Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Impaired SARS-CoV-2-specific T cell reactivity in patients with cirrhosis following mRNA COVID-19 vaccination

Samer Al-Dury, Johan Waern, Jesper Waldenström, Marko Alavanja, Hevar Hamah Saed, Andreas Törnell, Mohammad Arabpour, Hanna Grauers Wiktorin, Sigrun Einarsdottir, Johan Ringlander, Gisela Ringström, Kristoffer Hellstrand, Anna Martner, Martin Lagging

PII: S2589-5559(22)00068-4
DOI: https://doi.org/10.1016/j.jhepr.2022.100496
Reference: JHEPR 100496

To appear in: JHEP Reports

Received Date: 11 January 2022
Revised Date: 15 March 2022
Accepted Date: 13 April 2022

Please cite this article as: Al-Dury S, Waern J, Waldenström J, Alavanja M, Saed HH, Törnell A, Arabpour M, Wiktorin HG, Einarsdottir S, Ringlander J, Ringström G, Hellstrand K, Martner A, Lagging M, Impaired SARS-CoV-2-specific T cell reactivity in patients with cirrhosis following mRNA COVID-19 vaccination, JHEP Reports (2022), doi: https://doi.org/10.1016/j.jhepr.2022.100496.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 The Author(s). Published by Elsevier B.V. on behalf of European Association for the Study of the Liver (EASL).
Cirrhosis

2 doses mRNA COVID-19 vaccine

Healthy
Impaired SARS-CoV-2-specific T cell reactivity in patients with cirrhosis following mRNA COVID-19 vaccination

Samer Al-Dury†, Johan Waern†, Jesper Waldenström, Marko Alavanja, Hevar Hamah Saed, Andreas Törnell, Mohammad Arabpour, Hanna Grauers Wiktorin, Sigrun Einarsdottir, Johan Ringlander, Gisela Ringström, Kristoffer Hellstrand, Anna Martner, and Martin Lagging*

†Department of Medicine, Gastroenterology and Hepatology Unit, Sahlgrenska University Hospital, Gothenburg, Sweden, ‡Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, TIMM Laboratory, Sahlgrenska Center for Cancer Research, Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, Department of Hematology and Coagulation, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden, Region Västra Götaland, Sahlgrenska University Hospital, Department of Clinical Microbiology, Gothenburg, Sweden

†Contributed equally

*Correspondence to:
Martin Lagging
Department of Infectious Diseases/Virology
Sahlgrenska University Hospital and University of Gothenburg
413 45 Gothenburg, Sweden
martin.lagging@gu.se
Key Words: Cirrhosis, SARS-CoV-2, COVID-19, vaccination, T cells response, antibody response

Word count: 2343

Number of figures: 2

Number of tables: 4

Conflict of interest statement: The authors have nothing to disclose.

Financial disclosure: This work was supported by the Swedish Medical Research Council (Vetenskapsrådet; grant no. 2021-04779 and 2020-01437), ALF Funds at Sahlgrenska University Hospital (ALFGBG-438371), the Swedish Society of Medicine (SLS-961779 and SLS-961159) and the Gothenburg Society of Medicine (GLS-961772).

Author Contributions: SAD, JWae and ML were responsible for designing and writing the protocol, conducting the study, extracting, and analysing data, interpreting results, writing the manuscript, updating reference lists, and creating the table and figure. JW, AM and KH were responsible for designing and writing the protocol, extracting, and analysing data, interpreting results, writing the manuscript, updating reference lists, and creating the table and figure. MA, HHS, SE, JR, and GR participated in interpreting results and writing the manuscript. AT, MA, and HGW were responsible for performed the T cell as well as participated in extracting and analysing data and interpreting results.

Clinical trial registration: EudraCT 2021-000349-42
Abstract

**Background & Aims:** Liver cirrhosis entails elevated risk of COVID-19-associated mortality. This study determined T cell-mediated and antibody reactivity against the spike 1 (S1) protein of SARS-CoV-2 among 48 cirrhotic patients and 39 healthy controls after mRNA COVID-19 vaccination.

**Methods:** SARS-CoV-2-specific T cell reactivity was measured by induced level of T cell-derived interferon-γ (IFN-γ) in blood cells stimulated *ex vivo* with multimeric peptides spanning the N-terminal portion of S1. S1-induced IFN-γ was quantified before and after the 1st and 2nd vaccination (BNT162b2, Pfizer-BioNTech or mRNA-1273, Moderna) alongside serum IgG against the receptor-binding domain (RBD) within S1 (anti-RBD-S1 IgG).

**Results:** T cell reactivity against S1 was reduced in cirrhotic patients after the 1st (P<0.001 vs controls) and 2nd (P<0.001) vaccination. Sixty-eight % of patients lacked detectable S1-specific T cell reactivity after the 1st vaccination vs. 19% in controls (OR 0.11, HR 0.03-0.48, P=0.003) and 36% remained devoid of reactivity after the 2nd vaccination vs. 6% in controls (OR 0.12, HR 0.03-0.59, P=0.009). T cell reactivity in cirrhosis remained significantly impaired after correction for potential confounders in multivariable analysis. Advanced cirrhosis (Child-Pugh class B) was associated with absent or lower T cell responses (P<0.05 vs. Child-Pugh class A). The deficiency of T cell reactivity was paralleled by lower levels of anti-RBD-S1 IgG after the 1st (P<0.001 vs. controls) and 2nd (P<0.05) vaccination.

**Conclusions:** Cirrhotic patients show deficient T cell reactivity against SARS-CoV-2 antigens along with diminished levels of anti-RBD-S1 IgG after dual COVID-19 vaccination, highlighting the need for vigilance and additional preventative measures.
Lay summary: T cells are a pivotal component in the defence against viruses. We show that patients with liver cirrhosis have impaired SARS-CoV-2-specific T cell responses and lower antibody levels after mRNA vaccination against COVID-19 compared with healthy controls. Cirrhotic patients with more advanced liver disease exhibited particularly inferior vaccine responses. These results call for additional preventive measures in these patients.

Highlights:

- After COVID vaccination, cirrhotics had impaired T cell and antibody responses.
- Child-Pugh class B cirrhosis was associated with poorer immune responses than class A.
- Multivariate analyses excluded potential confounding variables.
Introduction

Regardless of aetiology, end-stage liver disease is characterized by impaired immunity. Cirrhosis-associated immune dysfunction (CAID) is believed to arise secondary to injury of hepatic reticuloendothelial cells, reduced hepatic production of proteins crucial for innate immunity [1] along with systemic inflammation [2] and translates into a perturbing propensity for severe and life-threatening infections. Although CAID is mostly associated with flawed innate responses [3-5], recent studies report that subsets of T cells in cirrhotic patients express markers of exhaustion, as reflected by expression of TIM-3, CTLA-4, and PD-1, suggesting that T cell deficiency may contribute to the observed susceptibility to infection [6, 7].

SARS-CoV-2-infected patients with cirrhosis are at elevated risk of decompensation, severe morbidity, and death [8, 9]. Thus far scarce data regarding the immunogenicity of COVID-19 vaccines have been reported in these patients. Forty days after immunization with 1 dose of viral vector (Johnson & Johnson) or 2 doses of mRNA (Pfizer-BioNTech or Moderna) COVID-19 vaccines, Thuluvath et al. reported that 19% of cirrhotic patients had suboptimal antibody levels [10]. Similarly, after 2 doses of viral vector (AstraZeneca) or mRNA (Pfizer-BioNTech or Moderna) vaccines, Ruether et al. detected T cell responses using a cytokine release assay in 17/26 (65%) of vaccinated patients as compared with 19/19 (100%) of healthy controls. In the latter study anti-RBD-S1 IgG levels were similar among cirrhotic patients and controls [11].

We aimed at evaluating the immunogenicity of mRNA-based COVID-19 vaccines in cirrhotic patients by concurrent quantification of T cell reactivity and anti-S1-RBD IgG.
Our results unravel a profound deficiency of T cell responsiveness against SARS-CoV-2 antigens in cirrhosis paralleled by impaired humoral immunity.
Patients and Methods

Study population and design

This prospective cohort study was conducted between April and October 2021 at Sahlgrenska University Hospital, Gothenburg, Sweden. Forty-eight subjects with cirrhosis of various aetiologies were enrolled among patients attending the outpatient clinic at the Department of Gastroenterology and Hepatology at this hospital (Supplemental Figure S1). Patients were diagnosed and examined by a specialist in clinical hepatology. Thirty-nine healthy controls were recruited among healthcare personnel at the Sahlgrenska University hospital as well as their family and friends. The baseline characteristics of patients and controls are detailed in Table 1. Patients or controls with PCR-verified COVID-19 at screening or presence of antibodies against SARS-CoV-2 at initial sampling were not included.

The participants received two doses of intramuscular mRNA vaccine (BNT162b2, Comirnaty, Pfizer-BioNTech or mRNA-1273, Spikevax, Moderna), at a median 36 (range 26 – 62) day interval. Peripheral blood was collected at baseline, i.e., 0-10 days before the 1st vaccination, as well as after the 1st (median 35 days (IQR 25-40 days)) and 2nd (median 89 days (IQR 67-96)) vaccine dose. Serum levels of anti-RBD-S1 IgG and the magnitude of T cell-derived IFN-γ production in response to multimeric S1 peptides after vaccination were predefined primary study endpoints.
Adverse events
Participants completed a questionnaire regarding adverse events after the 2nd vaccine dose. Adverse events were categorized per the CTCAE (Common Terminology Criteria for Adverse Events) standards.

Serology
Chemiluminescent microparticle immunoassays (CMIA)s were performed on serum using the automated Alinity system for the quantitative measurement of IgG antibodies against the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 (SARS-CoV-2 IgG II Quant, Abbott, Abbott Park, Illinois, USA) with levels reported in the WHO international standard Binding Antibody Units (BAU) / ml [12] (quantitative detection range of 14 to 5680 BAU/ml; samples reaching 5680 BAU/ml were diluted with seronegative serum and reanalysed allowing for an upper detection limit of >5680 BAU/ml).

SARS-CoV-2-specific T cell reactivity in blood
Vacutainer lithium-heparin tubes (BD, Plymouth, UK) were used to collect peripheral whole blood for assessment of SARS-CoV-2-specific T cell reactivity. One ml of whole blood was transferred to 10 ml-tubes (Sarstedt) and stimulated or not with 1 µg/ml/peptide of 15-mer peptides with 11-amino acid overlap spanning the N-terminal S1 domain of the SARS-CoV-2 S1 (130-127-041, Miltenyi Biotec). After two days of incubation at 37°C and 5% CO2, the tubes were centrifuged for 5 minutes at 1,500 rpm and plasma recovered. Plasma was stored at -80°C until analysis of released IFN-γ.
The 15-mer peptides used as stimuli in this assay can be presented on MHC class I and II to activate spike-specific CD8⁺ T cells and CD4⁺ T cells, respectively.

**IFN-γ ELISA**

Plasma collected from blood samples with or without S1 peptide stimulation was assessed for IFN-γ content by ELISA (DY285B, R&D systems) according to the manufacturer’s instructions. To minimize nonspecific reactivity, plasma was diluted (1:2) in PBS containing 1% BSA and 10% mouse serum (Invitrogen). Plates were analysed for optical densities at 450 nm and 570 nm using a FLUOstar Omega plate reader (BMG, Ortenberg, Germany). Levels of IFN-γ induced in response to S1 peptides, with background IFN-γ production in unstimulated samples subtracted, are presented throughout the manuscript. The limit of detection (LOD) of the assay was 10 pg/ml as reported elsewhere [13], and thus this threshold was used in the study.

**ARFI elastography**

Liver cirrhosis was confirmed at baseline using acoustic radiation force impulse (ARFI) measurement by the ultrasound system Acuson S2000 (Siemens Medical Solutions, Erlangen, Germany). Values were documented in median and interquartile range to median ratio (IQR: median).

**Ethical considerations**

All participants gave written informed consent before enrolment. This study was part of the DurIRVac study approved by the Swedish Ethical Review Authority (permit nos. 2021-00539) and by the Swedish Medical Products Agency (Dnr: 5.1-2021-11118).
The trial is registered at the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT no. 2021-000349-42).

**Statistical analysis**

Continuous variables were described as mean, median, and range of values, as applicable. Categorical data were described with contingency tables including frequency and percent. Mann-Whitney U-test was applied to calculate differences in serologic/cellular response between groups. The association between various continuous parameters was determined using Spearman’s correlation. Logistic regression was used to calculate the impact of various parameters on cellular and serological immune responses. Parameters with univariate P-values below 0.1 were included in multivariate analysis, and the magnitude of response presented as odds ratios with 95% confidence intervals. For some figures, the data were log-transformed, as indicated in the figure text. Values of BAU/ml and pg/ml below the limit of detection (LOD) were set to 50% of LOD. Data analyses were performed using SPSS for MacOS and GraphPad Prism 8 for macOS. Statistical significance was set to P<0.05. P-values are designated as follows: *P<0.05, **P<0.01, and ***P<0.001. All indicated P-values are two-sided.
Results

S1-specific T cell responses after COVID-19 vaccination

To determine the reactivity of SARS-CoV-2-specific T cells in cirrhotic patients after COVID-19 vaccination, blood samples collected after the 1st and 2nd vaccine doses were stimulated with multimeric peptides spanning the S1-region of spike 1 followed by analysis of induced levels of T cell-derived IFN-γ. This assay was previously shown to reflect presence of CD4+ and CD8+ T cells with specificity for S1-antigens [13]. The induction of IFN-γ in response to SARS-CoV-2 S1 peptides was impaired in cirrhotic patients after the 1st (median <10 vs. 79 pg/ml in controls, P<0.001) and 2nd vaccination (median 63 vs. 243 pg/ml, P<0.001) (Figure 1A). Similarly, the proportion of cirrhotic patients with IFN-γ levels below the level of detection (10 pg/ml) [13] was higher after the 1st (68 % vs. 19%, P<0.01 vs. controls) and 2nd vaccination (36% vs. 6%, P<0.01; Figure 1C).

Serological responses after vaccination

Similar to the impaired T cell response, anti-RBD-S1 IgG levels were lower in patients with cirrhosis as compared with healthy controls after the 1st (median 31 vs. 151 BAU/ml, P<0.001) and 2nd (median 514 vs. 1044 BAU/ml, P<0.05) vaccinations (Figure 1B). Additionally, after the 1st vaccination a higher proportion of patients with cirrhosis (35%) lacked detectable levels of anti-RBD-S1 compared with controls (6%) (P<0.05; Figure 1D). The characteristics of participants achieving or not achieving detectable cellular immune responses (≥10 pg/ml) and >100 BAU/ml of anti-RBD-S1 IgG after two vaccine doses are detailed in Table 2.
Impact of Child-Pugh class and concurrent therapy on immune reactivity after vaccination

The Child-Pugh classification (A-C) determines the severity and prognosis of cirrhosis where patients with class A have less pronounced liver disease and more favourable prospects of long-term survival [14, 15]. Levels of S1-induced IFN-γ were lower in patients with Child-Pugh class B compared with class A after the 1st (P<0.05) and 2nd (P<0.01) vaccination (Figure 2A). Similarly, anti-RBD-S1 IgG levels were lower in patients with Child-Pugh class B cirrhosis after the 1st vaccination (P<0.05 vs. class A; Figure 2B). The sample size of patients with Child-Pugh class C (n=2) was insufficient for analysis. No differences were observed regarding T or B cell responses among patients with or without ongoing immunosuppressive therapy or with intercurrent disease (Table 2). The aetiology of cirrhosis was diverse and multifactorial, but insufficient sample size prevented meaningful subgroup analyses.

Multivariable analysis

Logistic regression was performed to determine the impact of potential confounders on the observed differences of vaccine responses. The T cell-derived S1-induced IFN-γ levels were dichotomized based on above or below 10 pg/ml, which reportedly discriminates infected and uninfected subjects with >95% specificity and sensitivity [13]. Anti-RBD S1-IgG levels were dichotomized above or below 100 BAU/ml [16]. The S1-induced IFN-γ response remained significantly inferior in cirrhotic patients vs. controls after the 1st and 2nd vaccination when taking gender, age, vaccine type, intercurrent disease, immunosuppressive therapy, and time from vaccination to sampling into account (Table 3). Similarly, the antibody response after the 1st
vaccination remained significantly reduced in cirrhotic patients in multivariable analysis (Table 4).

**Documented COVID-19 during the study**
There were no reported cases of COVID-19 among the participants during the study period (April-October 2021).

**Tolerability and safety**
The most commonly reported adverse events were reaction at the injection site (64%) and fatigue (22%). The frequency or severity of adverse events did not differ between patients and controls and no serious adverse events were reported or recorded.
Discussion

The main finding of this study was that patients with liver cirrhosis show significantly abated antigen-specific T cell responses after COVID-19 vaccination. We thus observed that 68% of cirrhotic patients lacked T cell reactivity against S1 antigens after the 1st vaccination and that 36% remained non-reactive after the 2nd vaccination. These results confirm and extend those reported by Ruether et al. evaluating spike-specific T cell responses after COVID-19 vaccination [17]. Multivariable analyses showed that the observed T cell deficiency was independent of potential confounders, including intercurrent disease or immunosuppressive therapy. However, the limited sample size may have impacted these analyses. We also observed that T cell dysfunction was significantly more pronounced in Child-Pugh class B cirrhosis than in class A. Thus 9/9 of evaluable patients with class B cirrhosis were completely devoid of T cell reactivity against S1 antigens after the 1st vaccination, and 8/12 of class B cirrhosis patients (67%) remained non-reactive after the 2nd vaccination. Overall, our findings establish that T cells of cirrhotic patients respond poorly to SARS-CoV-2 antigens and that the degree of T cell deficiency is proportional to the severity of liver dysfunction. The serological findings are coherent with those reported by Thuluvath et al. [10] to support diminished humoral responses after COVID-19 vaccination in cirrhotic patients. However, this difference in antibody responses was less pronounced after the second vaccine dose, which may account for the lack of significance noted in some studies [11, 18].

The immune dysfunction in cirrhosis is primarily associated with flawed innate responses leading to risk of severe and potentially life-threatening bacterial infections
[3-5]. However, also T cell defects including impaired cytokine production elicited by broad T cell stimulation of blood samples from cirrhotic patients have been reported [6, 7]. These findings support that the here reported T cell deficiency against SARS-CoV-2 antigens may reflect a generic incapacity to mount T cell-mediated responses to infectious agents in cirrhotic patients.

Our findings have additional clinical implications including the observation that cirrhotic patients were largely unprotected after one dose of mRNA vaccine although a catch-up effect regarding antibody responses was noted after the second dose. This is important when considering the waning of immune responses over time after COVID-19 vaccination [19, 20]. In USA and many European countries, a third dose of the vaccine has been administered to most patients with chronic liver disease. In Sweden only patients with decompensated cirrhosis thus far have been prioritized, whereas patients with compensated cirrhosis have not been considered a vulnerable population. Our results suggest that additional vaccination be recommended to cirrhotic patients regardless of decompensated liver disease.

This study has strengths and limitations. The strengths include the diverse immunological methods and sampling after each vaccine dose along with the possibility of measuring aspects of immunity against SARS-CoV-2 among patients with variable severity of cirrhosis. The limitations embrace a small sample size and that samples from all patients were not always available for each time-point of analysis, e.g., the sample size may have been insufficient to rule out possible associations between vaccine responses and vaccine manufacturer (Pfizer vs. Moderna) or immunosuppressive therapy. It should be noted that increasing time
between the second vaccine dose and subsequent sampling had a weak, albeit
significant impact on both diminished humoral and cellular immune responses in
univariate but not in multivariate analyses as demonstrated in Tables 3 and 4, likely
reflecting waning immunity. Further studies are required to clarify if the observed T cell
impairment is generic to cirrhosis or to distinct aetiologies of this disease and also if T
cell deficiency impacts on the susceptibility to SARS-CoV-2 infection or on the severity
of COVID-19. Also, to define an insufficient humoral response, we used a cut-off anti-
RBD-S1 IgG level of 100 BAU/mL, which was previously utilized as a pre-specified
marker of poor response in a human therapeutic SARS-CoV-2 vaccine trial [16] where
antibody levels were reported in U/mL equivalent to BAU/mL with no conversion
required [21]. However, this threshold remains to be prospectively validated as a
correlate of protection against severe COVID-19 or across variants of SARS-CoV-2.

In conclusion, liver cirrhosis entails diminished humoral and in particular T cell-
mediated responses to dual COVID-19 vaccination. Our findings highlight the need for
continued vigilance and pre-emptive measures in this vulnerable population.

Acknowledgment

We thank all participants and the nursing staff at the Gastroenterology/Hepatology
Unit, Sahlgrenska University Hospital.

Data Availability Statement: For original data, please contact
martin.lagging@medfak.gu.se. As per Swedish law, individual participant data will not
be shared.
References

[1] Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. J Hepatol 2014;61:1385-1396.

[2] Albillos A, Martin-Mateos R, Van der Merwe S, Wiest R, Jalan R, Alvarez-Mon M. Cirrhosis-associated immune dysfunction. Nat Rev Gastroenterol Hepatol 2021.

[3] Irvine KM, Ratnasekera I, Powell EE, Hume DA. Causes and Consequences of Innate Immune Dysfunction in Cirrhosis. Front Immunol 2019;10:293.

[4] Tuchendler E, Tuchendler PK, Madej G. Immunodeficiency caused by cirrhosis. Clin Exp Hepatol 2018;4:158-164.

[5] Noor MT, Manoria P. Immune Dysfunction in Cirrhosis. J Clin Transl Hepatol 2017;5:50-58.

[6] Lebosse F, Gudd C, Tunc E, Singanayagam A, Nathwani R, Triantafyllou E, et al. CD8(+)T cells from patients with cirrhosis display a phenotype that may contribute to cirrhosis-associated immune dysfunction. EBioMedicine 2019;49:258-268.

[7] Rueschenbaum S, Ciesek S, Queck A, Widera M, Schwarzkopf K, Brune B, et al. Dysregulated Adaptive Immunity Is an Early Event in Liver Cirrhosis Preceding Acute-on-Chronic Liver Failure. Front Immunol 2020;11:534731.

[8] Choudhary NS, Dhampalwar S, Saraf N, Soin AS. Outcomes of COVID-19 in Patients with Cirrhosis or Liver Transplantation. J Clin Exp Hepatol 2021;11:713-719.

[9] Bajaj JS, Garcia-Tsao G, Biggins SW, Kamath PS, Wong F, McGeorge S, et al. Comparison of mortality risk in patients with cirrhosis and COVID-19 compared with patients with cirrhosis alone and COVID-19 alone: multicentre matched cohort. Gut 2021;70:531.
[10] Thuluvath PJ, Robarts P, Chauhan M. Analysis of antibody responses after COVID-19 vaccination in liver transplant recipients and those with chronic liver diseases. J Hepatol 2021;75:1434-1439.

[11] Ruether DF, Schaub GM, Duengelhoef PM, Haag F, Brehm TT, Fathi A, et al. SARS-CoV2-specific Humoral and T-cell Immune Response After Second Vaccination in Liver Cirrhosis and Transplant Patients. Clin Gastroenterol Hepatol 2022;20:162-172 e169.

[12] Kristiansen PA, Page M, Bernasconi V, Mattiuzzo G, Dull P, Makar K, et al. WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Lancet 2021;397:1347-1348.

[13] Törnell A, Grauers Wiktorin H, Ringlander J, Arabpour M, Nilsson MR, Nilsson S, et al. Rapid cytokine release assay for analysis of SARS-CoV-2-specific T cells in whole blood. J Inf Dis 2022;In press.

[14] Durand F, Valla D. Assessment of the prognosis of cirrhosis: Child-Pugh versus MELD. J Hepatol 2005;42 Suppl:S100-107.

[15] Albers I, Hartmann H, Bircher J, Creutzfeldt W. Superiority of the Child-Pugh classification to quantitative liver function tests for assessing prognosis of liver cirrhosis. Scand J Gastroenterol 1989;24:269-276.

[16] Hall VG, Ferreira VH, Ku T, Ierullo M, Majchrzak-Kita B, Chaparro C, et al. Randomized Trial of a Third Dose of mRNA-1273 Vaccine in Transplant Recipients. N Engl J Med 2021;385:1244-1246.

[17] Ruether DF, Schaub GM, Duengelhoef PM, Haag F, Brehm TT, Fathi A, et al. SARS-CoV2-specific Humoral and T-cell Immune Response After Second Vaccination in Liver Cirrhosis and Transplant Patients. Clin Gastroenterol Hepatol 2021.
[18] Bakasis AD, Bitzogli K, Mouziouras D, Pouliakis A, Roumpoutsou M, Goules AV, et al. Antibody Responses after SARS-CoV-2 Vaccination in Patients with Liver Diseases. Viruses 2022;14.

[19] Science Brief: SARS-CoV-2 Infection-induced and Vaccine-induced Immunity. CDC COVID-19 Science Briefs. Atlanta (GA); 2020.

[20] Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J Med 2021;385:e84.

[21] Jochum S, Kirste I, Hortsch S, Grunert VP, Legault H, Eichenlaub U, et al. Clinical utility of Elecsys Anti-SARS-CoV-2 S assay in COVID-19 vaccination: An exploratory analysis of the mRNA-1273 phase 1 trial. medRxiv 2021.
Table 1. Baseline characteristics of the population.

| Characteristics                                      | Patients with cirrhosis (n = 48) | Healthy controls (n = 39) | P-value |
|------------------------------------------------------|----------------------------------|---------------------------|---------|
| Age, median, years (range)                           | 63.5 (26–76)                    | 60 (25–86)                |         |
| Gender                                               |                                  |                           |         |
| Male, n (%)                                          | 21 (44)                          | 15 (38)                   |         |
| Female, n (%)                                        | 27 (56)                          | 24 (62)                   |         |
| Vaccine, Moderna/ Pfizer-BioNTech, n (%)             | 4(8) / 44 (92)                   | 2(5) / 37(95)             |         |
| Days after dose 2 to sampling, median (range)        | 89 (32–138)                      | 34 (14–147)               | <0.001  |
| Aetiology of cirrhosis                               |                                  |                           |         |
| Alcohol without other aetiology, n (%)               | 26 (54)                          |                           |         |
| NASH without other aetiology, n (%)                  | 6 (13)                           |                           |         |
| Combined NASH and alcohol, n (%)                     | 4 (8)                            |                           |         |
| Past hepatitis C without other aetiology, n (%)      | 1 (2)                            |                           |         |
| Combined past hepatitis C and alcohol, n (%)         | 2 (4)                            |                           |         |
| Autoimmune hepatitis, n (%)                          | 2 (4)                            |                           |         |
| Cholestatic liver disease (primary biliary cholangitis and primary sclerosing cholangitis), n (%) | 4 (8) |                     |         |
| Cryptogenic, n (%)                                   | 2 (4)                            |                           |         |
| Child-Pugh Score                                     |                                  |                           |         |
| Class A, score 5–6, n (%)                            | 31 (65)                          |                           |         |
| Class B, score 7–9, n (%)                            | 15 (31)                          |                           |         |
| Class C, score 10–15, n (%)                          | 2 (4)                            |                           |         |
| Comorbidities                                        |                                  |                           |         |
| Patient with at least 1 comorbidity, n (%)           | 37 (77)                          | 8 (21)                    |         |
| Hypertension, n (%)                                  | 17 (35)                          | 4 (10)                    |         |
| Type 2 Diabetes, n (%)                               | 13 (27)                          |                           |         |
| Osteoporosis, n (%)                                  | 6 (13)                           |                           |         |
| Hypothyroidism, n (%)                                | 6 (13)                           |                           |         |
| Hyperlipidaemia, n (%)                               | 6 (13)                           |                           |         |
| Condition                  | n (%) | n (%) |
|----------------------------|-------|-------|
| Asthma/allergy             | 5 (10)| 3 (8) |
| Stroke, n (%)              | 2 (4) |       |

**Use of immunosuppressive agents**

| Medication                  | n (%) |
|-----------------------------|-------|
| Corticosteroids, 4 (8)      |       |
| Azathioprine, 3 (6)         |       |
| Vedolizumab, 1 (2)          |       |
| Total 5, (10)               |       |

**Use of 2 or more suppressive medications, n (%)**

| Medication                  |       |
|-----------------------------|-------|
| Corticosteroids and azathioprine, 3 (6) | |
Table 2. Characteristics of patients with cirrhosis (n=48) grouped according to anti-RBD-S1 (<100 vs. ≥100 BAU/ml) or IFN-γ (<10 vs. ≥10 pg/ml) levels after two doses of mRNA COVID-19 vaccine.

| Characteristics                              | Anti RBD IgG <100 BAU/ml (n=7) | Anti RBD IgG ≥100 BAU/ml (n=41) | OR (95%CI) | P-value | Interferon-γ production <10 pg/ml (n=15) | OR (95%CI) | P-value | Interferon-γ production ≥10 pg/ml (n=27) | OR (95%CI) | P-value |
|----------------------------------------------|--------------------------------|---------------------------------|------------|---------|----------------------------------------|------------|---------|----------------------------------------|------------|---------|
| Median age at vaccination, year (range)      | 61 (48 – 73)                   | 64 (26 – 76)                   | 1.06       | 0.27    | 67 (43-76)                             | 63 (26-71) | 0.96    | 0.90-1.02                              | 0.2        |
| Gender female/male, n (%)                    | 5 (71) / 2 (29)                | 22 (54) / 19 (46)              | 0.60       | 0.55    | 6 (40) / 9 (60)                        | 17 (63) / 10 (37) | 2.55 | 0.7-9.31                             | 0.16       |
| Child-Pugh Class (A-C)                        | 4/3/00                        | 27/12/2                        | 0.96       | 0.95    | 7/8/00                                 | 21/4/2     | 0.50    | 0.17-1.49                             | 0.21       |
| Median ARFI value, kPa (range)               | 27.2 (14.8-40.2)              | 22.4 (2.2-56.9)                | 0.93       | 0.17    | 22.8 (6.2-40.2)                       | 22.5 (5.2-56.9) | 1.00  | 0.94-1.06                             | 0.95       |
| Vaccine (Pfizer-BioNTech / Moderna), n (%)   | 7 (100) / 0                    | 37 (90) / 4 (10)               | 1.321      | 1.002   | 15 (100) / 0                          | 24 (89) / 3 (11) | 2.781 | 0.542                                 |           |
| Days from vaccine dose 2 to sampling, days, median (range) | 90 (41-138)                   | 88 (32-138)                    | 0.98       | 0.28    | 90 (41-109)                           | 88 (14-95) | 0.96 | 0.92-1.00                             | 0.07       |
| Imunosuppression, n (%)                       | 0                             | 5 (12)                         | 1.711      | 1.002   | 0                                      | 5 (19)     | 4.791 | 0.142                                 |           |
Table 3. Logistic regression of S1-IFN-γ production ≥10 pg/mL in the controls and patients with cirrhosis.

|                         | First vaccine dose |                                                                 | Second vaccine dose |                                                                 |
|-------------------------|--------------------|----------------------------------------------------------------|--------------------|----------------------------------------------------------------|
|                         | OR (95%CI)         | Univariate p-value | Adjusted OR (95%CI) | Adjusted p-value | OR (95%CI)         | Univariate p-value | Adjusted OR (95%CI) | Adjusted p-value |
| Cirrhosis               | 0.11 (0.03-0.48)   | <0.001             | -                   | -                | 0.11 (0.02-0.54)   | 0.006               | 0.09 (0.02-0.57)   | 0.01               |
| Gender (female)         | 0.65 (0.20-2.12)   | 0.48               | -                   | -                | 2.02 (0.69-5.94)   | 0.2                 | -                   | -                   |
| Age (years)             | 0.97 (0.92-1.02)   | 0.22               | -                   | -                | 0.97 (0.92-1.03)   | 0.29                | -                   | -                   |
| Vaccine (Pfizer-BioNTech) | 1.451              | 0.491              | -                   | -                | 2.951              | 0.321               | -                   | -                   |
| Immunosuppression       | 2.22 (0.13-39.6)   | 0.59               | -                   | -                | 5.21               | 0.141               | -                   | -                   |
| Time FU-test vaccine 2 (days) | -                 | -                  | -                   | -                | 0.98 (0.97-1.00)   | 0.048               | 0.98 (0.96-1.00)   | 0.06               |

Odds ratio (OR). Follow-Up (FU). Statistics using logistic regression. ¹Fischer's exact test and likelihood ratio used.
Table 4. Logistic regression of anti-RBD-S1 IgG ≥100 BAU/mL in the controls and patients with cirrhosis.

|                     | First vaccine dose |     |     |     |     | Second vaccine dose |     |     |     |
|---------------------|-------------------|-----|-----|-----|-----|---------------------|-----|-----|-----|
|                     | OR(95%CI)         | p-value | OR(95%CI) | p-value | OR(95%CI) | Univariate p-value | Adjusted OR(95%CI) | Adjusted p-value | OR(95%CI) | Univariate p-value | Adjusted OR(95%CI) | Adjusted p-value |
| Cirrhosis           | 0.16 (0.04-0.58)  | 0.005 | -   | -   | -   | 0.50 (0.12-2.09)  | 0.34 | -   | -   |
| Gender (female)     | 0.93 (0.28-3.06)  | 0.93  | -   | -   | -   | 0.92 (0.24-3.52)  | 0.9  | -   | -   |
| Age (years)         | 0.98 (0.93-1.02)  | 0.33  | -   | -   | -   | 0.98 (0.92-1.04)  | 0.51 | -   | -   |
| Vaccine (Pfizer-    | 0.841             | 1.01  | -   | -   | -   | 1.541             | 1.01 | -   | -   |
| BioNTech)           |                   |       |     |     |     |                   |     |     |     |
| Immunosuppression   | 2.08 (0.16-29.96) | 0.57  | -   | -   | -   | 1.671             | 1.01 | -   | -   |
| Time FU-test vaccine 2 (days) |                   |       |     |     |     | 0.98 (0.96-1.00)  | 0.02 | -   | -   |

Odds ratio (OR). Follow-Up (FU). Statistics using logistic regression. ¹Fischer's exact test and likelihood ratio used.
Figure Legends

**Figure 1. B and T cell responses to COVID-19 vaccination in cirrhotic patients and controls.** Scatter plots with interquartile range demonstrating IgG antibody levels in serum (A) against the receptor-binding domain (RBD) within spike-1 (S1), and IFN-γ in supernatant plasma following stimulation of whole blood with multimeric peptides from the SARS-CoV-2 S1 protein (B) for patients with cirrhosis and healthy controls. Bar charts with percentages of cirrhotic patients and healthy controls with serum anti-RBD-S1 IgG levels below the limit of detection (C) and undetectable IFN-γ (<10 pg/ml) in supernatant plasma following stimulation with spike 1 peptides (D). Statistics were calculated by Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.001, ns = not significant.

**Figure 2. B and T cell responses to COVID-19 vaccination in cirrhotic patients stratified by Child-Pugh classification A or B.** Scatter plots with interquartile range demonstrating serum anti-RBD-S1 IgG levels (A) as well as IFN-γ in supernatant plasma following stimulation of whole blood with multimeric spike 1 peptides (B) in cirrhotic patients with Child-Pugh class A or B. Two patients had decompensated cirrhosis (class C) and were not included in these analyses. Statistics were calculated by Mann-Whitney U test. *p<0.05, **p<0.01, ns=not significant.
A

B

C

D

IFN-γ log10 pg/mL

anti-RBD S1 IgG (Log10 BAU/mL)

After 1st dose After 2nd dose

After 1st dose After 2nd dose

Controls Cirrhosis

Percentage IFN-γ <10 pg/mL

Percentage anti-RBD S1 IgG <LOD

After 1st dose After 2nd dose

After 1st dose After 2nd dose

n 16 31 31 42

n 16 34 38 48

*** ** ns
• After COVID vaccination, cirrhotics had impaired T cell and antibody responses.

• Child-Pugh class B cirrhosis was associated with poorer immune responses than class A.

• Multivariate analyses excluded potential confounding variables.