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Liver, Pancreas and Biliary Tract

Spike-specific humoral and cellular immune responses after COVID-19 mRNA vaccination in patients with cirrhosis: A prospective single center study

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ABSTRACT

Background and Aims: COVID-19 mRNA vaccines were approved to prevent severe forms of the disease, but their immunogenicity and safety in cirrhosis is poorly known.
Method: In this prospective single-center study enrolling patients with cirrhosis undergoing COVID-19 vaccination (BNT162b2 and mRNA-1273), we assessed humoral and cellular responses vs healthy controls, the incidence of breakthrough infections and adverse events (AEs). Antibodies against spike- and nucleocapsid-protein (anti-S and anti-N) and Spike-specific T-cells responses were quantified at baseline, 21 days after the first and second doses and during follow-up.
Results: 182 cirrhotics (85% SARS-CoV-2-naïve) and 38 controls were enrolled. After 2 doses of vaccine, anti-S titres were significantly lower in cirrhotics vs controls [1,751 (0.4–25,000) U/mL vs 4,523 (259–25,000) U/mL, p=0.012] and in SARS-CoV-2-naïve vs previously infected cirrhotics [999 (0.4–17,329) U/mL vs 7,500 (12.5–25,000) U/mL, (p<0.001)]. T-cell responses in cirrhotics were similar to controls, although with different kinetics. In SARS-CoV-2-naïve cirrhotics, HCC, Child-Pugh B/C and BNT162b2 were independent predictors of low response. Neither unexpected nor severe AEs emerged. During follow-up, 2% turned SARS-CoV-2 positive, all asymptomatic.
Conclusion: Humoral response to COVID-19 vaccines appeared suboptimal in patients with cirrhosis, particularly in SARS-CoV-2-naïve decompensated cirrhotics, although cellular response appeared preserved, and low breakthrough infections rate was registered.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represented one of the worst pandemics in history and is still ongoing worldwide [1]. It has been shown that patients with cirrhosis, and particularly those with a more advanced disease and comorbidities, have worse outcomes for COVID-19 [2–4]. Vaccination has been shown to be one of the most effective ways not

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only to control SARS-CoV-2 pandemic but also to reduce hospitalization rates, major complications, and deaths [5–7]. With these premises, scientific societies have recommended priority vaccination of these vulnerable patients [8,9]. However, despite the inclusion of over 100,000 participants in the phase 3 trials, data in patients with chronic liver disease are limited, as these patients represent less than 0.5% of those enrolled [5–7]. The evaluation of efficacy and safety of vaccination in patients with cirrhosis is important not only for the severity of COVID-19 in these patients but also because the immune response to COVID-19 vaccination might be dampened, as it has previously shown with vaccines against *S. pneumoniae*, influenza and hepatitis B virus [10–12]. A few preliminary data on COVID-19 vaccination in patients with chronic liver disease, cirrhosis and liver transplant suggested a suboptimal antibody response [13–15]. However, these studies have several limitations: lack of healthy control group; the enrolment of only few patients with compensated and decompensated cirrhosis and none with hepatocellular carcinoma (HCC), a short post-vaccination follow-up, no information on post-vaccination infection rate and durability of response.

In addition, no studies have assessed so far, the specific anti-Spike T-cell response in patients with cirrhosis undergoing vaccination. While tests for antibodies are routinely performed, the technical complexity of SARS-CoV-2-specific T-cell measurements has so far limited this analysis. The rapid tests based on release of T-cells cytokines like IFN-gamma and IL-2 in the supernatants of whole blood pulsed with peptides covering immunogenic regions of Spike could represent a reliable approach to evaluate quantity and function of vaccine-induced Spike specific T-cells [16].

The present study aimed to assess the immunogenicity, incidence of breakthrough infections, and safety of COVID-19 mRNA vaccines through 180 days from first dose in patients with compensated and decompensated cirrhosis with different aetiologies, compared to healthy subjects.

2. Patients and methods

2.1. Study design

This is a prospective, single-center, pharmacological observational study including all patients with cirrhosis of any etiology followed in our center who agreed to receive COVID-19 vaccination. The study is part of an hospital-based protocol, the PolImmune-COVID study (#286_2021), started on Jan 25th, 2021, after the approval of the INMI "Lazzaro Spallanzani" Ethics Committee (Roma, Italy).

The primary endpoint of the study was the immunogenicity of COVID-19 mRNA vaccines (BNT162b2 Pfizer BioNTech or mRNA-1273Moderna), measured as SARS-CoV-2 anti-Spike protein antibodies (anti-S) titres after 21 days from the second dose and predictors of response (V2). Secondary endpoints were durability of humoral response to COVID-19 vaccines measured at different time-points; evaluation of T-cell responses in a subgroup of patients and controls at different time-points; predictors of humoral response; evaluation of incidence and severity of post-vaccination SARS-CoV-2 infections in patients with cirrhosis; and safety defined by the incidence and grade of adverse events following vaccination.

All subjects gave their written consent to participate to the study which was carried out in conformity with the 2013 revision of the Declaration of Helsinki.

2.2. Patients and healthy controls

From March 2021, Italy prioritized immunization with COVID-19 vaccines of patients with chronic diseases, including those with cirrhosis. Patients with history of COVID-19 within 3–6 months before availability of vaccine might have received either a single dose or two doses according to physician decision, while all the others received 2 doses of vaccine. The study design also included a control group of healthcare workers who received COVID-19 mRNA vaccines with no major co-morbidities, unmatched for age, gender prevalence and previous COVID-19.

Patients and controls were also stratified according to the history of previous COVID-19 (either by a known diagnosis by nasopharyngeal swab for SARS-CoV-2 by PCR before enrolment or a positive result at baseline for antibodies against SARS-CoV-2 nucleocapsid protein (anti-N). Exclusion criteria included age <18 years and inability to provide informed consent.

2.3. Patients' information

Clinical data was obtained from patients' medical records and routine blood tests up to 3 months prior to the date of the first vaccination. Liver function was evaluated by means of both biochemical [i.e. bilirubin, international normalized ratio (INR), albumin, creatinine] and clinical variables (i.e. presence and degree of ascites, encephalopathy, esophagogastric varices). For each patient Child-Pugh and the Model for End-Stage Liver Disease (MELD) scores were assessed with last available data. Data on immunosuppressive drugs, HCC and comorbidities were also collected.

Participants filled in a questionnaire on vaccination side effects after 7 days following first and second doses: local (pain, redness, swelling, and lymphadenopathy) and systemic (fever, chills, headache, fatigue, myalgia, arthralgia, nausea and vomiting, diarrhea) adverse events (AE) were reported and graded according to the Common Terminology Criteria for AEs (CTCAE) grade scale. Any other adverse events occurring thereafter, or any other event or positivity for SARS-CoV-2 to the nasopharyngeal swab or any hospitalizations for COVID-19 were collected during normal visits and reported.

2.4. Humoral response

The anti-S titres were quantified by the anti–SARS-CoV-2 S enzyme immunoassay (Roche Elecsys, Roche, Monza) that tests for antibodies against the receptor-binding domain of the SARS-CoV-2 spike protein. According to Manufacturer's instruction, the antibody-positive cut-off was 0.8 U/mL. A low positive response was defined from 0.8 to 100 U/mL, based on thresholds of validating studies and on cut-offs used in previous trials. The anti-N antibodies (Roche Diagnostics, Monza; the antibody-positive cut-off was 0.08 Cut-off Index) were quantified at the same time-points to either confirm or detect SARS-CoV-2 infections in both patients and controls. Both tests were performed on Cobas e801 automatic analyzer (Roche Diagnostics, Monza, Italy) by ECLIA.

Blood samples were taken 1–3 days before the first dose (V0, Fig. 1), 21 days after the first dose of vaccine (V1) and 21 days after the second dose (V2). A pre-planned fourth test at 180 days from the second dose was anticipated before the third dose of vaccine (V3), due to change in vaccination policy in Italy [133 (70–208) days].

2.5. Spike-specific T cellular response

The spike-specific T-cell response was analyzed by a cytokine release assay (CRA) of whole peripheral blood stimulated using a SARS-CoV-2 spike-derived 15-mers peptides (S) pool described previously, that covers the immunogenic regions of SARS-CoV-2 Spike protein and represents 41% of the whole Spike protein [16,17]. Freshly drawn whole blood (320µL; within 6 h since venipuncture) was mixed with 80µL RPMI and stimulated with S

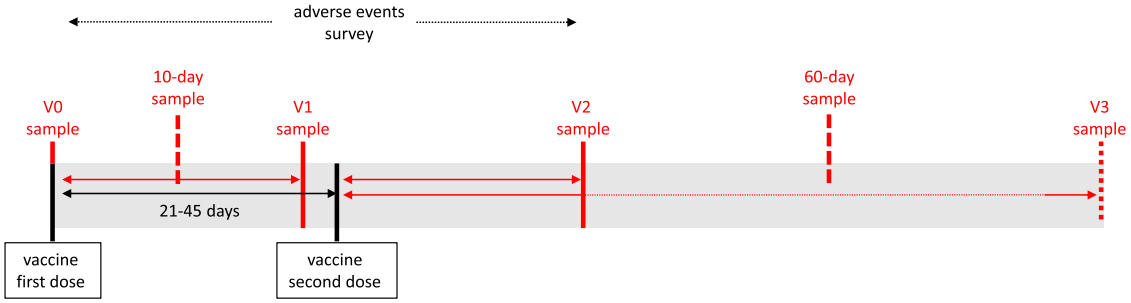

Fig. 1. Study's timeline. Humoral response (anti-S and anti-N) was evaluated at 4 time-points: before (V0) the first dose of anti-SARS-CoV-2 mRNA vaccines, 21 days after the first dose (V1), 21 days after the second dose (V2) and 133 (70–182) days from the second dose (V3). Anti-spike specific T-cell response was evaluated in two additional time-points also: 10 days after the first dose and 60 days after the second dose.

pool peptides to a final peptide concentration of 2 μg/mL or mixed with an equivalent amount of DMSO as control. Culture supernatants were collected 16 h after culture and stored at −80 °C until cytokine quantification. IFN-γ/IL-2 concentrations in plasma were quantified using an Ella machine (ProteinSimple) with microfluidic multiplex cartridges following the manufacturer's instructions. Background cytokine levels quantified from DMSO controls were subtracted from the corresponding peptide pool stimulated samples. The threshold for a positive response was set at 10 times the lower limit of quantification for each cytokine (IFN-γ =1.7 pg/mL; IL-2 = 5.4 pg/mL).

Whole blood collected before (Day 0), 10 and 21 days after the first dose and 21 (day 42), 39 (day 60) and 133 days after the second dose were used for these analyses.

2.6. Statistical analysis

Data is displayed as median (range) for continuous variables and as number and percentage for categorical variables. For categorical variables, the Chi-Square statistic was used to assess the statistical significance between groups. Continuous variables were first tested for normal distribution using the Kolmogorov-Smirnov test and Q-Q plots and then compared by *t*-test if normally distributed or by Kruskal Wallis/Mann-Whitney test if non-normally distributed. Patients and controls were stratified according to previous SARS-CoV-2 infection (anti-N positivity at baseline sample); patients were stratified according to liver function (according to Child-Pugh classification and MELD score), anti-S levels after first dose and presence of HCC and immunosuppressive drugs. Interval regression models were fitted to take into account left- and right-censored anti-S titres due to limits of essay detection, in both univariable and multivariable models. Factors putatively associated to anti-S titres were initially selected based on their clinical significance; to obtain a parsimonious model, the AIC criterion was used to select the best fitting multivariable model. Models for repeated measures within patient were applied whenever relevant, with time as fixed effect and patient as random effect. Anti-S titre was log-transformed to achieve normality. Analyses were performed using STATA software (release 16.0, Stata Corporation, College Station, TX).

3. Results

3.1. Baseline features

A total of 182 patients with cirrhosis and 38 healthy unmatched controls entered the study (Fig. 1) and were studied accordingly for humoral and T-cell response at pre-defined time-points (Fig. 2). Patients with cirrhosis had a median age of 61 (20–89) years and were predominantly males (75%). Detailed baseline demographic and clinical features are reported in Table 1. One hundred and thirty-five patients (74%) had a well-compensated cirrhosis (Child-Pugh A), 50 (31%) had a diagnosis of HCC (16 with active HCC). Among cirrhotic patients, 28 (16%) had a previous SARS-CoV-2 infection [20 symptomatic COVID-19, the median time elapsing between recovery and first dose of vaccine was 208 (26–432) days] and 154 (84%) were SARS-CoV-2 naïve. Between

March and July 2021, 180 (99%) patients received 2 doses of COVID-19 mRNA vaccines: 119 (66%) BNT162b2 and 61 (34%) mRNA-1273; while 2 patients received a single dose of BNT162b2, because of recent history of COVID-19.

Baseline features of 38 healthy subjects (control group) vaccinated with mRNA-vaccines are presented in Table 1: 12 (31%) had previous exposure to SARS-CoV-2 (10 symptomatic COVID-19, the median time elapsing between recovery and first dose of vaccine was 258 (88–348) days) and 29 (76%) received BNT162b2 vaccine and 9 (24%) the mRNA-1273 one.

3.2. Humoral response (anti-S titres) at different time-points and predictors of response

After the first vaccine dose (V1), 102 (60%) patients with cirrhosis were positive for anti-S compared to 31 (97%) healthy controls ($p<0.001$), the median antibody titres being significantly lower in the former group [23.5 (0.4–25,000) U/mL vs 84.8 (0.4–25,500) U/mL, $p = 0.0001$]. Anti-S titres were significantly lower among SARS-CoV-2 naïve cirrhotics compared to those with previous SARS-CoV-2 infection 13.9 (0.4–14,336) U/mL vs 7500 (273–25,000) U/mL ($p<0.001$) and the same held true for healthy controls [43.1 (0.4–345) U/mL vs 17,300 (8962–25,000) U/mL ($p<0.001$)] (Fig. 3). Among 145 SARS-CoV-2 naïve patients, 76 (52%) resulted anti-S Ab positive after the first vaccine dose compared to 22 (96%) healthy SARS-CoV-2 naïve subjects ($p<0.001$), the median anti-S Ab titres were 13.9 (0.4–14,336) U/mL vs 43.1 (0.4–345) U/mL, $p = 0.001$.

The primary endpoint of the study was humoral response 21 days after the second dose of vaccine (V2): 163 patients with cirrhosis resulted positive for anti-S (97%) compared to 33 healthy controls (100%) ($p = 0.07$), the anti-S titres being significantly lower in the former group [1751 (0.4–25,000) U/mL vs 4523 (259–25,000) U/mL, $p = 0.012$] (Fig. 3). Among 140 SARS-CoV-2 naïve patients, 135 (96%) were positive for anti-S after the second dose of vaccine compared to 22 (100%) healthy subjects ($p = 0.10$): the median anti-S titres were 999 (0.4–17,329) U/mL vs 1520 (259–13,500) U/mL, $p = 0.05$. Among patients with cirrhosis, anti-S titres were significantly lower in the SARS-CoV-2 naïve compared to those with previous SARS-CoV-2 infection [999 (0.4–17,329) U/mL vs 7500 (12.5–25,000) U/mL, ($p<0.001$)].

In SARS-CoV-2 naïve cirrhotics, independent baseline predictors of lower anti-S titres 21 days after the second dose of mRNA vac-

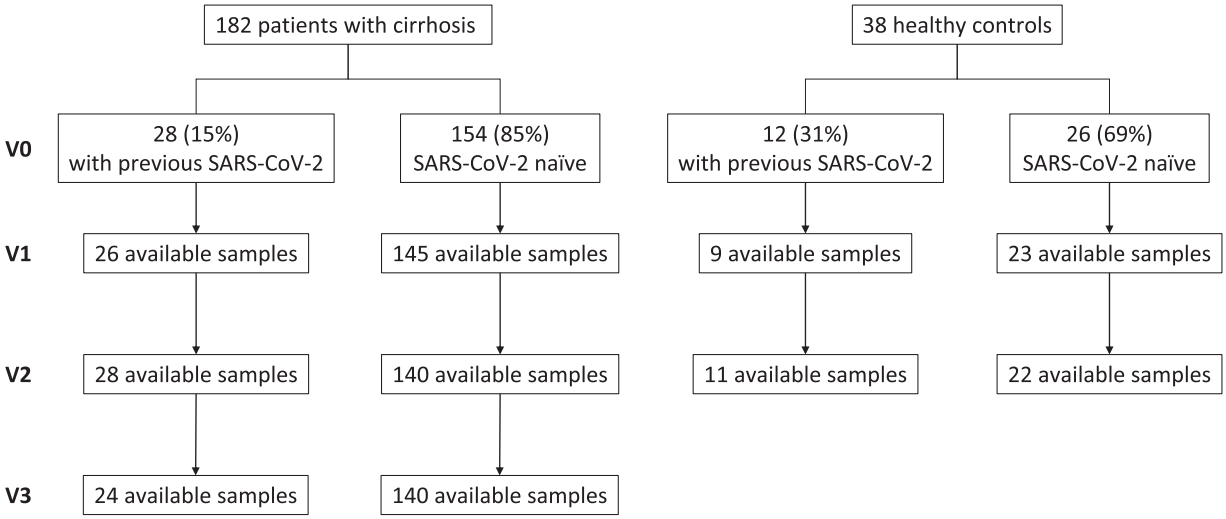

Fig. 2. Disposition of patients with cirrhosis and healthy controls over time and according to the previous SARS-CoV-2 exposure.

Table 1
Demographic and clinical characteristics of the 182 patients with cirrhosis and 38 healthy controls enrolled in the study.

Characteristics	Patients (n = 182)	Healthy controls (n = 38)
Age, years	61 (20–89)	56 (29–73)
Males	136 (75)	11 (29)
Ethnicity, Caucasian	168 (92)	38 (100)
Etiology of liver disease		
Viral*	82 (45)	
Alcohol	26 (14)	
Metabolic	14 (8)	
Multiple	36 (20)	
Other	24 (13)	
Albumin, g/dl	4.0 (2.5–4.0)	
Bilirubin, mg/dl	1.0 (0.2–12.0)	
INR	1.1 (0.9–2.0)	
Ascites	65 (36)	
Encephalopathy	25 (14)	
Platelet count/mm^3	119,000 (24,000–389,000)	
White blood cell count/mm^3	5175 (1100–14,510)	
Creatinine, mg/dl	0.9 (0.5–7.1)	
Child-Pugh score		
A (5–6)	135 (74)	
B (7–9)	30 (16)	
C (10–15)	17 (10)	
MELD score	8 (6–24)	
MELD score ≥15	14 (8)	
HCC	56 (31)	
previous	26 (46)	
active	30 (54)	
Esophagogastric Varices		
Absent	79 (44)	
Small	37 (20)	
Medium/Large	66 (36)	
Enlisted for liver transplantation	27 (15)	
BMI, kg/m^2	26 (16–43)	
Recent or current steroid therapy	9 (5)	
Comorbidity:		
Obesity	43 (24)	
Diabetes	39 (21)	
Arterial hypertension	80 (44)	
HIV infection	2 (1)	
Chronic kidney disease**	5 (3)	
Comorbidities ≥ 2***	45 (25)	
Previous SARS-CoV-2 exposure (%)	28 (16)	12 (31)
Vaccine type (%)		
mRNABNT162b2 (Comirnaty, Pfizer BioNTech)	121 (66)	29 (76)
mRNA-1273 (Spikevax, Moderna)	61 (34)	9 (24)

Values are reported as n (%) or median (range). INR, international normalized ratio; MELD, model for end-stage liver disease; HCC, hepatocellular carcinoma; BMI, body mass index; COVID-19, coronavirus disease 2019. *All patients but three with HCV achieved a sustained virological response and all patients with HBV were on effective nucleotide analogue therapy. **One patient in dialytic treatment. ***Comorbidities ≥2 were diabetes, obesity, arterial hypertension, chronic bronchopneumopathies; chronic kidney disease, HIV.

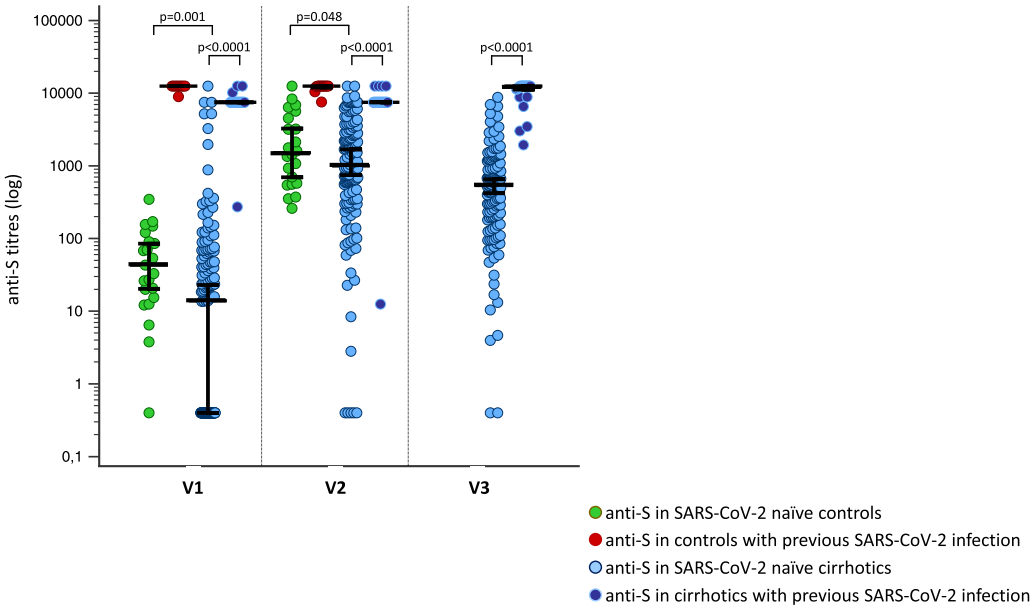

Fig. 3. Humoral response to SARS-CoV-2 vaccination at different time-points in patients with cirrhosis (V1, V2 and V3) and healthy controls (V1 and V2), according to previous SARS-CoV-2 infection. Statistical analysis by Kruskal-Wallis Test. Solid horizontal lines indicate medians and interquartile range.

Table 2
Univariate and multivariable interval regression models to predict lower response after two doses of COVID-19 mRNA vaccine in 140 SARS-CoV-2-naïve patients with cirrhosis and available V2 sample.

Variable	Categories	Univariable Model			Multivariable Model		
		anti-S titre difference (log scale)	95% CI	p-value	anti-S titre difference (log scale)	95% CI	p-value
Age, years	Continuous	−0.014	−0.035 to 0.007	0.191			
Gender	Female vs Male	0.000	−0.56 to 0.559	0.998			
Ethnicity	Other vs Caucasian	−0.492	−1.402 to 0.417	0.289			
Child-Pugh score	B/C vs A	−1.022	−1.53 to −0.514	<0.001	−0.480	−0.840 to −0.110	0.011
MELD score	Continuous	−0.082	−0.147 to −0.017	0.013			
Platelet count/mm^3	Continuous	0.004	0.001 to 0.007	0.004			
White blood cell, count/mm^3	Continuous	0.000	0.000 to 0.000	0.239			
Esophageal varices	Yes vs No	−0.529	−0.975 to −0.083	0.020			
Active HCC	Yes vs No	−0.917	−1.533 to −0.300	0.004	−0.480	−0.820 to −0.140	0.005
Immunocompromised conditions	Yes vs No	−0.937	−2.152 to 0.277	0.130			
Comorbidities ≥2	Yes vs No	−0.206	−0.703 to 0.291	0.416			
Vaccine type	mRNA-BNT162b2 vs mRNA-1273	−0.457	−0.963 to 0.049	0.076	−0.500	−0.840 to 0.15	<0.001

Interval regression analysis was used to take into account left- and right-censored anti-S titres due to limits of assay detection, in both univariable and multivariable models, the AIC criterion was used to select the best fitting multivariable model. Anti-S titre was log-transformed to achieve normality. MELD, model for end-stage liver disease; HCC, hepatocellular carcinoma; immunocompromised conditions were: active steroid or other immunosuppressive treatment for any reasons, lymphoproliferative disorders, chemotherapy for any reasons, HIV; Comorbidities ≥2 were: diabetes, obesity, arterial hypertension, chronic bronchopneumopathies; chronic kidney disease, HIV; mRNA-1273, Spikevax Moderna mRNA vaccine; mRNA-BNT162b2, Comirnaty Pfizer BioNTech mRNA vaccine.

cines (V2, Table 2) were Child-Pugh B/C, active HCC, and BNT162b2 vaccine. Based on this model, we compared anti-S titres of SARS-CoV-2 in naïve cirrhotics with either Child-Pugh B/C or active HCC with healthy naïve subjects: 632 (0.4–13,547) and 538 (0.4–5766) U/mL and vs 1520 (259–13,500) U/mL, $p<0.001$ and $p = 0.002$, respectively. While Child-Pugh A or HCC negative patients reached similar levels of anti-S Ab compared to healthy subjects at V2 [1377 (0.4–17,329) $p = 0.179$ and 1173 (0.4–17,329) $p = 0.178$, respectively].

When humoral responses after the first dose of vaccine (V1) were included in a time-related prediction model, independent predictors were active HCC, immunocompromised conditions, BNT162b2 vaccine and lower anti-S titres at V1 (Supplementary Table 1).

Another secondary endpoint was the kinetics of humoral response at different timepoints: the anti-S titres were significantly lower at the second (V3) compared to the first timepoint (V2): 657 (0.4–25,000) vs 1751 (0.4–25,000) U/mL, $p<0.001$ [median time elapsed from V2 to V3 111 (91–171) days]. In details, SARS-CoV-2 naïve patients with cirrhosis anti-S titres were significantly higher at V2 [999 (0.4–17,329) U/mL] compared to both V1 [13.9 (0.4–14,336) U/mL] and V3 [528 (0.4–8777)] ($p<0.001$ and $p<0.001$, respectively). In patients with previous SARS-CoV-2 infection, similar anti-S titres were observed in the three time-points. The trend

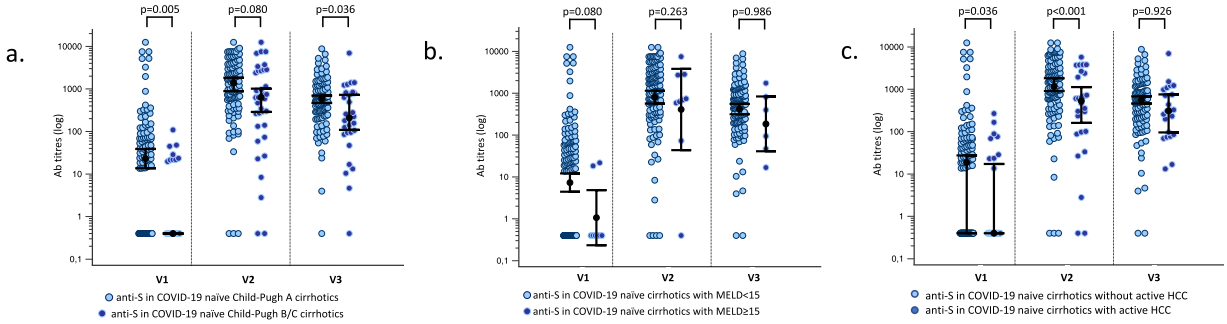

Fig. 4. Humoral response to SARS-CoV-2 vaccination at different time-points (V1, V2 and V3) in SARS-CoV-2-naïve cirrhotics, stratified according to different conditions: panel A: patients with Child-Pugh class A vs B/C [at V2: 1377 (0.4–17,329) vs 632 (0.4–13,547)]; panel B: patients with MELD<15 vs MELD≥15 [at V2: 1116 (0.4–17,329) vs 692 (0.4–7500)]; panel C: patients with active HCC vs negative/previous HCC [at V2: 538 (0.4–5766 vs 1377 (0.4–17,329)]. Statistical analysis by Kruskal-Wallis Test.

over time of antibodies titres for both patients and controls at different timepoints is presented in Fig. 3. The proportion of patients with anti-S titres above the threshold considered protective (i.e. ≥100 U/mL) was 29%, 89% and 87% at V1, V2 and V3 in the overall patients' population and 17%, 88% and 85%, among the SARS-CoV-2 naïve ones.

Considering both SARS-CoV-2 naïve and experienced patients, independent predictors of lower anti-S titres at V3 (median time 133 days from the second dose) were SARS-CoV-2 naïve condition and infection and BNT162b2 vaccine (Supplementary Table 2).

Among SARS-CoV-2 naïve patients, the response to vaccine at different time-points have been also reported in Fig. 4, according to Child-Pugh class, MELD score and presence of active HCC (Supplementary Table 3). The clinical characteristics of the 5 (3%) patients who tested negative for anti-S Ab after two doses of vaccine are presented in Supplementary Table 4.

Among the 20 patients with previous symptomatic SARS-CoV-2 infection, the median anti-S titres were 277 (19.4–3422) U/mL at V0, 7500 (273–25,000) U/mL at V1, 7500 (7500–25,000) U/mL at V2 and 13,227 (1936–25,000) U/mL at V3; among the 8 patients with previous asymptomatic SARS-CoV-2 infection, the median anti-S titres were: 311 (45.5–781) U/mL at V0, 7500 (7500–10,187) U/mL at V1, 7500 (12.5–7500) U/mL at V2 and 12,880 (3471–25,000) U/mL at V3.

3.3. Spike-specific T cellular response

The kinetics of Spike-specific T-cell immune responses induced by two doses of mRNA vaccines was assessed in a subset of 25 patients with cirrhosis: median age was 63 (48–81) years; 72% were males, 68% with viral etiology, 72% with Child-Pugh A cirrhosis and 12% had experienced a previous SARS-CoV-2 infection.

The ability of mRNA vaccination to induce Spike-specific T-cells in cirrhotic patients was compared with the one obtained in 29 matched healthy controls (Fig. 5a). Vaccination induced Spike-specific T-cell response in all cirrhotic patients, but the kinetics of expansion and contraction of Spike-specific cytokines production was different to what observed in healthy controls. First and second doses of vaccine induced a quantity of Spike peptide pool induced T-cell cytokines inferior to what detected in healthy individuals, although not statistically significant. While the level of IFN-gamma and IL-2 peptide induced cytokines in healthy controls was stable at day 60 and slightly decreased at V3, cirrhotic patients displayed a different kinetic of the T-cell response with delayed response with similar levels of IFN-gamma and IL-2 production at day 133. The three cirrhotic patients who were vaccinated after an episode of SARS-coV-2 infection, displayed a level of Spike-specific T-cell response that was higher than the one detected in cirrhotic naïve. Different kinetics according to previous infection and degree of liver decompensation are exemplified in Fig. 5B.

Among the 25 patients with available data for both SARS-CoV-2–specific T-cell -mediated and antibody responses elicited by mRNA vaccines, a statistically significant positive correlation between the anti-S titres and INF-gamma levels were found both at V2 (Spearman's rho, 0.510 $p = 0.0026$) and V3 (Spearman's rho, 0.435, $p = 0.0025$).

3.4. Post-vaccination infection rate

After a median follow-up of 4.8 (1.5–5.8) months since the first dose of vaccine, 4 cirrhotic patients turned anti-N positive (2%). One patient had a pauci-symptomatic COVID-19 after 2 doses of vaccine (fever and anosmia with PCR nasopharyngeal swab for SARS-CoV-2 positive) while the other 3 patients reported no symptoms related to infection. No patient was hospitalized for COVID-19. The anti-S titres at V2 (3.9 ± 0.6 months before anti-N positivity) were 8637, 7500 and 7500 U/mL in the three asymptomatic patients and 2665 U/mL in the pauci-symptomatic one, respectively.

3.5. Adverse events and clinical outcomes

No serious and unexpected adverse events were reported in patients with cirrhosis (Table 3). During the study period, 9 patients were liver transplanted (5 for end-stage liver disease and 4 for HCC): two patients with 1 dose only before transplant, while 7 patients were fully vaccinated with median time interval between second dose and transplant of 102 (3–202) days. Finally, 3 patients died (2 for end-stage liver disease and 1 for HCC progression): none with COVID-19-related symptoms.

4. Discussion

In this prospective study, we evaluated both humoral and T-cell spike-specific response at different time-points, incidence of breakthrough infections after vaccination and safety of COVID-19 mRNA vaccines in patients with compensated and decompensated cirrhosis with different aetiologies, compared to healthy subjects. We demonstrated that patients with cirrhosis had lower titres of anti-S compared to healthy controls and this was more evident in SARS-CoV-2 naïve patients with either a decompensated cirrhosis or an active HCC. Vaccination-induced spike-specific T-cell responses appeared slow but similar to healthy controls. Finally, in the 4 months following the second dose, there were a limited number of breakthrough infections; mRNA SARS-CoV-2 vaccines were well tolerated with no unexpected or severe adverse events.

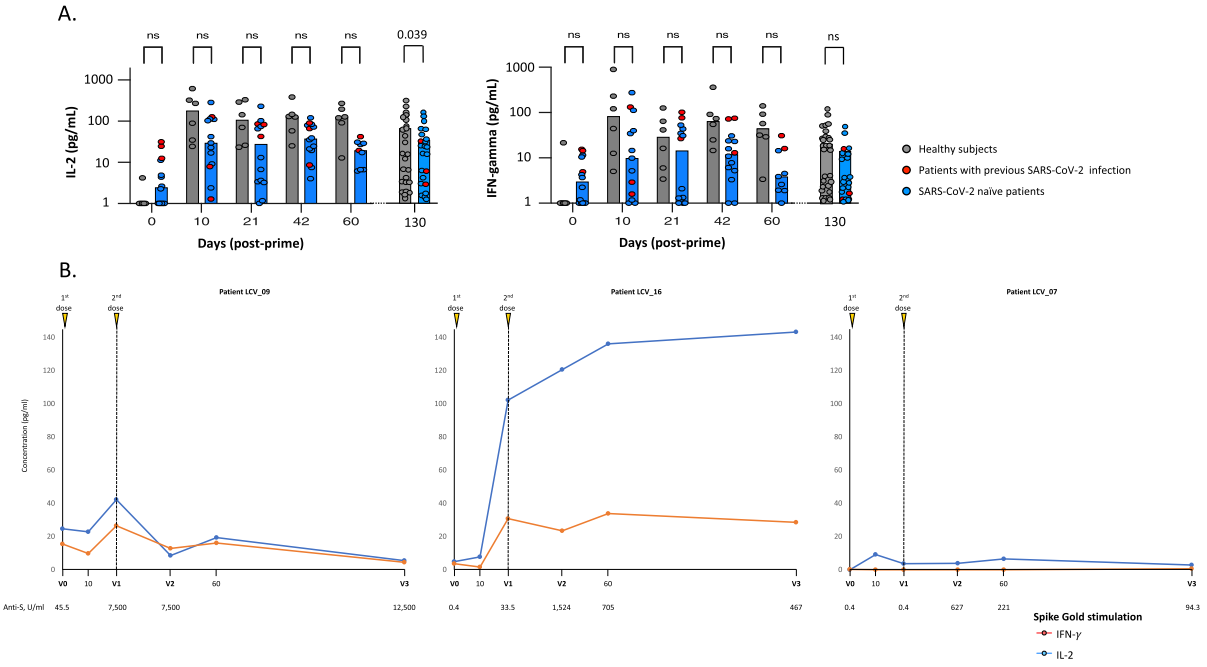

Fig. 5. A. Longitudinal analysis of SARS-CoV-2 Spike-specific T-cell response in 29 healthy individuals (gray dots) and 25 patients with cirrhosis (blue dots are SARS-CoV-2 naïve patients; red dots are patients with previous SARS-CoV-2 exposure), immunized with COVID-19 mRNA vaccines. The quantity of secreted IFN-γ and IL-2 after stimulation with peptide pool covering immunodominant region of Spike-protein at indicated time-points before and after second dose is indicated. Statistical analysis by one-way ANOVA. ns=not statistically significant. B. Longitudinal kinetics of SARS-CoV-2 Spike-specific T-cell response in 3 patients with different clinical characteristics: patient LCV_09 has a compensated cirrhosis and previous SARS-CoV-2 exposure; patient LCV_16 is a SARS-CoV-2-naïve with compensated cirrhosis; patient LCV_07 is a SARS-CoV-2-naïve with decompensated cirrhosis (Child-Pugh class C, MELD score 17). Reported are also the anti-S levels at different timepoints for each patient.

Table 3
Vaccine adverse events of the 182 patients with cirrhosis and 38 healthy subjects enrolled in the study.

Variables	First dose vaccine						Second dose vaccine					
	Patients with cirrhosis			Healthy controls			Patients with cirrhosis			Healthy controls		
	All (N = 182)	SARS-CoV-2 naïve (N = 154)	SARS-CoV-2 experienced (N = 28)	All (N = 38)	SARS-CoV-2 naïve (N = 25)	SARS-CoV-2 experienced (N = 13)	All (N = 180)	SARS-CoV-2 naïve (N = 154)	SARS-CoV-2 experienced (N = 26)	All (N = 38)	SARS-CoV-2 naïve (N = 25)	SARS-CoV-2 experienced (N = 13)
Subjects with side effects	70 (38)	55 (36)	15 (54)	14 (37)	8 (32)	6 (46)	80 (44)	66 (43)	14 (54)	17 (45)	10 (40)	6 (54)
Side effects	113	86	27	34	20	14	136	109	27	50	31	21
Side effects severity*												
- G1	108	83	25	33	20	13	126	101	25	48	29	19
- G2	5	3	2	1	0	1	10	8	2	4	2	2
- G3/4	0	0	0	0	0	0	0	0	0	0	0	0
Side effects type												
-Vaccine site pain	57	48	9	15	9	6	48	43	5	15	9	6
-Vaccine site redness	7	7	0	0	0	0	6	6	0	2	1	1
-Vaccine site swell	7	6	1	2	1	1	4	3	1	2	1	1
-Fever	9	2	7	6	4	2	31	20	11	12	8	4
-Headache	3	1	2	1	1	0	9	8	1	1	1	0
-Nausea	5	2	3	1	0	1	6	3	3	1	0	1
-Myalgia	6	4	2	4	2	2	6	4	2	6	4	2
-Arthralgia	5	3	2	1	1	0	8	7	1	5	3	2
-Anaphylaxis	0	0	0	0	0	0	0	0	0	0	0	0
-Other	14	13	1	3	2	1	18	15	3	6	4	2

Values are reported as N (%); *severity of side effects graded according to the Common Terminology Criteria for Adverse Events (CTCAE) grade scale.

The current knowledge on the long-term humoral response to vaccination in patients with cirrhosis is hampered by the fact that previous studies analysed only a single time-point after a full cycle of vaccination [13,14,18]. In contrast, in our study, we were able to assess the kinetics of humoral response over time, i.e. at three different time-points up to 133 days, comparing these findings in healthy controls and patients with different liver disease severity. We observed a weaker response in SARS-CoV-2-naïve patients after the first dose as compared to both controls in the same conditions and patients with a previous SARS-CoV-2 exposure. Moreover, a waning antibody's response was observed in SARS-CoV-2-naïve patients with cirrhosis, with a statistically significant decrease from the V2 to the V3 values, while patients with previous SARS-CoV-2 infection maintained high anti-S titres throughout the study pe-

riod, which could not be described in previous studies, while similar results have been recently shown in liver transplanted patients [13,14,18,19].

Since previous studies had either excluded patients with a known history of COVID-19 or did not screen patients for anti-N at baseline, our study is the first describing response to anti-COVID-19 vaccination in both patients and healthy controls with and without a previous SARS-CoV-2 exposure, without relying on symptoms but on anti-N positivity to rule out previous asymptomatic exposure. We have shown that, in patients with previous SARS-CoV-2 infection, titres of anti-S were comparable to those observed in healthy individuals, thus confirming also in cirrhotics that the "hybrid immunity" (infection and vaccination) grants for a rapid and durable protection against SARS-CoV-2. Ruether et al. tested 2 cirrhotic patients with a past symptomatic COVID-19, showing very high anti-S titres following the two doses of COVID-19 mRNA vaccine, similar to those obtained in healthy controls [18]. These data might also suggest an enhanced immunological response even in cirrhotics following booster vaccination [20,21].

Among SARS-CoV-2-naïve patients with cirrhosis, who had in general a lower humoral response to vaccine compared to healthy individuals, those staged Child-Pugh B/C, with active HCC or immunization with mRNA BNT162b2 had lower anti-S titres after two doses of COVID-19 vaccine, according to the multivariable analysis with baseline variables only. In a second model which include also anti-S titres at V1 in a time-dependent mode, immunocompromised conditions and lower anti-S titres following the first dose emerged as other independent predictors of lower anti-S titres at V2. Previous studies excluded any statistically significant difference in terms of humoral (and T-cell response) in patients with compensated cirrhosis compared to those with a more advanced disease [13,14,18]. Thuluvath reported an "adequate response" 77% of 79 patients with cirrhosis, independently of disease severity [13]. Ai described similar humoral response in 11 Child-Pugh B/C compared to 142 Child-Pugh A patients following inactivated whole-virion vaccines [14]. Evaluations of the differences in immunogenicity between the mRNA vaccines are limited, however our results are in line with previous studies in general population reporting that SARS-CoV-2 anti-spike levels were significantly higher with mRNA-1273 than with BNT162b2 at 6–10 weeks after 2-dose vaccination [22]. Long-term response was independently predicted by previous SARS-CoV-2 infection and immunization by mRNA-1273 vaccine.

As a unique feature of our study in patients with cirrhosis, T-cell Spike-specific response was evaluated in a subgroup of patients and controls. Vaccination induced Spike-specific T-cell responses in all cirrhotic patients, but the kinetic of expansion and contraction was different to the one detected in healthy controls. Despite the level of Spike-specific T-cell response before vaccination was superior in cirrhotic than healthy individuals (likely due to the presence of T-cells cross-reactive to other Coronaviruses) [23,24], first and second dose vaccination induce a quantity of Spike specific T-cell response inferior to what detected in healthy individuals. While Spike-specific T-cell response in healthy controls was stable at day 60 and slightly reduced at day 133, cirrhotic patients displayed a different kinetic of the T-cell response with slower growth reaching similar IFN-gamma and IL-2 production at day 133. These data, generated in a small proportion of patients with a few with decompensated cirrhosis, should be confirmed in larger cohort of patient. Of note, in the three SARS-CoV-2 experienced cirrhotics the level of Spike-specific T-cell response was superior to the one detected in naïve patients, showing that also in cirrhosis heterologous immunity elicits a superior level of antiviral immunity than vaccination alone [20,21].

At the time of reporting, the proportion of patients who seroconverted to anti-N was quite low, only 2%. Moreover, these few patients remained fully asymptomatic or developed mild symptoms. The only and largest study on breakthrough infection in patients with cirrhosis after anti-COVID-19 vaccination reported a reduced risk of death by multivariable analysis of a 1:2 propensity matched US cohort including vaccinated and unvaccinated patients [25]. However, in this retrospective study patients were not tested for humoral response to vaccines, thus limiting the comparability to our study.

Safety remains an important endpoint of any study in which patients with severe liver disease are enrolled: no serious safety signals were reported in our patients and hence we suggest continuing vaccination of our patients, even with the third dose. Similar data has been previously shown in patients chronic liver disease with inactivated whole-virion SARS-CoV-2 vaccination [14].

We acknowledge that the current study has some limitations. First, these are preliminary results with a follow-up limited to 4 months. Larger multicenter studies are deemed necessary to define the added value of the third dose of vaccine, as currently recommended in Italy and worldwide. Secondly, the number of patients enrolled in the study, and those analyzed for T-cell responses is limited and data on long-term cellular response is lacking, thus deserving further and larger studies to confirm our findings. Third, we do not have long term follow-up data on antibody titers for healthy controls. The fact that this first data-analysis was conducted both before the fourth wave of pandemic and before the third dose of anti-SARS-CoV-2 vaccine, favored a simplified and clearer analysis of the results which otherwise would be made extremely heterogeneous by the timing of the third dose together with any silent or paucisymptomatic infections, making the results more difficult to interpret. Moreover, this is the first study including both patients and controls, SARS-CoV-2-naïve and experienced subjects, and multiple samples collection to study both humoral and cellular responses, thus giving the most comprehensive view to date published in vaccinated patients with cirrhosis. Finally, the number of patients with breakthrough infection might be underestimated in other studies because asymptomatic patients might be less likely to report to medical attention and undergo testing. In our study we were able to detect all seroconversion independently on symptoms of COVID-19, thanks to the design of the study itself which provided for the evaluation of anti-N at pre-established time-points.

In conclusion, this study demonstrates that COVID-19 mRNA vaccination is safe in patients with cirrhosis but elicits weaker humoral and slower cellular responses compared to healthy subjects, particularly in SARS-CoV-2-naïve cirrhotics with decompensated cirrhosis and active HCC. These patients should be counselled to follow self-protective behavior to minimize the risk of infection, since post-vaccination COVID-19 in patients with cirrhosis maintains a residual risk of serious outcomes [25].

Conflict of interest

MI: Speaking/Teaching, consultant and advisory board for Bayer, Gilead Sciences, BMS, Janssen, Ipsen, MSD, BTG-Boston Scientific, AbbVie, Guerbet, EISAI, Roche.

BA: A patent for a method to monitor SARS-CoV-2-specific T-cells in biological samples pending.

PL: Advisory Board/Speaker Bureau for BMS, ROCHE, GILEAD SCIENCES, GSK, ABBVIE, MSD, ARROWHEAD, ALNYLAM, JANSSEN, SBRING BANK, MYR, EIGER, ALIGOS, ANTIOS, VIR.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2022.09.010.

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