsDNA, double-stranded DNA; ASMA, anti-smooth muscle antibody; V, vessel; G, glomerulus; T, tubulus; VSM47, vascular smooth muscle; AMA, anti-mitochondrial antibody; M2-3E, AMA targeting 3E subunit of M2 complex; M2, AMA targeting M2 complex; SLA/LP, soluble liver antigen/liver-pancreas antigen; LKM-1, liver-kidney microsomal type 1; LC-1, liver cytosol type 1; ANCA, anti-neutrophil cytoplasmic antibody; cANCA, cytoplasmic ANCA; pANCA, perinuclear ANCA.

PR3, Proteinase 3; MPO, Myeloperoxidase; CCP, cyclic citrullinated peptide; TPO, thyroid peroxidase; tTG, anti-tissue transglutaminase.

autoantibodies (OR = 10.62, 95 % CI [2.1; 82.6], $p = 0.006$) followed by AIH patients (OR = 5, 95 % CI [1.6; 19.7], $p = 0.011$) and PBC patients (OR = 4.66, 95 % CI [1.5; 17.6], $p = 0.011$).

The proportion of seropositive subjects who had at least one autoantibody negativization after vaccination was 25 % for HW, 57 % for AIH, 40 % for PBC and 50 % for PSC. AIH patients had a significantly higher frequency of negativization as compared to HW (Kruskal-Wallis test: $p = 0.002$; Wilcoxon rank sum test AIH-HW $p = 0.001$). In the AIH group, the number of autoantibody negativizations was higher than the number of positivizations (Table 2).

The effect of sex, age, presence of immunosuppression and type of vaccine in AILD patients assessed by multivariate logistic regression showed that patients who received the BNT162b2 vaccine were more likely to develop new autoantibodies and to have increasing autoantibody titers as compared to patients who received the mRNA-1273 vaccine (OR = 2.71, 95 % CI [1.09; 7.04], $p = 0.035$), the likelihood of these events being almost 22 % higher (Table 4).

Data on COVID-19 infection before vaccination was not available in the database. Anti-spike antibody measured in the serum sample taken before vaccination is a marker of previous SARS-CoV-2 infection; the time elapsed from sample collection to vaccination was reasonably short (median: 38 days, Table 1) only for HW. In this subgroup, the rate of no autoantibody changes after vaccination was higher (24/31, 77 %) in those who were anti-spike-negative before vaccination, than in those who were anti-spike positive before vaccination (4/7, 57 %).

None of the AILD patients developed new autoimmune diseases at one year after vaccination. The only patient having worsening of the pre-existing liver disease after vaccination was an 82-year-old woman with AIH type 1 (ANA 1:80 on HEp-20-10 cells and SMA 1:5120, tested locally), diagnosed in 2019 with advanced liver fibrosis at presentation, who received two doses of the BNT162b2 vaccine. She had a pre-existing diagnosis of autoimmune thyroiditis and was in AIH biochemical

remission on 6-mercaptopurine monotherapy before vaccination; ANA and SMA were negative in the pre-pandemic serum sample. She developed ANA (1:160 AC-1 and 1:320 AC-8 on HEp-20-10 cells) and SMA-V (1:80) six months after vaccination, in association with the appearance of anti-PML-antibody (anti-sp100 and ANA AC-6 were negative before and after vaccination) and anti-TPO; both before and after vaccination, anti-actin tested by immunoblot was positive, but negative by IIF on VSM 47 cells. She developed progressively increasing transaminase levels starting from one month after the second vaccine dose, requiring prednisone treatment in addition to 6-mercaptopurine with rapid transaminase level normalization (Table 3a).

5.1. Autoimmune hepatitis

Thirteen of the 35 (37 %) AIH patients developed new autoantibodies after vaccination, compared to 4 of 38 (11 %) HW ($p < 0.01$) (Tables 2 and 3a). Sex, age, and type of vaccine were not associated with an increased risk of developing new autoantibodies. Three patients had increasing titers of autoantibodies after vaccination: one of AMA and AC-21; one of AC-21; one of ANA with homogeneous pattern on primate liver.

Autoantibodies disappeared in 19 AIH patients (54 %) after vaccination (Table 2, Table 3b, Fig. 1). Six patients had decreasing titers of nine autoantibodies after vaccination: one of anti-actin tested on VSM 47 cells, of SMA-V and of SMA-G; one of anti-actin tested on VSM 47 cells and of ANA with nucleolar pattern on primate liver; two of atypical pANCA; and one each of AC-8 and of the granular cytoplasm pattern on primate liver, suggesting AMA positivity.

5.2. Primary biliary cholangitis

Seventeen of the 49 (35 %) patients developed new autoantibodies after vaccination, including 14 PBC and three isPBC; all maintained normal liver biochemistry and none developed new autoimmune diseases after vaccination (Table 3a). The proportion of PBC/isPBC patients developing new autoantibodies was significantly higher compared to healthy controls (35 % vs 11 %, $p = 0.01$). While sex and age older than 50 years were not associated with development of new autoantibodies, patients who received the BNT162b2 vaccine developed more frequently new autoantibodies as compared to those receiving the mRNA-1273 vaccine ($p = 0.025$; OR = 4.9). One PBC patient had an increasing AMA titer after vaccination (from 1:160 to 1:640), maintaining normal ALP serum levels till last follow-up three years after vaccination (Table 3a).

Autoantibodies disappeared in 19 patients (39 %) after vaccination (Table 2, Table 3b, Fig. 1). Five PBC patients had decreasing autoantibody titers after vaccination: one had decreasing titers of AMA, of the cytoplasmic granular pattern on primate liver, of ANA on liver rat substrate and of anti-actin antibody on VSM 47 cells; one had decreasing titers of AC-11 and of ANA with membrane pattern on primate liver; one of AMA and of ANA on liver rat; and one each of AC-11 and of AC-8.

Table 2

Number of subjects showing autoantibody determination positivization or negativization.

Number of changes in autoantibody determinations	AIH (%) (n = 35)	PBC (%) (n = 49)	PSC (%) (n = 9)	HW (%) (n = 38)
+6	1 (3)	–	–	–
+3	3 (9)	–	–	–
+2	3 (9)	4 (8)	2 (22)	2 (5)
+1	6 (17)	13 (27)	3 (33)	2 (5)
No changes	10 (29)	19 (39)	3 (33)	28 (74)
–1	7 (20)	16 (33)	0 (0)	5 (13)
–2	6 (17)	1 (2)	1 (11)	1 (3)
–3	1 (3)	1 (2)	2 (22)	–
–5	2 (6)	1 (2)	–	–
–6	1 (3)	–	–	–
–8	1 (3)	–	–	–
–11	1 (3)	–	–	–

AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; HW, healthcare workers.

Subjects can be included both in the positivizations (+) and in the negativizations (–) at the same time if they show various changes.

Table 3a

Clinical information on patients and controls developing new specificities or showing worsening of their baseline autoantibody profile after SARS-CoV-2 mRNA vaccine.

Disease	Age	Sex	Vaccine	Autoantibodies appearing after vaccine	Comments	Concomitant autoimmune diseases	Relapse/worsening of ALLD after vaccine
AIH	74	M	M	Atypical p-ANCA 1:10	<i>De novo</i>	PBC	no
AIH	74	F	M	Anti-actin on VSM 47 cells (1:40 before vaccine, 1:100 after vaccine)	SMA-V positive 1:100 before and after vaccine	none	no
AIH	69	F	P	Atypical p-ANCA 1:320	<i>De novo</i>	none	No, no inflammation (Ishak 1) at liver biopsy 1.5 years after vaccine
AIH	69	M	P	AC-27 pattern on HEp-20-10 cells 1:160	<i>De novo</i> ANA on HEp-20-10 cells: AC-1 1:640 (before vaccine, 1:40 after vaccine) AC-8 1:320 (before and after vaccine)	Celiac disease	no
AIH	75	F	P	Anti-Ku	<i>De novo</i>	none	no
AIH	78	M	M	Anti-CCP	<i>De novo</i>	none	no
				ANA on HEp-20-10 cells AC-8 1:100	ANA on HEp-20-10 cells AC-8 positive at presentation, negative on IS treatment		
AIH	60	F	P	Anti-Ro52, anti-SSA, anti-dsDNA	<i>De novo</i> AC-3 on HEp-20-10 cells 1:640 before and after vaccine	Autoimmune thyroiditis, psoriasis, PSC	No, mild (Ishak 4) inflammation at liver biopsy 1 year after vaccine
AIH	56	F	P	AMA 1:100, ANA on HEp-20-10 cells AC-21 1:100	<i>De novo</i> Anti-M2 and anti-M2-3E negative before and after vaccine	none	Normal liver biopsy 2 years after vaccine AIH relapse after SARS-CoV-2 infection 2.5 years after vaccine
AIH	19	M	P	ANA liver rat 1:160, ANA on HEp-20-10 cells AC-3/AC-4 1:100, AC-5 1:160	ANA positive on HEp-20-10 cells AC-4 1:320 at diagnosis, negative on IS treatment	none	no
AIH	63	F	P	Anti-gp210, ANA primate liver homogeneous 1:100, anti-TPO	ANA on HEp-20-10 cells 1:80 at diagnosis, negative on IS treatment Anti-TPO borderline before vaccine	Autoimmune thyroiditis	No Absence of cholestasis 1 year after vaccine
AIH	56	M	M	AMA 1:160	<i>De novo</i> Anti-M2 and anti-M2-3E negative before and after vaccine	none	Slight gGT elevation since 2017, unchanged after vaccine
AIH	82	F	P	Anti-PML11, anti-PML anti-TPO	<i>De novo</i> <i>De novo</i> SMA 1:5120 at diagnosis	Autoimmune thyroiditis	AIH relapse
				ANA on HEp-20-10 cells AC-1 1:160 and AC-8 1:320, SMA-V 1:100			
AIH	71	F	M	Anti-actin on VSM 47 cells 1:100, ANA on HEp-20-10 cells AC-1 1:100	<i>De novo</i> SMA negative before and after vaccine ANA on HEp-20-10 cells AC-1 1:80 before vaccine	Autoimmune thyroiditis, autoimmune gastritis	no
PBC	72	F	P	Anti-dsDNA	ANA on HEp-20-10 cells AC-1 1:320 before and after vaccine	none	no
PBC	78	F	M	atypical p-ANCA 1:10	<i>De novo</i>	none	no
PBC	49	F	P	primate liver nuclear membrane 1:160	ANA on HEp-20-10 cells AC-8 1:1000 before and after vaccine, anti-gp210 RUO and Euroline negative before and after vaccine	Systemic sclerosis	no
PBC	60	F	P	Anti-M2	Anti-M2-3E and AMA positive before and after vaccine	none	no
PBC	75	F	M	Anti-CENP-A, anti-CENP-B	<i>De novo</i> ANA on HEp-20-10 cells AC-6 1:32000 before and after vaccine	none	
PBC	82	F	M	Anti-dsDNA	Anti-dsDNA near the cut-off before vaccine ANA on HEp-20-10 cells AC-3 1:640 before and after vaccine	Autoimmune thyroiditis	no
PBC	69	F	P	ANA liver rat 1:100	ANA on liver rat 1:80 before vaccine	none	no
PBC	62	F	M	Anti-PML11, AMA 1:160	ANA on HEp-20-10 cells AC-6 1:3200 before and after vaccine AMA 1:80 before vaccine Anti-M2 and anti-M2-3E positive before and after vaccine	none	no
PBC	72	F	P	Anti-sp100	<i>De novo</i> ANA on HEp-20-10 cells AC-6 negative before and after vaccine	none	no
PBC	72	M	P	Anti-F-actin	SMA negative before and after vaccine Anti-F-actin before vaccine borderline	none	no
PBC	29	F	M	Anti-F-actin	SMA negative before and after vaccine Anti-F-actin before vaccine borderline	Autoimmune thyroiditis, juvenile arthritis, autoimmune urticaria	no

(continued on next page)

Table 3a (continued)

Disease	Age	Sex	Vaccine	Autoantibodies appearing after vaccine	Comments	Concomitant autoimmune diseases	Relapse/worsening of AILD after vaccine
PBC	53	F	P	ANA liver rat 1:160	ANA liver rat 1:80 before vaccine ANA on HEp-20-10 cells 1:1000 before and after vaccine AC-3	none	no
PBC	49	F	P	Anti-F-actin	SMA negative before and after vaccine Anti-F-actin borderline before vaccine	Sjögren syndrome	no
PBC	67	F	P	ANA liver rat 1:1000	ANA on HEp-20-10 cells AC-3 1:3200 before and after vaccine	none	no
PBC	57	F	M	Anti-Ro52	ANA on HEp-20-10 cells AC-3 1:32000 before and after vaccine	Systemic scleroderma	no
PBC	60	F	P	ANA liver rat 1:100, primate liver nuclear membrane 1:320	ANA on HEp-20-10 cells AC-3 before vaccine 1:3200, after vaccine 1:10000 Anti-gp210 negative before and after vaccine	Lichen	no
PBC	73	F	M	Anti-SSA	Anti-SSA borderline before vaccine		
PSC	56	F	M	Anti-LKM-1 1:320	Only kidney rat substrate positive, liver rat and Euroline negative	none	no
PSC	64	F	P	HEp-20-10 cells AC-16 1:100, SMA-V 1:100	Anti-F-actin negative before and after vaccine, SMA-V and AC-16 on HEp-20-10 cells 1:80 before vaccine	Inflammatory bowel disease	no
PSC	69	M	P	Anti-PM100 ANA on HEp-20-10 cells AC-1 1:640	<i>De novo</i> ANA on HEp-20-10 cells 1:40 before vaccine	Inflammatory bowel disease	no
PSC	59	F	M	ANA liver rat 1:100	<i>De novo</i>	Inflammatory bowel disease	no
PSC	61	M	P	SMA on rat stomach substrate 1:160	SMA on kidney substrate and anti-F-actin negative before and after vaccine	Pemphigus vulgaris	no
HW	60	F	P	Anti-RP155	Anti-RP155 borderline before vaccine ANA on HEp-20-10 cells negative before and after vaccine	No information	NA
HW	61	F	P	ANA on HEp-20-10 cells AC-4 1:100	ANA on HEp-20-10 cells AC-4 1:80 before vaccine	No information	NA
HW	50	F	P	Anti-F-Actin	Anti-F-actin Euroline borderline before vaccine SMA-V 1:40 before vaccine, 1:80 after vaccine	No information	NA
HW	63	F	P	anti-TPO ANA liver rat 1:160	<i>De novo</i> ANA on liver rat 1:80 before vaccine ANA on HEp-20-10 cells AC-1 1:100 before vaccine, 1:160 after vaccine	No information	NA

Pt patient; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis, HW, healthcare workers; P, BNT162b2 vaccine; M, mRNA-1273 vaccine; AILD, autoimmune liver disease; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; isPBC, isolated PBC serology; PSC, primary sclerosing cholangitis; IS, immunosuppressive; HC, healthy control; gGT, gamma-glutamyltransferase; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; ANA, anti-nuclear antibody; CCP, cyclic citrullinated peptide; SSA, Sjögren's-syndrome-related antigen A; dsDNA, double stranded DNA; AMA, anti-mitochondrial antibody; gp210, glycoprotein-210; SMA-V, anti-smooth muscle antibody vessel; Anti-TPO, anti-thyroidperoxidase; LKM-1, liver kidney microsomal type 1; NA, not applicable.

5.3. Primary sclerosing cholangitis

Five (56 %) PSC patients developed new autoantibodies after vaccination, the proportion being significantly higher compared to HW (56 % vs 11 %, $p = 0.007$). One patient had an increasing titer of ANA with nucleolar and homogeneous pattern on primate liver. None had worsening of PSC or of concomitant inflammatory bowel disease, none developed new autoimmune diseases (Tables 1 and 3a).

Autoantibodies disappeared in three patients after vaccination (33 %) (Table 2, Table 3b, Fig. 1).

5.4. Healthy controls

Four (11 %) of the 38 HW developed autoantibodies after vaccination (Table 2, Table 3a, Fig. 1). One developed ANA on liver rat substrate and anti-TPO, one anti-actin on VSM cells and SMA on stomach substrate, one AC-4 and one anti-RP155. Sex, age older than 50 years and vaccine type were not associated with development of new specificities. Autoantibodies disappeared after vaccination in six of the 24 subjects who had positivities before vaccination (Table 2, Table 3b, Fig. 1).

6. Discussion

In this study, we have centrally tested a large panel of liver- and non-liver-related autoantibodies before and after mRNA SARS-CoV-2 vaccines in patients with AILD and in healthcare workers: while AILD patients develop autoantibodies more frequently than HW after mRNA vaccination, we show that autoantibodies also frequently disappear after mRNA vaccination in these patients, suggesting that in this autoimmunity-prone population mRNA vaccines do not induce worsening of autoimmunity. This is confirmed by the clinical course, which was not worsened after vaccination, only one AIH patient experiencing a relapse following a second BNT162b vaccine, which was easily controlled with steroid therapy.

In the present study, autoantibodies were tested using two complementary approaches. The Euroline western blots allow detection of a large variety of reactivities, nuclear- or liver disease-specific. The knowledge of the specific antibodies acquired by this technique, however, is limited by its inability to detect accurately reactivity to conformational epitopes. This limitation, intrinsic to western blots, is addressed using immunofluorescence, where the substrate maintains conformational antigenic determinants. Thus, employing these two methodological approaches in parallel provides wider information, though occasional discrepancies between the two techniques are to be

Table 3b

Summary of patients and controls showing disappearance of autoantibodies after SARS-CoV-2 mRNA vaccine.

Disease	Age	Sex	Vaccine	Autoantibodies disappearing after vaccine	Comments	Concomitant autoimmune diseases
AIH	73	F	P	SMA and SMA-V (1:100 before vaccine 1:40 after vaccine)	Anti-F-actin and anti-actin on VSM 47 cells negative before and after vaccine	Thyroiditis
AIH	70	F	P	Anti-dsDNA	ANA on HEp-20-10 cells 1:640 AC-1 before and after vaccine	None
AIH	28	F	P	ANA on HEp-20-10 cells AC-1 (1:160 before vaccine, 1:80 after vaccine)	ANA on liver rat positive before (1:160) and after vaccine (1:100)	Inflammatory bowel disease
AIH	57	F	M	Anti-F-Actin, SMA-V Anti-gp210 Euroline anti-Mi-2b atypical ANCA (1:10 before vaccine, negative after vaccine)	Anti-actin on VSM 47 cells negative before and after vaccine anti-gp210 RUO and ANA on HEp-20-10 cells negative before and after vaccine	None
AIH	69	M	P	Anti-F-Actin, anti-actin on VSM 47 cells, SMA-V, SMA on rat stomach ANA nucleolar pattern on primate liver (1:640 before vaccine, 1:40 after vaccine) ANA on HEp-20-10 cells AC-1 (1:640 before vaccine, 1:40 after vaccine) p-ANCA (1:10 before vaccine, negative after vaccine)	SMA-G and SMA-T negative before and after vaccine ANA on HEp-20-10 cells AC-8 positive before (1:640) and after (1:320) vaccine ANA on liver rat positive before (1:320) and after vaccine (1:320) Anti-PR3 and anti-MPO negative before and after vaccine, atypical pANCA positive before (1:320) and after (1:320) vaccine	Celiac disease
AIH	81	M	M	Anti-gp210 RUO, anti-gp210 Euroline, ANA on HEp-20-10 cells AC-11 (1:10000 before vaccine, negative after vaccine) AMA (1:320 before vaccine, 1:80 after vaccine), anti-M2-3E, anti-M2	ANA on liver rat negative before and after vaccine	Primary biliary cholangitis
AIH	78	M	M	Anti-gp210 RUO, anti-gp210 Euroline, ANA nuclear membrane on primate liver (1:3200 before vaccine, negative after vaccine), ANA AC-11 on HEp-20-10 cells (1:10000 before vaccine, negative after vaccine) Anti-M2-3E, anti-M2, AMA (1:3200 before vaccine, negative after vaccine), AC-21 on HEp-20-10 cells (1:3200 before vaccine, negative after vaccine), granular cytoplasm on primate liver 81:640 before vaccine, negative after vaccine) DFS70		None
AIH	60	F	P	Anti-RP11 Anti-CENP-B SMA on rat stomach from 1:100 to negative	AC-24 on HEp-20-10 negative before and after vaccine SMA-V from 1:80 to negative, SMA-G and SMA-T negative Anti-F-actin and anti-actin on VSM 47 cells negative before and after vaccine	None
AIH	70	M	M	ANA on HEp-20-10 cells AC-4 (1:160 before vaccine, 1:80 after vaccine)	ANA on rat liver from 1:80 to negative	None
AIH	56	F	P	ANA on HEp-20-10 cells AC-4 from 1:100 to 1:20	ANA on primate liver negative before and after vaccine ANA on liver rat and primate liver negative before and after vaccine	None
AIH	70	F	P	SMA-V and SMA-G (1:000 before vaccine, negative after vaccine)	SMA-T negative before and after vaccine Anti-F-actin, anti-actin on VSM 47 and SMA on rat stomach positive before and after vaccine	None
AIH	28	F	P	ANA homogeneous pattern on primate liver (1:160 before vaccine, 1:80 after vaccine)	ANA on HEp-20-10 cells AC-1 and on liver rat positive before and after vaccine	None
AIH	19	M	P	SMA-T (1:320 before vaccine, negative after vaccine)	SMA-V 1:1000 before vaccine, 1:320 after vaccine) SMA-G 1:1000 before vaccine, 1:160 after vaccine Anti-F-actin and anti-actin on VSM 47 positive before and after vaccine	None
AIH	68	F	M	SMA-V (1:100 before vaccine, negative after vaccine)	SMA-G and SMA-T negative before and after vaccine Anti-F-actin and anti-actin on VSM 47 positive before and after vaccine	None
AIH	74	F	M	Anti-actin on VSM 47 cells, anti-F-Actin ANA on HEp-20-10 cells AC-4 (1:100 before vaccine, negative after vaccine)	SMA-V and on stomach 1:100 before and after vaccine, SMA-G and SMA-T negative before and after vaccine ANA on liver rat 1:100 before and after vaccine ANA on primate liver negative before and after vaccine	None
AIH	68	M	P	Anti-Ku ANA on HEp-20-10 cells AC-8 (1:160 before vaccine, negative after vaccine)	ANA on liver rat and on primate liver negative before and after vaccine	Multiple sclerosis
AIH	63	F	P	ANA on HEp-20-10 AC-15 (1:100 before vaccine, negative after vaccine) SMA-G (1:160 before vaccine, 1:80 after vaccine), SMA-T (1:100 before vaccine, negative after vaccine) Bile canaliculi pattern on primate liver (1:100 before vaccine, 1:40 after vaccine) Anti-CCP	ANA on HEp20-10 cells AC-1 and AC-8 positive before and after vaccine, ANA on liver rat 1:320 before and after vaccine, ANA on primate liver nucleolar pattern positive before and after vaccine SMA-V, anti-F-actine and anti-actineon VSM 47 positive before and after vaccine	Thyroiditis
AIH	82	F	P	ANA on HEp-20-10 AC-21 (1:160 before vaccine, negative after vaccine)	AMA negative before and after vaccine	None
AIH	64	F	P	ANA on liver rat (1:100 before vaccine, negative after vaccine) atypical ANCA (1:10 before vaccine, negative after vaccine)	ANA on HEp-20-10 cells AC-4 from 1:80 to 1:40 ANA on primate liver negative before and after vaccine	None

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Table 3b (continued)

Disease	Age	Sex	Vaccine	Autoantibodies disappearing after vaccine	Comments	Concomitant autoimmune diseases
PBC	72	F	P	pANCA (1:10 before vaccine, negative after vaccine)	Atypical pANCA positive before and after vaccine, anti-MPO and anti-PR3 negative before and after vaccine	None
PBC	49	F	P	SMA on rat stomach (1:160 before vaccine, 1:80 after vaccine)	SMA-V positive before (1:160) and after (1:100) vaccine, SMA-G, SMA-T, anti-actin on VSM 47 and anti-F-actine negative before and after vaccine	Systemic sclerosis
PBC	66	F	M	Anti-dsDNA	ANA on HEp-20-10 cells positive AC-11 before and after vaccine	Sjögren syndrome
PBC	55	F	M	Anti-Ro52 RUO	Anti-gp210 RUO and Euroline negative before and after vaccine	Systemic sclerosis
PBC	65	F	M	AMA (1:1000 before vaccine, 1:20 after vaccine)	Anti-Ro52 Euroline negative before and after vaccine	Rheumatoid arthritis
PBC	72	F	P	ANA on HEp-20-10 cells AC-11 (1:320 before vaccine, 1:80 after vaccine) ANA liver rat (1:1000 before vaccine, 1:80 after vaccine) ANA on primate liver nuclear membrane pattern (1:320 before vaccine, negative after vaccine)	Anti-gp210 RUO and Euroline negative before and after vaccine	None
PBC	50	F	P	Anti-gp210 Euroline	Anti-gp210 RUO positive before and after vaccine	None
PBC	77	F	M	ANA on liver rat (1:1000 before vaccine, 1:40 after vaccine) ANA on primate liver nuclear membrane pattern (1:100 before vaccine, 1:40 after vaccine)	ANA on HEp-20-10 AC-11 positive before and after vaccine ANA on HEp-20-10 AC-4 and AC-11 positive before and after vaccine	None
PBC	53	F	P	Anti-dsDNA	Anti-gp210 RUO and Euroline negative before and after vaccine	None
PBC	88	F	M	AMA (1:100 before vaccine, negative after vaccine)	ANA on Hep-20-10 AC-3 positive before and after vaccine	Sjögren syndrome
PBC	55	F	M	Anti-DFS70	Anti-M2-3E positive before and after vaccine	Sjögren syndrome
PBC	72	F	M	ANA on HEp-20-10 AC-23 1:100 to 1:80	Anti-M2 negative before and after vaccine	None
PBC	71	F	P	AMA (1:640 before vaccine, negative after vaccine)	ANA on HEp-20-10 cells AC-3 positive before and after vaccine	None
PBC	67	F	M	Smooth cytoplasm pattern on primate liver (1:640 before vaccine, negative after vaccine)	Anti-M2-3E positive before and after vaccine	Cutaneous lupus erythematosus
PBC	61	F	M	Anti-dsDNA	ANA on HEp-20-10 AC-11 positive before and after vaccine	Celiac disease
PBC	57	F	M	AMA (1:1000 before vaccine, negative after vaccine)	Anti-M2 and anti-M2-3E negative before and after vaccine	None
PBC	48	F	M	Anti-M2-3E HEp-20-10 cells AC-18 (1:320 before vaccine, 1:80 after vaccine)	AMA and anti-M2 positive before and after vaccine	None
PBC	65	F	M	Anti-M2	AMA and anti-M2-3E positive before and after vaccine	Rheumatoid arthritis
PBC	73	F	M	HEp-20-10 AC-21 from 1:100 to 1:80	AMA 1:80 before and after vaccine, anti-M2 and anti-M2-3E positive before and after vaccine	None
PSC	56	F	M	Anti-PR3 ANA on liver rat (1:1000 before vaccine, 1:80 after vaccine) ANA on primate liver homogeneous pattern (1:640 before vaccine, 1:40 after vaccine)	Atypical ANCA and pANCA positive before and after vaccine, cANCA negative before and after vaccine	None
PSC	69	M	P	Anti-PR3 ANA on HEp-20-10 AC-5 (1:1000 before vaccine, negative after vaccine) Mitotic pattern AC-28 on HEp-20-10 cells (1:1000 before vaccine, negative after vaccine)	ANA on HEp-20-10 AC-1 positive before and after vaccine	Morbus Crohn
PSC	54	F	P	Anti-F-Actin Anti-TPO	Atypical ANCA positive before and after vaccine	Ulcerative colitis
HW	23	F	P	LC-1 RUO	SMA on rat stomach, SMA on rat kidney and anti-actin on VSM 47 negative before and after vaccine	No information
HW	61	F	P	ANA on HEp-20-10 cells AC-4 (1:100 before vaccine, negative after vaccine)	Anti-LC1 negative at indirect immunofluorescence on triple rodent tissue	No information
HW	64	F	P	Cytoplasmatic pattern on HEp-20-10 AC-22 (1:100 before vaccine, 1:40 after vaccine)	ANA on liver rat and on primate liver negative before and after vaccine	No information
HW	61	F	P	ANA on HEp-20-10 cells AC-4 (1:100 before vaccine, 1:80 after vaccine) Cytoplasmic pattern on HEp-20-10 cells AC-23 (1:100 before vaccine, negative after vaccine)	ANA on liver rat and on primate liver negative before and after vaccine	No information
HW	47	M	P	Cytoplasmic pattern on HEp-20-10 cells AC-18 (1:100 before vaccine, 1:80 after vaccine)		No information
HW	52	F	M	ANA on HEp-20-10 cells AC-4 (1:100 before vaccine, 1:80 after vaccine)		No information

P, BNT162b2 vaccine; M, mRNA-1273 vaccine; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; HW, health care workers; gp210, glycoprotein 210; AMA, anti-mitochondrial antibodies; M2-3E, recombinant antigen fusion protein for the detection of AMA-M2; M2, AMA targeting M2 antigen; SMA, anti-smooth muscle antibody; SMA-V, SMA vascular staining pattern; dsDNA, double-stranded DNA; Mi-2b, Mi-2b protein; ANCA, anti-neutrophil cytoplasmatic antibodies; VSM47, vascular smooth muscle; p-ANCA, perinuclear ANCA; DFS70, dense fine speckled; RP, ribosomal protein; CB, centromeric protein B;

SMA-G, SMA glomerular staining pattern; SMA-T, SMA tubular staining pattern; Ku, Ku-protein; Anti CCP, anti citrullinated protein antibodies; Ro 52, Ro-protein; PR3, Proteinase 3; Anti-TPO, tyreoperoxidase-antibodies; LC-1, liver cytosol antigen 1.

Table 4
Multivariate logistic regression model testing associations between different sets of sociodemographic and clinical variables and the positivization of at least one autoantibody of autoimmune liver disease patients.

	OR (95 % CI)	p	Marginal effect
Age	1.02 (0.99–1.05)	0.196	0.004
Sex			
F	–	–	–
M	1.80 (0.58–5.66)	0.306	0.129
Immunosuppression	0.70 (0.27–1.75)	0.453	–0.078
Vaccine			
mRNA-1273	–	–	–
BNT162b2	2.71 (1.09–7.04)	0.035	0.219

expected.

In a large prospective study of multiple sclerosis patients who received mRNA vaccines no development of autoantibodies against central nervous system autoantigens or increased relapse rate were observed [21]. Similarly, in a cohort of inflammatory arthritis patients, the rate of ANA seroconversion after mRNA SARS-CoV-2 vaccines was similar to healthy controls, and not associated with disease flares; interestingly, patients who had previously been infected with the SARS-CoV-2 virus had a higher ANA seroconversion rate after mRNA vaccines as compared to uninfected patients [22]. An Italian study tested autoantibodies in healthcare workers before and after two and three doses of mRNA SARS-CoV-2 vaccines: the authors found that ANA seroconversion (tested by indirect immunofluorescence on HEp2 cells) directly correlated with the number of vaccine expositions; no clinical data are available [11]. The same observation has been reported in patients with autoimmune rheumatic diseases, with autoantibodies appearance correlating with the risk of flares, without new onset autoimmune diseases after vaccination [23]. Finally, a study from Israel including 101 general population individuals did not find an association between the BNT162b2 vaccine and ANA or anti-phospholipids antibodies appearance [24].

Interestingly, we found that the BNT162b2 vaccine was associated with a higher rate of new autoantibody development and titer increase as compared to the mRNA-1273 vaccine in AILD. This observation is new and somehow counterintuitive, since the BNT162b2 contains less mRNA (30 µg) than the mRNA-1273 vaccine (100 µg). This difference between the two mRNA vaccines has not been reported in earlier studies probably because the populations analysed had received prevalently the BNT162b2 vaccine, which had been approved first in most countries [7]. While both vaccines are based on poly uracil modified mRNA in lipid nanoparticles as a delivery system, both components acting as adjuvants, the ionizable cationic lipids of the lipid nanoparticles used in the two vaccines are different, possibly explaining our observation [25].

Our study has a few limitations. First, the PSC group was smaller, due to the rarity of this condition, therefore limiting the generalization of our results on PSC patients. Second, we were not able to include unvaccinated pathological control groups, since the vaccination rate in older patients with chronic diseases, as our study population was, is very high in Switzerland [26]. Third, we used healthcare workers as controls, since we did not have data on vaccination, demographics and sera available of other controls. Healthcare workers are exposed to infections and may have asymptomatic or oligosymptomatic autoimmune diseases, therefore they may be more often positive for autoantibodies as compared to healthy subjects. Consequently, the difference between patients and controls in autoantibodies appearance after vaccination would have been even more pronounced if we had had healthy controls available.

In conclusion, our data are reassuring concerning safety of mRNA

vaccines in AILD patients, since we did not observe a worsening of the humoral autoimmunity or of the clinical course in our large cohort. In this context, it should be remembered that SARS-CoV-2 infection itself has been shown to be associated with appearance of autoantibodies and with flares of pre-existing autoimmune diseases, further highlighting the suitability of SARS-CoV-2 vaccination also in patients with autoimmune liver disorders [27,28].

Founding

This work was supported by EUROIMMUN.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Tobias Kälén: Writing – review & editing, Formal analysis, Data curation. **Katia Passarin:** Writing – review & editing, Software, Formal analysis. **Magdalena Filipowicz-Sinnreich:** Writing – review & editing, Data curation. **David Semela:** Writing – review & editing, Data curation. **Tanja Seifert:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Federica Sallusto:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization. **Diego Vergani:** Writing – review & editing, Validation, Supervision, Conceptualization. **Andreas Cerny:** Writing – review & editing, Conceptualization. **Giorgina Mieli-Vergani:** Writing – review & editing, Validation, Supervision, Conceptualization. **Benedetta Terziroli Beretta-Piccoli:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Acknowledgment

Fondazione Epatocentro Ticino, for the biobank management and biosamples handling; Martina Lehmann, Silvia Jacobsen, Christine Maaßen, Fabian Lindhorst, Dr. Wolfgang Meyer for coordinating and conducting the serological tests.

Data availability

Data will be made available on request.

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