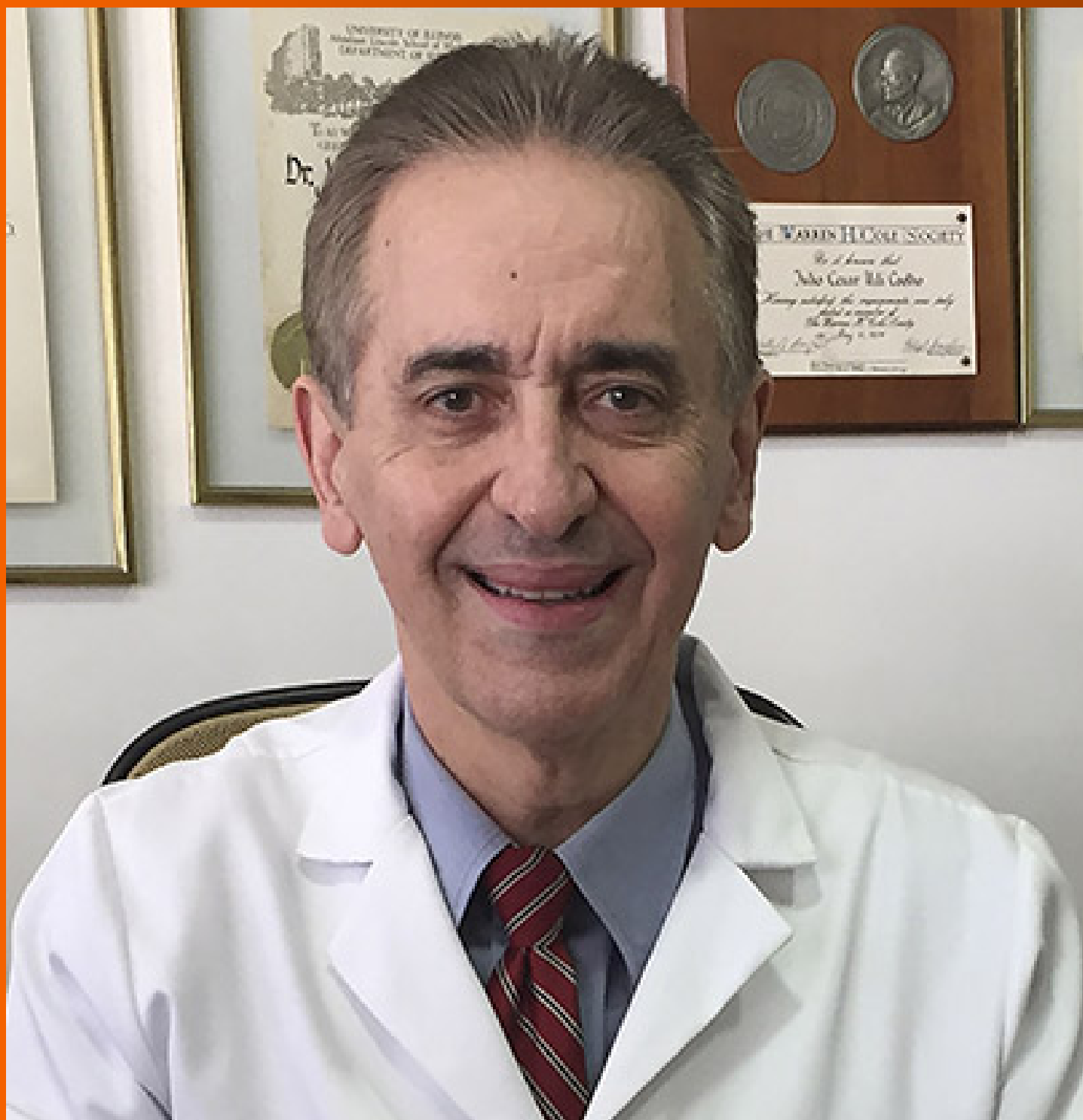


# World Journal of *Hepatology*

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### EDITORIAL

- 1203** Transition of an acronym from nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease  
*Alam S, Fahim SM*

### OPINION REVIEW

- 1208** Non-invasive real-time assessment of hepatic macrovesicular steatosis in liver donors: Hypothesis, design and proof-of-concept study  
*Rajamani AS, Rammohan A, Sai VR, Rela M*

### REVIEW

- 1215** Impact of COVID-19 pandemic on liver, liver diseases, and liver transplantation programs in intensive care units  
*Omar AS, Kaddoura R, Orabi B, Hanoura S*
- 1234** In the era of rapid mRNA-based vaccines: Why is there no effective hepatitis C virus vaccine yet?  
*Echeverría N, Comas V, Aldunate F, Perbolianachis P, Moreno P, Cristina J*
- 1269** Pediatric non-cirrhotic portal hypertension: Endoscopic outcome and perspectives from developing nations  
*Sarma MS, Seetharaman J*

### MINIREVIEWS

- 1289** Acute-on-chronic liver failure in children  
*Islek A, Tumgor G*
- 1299** Coronavirus disease 2019 in liver transplant patients: Clinical and therapeutic aspects  
*Loinaz-Seguro C, Marcacuzco-Quinto A, Fernández-Ruiz M*
- 1316** Pediatric vascular tumors of the liver: Review from the pathologist's point of view  
*Cordier F, Hoorens A, Van Dorpe J, Creyten D*
- 1328** Autoimmune hepatitis in genetic syndromes: A literature review  
*Capra AP, Chiara E, Briuglia S*
- 1341** Assessing the prognosis of cirrhotic patients in the intensive care unit: What we know and what we need to know better  
*da Silveira F, Soares PHR, Marchesan LQ, da Fonseca RSA, Nedel WL*
- 1351** Liver transplantation for pediatric inherited metabolic liver diseases  
*Vimalesvaran S, Dhawan A*



- 1367 Liver and COVID-19: From care of patients with liver diseases to liver injury

*Gaspar R, Castelo Branco C, Macedo G*

## ORIGINAL ARTICLE

### Basic Study

- 1378 Direct modulation of hepatocyte hepcidin signaling by iron

*Yu LN, Wang SJ, Chen C, Rausch V, Elshaarawy O, Mueller S*

- 1394 Serum zonulin levels in patients with liver cirrhosis: Prognostic implications

*Voulgaris TA, Karagiannakis D, Hadziyannis E, Manolakopoulos S, Karamanolis GP, Papatheodoridis G, Vlachogiannakos J*

### Retrospective Cohort Study

- 1405 Impact of biliary complications on quality of life in live-donor liver transplant recipients

*Guirguis RN, Nashaat EH, Yassin AE, Ibrahim WA, Saleh SA, Bahaa M, El-Meteini M, Fathy M, Dabbous HM, Montasser IF, Salah M, Mohamed GA*

### Retrospective Study

- 1417 Machine learning models for predicting non-alcoholic fatty liver disease in the general United States population: NHANES database

*Atsawarungruangkit A, Laoveeravat P, Promrat K*

- 1428 Acute liver failure with hemolytic anemia in children with Wilson's disease: Genotype-phenotype correlations?

*Pop TL, Grama A, Stefanescu AC, Willheim C, Ferenci P*

### Observational Study

- 1439 Clinical outcomes of patients with two small hepatocellular carcinomas

*Pham AD, Vaz K, Ardalan ZS, Sinclair M, Apostolov R, Gardner S, Majeed A, Mishra G, Kam NM, Patwala K, Kutaiba N, Arachchi N, Bell S, Dev AT, Lubel JS, Nicoll AJ, Sood S, Kemp W, Roberts SK, Fink M, Testro AG, Angus PW, Gow PJ*

## CASE REPORT

- 1450 Focal nodular hyperplasia associated with a giant hepatocellular adenoma: A case report and review of literature

*Gaspar-Figueiredo S, Kefleyesus A, Sempoux C, Uldry E, Halkic N*

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## Transition of an acronym from nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease

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### Abstract

Nonalcoholic fatty liver disease (NAFLD) is a global public health concern owing to its substantial contribution to chronic liver diseases. The disease is closely linked to metabolic syndrome (MS), suggesting a common biological pathway and shared disease mechanism for both ailments. Previous studies revealed a close relationship of NAFLD with the components of MS including abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. Hence, a group of experts recently renamed NAFLD as metabolic dysfunction-associated fatty liver disease (MAFLD) in order to encompass a more appropriate pathogenesis of the disease. NAFLD was first named to describe a condition similar to alcoholic hepatitis in absence of significant alcohol consumption. However, knowledge pertaining to the etiopathogenesis of the disease has evolved over the past four decades. Recent evidence endorses NAFLD as a terminology of exclusion and suggests that it may often leads to misdiagnosis or inappropriate management of patients, particularly in clinical practice. On the other hand, the new definition is useful in addressing hepatic steatosis with metabolic dysfunction, which ultimately covers most of the patients with such illness. Therefore, it seems to be helpful in improving clinical diagnosis and managing high-risk patients with fatty liver disease. However, it is imperative to validate the new terminology at the population level to ensure a holistic approach to reduce the global burden of this heterogeneous disease condition.

**Key Words:** Nonalcoholic fatty liver disease; Metabolic dysfunction-associated fatty liver disease; Redefining; Redefinition of fatty liver disease

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**Core Tip:** A consensus of experts recently renamed nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease. The new definition is advantageous for improving clinical diagnosis and managing high-risk patients with fatty changes in liver.

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## INTRODUCTION

The rising burden of nonalcoholic fatty liver disease (NAFLD) is a global public health concern. This progressive liver disease is a leading cause of chronic hepatic ailments worldwide[1,2]. Recent reports confirm that NAFLD accounts for approximately 8% of the annual 2.14 million global deaths from liver disease[3]. Over the past two decades a substantial elevation in the prevalence of NAFLD has been reported, with strong evidence of a close link between NAFLD and metabolic syndrome (MS)[4]. NAFLD is often found to be associated with the components of MS, such as abdominal obesity, dyslipidemia, hypertension, and hyperglycemia[5]. In addition, the risk factors of NAFLD and MS have also been found to be identical in many studies[1]. Therefore, it has been suggested that both NAFLD and MS follow a common biological pathway as well as a shared disease mechanism. In line with that, a consensus of experts recently renamed NAFLD to metabolic dysfunction-associated fatty liver disease (MAFLD) so that the term could accurately reflect the pathogenesis of the disease[6]. According to the new definition, MAFLD would be diagnosed if there was evidence of hepatic steatosis in addition to any of the following three conditions, overweight/obesity, type 2 diabetes mellitus, or metabolic dysregulation[7]. The expert opinion was that the new definition is superior for diagnosing NAFLD patients with severe liver injury. Moreover, it is more practical to diagnose high-risk patients and evaluate disease progression in clinical settings[8,9].

## DISCUSSION

Nonalcoholic steatohepatitis (NASH) was first used nearly four decades ago to describe a condition that mimics alcoholic hepatitis in absence of significant alcohol consumption[10]. Initially, the pathology was found to be linked to obesity or obesity-associated disorders. Subsequently, the disease was renamed NAFLD, referring to the absence of any known etiology of liver disease. In the meantime, a detailed understanding of the etiopathogenesis of the disease has evolved as the link between NAFLD, insulin resistance, and other components of MS was explored. Molecular-level investigations explored the role of multiple genetic and cellular mechanisms in the pathogenesis of NAFLD[11]. Epidemiological studies also revealed a number of social, demographic, and clinical determinants responsible for development of NAFLD[12]. Results of the studies described NAFLD as a heterogeneous condition. However, the archaic NAFLD nomenclature, which is a terminology of exclusion, remained unchanged over the years. The inclusion of alcohol in the name and definition is also problematic. In real-life clinical practice, the features of NAFLD often overlap with the characteristics of patients who consume alcohol. Moreover, there is no accepted method to appropriately measure alcohol intake in clinical facilities. Hence, there remains a possibility of misdiagnosis or inappropriate management of patients. Considering the above context, there has been a proposal to change the name since the beginning of this century. As the disease was found to be closely associated with metabolic dysfunction and insulin resistance, the scientific community proposed several names related to metabolic dysfunction, for example, metabolic steatohepatitis, metabolic fatty liver disease, and metabolic-associated fatty liver[10]. Eventually, a consensus of global experts opted for MAFLD.

**Table 1 Potential positive implications and challenges related to transition of nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease**

Positive implications	Challenges
Useful in overcoming the dichotomization of NASH and non-NASH in clinical practices	Obtain a global acceptance as some researchers consider the name change premature and inappropriate
Facilitate diagnosis and evaluation of disease progression in high-risk patients	Underestimation of actual prevalence of the disease using the criteria of MAFLD
Improve awareness of physicians, healthcare providers and patients	Further clarification and stratification of the definition to guide decision-making and assess prognosis of the disease
Improve physician-patient communication	Address the patients with fatty changes in liver in absence of metabolic derangements
Improve clinical diagnosis and patient care	Deal with lean or undernourished individuals with hepatic fatty changes
Reduce confusion and stigma regarding the disease	Lack of information regarding genetic risk factors, phenotyping measurements, body fat content, and alterations in gut microbiota in the new definition
Increase public attention and improve health policy actions	Determine the outcome variable of ongoing clinical trials where “improvement in NASH” is one of the endpoints

MAFLD: Metabolic dysfunction-associated fatty liver disease; NASH: Nonalcoholic steatohepatitis.

It is assumed that the new definition would improve clinical diagnosis (Table 1). The term MAFLD annulled two different NAFLD entities, simple steatosis and NASH, and conceptualized the fatty changes in the liver as a disease process. Therefore, the redefinition of MAFLD would help to overcome the dichotomization of NASH and non-NASH, and facilitate the assessment of disease severity in clinical practice[13]. A recent study reported that the switch from NAFLD to MAFLD increased the awareness of physicians regarding the management of the disease[14]. However, changes in nomenclature may have potential implications for ongoing clinical trials in which “improvement in NASH” is an outcome variable. It is possible to redefine the outcomes of clinical trials based on the existing MAFLD framework, but there remains certain disagreement regarding the new terminology and its definition that need to be addressed[15]. The new criteria may underestimate the actual prevalence of the disease, as reported in a recent study[8]. It may also exclude patients without metabolic disturbances. A recent review found that metabolic derangements may be absent in 30% of the patients diagnosed with NAFLD[5]. The new definition is also not clear regarding concomitant liver diseases such as drug-induced, viral or auto-immune liver disease. Apart from individuals with high body mass index, NAFLD has also been reported in lean and nonobese adults. It is assumed that visceral adiposity and differences of metabolic adaptations may play a potential role in the pathogenesis of hepatic steatosis in lean adults[16]. Alterations in gut microbiota can also be a contributing factor in developing NAFLD in lean and undernourished adults[16]. Moreover, there is evidence in support of a significant relationship between a positive family history of metabolic traits and NAFLD, particularly in lean patients with a fatty liver[17]. Individuals with a family history of metabolic traits are likely to develop complications of NAFLD at a younger age[18]. Therefore, body fat content, rate of weight gain, and family history of metabolic traits need to be considered when constructing a new conceptual framework to define MAFLD. It seems that diagnosis of cryptogenic cirrhosis attributable to metabolic derangements would be easier using the new definition of MAFLD, as cryptogenic cirrhosis was found to be associated with obesity and diabetes[19]. Nevertheless, a more insightful opinion is required to establish an accurate definition so that the term incorporates individuals with hepatic fatty changes in the absence of metabolic derangements. Moreover, there should be definitive guidelines regarding inclusion of genetic risk factors, phenotypic measurements, dietary intake, visceral adiposity, and alterations in gut microbiota in the definition.

## CONCLUSION

As more than one-fourth of the global population have NAFLD. Emphasis should be given to appropriate understanding of etiopathogenesis of the ailment[20]. To that end, an appropriate term is required so that it can reflect the entire pathophysiology of

the disease and cover the whole population with perturbed accumulation of hepatic fat. The new definition seems to address hepatic steatosis with metabolic dysfunction, which ultimately covers most of the cases with such illness. It is also useful for improving clinical diagnosis and managing high-risk patients with fatty changes in the liver. Therefore, the shift in terminology from NAFLD to MAFLD has already attained global endorsement. However, validation of the new term at the population level is warranted to ensure a holistic approach to reduce the global burden.

## REFERENCES

- Bellentani S.** The epidemiology of non-alcoholic fatty liver disease. *Liver Int* 2017; **37**: 81-84 [PMID: [28052624](#) DOI: [10.1111/liv.13299](#)]
- Golabi P, Otgonsuren M, de Avila L, Sayiner M, Rafiq N, Younossi ZM.** Components of metabolic syndrome increase the risk of mortality in nonalcoholic fatty liver disease (NAFLD). *Medicine (Baltimore)* 2018; **97**: e0214 [PMID: [29595666](#) DOI: [10.1097/MD.00000000000010214](#)]
- Paik JM, Golabi P, Younossi Y, Mishra A, Younossi ZM.** Changes in the Global Burden of Chronic Liver Diseases From 2012 to 2017: The Growing Impact of NAFLD. *Hepatology* 2020; **72**: 1605-1616 [PMID: [32043613](#) DOI: [10.1002/hep.31173](#)]
- Kumar R, Priyadarshi RN, Anand U.** Non-alcoholic Fatty Liver Disease: Growing Burden, Adverse Outcomes and Associations. *J Clin Transl Hepatol* 2020; **8**: 76-86 [PMID: [32274348](#) DOI: [10.14218/JCTH.2019.00051](#)]
- Kim D, Touros A, Kim WR.** Nonalcoholic Fatty Liver Disease and Metabolic Syndrome. *Clin Liver Dis* 2018; **22**: 133-140 [PMID: [29128053](#) DOI: [10.1016/j.cld.2017.08.010](#)]
- Eslam M, Sanyal AJ, George J; International Consensus Panel.** MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: [32044314](#) DOI: [10.1053/j.gastro.2019.11.312](#)]
- Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbaek H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratzliff V, George J.** A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: [32278004](#) DOI: [10.1016/j.jhep.2020.03.039](#)]
- Lin S, Huang J, Wang M, Kumar R, Liu Y, Liu S, Wu Y, Wang X, Zhu Y.** Comparison of MAFLD and NAFLD diagnostic criteria in real world. *Liver Int* 2020; **40**: 2082-2089 [PMID: [32478487](#) DOI: [10.1111/liv.14548](#)]
- Niriella MA, Ediriweera DS, Kasturiratne A, De Silva ST, Dassanayaka AS, De Silva AP, Kato N, Pathmeswaran A, Wickramasinghe AR, de Silva HJ.** Outcomes of NAFLD and MAFLD: Results from a community-based, prospective cohort study. *PLoS One* 2021; **16**: e0245762 [PMID: [33534815](#) DOI: [10.1371/journal.pone.0245762](#)]
- Fouad Y, Waked I, Bollipo S, Goma A, Ajlouni Y, Attia D.** What's in a name? *Liver Int* 2020; **40**: 1254-1261 [PMID: [32301554](#) DOI: [10.1111/liv.14478](#)]
- Kuchay MS, Misra A.** From non-alcoholic fatty liver disease (NAFLD) to metabolic-associated fatty liver disease (MAFLD): A journey over 40 years. *Diabetes Metab Syndr* 2020; **14**: 695-696 [PMID: [32442920](#) DOI: [10.1016/j.dsx.2020.05.019](#)]
- Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A.** Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol* 2017; **23**: 8263-8276 [PMID: [29307986](#) DOI: [10.3748/wjg.v23.i47.8263](#)]
- Yilmaz Y, Byrne CD, Musso G.** A single-letter change in an acronym: signals, reasons, promises, challenges, and steps ahead for moving from NAFLD to MAFLD. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 345-352 [PMID: [33270482](#) DOI: [10.1080/17474124.2021.1860019](#)]
- Fouad Y, Goma A, Semida N, Ghany WA, Attia D.** Change from NAFLD to MAFLD increases the awareness of fatty liver disease in primary care physicians and specialists. *J Hepatol* 2021; **74**: 1254-1256 [PMID: [33582129](#) DOI: [10.1016/j.jhep.2020.12.035](#)]
- Younossi ZM, Rinella ME, Sanyal AJ, Harrison SA, Brunt EM, Goodman Z, Cohen DE, Loomba R.** From NAFLD to MAFLD: Implications of a Premature Change in Terminology. *Hepatology* 2021; **73**: 1194-1198 [PMID: [32544255](#) DOI: [10.1002/hep.31420](#)]
- Chen F, Esmaili S, Rogers GB, Bugianesi E, Petta S, Marchesini G, Bayoumi A, Metwally M, Azadaryany MK, Coulter S, Choo JM, Younes R, Rosso C, Liddle C, Adams LA, Craxi A, George J, Eslam M.** Lean NAFLD: A Distinct Entity Shaped by Differential Metabolic Adaptation. *Hepatology* 2020; **71**: 1213-1227 [PMID: [31442319](#) DOI: [10.1002/hep.30908](#)]
- Fahim SM, Chowdhury MAB, Alam S.** Non-alcoholic fatty liver disease (NAFLD) among underweight adults. *Clin Nutr ESPEN* 2020; **38**: 80-85 [PMID: [32690182](#) DOI: [10.1016/j.clnesp.2020.06.002](#)]
- Bhadoria AS, Kedarisetty CK, Bihari C, Kumar G, Jindal A, Bhardwaj A, Shasthry V, Vyas T, Benjamin J, Sharma S, Sharma MK, Sarin SK.** Impact of family history of metabolic traits on severity of non-alcoholic steatohepatitis related cirrhosis: A cross-sectional study. *Liver Int* 2017; **37**: 1397-



- 1404 [PMID: [28231412](#) DOI: [10.1111/liv.13396](#)]
- 19 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669 [PMID: [10051466](#) DOI: [10.1002/hep.510290347](#)]
- 20 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: [26707365](#) DOI: [10.1002/hep.28431](#)]



## Non-invasive real-time assessment of hepatic macrovesicular steatosis in liver donors: Hypothesis, design and proof-of-concept study

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### Abstract

Macrovesicular Steatosis (MS) is an independent risk factor for adverse post-liver transplant (LT) outcomes. The degree of MS is intimately related to the viability of the liver graft, which in turn is crucial to the success of the operation. An ideal liver graft should have no MS and most centres would find it unacceptable to use a donor liver with severe MS for LT. While a formal liver biopsy is the gold-standard diagnostic test for MS, given the logistical and time constraints it is not universally feasible. Other tests like a frozen section biopsy are plagued by issues of fallibility with reporting and sampling bias making them inferior to a liver biopsy. Hence, the development of an accurate, non-invasive, easy-to-use, handheld, real-time device for quantification of MS would fill this lacuna in the deceased donor selection process. We present the hypothesis, design and proof-of-concept of a study, which aims to standardise and determine the feasibility and accuracy of a novel handheld device applying the principle of diffuse reflectance spectroscopy for real-time quantification of MS.

**Key Words:** Macrovesicular steatosis; Deceased donors; Liver transplantation; Real-time devices; Diffuse reflectance spectroscopy

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**Core Tip:** The degree of macrovesicular steatosis (MS) is intimately related to the viability of the liver graft, which in turn is crucial to the success of the liver transplant operation. The development of an accurate, non-invasive, easy-to-use, handheld, real-time device for quantification of MS would fill a lacuna in the deceased donor selection process. We present the hypothesis, design and proof-of-concept study for a novel handheld device for real-time quantification of MS.

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## INTRODUCTION

Macrovesicular Steatosis (MS) is an independent risk factor for adverse post-liver transplant (LT) outcomes. The degree of MS is intimately related to the viability of the liver graft, which in turn is crucial to the success of the operation. An ideal liver graft should have no MS and most centres would find it unacceptable to use a donor liver with severe MS for LT. While a formal liver biopsy is the gold-standard diagnostic test for MS, given the logistical and time constraints it is not universally feasible. Other tests like a frozen section biopsy are plagued by issues of fallibility with reporting and sampling bias making them inferior to a liver biopsy. Hence, the development of an accurate, non-invasive, easy-to-use, handheld, real-time device for quantification of MS would fill this much vaunted lacuna in the deceased donor selection process. We present the hypothesis, design and proof-of-concept of a study, which aims to standardise and determine the feasibility and accuracy of a novel handheld device applying the principle of diffuse reflectance spectroscopy for real-time quantification of MS.

## AIM

The objective of the present investigation is to apply the principle of diffuse reflectance spectroscopy (DRS) to standardize and determine the feasibility and accuracy of a handheld device for real-time quantification of MS.

## PRINCIPLE AND HYPOTHESIS

DRS is an optical measurement method which is based on the principle of tissue illumination and the measurement of reflectance[8]. Briefly, the tissue is illuminated with light from a broadband light source, and after interacting with the tissue the diffusely reflected light is collected and analyzed. By fitting the analyzed data to a mathematical model, tissue characteristics such as its structure and composition can be estimated. Quantification of MS with infrared (IR) spectroscopy directly depends on the absorption of IR light due to vibrational excitation in molecular groups[9]. In liver tissues, the absorption in the visible wavelength range is dominated by bile and hemoglobin, whereas lipid, water and collagen are the main source of absorption in the near-infrared wavelength range. Hence IR spectra is the wavelength of interest for this study. Recent studies on the human liver show that the absorption of light around 1200 nm is dominated by the lipid and this can be used for the assessment of steatosis [9-11].

We hypothesize that the broadband light source can be replaced with a narrow band light emitting diode (LED) of 1200 nm and the spectrometer with a highly sensitive photodetector. Using the absorption characteristics, a calibration curve can be determined based on the fat content on the liver; allowing for the development of a mathematical model and a real-time quantitative analysis of MS. We also hypothesize that once the difference in absorbance spectrum between normal and MS liver is

established, the optical device can be miniaturized further. This novel optic-based handheld device for MS detection will retain its accuracy whilst being portable and affordable as well.

## DEVICE SETUP AND METHOD

A handheld device was designed and developed with a single infrared LED (IR-LED)-photodetector (PD) arrangement coupled through a fibre optic reflection probe bundle. One end of the reflection probe was coupled to a LED, and the other end to a highly sensitive photodetector. These optoelectronic components were placed in a custom-made plastic block to avoid ambient noise or cross-coupling between the LED and PD. The optoelectronic circuitry comprised of a 5 V linear voltage regulator followed by a constant current circuit using two bipolar junction transistors to drive IR-LED and a trans-impedance amplifier circuit for the PD to convert photocurrent into photovoltage. This circuit was powered by a 9 V battery placed within the handheld device (Figure 1A). The obtained photovoltage was then transmitted to a low-power system on a chip microcontroller *via* a buffer integrated circuit[12]. The device has an LED display that shows voltage response corresponding to the diffused reflectance data from the liver (Figure 1B).

The measurement was carried out with the handheld device employing a mathematical model. With the device powered on, the fiberoptic reflection probe was placed on the diffuse reflectance standard (WS-1 ocean optics, United States) and the initial voltage value made note of. This was taken as the reference value; the probe was then placed on the test sample to record its voltage value. An algorithm was formulated to calculate the resultant fat absorbance value ( $A_f$ ) with this reference ( $V_r$ ) and test ( $V_t$ ) voltage values from the below equation.

$$A_f = -\log_{10} \frac{V_t}{V_r}$$

### Proof-of-concept

For a practical assessment of the above hypothesis, an initial proof of concept analysis was done using 50 abattoir retrieved large animal livers, with varying percentage of fat (Figure 2). Calibration of the device was initially done with 100% fat and normal liver. The results from fat and normal liver were compared to determine the fat composition. Absorbance data was normalized by taking the closest valley to 1300 nm to improve its sensitivity towards estimation of fat percentage [13,14].

$$\text{Normalized absorbance} = 1 - \frac{a_1 \times \lambda_2}{a_2 \times \lambda_1}$$

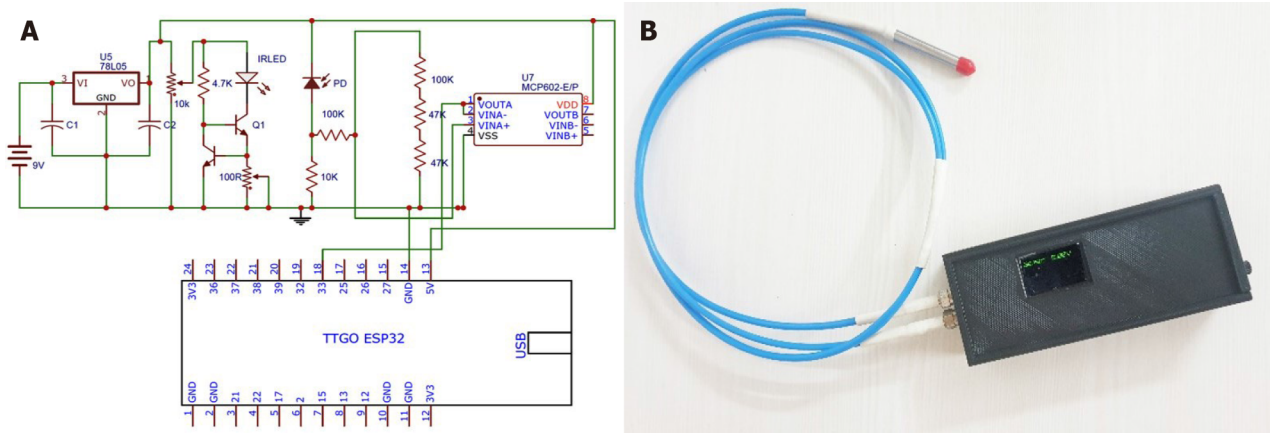
The above equation was used to calculate the normalized absorbance value. This was done by taking the ratio of absorbance responses ( $a_1$ ,  $a_2$ ) at two wavelengths ( $\lambda_1$  and  $\lambda_2$ ) and subtracting it from 1. The specific absorption spectrum of fat peaked at approximate 1200 nm and the normal liver had a Gaussian response at 1200 nm (Figure 3A). Figure 3B shows the calculated absorbance response of fat and liver was noted to be  $0.3203 \pm 0.09$  and  $0.058 \pm 0.01$  respectively. The absorbance values obtained were evaluated against the gold standard biopsy results of these animal livers.

## STUDY DESIGN

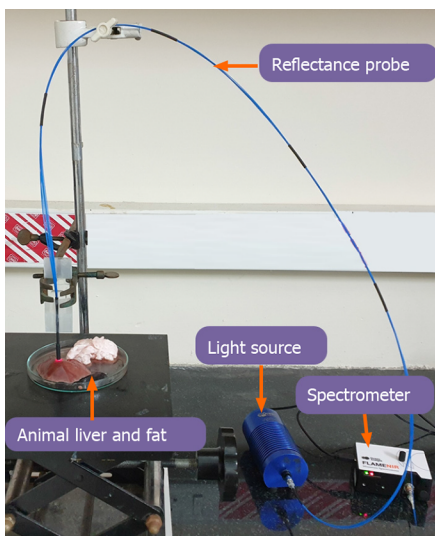
It is an observational study where the point-of-care device is used to assess MS in a non-invasive manner. The study is to be conducted at organ retrieval centres across the city of Chennai, India. The study design is presented in Figure 4.

### Calibration cohort

Initial calibration of the device is to be conducted on 50 livers. Fifteen live liver donors will be assessed for levels of MS. Ten recordings with the device per liver will be noted across the right lobe. As a standard unit protocol, all live liver donors undergo an intraoperative liver biopsy which will be used for comparison. 100% fat as a baseline calibration will be used by analyzing the excised falciform ligament from each of these patients. 35 livers in the real-world deceased donor situation will be analyzed using the device to correlate the estimated MS content with a standard biopsy estimation.



**Figure 1** Principle and set-up of the hand-held real time device to measure macrovesicular steatosis. A: Optoelectronics circuit, B: Handheld point of care device.



**Figure 2** Proof-of-concept study using the prototype model of the device.

These observations will enable the development of a calibrated algorithm based on the reflectance for MS. Optimum conditions for use, including lighting, temperature, distance from the liver, will also be standardized.

### Validation cohort

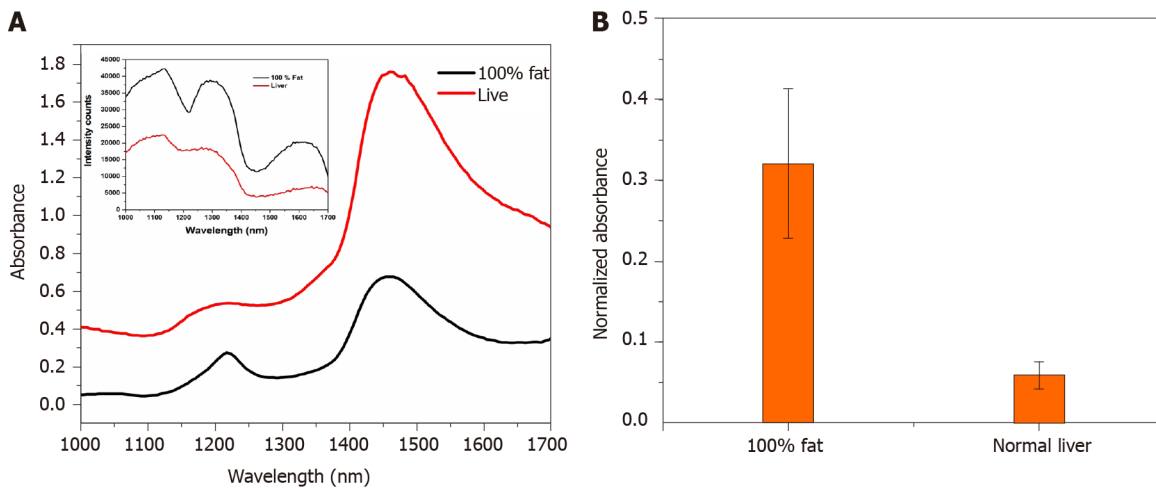
Analysis will be performed on 50 deceased donor livers to test and evaluate the accuracy of the developed algorithm and the point-of-care device.

### Inclusion criteria

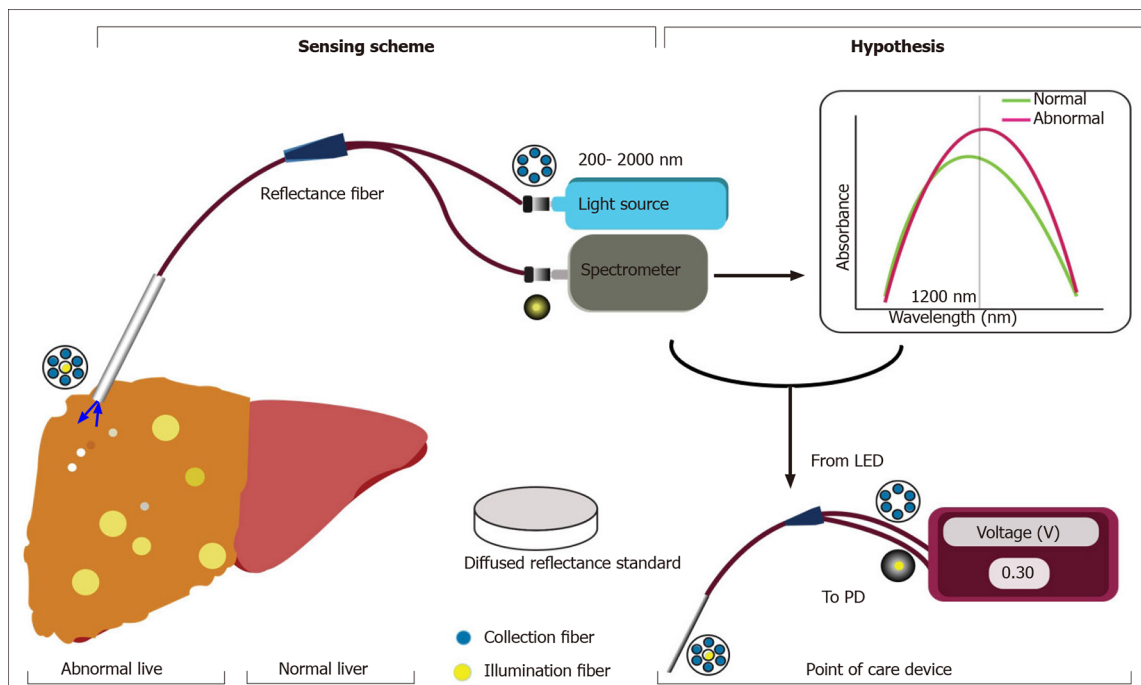
For the calibration cohort, all living liver donors will be included. The standard selection criteria for these living donors are include: (1) Age 18-50 years; (2) ABO compatible blood group with the recipient; (3) No comorbidities, or 1 comorbidity; (4) Liver attenuation index  $\geq +6$ ; (5) Body mass index  $< 30 \text{ kg/m}^2$ ; (6) Graft to recipient weight ratio  $> 0.8$ ; (7) Functional liver remnant volume  $> 30\%$ ; (8) Anatomically suitable for donation; and (9) Any other donor who beyond the above criteria but approved for donation based on the decision of the multi-disciplinary team meeting.

The deceased donors include all brain-dead donors consented for organ donation: (1) Adults between 18 years and 75 years of age; and (2) Donation after brain death. For the validation cohort, all brain-dead donors consented for organ donation will be included: (1) Adults between 18 years and 75 years of age; and (2) Donation after brain death.

As the device analyses the fat content of the donor liver, no specific recipient-based inclusion criteria were defined. Donors of all recipients who underwent the LT



**Figure 3 Comparison between large animal liver retrieved from abattoir and 100% fat.** A: Absorbance spectrum of abattoir retrieved large animal liver and 100% fat (inset: intensity spectra of liver and 100% fat); B: Calculated absorbance response of abattoir retrieved large animal liver and 100% fat.



**Figure 4 Schematic representation of the proposed study design, experimental setup, and hypothesis towards the development of handheld device.** It consists of reflectance probe bundle with home-made plastic block to house light emitting diode, photodetector, optoelectronic circuitry, and display. PD: Photodetector.

operation and recipients of all etiologies were included.

#### Exclusion criteria

(1) Paediatric deceased donors; (2) Donation after cardiac death; (3) Donations where a frozen section/standard biopsy could not be performed; and (4) Discarded organs.

#### Concerns and untested variables

Liver with underlying fibrosis, cholestasis, sinusoidal obstruction syndrome (blue color) and those which could possibly bias the spectral analyses.

#### Ethics, informed consent, safety, and registration of trial

The study will be conducted in accordance with the principles of the Declaration of Helsinki and “good clinical practice” guidelines. Approval from the institutional ethics committee has been obtained. As a testimonial to its bona fide nature, the study has

also been registered with the Clinical Trials Registry of India, National Institute of Medical Statistics, Indian Council of Medical Research, India. CTRI No: CTRI/2021/01/030223.

## STATISTICAL ANALYSIS

Statistical analysis will be performed with the SPSS V.20.0. To compare specific variables, the extended  $\chi^2$  test will be used. For non-parametric analysis of continuous distributed variables, the Mann-Whitney *U* test and the Kruskal-Wallis test will be used.  $P < 0.05$  is considered statistically significant.

## DISCUSSION

The need for a quick, portable, efficacious and economical device to diagnose MS is evident by the number of proof-of-concept studies available in this regard[7,9,11,15]. DRS as a diagnostic modality has been used in endoluminal studies of upper and lower gastrointestinal endoscopies[16,17]. Using the absorption and scattering patterns of biological tissues, DRS allows for accurate differentiation of polyps and subendothelial pathology. Reports on the use of DRS in the identification of MS in murine and porcine liver models show promising results[10,11]. Clinical studies are however sparse and those attempted involve using a micro-spectrometer placed directly over the liver graft[8,10,15]. Nonetheless, there are several drawbacks to these devices. The micro-spectrometers require a sophisticated optical setup, which included an optical spectrometer and other expensive optical components. In addition, due to concerns of sterility, a spectrometer cannot be used on multiple patients. Moreover, these devices require network access, without which the diagnostic algorithm may not be useful. Put together these devices have proved cumbersome to the organ-retrieving surgeon.

To overcome the pitfalls of these prototype models, our device uses IR light guided *via* an optical fibre, and the diffuse reflections are obtained from the tissue sample by measuring the steady-state spectrum. The broadband light source is replaced with a narrow band LED and the spectrometer with a photodetector. Once the algorithm is standardized this optical setup can be miniaturized further, and linked to the internet allowing for remote viewing by the concerned teams.

To push the envelope further, should our device be validated in the current study, we propose that there is potential to link our device with a smartphone application incorporating the algorithm and make use of the current generation of high-resolution smartphone cameras. This would allow for a real-time high-resolution image along with MS percentage to be remotely transmitted using the mobile network to the concerned senior members of the transplant team.

## CONCLUSION

We hypothesize that once validated, our device can potentially prove to be an invaluable apparatus at the hands of the organ retrieving surgeon. It will be non-invasive, portable (hand-held), economical, provide real-time readings of the percentage of MS with image reference and be efficaciously handled by junior surgeons, while not requiring any special network capabilities apart from the presence of the now ubiquitous smartphone. This will dramatically ease the currently available circuitous and subjective process of determining MS and decision making in selecting deceased donor organs for LT. Nonetheless, ours is a hypothesis and initial proof-of-concept study which requires real-world validation across multiple centres and in a large cohort of patients before it can become an integral part of the liver retrieval algorithm.

## REFERENCES

- 1 Zhang QY, Zhang QF, Zhang DZ. The Impact of Steatosis on the Outcome of Liver Transplantation: A Meta-Analysis. *Biomed Res Int* 2019; **2019**: 3962785 [PMID: 31218224 DOI: 10.1155/2019/3962785]



- 2 **Ng M**, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA, Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwar P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Meriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX, Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang X, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ, Gakidou E. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; **384**: 766-781 [PMID: [24880830](#) DOI: [10.1016/S0140-6736\(14\)60460-8](#)]
- 3 **NCD Risk Factor Collaboration (NCD-RisC)**. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet* 2017; **390**: 2627-2642 [PMID: [29029897](#) DOI: [10.1016/S0140-6736\(17\)32129-3](#)]
- 4 **McCormack L**, Dutkowski P, El-Badry AM, Clavien PA. Liver transplantation using fatty livers: always feasible? *J Hepatol* 2011; **54**: 1055-1062 [PMID: [21145846](#) DOI: [10.1016/j.jhep.2010.11.004](#)]
- 5 **Yersiz H**, Lee C, Kaldas FM, Hong JC, Rana A, Schnickel GT, Wertheim JA, Zarrinpar A, Agopian VG, Gornbein J, Naini BV, Lassman CR, Busuttil RW, Petrowsky H. Assessment of hepatic steatosis by transplant surgeon and expert pathologist: a prospective, double-blind evaluation of 201 donor livers. *Liver Transpl* 2013; **19**: 437-449 [PMID: [23408461](#) DOI: [10.1002/lt.23615](#)]
- 6 **Holówko W**, Mazurkiewicz M, Grąt M, Koperski L, Lewandowski Z, Smoter P, Ziarkiewicz-Wróblewska B, Górnicka B, Zborowska H, Krawczyk M. Reliability of frozen section in the assessment of allograft steatosis in liver transplantation. *Transplant Proc* 2014; **46**: 2755-2757 [PMID: [25380910](#) DOI: [10.1016/j.transproceed.2014.09.102](#)]
- 7 **Schwenzer NF**, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *J Hepatol* 2009; **51**: 433-445 [PMID: [19604596](#) DOI: [10.1016/j.jhep.2009.05.023](#)]
- 8 **Nilsson JH**, Reistad N, Brange H, Öberg CF, Stureson C. Diffuse Reflectance Spectroscopy for Surface Measurement of Liver Pathology. *Eur Surg Res* 2017; **58**: 40-50 [PMID: [27658312](#) DOI: [10.1159/000449378](#)]
- 9 **Le Naour F**, Gadea L, Danulot M, Yousef I, Vibert E, Wavelet M, Kaščáková S, Castaing D, Samuel D, Dumas P, Guettier C. Quantitative assessment of liver steatosis on tissue section using infrared spectroscopy. *Gastroenterology* 2015; **148**: 295-297 [PMID: [25461852](#) DOI: [10.1053/j.gastro.2014.11.038](#)]
- 10 **Evers DJ**, Westerkamp AC, Spliethoff JW, Pully VV, Hompes D, Hendriks BH, Prevoo W, van Velthuisen ML, Porte RJ, Ruers TJ. Diffuse reflectance spectroscopy: toward real-time quantification of steatosis in liver. *Transpl Int* 2015; **28**: 465-474 [PMID: [25556291](#) DOI: [10.1111/tri.12517](#)]
- 11 **Westerkamp AC**, Pully VV, Karimian G, Bomfati F, Veldhuis ZI, Wiersema-Buist J, Hendriks BH, Lisman T, Porte RJ. Diffuse reflectance spectroscopy accurately quantifies various degrees of liver steatosis in murine models of fatty liver disease. *J Transl Med* 2015; **13**: 309 [PMID: [26388419](#) DOI: [10.1186/s12967-015-0671-1](#)]
- 12 **Rajamani AS**, M D, Sai VVR. Plastic fiber optic sensor for continuous liquid level monitoring. *Sensors Actuators A Phys* 2019; **296**: 192-199 [DOI: [10.1016/j.sna.2019.07.021](#)]
- 13 **Cho MJ**, Pernarowski M. Application of absorbance ratios to analysis of pharmaceuticals. VI. Analysis of binary mixture using a reference spectrum. *J Pharm Sci* 1970; **59**: 1333-1335 [PMID: [5469801](#) DOI: [10.1002/jps.2600590926](#)]
- 14 **Cleverley B**, Dolby R. Absorbance Ratio Calculations from the Infrared Spectra of Thick Liquid Films. *Appl Spectrosc* 1978; **32**: 187-189 [DOI: [10.1366/000370278774331594](#)]
- 15 **Golse N**, Cosse C, Allard MA, Laurenzi A, Tedeschi M, Guglielmo N, Fernandez-Sevilla E, Robert M, Tréchet B, Pietrasz D, Pittau G, Ciacio O, Sa Cunha A, Castaing D, Cherqui D, Adam R, Samuel D, Sebag M, Vibert E. Evaluation of a micro-spectrometer for the real-time assessment of liver graft with mild-to-moderate macrosteatosis: A proof of concept study. *J Hepatol* 2019; **70**: 423-430 [PMID: [30399385](#) DOI: [10.1016/j.jhep.2018.10.034](#)]
- 16 **Coda S**, Siersema PD, Stamp GW, Thillainayagam AV. Biophotonic endoscopy: a review of clinical research techniques for optical imaging and sensing of early gastrointestinal cancer. *Endosc Int Open* 2015; **3**: E380-E392 [PMID: [26528489](#) DOI: [10.1055/s-0034-1392513](#)]
- 17 **Mundo AI**, Greening GJ, Fahr MJ, Hale LN, Bullard EA, Rajaram N, Muldoon TJ. Diffuse reflectance spectroscopy to monitor murine colorectal tumor progression and therapeutic response. *J Biomed Opt* 2020; **25**: 1-16 [PMID: [32141266](#) DOI: [10.1117/1.JBO.25.3.035002](#)]

# Impact of COVID-19 pandemic on liver, liver diseases, and liver transplantation programs in intensive care units

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## Abstract

Emerging worldwide data have been suggesting that coronavirus disease 2019 (COVID-19) pandemic consequences are not limited to the respiratory and cardiovascular systems but encompass adverse gastrointestinal manifestations including acute liver injury as well. Severe cases of liver injury associated with higher fatality rates were observed in critically ill patients with COVID-19. Intensive care units (ICU) have been the center of disposition of severe cases of COVID-19. This review discusses the pathogenesis of acute liver injury in ICU patients with COVID-19, and analyzes its prevalence, consequences, possible drug-induced liver injury, and the impact of the pandemic on liver diseases and transplantation programs.

**Key Words:** COVID-19; Critical care; Drugs; Liver; Liver transplantation; Outcome; Severe liver injury

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**Core Tip:** In this manuscript, liver dysfunction is seen more in patients with more severe disease upon presentation. It is difficult to separate the independent effect of viral infection from various treatment modalities, including antibiotics and experimental antiviral drugs that are used in these patients.

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## INTRODUCTION

More than a year ago, the global pandemic started from its epicentre in Wuhan. In coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the lung is the main organ targeted by the virus[1]. The organism exhibits a wide range of severity and a diverse disruption of extra-pulmonary systems, including gastrointestinal, renal, cardiac[2,3], hepatic[4], and even multi-organ damage[2,5]. Moderate or severe symptoms have been reported in almost 20% of all COVID-19 patients, while 5% progress into critical stages of the disease[6].

The rate of intensive care unit (ICU) admission due to COVID-19 is quite variable, ranging from 3% to 100% in literature[7]. The liver could be affected in COVID-19 through several mechanisms, including virus-related liver cell injury, disorganized immune response, drug-induced liver injury (DILI) and ischemic liver dysfunction in the settings of multisystem organ failure[8]. The reported rate of COVID-19-induced liver injury ranged from 14.8% in one study[9] and up to 74% in another[10]. In a case series of critically ill patients with COVID-19, liver injury was frequent but transient and non-severe[11]. Patients may not be equally affected by the pandemic, certain patient populations are potentially more vulnerable. Immunocompromised patients and patients with cirrhosis are probably more susceptible to worse outcomes after SARS-CoV-2 infection[5]. The data in literature on how chronic immunosuppression can influence COVID-19 outcomes is scarce[6]. This minireview will discuss the pathogenesis of acute liver injury in ICU patients with COVID-19, focusing on its prevalence, consequences, DILI, and its impact on existing liver diseases and liver transplantation programs.

## PATHOGENESIS OF LIVER INJURY IN COVID-19

Liver injury in COVID-19 can be related to the direct cytopathic effect of the virus, DILI, uncontrolled immune reaction, or sepsis[12]. SARS-CoV-2 ribonucleic acid has been detected in blood and stool samples of COVID-19 patients who presented with diarrhoea, indicating the liver's probable involvement in the disease pathogenesis[13, 14]. It has been suggested that there is a considerable expression of angiotensin-converting enzyme 2 (ACE2) receptors in cholangiocytes, where SARS-CoV-2 binding may adversely affect liver function. Moreover, COVID-19 may worsen the underlying chronic liver disease(s) (CLD), leading to hepatic decompensation or acute-on-chronic liver failure and increasing the risk of mortality, particularly in critically ill patients[12, 15-17]. However, in severe COVID-19, liver damage is more likely due to the inflammatory cytokine storm[12,18] rather than the direct cytopathic effects of the virus[12].

The progression of SARS-CoV-2 infection has been divided into four phases: Upper and lower respiratory tract infection, usually treated as outpatients, COVID-19 associated lung injury, usually treated as inpatients, systemic inflammatory response syndrome (SIRS), and systemic failure. Liver involvement is often observed in the latter phases but can also occur in the earlier ones. In SIRS, pro-thrombotic factors accumulate due to bone marrow and liver acute phase response causing thrombosis, whereas in the last phase, multi-organ vascular dysfunction and cytokine storm occur in view of the ongoing interaction between the lung and systemic inflammation[19].



Hypoxic hepatitis (HH), known as shock liver or ischemic hepatitis, is an acute liver injury resulting from liver hypoxia[20]. The extensive complex vascular supply together with high metabolic efficacy results in a liver vulnerable to circulatory disturbances. Critically ill patients with circulatory or respiratory manifestations which may influence liver perfusion are at higher risk of HH[3,21,22]. The mechanism by which SARS-CoV-2 infection leads to HH is not fully understood. Multiple theories have been postulated, including hypoxemia developed due to COVID-19 pneumonia [2] and systemic stress caused by SIRS[19]. Both may provide a route to a compensatory decrease in peripheral and splanchnic blood flow, resulting in decreased hepatic blood flow leading to hepatocellular hypoxia[23]. Reperfusion injury is mediated by the generation of reactive oxygen species when ischemic hepatocytes are re-exposed to oxygen, leading to cell injury *via* lipid peroxidation[24]. Waseem and Chen[21] defined the diagnostic criteria for HH as circulatory or respiratory failure with a dramatic but transient rise in serum aminotransferases activity when excluding other causes of liver cell necrosis, especially viral or drug-induced hepatitis[21]. A visual summary of liver injury in COVID-19 is presented in Figure 1.

## PREVALENCE AND CONSEQUENCES OF COVID-19-ASSOCIATED LIVER INJURY

The liver injury induced by COVID-19, including its pattern and severity, has not been uniformly defined or well characterized[25,26]. Some definitions reported in the literature, including DILI, are presented in Table 1. Secondary liver injury was the most common, being the first occurrence[3]. Liver injury has been reported as the elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels[25,26]. Thus, the liver injury appears to be of a hepatocellular (56%) rather than cholestatic (24%) or mixed (19%) pattern[3,25-28], while jaundice is uncommon[3]. Alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) as markers of bile duct injury have not increased significantly in the respective studies[3,25]. However, not all liver function tests (LFTs) have been strictly reported[29].

Liver injury in COVID-19, manifested as changes in LFTs, is usually mild and transient[26,30-33] and does not require treatment[30,32]. However, severe cases have been reported[26,30,32]. Mild, moderate, and severe injuries were reported in 45%-65%, 21%, and 6.4% of the cases, respectively[25-27]. In one study, mild elevation in LFTs levels was reported in 90% of patients admitted to hospital with marked elevation during hospital stay[34]. Elevation in AST levels is more common than ALT and other LFTs[29,30]. In one report, the acute liver injury occurred on day 17 after the onset of symptoms[28]. In patients with severe COVID-19, the elevation in the transaminases and bilirubin levels was at least double that in patients with mild and moderate disease[33]. Elevation in GGT levels was more noticeable in severe cases, while ALP levels usually remained normal in both mild and severe cases[35]. Variable and inconsistent degrees of LFTs abnormalities, ranging from 3.75% to more than 50% of all patients, have been described[5,25,33,36]. A meta-analysis found a pooled incidence of elevated liver enzymes by 23.1%[37]. Although some studies did not show a statistical difference in abnormal LFTs between patients with severe and non-severe disease[37,38] or between survivors and non-survivors[39], many other studies have consistently shown elevated LFTs to be more prevalent in fatal or severe disease[1,2,14,28,34,40-43] in up to 58%-78% of cases[40,44,45].

Patients with LFTs abnormalities had a more severe inflammation[25-27] and degree of organ dysfunction[27]. At least two meta-analyses have confirmed the association between liver injury and the severity of COVID-19[46,47]. Liver injury had prognostic implications in patients with COVID-19. Liver injury or abnormal LFTs were associated with increased risk of ICU admission[25,27,48,49], intubation[25,49], mechanical ventilation need[27], acute renal injury, vasopressor use[25,27], long hospital stays[27], mortality[25,27,28,37,48,49], and composite of ICU admission and mortality[27,50]. Tables 2 and 3 present selected liver injury-related markers and clinical outcomes of non-survivors[39,43,44,51-55], or patients with severe disease[1,2,9,28,34,40,42,56-63], including those admitted to ICU due to COVID-19[1,2,57].

## PRE-EXISTING LIVER DISEASE IN COVID-19-ASSOCIATED LIVER INJURY

Underlying CLD in patients with COVID-19 have been reported in several studies and

**Table 1** Reported definitions for liver injury in coronavirus disease 2019

Term	Definition(s)
Liver disorder	Serum ALT or AST > 2 × ULN, TB > 2 × ULN, ALP ≥ 2 ULN[75]
Liver injury or acute liver injury	ALT and/or AST above 3 × ULN, ALP, GGT, and/or TB above 2 × ULN[9,34] ALT and/or AST ≥ 2 × ULN, with TB ≥ 2 × ULN and/or INR ≥ 1.7[70] ALT levels above 3 × the ULN[28]
Mild liver injury	ALT above the ULN and below 2 × the ULN[25]
Moderate liver injury	ALT between 2-5 × the ULN[25]
Severe liver injury	ALT above 5 × the ULN[25] Any elevation of enzymes above 3 × the ULN and bilirubin above 2 × the ULN[5]
Liver test abnormalities	Elevation of the following serum liver enzymes: ALT > 40 U/L, AST > 40 U/L, GGT > 49 U/L, ALP > 135 U/L, and TB > 17.1 μmol/L[34]
De novo LFTs abnormality	The occurrence of abnormal LFTs in patients with normal LFTs at admission[27]
LFTs elevation	Increase in serum liver enzyme levels above the ULN[27,28]
Mild LFTs elevations	Elevation 1-2 times above the ULN[25,34]
Hepatocellular or hepatocyte type	The pattern of abnormal LFTs with predominantly elevated ALT and AST[27] Patients with raised ALT and/or AST more than 3 × the ULN[34] AST/ALT activity is higher than the ALP/GGT activity, with liver enzyme activities calculated by multiples of their ULN[34]
Cholestatic or cholangiocyte type	Pattern of abnormal LFTs with predominantly elevated ALP and GGT[27] Patients with raised ALP or GGT 2 × the ULN[34] ALP/GGT activity was higher than the AST/ALT activity, with the liver enzyme activities calculated by multiples of their ULN[34]
Mixed type	Mixed pattern when the extents of AST/ALT and ALP/GGT are similar[27] A combination of both ALT/AST elevated more than 3 × the ULN and ALP/GGT twice the ULN[34]
Drug-induced liver injury	Any elevation in liver enzymes or TB after the initiation of the drug in the absence of identified common causes of liver disease[5]

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio; LFTs: Liver function tests; TB: Total bilirubin; ULN: Upper limit of normal.

ranged from 2% to 11%[30,36,64], up to 19% in one study[65]. Pooled prevalence of pre-existing CLD in one meta-analysis was 3%[66], which was comparable to that of another meta-analysis (3.6%)[5]. The latter reported pooled prevalence of CLD of 3.9% and 4.7% among severely infected patients and the non-survivors, respectively[5]. Compared with patients without underlying liver diseases, the odds ratio (OR) of developing severe disease was 0.81 [95% confidence interval (CI): 0.31-2.09,  $P = 0.67$ ][67]. The presence of underlying liver disease was associated with increased the risk of mortality and hospitalization, before {[risk ratio (RR): 2.8, 95%CI: 1.9-4.0,  $P < 0.001$ ]; (RR: 1.7, 95%CI: 1.2-2.0,  $P < 0.001$ )} and after propensity matching [(RR: 3.0, 95%CI: 1.5-6.0,  $P = 0.001$ ); (RR: 1.3, 95%CI: 1.1-1.6,  $P = 0.006$ )], when compared to those without liver diseases, respectively[68].

The presence of CLD was also found to be an independent predictor for ICU admission (adjusted OR 1.77, 95%CI: 1.03-3.04,  $P = 0.04$ ) and mechanical ventilation need (adjusted OR 2.08, 95%CI: 1.20-3.60,  $P = 0.0092$ )[65]. The reported etiologies of the pre-existing liver diseases before COVID-19 included chronic viral hepatitis B and C, alcoholic and metabolic liver disease, cirrhosis of any cause, and others[5,26,31]. Liver cirrhosis is the end-stage of these liver-related diseases[31]. In one study ( $n = 363$ ), 19% of patients had a pre-existing liver disease with the predominance of non-alcoholic fatty liver disease (NAFLD) (79.7%). Compensated cirrhosis, decompensated cirrhosis, and viral hepatitis B and C accounted for 8.7%, 4.3%, 2.9%, and 8.7% of all patients, respectively[65]. In contrast, the reported rates in one meta-analysis of 107 studies ( $n = 20874$ ) were, CLD/cirrhosis in 61.1%, NAFLD in 19.5%, hepatitis B in 17.8%, and hepatitis C in 0.73% of patients[5].

Table 2 Reported data on survivors versus non-survivors in coronavirus disease 2019

Ref.	N (all) n (non-survivors)	Age (year)	Male	Pre-existing CLD	Type of liver disease	Elevated LFTs on admission (%)	LFTs levels on admission. ALT/AST/ALP/GGT (U/L)/TB (μmol)	Selected complications or clinical outcomes
Cao <i>et al</i> [51]. China	N = 102 (n = 17)	53 vs 72	47.1% vs 76.5%	2.4% vs 5.9%	-	ALT: NR vs 41.1%	ALT: NR vs 40	ALI: 24.7% vs 76.5%; ARDS: 5.9% vs 88.2%; Shock: 3.5% vs 41.1%; MV: 2.4% vs 70.6%
Chen <i>et al</i> [52]. China	N = 274 (n = 113)	51 vs 68	55% vs 73%	-	HBV surface antigen positivity	ALT: 19% vs 27%; AST: 16% vs 52%	ALT: 20 vs 28; AST: 25 vs 45; ALP: 64 vs 76; GGT: 28 vs 42; TB: 8.4 vs 12.6	ALI: 2% vs 9%; ARDS: 52% vs 100%; Shock: 0% vs 41%; MV: 82% vs 16%
Chen <i>et al</i> [53]. China	N = 55 (n = 19) <sup>1</sup>	72 vs 77	50% vs 84.2%	2.8% vs 5.3%	-	ALT: 19.4% vs 31.6%; AST: 50% vs 73.7%	ALT: 40 vs 44; AST: 55 vs 78	MV: 30.6% vs 68.4%
Du <i>et al</i> [54]. China	N = 85 <sup>2</sup>	65.8	72.9%	5.9%	-	ALT: 16.5%; AST: 32.9%; TB: 35.3%	ALT: 72.9; AST: 94.4; TB: 18.4	ALI: 35.3%; ARDS: 74.1%; Shock: 81.2%; MV: 93% <sup>3</sup>
Wu <i>et al</i> [42]. China	N = 84 (n = 44) <sup>4</sup>	50 vs 68.5	77.5% vs 65.9%	3.5% <sup>5</sup>	-	-	ALT: 35 vs 39; AST: 38.5 vs 37; TB: 11.6 vs 14.5	MV: 57.5% vs 97.8% <sup>3</sup> ; Others reported as association
Yang <i>et al</i> [55]. China	N = 92 <sup>2</sup>	69.8	53.3%	3.3%	-	-	ALT: 27; AST: 31; TB: 13.6	ALI: 16.5%; ARDS: 80.2%; MODS: 15.4%
Yang <i>et al</i> [39]. China	N = 52 (n = 32)	51.9 vs 64.6	70% vs 66%	-	-	-	TB: 13.1 vs 19.5	ALI: 30% vs 28%; ARDS: 45% vs 81%; MV: 35% vs 94%
Zhang <i>et al</i> [44]. China	N = 82 <sup>2</sup>	72.5	65.9%	2.4%	-	ALT: 30.6%; AST: 61.1%; TB: 30.6%	ALT: 26; AST: 72; TB: 13.6	Hepatic damage: 78%; Liver-associated death: 1.2%; MV: 40.2%
Zhou <i>et al</i> [43]. China	N = 191 (n = 54)	52 vs 69	59% vs 70%	-	-	ALT: 24% vs 48%	ALT: 27 vs 40	ARDS: 7% vs 93%; Shock: 0% vs 70%; MV: 2% vs 100% <sup>3</sup>

<sup>1</sup>Patients ≥ 65 years subgroup (55 of 203 patients).<sup>2</sup>Reported fatal cases only.<sup>3</sup>Invasive and non-invasive mechanical ventilation.<sup>4</sup>Subgroup of patients who developed acute respiratory distress syndrome (ARDS) after admission and those who progressed from ARDS to death (total patients = 201).<sup>5</sup>Reported for all patients.

ALT: Alanine aminotransferase; ALI: Acute liver injury; ALP: Alkaline phosphatase; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; CLD: Chronic liver disease; GGT: Gamma-glutamyl transpeptidase; HBV: Hepatitis B virus; LFTs: Liver function tests; MODS: Multiple organ dysfunction syndrome; MV: Mechanical ventilation; N and n: Number of patients; NR: Not reported; TB: Total bilirubin.

Hepatitis B virus co-infection may subject COVID-19 patients to an exacerbated liver injury[30] and a more severe disease[69]. Acute liver injury in COVID-19 patients with hepatitis was significantly higher than that in patients without chronic hepatitis (15.0% vs 7.0%,  $P < 0.001$ )[70]. Patients with NAFLD, renamed as metabolic-associated fatty liver disease[26,31], had a significantly higher likelihood of abnormal LFTs, longer viral shedding time, and higher rate of COVID-19 progression (OR: 6.4, 95%CI: 1.5-31.2), compared to those without NAFLD[71]. NAFLD was significantly associated with ICU admissions (adjusted OR: 2.30, 95%CI: 1.27-4.17,  $P = 0.03$ ) and mechanical ventilation need, (adjusted OR: 2.15, 95%CI: 1.18-3.91,  $P = 0.02$ ) but not with mortality [65]. Furthermore, NAFLD in younger patients (< 60 years) was associated with the prevalence of severe COVID-19 (adjusted OR: 2.67, 95%CI: 1.13-6.34,  $P = 0.03$ )[72]. COVID-19 patients with liver cirrhosis were found to be at increased risk of mortality compared with those without the disease (RR: 4.6, 95%CI: 2.6-8.3,  $P < 0.0001$ )[68,71]. Multivariate analysis showed that liver cirrhosis was an independent predictor for mortality (adjusted OR: 12.5, 95%CI: 2.16-72.5,  $P = 0.009$ ) but not for ICU admission or mechanical ventilation need[65].

**Table 3** Reported data on critically ill, intensive care units, or severe coronavirus disease 2019 patients

Ref.	N (all), n (severe disease). Patient population	Age (year)	Male	Pre-existing CLD	Type of pre-existing CLD	Elevated LFTs on admission (%)	LFTs levels on admission. ALT/AST/ALP/GGT (U/L)/TB (μmol)	Selected complications or clinical outcomes
Arentz <i>et al</i> [56]. United States	N = 21. Critically ill	70	52%	4.8%	Cirrhosis	-	ALT: 108; AST: 273; ALP: 80; TB: 0.6 mg/dL	ALI: 14.3%; Severe ARDS: 57.1%; MV: 71%; Death: 52.4%
Cai <i>et al</i> [34]. China	N = 318 (n = 85) <sup>1</sup> . Non-severe vs severe	47. All patients	47.5%. All patients	5%. All patients	ALD, NAFLD, HVB	ALT: 6.4% vs 21.1%; AST: 0.68% vs 18.8%; GGT: 5.1% vs 29.4%; TB: 1.2% vs 7%	-	MOF: 0% vs 11.7%
Cai <i>et al</i> [9]. China	N = 298 (n = 58). Non-severe vs severe	41 vs 62.5	44.1% vs 67.2%	8.3% vs 13.7%	NAFLD: 3.3% vs 10.3%; ALD: 3.3% vs 1.7%; HBV: 1.7% vs 1.7%	-	ALT: 20 vs 26.8; AST: 26 vs 36; ALP: 61 vs 58; GGT: 21 vs 35.2; TB: 10.9 vs 11.2	ALI: 9.6% vs 36.2%; Discharge: 93.3% vs 75.9%; Hospital-stay: 19 d vs 27 d; Death: 0% vs 5.2%
Du <i>et al</i> [57]. China	N = 109. Non-ICU vs ICU	72.7 vs 68.4	65.5% vs 70.6%	3.4% vs 0%	-	ALT: 13.8% vs 19.6%; AST: 49% vs 43.1%	ALT: 21.6 vs 27; AST: 32 vs 40	Invasive MV: 0% vs 64.7%; Hospital-stay: 12.5 d vs 15.9 d
Guan <i>et al</i> [40]. China	N = 1099 (n = 173). Non-severe vs severe	45 vs 52	58.2% vs 57.8%	2.4% vs 0.6%	HBV	ALT: 19.8% vs 28.1%; AST: 18.2% vs 39.4%; TB: 9.9% vs 13.3%	-	ARDS: 1.1% vs 15.6%; MV: 0% vs 38.7%; Discharge: 5.4% vs 2.9%; Hospital-stay: 11 d vs 13 d; Death: 0.1% vs 8.1%
Huang <i>et al</i> [2]. China	N = 41 (n = 13). Non-ICU vs ICU	49 vs 49	68% vs 85%	4% vs 0%	-	AST: 25% vs 62%	ALT: 27 vs 49; AST: 34 vs 44; TB: 10.8 vs 14	ARDS: 4% vs 85%; Shock: 0% vs 23%; Invasive MV: 0% vs 15%; Discharge: 75% vs 54%; Death: 4% vs 38%
Lei <i>et al</i> [28]. China	N = 5771 (n = 1186). Non-severe vs severe	55 vs 59	45.1% vs 55.3%	1.2% vs 2.1%	Viral hepatitis Cirrhosis	-	ALT: 23 vs 26; AST: 22 vs 31; ALP: 65 vs 63; TB: 10.3 vs 10.6	Reported as association not absolute values
Li <i>et al</i> [58]. China	N = 548 (n = 269). Non-severe vs severe	56 vs 65	45.2% vs 56.9%	1.1% vs 0.7%	HBV	ALT: 22.3% vs 24.1%; AST: 23.3% vs 43.4%; TB: 2.3% vs 6.4%	-	ALI: 15.8% vs 23%; ARDS: 9.7% vs 68%; MV: 4% vs 34.2% <sup>2</sup> ; Discharge: 72.9% vs 31.7%; Death: 1.1% vs 32.5%
Mo <i>et al</i> [59]. China	N = 155 (n = 85) <sup>3</sup> . General vs refractory	47 vs 61	44.3% vs 64.7%	2.9% vs 5.9%	-	-	ALT: 20 vs 28; AST: 32 vs 37	Critical case: 4.3% vs 40%; MV: 0% vs 41.2%; Others reported as association
Wan <i>et al</i> [60]. China	N = 135 (n = 40). Mild vs severe	44 vs 56	54.7% vs 52.5%	1% vs 2.5%	-	AST: 16% vs 37.5%	ALT: 21.7 vs 26.6; AST: 22.4 vs 33.6; TB: 8.6 vs 9.8	ARDS: 1.1% vs 50%; Shock: 0% vs 2.5%; Discharge: 10.5% vs 12.5%; Death: 0% vs 2.5%
Wang <i>et al</i> [1]. China	N = 138 (n = 36). Non-ICU vs ICU	51 vs 66	52% vs 61.1%	3.9% vs 0%	-	-	ALT: 23 vs 35; AST: 29 vs 52; TB: 9.3 vs 11.5	ARDS: 4.9% vs 61.1%; Shock: 1% vs 30.6%; Invasive MV: 0% vs 47.2%
Wu <i>et al</i> [42]. China	N = 201 (n = 84) <sup>4</sup> . Non-ARDS vs ARDS	48 vs 58.5	58.1% vs 71.4%	3.5% <sup>5</sup>	-	-	ALT: 27 vs 35; AST: 30 vs 38; TB: 10.5 vs 12.9	MV: 0% vs 78.6% <sup>2</sup> ; Others reported as association
Zhang <i>et al</i> [61]. China	N = 221 (n = 55). Non-severe vs severe	51 vs 62	44% vs 63.6%	1.8% vs 7.3%	-	-	ALT: 22 vs 32; AST: 27 vs 51; TB: 9.6 vs 11.4	ARDS: 0% vs 87.3%; Shock: 0% vs 27.3%; MV: 1.2% vs 74.6% <sup>2</sup> ; Discharge: 21.1% vs 12.7%; Death: 0% vs 21.8%
Zhang <i>et al</i> [62]. China	N = 140 (n = 58). Non-severe vs severe	51.1 vs 64	46.3% vs 56.9%	5% vs 6.9%	Fatty liver and abnormal liver function	-	-	-

Zheng <i>et al</i> [63]. China	N = 161 (n = 40 vs 57 30). Non-severe vs severe	50.4% vs 46.7%	3.1% vs 0%	-	ALT: 6.1% vs 16.7%; AST: 7.6% vs 40%; TB: 4.6% vs 10%	ALT: 19.3 vs 23.9; AST: 23.4 vs 31.6; TB: 10.7 vs 12.7	-
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<sup>1</sup>Total number of patients is 417 and 318 is the number for patients with liver injury (for which the comparison between severe and non-severe disease was done).

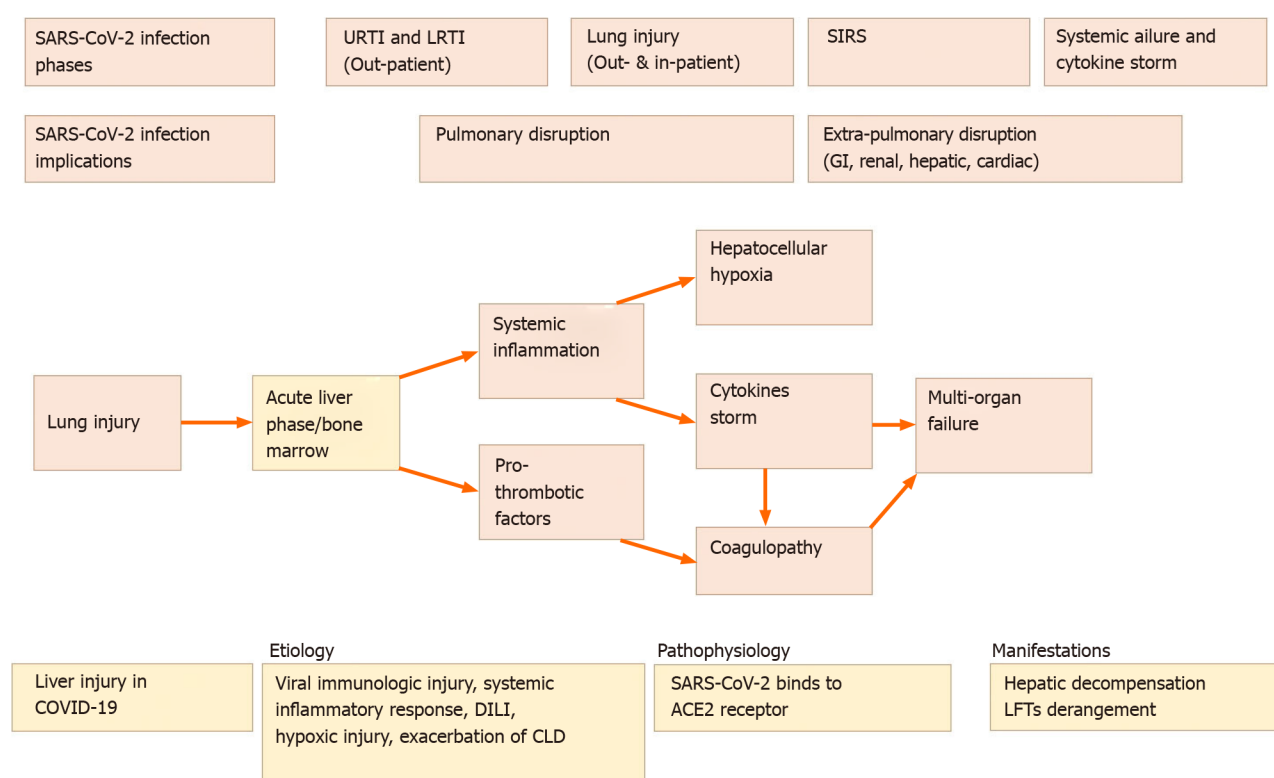
<sup>2</sup>Invasive and non-invasive mechanical ventilation.

<sup>3</sup>Reported patients with refractory and critical illness and  $\geq 10$  d of treatment in hospital.

<sup>4</sup>Subgroup of patients who developed acute respiratory distress syndrome (ARDS) after admission and those who progressed from ARDS to death.

<sup>5</sup>Reported for all patients.

ICU: Intensive care units; ALD: Alcoholic liver disease; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ALI: Acute liver injury; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; CLD: Chronic liver disease; GGT: Gamma-glutamyl transpeptidase; HBV: Hepatitis B virus; LFTs: Liver function tests; MOF: Multiorgan failure; MV: Mechanical ventilation; N and n: Number of patients; NAFLD: Non-alcoholic fatty liver disease; TB: Total bilirubin.



**Figure 1 Pathogenesis of liver injury in coronavirus disease 2019.** ACE2: Angiotensin-converting enzyme 2; COVID-19: Coronavirus disease 2019; CLD: Chronic liver disease(s); DILI: Drug-induced liver injury; GI: Gastrointestinal; LRTI: Lower respiratory tract infection; LFTs: Liver function tests; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; SIRS: Systemic inflammatory response syndrome; URTI: Upper respiratory tract infection.

## DILI IN PATIENTS WITH COVID-19

Numerous medications that are currently used to treat SARS-CoV-2 infection carry the risk of hepatotoxicity. Given that many medications are being used in combination, the interpretation of the commonly seen raised liver transaminases in patients with COVID-19 can be biased. While the efficacy of these medications towards improving COVID-19's morbidity and mortality is still to be proven, their safety should be monitored closely[73]. A retrospective study aimed to investigate adverse drug reactions (ADRs) in 217 COVID-19 patients using a hospital pharmacovigilance system in China found that 82 patients experienced 94 ADRs, with 13.8% of them were categorized as liver disorders. A multivariate analysis showed that the occurrence of ADRs has been associated with the length of stay (OR: 2.02, 95%CI: 1.03–3.96,  $P = 0.04$ ), number of drugs used in hospital (OR: 3.12, 95%CI: 1.60–6.27,  $P = 0.001$ ) and underlying diseases (OR: 2.07, 95%CI: 1.02–4.23,  $P = 0.045$ )[73]. In a prospective study using pharmacovigilance system in Spain, patients with COVID-19 had a higher incidence of hepatitis as a serious ADR than that in non-COVID-19 patients (45.1% vs 23.7%)[74]. In a meta-analysis of 10 studies, DILI in COVID-19 was reported in 25.4%



of the total patients[5]. Therapies that have been implicated in hepatotoxicity included remdesivir, lopinavir/ritonavir, oseltamivir, hydroxychloroquine, paracetamol[5], tocilizumab[74], in addition to antibiotics, non-steroidal anti-inflammatory drugs, herbal medications, and interferon[34]. In a retrospective, observational cohort study ( $n = 1827$ ), the use of lopinavir/ritonavir, hydroxychloroquine, remdesivir, and tocilizumab was associated with statistically significant abnormal ALT and AST levels (*i.e.*,  $> 5 \times$  upper limit of normal)[75].

Data on DILI's clinical significance have not been consistent. Sun *et al*[73] reported 18.1% of ADRs to be of serious severity, with 82.3% of them related to liver injury[73]. Ramirez *et al*[74] reported a mortality rate of 30.5% in COVID-19 patients with serious ADRs compared with 3.9% in non-COVID-19 patients with serious ADRs[74]. However, Kulkarni *et al*[5] concluded that remdesivir and lopinavir/ritonavir DILI was not life-threatening[5]. A systematic review and network meta-analysis of 110 studies reported no association between a regimen or an agent with non-cardiac severe adverse events[76]. In a multicenter and retrospective study ( $n = 565$ ) on hospitalized COVID-19 patients, *de novo* LFTs abnormality was noted with tocilizumab (82% *vs* 52%;  $P = 0.009$ ) and lopinavir/ritonavir (64% *vs* 48%;  $P = 0.045$ ). Moreover, there was a trend towards an increased composite endpoint of death or transfer to ICU associated with *de novo* LFTs abnormality with an incidence of 14% *vs* 5% ( $P = 0.069$ )[27]. Although published data regarding the incidence, severity and clinical significance of DILI have not been consistent, it warrants close monitoring of LFTs. Table 4 summarizes the reported DILI of selected therapies against COVID-19[5,27,34,74,75,77-95].

## DRUG-DRUG INTERACTIONS BETWEEN IMMUNOSUPPRESSIVE THERAPY AND COVID-19 AGENTS

Calcineurin inhibitors (CNIs), such as cyclosporine and FK506 (tacrolimus), antimetabolite drugs, such as mycophenolate mofetil (MMF), mycophenolic acid, and corticosteroids are commonly used for immunosuppression after liver transplantation (LTX) [96]. Some centres adopted dose modifications based on expert opinion with many uncertainties regarding the best approach for combination therapies and immunosuppressive agents against COVID-19. In two large academic centers in New York City including 90 patients with solid organ transplant, antimetabolite drugs doses were reduced or held in 88% of patients, steroids in 7%, and CNIs in 18%, with no reported acute rejection cases at 20-day follow-up[6]. In a prospective European study of 57 liver transplant patients with COVID-19, immunosuppression therapy doses were reduced in 39% of patients and discontinued in 7%. Reduction or continuation of therapy did not affect mortality, while the discontinuation effect was not assessed[97]. Drug interactions between COVID-19 medications and immunosuppression therapy were also considered. For instance, lopinavir-ritonavir combination interacts with CNIs and MMF, it is not recommended to be used with steroids[98] and has been reported to interact with mechanistic target of rapamycin (mTOR) as well[18]. Moreover, tocilizumab may decrease CNIs plasma concentration, unlike remdesivir which does not interact with the immunosuppressive drugs[98]. Hydroxychloroquine has been reported to interact with CNIs and mTOR[18]. Relevant recommendations included checking for drug interactions[18,98], dose reduction of steroids, CNIs and MMF[98,99], switching mTOR to CNIs[18], switching MMF and CNIs to steroids, and withdrawal of agents such as CNIs and MMF in severe COVID-19[99]. Monitoring of immunosuppressive drug levels should be warranted when possible[98,99]. The European Society of Clinical Microbiology and Infectious Diseases advised not to reduce the doses of immunosuppressive drugs in liver transplanted patients and raised the importance of considering vaccination with *Streptococcus pneumonia* and influenza vaccines[100].

## IMPACT OF COVID-19 ON LIVER TRANSPLANTATION PROGRAMS

### General measures

The unprecedented disturbance created by the COVID-19 pandemic has impacted different sectors of health care systems worldwide. For instance, elective services were cancelled or postponed while lifesaving transplant programs, including those for a liver transplant, have been continued. However, the non-lifesaving transplant services

**Table 4** Reported effects of selected coronavirus disease 2019 therapies on liver

Medication (class)	Pattern of liver injury	Evidence
Corticosteroids (Anti-inflammatory agent)	Acute liver injury[77]	Multicenter cohort study ( $n = 774$ ); COVID-19 with ARDS: Incidence of ALI versus control (18.3% <i>vs</i> 9.9%; $P = 0.001$ ) [77]  Meta-analysis; critically ill COVID-19 patients: No association with serious adverse effects[78]  RECOVERY trial: No reported serious ADRs or DILI[79]
Favipiravir (RdRp inhibitor)	Abnormal LFTs[80]  Elevation of transaminases levels[81]	RCT ( $n = 150$ ); mild-to-moderate COVID-19: Abnormal LFTs versus control 6.8% <i>vs</i> 2.7%[80]  RCT; moderate COVID-19: Elevated ALT and AST were reported[81]
Hydroxychloroquine (Antimalarial agent)	Liver toxicity is not common[82]. Elevation of transaminases levels[74,75,82-84]	Retrospective study ( $n = 153$ ): Elevation in AST (11%) and ALT (9%)[82]  RCT ( $n = 504$ ); mild-to-moderate COVID-19: Elevation in ALT or AST elevation 10.6% in HCQ plus azithromycin, 9% in HCQ, and 3.5% in control arm ( $P = 0.008$ )[83]  Systematic review: Elevations of LFTs was transient[84]  Recovery trial: No reported DILI[85]
Interferon	-	Data on safety in COVID-19 patients is scarce
Lopinavir/ritonavir (Protease inhibitor)	Rise in liver function parameters[5,27,34,74,86]  Hyperbilirubinemia[5,34]	RCT ( $n = 199$ ): Elevated AST versus control (2.1% <i>vs</i> 5.1%), elevated ALT (1.1% <i>vs</i> 1%), elevated TB (3.2% <i>vs</i> 3%)[86]  Meta-analysis: DILI in 37.2% of patients (as hyperbilirubinemia followed by elevation of transaminases)[5]
Remdesivir (RdRp inhibitor)	Not well established. Elevation of transaminases levels[5,75,87-89]. Elevation of TB levels[88]. Hypoalbuminemia[88]	Case series: Elevated aminotransferases in 23 % discontinuation in 4% of patients[87]  RCT ( $n = 237$ ) in severe COVID-19: Elevated TB versus placebo (10% <i>vs</i> 9%) and AST (5% <i>vs</i> 12%), hypoalbuminemia (13% <i>vs</i> 15%). Discontinuation in 1% of patients[88]  Open-label, phase 3 trial: Elevated ALT (5%-6%) and AST (7%-8%)[89]  Meta-analysis: Pooled incidence of DILI of 15.2%[5]  Meta-analysis: No difference as compared to placebo in liver enzymes elevation[90]
Tocilizumab (Humanized recombinant monoclonal antibody)	Elevation of transaminases levels[27,75,91-94]. Liver injury as early as 24 h with a 40-fold increase in transaminases that normalized in 10 d[91]	Case series; 7 severe COVID-19 patients: Up to 4.5 folds elevated baseline ALT and AST. Transaminases normalized in 3 wk[92]  Retrospective study ( $n = 1827$ ): AST $> 5 \times$ ULN in 69.1%, and ALT $> 5 \times$ ULN in 72.1% of patients[75]  Observational study ( $n = 104$ ): Minor increase of AST, ALT ( $P < 0.001$ ) and GGT ( $P = 0.003$ ; no safety concerns on follow up[93]  RCT ( $n = 243$ ): ALT elevation versus placebo (5% <i>vs</i> 4.9%), AST elevation in 3.7%[94]  RCT ( $n = 130$ ); moderate or severe COVID-19: No increase in hepatitis risk[95]

COVID-19: Coronavirus disease 2019; ADRs: Adverse drug reactions; ALI: Acute liver injury; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ALI: Acute liver injury; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; DILI: Drug-induced liver injury; GGT: Gamma-glutamyl transpeptidase; HCQ: Hydroxychloroquine; LFTs: Liver functions tests; RCT: Randomized controlled trial; RdRp: RNA-dependent RNA polymerase; TB: Total bilirubin; ULN: Upper limit of normal.

were frequently delayed, exposing patients to emergency situations[101]. LTX is the most common solid organ transplantation procedure after the kidney, with a global rate of 3.7 *per* million population[102]. The indication of LTX in the acute phases of liver diseases includes acute liver cell failure, metabolic liver diseases, advanced

complicated cirrhosis, and CLD associated with systemic complications[103]. Elective LTX indications include advanced cirrhosis associated with deteriorating synthetic function, renal function, and the related complications[104]. The general precautions before LTX currently comprise a COVID-19 testing for both donors and recipients awaiting transplant and consenting for the possible hazard of acquiring nosocomial COVID-19[100]. The standard method of COVID-19 testing is through a nasopharyngeal swab or intraoperative bronchoalveolar lavage. Viral load should be measured in positive cases. The transplant team should be adequately screened, and the risk of exposure identified[101]. Hollander and Carr[105] advised on the use of telemedicine, such as virtual clinics or *via* phone calls, to minimize both healthcare providers and patient's exposure to COVID-19[105]. The success of telemedicine in the Chinese territory during the peak of the pandemic could be transposed to future networking to use information and communication technology extensively during the care of patients with COVID-19[106].

### ***ICU care of liver transplant patients in the era of COVID-19***

Strict infection control measures are required in the post-operative care of LTX patients to prevent nosocomial infections that include COVID-19[107]. During the admission of LTX patients, they should be directed to separate rooms away from the general wards, and strict disinfection and isolation practices should be in place. Medical and surgical rounds should be minimized, and laboratory testing and radiological studies should be reduced to the least required[108]. Acquiring symptoms suggestive of COVID-19 in a LTX patient should prompt urgent evaluation with the relevant investigations[105]. Other challenges prompted by the COVID-19 pandemic include the increased demand for ICU beds, requiring health care practitioners to work in a dynamic way to maximize ICU bed utilization[109]. During the pandemic, the settings required for ICU should include separate units equipped with high-efficiency particulate air filters[110]. The goals of ICU disposition for LTX patients comprise neurological monitoring, hemodynamic monitoring and support, early weaning from the mechanical ventilator, preventing nosocomial infections and graft-related complications and enhancing early graft recovery[111]. Some institutes screen for COVID-19 in LTX recipients[112]. Simple and effective measures could be implemented to shorten the ICU stay for LTX patients through fast-track procedures, including operating room early extubation, reduction of ventilation time, and direct transfer from the recovery room to surgical wards[113,114]. Transplant services constantly demand resources, which have become extremely limited with the emergence of the COVID-19 pandemic due to staff shortage, saturation of the ICU, and drainage of supplies. Exceptional scarcity of donors and demand for organs also aggravate this problem[115,116].

### ***Transplantation outcome during COVID-19 pandemic***

Reports regarding the outcomes of LTX patients have been inconsistent. Although early reports have not found more severe or worse outcomes among immunosuppressed patients[117], subsequent data showed that solid organ transplant recipients diagnosed with COVID-19, including LTX, seemed to be at increased risk of severe disease, morbidity, and poor outcomes[6,118], such as high mortality with an in-hospital mortality rate of 29%[119]. Bossini *et al*[120] reported a higher rate of acute respiratory distress syndrome (ARDS) and death among patients who received solid organs[120], while others reported similar outcomes in COVID-19 patients with and without solid organ transplant[121]. In a multi-centre study of ICU patients after solid organ transplant, the rate of ARDS, duration of mechanical ventilation, vasopressors requirements, and death were similar between groups[122].

### ***Vaccination considerations***

It was reported that in liver transplant recipients, COVID-19 infection was not associated with increased mortality. However, these patients are subjected to severe disease, as evidenced by a higher rate of both ICU admission and mechanical ventilation use[123]. The European Association for the Study of the Liver (EASL) suggested a particular form of judging the vaccination decision based on patient's morbidities[124]. The immune response to COVID-19 vaccination could be lower in LTX patients when compared with healthy subjects. Poor response to vaccination is affected by age, renal function, and enhanced immune suppression[125].



## IMPLICATIONS AND FUTURE DIRECTIONS

The COVID-19 pandemic has been presented as an unprecedented global health care crisis, causing significant setbacks among various health care services including the management of CLD. Besur *et al*[35] reported that screening for CLD, its complications and regular follow up visits were deferred which affected slowing or reversing the progression of CLD and worsened the prognosis of patients with CLD. Late identification of CLD complications such as hepatocellular carcinoma (HCC) could also affect the clinical outcomes in these patients. Social distancing measures have put CLD patients at risk of malnutrition, reduced mental health capacity, and decompensation [35]. The evidence associating acute liver injury with poor patients' outcomes and increased severity of COVID-19 is growing, and more research is necessary to further explain the relation between liver biomarkers changes and patients' outcomes in COVID-19[126]. Various factors influence the course of COVID-19, and there is a need for international collaborative registries to clarify the full spectrum of the disease. The registries, Surveillance Epidemiology of Coronavirus (COVID-19) Under Research Exclusion (SECURE-Cirrhosis) and Coronavirus (COVID-19) in liver disease reporting registry (COVID-HEP) were established to report data on patients with liver disease. The last report published in August 2020 by SECURE-Cirrhosis and the EASL supported COVID-Hep, reported 158 deaths (31%) among patients who had cirrhosis and developed SARS-CoV-2 infections[127]. When this article was written, the latest update from both COVID-Hep and SECURE-Cirrhosis registries reported 1341 cases that included 645 cases with cirrhosis, 205 liver transplant recipients and 270 deaths as of February 12, 2021[128].

COVID-19 pandemic has disrupted various healthcare services worldwide, limiting the services offered to urgent and emergent cases. These changes in services, clinician behaviour and re-organization of hospital activities can indirectly affect morbidity and mortality[129]. delaying or halting diagnostic and therapeutic services for diseases with a high global burden such as cardiovascular diseases can contribute to long-term and indirect adverse health outcomes. For example, cardiac diagnostics procedures, stress tests[129], emergency department (ED) and hospital admissions, procedures and treatments were markedly declined during the pandemic year as compared with that of the previous years[130]. In 909 inpatient and outpatient centres from 108 countries, the rate of cardiac diagnostic procedures decreased by 42% and 64% as of March and April 2020, respectively, with the highest reduction of 78% observed for the stress tests, as compared with March 2019.[130]. A further 22% reduction was noted in low and low-middle income countries, which might be attributed to inaccessible personal protective equipment and telehealth services[130].

In United Kingdom, a cross-sectional study conducted in nine hospitals compared the hospitals' cardiovascular activity data between October 2019 and May 2020 with the respective weeks in 2018 and 2019. There was a marked decline in ED attendances, admissions and hospital procedures and treatments[129]. Patients with other chronic diseases which require close follow up have been negatively affected as well. A cross-sectional study of six referral centres in France showed that in 2020 significantly fewer patients with HCC were referred to the multidisciplinary tumour board ( $P = 0.034$ ) and fewer received the first diagnosis of HCC ( $P = 0.083$ ) compared with 2019[131]. Therapy optimization and frequency of follow-up visits were also affected by the global pandemic in response to social distancing and re-allocation of services towards fighting COVID-19. A delay in therapy modification for more than one month was noted in 21.5% *vs* 9.5% of patients during 2020 compared with 2019 ( $P < 0.001$ ), respectively[131]. In patients with hepatitis C virus who were following up for HCC, there was significant reduction in their scheduled visits, *i.e.*, by before 75%, 63.0%, and 49.1% in March to May 2020, respectively, compared with 97% before February 2020 [132]. Surgical interventions for HCC have significantly declined or stopped across many centers in the world due to increased risk of blood transfusion, ICU stay, prolonged hospitalization and developing COVID-19 after surgery[133]. In a national survey by the Italian Association for the Study of the Liver, HCC treatment was affected; where surgical treatment was reduced in 44% and suspended in 44% of the participating centres, while the loco-regional treatment was reduced in 34% and suspended in 8% of the centres[134].

## CONCLUSION

The pathogenesis and characteristics of COVID-19-related multifactorial liver injury

can be explained by multiple mechanisms. The knowledge about the full spectrum of SARS-CoV-2 infection is being accumulated, given the novelty of the disease and the constantly reported new data. Liver dysfunction is commonly seen in patients presenting with the severe form of COVID-19. Various therapeutic options used for COVID-19 can lead to DILI and contribute to the exacerbation of the existing liver injury. It is challenging to identify the causal factor in the settings of infection, sepsis, and/or hypoxia, especially when the liver enzymes abnormalities are non-specific. The underlying liver disease has not been linked with poor outcomes. Hospitalized patients or those with liver comorbidities should be monitored closely. Patients with COVID-19 and LTX must maintain strict infection control and monitor drug interactions while maintaining immunosuppressive therapy at regular doses. Future research would help explain liver injury associated with SARS-CoV-2 infection and design specific guidelines for the management of COVID-19 in these patients.

## REFERENCES

- 1 **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020; **323**: 1061-1069 [PMID: [32031570](#) DOI: [10.1001/jama.2020.1585](#)]
- 2 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: [31986264](#) DOI: [10.1016/S0140-6736\(20\)30183-5](#)]
- 3 **Yang RX**, Zheng RD, Fan JG. Etiology and management of liver injury in patients with COVID-19. *World J Gastroenterol* 2020; **26**: 4753-4762 [PMID: [32921955](#) DOI: [10.3748/wjg.v26.i32.4753](#)]
- 4 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: [32085846](#) DOI: [10.1016/S2213-2600\(20\)30076-X](#)]
- 5 **Kulkarni AV**, Kumar P, Tevethia HV, Premkumar M, Arab JP, Candia R, Talukdar R, Sharma M, Qi X, Rao PN, Reddy DN. Systematic review with meta-analysis: liver manifestations and outcomes in COVID-19. *Aliment Pharmacol Ther* 2020; **52**: 584-599 [PMID: [32638436](#) DOI: [10.1111/apt.15916](#)]
- 6 **Pereira MR**, Mohan S, Cohen DJ, Husain SA, Dube GK, Ratner LE, Arcasoy S, Aversa MM, Benvenuto LJ, Dadhania DM, Kapur S, Dove LM, Brown RS Jr, Rosenblatt RE, Samstein B, Uriel N, Farr MA, Satlin M, Small CB, Walsh TJ, Kodiyanplakkal RP, Miko BA, Aaron JG, Tsapepas DS, Emond JC, Verna EC. COVID-19 in solid organ transplant recipients: Initial report from the US epicenter. *Am J Transplant* 2020; **20**: 1800-1808 [PMID: [32330343](#) DOI: [10.1111/ajt.15941](#)]
- 7 **Tuletaeva GA**. [Dynamics of clinico-immunological indicators in children with neurodermatitis during climatotherapy]. *Vopr Kurortol Fizioter Lech Fiz Kult* 1988; **29**: 29-31 [PMID: [3264966](#) DOI: [10.1371/journal.pone.0235653](#)]
- 8 **Guan GW**, Gao L, Wang JW, Wen XJ, Mao TH, Peng SW, Zhang T, Chen XM, Lu FM. [Exploring the mechanism of liver enzyme abnormalities in patients with novel coronavirus-infected pneumonia]. *Zhonghua Gan Zang Bing Za Zhi* 2020; **28**: 100-106 [PMID: [32077659](#) DOI: [10.3760/cma.j.issn.1007-3418.2020.02.002](#)]
- 9 **Cai Q**, Huang D, Ou P, Yu H, Zhu Z, Xia Z, Su Y, Ma Z, Zhang Y, Li Z, He Q, Liu L, Fu Y, Chen J. COVID-19 in a designated infectious diseases hospital outside Hubei Province, China. *Allergy* 2020; **75**: 1742-1752 [PMID: [32239761](#) DOI: [10.1111/all.14309](#)]
- 10 **Fan Z**, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z, Cheng J. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; **18**: 1561-1566 [PMID: [32283325](#) DOI: [10.1016/j.cgh.2020.04.002](#)]
- 11 **Cardoso FS**, Pereira R, Germano N. Liver injury in critically ill patients with COVID-19: a case series. *Crit Care* 2020; **24**: 190 [PMID: [32366282](#) DOI: [10.1186/s13054-020-02924-4](#)]
- 12 **Jothimani D**, Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. *J Hepatol* 2020; **73**: 1231-1240 [PMID: [32553666](#) DOI: [10.1016/j.jhep.2020.06.006](#)]
- 13 **Yeo C**, Kaushal S, Yeo D. Enteric involvement of coronaviruses: is faecal-oral transmission of SARS-CoV-2 possible? *Lancet Gastroenterol Hepatol* 2020; **5**: 335-337 [PMID: [32087098](#) DOI: [10.1016/S2468-1253\(20\)30048-0](#)]
- 14 **Zhang C**, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020; **5**: 428-430 [PMID: [32145190](#) DOI: [10.1016/S2468-1253\(20\)30057-1](#)]
- 15 **Kuhn JH**, Li W, Choe H, Farzan M. Angiotensin-converting enzyme 2: a functional receptor for SARS coronavirus. *Cell Mol Life Sci* 2004; **61**: 2738-2743 [PMID: [15549175](#) DOI: [10.1007/s00018-004-4242-5](#)]
- 16 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: [15141377](#) DOI: [10.1002/path.1570](#)]

- 17 **Walls AC**, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 2020; **181**: 281-292.e6 [PMID: [32155444](#) DOI: [10.1016/j.cell.2020.02.058](#)]
- 18 **Alqahtani SA**, Schattenberg JM. Liver injury in COVID-19: The current evidence. *United European Gastroenterol J* 2020; **8**: 509-519 [PMID: [32450787](#) DOI: [10.1177/2050640620924157](#)]
- 19 **Polidoro RB**, Hagan RS, de Santis Santiago R, Schmidt NW. Overview: Systemic Inflammatory Response Derived From Lung Injury Caused by SARS-CoV-2 Infection Explains Severe Outcomes in COVID-19. *Front Immunol* 2020; **11**: 1626 [PMID: [32714336](#) DOI: [10.3389/fimmu.2020.01626](#)]
- 20 **Henrion J**. Hypoxic hepatitis: the point of view of the clinician. *Acta Gastroenterol Belg* 2007; **70**: 214-216 [PMID: [17715637](#)]
- 21 **Waseem N**, Chen PH. Hypoxic Hepatitis: A Review and Clinical Update. *J Clin Transl Hepatol* 2016; **4**: 263-268 [PMID: [27777895](#) DOI: [10.14218/JCTH.2016.00022](#)]
- 22 **Lightsey JM**, Rockey DC. Current concepts in ischemic hepatitis. *Curr Opin Gastroenterol* 2017; **33**: 158-163 [PMID: [28346236](#) DOI: [10.1097/MOG.0000000000000355](#)]
- 23 **Dunn GD**, Hayes P, Breen KJ, Schenker S. The liver in congestive heart failure: a review. *Am J Med Sci* 1973; **265**: 174-189 [PMID: [4573728](#) DOI: [10.1097/00000441-197303000-00001](#)]
- 24 **Rosser BG**, Gores GJ. Liver cell necrosis: cellular mechanisms and clinical implications. *Gastroenterology* 1995; **108**: 252-275 [PMID: [7806049](#) DOI: [10.1016/0016-5085\(95\)90032-2](#)]
- 25 **Phipps MM**, Barraza LH, LaSota ED, Sobieszczyk ME, Pereira MR, Zheng EX, Fox AN, Zucker J, Verna EC. Acute Liver Injury in COVID-19: Prevalence and Association with Clinical Outcomes in a Large U.S. Cohort. *Hepatology* 2020; **72**: 807-817 [PMID: [32473607](#) DOI: [10.1002/hep.31404](#)]
- 26 **El Ouali S**, Romero-Marrero C, Regueiro M. Hepatic manifestations of COVID-19. *Cleve Clin J Med* 2020 [PMID: [32855179](#) DOI: [10.3949/ccjm.87a.ccc061](#)]
- 27 **Piano S**, Dalbeni A, Vettore E, Benfaremo D, Mattioli M, Gambino CG, Framba V, Cerruti L, Mantovani A, Martini A, Luchetti MM, Serra R, Cattelan A, Vettor R, Angeli P; COVID-LIVER study group. Abnormal liver function tests predict transfer to intensive care unit and death in COVID-19. *Liver Int* 2020; **40**: 2394-2406 [PMID: [32526083](#) DOI: [10.1111/liv.14565](#)]
- 28 **Lei F**, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, Zhang XJ, Cai J, Lin L, Ouyang S, Wang X, Yang C, Cheng X, Liu W, Li H, Xie J, Wu B, Luo H, Xiao F, Chen J, Tao L, Cheng G, She ZG, Zhou J, Wang H, Lin J, Luo P, Fu S, Ye P, Xiao B, Mao W, Liu L, Yan Y, Chen G, Huang X, Zhang BH, Yuan Y. Longitudinal Association Between Markers of Liver Injury and Mortality in COVID-19 in China. *Hepatology* 2020; **72**: 389-398 [PMID: [32359177](#) DOI: [10.1002/hep.31301](#)]
- 29 **Labenz C**, Toenges G, Wörns MA, Sprinzl MF, Galle PR, Schattenberg JM. Liver injury in patients with severe acute respiratory syndrome coronavirus-2 infection: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2021; **33**: 1194-1200 [PMID: [32796355](#) DOI: [10.1097/MEG.0000000000001827](#)]
- 30 **Su TH**, Kao JH. The clinical manifestations and management of COVID-19-related liver injury. *J Formos Med Assoc* 2020; **119**: 1016-1018 [PMID: [32345544](#) DOI: [10.1016/j.jfma.2020.04.020](#)]
- 31 **Konturek PC**, Harsch IA, Neurath MF, Zopf Y. COVID-19 - more than respiratory disease: a gastroenterologist's perspective. *J Physiol Pharmacol* 2020; **71** [PMID: [32633236](#) DOI: [10.26402/jpp.2020.2.02](#)]
- 32 **Cheong J**, Bartell N, Peeraphatdit T, Mosli M, Al-Judaibi B. Gastrointestinal and liver manifestations of COVID-19. *Saudi J Gastroenterol* 2020; **26**: 226-232 [PMID: [32367837](#) DOI: [10.4103/sjg.SJG\\_147\\_20](#)]
- 33 **Zhong P**, Xu J, Yang D, Shen Y, Wang L, Feng Y, Du C, Song Y, Wu C, Hu X, Sun Y. COVID-19-associated gastrointestinal and liver injury: clinical features and potential mechanisms. *Signal Transduct Target Ther* 2020; **5**: 256 [PMID: [33139693](#) DOI: [10.1038/s41392-020-00373-7](#)]
- 34 **Cai Q**, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; **73**: 566-574 [PMID: [32298767](#) DOI: [10.1016/j.jhep.2020.04.006](#)]
- 35 **Besur S**, Begum R, Gunabushanam V, Bonkovsky HL. Liver disease in the Era of Coronavirus Disease 19 (COVID-19) pandemic. *Ann Clin Gastroenterol Hepatol* 2020; **4**: 052-057
- 36 **Papadopoulos N**, Vasileiadi S, Deutsch M. COVID-19 and liver injury: where do we stand? *Ann Gastroenterol* 2020; **33**: 459-464 [PMID: [32879591](#) DOI: [10.20524/aog.2020.0522](#)]
- 37 **Wu J**, Li W, Shi X, Chen Z, Jiang B, Liu J, Wang D, Liu C, Meng Y, Cui L, Yu J, Cao H, Li L. Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19). *J Intern Med* 2020; **288**: 128-138 [PMID: [32220033](#) DOI: [10.1111/joim.13063](#)]
- 38 **Cao W**, Shi L, Chen L, Xu X, Wu Z. Clinical features and laboratory inspection of novel coronavirus pneumonia (COVID-19) in Xiangyang, Hubei. 2020 Preprint. Available from: medRxiv:20026963 [DOI: [10.1101/2020.02.23.20026963](#)]
- 39 **Yang X**, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; **8**: 475-481 [PMID: [32105632](#) DOI: [10.1016/S2213-2600\(20\)30079-5](#)]
- 40 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical

- Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: [32109013](#) DOI: [10.1056/NEJMoa2002032](#)]
- 41 **Shi H**, Han X, Jiang N, Cao Y, Alwalid O, Gu J, Fan Y, Zheng C. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis* 2020; **20**: 425-434 [PMID: [32105637](#) DOI: [10.1016/S1473-3099\(20\)30086-4](#)]
  - 42 **Wu C**, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J, Song Y. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* 2020; **180**: 934-943 [PMID: [32167524](#) DOI: [10.1001/jamainternmed.2020.0994](#)]
  - 43 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: [32171076](#) DOI: [10.1016/S0140-6736\(20\)30566-3](#)]
  - 44 **Zhang B**, Zhou X, Qiu Y, Song Y, Feng F, Feng J, Song Q, Jia Q, Wang J. Clinical characteristics of 82 cases of death from COVID-19. *PLoS One* 2020; **15**: e0235458 [PMID: [32645044](#) DOI: [10.1371/journal.pone.0235458](#)]
  - 45 **Huang Y**, Zhou H, Yang R, Xu Y, Feng X, Gong P. Clinical characteristics of 36 non-survivors with COVID-19 in Wuhan, China. 2020 Preprint. Available from: MedRxiv:20029009 [DOI: [10.1101/2020.02.27.20029009](#)]
  - 46 **Parohan M**, Yaghoubi S, Seraji A. Liver injury is associated with severe coronavirus disease 2019 (COVID-19) infection: A systematic review and meta-analysis of retrospective studies. *Hepatol Res* 2020; **50**: 924-935 [PMID: [32386449](#) DOI: [10.1111/hepr.13510](#)]
  - 47 **Youssef M**, H Hussein M, Attia AS, M Elshazli R, Omar M, Zora G, S Farhoud A, Elnahla A, Shihabi A, Toraih EA, S Fawzy M, Kandil E. COVID-19 and liver dysfunction: A systematic review and meta-analysis of retrospective studies. *J Med Virol* 2020; **92**: 1825-1833 [PMID: [32445489](#) DOI: [10.1002/jmv.26055](#)]
  - 48 **Ponziani FR**, Del Zompo F, Nesci A, Santopaolo F, Ianiro G, Pompili M, Gasbarrini A; "Gemelli against COVID-19" group. Liver involvement is not associated with mortality: results from a large cohort of SARS-CoV-2-positive patients. *Aliment Pharmacol Ther* 2020; **52**: 1060-1068 [PMID: [32628793](#) DOI: [10.1111/apt.15996](#)]
  - 49 **Ferm S**, Fisher C, Pakala T, Tong M, Shah D, Schwarzbaum D, Cooley V, Hussain S, Kim SH. Analysis of Gastrointestinal and Hepatic Manifestations of SARS-CoV-2 Infection in 892 Patients in Queens, NY. *Clin Gastroenterol Hepatol* 2020; **18**: 2378-2379.e1 [PMID: [32497637](#) DOI: [10.1016/j.cgh.2020.05.049](#)]
  - 50 **Hajifathalian K**, Krisko T, Mehta A, Kumar S, Schwartz R, Fortune B, Sharaiha RZ; WCM-GI research group. Gastrointestinal and Hepatic Manifestations of 2019 Novel Coronavirus Disease in a Large Cohort of Infected Patients From New York: Clinical Implications. *Gastroenterology* 2020; **159**: 1137-1140.e2 [PMID: [32389667](#) DOI: [10.1053/j.gastro.2020.05.010](#)]
  - 51 **Cao J**, Tu WJ, Cheng W, Yu L, Liu YK, Hu X, Liu Q. Clinical Features and Short-term Outcomes of 102 Patients with Coronavirus Disease 2019 in Wuhan, China. *Clin Infect Dis* 2020; **71**: 748-755 [PMID: [32239127](#) DOI: [10.1093/cid/ciaa243](#)]
  - 52 **Chen T**, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D, Yu H, Wang H, Wang T, Guo W, Chen J, Ding C, Zhang X, Huang J, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 2020; **368**: m1091 [PMID: [32217556](#) DOI: [10.1136/bmj.m1091](#)]
  - 53 **Chen T**, Dai Z, Mo P, Li X, Ma Z, Song S, Chen X, Luo M, Liang K, Gao S, Zhang Y, Deng L, Xiong Y. Clinical Characteristics and Outcomes of Older Patients with Coronavirus Disease 2019 (COVID-19) in Wuhan, China: A Single-Centered, Retrospective Study. *J Gerontol A Biol Sci Med Sci* 2020; **75**: 1788-1795 [PMID: [32279081](#) DOI: [10.1093/gerona/glaa089](#)]
  - 54 **Du Y**, Tu L, Zhu P, Mu M, Wang R, Yang P, Wang X, Hu C, Ping R, Hu P, Li T, Cao F, Chang C, Hu Q, Jin Y, Xu G. Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan. A Retrospective Observational Study. *Am J Respir Crit Care Med* 2020; **201**: 1372-1379 [PMID: [32242738](#) DOI: [10.1164/rccm.202003-0543OC](#)]
  - 55 **Yang F**, Shi S, Zhu J, Shi J, Dai K, Chen X. Analysis of 92 deceased patients with COVID-19. *J Med Virol* 2020; **92**: 2511-2515 [PMID: [32293741](#) DOI: [10.1002/jmv.25891](#)]
  - 56 **Arentz M**, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M. Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State. *JAMA* 2020; **323**: 1612-1614 [PMID: [32191259](#) DOI: [10.1001/jama.2020.4326](#)]
  - 57 **Du RH**, Liu LM, Yin W, Wang W, Guan LL, Yuan ML, Li YL, Hu Y, Li XY, Sun B, Peng P, Shi HZ. Hospitalization and Critical Care of 109 Decedents with COVID-19 Pneumonia in Wuhan, China. *Ann Am Thorac Soc* 2020; **17**: 839-846 [PMID: [32255382](#) DOI: [10.1513/AnnalsATS.202003-225OC](#)]
  - 58 **Li X**, Xu S, Yu M, Wang K, Tao Y, Zhou Y, Shi J, Zhou M, Wu B, Yang Z, Zhang C, Yue J, Zhang Z, Renz H, Liu X, Xie J, Xie M, Zhao J. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol* 2020; **146**: 110-118 [PMID: [32294485](#) DOI: [10.1016/j.jaci.2020.04.006](#)]
  - 59 **Mo P**, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, Xiong Y, Cheng Z, Gao S, Liang K, Luo M, Chen T, Song S, Ma Z, Chen X, Zheng R, Cao Q, Wang F, Zhang Y. Clinical characteristics of



- refractory COVID-19 pneumonia in Wuhan, China. *Clin Infect Dis* 2020 [PMID: [32173725](#) DOI: [10.1093/cid/ciaa270](#)]
- 60 **Wan S**, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, Lang C, Huang D, Sun Q, Xiong Y, Huang X, Lv J, Luo Y, Shen L, Yang H, Huang G, Yang R. Clinical features and treatment of COVID-19 patients in northeast Chongqing. *J Med Virol* 2020; **92**: 797-806 [PMID: [32198776](#) DOI: [10.1002/jmv.25783](#)]
  - 61 **Zhang G**, Hu C, Luo L, Fang F, Chen Y, Li J, Peng Z, Pan H. Clinical features and short-term outcomes of 221 patients with COVID-19 in Wuhan, China. *J Clin Virol* 2020; **127**: 104364 [PMID: [32311650](#) DOI: [10.1016/j.jcv.2020.104364](#)]
  - 62 **Zhang JJ**, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020; **75**: 1730-1741 [PMID: [32077115](#) DOI: [10.1111/all.14238](#)]
  - 63 **Zheng F**, Tang W, Li H, Huang YX, Xie YL, Zhou ZG. Clinical characteristics of 161 cases of corona virus disease 2019 (COVID-19) in Changsha. *Eur Rev Med Pharmacol Sci* 2020; **24**: 3404-3410 [PMID: [32271459](#) DOI: [10.26355/eurrev\\_202003\\_20711](#)]
  - 64 **Lee IC**, Huo TI, Huang YH. Gastrointestinal and liver manifestations in patients with COVID-19. *J Chin Med Assoc* 2020; **83**: 521-523 [PMID: [32243269](#) DOI: [10.1097/JCMA.0000000000000319](#)]
  - 65 **Hashemi N**, Viveiros K, Redd WD, Zhou JC, McCarty TR, Bazarbashi AN, Hathorn KE, Wong D, Njie C, Shen L, Chan WW. Impact of chronic liver disease on outcomes of hospitalized patients with COVID-19: A multicentre United States experience. *Liver Int* 2020; **40**: 2515-2521 [PMID: [32585065](#) DOI: [10.1111/liv.14583](#)]
  - 66 **Mantovani A**, Beatrice G, Dalbeni A. Coronavirus disease 2019 and prevalence of chronic liver disease: A meta-analysis. *Liver Int* 2020; **40**: 1316-1320 [PMID: [32329563](#) DOI: [10.1111/liv.14465](#)]
  - 67 **Pongpirul WA**, Mott JA, Woodring JV, Uyeki TM, MacArthur JR, Vachiraphan A, Suwanvattana P, Uttayamakul S, Chunsuttiwat S, Chotpitayasunondh T, Pongpirul K, Prasithsirikul W. Clinical Characteristics of Patients Hospitalized with Coronavirus Disease, Thailand. *Emerg Infect Dis* 2020; **26**: 1580-1585 [PMID: [32267826](#) DOI: [10.3201/eid2607.200598](#)]
  - 68 **Singh S**, Khan A. Clinical Characteristics and Outcomes of Coronavirus Disease 2019 Among Patients With Preexisting Liver Disease in the United States: A Multicenter Research Network Study. *Gastroenterology* 2020; **159**: 768-771.e3 [PMID: [32376408](#) DOI: [10.1053/j.gastro.2020.04.064](#)]
  - 69 **Chen X**, Jiang Q, Ma Z, Ling J, Hu W, Cao Q, Mo P, Yao L, Yang R, Gao S, Gui X, Hou W, Xiong Y, Li J, Zhang Y. Clinical Characteristics of Hospitalized Patients with SARS-CoV-2 and Hepatitis B Virus Co-infection. *Virol Sin* 2020; **35**: 842-845 [PMID: [32839868](#) DOI: [10.1007/s12250-020-00276-5](#)]
  - 70 **Yip TC**, Lui GC, Wong VW, Chow VC, Ho TH, Li TC, Tse YK, Hui DS, Chan HL, Wong GL. Liver injury is independently associated with adverse clinical outcomes in patients with COVID-19. *Gut* 2021; **70**: 733-742 [PMID: [32641471](#) DOI: [10.1136/gutjnl-2020-321726](#)]
  - 71 **Ji D**, Qin E, Xu J, Zhang D, Cheng G, Wang Y, Lau G. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020; **73**: 451-453 [PMID: [32278005](#) DOI: [10.1016/j.jhep.2020.03.044](#)]
  - 72 **Zhou YJ**, Zheng KI, Wang XB, Yan HD, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, George J, Zheng MH. Younger patients with MAFLD are at increased risk of severe COVID-19 illness: A multicenter preliminary analysis. *J Hepatol* 2020; **73**: 719-721 [PMID: [32348790](#) DOI: [10.1016/j.jhep.2020.04.027](#)]
  - 73 **Sun J**, Deng X, Chen X, Huang J, Huang S, Li Y, Feng J, Liu J, He G. Incidence of Adverse Drug Reactions in COVID-19 Patients in China: An Active Monitoring Study by Hospital Pharmacovigilance System. *Clin Pharmacol Ther* 2020; **108**: 791-797 [PMID: [32324898](#) DOI: [10.1002/cpt.1866](#)]
  - 74 **Ramírez E**, Urroz M, Rodríguez A, González-Muñoz M, Martín-Vega A, Villán Y, Seco E, Monserrat J, Frias J, Carcas AJ, Borobia AM. Incidence of Suspected Serious Adverse Drug Reactions in Corona Virus Disease-19 Patients Detected by a Pharmacovigilance Program by Laboratory Signals in a Tertiary Hospital in Spain: Cautionary Data. *Front Pharmacol* 2020; **11**: 602841 [PMID: [33343374](#) DOI: [10.3389/fphar.2020.602841](#)]
  - 75 **Hundt MA**, Deng Y, Ciarleglio MM, Nathanson MH, Lim JK. Abnormal Liver Tests in COVID-19: A Retrospective Observational Cohort Study of 1,827 Patients in a Major U.S. Hospital Network. *Hepatology* 2020; **72**: 1169-1176 [PMID: [32725890](#) DOI: [10.1002/hep.31487](#)]
  - 76 **Kim MS**, An MH, Kim WJ, Hwang TH. Comparative efficacy and safety of pharmacological interventions for the treatment of COVID-19: A systematic review and network meta-analysis. *PLoS Med* 2020; **17**: e1003501 [PMID: [33378357](#) DOI: [10.1371/journal.pmed.1003501](#)]
  - 77 **Liu J**, Zhang S, Dong X, Li Z, Xu Q, Feng H, Cai J, Huang S, Guo J, Zhang L, Chen Y, Zhu W, Du H, Liu Y, Wang T, Chen L, Wen Z, Annane D, Qu J, Chen D. Corticosteroid treatment in severe COVID-19 patients with acute respiratory distress syndrome. *J Clin Invest* 2020; **130**: 6417-6428 [PMID: [33141117](#) DOI: [10.1172/JCI140617](#)]
  - 78 **WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group**, Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, Annane D, Azevedo LCP, Berwanger O, Cavalcanti AB, Dequin PF, Du B, Emberson J, Fisher D, Giraudeau B, Gordon AC, Granholm A, Green C, Haynes R, Heming N, Higgins JPT, Horby P, Jüni P, Landray MJ, Le Gouge A, Leclerc M, Lim WS, Machado FR, McArthur C, Meziani F, Möller MH, Perner A, Petersen MW, Savovic J,

- Tomazini B, Veiga VC, Webb S, Marshall JC. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. *JAMA* 2020; **324**: 1330-1341 [PMID: [32876694](#) DOI: [10.1001/jama.2020.17023](#)]
- 79 **RECOVERY Collaborative Group**, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021; **384**: 693-704 [PMID: [32678530](#) DOI: [10.1056/NEJMoa2021436](#)]
- 80 **Udwadia ZF**, Singh P, Barkate H, Patil S, Rangwala S, Pendse A, Kadam J, Wu W, Caracta CF, Tandon M. Efficacy and safety of favipiravir, an oral RNA-dependent RNA polymerase inhibitor, in mild-to-moderate COVID-19: A randomized, comparative, open-label, multicenter, phase 3 clinical trial. *Int J Infect Dis* 2021; **103**: 62-71 [PMID: [33212256](#) DOI: [10.1016/j.ijid.2020.11.142](#)]
- 81 **Ivashchenko AA**, Dmitriev KA, Vostokova NV, Azarova VN, Blinow AA, Egorova AN, Gordeev IG, Ilin AP, Karapetian RN, Kravchenko DV, Lomakin NV, Merkulova EA, Papazova NA, Pavlikova EP, Savchuk NP, Simakina EN, Sitdekov TA, Smolyarchuk EA, Tikhomolova EG, Yakubova EV, Ivachtchenko AV. AVIFAVIR for Treatment of Patients With Moderate Coronavirus Disease 2019 (COVID-19): Interim Results of a Phase II/III Multicenter Randomized Clinical Trial. *Clin Infect Dis* 2021; **73**: 531-534 [PMID: [32770240](#) DOI: [10.1093/cid/ciaa1176](#)]
- 82 **Satlin MJ**, Goyal P, Magleby R, Maldarelli GA, Pham K, Kondo M, Schenck EJ, Rennert H, Westblade LF, Choi JJ, Safford MM, Gulick RM. Safety, tolerability, and clinical outcomes of hydroxychloroquine for hospitalized patients with coronavirus 2019 disease. *PLoS One* 2020; **15**: e0236778 [PMID: [32701969](#) DOI: [10.1371/journal.pone.0236778](#)]
- 83 **Cavalcanti AB**, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A, Damiani LP, Marcadenti A, Kawano-Dourado L, Lisboa T, Junqueira DLM, de Barros E Silva PGM, Tramujas L, Abreu-Silva EO, Laranjeira LN, Soares AT, Echenique LS, Pereira AJ, Freitas FGR, Gebara OCE, Dantas VCS, Furtado RHM, Milan EP, Golin NA, Cardoso FF, Maia IS, Hoffmann Filho CR, Kormann APM, Amazonas RB, Bocchi de Oliveira MF, Serpa-Neto A, Falavigna M, Lopes RD, Machado FR, Berwanger O; Coalition Covid-19 Brazil I Investigators. Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. *N Engl J Med* 2020; **383**: 2041-2052 [PMID: [32706953](#) DOI: [10.1056/NEJMoa2019014](#)]
- 84 **Das S**, Bhowmick S, Tiwari S, Sen S. An Updated Systematic Review of the Therapeutic Role of Hydroxychloroquine in Coronavirus Disease-19 (COVID-19). *Clin Drug Investig* 2020; **40**: 591-601 [PMID: [32468425](#) DOI: [10.1007/s40261-020-00927-1](#)]
- 85 **RECOVERY Collaborative Group**, Horby P, Mafham M, Linsell L, Bell JL, Staplin N, Emberson JR, Wiselka M, Ustianowski A, Elmahi E, Prudon B, Whitehouse T, Felton T, Williams J, Faccenda J, Underwood J, Baillie JK, Chappell LC, Faust SN, Jaki T, Jeffery K, Lim WS, Montgomery A, Rowan K, Tarning J, Watson JA, White NJ, Juszczak E, Haynes R, Landray MJ. Effect of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* 2020; **383**: 2030-2040 [PMID: [33031652](#) DOI: [10.1056/NEJMoa2022926](#)]
- 86 **Cao B**, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 2020; **382**: 1787-1799 [PMID: [32187464](#) DOI: [10.1056/NEJMoa2001282](#)]
- 87 **Grein J**, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, Feldt T, Green G, Green ML, Lescure FX, Nicastri E, Oda R, Yo K, Quiros-Roldan E, Studemeister A, Redinski J, Ahmed S, Bernett J, Chelliah D, Chen D, Chihara S, Cohen SH, Cunningham J, D'Arminio Monforte A, Ismail S, Kato H, Lapadula G, L'Her E, Maeno T, Majumder S, Massari M, Mora-Rillo M, Mutoh Y, Nguyen D, Verweij E, Zoufaly A, Osinusi AO, DeZure A, Zhao Y, Zhong L, Chokkalingam A, Elboudwarej E, Telep L, Timbs L, Henne I, Sellers S, Cao H, Tan SK, Winterbourne L, Desai P, Mera R, Gaggari A, Myers RP, Brainard DM, Childs R, Flanagan T. Compassionate Use of Remdesivir for Patients with Severe Covid-19. *N Engl J Med* 2020; **382**: 2327-2336 [PMID: [32275812](#) DOI: [10.1056/NEJMoa2007016](#)]
- 88 **Wang Y**, Zhang D, Du G, Du R, Zhao J, Jin Y, Fu S, Gao L, Cheng Z, Lu Q, Hu Y, Luo G, Wang K, Lu Y, Li H, Wang S, Ruan S, Yang C, Mei C, Wang Y, Ding D, Wu F, Tang X, Ye X, Ye Y, Liu B, Yang J, Yin W, Wang A, Fan G, Zhou F, Liu Z, Gu X, Xu J, Shang L, Zhang Y, Cao L, Guo T, Wan Y, Qin H, Jiang Y, Jaki T, Hayden FG, Horby PW, Cao B, Wang C. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020; **395**: 1569-1578 [PMID: [32423584](#) DOI: [10.1016/S0140-6736\(20\)31022-9](#)]
- 89 **Goldman JD**, Lye DCB, Hui DS, Marks KM, Bruno R, Montejano R, Spinner CD, Galli M, Ahn MY, Nahass RG, Chen YS, SenGupta D, Hyland RH, Osinusi AO, Cao H, Blair C, Wei X, Gaggari A, Brainard DM, Towner WJ, Muñoz J, Mullane KM, Marty FM, Tashima KT, Diaz G, Subramanian A; GS-US-540-5773 Investigators. Remdesivir for 5 or 10 Days in Patients with Severe Covid-19. *N Engl J Med* 2020; **383**: 1827-1837 [PMID: [32459919](#) DOI: [10.1056/NEJMoa2015301](#)]
- 90 **Piscocya A**, Ng-Sueng LF, Parra Del Riego A, Cerna-Viacava R, Pasupuleti V, Roman YM, Thota P, White CM, Hernandez AV. Efficacy and harms of remdesivir for the treatment of COVID-19: A

- systematic review and meta-analysis. *PLoS One* 2020; **15**: e0243705 [PMID: [33301514](#) DOI: [10.1371/journal.pone.0243705](#)]
- 91 **Muhović D**, Bojović J, Bulatović A, Vukčević B, Ratković M, Lazović R, Smolović B. First case of drug-induced liver injury associated with the use of tocilizumab in a patient with COVID-19. *Liver Int* 2020; **40**: 1901-1905 [PMID: [32478465](#) DOI: [10.1111/liv.14516](#)]
  - 92 **Serviddio G**, Villani R, Stallone G, Scioscia G, Foschino-Barbaro MP, Lacedonia D. Tocilizumab and liver injury in patients with COVID-19. *Therap Adv Gastroenterol* 2020; **13**: 1756284820959183 [PMID: [33101458](#) DOI: [10.1177/1756284820959183](#)]
  - 93 **Corominas H**, Castellví I, Pomar V, Antonijoan R, Mur I, Matas L, Gich I, de Benito N, Laiz A, Castillo D, Villamarin L, Filella D, Millán AM, Quijada MÁ, Puig M, Casademont J, Domingo P. Effectiveness and safety of intravenous tocilizumab to treat COVID-19-associated hyperinflammatory syndrome: Covizumab-6 observational cohort. *Clin Immunol* 2021; **223**: 108631 [PMID: [33189888](#) DOI: [10.1016/j.clim.2020.108631](#)]
  - 94 **Stone JH**, Frigault MJ, Serling-Boyd NJ, Fernandes AD, Harvey L, Foulkes AS, Horick NK, Healy BC, Shah R, Bensaci AM, Woolley AE, Nikiforow S, Lin N, Sagar M, Schrager H, Huckins DS, Axelrod M, Pincus MD, Fleisher J, Sacks CA, Dougan M, North CM, Halvorsen YD, Thurber TK, Dagher Z, Scherer A, Wallwork RS, Kim AY, Schoenfeld S, Sen P, Neilan TG, Perugino CA, Unizony SH, Collier DS, Matza MA, Yin H, JM, Bowman KA, Meyerowitz E, Zafar A, Drobní ZD, Bolster MB, Kohler M, D'Silva KM, Dau J, Lockwood MM, Cubbison C, Weber BN, Mansour MK; BACC Bay Tocilizumab Trial Investigators. Efficacy of Tocilizumab in Patients Hospitalized with Covid-19. *N Engl J Med* 2020; **383**: 2333-2344 [PMID: [33085857](#) DOI: [10.1056/NEJMoa2028836](#)]
  - 95 **Hermine O**, Mariette X, Tharaux PL, Resche-Rigon M, Porcher R, Ravaud P; CORIMUNO-19 Collaborative Group. Effect of Tocilizumab vs Usual Care in Adults Hospitalized With COVID-19 and Moderate or Severe Pneumonia: A Randomized Clinical Trial. *JAMA Intern Med* 2021; **181**: 32-40 [PMID: [33080017](#) DOI: [10.1001/jamainternmed.2020.6820](#)]
  - 96 **Parente A**, Manzia TM, Angelico R, Tirota F, Muiesan P, Tisone G, Framarino Dei Malatesta M. COVID-19, liver transplant, and immunosuppression: Allies or foes? *Transpl Infect Dis* 2021; **23**: e13417 [PMID: [32666588](#) DOI: [10.1111/tid.13417](#)]
  - 97 **Becchetti C**, Zambelli MF, Pasulo L, Donato MF, Invernizzi F, Detry O, Dahlqvist G, Ciccarelli O, Morelli MC, Fraga M, Svegliati-Baroni G, van Vlierberghe H, Coenraad MJ, Romero MC, de Gottardi A, Toniutto P, Del Prete L, Abbati C, Samuel D, Pirenne J, Nevens F, Dufour JF; COVID-LT group. COVID-19 in an international European liver transplant recipient cohort. *Gut* 2020; **69**: 1832-1840 [PMID: [32571972](#) DOI: [10.1136/gutjnl-2020-321923](#)]
  - 98 **El Kassas M**, Alborae M, Al Balakosy A, Abdeen N, Afify S, Abdalgaber M, Sherief AF, Madkour A, Abdallah Ahmed M, Eltabbakh M, Salaheldin M, Wifi MN. Liver transplantation in the era of COVID-19. *Arab J Gastroenterol* 2020; **21**: 69-75 [PMID: [32439237](#) DOI: [10.1016/j.ajg.2020.04.019](#)]
  - 99 **Forns X**, Navasa M. Liver transplant immunosuppression during the covid-19 pandemic. *Gastroenterol Hepatol* 2020; **43**: 457-463 [PMID: [32646657](#) DOI: [10.1016/j.gastrohep.2020.06.003](#)]
  - 100 **Boettler T**, Newsome PN, Mondelli MU, Maticic M, Cordero E, Cornberg M, Berg T. Care of patients with liver disease during the COVID-19 pandemic: EASL-ESCMID position paper. *JHEP Rep* 2020; **2**: 100113 [PMID: [32289115](#) DOI: [10.1016/j.jhepr.2020.100113](#)]
  - 101 **Spoletini G**, Bianco G, Graceffa D, Lai Q. Transplantation during the COVID-19 pandemic: nothing noble is accomplished without danger. *BMC Gastroenterol* 2020; **20**: 259 [PMID: [32762651](#) DOI: [10.1186/s12876-020-01401-0](#)]
  - 102 **Carmona M**, Álvarez M, Marco J, Mahillo B, Domínguez-Gil B, Núñez JR, Matesanz R. Global organ transplant activities in 2015. Data from the Global Observatory on Donation and Transplantation (GODT). *Transplantation* 2017; **101**: S29 [DOI: [10.1097/01.tp.0000525015.43613.75](#)]
  - 103 **Martin P**, DiMartini A, Feng S, Brown R Jr, Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014; **59**: 1144-1165 [PMID: [24716201](#) DOI: [10.1002/hep.26972](#)]
  - 104 **Yu L**, Ioannou GN. Survival of liver transplant recipients with hemochromatosis in the United States. *Gastroenterology* 2007; **133**: 489-495 [PMID: [17681170](#) DOI: [10.1053/j.gastro.2007.05.054](#)]
  - 105 **Hollander JE**, Carr BG. Virtually Perfect? *N Engl J Med* 2020; **382**: 1679-1681 [PMID: [32160451](#) DOI: [10.1056/NEJMp2003539](#)]
  - 106 **Hong Z**, Li N, Li D, Li J, Li B, Xiong W, Lu L, Li W, Zhou D. Telemedicine During the COVID-19 Pandemic: Experiences From Western China. *J Med Internet Res* 2020; **22**: e19577 [PMID: [32349962](#) DOI: [10.2196/19577](#)]
  - 107 **Kumar D**, Manuel O, Natori Y, Egawa H, Grossi P, Han SH, Fernández-Ruiz M, Humar A. COVID-19: A global transplant perspective on successfully navigating a pandemic. *Am J Transplant* 2020; **20**: 1773-1779 [PMID: [32202064](#) DOI: [10.1111/ajt.15876](#)]
  - 108 **American Association for the Study of Liver Diseases**. AASLD clinical insights for hepatology and liver transplant providers during the COVID-19 pandemic [online]. [cited 31 January 2021]. Available from: <https://www.aasld.org/sites/default/files/2020-04/AASLD-COVID19-ClinicalInsights-4.07.2020-Final.pdf>
  - 109 **Dwosh HA**, Hong HH, Austgarden D, Herman S, Schabas R. Identification and containment of an outbreak of SARS in a community hospital. *CMAJ* 2003; **168**: 1415-1420 [PMID: [12771070](#)]

- 110 **Al-Dorzi HM**, Aldawood AS, Khan R, Baharoon S, Alchin JD, Matroud AA, Al Johany SM, Balkhy HH, Arabi YM. The critical care response to a hospital outbreak of Middle East respiratory syndrome coronavirus (MERS-CoV) infection: an observational study. *Ann Intensive Care* 2016; **6**: 101 [PMID: [27778310](#) DOI: [10.1186/s13613-016-0203-z](#)]
- 111 **Feltracco P**, Barbieri S, Galligioni H, Michieletto E, Carollo C, Ori C. Intensive care management of liver transplanted patients. *World J Hepatol* 2011; **3**: 61-71 [PMID: [21487537](#) DOI: [10.4254/wjh.v3.i3.61](#)]
- 112 **Cardoso FS**. Liver Transplantation in an ICU Dominated by COVID-19. *Liver Transpl* 2020; **26**: 1064-1065 [PMID: [32246576](#) DOI: [10.1002/lt.25770](#)]
- 113 **Mandell MS**, Stoner TJ, Barnett R, Shaked A, Bellamy M, Biancofiore G, Niemann C, Walia A, Vater Y, Tran ZV, Kam I. A multicenter evaluation of safety of early extubation in liver transplant recipients. *Liver Transpl* 2007; **13**: 1557-1563 [PMID: [17969193](#) DOI: [10.1002/Lt.21263](#)]
- 114 **Glanemann M**, Busch T, Neuhaus P, Kaisers U. Fast tracking in liver transplantation. Immediate postoperative tracheal extubation: feasibility and clinical impact. *Swiss Med Wkly* 2007; **137**: 187-191 [PMID: [17525870](#)]
- 115 **Schaubel DE**, Guidinger MK, Biggins SW, Kalbfleisch JD, Pomfret EA, Sharma P, Merion RM. Survival benefit-based deceased-donor liver allocation. *Am J Transplant* 2009; **9**: 970-981 [PMID: [19341419](#) DOI: [10.1111/j.1600-6143.2009.02571.x](#)]
- 116 **Vitale A**, Volk ML, De Feo TM, Burra P, Frigo AC, Ramirez Morales R, De Carlis L, Belli L, Colledan M, Fagioli S, Rossi G, Andorno E, Baccarani U, Regalia E, Vivarelli M, Donatuccio M, Cillo U; Liver Transplantation North Italy Transplant program (NITp) working group. A method for establishing allocation equity among patients with and without hepatocellular carcinoma on a common liver transplant waiting list. *J Hepatol* 2014; **60**: 290-297 [PMID: [24161408](#) DOI: [10.1016/j.jhep.2013.10.010](#)]
- 117 **D'Antiga L**. Coronaviruses and Immunosuppressed Patients: The Facts During the Third Epidemic. *Liver Transpl* 2020; **26**: 832-834 [PMID: [32196933](#) DOI: [10.1002/lt.25756](#)]
- 118 **Cravedi P**, Mothi SS, Azzi Y, Haverly M, Farouk SS, Pérez-Sáez MJ, Redondo-Pachón MD, Murphy B, Florman S, Cyrino LG, Grafals M, Venkataraman S, Cheng XS, Wang AX, Zaza G, Ranghino A, Furian L, Manrique J, Maggiore U, Gandolfini I, Agrawal N, Patel H, Akalin E, Riella LV. COVID-19 and kidney transplantation: Results from the TANGO International Transplant Consortium. *Am J Transplant* 2020; **20**: 3140-3148 [PMID: [32649791](#) DOI: [10.1111/ajt.16185](#)]
- 119 **Lee BT**, Perumalswami PV, Im GY, Florman S, Schiano TD. COVID-19 in Liver Transplant Recipients: An Initial Experience from the U.S. Epicenter. *Gastroenterology* 2020; **S0016**: 5085(20)34703-X [DOI: [10.1053/j.gastro.2020.05.050](#)]
- 120 **Bossini N**, Alberici F, Delbarba E, Valerio F, Manenti C, Possenti S, Econimo L, Maffei C, Pola A, Terlizzi V, Salviani C, Moscato M, Pasquali S, Zambetti N, Tonoli M, Affatato S, Pecchini P, Viola FB, Malberti F, Depetri G, Gaggiotti M, Scolari F; Brescia Renal COVID task force. Kidney transplant patients with SARS-CoV-2 infection: The Brescia Renal COVID task force experience. *Am J Transplant* 2020; **20**: 3019-3029 [PMID: [32627319](#) DOI: [10.1111/ajt.16176](#)]
- 121 **Favà A**, Cucchiari D, Montero N, Toapanta N, Centellas FJ, Vila-Santandreu A, Coloma A, Meneghini M, Manonelles A, Sellarés J, Torres I, Gelpi R, Lorenzo I, Ventura-Aguir P, Cofan F, Torregrosa JV, Perelló M, Facundo C, Seron D, Oppenheimer F, Bestard O, Cruzado JM, Moreso F, Melilli E. Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: A multicentric cohort study. *Am J Transplant* 2020; **20**: 3030-3041 [PMID: [32777153](#) DOI: [10.1111/ajt.16246](#)]
- 122 **Molnar MZ**, Bhalla A, Azhar A, Tsujita M, Talwar M, Balaraman V, Sodhi A, Kalaria D, Eason JD, Hayek SS, Coca SG, Shaei S, Neyra JA, Gupta S, Leaf DE, Kovesdy CP; STOP-COVID Investigators. Outcomes of critically ill solid organ transplant patients with COVID-19 in the United States. *Am J Transplant* 2020; **20**: 3061-3071 [PMID: [32844546](#) DOI: [10.1111/ajt.16280](#)]
- 123 **Webb GJ**, Moon AM, Barnes E, Barritt AS, Marjot T. Determining risk factors for mortality in liver transplant patients with COVID-19. *Lancet Gastroenterol Hepatol* 2020; **5**: 643-644 [PMID: [32339474](#) DOI: [10.1016/S2468-1253\(20\)30125-4](#)]
- 124 **Cornberg M**, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. *J Hepatol* 2021; **74**: 944-951 [PMID: [33563499](#) DOI: [10.1016/j.jhep.2021.01.032](#)]
- 125 **Rabinowich L**, Grupper A, Baruch R, Ben-Yehoyada M, Halperin T, Turner D, Katchman E, Levi S, Houry I, Lubezky N, Shibolet O, Katchman H. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. *J Hepatol* 2021; **75**: 435-438 [PMID: [33892006](#) DOI: [10.1016/j.jhep.2021.04.020](#)]
- 126 **Sharma A**, Jaiswal P, Kerakhan Y, Saravanan L, Murtaza Z, Zergham A, Hanganur NS, Akbar A, Deol A, Francis B, Patel S, Mehta D, Jaiswal R, Singh J, Patel U, Malik P. Liver disease and outcomes among COVID-19 hospitalized patients - A systematic review and meta-analysis. *Ann Hepatol* 2021; **21**: 100273 [PMID: [33075578](#) DOI: [10.1016/j.aohp.2020.10.001](#)]
- 127 **Combined Update**. (2020, August 25). Retrieved March 5, 2021, from SECURE-Cirrhosis and EASL supported COVID-Hep. [cited 4 April 2021]. Available from: [https://www.covid-hep.net/img/update\\_20200825.pdf](https://www.covid-hep.net/img/update_20200825.pdf)
- 128 **COVID-Hep**. net. Updates. [cited 5 March 2021]. Available from: <https://www.covid-hep.net/updates.html>
- 129 **Ball S**, Banerjee A, Berry C, Boyle JR, Bray B, Bradlow W, Chaudhry A, Crawley R, Danesh J,



- Denniston A, Falter F, Figueroa JD, Hall C, Hemingway H, Jefferson E, Johnson T, King G, Lee KK, McKean P, Mason S, Mills NL, Pearson E, Pirmohamed M, Poon MTC, Priedon R, Shah A, Sofat R, Sterne JAC, Strachan FE, Sudlow CLM, Szarka Z, Whiteley W, Wyatt M; CVD-COVID-UK Consortium. Monitoring indirect impact of COVID-19 pandemic on services for cardiovascular diseases in the UK. *Heart* 2020; **106**: 1890-1897 [PMID: [33020224](#) DOI: [10.1136/heartjnl-2020-317870](#)]
- 130 **Einstein AJ**, Shaw LJ, Hirschfeld C, Williams MC, Villines TC, Better N, Vitola JV, Cerci R, Dorbala S, Raggi P, Choi AD, Lu B, Sinitsyn V, Sergienko V, Kudo T, Nørgaard BL, Maurovich-Horvat P, Campisi R, Milan E, Louw L, Allam AH, Bhatia M, Malkovskiy E, Goebel B, Cohen Y, Randazzo M, Narula J, Pascual TNB, Pynda Y, Dondi M, Paez D; the; INCAPS COVID Investigators Group. International Impact of COVID-19 on the Diagnosis of Heart Disease. *J Am Coll Cardiol* 2021; **77**: 173-185 [PMID: [33446311](#) DOI: [10.1016/j.jacc.2020.10.054](#)]
- 131 **Amaddeo G**, Brustia R, Allaire M, Lequoy M, Hollande C, Regnault H, Blaise L, Ganne-Carrié N, Séror O, Larrey E, Lim C, Scatton O, El Mouhadi S, Ozenne V, Paye F, Balladur P, Dohan A, Massault PP, Pol S, Dioguardi Burgio M, Vilgrain V, Sepulveda A, Cauchy F, Luciani A, Sommacale D, Leroy V, Roudot-Thoraval F, Bouattour M, Nault JC; Paris Liver Cancer Group. Impact of COVID-19 on the management of hepatocellular carcinoma in a high-prevalence area. *JHEP Rep* 2021; **3**: 100199 [PMID: [33163949](#) DOI: [10.1016/j.jhepr.2020.100199](#)]
- 132 **Toyoda H**, Yasuda S, Kiriya S, Tanikawa M, Hisanaga Y, Kanamori A, Kitabatake S, Yamamoto S, Shiota S, Furoi M, Koyabu T, Furukawa D, Kumada T, Sumida Y. Impact of COVID-19 pandemic on surveillance of hepatocellular carcinoma: a study in patients with chronic hepatitis C after sustained virologic response. *GastroHep* 2020 [PMID: [32837333](#) DOI: [10.1002/ygh.2.418](#)]
- 133 **Fancellu A**, Sanna V, Scognamiglio F, Feo CF, Vidili G, Nigri G, Porcu A. Surgical treatment of hepatocellular carcinoma in the era of COVID-19 pandemic: A comprehensive review of current recommendations. *World J Clin Cases* 2021; **9**: 3517-3530 [PMID: [34046452](#) DOI: [10.12998/wjcc.v9.i15.3517](#)]
- 134 **Aghemo A**, Masarone M, Montagnese S, Petta S, Ponziani FR, Russo FP; Associazione Italiana Studio Fegato (AISF). Assessing the impact of COVID-19 on the management of patients with liver diseases: A national survey by the Italian association for the study of the Liver. *Dig Liver Dis* 2020; **52**: 937-941 [PMID: [32703730](#) DOI: [10.1016/j.dld.2020.07.008](#)]

## In the era of rapid mRNA-based vaccines: Why is there no effective hepatitis C virus vaccine yet?

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### Abstract

Hepatitis C virus (HCV) is responsible for no less than 71 million people chronically infected and is one of the most frequent indications for liver transplantation worldwide. Despite direct-acting antiviral therapies fuel optimism in controlling HCV infections, there are several obstacles regarding treatment accessibility and reinfection continues to remain a possibility. Indeed, the majority of new HCV infections in developed countries occur in people who inject drugs and are more plausible to get reinfected. To achieve global epidemic control of this virus the development of an effective prophylactic or therapeutic vaccine becomes a must. The coronavirus disease 19 (COVID-19) pandemic led to auspicious vaccine development against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus, which has renewed interest on fighting HCV epidemic with vaccination. The aim of this review is to highlight the current situation of HCV vaccine candidates designed to prevent and/or to reduce HCV infectious cases and their complications. We will emphasize on some of the crossroads encountered during vaccine development against this insidious virus, together with some key aspects of HCV immunology which have, so far, hampered the progress in this area. The main focus will be on nucleic acid-based as well as recombinant viral vector-based vaccine candidates as the most novel vaccine approaches, some of which have been recently and successfully employed for SARS-CoV-2 vaccines. Finally, some ideas will be presented on which methods to explore for the design of live-attenuated vaccines against HCV.

**Key Words:** Hepatitis C virus; Vaccine candidates; Nucleic acid-based vaccines;

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**Core Tip:** Hepatitis C virus (HCV) remains a global health burden despite the successful introduction of direct-acting antiviral therapies. In order to achieve global control of HCV epidemic a vaccine is necessary. Its development has faced many hurdles, reason why it is still elusive. Herein, we describe all the challenges during HCV vaccine research, focusing on HCV immunology and emphasizing on current vaccine candidates, particularly nucleic acid-based as well as recombinant vector-based vaccines. We also highlight the impact of severe acute respiratory syndrome coronavirus-2 vaccine race on the renewed interest on HCV vaccine production. Finally, we present ideas on live-attenuated vaccine approaches against HCV.

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## INTRODUCTION

### *Hepatitis C virus infection and the need for a vaccine*

Hepatitis C virus (HCV), discovered in 1989[1], represents an important health burden. In 2015, the World Health Organization (WHO) estimated that there were at least 71 million people chronically infected with HCV, which represents a global prevalence of approximately 1%[2]. Additionally, around 400000 deaths occurred from infection complications.

Infections with HCV cause both acute as well as chronic liver disease in 60%-80% of the cases. Chronicity is associated with the development of cirrhosis (15%-30%) and hepatocellular carcinoma (HCC)[3]. Liver damage resulting from this infection makes it one of the most frequent indications for liver transplantation worldwide[4-8].

The problem of HCV infections worldwide has led the WHO to propose the elimination of viral hepatitis as a public health burden by 2030[2]. However, in order to achieve this goal, big scale interventions are needed, such as screening testing, effective treatment and hopefully vaccination, the latter still non-existing for HCV.

Access to widely available screening tests is uncommon and is hindered by economic reasons, particularly given the fact that new HCV infections are mainly asymptomatic[9]. This leads to an underestimation of the disease prevalence and does not contribute to the eradication goal. Concerning treatment, the development of interferon-free (IFN-free) regimens based in direct-acting antivirals (DAAs) has revolutionized HCV therapy. These antivirals have significantly increased response rates (up to 98%) and greatly reduced treatment duration to only 8-12 wk of oral treatment. DAAs have generated optimism on the global control front, and some consider that this pathogen can now be effectively controlled solely by means of antiviral therapy[10,11]. However, there are some limitations and obstacles to keep the virus in check, in particular, the cost and practical aspects of treatment access, which is uneven among different countries and leaves underdeveloped regions without treatment[11]. Additionally, resistance to DAAs emerged concomitantly with their development and implementation. Resistance-associated substitutions have been detected both before as well as during and after treatment with DAAs[12]. Another interesting aspect to consider is that eliminating HCV infection with DAAs does not eradicate the risk of developing liver cancer. Also, protective immunity is usually insufficient after natural or treatment-induced viral clearance, thus, the possibility of reinfection remains[13]. Together, these facts make HCV elimination in high-risk groups a very challenging task and the need for an effective prophylactic vaccine remains the greatest uncovered medical problem in the hepatitis C field[14]. Vaccination against HCV infection would reduce public healthcare resources by avoiding expensive DAA-based regimens or medical treatments for any liver or metabolic

complications derived from long-term infections[15-17], especially in low- or middle-income countries, where HCV prevalence is still moderate-high and access to diagnosis and treatment uneven and costly[18].

Proper immune responses are able to clear HCV acute infections, preventing the progression to chronicity (in 20%-40% of infected individuals). This fact suggests that vaccination could be a reasonable goal[19] provided we grasp a better understanding of immune responses against HCV in order to develop different vaccine candidates that allow for appropriate protection.

Global epidemic control will only be possible if the number of new HCV infections is reduced alongside with an increased number of cured patients[11,14]. However, a recent report showed that almost 60% of 91 surveyed countries had, in 2016, higher rates of infection than cures, making the goal of HCV elimination as a health burden by 2030, difficult to achieve[20].

For all the reasons previously mentioned, safe and effective prophylactic and/or therapeutic vaccines are necessary for the global control of HCV epidemic[11,21-24]. Indeed, no infectious disease has been controlled and eradicated with antimicrobial treatment, while it has in fact been possible by vaccination[10]. Furthermore, effective vaccination strategies widely available have been the only unfailing method to keep viral transmission at bay by providing herd immunity[25]. Modelling studies have indicated that, even with the introduction of new DAA treatments, only a quasi-eradication of HCV would be possible[26,27], highlighting the need for a vaccine against HCV.

Two extraordinary and unique situations that took place during this last year have fueled optimism on vaccine development against HCV. First, the Nobel Prize in Physiology or Medicine 2020 for the discovery of HCV which was awarded last October [28]. Three distinguished researchers, Harvey J. Alter, Michael Houghton and Charles M. Rice, received the prize for their contribution in identifying the etiological agent of the hepatitis formerly known as non-A non-B, and enabling the development of screening tests and antiviral drugs for its treatment. All of them expressed their hopes for a future vaccine against hepatitis C in their Nobel lectures, and Charles M. Rice specifically stated that he hoped we can learn from all the efforts that are being put into developing coronavirus disease 19 (COVID-19) vaccines[29]. This last state-ment refers to the second event from last year that has renewed interest on HCV vaccines: The COVID-19 pandemic and the remarkable development of several vac-cines to fight it. In the same line, in June 2020, the National Institutes of Health (NIH) opened a grant opportunity for the design of vaccines against HCV assigning USD 8 million to this aim[30].

This review focuses on different vaccine candidates designed to prevent or diminish HCV infection cases, and summarizes all the pitfalls encountered during vaccine development against this virus, including some key aspects of HCV immunology. We make special emphasis on nucleic acid-based vaccines as well as recombinant viral vectors and provide information on severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccines as examples of approaches that might be important in HCV vaccine development.

### ***Prophylactic vs therapeutic vaccines***

Vaccine candidates with two different goals have been considered to control HCV epidemic: Prophylactic and therapeutic (primary and secondary prevention, respectively). The most widespread use of vaccination has always been to prevent a particular disease (prophylactic vaccination)[31] by building immunity in an individual prior to the first encounter with the pathogen, and thus becoming immune to a particular illness. On the other hand, therapeutic vaccination is meant to induce immune responses against a disease that is already in course in a given individual[32].

As we will later discuss in detail, the challenges for designing an effective prophylactic vaccine are vast (HCV variability and diversity, limited animal models and a complex immunological response). Many preventive vaccines against other viral patho-gens are able to induce neutralizing antibodies (nAbs) that correlate with protection, which seems to be difficult to achieve for HCV[14]. Nevertheless, even a low efficacy prophylactic vaccine might be useful to decrease the epidemic impact in high-risk populations by reducing the number of new infections[33-35].

Therapeutic vaccines against HCV have great potential to aid in controlling chronic infections by increasing curing rates or reducing therapy duration[36]. In this new DAA era, sustained virological response (SVR) rates are extremely high (above 98%) and treatment duration has already been shortened compared to classic dual therapy (pegylated IFN- $\alpha$  plus ribavirin). However, there are difficult to treat patients (with active HCC or severe liver decompensation, those experiencing multiple DAA

treatment failures, or those infected with HCV genotype 3)[37] for which this therapeutic approach would be beneficial. These vaccines would boost HCV-specific T cell responses and would help in three different ways: (1) Preventing viral relapse if therapeutic vaccines were to be administered in conjunction with DAA therapy; (2) Maximizing early viral clearance and thus increasing SVR rates by first employing a therapeutic vaccine followed by the antiviral treatment; and (3) Producing partial control of HCV infection just by means of therapeutic immunization and thus reducing viral load[38]. Despite promising results in decreasing viral titers, rebounds have been observed, most likely due, either to immune escape or the inability of properly inhibiting viral replication or eliminating most of HCV-infected hepatocytes[21].

### **Expected outcome of effective vaccine candidates**

In general, effective vaccine candidates should stimulate generation of nAbs and a proper cellular immune response. In order to design vaccines that elicit protective immunity against HCV, it is of utmost importance to consider the virus tropism (mainly hepatocytes), transmission route (parenteral transmission through contaminated blood) and pathogenesis[39].

A vaccine that induces immune responses similar to those produced by individuals which have successfully cleared the virus after an acute HCV infection, might prove valuable[19]. As we will discuss in the next section, vigorous responses of broadly cross-reactive CD4+, CD8+ T cells to conserved epitopes[40–42], as well as nAbs contribute to HCV spontaneous clearance[43,44].

## **ADAPTIVE IMMUNE RESPONSE IN HCV INFECTION**

Approximately 20%–40% of HCV-infected patients clear the virus spontaneously, while the rest develop a persistent infection that will result in severe fibrosis, cirrhosis and HCC[3,45]. Thus, it is essential to understand the immune protection induced during acute infections in patients that achieved spontaneous viral clearance in order to determine the immune parameters that a successful vaccine has to reach.

Multiple evidences in human and animal models have demonstrated the undoubted association of spontaneous viral clearance with a broad, sustained HCV-specific T cell-mediated immunity (CMI) to conserved HCV non-structural proteins[46,47] and nAb targeting conserved regions of viral envelope glycoproteins E1E2[48].

As will be detailed below, both arms of the immune response are primed during HCV infection, but the characteristics vary depending on whether an acute infection is spontaneously resolved or if it evolves to chronicity.

### **Cellular immune protection**

While HCV-specific CD8+ T cells are the main effector cells, the outcome of infection depends on eliciting efficient virus-specific CD4+ T cell responses[49]. These cells are the central regulators of adaptive immunity providing help for priming CD8+ T cell response as well as antibody response during viral infections. The breadth of the T cell response is a key determinant to spontaneously clear HCV. High numbers of CD4+ and CD8+ T cells targeting different epitopes were observed in individuals who resolved acute infections in comparison to those who evolve to chronicity[42,50,51]. These cells are multi-specifically targeting both structural and non-structural HCV proteins[46,52,53]. However, CD8+ T cells targeting non-structural proteins are immunodominant and associate with spontaneous clearance[54].

The strength of the CMI is also important for HCV infection outcome. Indeed, a robust HCV-specific CD8+ T cell response is associated with the resolution of acute HCV infection[55]. In an acute infection, cytotoxic T lymphocytes (CTLs) have cytolytic and noncytolytic functions which mediate viral eradication[56]. They traffic from the lymph nodes to the liver, where they recognize HCV-antigenic peptides loaded on human leukocyte antigen class I in infected hepatocytes. These infected cells can be lysed through the action of perforins and granzymes, or, killed *via* Fas/FasL interactions that activate the caspase cascade and end up in the apoptosis of the target cell. The noncytolytic function occurs without destroying infected cells, where viral replication is inhibited by cytokines released by CTLs which generate an antiviral environment.

Broad specific CD4+ T cells are detected during the acute phase regardless of the final outcome. However, these cells undergo an early decrease in frequency and breadth in persistent HCV infection compared to patients who clear the infection spontaneously[57]. Thus, spontaneous resolution is associated with a CD4+ T cell



response significantly stronger in comparison to persistently, or chronically infected individuals[58,59].

In chronic infections, the limited functionality of specific CD4<sup>+</sup> T cells due to the lack of proliferative capacity and cytokines production[59-61] leads to a dysregulated CD8<sup>+</sup> T cell response which facilitates the emergence of escape viral variants[62]. Dysfunctional CD8<sup>+</sup> T cells are unable to control the viral load and become exhausted because of the persistent exposure to HCV epitopes which have not mutated[63]. Thus, these exhausted T cells undergo a progressive loss of their cytotoxic activity, proliferative capacity and proinflammatory cytokines production[64,65]. However, it is of note, that the cytolytic activity, and in particular the Fas/FasL dependent function, are associated with HCV immunopathology. Fas expression is up-regulated in hepatocytes of an infected liver whereas FasL is expressed in CTLs. This leads to liver damage by apoptosis of both infected and bystander hepatocytes, and subsequent liver fibrosis development[66].

### ***Humoral immune protection***

During acute HCV infection antibodies are produced and target epitopes in both structural and non-structural proteins, however, the envelope glycoproteins E1 and E2 are the main targets of the humoral immune response. Located at the N-terminal end of E2, the hypervariable region 1 (HVR1) is an immunodominant motif[67], which is the most variable region of the HCV genome[68]. Mutation in neutralizing epitopes allow the virus to escape from isolate-specific nAbs[69-71].

Early studies reported that nAbs developed against HCV target the HVR1 region of E2, however these nAbs were isolate-specific[67,69]. Thus, diverse studies have identified monoclonal antibodies (mAbs) that target conserved sites across multiple HCV genotypes located on either linear[72,73] or conformational[74,75] epitopes on E2 ectodomain.

Analyzing sera from different patients who were infected with the same HCV isolate showed that 43% of those who resolved their infections had nAbs against the main HVR1 variant, whereas these antibodies were present only in 13% of patients who evolved to chronicity[76]. Interestingly, plasma isolated from HCV-infected patients immediately prior to clearance has a better capacity to neutralize HCV strains from different genotypes compared to acute infection plasma from patients who subsequently evolve to persistence[77,78]. Furthermore, analysis from patients who cleared HCV infection showed detectable level of nAbs at earlier time points in comparison with acute infections that proceed to chronicity[79]. Chronic infections have been associated with a delayed cross-reactive nAbs response[43,77,78,80]. Although cross-reactive nAbs elicited during chronicity are not able to clear the infection, these have been associated with reduced liver fibrosis[81].

Despite the high genetic diversity of HCV, it was possible to isolate broadly neutralizing human Abs (bNAbs) from HCV-infected individuals, capable of neutralizing diverse HCV genotypes targeting relatively conserved regions on envelope glycoproteins[48,75,82]. These bNAbs have shown to be protective against infection in animal models of HCV[75] and are capable of abrogating established HCV infection in a humanized transgenic mouse model[48]. These findings underscore the protective role of the antibody response.

### ***Evidence of protective immunity against HCV reinfection***

The resolution of the initial HCV infection does not lead to sterilizing immunity so patients who previously controlled the primary HCV infection can be infected again [83]. However, differential rates of reinfection and/or chronicity have been reported among people who inject drugs (PWIDs) with the same risk of exposure, being reduced in people previously infected in comparison with people without previous infection[84]. Resolution is achieved in about 80% of HCV-reinfected patients[85].

Reinfection was characterized by a significant reduction in duration and magnitude of viremia compared with the primary infection and it was also shown to protect against persistence[85]. Moreover, clearance of reinfection was associated with an earlier and higher frequency of broadened T cells secreting IFN- $\gamma$  as compared to primary infection[86-89] and an early induction of nAbs[85,90].

Long-lived memory HCV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells are detected in the peripheral blood in humans following spontaneous resolution of the primary infection for up to 20 years[89,91]. CD4<sup>+</sup> T cell depletion before reinfection leads to viral persistence even in the presence of functional CD8<sup>+</sup> T cells which evidences the protective role of memory T cells upon re-exposure to HCV. While CD8<sup>+</sup> T cells are the main effector cells in viral control, CD4<sup>+</sup> T cells are essential for CD8<sup>+</sup> T cell function and prevent viral escape within epitopes targeted by CD8<sup>+</sup> T cells.

## CHALLENGES FOR DEVELOPING ANTI-HCV VACCINES

A number of difficulties have hindered the development of vaccines against HCV throughout the years (Figure 1). Despite all the knowledge acquired on the biology of this virus in recent years, a full understanding of key aspects of its pathogenesis and the host's immune response remains elusive. Taking into account the correlate of protection, an effective vaccine needs to be able to prime both arms of the adaptive immune response. Thus, vaccination has to induce an early and sustained expansion of specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell response. Alongside cellular immunity, cross-reactive nAbs need to be elicited to provide protection against different variants and genotypes.

In this section we will go over the most important challenges on the design and validation of an effective vaccine against HCV.

### **Lack of economic incentive**

Despite the fact that vaccines are great tools to prevent diseases, usually they are not as profitable as are drugs and other health services, and therefore investing in vaccine development is less appealing for the pharmaceutical industry[92]. Additionally, the development of vaccines with two different aims (prophylactic and therapeutic) would probably be expensive, and including prime/boost vaccination strategies may result impractical[19]. On another front, most newly infected individuals are PWID which mainly belong to populations with limited financial resources. This represents another discouraging aspect for companies interested in vaccine development[19].

From an economic perspective, though, there is well-reported evidence that vaccines are, in the long run, the most cost-effective public health measure after access to clean water[93,94]. A vaccine to fight HCV will, most likely, not be an exception.

### **Viral genetic diversity and variability**

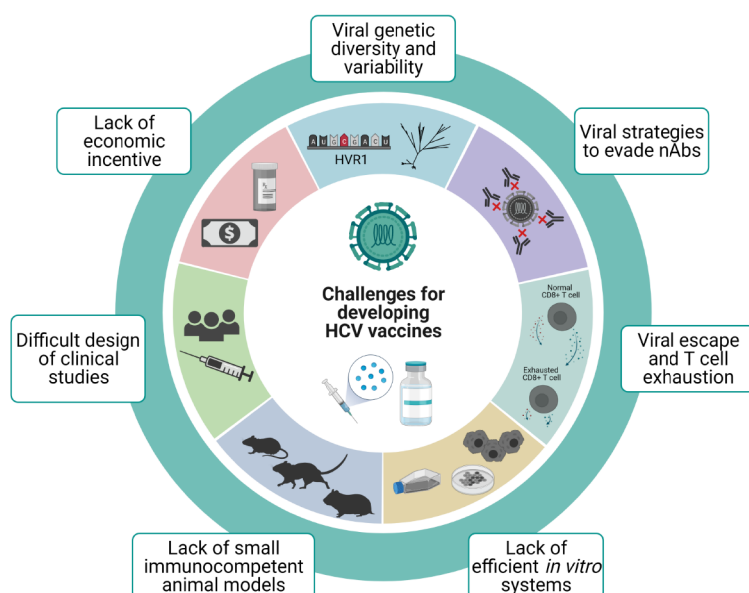
HCV is an enveloped virus with a single-stranded positive RNA genome which has a single open reading frame (ORF) flanked by non-coding regions at both ends (5' and 3'). For these features, it is classified as the prototype member of the Hepacivirus genus within the *Flaviviridae* family[95]. The ORF codes for a polyprotein of around 3000 amino acids which is co- and post-translationally processed into three structural (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B)[96].

Mutation is a key mechanism contributing to HCV genetic diversity and it is mainly driven by the error prone NS5B RNA-dependent RNA-polymerase[97]. HCV has an approximate mutation rate of 10<sup>-5</sup> mutations/nucleotide/replicative cycle[98,99], a characteristic which together with big population sizes, short generation times, and high replication rates generates the intra-host circulation of a complex population of closely related genome variants, usually termed as viral quasispecies[100,101]. Of utmost importance is the N-terminus of the envelope protein E2[67]. It contains the HVR1 region of about 30 amino acids which exhibits a huge variation among different isolates, and it is the most variable region of the entire HCV genome[68]. Even though most HCV-infected individuals develop nAbs against the virus, this high variability represents a problem as it allows the virus to escape immunologic surveillance and prevents the development of vaccines that induce cross-reactive nAbs[21]. Thus, a major challenge for the development of a broadly reactive vaccine for the control of HCV infection is identifying conserved neutralizing epitopes outside of HVR1.

Notably, mutations within HVR1 have also been associated with resistance to cross-neutralizing antibody response even if their epitopes are conserved, which highlights again the difficulties in achieving HCV neutralization as HCV could persist even in the presence of an antibody response to conserved epitopes[102,103]. This finding suggests that the neutralizing capacity of an antibody should not only consider the degree of conservation of its epitope.

Mutation rates coupled with the selective pressure exerted by the host's immune system has steered HCV diversification into 8 genotypes and 90 subtypes[104,105]. HCV strains from different genotypes differ by 30% in their nucleotide positions within the coding region, whereas subtypes exhibit 15% nucleotide variation[106]. Genotypes 1 and 3 are the most prevalent worldwide (accounting for 49.1% and 17.9% of diagnosed cases, respectively), and are most frequently found in developed countries[107].

The quasispecies dynamic as well as the resulting viral diversity confers HCV an amazing ability to adapt which in turn implies the possibility to escape from different therapeutic or preventive approaches such as antiviral drugs or vaccines[108-112].



**Figure 1 Challenges in hepatitis C virus vaccine research.** Graphical representation of all the hurdles yet to be overcome in order to develop effective vaccines against hepatitis C virus. Image created with BioRender.com. HCV: Hepatitis C virus. HVR1: Hypervariable region 1.

Thus, T cell-based vaccines intended to induce broadly reactive immune responses by targeting more conserved regions/proteins of the virus are desirable if the aim is to protect against new infections and/or persistence[11,21].

#### ***Viral strategies to evade neutralization by antibodies***

Viral entry to host cells and viral interactions with different host factors could theoretically be blocked by nAbs targeting HCV envelope glycoproteins E1 and E2. However, the virus has evolved several mechanisms which affect the host's ability to neutralize the virus. One of the mechanisms has been described extensively above (genetic diversity, particularly in HVR1 region), yet there are a number of other strategies employed by this virus to evade neutralization: (1) Glycosylation of structural proteins; (2) Cell-to-cell transmission; (3) Interfering antibodies; (4) Association with lipoproteins; (5) Antibody decoy; (6) Flexible conformational epitopes; and (7) Enhancing of viral entry.

**Glycosylation of structural proteins:** This feature reduces their immunogenicity as they are recognized as selfstructures. This is an important mechanism used by HCV to escape host humoral immune response. Glycans act by masking antigenic sites targeted by nAbs, interfering sterically with antibody neutralization[113]. Indeed, the deletion of N-glycans leads to an increase in E1E2 immunogenicity and can induce a more potent antibody response against HCV[114-116]. Glycan shift is another mechanism to induce neutralization resistance through glycosylation. Single point mutations which result in deleting a glyco-sylation site or generating a new glycosylation site in another part of the protein could facilitate viral resistance to neutralization. It has been reported that a new glycosylation site arose after incubating for 5 d a cell-culture derived HCV with nAbs obtained from mice. As a result, those broadly nAbs showed a decrease in their efficacy[117].

**Cell-to-cell transmission:** It is another mechanism for viral dissemination, which avoids the extracellular compartment and favors escaping host humoral immune responses[118,119].

**Interfering antibodies:** When non-nAb bind to sequences in the C-terminal region of HVR1, they disrupt the recognition of conserved epitopes by antibodies with neutralizing capability. Indeed, the remotion of interfering antibodies in chronic patients and vaccinated chimpanzees increases virus susceptibility to neutralization highlighting the role of interfering antibody in viral escape[120]. Similarly, when HVR1 was removed, enhanced and broad cross-neutralizing activity was observed[121,122].

**Association with lipoproteins:** HCV circulates in the blood in association with triglyceride-rich lipoproteins and low-density lipoproteins forming hybrid lipoviral

particles, which are a hallmark of infectious HCV particles. Several host-derived factors play a role in evading antibody neutralization. Lipoproteins such as apolipoprotein E contribute to humoral immune escape by hiding relevant neutralization epitopes in E2 protein, preventing them to be exposed during HCV assembly and maturation, hence, abrogating antibody neutralization[123,124].

**Antibody decoy:** Interestingly, *in vitro* studies have reported that HCV-infected cells release E2-containing exosomes that act as antibody bait making HCV virions less susceptible to neutralization[125].

**Flexible conformational epitopes:** The capacity of some conserved neutralizing epitopes in E2 to adopt different conformations when complexed with diverse antibodies contributes to evade neutralization by antibodies. This conformational flexibility must be taken into account during vaccine design[126].

**Enhancing of viral entry:** It has been shown that host mutations that alter the interaction of serum components like high-density lipoprotein with scavenger receptor BI enhance viral entry to the cell[127]. This, in turn, protects the virus against humoral response as the time window in which nAbs can bind and act is reduced[128,129]. Fofana *et al*[130] (2012) also showed that mutations in the E2 glycoprotein, conferred viral escape to humoral responses by altering the use of the T cell receptor CD81[130].

Despite these challenges, it has been possible to isolate broadly cross-neutralizing mAbs with the ability to block HCV infection of various genotypes and thus, protect against heterologous viral infection[75,131-134]. These findings suggest that a prophylactic vaccine against HCV may indeed be achievable.

The elucidation of the crystal structure of E2 has provided a better insight into different antigenic domains and regions that allow a rational vaccine design. A study showed that epitopes within E2, exhibiting moderate or conserved variability, were efficiently targeted by bNAbs[135,136]. Unfortunately, despite the relative conservation of some bNAbs epitopes, escape mutations have been identified[137,138].

### **Escape mechanisms from T cell responses: Viral escape and T cell exhaustion**

Several studies have evidenced the key role of cellular immunity in the clearance of infection. An effective vaccine has to induce a rapid recall of the memory T cell responses that is associated with reduced viraemia and a higher likelihood of spontaneous resolution. However, the virus has developed different mechanisms to lead to an inefficient cellular response even when re-exposed with homologous virus: (1) Viral escape T cell recognition; and (2) T cell exhaustion.

(1) Escape mutations within major histocompatibility (MHC) class I-restricted HCV epitopes represent the main mechanism used by HCV to evade CTL responses and thus it is associated with persistence. Unlike CD8<sup>+</sup> epitopes, escape mutations within targeted CD4<sup>+</sup> T cell epitopes are not common, suggesting that CD4<sup>+</sup> T cells failure mechanisms cannot be completely explained by viral escape[139]. Escape mutations occur early in infection and they are rare during long-term chronic infection, possibly due to the lack of T cell-mediated selective pressure[140]. Interestingly, escape variants show an impaired replicative fitness[141,142] and this contributes to limiting the variability within some epitopes[143,144]. As a consequence, the ideal target for T cell-based vaccines are conserved epitopes less likely to mutate because of viral fitness cost [141,142]. Another effect of escape variants results in impaired recognition by T cells receptors and thus prevents CD8<sup>+</sup> T cell recognition. Moreover, CD8<sup>+</sup> T cells from infected patients with genotype 4 were not able to recognize epitopes from other genotypes[52]. This finding highlights the challenging task of choosing vaccine targets that protect against multiple HCV genotypes. Hence, identifying conserved epitopes recognizable by specific CD8<sup>+</sup> T cells is a key point to develop efficient T cell-based vaccines.

(2) T cell exhaustion: While T cell-based vaccines likely provide protection against chronic virus infections, they also have the potential to generate immunopathology following subsequent virus infection. This is illustrated by the fact that during chronic infection an impaired HCV-specific CD8<sup>+</sup> T cell response develops, known as T cell exhaustion. This phenotype is associated with the inability of the immune system to control viraemia during chronic infection. These exhausted T cells undergo a progressive loss of their ability to proliferate, to secrete cytokines (such as IFN- $\gamma$ ), and to be cytotoxic[64,65].

Long-lived memory T cell response is only induced following spontaneous clearance and it can provide some protection. However, individuals who cannot maintain such long-lived memory T cell response due to T cell exhaustion are not



protected upon re-exposure.

One of the major challenges for immunogenic T cell vaccines refers to the recovery of T cell immunity through vaccination in people with persistent HCV infection. Kelly *et al*[145] (2016) demonstrated that when an HCV T cell vaccine based on chimpanzee adenoviruses (ChAd3) are given to patients with chronic disease, the immune response is not able to restore T cell function[145]. Failure to respond to this vaccine approach may be the result of T cell exhaustion, as vaccination is stimulating memory responses that were induced early in infection but that ended up partially dysfunctional following viral exposure[145].

### **Lack of efficient *in vitro* systems**

An essential step in vaccine research is the evaluation of antibodies generated as a result of natural infections or experimental immunizations, as well as the evaluation of vaccine candidates. For those purposes using different *in vitro* and animal models becomes a must[23].

As we will exemplify in a later section on vaccines against SARS-CoV-2, the generation of live-attenuated and/or inactivated whole virus vaccines has been possible against a number of different viruses (measles, mumps, rubella, rotavirus, hepatitis A virus, poliovirus, among others), however this strategy is not achievable to generate HCV vaccines. Since HCV was discovered[1], and only until recently, research has been thwarted by the inability to culture the virus both *in vitro* and *in vivo*[23, 146].

As for *in vitro* models, propagating HCV in cultured cells remained limited for several years since inoculation of patient sera or plasma in different cell lines resulted in limited or no viral replication[147]. The first report of efficient replication came from working with HCV subgenomic replicons (where the structural region was replaced by a neomycin-encoding gene)[148]. However, the challenge was to generate an *in vitro* system that was able to produce infectious HCV particles at high titers that would allow further research[23]. The production of cell-culture derived viral particles (HCVcc) was only achieved in 2003 with the discovery of a genotype 2a isolate (strain JFH-1) derived from a Japanese patient with a fulminant hepatitis[149,150]. Transfecting replicon HCV RNA from isolate JFH-1 into human hepatoma-derived Huh7 cells resulted in efficient RNA replication without the need of any adaptive mutations[150, 151]. Nevertheless, despite this breakthrough, efforts to replicate this with other isolates corresponding to different genotypes were only partially successful. On the one hand, some of these cloned full-length RNAs were able to produce infection *in vivo* (in chimpanzees), but on the other hand, even in the presence of multiple adaptive mutations, they failed to produce infectious viral particles in cell culture, despite some being able to efficiently replicate (details on the history of HCV cell culture systems are thoroughly reviewed elsewhere[147,152-154]).

Further studies on HCVcc led to the discovery of more permissive cell clones derived from Huh7 cells (*e.g.*, Huh7.5 and Huh7.5.1)[155,156] as well as to the generation of inter- and intragenotypic recombinant genomes that are able to recapitulate the complete HCV life cycle and produce high titers of infectious particles *in vitro*. These recombinants have been shown to be optimal *in vitro* models to study the neutralization ability both of mAbs as well as of sera from infected patients[82,157-160]. They have also been used to characterize antibody escape mutations[71,137,161]. Additionally, reporter and flag-tagged JFH-1-based genomes (J6/JFH1) have been generated[162-164] and used in vaccine development[165], the latter in particular to facilitate large-scale purification of viral particles[163]. However, the most important aim in this field would be to efficiently grow any virus derived from HCV infected patients, which unfortunately has not yet been achieved[153]. For now, we depend on the constructs described above as well as a few full-length consensus clones, which have been developed after a lot of research effort and had to be designed including numerous adaptive mutations[166-170], therefore, not quite resembling natural circulating isolates. In spite of the setbacks, all these constructs have the potential to be employed for producing inactivated whole-virus vaccines.

Another *in vitro* approach to assess the neutralizing ability of sera and mAbs, in addition to HCVcc, relies on the generation of HCV pseudoparticles (HCVpp). These are generated by cotransfecting HCV E1 and E2 genes together with a retroviral packing and reporter system[171]. Due to the struggles imposed by the generation of different HCVcc derived viral particles, HCVpps were actually developed earlier[172, 173] but continue to be used in vaccine research nowadays[157,174-176].



### **Lack of small immunocompetent animal models**

Humans are the natural hosts of HCV, and in order to test the efficacy and safety of vaccine candidates in pre-clinical studies, *in vivo* animal models are needed. Foremost, *in vivo* studies on pathogenesis of HCV chronic infections have been problematic since HCV only infects humans and, under experimental conditions, also chimpanzees. The first and most successful immunocompetent animal model has indeed been the chimpanzee. However, ethical concerns and its inclusion on the United States Fish and Wildlife Service's Endangered Species have led to a ban in its use for biomedical research[177]. Even before this prohibition, the continued use of these animals faced many issues such as high costs, small cohort sizes which made statistically significant results difficult to achieve, and the inability to genetically manipulate chimpanzees. Furthermore, it would require the need to have special and expensive facilities to breed and keep them under study[178].

Small animal models are frequently very useful tools to test potential vaccine candidates, but, since HCV does not infect rodents, a lot of effort has been devoted into developing strategies to adapt mice to evaluate HCV vaccines. This led to the use of chimeric humanized or transgenic mice with humanized livers[179] or expressing human CD81 and occludin[180], two cellular proteins that HCV uses as receptors for cell entry. However, mouse models are difficult to produce, and most are immunocompromised, which makes them inappropriate to study virus-host interactions and immune responses. Additionally, they do not exhibit cirrhosis or HCC[181]. In spite of this, genetically humanized fully immunocompetent inbred mice expressing human orthologs of HCV entry factors were developed[182], which have allowed the study of viral entry, yet not the full viral cycle. To address the latter, Chen *et al*[183] (2014), developed an immune-competent humanized mice model that is capable of developing persistent HCV infections and hepatopathological manifestations[183], yet the mice stock are outbred and genetically not well defined. More recently, Keng *et al* [184] (2016) were able to establish a new humanized mouse model including human hepatocytes as well as human immune system[184], which was able to recapitulate HCV infection and immunopathogenesis[181], although low levels of B cells were detected when compared to clinical settings.

For the difficulties in getting broad access to small immunocompetent mouse models, alternative experimental non-human primate models have been explored. However, no signs of infection were detected (for a detailed review see Ploss and Kapoor[178], 2020), with the exception of tree shrews (now classified in a separate order Scandentia, but previously designated as small squirrel-like primates) which can become symptomatic and even progress to chronicity[185]. Despite this encouraging finding, keeping these animals in captivity is a difficult task, and additionally they are genetically diverse for being an outbred species, which again poses issues to be widely used in HCV biomedical research[178].

Altogether, this shows us the difficulty we face when we need animals that can be employed for vaccine development but also to study HCV-associated pathogenesis. An alternative could be the use of substitutes and analogue viral models that can be propagated in mice lab strains and that appear to share basic immunological features with HCV. Recently, the discovery of non-primate hepaciviruses has raised interest since they can be used as analogues of HCV infection[23]. A rodent Hepacivirus discovered in Norway rats[186] has been shown to establish high-titer liver infections when inoculated in immunocompetent mice, and thus, provides insight into hepatic immune responses[187]. However, the main drawback of this model is the limited sequence homology to HCV[186]. On the other hand, equine hepacivirus (eqHV), formerly known as non-primate Hepacivirus, is the closest relative of HCV and both species share some important features such as the level of E1E2 glycosylation or the presence of miR-122 seed sites in their 5' non-coding regions (2 sites in HCV and 1 site in eqHV)[188,189]. These approaches of using alternative and analogue viral models for vaccine development is extremely valuable, yet it is worth acknowledging that different mammalian immune systems might respond in different ways and this should be taken into consideration at the moment of interpreting data[23].

### **Difficulty in designing clinical studies**

The design of clinical studies for HCV vaccine candidates poses its own hurdles. It must be considered that, in order for an effective vaccine to be validated, it should be tested in populations at risk for HCV infection[11,36]. This is an issue in developed countries where HCV infection incidence is low other than in PWID populations. Targeting this group of patients has ethical concerns and practical difficulties to be overcome[190]. Despite this, there are a few studies which have been successful in

identifying, enrolling and monitoring PWID before developing an acute HCV infection [191,192], the latest completed phase I/II clinical trial with outcome results was able to enroll 548 active intravenous drug users (ClinicalTrials.gov Identifier: NCT01436357 [193])[194]. On the other hand, large studies could be conducted where incidence is higher, such as some developing countries. However, logistical problems may arise due to the large number of patients needed and their appropriate follow up, specifically to detect acute cases of hepatitis, which usually course without any symptoms[36].

## APPROACHES TO DESIGN VACCINE CANDIDATES FOR HCV

There are several traditional and newer approaches in vaccine development, and most of them have been explored for the design of HCV vaccine candidates (Figure 2), albeit the majority only directed at genotype 1.

Traditional vaccine approaches include whole-organisms vaccines containing either inactivated whole or live attenuated viruses. Live attenuated vaccines are potent in inducing CMI and humoral immunity and have been successful for many viral infections because they resemble what occurs naturally. Nevertheless, they have the potential risk of reverting to virulent wild-type strains. In contrast, inactivated viruses are noninfectious but have the downside of being less immunogenic than attenuated viruses. Therefore, when inactivated whole viruses are developed as vaccine candidates, they often include adjuvants and/or booster injections in order to enhance the immunogenicity[195].

Newer methods involve the use of one or more genes of the virus of interest to be incorporated into the genome of a nonpathogenic organism for amplification. In this way, mainly three different approaches have been developed: Subunits vaccines (by purifying the protein/s of interest generated in the heterologous organisms), DNA vaccines (usually by isolating a plasmid containing the gene/s of interest), and recombinant viruses (by using the entire host virus as a live vector)[195].

The latest method successfully explored has been the use of RNA-based vaccines, whose development is faster than other technologies, easily scalable, and of lower cost to manufacture. These characteristics have been essential to the development and recent authorization for emergency use of some of the vaccines currently available to control the COVID-19 pandemic[196].

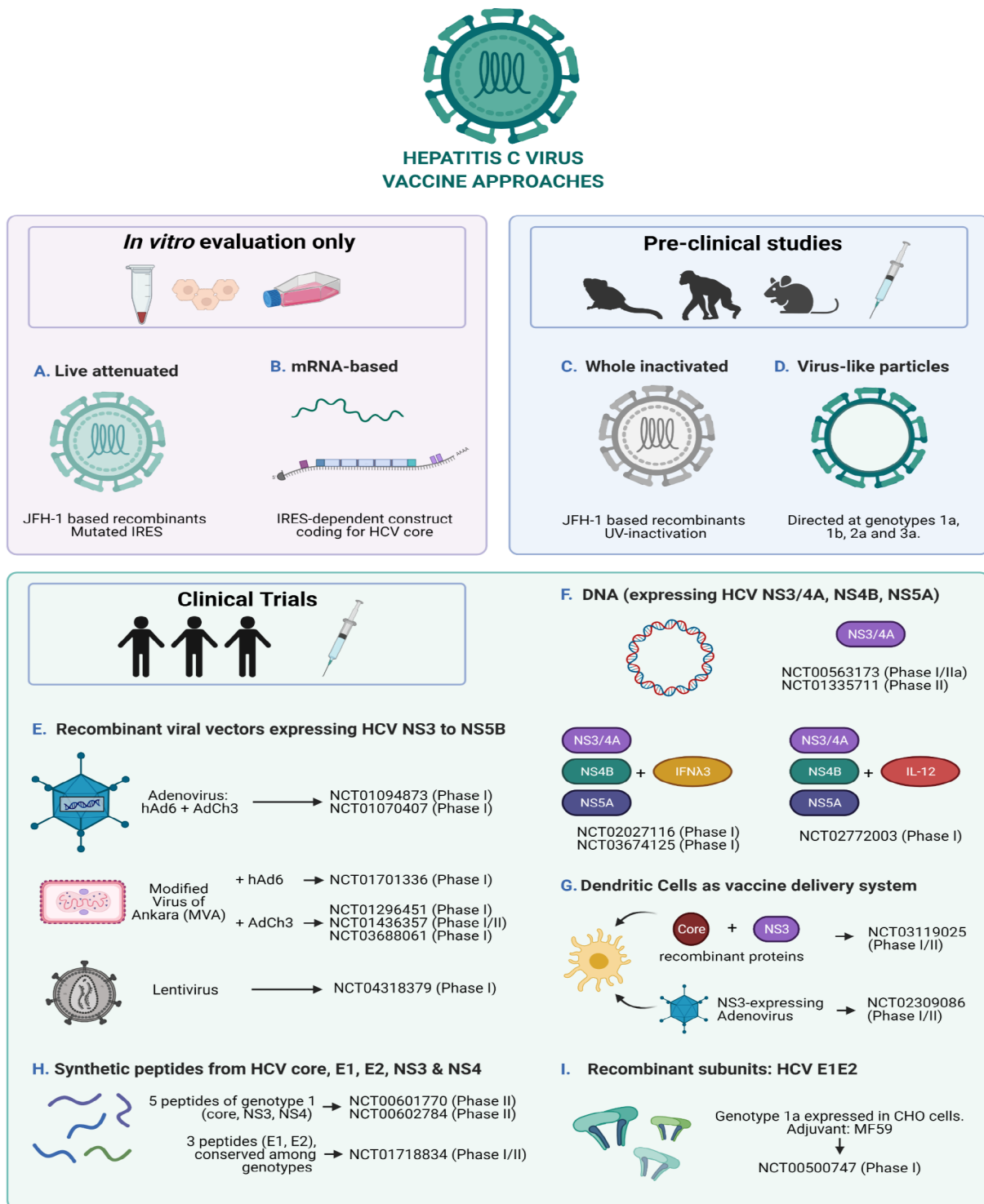
In this section we will go over some of the vaccine candidates explored against HCV, and we will delve into nucleic acid-based and recombinant viral vector approaches.

### *Inactivated whole virus (HCVcc)*

This traditional approach of inactivated virus was only feasible after the development of cell culture systems, with all the challenges that they impose even nowadays. This is partly the reason why there are only a few pre-clinical studies assessing the immunogenicity of inactivated HCVcc as vaccine candidates[197,198]. Both studies have shown the induction of humoral immune responses in chimeric mice[198] as well as in a non-human primate model[197]. The latter also elicited T cell responses. These findings are promising, but there are still some developmental challenges to overcome if this approach is to be considered for clinical trials, such as production in serum-free culture conditions and scalable and cost-efficient downstream processes. Fortunately, there are a few studies which have addressed these difficulties, and have shown that high titer serum-free HCVcc is possible for different intra and intergenotypic recombinants based on JFH-1 isolate[199] and that more efficient downstream processes based on ultracentrifugation and chromatography can be applied[200]. Nevertheless, the challenge of generating high titers of HCVcc of the most widespread genotypes and subtypes still remains.

### *Recombinant subunits and synthetic peptides*

Recombinant E1/E2 proteins were the first prophylactic vaccine candidates being tested since they are the major targets for nAb, in particular HVR1 region within E2. They were shown to be able to induce the generation of nAb in chimpanzees[201], yet only one candidate reached clinical trials in 2007 (ClinicalTrials.gov Identifier: NCT00500747[202]). Results of the phase I trial in healthy volunteers showed the vaccine was well-tolerated at different doses used, and that it was able to induce antibody production[203,204].



**Figure 2 Summary of all hepatitis C virus vaccine approaches explored to date.** The studies are divided in three categories depending on the highest stage of research achieved: *In vitro* evaluation only (in lilac background), pre-clinical studies in different animal models (in light blue background) and clinical trials in healthy volunteers and/or chronically hepatitis C virus-infected patients (in green background). For each approach (A to I) key characteristics on the vaccine candidates are provided. In addition, for all the technologies that have reached clinical trials, the ClinicalTrials.gov Identifier and the phase of the trial are indicated. Image created with BioRender.com.

Whereas recombinant E1E2 vaccines were designed to elicit humoral immune response, synthetic peptide vaccines are more attractive since they can be designed to prime both arms of the immune response. Some peptide combinations targeting both cytotoxic lymphocytes and CD4<sup>+</sup> T cell epitopes (core, NS3, NS4) have entered clinical trials. Results for the phase 2 trial NCT00602784[205] have shown that the peptide vaccine IC41 can trigger T cell responses in relapse patients after dual therapy, yet viral clearance was not achieved[206]. Unfortunately, humoral response was not analyzed. The results of the other studies remain to be published (ClinicalTrials.gov

Identifier: NCT01718834[207] and NCT00601770[208]).

Of interest, computational identification of B and T cell epitopes has been explored as an alternative for the rational design of effective vaccine candidates. By means of different immune-bioinformatic and population dynamics simulation approaches, many predicted epitopes in E2, NS3/4A, NS5A and NS5B have been identified[209-212]. These approaches provided valuable information and *in silico* screening methods for highly conserved immunogen candidates with the putative ability to block escape mutations (for a detailed review please see[213]). These computational designs can help speed up vaccine development at the experimental stages by rationally selecting the most promising epitopes for subunit vaccine *in vitro* and *ex vivo* evaluation.

### **Virus-like particles**

Virus-like particles (VLPs) are particles that resemble a virion but do not contain the viral genome, rather they are generated by the auto assembly of structural proteins in a manner that is genome-independent. In this way, the particle is similar to the native virus but it lacks the ability to replicate and for vaccine candidates is a very attractive technology since they are more immunogenic than soluble proteins and can prime both arms of the immune response[214].

The rationale behind this type of vaccines is supported by the successful development of vaccines against hepatitis B virus and human papilloma virus, currently commercially available[23]. Unfortunately, despite having shown promising pre-clinical results[215,216], to the best of our knowledge, HCV VLPs have not yet reached clinical trials.

### **Recombinant vector-based vaccines**

The use of live recombinant viral-based HCV vaccines as a genetic immunization approach has shown to be powerful for eliciting CMI[217]. For this purpose, different modified viruses are used as vectors to carry HCV genetic information[19].

Adenoviral vectors are the most widespread used in the vaccine developing industry. They are attractive models for different reasons: Adenoviral genomes are well characterized and are relatively easy to modify into replication-defective viruses, most human adenoviruses cause mild infections, they infect a broad number of cell types (dividing and non-dividing), they can be grown to high titers in tissue culture, and by deleting essential genes, genetic information of interest can be inserted[218]. The most frequently used in immunization studies is the human adenovirus serotype 5 (hAd5), which is included in at least 12 of the vaccines against SARS-CoV-2 that are currently on clinical trials and in one that already had authorization for emergency use (Sputnik V vaccine)[219,220]. Despite their benefits, individuals might exhibit preexisting anti hAd5 Abs, which could diminish the immune response to vaccines based on this viral vector. For this reason, less frequent serotypes such as hAd24, hAd6 or hAd26 have been employed in pre-clinical and clinical studies of vaccine candidates against different viruses[221-223]. Additionally, adenoviruses that infect chimpanzees (AdCh3) have been tested in conjunction with hAd6, both carrying HCV non-structural proteins NS3 to NS5B of genotype 1b, yet despite reaching clinical trials, they have only been evaluated in phase I studies (ClinicalTrials.gov Identifiers: NCT01094873[224] and NCT01070407[225]). The reason for not continuing these studies seemed to be the inability to restore CMI, and as a result, a non-significant effect on HCV viral load was observed[145].

In light of these drawbacks, another viral vector has been employed in prime/boost vaccination strategies against HCV: The Modified Virus of Ankara (MVA), an attenuated poxvirus strain which is immunogenic and safe since it lacks several immunomodulatory genes[226]. MVA vector together with hAd6, both expressing HCV non-structural proteins NS3 to NS5B have entered phase I clinical trials to evaluate the combination as a therapeutic vaccine to be used in conjunction with dual therapy (ClinicalTrials.gov Identifier: NCT01701336[227]). Even though the study is complete, no results have been disclosed, presumably due to the newer DAA treatments which have completely substituted classical therapy. The most promising trials currently in phase I and II use the combination of ChAd and MVA vectors harboring HCV NS3-NS5B genomic regions. A phase I study in healthy volunteers showed promising results in terms of eliciting T cell responses (ClinicalTrials.gov Identifier: NCT01296451[228])[229]. Unfortunately, a phase I/II study in PWID population showed that this vaccination strategy was not effective for preventing chronic infections since T cell exhaustion was not reversed (ClinicalTrials.gov Identifier: NCT01436357[193])[194,230]. These results highlight the need for a vaccine strategy that stimulates both humoral and T cell immunity[23,231]. However, attempts to enhance CMI without the need of boosting the generation of Abs, have been



addressed in pre-clinical studies on non-human primates by fusing the HCV non-structural antigen to MHC class II-associated invariant chain[232]. The results showed enhanced and accelerated CD8+ T cell responses and paved the way to reach clinical trials. At the time of writing this manuscript, there is an actively recruiting phase I clinical trial (ClinicalTrials.gov Identifier: NCT03688061[233]) that seeks to enroll 25 healthy participants to assess the safety and immunogenicity of HCV prime/boost vaccination with both ChAd and MVA vectors expressing HCV non-structural antigens fused to a class II-invariant gene. Results from only 15 individuals seem promising, largely mimicking pre-clinical studies, but more participants are still needed and assessment of durability of the enhanced CMI needs to be further addressed[234].

The most recent vector-based therapeutic vaccine candidate entering phase I clinical trials is a lentiviral based HCV immunotherapy (HCVax) which aims to evaluate both the safety and the immune response in chronic HCV patients (ClinicalTrials.gov Identifier: NCT04318379[235]). Last generation lentiviral vectors are safer than first generation ones (previously used for gene therapy) and like adenoviral vectors, are capable of infecting both dividing and nondividing cells, and since they integrate into the host's genome, expression of the transgene can be long-term, a characteristic which makes them attractive as vaccine strategy[236].

### ***Nucleic acid-based vaccines***

Nucleic acid based-vaccines present numerous advantages over traditional vaccine approaches: (1) No issues associated with misfolding of proteins in recombinant protein vaccines or with high manufacture costs; (2) No infectious risks that might be associated with live-attenuated or inactivated whole virus vaccines; (3) They are able to activate both arms of the immune response (humoral and cellular); (4) The expression of antigens resembles natural epitopes; (5) In a single injection, multiple genes can be delivered; and (6) If multiple doses are needed, unlike the use of recombinant virus-based vaccines, there is no risk of anti-vector immunity[39,237,238].

DNA-based vaccines have been in the picture for nearly 40 years now[239]. They usually consist of purified plasmids which harbor sequences of interest that are expressed under the control of a eukaryotic promoter for a robust expression in mammalian cells. They are inexpensive, easy to manufacture, and also important, stable at room temperature. All of which are features that make them an ideal technology in vaccine research, as distribution and access could be granted effortlessly even to developing countries[39].

RNA vaccines have been explored for around 25 years, beginning with studies of self-amplifying RNA vectors (modified RNA from viruses) as well as mRNA pulsed into dendritic cells (DCs)[240,241], and have been largely assessed for tumor vaccination[242]. They share some features with DNA vaccines, but they do not need to enter the nucleus to translate the genetic information into antigen proteins, which represents an advantage over DNA immunization since the barrier of the nuclear envelope is removed, and thus, their efficacy is higher[238]. However, RNA is more labile than DNA, which might yield less robust vaccines than DNA-based formulations due to RNA shorter shelf life, reason why modified nucleosides have been used to enhance stability and therefore induce a higher antigen production[238], as it is the case of the COVID-19 mRNA Vaccine (nucleoside modified)[243].

The first approach for delivery of nucleic acid-based vaccines, was direct injection of naked DNA plasmid or mRNA (transdermally or intramuscularly), however, efficiency seemed to be very low, in part due to the negative charge of these molecules. Therefore, several delivery methods were developed to improve uptake and immunogenicity in different organisms: (1) Gene gun: DNA is loaded on the surface of tungsten or gold particles and then fired at target cells; (2) Electroporation: Transient pores in cell membranes are created by electrical impulses allowing DNA delivery inside the cell; and (3) Nanoparticles: Non-viral vectors made up from lipids, inorganic molecules and polymers can safely carry DNA and RNA into a cell by encapsulating the negatively charged nucleic acid, preventing its digestion by endonucleases and facilitating intracellular release[36,238].

### ***DNA-based vaccines***

Multiple pre-clinical studies in different animal models have been performed throughout the years to assess the efficiency of several DNA-based formulations against HCV to elicit immune responses. Nevertheless, only a few have entered phase I or II clinical trials.



The use of core as antigen, directly injected as naked DNA plasmid intramuscularly (IM) or intraperitoneally (IP) into different mice models, has evidenced a weak immunogenic capacity in terms of humoral response but strong CMI, even though at least 2 doses 2-4 wk apart were administered[244-248]. Using the same delivery method and injection scheme, HCV core and E2 sequences were fused to immunogenic proteins (hepatitis B surface antigen or gD protein from herpes simplex virus type-1) to address the weak Ab response, and both arms of the immune response were detected in mice as well as in rats[249-251]. Others have attempted to evaluate if different localizations of HCV antigens within the cell and the CpG content of the plasmid backbone might influence the Ab response. Results indicated that membrane-bound and secreted E2 forms as well as the addition of immunostimulatory CpG motifs elicited a better humoral response in mice[252]. Low doses of IFN- $\alpha$  have also shown to augment CTL response after DNA immunization with a plasmid encoding HCV core protein in mice models[253].

Targeting structural proteins in DNA-based formulations employing injection of naked plasmid as the delivery method was thoroughly tested in animal models but the vast majority failed to enter clinical trials. With the increasing knowledge on immune correlates during acute infections, it became clear that non-structural proteins are the target of CMI during acute resolutions, and that other delivery methods such as electroporation or gene gun rendered broadly reactive CTL responses[254].

As a consequence, DNA-based vaccines encoding HCV non-structural proteins have become widely used approaches. Transdermal gene gun injection of DNA plasmid encoding NS3/4A proteins into mice has shown high titers of Abs and the ability to prime CD4<sup>+</sup> T helper cells[255] and also a CD8<sup>+</sup> T cells that were able to clear HCV protein-expressing hepatocytes and persist up to 12-18 mo after immunization[256, 257]. When NS3 DNA vaccine was co-administered with interleukin-12 as adjuvant, strong immunogenicity was also displayed in murine models[258]. Several other adjuvants have also been employed in NS3-based DNA vaccination in order to enhance their potency (for a detailed review see Sepulveda-Crespo *et al*[231] 2020). In addition, constructs encoding a codon-optimized NS5A injected IM into mice, in combination with *in vivo* electroporation, were also able to prime specific T cell responses[259]. Two clinical trials in chronic HCV patients (naïve to treatment, infected with genotype 1) have entered phase I/IIa and phase II to evaluate a potential therapeutic vaccine based on a plasmid encoding NS3/4A (ChronVac-C) (ClinicalTrials.gov Identifier: NCT00563173[260] and NCT01335711[261]). Results have shown that high doses of ChronVac-C were able to activate HCV-specific T cell responses which led to a transient reduction in viral loads[262]. When 8 of the 12 patients enrolled also received dual therapy after the vaccine doses, 6 were able to achieve SVR, which might indicate that immunization had a beneficial effect on the response to therapy. However, these results seem irrelevant at present with the advent of DAA treatments.

Even though pre-clinical results were promising, full-length NS3 protein exhibits immunosuppressive effects and it is possibly involved in the development of HCC due to its enzymatic activity which deregulates the normal functions of the host cells[263]. Even though DNA immunization renders antigen expression only transiently, and the adverse effects possibly caused by NS3 enzymatic activity would be marginal, alternative plasmids for DNA vaccination encoding modified NS3 sequences have been tested in animal models. Ratnoglik *et al*[264] (2014) showed that vaccinating mice with a non-enzymatic version of NS3 (with its catalytic site and NTPase/RNA helicase domains mutated to abrogate their functions) induced strong CMI, indicating that mutations in this protein do not seem to interfere with its immunogenicity[264]. Additionally, a plasmid with a truncated form of NS3, only encoding immunogenic epitopes (1095-1379 amino acids positions), succeeded in eliciting a strong Ab response after repeated intra-dermal inoculation in mice[265]. However, none of these candidates has reached clinical trials.

These findings seem to indicate that immunizing only with DNA-based formulations coding for NS3/4A or NS5A might not be sufficient to control viremia in HCV-infected patients, despite encouraging pre-clinical results in animal models.

In addition to NS3/4A or NS5A plasmid vaccination, IM injections followed by electroporation of constructs encoding NS3 to NS5B into *Rhesus macaques* and chimpanzees, in multiple-dose boosting schemes, evidenced HCV-specific effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells and effector memory-like CTLs after immunization[266,267]. More recently, studies in mice have shown that adding a plasmid expressing cytokine IFN- $\lambda$ 3 (formerly known as IL28B) to the immunization with plasmids expressing NS3/4A, NS4b and NS5A provided significant immunoadjuvant activity[268]. These encouraging results led to a phase I clinical trial to evaluate the safety, tolerability and immunogenicity of this strategy in chronic hepatitis C patients infected with HCV

genotypes 1a or 1b, which had previously exhibited treatment failure to dual therapy alone or in combination with DAAs (ClinicalTrials.gov Identifier: NCT02027116[269]). The vaccination strategy comprised a combination of 3 plasmids each encoding NS3/4A, NS4B or NS5A (formerly known as VGX-6150) and a fourth plasmid encoding IFN- $\lambda$ 3 as an efficacy enhancer (the mixture of 4 plasmids has been renamed to GLS-6150). Three different doses were tested in a prime-vaccination scheme of 4 doses every 4 wk, and then a booster immunization at week 36, all injected IM followed by electroporation. Results of this trial have been recently published and they showed that GLS-6150 is safe and was overall well tolerated with no serious adverse events identified[270]. More importantly, vaccination increased the HCV-specific T cell responses, although, surprisingly, RNA viral titers did not decrease. Therefore, considering the reinfection possibility of patients who achieved SVR after DAA treatment, a new phase I clinical trial is ongoing in order to assess immunogenicity of GLS-6150 in this population and in healthy volunteers (ClinicalTrials.gov Identifier: NCT03674125[271]). Another clinical trial employing DNA vaccination of plasmids encoding NS3 to NS5A (INO-8000) but with the co-administration of a different adjuvant (interleukin-12) is currently active as a phase I study in chronically HCV infected patients (genotype 1) (ClinicalTrials.gov Identifier: NCT02772003[272]) which highlights the potential of these approaches including immunostimulatory molecules as adjuvants. The main takeaway of these approaches is that, the addition of more nonstructural genes as well as the co-administration of immunostimulatory adjuvants, might still be insufficient to clear an established infection. The question remains if they might be useful to prevent reinfections.

Therefore, as an alternative, heterologous prime/boost vaccination strategies have also been explored in mice, in which immunization with DNA-based vaccines is followed by immunization with viral vectors such as MVA to enhance response levels [273]. Even though results provided proof-of-concept that 2 different HCV vaccine technologies can improve immunogenicity when used in combination, to the best of our knowledge, so far, no clinical trial has tested this approach.

### **RNA-based vaccines**

As will be detailed in the section about vaccines against SARS-CoV-2, several mRNAs-based vaccine candidates have been intensely explored in clinical trials, in particular to fight the COVID-19 pandemic. However, so far none have been approved for human use, with the exception of some of the vaccines currently in phase 3 clinical trials which are undergoing assessment for WHO emergency use listing and prequalification[274-277] (ClinicalTrials.gov Identifier: NCT04368728[278] and NCT04713553 [279]–Pfizer/BioNTech SE, ClinicalTrials.gov Identifier: NCT04470427[280] and NCT04649151[281]–Moderna TX, Inc).

On the contrary, with the exception of using mRNA to transfect DCs (which will be discussed in the next section), there have been no pre-clinical or clinical trials using mRNA-based vaccines against HCV. Interestingly, Sharifnia *et al*[282] (2019) have proposed for the first time that an RNA-based vaccine against HCV could be feasible since after *in vitro* generation of an mRNA coding for the core protein, they were able to detect core protein in monocyte-derived DCs which were previously transfected with this construct[282]. Unfortunately, no further animal studies were performed to assess the immunogenicity of this approach.

### **DCs as vaccine delivery system**

DCs are one of the most potent antigen-presenting cells needed to induce and maintain immune responses. Given their fundamental roles, DC-based vaccination strategies have been given special attention, in particular for cancer immunotherapy [283]. However, different approaches have also been explored in HCV vaccination both in pre-clinical studies as well as in clinical trials[284]. Strategies involve loading DCs with HCV core, NS3 or NS5 proteins[285,286], pulsing them with HCVpp[287], transfecting them with DNA[288] or mRNA[289], or transducing them with adenoviral vectors expressing HCV non-structural proteins[290-293].

Two recently concluded phase I/II clinical trials have enrolled chronically HCV-infected patients (HCV genotype 1b) to evaluate the safety and clinical efficacy of therapeutic vaccination using autologous DCs. Despite employing different strategies (autologous DCs loaded with recombinant HCV core and NS3 proteins *vs* transduced with a recombinant adenovirus encoding NS3), both studies revealed similar results in terms of immunogenicity and ability to reduce viral titers: T cell responses were generated albeit weakly, and these were insufficient to clear the virus or reduce viral loads[286,293] (ClinicalTrials.gov Identifier: NCT03119025[294] and NCT02309086[295]). These findings are somewhat discouraging since in order to design better

vaccination strategies, attention will have to be placed on enhancing CMI so as to, at least partially, reduce viral titers.

## IS THERE A POTENTIAL USE OF ATTENUATED VIRUSES AS VACCINE CANDIDATES AGAINST HCV?

As with whole inactivated virus vaccines against HCV, the limited *in vitro* culture systems have hampered studies on attenuated vaccines. In particular, attenuation has been achieved by serial passaging of a given virus in non-primate cells, which leads to the emergence of mutations that have low fitness in human cells. Yet HCV does not replicate efficiently in non-human cells, which poses problems for the identification and production of attenuated strains. Additionally, there is also the risk of causing an infection after the use of these types of vaccines, which in principle, limits their potential use[11,14]. However, it is worth noting that live-attenuated viral vaccines are licensed for human use for prevention of several viral diseases such as dengue, hepatitis A, measles, mumps, varicella, yellow fever and gastrointestinal disorders caused by rotaviruses[296]. Therefore, if properly designed, this technology offers safe and effective vaccines.

Considering the issue of identifying attenuating mutations in non-human cultures, an alternative is to detect mutations occurring naturally within the human host, present only as minority variants within the quasispecies, and exhibiting an attenuated phenotype.

HCV, as many members of the *Flaviviridae* family (all except for those within the *Flavivirus* genus), translate its polyprotein in a CAP-independent manner by recruiting the ribosome directly to the internal ribosome entry site (IRES), which is found in the 5' non-coding region[297]. IRES structure and sequence are essential to its function, and any change can affect the translation process[298,299]. Therefore, investigating on mutations that might affect this process may enable an alternative approach for the design of live-attenuated vaccines against HCV. In this regard, our group has identified several mutations within the IRES of HCV isolates from chronically infected patients of genotype 1a and 3a, that are present in very low frequencies within the viral population, and that have evidenced a significant decrease in viral translation efficiency *in vitro*[300]. Studies in cell culture, using full-genome chimera replicons based on JFH-1 strain are underway in order to assess both translation efficiency as well as viral fitness.

It is important to mention, that one of the initial vaccines designed to fight polio was a formulation with poliovirus (PV) strains where, through successive passages in non-human cells, mutations were selected along the whole genome[301]. Of those, a mutation within PV IRES which drastically diminishes the translation efficiency, is the main responsible for the attenuated phenotype[302]. Unfortunately, live-attenuated PV vaccines have shown to be genetically unstable, and some of the mutations that confer the attenuated phenotype can reverse during replication in humans, causing rare cases of vaccine-associated paralytic poliomyelitis[303]. Thus, if the aim were to design a safe live-attenuated HCV vaccine with mutations in the IRES region, perhaps additional approaches would need to be considered so as to minimize the chances for reversion or enhancing the resulting immune response. One such approach could be constructing a bicistronic vector co-expressing an antiviral protein (for example IFN- $\beta$ ), which has already been proven effective to limit viral spread and to induce antiviral immunity in animal models when assessing a *Flavivirus* vaccine candidate[304].

On the other hand, a rational synthetic design of attenuated strains might be a new and achievable approach to employ based on the newest infectious replicons that harbor almost the entire genome sequence from non-JFH-1 strains, covering in this way most of the circulating HCV genotypes. This strategy has been successfully developed and tested in mice for other RNA viruses such as Influenza A virus and Coxsackievirus[305]. It consisted of engineering codons that were more prone to generate a Stop mutation after a single nucleotide change in as many positions as possible, without changing the amino acid identity. This strategy proved that the synthetic and rational generation of self-limiting vaccines is possible in different RNA viruses and thus, could represent an alternative way of generating HCV attenuated vaccines as well, provided that the issues with *in vitro* scaling-up production can be overcome in the near future.

## LESSONS LEARNT FROM ANTI-SARS-CoV-2 VACCINES

COVID-19, caused by the SARS-CoV-2[306], has become a major health concern all over the world and has spawned challenges to develop safe and effective antiviral drugs and vaccines for preventive use. Vaccine development is a complex and time-consuming process, that typically requires years of research and testing before reaching the clinic. But in 2020, in an unprecedented effort due to the synergy between academia, researchers, and pharmacists, added to financial support and guided by cumulative knowledge from many years of scientific work, scientists were able to produce safe and effective coronavirus vaccines in record time[307]. Coronavirus vaccine types include inactivated vaccines, nucleic acid vaccines, adenovirus vector - based vaccines, and recombinant subunits vaccines. Up until February 18<sup>th</sup> researchers were testing 70 vaccine candidates in clinical trials, and 20 have reached the final stages of testing. Over 10 have been approved for emergency use in several countries around the world. Among these, it seems important to highlight the Emergency Use Authorization for 2 highly effective mRNA COVID-19 vaccines from Pfizer-BioNTech and Moderna. This is the first time that mRNA-based vaccines have ever been approved for human use, and marks a critical milestone for achievement in both science and public health[275,308,309]. As previously mentioned, mRNA vaccines trigger immune responses by transfecting synthetic mRNA encoding viral antigens (in this case spike protein or protein motifs) into human cells. Once the nucleic acid enters the cytosol of the cell, the mRNA vaccine temporarily induces the cell to produce specific viral antigens coded by the mRNA[308,310]. The major breakthroughs of these two vaccines were: (1) The mRNA modifications and purification process to reduce the innate immune response and to improve mRNA stability; and (2) The effective intracellular delivery to facilitate cellular uptake of mRNA and to protect it from RNase degradation.

These RNA vaccines generate powerful antibody responses to the SARS-CoV-2 coronavirus, but they have not proven to be as good as the AstraZeneca/Oxford vaccine (adenoviral vector vaccine) at stimulating CD8<sup>+</sup> T cells. Recently animal studies suggest that a combination of an RNA coronavirus vaccine and a adenoviral vector vaccine (AstraZeneca/Oxford vaccine) could strengthen immune response by rousing CD8<sup>+</sup> T cells in mice better than either vaccine alone[311,312]. This preliminary data should be confirmed in upcoming clinical trials.

Thus, what can we learn about SARS-CoV-2 impressive vaccine development? Firstly, that when there is interest and resources, the development and production times of a vaccine can be significantly reduced. Secondly, that mRNA vaccines have a high potency, ability for rapid development, and cost-efficient production. Thirdly, that preliminary data suggests that mixing COVID vaccines technologies boosts the immune response at a cellular level.

Is it possible, therefore, to apply all the knowledge gained from COVID-19 vaccines to accelerate HCV vaccine development? Unfortunately, only partially. As mentioned in the section about challenges, many hurdles remain since HCV biology and immunology differ greatly from that of SARS-CoV-2. However, the so far unexplored possibility of an HCV mRNA-based vaccine could certainly benefit from the experiences and developments in the field of RNA-based vaccines against SARS-CoV-2.

## CONCLUSION

HCV is an insidious virus, which, since its discovery, has caused enormous difficulty to be kept under control. The successful introduction of DAAs has become a milestone in keeping the epidemic in line, however it has proven to be insufficient to achieve global eradication of this virus and all the health complications derived from the infection. Therefore, numerous approaches have been explored in order to design an effective vaccine, either prophylactic or therapeutic. Unfortunately, to date, none of these attempts have rendered a viable vaccine for human use. Several drawbacks have hampered its development, among which, to our understanding, one of the most difficult to override is T cell exhaustion, the main cause of therapeutic vaccines failure. However, many other challenges related to a still incomplete understanding of HCV immunology remain to be overcome. Noteworthy among these, is the insufficiency of CMI to control infections and the need for a joint humoral response, as well as the necessity for characterization of better epitopes for nAbs. An approach that might prove effective in the future, is the use of heterologous prime/boost vaccination,



where two different technologies can be employed to enhance the immune responses. Additionally, we believe that ongoing efforts to develop improved and more suitable *in vivo* systems should be a priority, since many of the successful pre-clinical studies have possibly failed in clinical trials due to the differences in immunopathology between the used animal models and humans. All of the hard work that has enabled the rapid and effective development of vaccines against SARS-CoV-2 should be taken as an example of what can be achieved if the interest and the efforts are focused on tackling a health burden. In particular, the advances on mRNA-based vaccine technology, which so far has not been explored in HCV vaccine candidates, would be a good starting point if the aim is to explore alternatives not investigated so far. Additionally, different methodologies which have been shown to be efficacious against other RNA viruses, are available for the design of live-attenuated strains as vaccines against HCV. Following this line of thought, and likely fueled both by the success of COVID-19 vaccines[313] and by the Nobel Prize in Physiology or Medicine 2020 (awarded to three scientists for the discovery of HCV)[28], last year, the NIH opened a grant opportunity for projects concerning HCV vaccine design[30]. As a result, it is expected that more research will be focused on this subject in the upcoming years, and hopefully, auspicious findings will follow. This renewed interest in funding HCV vaccines might be what is needed to achieve HCV global eradication, as has been proposed by the WHO a few years ago. Allocating funds for this purpose boosts the research area that has been left behind in terms of breakthroughs that can be effectively translated to public health benefits.

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## REFERENCES

- 1 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562 DOI: 10.1126/science.2523562]
- 2 **WHO**. Global Hepatitis Report 2017. Geneva: World Health Organization. [cited 10 February 2021]. Available from: <https://www.who.int/publications/i/item/global-hepatitis-report-2017>
- 3 **WHO**. Fact Sheet: Hepatitis C [Internet]. World Heal. Organ.2018. [cited 10 January 2021]. Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c>
- 4 **Cholankeril G**, Ahmed A. Alcoholic Liver Disease Replaces Hepatitis C Virus Infection as the Leading Indication for Liver Transplantation in the United States. *Clin Gastroenterol Hepatol* 2018; **16**: 1356-1358 [PMID: 29199144 DOI: 10.1016/j.cgh.2017.11.045]
- 5 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol* 2016; **64**: 433-485 [PMID: 26597456 DOI: 10.1016/j.jhep.2015.10.006]
- 6 **Graziadei I**, Zoller H, Fickert P, Schneeberger S, Finkenstedt A, Peck-Radosavljevic M, Müller H, Kohl C, Sperner-Unterwieser B, Eschertzhuber S, Hofer H, Öfner D, Tilg H, Vogel W, Trauner M, Berlakovich G. Indications for liver transplantation in adults: Recommendations of the Austrian Society for Gastroenterology and Hepatology (ÖGGH) in cooperation with the Austrian Society for Transplantation, Transfusion and Genetics (ATX). *Wien Klin Wochenschr* 2016; **128**: 679-690 [PMID: 27590261 DOI: 10.1007/s00508-016-1046-1]
- 7 **Debes JD**, Chan AJ, Balderramo D, Kikuchi L, Gonzalez Ballera E, Prieto JE, Tapias M, Idrovo V, Davalos MB, Cairo F, Barreyro FJ, Paredes S, Hernandez N, Avendaño K, Diaz Ferrer J, Yang JD, Carrera E, Garcia JA, Mattos AZ, Hirsch BS, Gonçalves PT, Carrilho FJ, Roberts LR. Hepatocellular carcinoma in South America: Evaluation of risk factors, demographics and therapy. *Liver Int* 2018; **38**: 136-143 [PMID: 28640517 DOI: 10.1111/liv.13502]
- 8 **Piñero F**, Costa P, Boteon YL, Duque SH, Marciano S, Anders M, Varón A, Zerega A, Poniachik J, Soza A, Padilla Machaca M, Menéndez J, Zapata R, Vilatoba M, Muñoz L, Maraschio M, Podestá LG, McCormack L, Gadano A, Boin ISFF, García P, Silva M; Latin American Liver Research, Education, Awareness Network (LALREAN). A changing etiologic scenario in liver transplantation for hepatocellular carcinoma in a multicenter cohort study from Latin America. *Clin Res Hepatol Gastroenterol* 2018; **42**: 443-452 [PMID: 29773419 DOI: 10.1016/j.clinre.2018.03.014]
- 9 **Gravitz L**. Introduction: a smouldering public-health crisis. *Nature* 2011; **474**: S2-S4 [PMID: 21666731 DOI: 10.1038/474S2a]
- 10 **Bartenschlager R**, Baumert TF, Bukh J, Houghton M, Lemon SM, Lindenbach BD, Lohmann V,



- Moradpour D, Pietschmann T, Rice CM, Thimme R, Wakita T. Critical challenges and emerging opportunities in hepatitis C virus research in an era of potent antiviral therapy: Considerations for scientists and funding agencies. *Virus Res* 2018; **248**: 53-62 [PMID: [29477639](#) DOI: [10.1016/j.virusres.2018.02.016](#)]
- 11 **Bailey JR**, Barnes E, Cox AL. Approaches, Progress, and Challenges to Hepatitis C Vaccine Development. *Gastroenterology* 2019; **156**: 418-430 [PMID: [30268785](#) DOI: [10.1053/j.gastro.2018.08.060](#)]
  - 12 **Sorbo MC**, Cento V, Di Maio VC, Howe AYM, Garcia F, Perno CF, Ceccherini-Silberstein F. Hepatitis C virus drug resistance associated substitutions and their clinical relevance: Update 2018. *Drug Resist Updat* 2018; **37**: 17-39 [PMID: [29525636](#) DOI: [10.1016/j.drug.2018.01.004](#)]
  - 13 **Midgard H**, Weir A, Palmateer N, Lo Re V 3rd, Pineda JA, Macías J, Dalgard O. HCV epidemiology in high-risk groups and the risk of reinfection. *J Hepatol* 2016; **65**: S33-S45 [PMID: [27641987](#) DOI: [10.1016/j.jhep.2016.07.012](#)]
  - 14 **Cox AL**. Challenges and Promise of a Hepatitis C Virus Vaccine. *Cold Spring Harb Perspect Med* 2020; **10**: a036947 [PMID: [31548228](#) DOI: [10.1101/cshperspect.a036947](#)]
  - 15 **Chaudhari R**, Fouda S, Sainu A, Pappachan JM. Metabolic complications of hepatitis C virus infection. *World J Gastroenterol* 2021; **27**: 1267-1282 [PMID: [33833481](#) DOI: [10.3748/wjg.v27.i13.1267](#)]
  - 16 **Ioannou GN**, Feld JJ. What Are the Benefits of a Sustained Virologic Response to Direct-Acting Antiviral Therapy for Hepatitis C Virus Infection? *Gastroenterology* 2019; **156**: 446-460.e2 [PMID: [30367836](#) DOI: [10.1053/j.gastro.2018.10.033](#)]
  - 17 **Tapper EB**, Catana AM, Sethi N, Mansuri D, Sethi S, Vong A, Afdhal NH. Direct costs of care for hepatocellular carcinoma in patients with hepatitis C cirrhosis. *Cancer* 2016; **122**: 852-858 [PMID: [26716758](#) DOI: [10.1002/cncr.29855](#)]
  - 18 **Bardach A**, Hernández-Vásquez A, Palacios A, Calderón M, Soto N, Balan D, Augustovski F. Epidemiología, consumo de recursos y costos del manejo médico de la Hepatitis C en Argentina, Colombia, Uruguay y Venezuela. *Value Health Reg Issues* 2019; **20**: 180-190 [PMID: [31654963](#) DOI: [10.1016/j.vhri.2019.06.004](#)]
  - 19 **Roohvand F**, Kossari N. Advances in hepatitis C virus vaccines, part two: advances in hepatitis C virus vaccine formulations and modalities. *Expert Opin Ther Pat* 2012; **22**: 391-415 [PMID: [22455502](#) DOI: [10.1517/13543776.2012.673589](#)]
  - 20 **Hill AM**, Nath S, Simmons B. The road to elimination of hepatitis C: analysis of cures versus new infections in 91 countries. *J Virus Erad* 2017; **3**: 117-123 [PMID: [28758018](#) DOI: [10.1016/S2055-6640\(20\)30329-0](#)]
  - 21 **Feinstone SM**, Hu DJ, Major ME. Prospects for prophylactic and therapeutic vaccines against hepatitis C virus. *Clin Infect Dis* 2012; **55** Suppl 1: S25-S32 [PMID: [22715210](#) DOI: [10.1093/cid/cis362](#)]
  - 22 **Massoud O**. Hepatitis C: looking into the future. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 367-374 [PMID: [32216467](#) DOI: [10.1080/17474124.2020.1746641](#)]
  - 23 **Duncan JD**, Urbanowicz RA, Tarr AW, Ball JK. Hepatitis C Virus Vaccine: Challenges and Prospects. *Vaccines (Basel)* 2020; **8**: 90 [PMID: [32079254](#) DOI: [10.3390/vaccines8010090](#)]
  - 24 **Roingeard P**, Beaumont E. Hepatitis C Vaccine: 10 Good Reasons for Continuing. *Hepatology* 2020; **71**: 1845-1850 [PMID: [32060946](#) DOI: [10.1002/hep.31182](#)]
  - 25 **Fine P**, Eames K, Heymann DL. "Herd immunity": a rough guide. *Clin Infect Dis* 2011; **52**: 911-916 [PMID: [21427399](#) DOI: [10.1093/cid/cir007](#)]
  - 26 **Mennini FS**, Marcellusi A, Andreoni M, Gasbarrini A, Salomone S, Craxi A. Health policy model: long-term predictive results associated with the management of hepatitis C virus-induced diseases in Italy. *Clin Outcomes Res* 2014; **6**: 303-310 [PMID: [24971024](#) DOI: [10.2147/CEOR.S62092](#)]
  - 27 **Cipriano LE**, Goldhaber-Fiebert JD. Population Health and Cost-Effectiveness Implications of a "Treat All" Recommendation for HCV: A Review of the Model-Based Evidence. *MDM Policy Pract* 2018; **3**: 2381468318776634 [PMID: [30288448](#) DOI: [10.1177/2381468318776634](#)]
  - 28 **Nobelprize.org**. Press release: The Nobel Prize in Physiology or Medicine 2020 [Internet]. Nobel Media AB 20212020. [cited 10 February 2021]. Available from: <https://www.nobelprize.org/prizes/medicine/2020/press-release/>
  - 29 **Nobelprize.org**. Streams during Nobel Week 2020 [Internet]. Nobel Lect. Physiol. or Med.2020 [cited 10 February 2021]. Available from: <https://www.nobelprize.org/nobel-week-live-streams-2020/>
  - 30 **National Institutes of Health (NIH)**. Funding Opportunity: Rational Design of Vaccines Against Hepatitis C Virus [Internet]. 2020. [cited 10 February 2021]. Available from: <https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-20-019.html>
  - 31 **Farlex Partner Medical Dictionary**. Prophylactic vaccination. (n.d.) [Internet]. 2012 [cited 10 February 2021]. Available from: <https://medical-dictionary.thefreedictionary.com/prophylactic+vaccination>
  - 32 **McGraw-Hill Dictionary of Scientific & Technical Terms 6E**. Therapeutic vaccination. (n.d.) [Internet]. 2003. [cited 10 February 2021]. Available from: <https://encyclopedia2.thefreedictionary.com/therapeutic+vaccination>
  - 33 **Major M**, Gutfraind A, Shekhtman L, Cui Q, Kachko A, Cotler SJ, Hajarizadeh B, Sacks-Davis R, Page K, Boodram B, Dahari H. Modeling of patient virus titers suggests that availability of a vaccine could reduce hepatitis C virus transmission among injecting drug users. *Sci Transl Med* 2018; **10**:

- eaao4496 [PMID: 29997251 DOI: 10.1126/scitranslmed.aao4496]
- 34 **Scott N**, McBryde E, Vickerman P, Martin NK, Stone J, Drummer H, Hellard M. The role of a hepatitis C virus vaccine: modelling the benefits alongside direct-acting antiviral treatments. *BMC Med* 2015; **13**: 198 [PMID: 26289050 DOI: 10.1186/s12916-015-0440-2]
- 35 **Stone J**, Martin NK, Hickman M, Hellard M, Scott N, McBryde E, Drummer H, Vickerman P. The Potential Impact of a Hepatitis C Vaccine for People Who Inject Drugs: Is a Vaccine Needed in the Age of Direct-Acting Antivirals? *PLoS One* 2016; **11**: e0156213 [PMID: 27224423 DOI: 10.1371/journal.pone.0156213]
- 36 **Halliday J**, Klenerman P, Barnes E. Vaccination for hepatitis C virus: closing in on an evasive target. *Expert Rev Vaccines* 2011; **10**: 659-672 [PMID: 21604986 DOI: 10.1586/erv.11.55]
- 37 **Huang CF**, Yu ML. Unmet needs of chronic hepatitis C in the era of direct-acting antiviral therapy. *Clin Mol Hepatol* 2020; **26**: 251-260 [PMID: 32188235 DOI: 10.3350/cmh.2020.0018]
- 38 **Torresi J**, Johnson D, Wedemeyer H. Progress in the development of preventive and therapeutic vaccines for hepatitis C virus. *J Hepatol* 2011; **54**: 1273-1285 [PMID: 21236312 DOI: 10.1016/j.jhep.2010.09.040]
- 39 **Mekonnen ZA**, Grubor-Bauk B, Masavuli MG, Shrestha AC, Ranasinghe C, Bull RA, Lloyd AR, Gowans EJ, Wijesundara DK. Toward DNA-Based T-Cell Mediated Vaccines to Target HIV-1 and Hepatitis C Virus: Approaches to Elicit Localized Immunity for Protection. *Front Cell Infect Microbiol* 2019; **9**: 91 [PMID: 31001491 DOI: 10.3389/fcimb.2019.00091]
- 40 **Wolski D**, Foote PK, Chen DY, Lewis-Ximenez LL, Fauvelle C, Aneja J, Walker A, Tonnerre P, Torres-Cornejo A, Kvistad D, Imam S, Waring MT, Tully DC, Allen TM, Chung RT, Timm J, Haining WN, Kim AY, Baumert TF, Lauer GM. Early Transcriptional Divergence Marks Virus-Specific Primary Human CD8<sup>+</sup> T Cells in Chronic versus Acute Infection. *Immunity* 2017; **47**: 648-663.e8 [PMID: 29045899 DOI: 10.1016/j.immuni.2017.09.006]
- 41 **Kared H**, Fabre T, Bédard N, Bruneau J, Shoukry NH. Galectin-9 and IL-21 mediate cross-regulation between Th17 and Treg cells during acute hepatitis C. *PLoS Pathog* 2013; **9**: e1003422 [PMID: 23818845 DOI: 10.1371/journal.ppat.1003422]
- 42 **Schulze zur Wiesch J**, Lauer GM, Day CL, Kim AY, Ouchi K, Duncan JE, Wurcel AG, Timm J, Jones AM, Mothe B, Allen TM, McGovern B, Lewis-Ximenez L, Sidney J, Sette A, Chung RT, Walker BD. Broad repertoire of the CD4<sup>+</sup> Th cell response in spontaneously controlled hepatitis C virus infection includes dominant and highly promiscuous epitopes. *J Immunol* 2005; **175**: 3603-3613 [PMID: 16148104 DOI: 10.4049/jimmunol.175.6.3603]
- 43 **Pestka JM**, Zeisel MB, Bläser E, Schürmann P, Bartosch B, Cosset FL, Patel AH, Meisel H, Baumert J, Viazov S, Rispeter K, Blum HE, Roggendorf M, Baumert TF. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc Natl Acad Sci U S A* 2007; **104**: 6025-6030 [PMID: 17392433 DOI: 10.1073/pnas.0607026104]
- 44 **Walker MR**, Leung P, Eltahla AA, Underwood A, Abayasingam A, Brasher NA, Li H, Wu BR, Maher L, Luciani F, Lloyd AR, Bull RA. Clearance of hepatitis C virus is associated with early and potent but narrowly-directed, Envelope-specific antibodies. *Sci Rep* 2019; **9**: 13300 [PMID: 31527718 DOI: 10.1038/s41598-019-49454-w]
- 45 **Micallef JM**, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat* 2006; **13**: 34-41 [PMID: 16364080 DOI: 10.1111/j.1365-2893.2005.00651.x]
- 46 **Smyk-Pearson S**, Tester IA, Klarquist J, Palmer BE, Pawlotsky JM, Golden-Mason L, Rosen HR. Spontaneous recovery in acute human hepatitis C virus infection: functional T-cell thresholds and relative importance of CD4 help. *J Virol* 2008; **82**: 1827-1837 [PMID: 18045940 DOI: 10.1128/JVI.01581-07]
- 47 **Baumert TF**, Fauvelle C, Chen DY, Lauer GM. A prophylactic hepatitis C virus vaccine: a distant peak still worth climbing. *J Hepatol* 2014; **61**: S34-S44 [PMID: 25443345 DOI: 10.1016/j.jhep.2014.09.009]
- 48 **Bailey JR**, Flyak AI, Cohen VJ, Li H, Wasilewski LN, Snider AE, Wang S, Learn GH, Kose N, Loerinc L, Lampley R, Cox AL, Pfaff JM, Doranz BJ, Shaw GM, Ray SC, Crowe JE Jr. Broadly neutralizing antibodies with few somatic mutations and hepatitis C virus clearance. *JCI Insight* 2017; **2**: e92872 [PMID: 28469084 DOI: 10.1172/jci.insight.92872]
- 49 **Kaplan DE**, Sugimoto K, Newton K, Valiga ME, Ikeda F, Aytaman A, Nunes FA, Lucey MR, Vance BA, Vonderheide RH, Reddy KR, McKeating JA, Chang KM. Discordant role of CD4 T-cell response relative to neutralizing antibody and CD8 T-cell responses in acute hepatitis C. *Gastroenterology* 2007; **132**: 654-666 [PMID: 17258733 DOI: 10.1053/j.gastro.2006.11.044]
- 50 **Cooper S**, Erickson AL, Adams EJ, Kansopon J, Weiner AJ, Chien DY, Houghton M, Parham P, Walker CM. Analysis of a successful immune response against hepatitis C virus. *Immunity* 1999; **10**: 439-449 [PMID: 10229187 DOI: 10.1016/S1074-7613(00)80044-8]
- 51 **Lechner F**, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, Robbins G, Phillips R, Klenerman P, Walker BD. Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 2000; **191**: 1499-1512 [PMID: 10790425 DOI: 10.1084/jem.191.9.1499]
- 52 **Luxemburger H**, Neumann-Haefelin C, Thimme R, Boettler T. HCV-Specific T Cell Responses During and After Chronic HCV Infection. *Viruses* 2018; **10**: 645 [PMID: 30453612 DOI: 10.3390/v10110645]
- 53 **Spada E**, Mele A, Berton A, Ruggeri L, Ferrigno L, Garbuglia AR, Perrone MP, Girelli G, Del

- Porto P, Piccolella E, Mondelli MU, Amoroso P, Cortese R, Nicosia A, Vitelli A, Folgori A. Multispecific T cell response and negative HCV RNA tests during acute HCV infection are early prognostic factors of spontaneous clearance. *Gut* 2004; **53**: 1673-1681 [PMID: [15479691](#) DOI: [10.1136/gut.2003.037788](#)]
- 54 **Smyk-Pearson S**, Tester IA, Lezotte D, Sasaki AW, Lewinsohn DM, Rosen HR. Differential antigenic hierarchy associated with spontaneous recovery from hepatitis C virus infection: implications for vaccine design. *J Infect Dis* 2006; **194**: 454-463 [PMID: [16845628](#) DOI: [10.1086/505714](#)]
- 55 **Badr G**, Bédard N, Abdel-Hakeem MS, Trautmann L, Willems B, Villeneuve JP, Haddad EK, Sékaly RP, Bruneau J, Shoukry NH. Early interferon therapy for hepatitis C virus infection rescues polyfunctional, long-lived CD8<sup>+</sup> memory T cells. *J Virol* 2008; **82**: 10017-10031 [PMID: [18667516](#) DOI: [10.1128/JVI.01083-08](#)]
- 56 **Chigbu DI**, Loonawat R, Sehgal M, Patel D, Jain P. Hepatitis C Virus Infection: Host Virus Interaction and Mechanisms of Viral Persistence. *Cells* 2019; **8**: 376 [PMID: [31027278](#) DOI: [10.3390/cells8040376](#)]
- 57 **Schulze Zur Wiesch J**, Ciuffreda D, Lewis-Ximenez L, Kasprowicz V, Nolan BE, Streeck H, Aneja J, Reyrol LL, Allen TM, Lohse AW, McGovern B, Chung RT, Kwok WW, Kim AY, Lauer GM. Broadly directed virus-specific CD4<sup>+</sup> T cell responses are primed during acute hepatitis C infection, but rapidly disappear from human blood with viral persistence. *J Exp Med* 2012; **209**: 61-75 [PMID: [22213804](#) DOI: [10.1084/jem.20100388](#)]
- 58 **Day CL**, Lauer GM, Robbins GK, McGovern B, Wurcel AG, Gandhi RT, Chung RT, Walker BD. Broad specificity of virus-specific CD4<sup>+</sup> T-helper-cell responses in resolved hepatitis C virus infection. *J Virol* 2002; **76**: 12584-12595 [PMID: [12438584](#) DOI: [10.1128/JVI.76.24.12584-12595.2002](#)]
- 59 **Chang KM**, Thimme R, Melpolder JJ, Oldach D, Pemberton J, Moorhead-Loudis J, McHutchison JG, Alter HJ, Chisari FV. Differential CD4(+) and CD8(+) T-cell responsiveness in hepatitis C virus infection. *Hepatology* 2001; **33**: 267-276 [PMID: [11124845](#) DOI: [10.1053/jhep.2001.21162](#)]
- 60 **Semmo N**, Day CL, Ward SM, Lucas M, Harcourt G, Loughry A, Klenerman P. Preferential loss of IL-2-secreting CD4<sup>+</sup> T helper cells in chronic HCV infection. *Hepatology* 2005; **41**: 1019-1028 [PMID: [15841456](#) DOI: [10.1002/hep.20669](#)]
- 61 **Semmo N**, Klenerman P. CD4<sup>+</sup> T cell responses in hepatitis C virus infection. *World J Gastroenterol* 2007; **13**: 4831-4838 [PMID: [17828814](#) DOI: [10.3748/wjg.v13.i36.4831](#)]
- 62 **Bowen DG**, Walker CM. Mutational escape from CD8<sup>+</sup> T cell immunity: HCV evolution, from chimpanzees to man. *J Exp Med* 2005; **201**: 1709-1714 [PMID: [15939787](#) DOI: [10.1084/jem.20050808](#)]
- 63 **Abdel-Hakeem MS**, Shoukry NH. Protective immunity against hepatitis C: many shades of gray. *Front Immunol* 2014; **5**: 274 [PMID: [24982656](#) DOI: [10.3389/fimmu.2014.00274](#)]
- 64 **Gruener NH**, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, Walker B, Sullivan J, Phillips R, Pape GR, Klenerman P. Sustained dysfunction of antiviral CD8<sup>+</sup> T lymphocytes after infection with hepatitis C virus. *J Virol* 2001; **75**: 5550-5558 [PMID: [11356962](#) DOI: [10.1128/JVI.75.12.5550-5558.2001](#)]
- 65 **Wedemeyer H**, He XS, Nascimbeni M, Davis AR, Greenberg HB, Hoofnagle JH, Liang TJ, Alter H, Rehermann B. Impaired effector function of hepatitis C virus-specific CD8<sup>+</sup> T cells in chronic hepatitis C virus infection. *J Immunol* 2002; **169**: 3447-3458 [PMID: [12218168](#) DOI: [10.4049/jimmunol.169.6.3447](#)]
- 66 **Sayers TJ**, Brooks AD, Seki N, Smyth MJ, Yagita H, Blazar BR, Malysguine AM. T cell lysis of murine renal cancer: multiple signaling pathways for cell death via Fas. *J Leukoc Biol* 2000; **68**: 81-86 [PMID: [10914493](#)]
- 67 **Farci P**, Shimoda A, Wong D, Cabezon T, De Gioannis D, Strazzer A, Shimizu Y, Shapiro M, Alter HJ, Purcell RH. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. *Proc Natl Acad Sci U S A* 1996; **93**: 15394-15399 [PMID: [8986822](#) DOI: [10.1073/pnas.93.26.15394](#)]
- 68 **Hijikata M**, Kato N, Ootsuyama Y, Nakagawa M, Ohkoshi S, Shimotohno K. Hypervariable regions in the putative glycoprotein of hepatitis C virus. *Biochem Biophys Res Commun* 1991; **175**: 220-228 [PMID: [1847805](#) DOI: [10.1016/S0006-291X\(05\)81223-9](#)]
- 69 **Farci P**, Alter HJ, Wong DC, Miller RH, Govindarajan S, Engle R, Shapiro M, Purcell RH. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. *Proc Natl Acad Sci U S A* 1994; **91**: 7792-7796 [PMID: [7519785](#) DOI: [10.1073/pnas.91.16.7792](#)]
- 70 **Cuevas JM**, González-Candelas F, Moya A, Sanjuán R. Effect of ribavirin on the mutation rate and spectrum of hepatitis C virus in vivo. *J Virol* 2009; **83**: 5760-5764 [PMID: [19321623](#) DOI: [10.1128/JVI.00201-09](#)]
- 71 **Gal-Tanamy M**, Keck ZY, Yi M, McKeating JA, Patel AH, Fong SK, Lemon SM. In vitro selection of a neutralization-resistant hepatitis C virus escape mutant. *Proc Natl Acad Sci U S A* 2008; **105**: 19450-19455 [PMID: [19052239](#) DOI: [10.1073/pnas.0809879105](#)]
- 72 **Broering TJ**, Garrity KA, Boatright NK, Sloan SE, Sandor F, Thomas WD Jr, Szabo G, Finberg RW, Ambrosino DM, Babcock GJ. Identification and characterization of broadly neutralizing human monoclonal antibodies directed against the E2 envelope glycoprotein of hepatitis C virus. *J Virol* 2009; **83**: 12473-12482 [PMID: [19759151](#) DOI: [10.1128/JVI.01138-09](#)]

- 73 **Bartosch B**, Bukh J, Meunier JC, Granier C, Engle RE, Blackwelder WC, Emerson SU, Cosset FL, Purcell RH. In vitro assay for neutralizing antibody to hepatitis C virus: evidence for broadly conserved neutralization epitopes. *Proc Natl Acad Sci U S A* 2003; **100**: 14199-14204 [PMID: [14617769](#) DOI: [10.1073/pnas.2335981100](#)]
- 74 **Keck ZY**, Li TK, Xia J, Gal-Tanamy M, Olson O, Li SH, Patel AH, Ball JK, Lemon SM, Fong SK. Definition of a conserved immunodominant domain on hepatitis C virus E2 glycoprotein by neutralizing human monoclonal antibodies. *J Virol* 2008; **82**: 6061-6066 [PMID: [18400849](#) DOI: [10.1128/JVI.02475-07](#)]
- 75 **Law M**, Maruyama T, Lewis J, Giang E, Tarr AW, Stamatakis Z, Gastaminza P, Chisari FV, Jones IM, Fox RI, Ball JK, McKeating JA, Kneteman NM, Burton DR. Broadly neutralizing antibodies protect against hepatitis C virus quasispecies challenge. *Nat Med* 2008; **14**: 25-27 [PMID: [18064037](#) DOI: [10.1038/nm1698](#)]
- 76 **Zibert A**, Meisel H, Kraas W, Schulz A, Jung G, Roggendorf M. Early antibody response against hypervariable region 1 is associated with acute self-limiting infections of hepatitis C virus. *Hepatology* 1997; **25**: 1245-1249 [PMID: [9141445](#) DOI: [10.1002/hep.510250530](#)]
- 77 **Dowd KA**, Netski DM, Wang XH, Cox AL, Ray SC. Selection pressure from neutralizing antibodies drives sequence evolution during acute infection with hepatitis C virus. *Gastroenterology* 2009; **136**: 2377-2386 [PMID: [19303013](#) DOI: [10.1053/j.gastro.2009.02.080](#)]
- 78 **Osburn WO**, Snider AE, Wells BL, Latanich R, Bailey JR, Thomas DL, Cox AL, Ray SC. Clearance of hepatitis C infection is associated with the early appearance of broad neutralizing antibody responses. *Hepatology* 2014; **59**: 2140-2151 [PMID: [24425349](#) DOI: [10.1002/hep.27013](#)]
- 79 **Strasak AM**, Kim AY, Lauer GM, de Sousa PS, Gnuino CF, Fernandes CA, Velloso CE, de Almeida AJ, de Oliveira JM, Yoshida CF, Schulze zur Wiesch J, Paranhos-Baccalá G, Lang S, Brant LJ, Ulmer H, Strohmaier S, Kaltenbach L, Lampe E, Lewis-Ximenez LL. Antibody dynamics and spontaneous viral clearance in patients with acute hepatitis C infection in Rio de Janeiro, Brazil. *BMC Infect Dis* 2011; **11**: 15 [PMID: [21226945](#) DOI: [10.1186/1471-2334-11-15](#)]
- 80 **Lavillette D**, Morice Y, Germanidis G, Donot P, Soulier A, Pagkalos E, Sakellariou G, Intrator L, Bartosch B, Pawlotsky JM, Cosset FL. Human serum facilitates hepatitis C virus infection, and neutralizing responses inversely correlate with viral replication kinetics at the acute phase of hepatitis C virus infection. *J Virol* 2005; **79**: 6023-6034 [PMID: [15857988](#) DOI: [10.1128/JVI.79.10.6023-6034.2005](#)]
- 81 **Deng K**, Liu R, Rao H, Jiang D, Wang J, Xie X, Wei L. Antibodies Targeting Novel Neutralizing Epitopes of Hepatitis C Virus Glycoprotein Preclude Genotype 2 Virus Infection. *PLoS One* 2015; **10**: e0138756 [PMID: [26406225](#) DOI: [10.1371/journal.pone.0138756](#)]
- 82 **Giang E**, Dorner M, Prentoe JC, Dreux M, Evans MJ, Bukh J, Rice CM, Ploss A, Burton DR, Law M. Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. *Proc Natl Acad Sci* 2012; **109**: 6205-6210 [PMID: [22492964](#) DOI: [10.1073/pnas.1114927109](#)]
- 83 **Thomas DL**, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, Nolt K, Nelson KE, Strathdee SA, Johnson L, Laeyendecker O, Boitnott J, Wilson LE, Vlahov D. The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 2000; **284**: 450-456 [PMID: [10904508](#) DOI: [10.1001/jama.284.4.450](#)]
- 84 **Mehta B**, Kumar V, Chawla S, Jindal H, Bhatt B. Hepatitis C: is a vaccine the solution? *Hum Vaccin Immunother* 2014; **10**: 417-419 [PMID: [24165512](#) DOI: [10.4161/hv.26970](#)]
- 85 **Osburn WO**, Fisher BE, Dowd KA, Urban G, Liu L, Ray SC, Thomas DL, Cox AL. Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. *Gastroenterology* 2010; **138**: 315-324 [PMID: [19782080](#) DOI: [10.1053/j.gastro.2009.09.017](#)]
- 86 **Grakoui A**, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghraieb J, Murthy KK, Rice CM, Walker CM. HCV persistence and immune evasion in the absence of memory T cell help. *Science* 2003; **302**: 659-662 [PMID: [14576438](#) DOI: [10.1126/science.1088774](#)]
- 87 **Lanford RE**, Guerra B, Chavez D, Bigger C, Brasky KM, Wang XH, Ray SC, Thomas DL. Cross-genotype immunity to hepatitis C virus. *J Virol* 2004; **78**: 1575-1581 [PMID: [14722311](#) DOI: [10.1128/JVI.78.3.1575-1581.2004](#)]
- 88 **Shata MT**, Tricoche N, Perkus M, Tom D, Brotman B, McCormack P, Pfahler W, Lee DH, Tobler LH, Busch M, Prince AM. Exposure to low infective doses of HCV induces cellular immune responses without consistently detectable viremia or seroconversion in chimpanzees. *Virology* 2003; **314**: 601-616 [PMID: [14554088](#) DOI: [10.1016/s0042-6822\(03\)00461-6](#)]
- 89 **Shoukry NH**, Grakoui A, Houghton M, Chien DY, Ghraieb J, Reimann KA, Walker CM. Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. *J Exp Med* 2003; **197**: 1645-1655 [PMID: [12810686](#) DOI: [10.1084/jem.20030239](#)]
- 90 **Sacks-Davis R**, Grebely J, Dore GJ, Osburn W, Cox AL, Rice TM, Spelman T, Bruneau J, Prins M, Kim AY, McGovern BH, Shoukry NH, Schinkel J, Allen TM, Morris M, Hajarizadeh B, Maher L, Lloyd AR, Page K, Hellard M; InC3 study group. Hepatitis C Virus Reinfection and Spontaneous Clearance of Reinfection--the InC3 Study. *J Infect Dis* 2015; **212**: 1407-1419 [PMID: [25883387](#) DOI: [10.1093/infdis/jiv220](#)]
- 91 **Takaki A**, Wiese M, Maertens G, Depla E, Seifert U, Liebetrau A, Miller JL, Manns MP, Rehmann B. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nat Med* 2000; **6**: 578-582 [PMID: [10802716](#) DOI: [10.1038/75063](#)]
- 92 **Institute of Medicine (US) Committee on the Evaluation of Vaccine Purchase Financing in the**



- United States.** Financing Vaccines in the 21st Century: Assuring Access and Availability. Washington (DC): National Academies Press (US) 2003 [PMID: 25057673]
- 93 **Rémy V**, Zöllner Y, Heckmann U. Vaccination: the cornerstone of an efficient healthcare system. *J Mark Access Heal Policy* 2015; **3**: 27041 [PMID: 27123189 DOI: 10.3402/jmahp.v3.27041]
  - 94 **Park M**, Jit M, Wu JT. Cost-benefit analysis of vaccination: a comparative analysis of eight approaches for valuing changes to mortality and morbidity risks. *BMC Med* 2018; **16**: 139 [PMID: 30180901 DOI: 10.1186/s12916-018-1130-7]
  - 95 **Bukh J**. The history of hepatitis C virus (HCV): Basic research reveals unique features in phylogeny, evolution and the viral life cycle with new perspectives for epidemic control. *J Hepatol* 2016; **65**: S2-S21 [PMID: 27641985 DOI: 10.1016/j.jhep.2016.07.035]
  - 96 **Bartenschlager R**, Lohmann V. Replication of hepatitis C virus. *J Gen Virol* 2000; **81**: 1631-1648 [PMID: 10859368 DOI: 10.1099/0022-1317-81-7-1631]
  - 97 **Echeverría N**, Moratorio G, Cristina J, Moreno P. Hepatitis C virus genetic variability and evolution. *World J Hepatol* 2015; **7**: 831-845 [PMID: 25937861 DOI: 10.4254/wjh.v7.i6.831]
  - 98 **Ribeiro RM**, Li H, Wang S, Stoddard MB, Learn GH, Korber BT, Bhattacharya T, Guedj J, Parrish EH, Hahn BH, Shaw GM, Perelson AS. Quantifying the diversification of hepatitis C virus (HCV) during primary infection: estimates of the in vivo mutation rate. *PLoS Pathog* 2012; **8**: e1002881 [PMID: 22927817 DOI: 10.1371/journal.ppat.1002881]
  - 99 **Geller R**, Estada Ú, Peris JB, Andreu I, Bou JV, Garijo R, Cuevas JM, Sabariego R, Mas A, Sanjuán R. Highly heterogeneous mutation rates in the hepatitis C virus genome. *Nat Microbiol* 2016; **1**: 16045 [PMID: 27572964 DOI: 10.1038/nmicrobiol.2016.45]
  - 100 **Martell M**, Esteban JI, Quer J, Genescà J, Weiner A, Esteban R, Guardia J, Gómez J. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. *J Virol* 1992; **66**: 3225-3229 [PMID: 1313927 DOI: 10.1128/JVI.66.5.3225-3229.1992]
  - 101 **Domingo E**, Baranowski E, Ruiz-Jarabo CM, Martín-Hernández AM, Sáiz JC, Escarmís C. Quasispecies structure and persistence of RNA viruses. *Emerg Infect Dis* 1998; **4**: 521-527 [PMID: 9866728 DOI: 10.3201/eid0404.980402]
  - 102 **Keck ZY**, Li SH, Xia J, von Hahn T, Balfe P, McKeating JA, Witteveldt J, Patel AH, Alter H, Rice CM, Fong SK. Mutations in hepatitis C virus E2 located outside the CD81 binding sites lead to escape from broadly neutralizing antibodies but compromise virus infectivity. *J Virol* 2009; **83**: 6149-6160 [PMID: 19321602 DOI: 10.1128/JVI.00248-09]
  - 103 **El-Diwany R**, Cohen VJ, Mankowski MC, Wasilewski LN, Brady JK, Snider AE, Osburn WO, Murrell B, Ray SC, Bailey JR. Extra-epitopic hepatitis C virus polymorphisms confer resistance to broadly neutralizing antibodies by modulating binding to scavenger receptor B1. *PLoS Pathog* 2017; **13**: e1006235 [PMID: 28235087 DOI: 10.1371/journal.ppat.1006235]
  - 104 **Smith DB**, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. HCV Classification: A web resource to manage the classification and genotype and subtype assignments of hepatitis C virus [Internet]. ICTV2017. [cited 10 January 2021]. Available from: [https://talk.ictvonline.org/ictv\\_wikis/flaviviridae/w/sg\\_flavi/56/hcv-classification](https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/56/hcv-classification)
  - 105 **Borgia SM**, Hedskog C, Parhy B, Hyland RH, Stamm LM, Brainard DM, Subramanian MG, McHutchison JG, Mo H, Svarovskaia E, Shafran SD. Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes. *J Infect Dis* 2018; **218**: 1722-1729 [PMID: 29982508 DOI: 10.1093/infdis/jiy401]
  - 106 **Smith DB**, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology* 2014; **59**: 318-327 [PMID: 24115039 DOI: 10.1002/hep.26744]
  - 107 **Petruzzello A**, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World J Gastroenterol* 2016; **22**: 7824-7840 [PMID: 27678366 DOI: 10.3748/wjg.v22.i34.7824]
  - 108 **Pawlotsky JM**. Hepatitis C Virus Resistance to Direct-Acting Antiviral Drugs in Interferon-Free Regimens. *Gastroenterology* 2016; **151**: 70-86 [PMID: 27080301 DOI: 10.1053/j.gastro.2016.04.003]
  - 109 **Farci P**, Shimoda A, Coiana A, Diaz G, Peddis G, Melpolder JC, Strazzer A, Chien DY, Munoz SJ, Balestrieri A, Purcell RH, Alter HJ. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science* 2000; **288**: 339-344 [PMID: 10764648 DOI: 10.1126/science.288.5464.339]
  - 110 **Farci P**, Bukh J, Purcell RH. The quasispecies of hepatitis C virus and the host immune response. *Springer Semin Immunopathol* 1997; **19**: 5-26 [PMID: 9266628 DOI: 10.1007/BF00945022]
  - 111 **Forns X**, Purcell RH, Bukh J. Quasispecies in viral persistence and pathogenesis of hepatitis C virus. *Trends Microbiol* 1999; **7**: 402-410 [PMID: 10498948 DOI: 10.1016/S0966-842X(99)01590-5]
  - 112 **Timm J**, Lauer GM, Kavanagh DG, Sheridan I, Kim AY, Lucas M, Pillay T, Ouchi K, Reyrol LL, Schulze zur Wiesch J, Gandhi RT, Chung RT, Bhardwaj N, Klenerman P, Walker BD, Allen TM. CD8 epitope escape and reversion in acute HCV infection. *J Exp Med* 2004; **200**: 1593-1604 [PMID: 15611288 DOI: 10.1084/jem.20041006]
  - 113 **Lavie M**, Hanouille X, Dubuisson J. Glycan Shielding and Modulation of Hepatitis C Virus Neutralizing Antibodies. *Front Immunol* 2018; **9**: article910 [PMID: 29755477 DOI: 10.3389/fimmu.2018.00910]



- 10.3389/fimmu.2018.00910]
- 114 **Helle F**, Goffard A, Morel V, Duverlie G, McKeating J, Keck ZY, Fong S, Penin F, Dubuisson J, Voisset C. The neutralizing activity of anti-hepatitis C virus antibodies is modulated by specific glycans on the E2 envelope protein. *J Virol* 2007; **81**: 8101-8111 [PMID: [17522218](#) DOI: [10.1128/JVI.00127-07](#)]
  - 115 **Ren Y**, Min YQ, Liu M, Chi L, Zhao P, Zhang XL. N-glycosylation-mutated HCV envelope glycoprotein complex enhances antigen-presenting activity and cellular and neutralizing antibody responses. *Biochim Biophys Acta* 2016; **1860**: 1764-1775 [PMID: [26278021](#) DOI: [10.1016/j.bbagen.2015.08.007](#)]
  - 116 **Helle F**, Vieyres G, Elrief L, Popescu C-I, Wychowski C, Descamps V, Castelain S, Roingeard P, Duverlie G, Dubuisson J. Role of N-Linked Glycans in the Functions of Hepatitis C Virus Envelope Proteins Incorporated into Infectious Virions. *J Virol* 2010; **84**: 11905-11915 [PMID: [20844034](#) DOI: [10.1128/JVI.01548-10](#)]
  - 117 **Pantua H**, Diao J, Ultsch M, Hazen M, Mathieu M, McCutcheon K, Takeda K, Date S, Cheung TK, Phung Q, Hass P, Arnott D, Hongo JA, Matthews DJ, Brown A, Patel AH, Kelley RF, Eigenbrot C, Kapadia SB. Glycan shifting on hepatitis C virus (HCV) E2 glycoprotein is a mechanism for escape from broadly neutralizing antibodies. *J Mol Biol* 2013; **425**: 1899-1914 [PMID: [23458406](#) DOI: [10.1016/j.jmb.2013.02.025](#)]
  - 118 **Timpe JM**, Stamataki Z, Jennings A, Hu K, Farquhar MJ, Harris HJ, Schwarz A, Desombere I, Roels GL, Balfe P, McKeating JA. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology* 2008; **47**: 17-24 [PMID: [17941058](#) DOI: [10.1002/hep.21959](#)]
  - 119 **Brimacombe CL**, Grove J, Meredith LW, Hu K, Syder AJ, Flores MV, Timpe JM, Krieger SE, Baumert TF, Tellinghuisen TL, Wong-Staal F, Balfe P, McKeating JA. Neutralizing antibody-resistant hepatitis C virus cell-to-cell transmission. *J Virol* 2011; **85**: 596-605 [PMID: [20962076](#) DOI: [10.1128/JVI.01592-10](#)]
  - 120 **Zhang P**, Zhong L, Struble EB, Watanabe H, Kachko A, Mihalik K, Virata ML, Alter HJ, Feinstone S, Major M. Depletion of interfering antibodies in chronic hepatitis C patients and vaccinated chimpanzees reveals broad cross-genotype neutralizing activity. *Proc Natl Acad Sci* 2009; **106**: 7537-7541 [PMID: [19380744](#) DOI: [10.1073/pnas.0902749106](#)]
  - 121 **Bankwitz D**, Steinmann E, Bitzegeio J, Ciesek S, Friesland M, Herrmann E, Zeisel MB, Baumert TF, Keck ZY, Fong SK, Pécheur EI, Pietschmann T. Hepatitis C virus hypervariable region 1 modulates receptor interactions, conceals the CD81 binding site, and protects conserved neutralizing epitopes. *J Virol* 2010; **84**: 5751-5763 [PMID: [20357091](#) DOI: [10.1128/JVI.02200-09](#)]
  - 122 **Prentoe J**, Verhoye L, Velázquez Moctezuma R, Buysschaert C, Farhoudi A, Wang R, Alter H, Meuleman P, Bukh J. HVR1-mediated antibody evasion of highly infectious in vivo adapted HCV in humanised mice. *Gut* 2016; **65**: 1988-1997 [PMID: [26589670](#) DOI: [10.1136/gutjnl-2015-310300](#)]
  - 123 **Fauvel C**, Felmlee DJ, Crouchet E, Lee J, Heydmann L, Lefèvre M, Magri A, Hiet MS, Fofana I, Habersetzer F, Fong SK, Milne R, Patel AH, Vercauteren K, Meuleman P, Zeisel MB, Bartenschlager R, Schuster C, Baumert TF. Apolipoprotein E Mediates Evasion From Hepatitis C Virus Neutralizing Antibodies. *Gastroenterology* 2016; **150**: 206-217.e4 [PMID: [26404951](#) DOI: [10.1053/j.gastro.2015.09.014](#)]
  - 124 **Bankwitz D**, Doepke M, Hueging K, Weller R, Bruening J, Behrendt P, Lee JY, Vondran FWR, Manns MP, Bartenschlager R, Pietschmann T. Maturation of secreted HCV particles by incorporation of secreted ApoE protects from antibodies by enhancing infectivity. *J Hepatol* 2017; **67**: 480-489 [PMID: [28438690](#) DOI: [10.1016/j.jhep.2017.04.010](#)]
  - 125 **Deng L**, Jiang W, Wang X, Merz A, Hiet MS, Chen Y, Pan X, Jiu Y, Yang Y, Yu B, He Y, Tu Z, Niu J, Bartenschlager R, Long G. Syntenin regulates hepatitis C virus sensitivity to neutralizing antibody by promoting E2 secretion through exosomes. *J Hepatol* 2019; **71**: 52-61 [PMID: [30880226](#) DOI: [10.1016/j.jhep.2019.03.006](#)]
  - 126 **Ströh LJ**, Nagarathinam K, Krey T. Conformational Flexibility in the CD81-Binding Site of the Hepatitis C Virus Glycoprotein E2. *Front Immunol* 2018; **9**: article1396 [PMID: [29967619](#) DOI: [10.3389/fimmu.2018.01396](#)]
  - 127 **Grove J**, Nielsen S, Zhong J, Bassendine MF, Drummer HE, Balfe P, McKeating JA. Identification of a residue in hepatitis C virus E2 glycoprotein that determines scavenger receptor BI and CD81 receptor dependency and sensitivity to neutralizing antibodies. *J Virol* 2008; **82**: 12020-12029 [PMID: [18829747](#) DOI: [10.1128/JVI.01569-08](#)]
  - 128 **Voisset C**, de Beeck AO, Horellou P, Dreux M, Gustot T, Duverlie G, Cosset FL, Vu-Dac N, Dubuisson J. High-density lipoproteins reduce the neutralizing effect of hepatitis C virus (HCV)-infected patient antibodies by promoting HCV entry. *J Gen Virol* 2006; **87**: 2577-2581 [PMID: [16894196](#) DOI: [10.1099/vir.0.81932-0](#)]
  - 129 **Dreux M**, Pietschmann T, Granier C, Voisset C, Ricard-Blum S, Mangeot PE, Keck Z, Fong S, Vu-Dac N, Dubuisson J, Bartenschlager R, Lavillette D, Cosset FL. High density lipoprotein inhibits hepatitis C virus-neutralizing antibodies by stimulating cell entry via activation of the scavenger receptor BI. *J Biol Chem* 2006; **281**: 18285-18295 [PMID: [16675450](#) DOI: [10.1074/jbc.M602706200](#)]
  - 130 **Fofana I**, Fafi-Kremer S, Carolla P, Fauvel C, Zahid MN, Turek M, Heydmann L, Cury K, Hayer J, Combet C, Cosset F, Pietschmann T, Hiet M, Bartenschlager R, Habersetzer F, Doffoël M, Keck Z, Fong SKH, Zeisel MB, Stoll-Keller F, Baumert TF. Mutations That Alter Use of Hepatitis C

- Virus Cell Entry Factors Mediate Escape From Neutralizing Antibodies. *Gastroenterology* 2012; **143**: 223-233 [PMID: [22503792](#) DOI: [10.1053/j.gastro.2012.04.006](#)]
- 131 **Tarr AW**, Owsianka AM, Timms JM, McClure CP, Brown RJ, Hickling TP, Pietschmann T, Bartenschlager R, Patel AH, Ball JK. Characterization of the hepatitis C virus E2 epitope defined by the broadly neutralizing monoclonal antibody AP33. *Hepatology* 2006; **43**: 592-601 [PMID: [16496330](#) DOI: [10.1002/hep.21088](#)]
  - 132 **Tarr AW**, Urbanowicz RA, Hamed MR, Albecka A, McClure CP, Brown RJ, Irving WL, Dubuisson J, Ball JK. Hepatitis C patient-derived glycoproteins exhibit marked differences in susceptibility to serum neutralizing antibodies: genetic subtype defines antigenic but not neutralization serotype. *J Virol* 2011; **85**: 4246-4257 [PMID: [21325403](#) DOI: [10.1128/JVI.01332-10](#)]
  - 133 **Costers S**, Vanhee M, Van Breedam W, Van Doorselaere J, Geldhof M, Nauwynck HJ. GP4-specific neutralizing antibodies might be a driving force in PRRSV evolution. *Virus Res* 2010; **154**: 104-113 [PMID: [20837070](#) DOI: [10.1016/j.virusres.2010.08.026](#)]
  - 134 **Keck ZY**, Xia J, Wang Y, Wang W, Krey T, Prentoe J, Carlsen T, Li AY, Patel AH, Lemon SM, Bukh J, Rey FA, Fong SK. Human monoclonal antibodies to a novel cluster of conformational epitopes on HCV E2 with resistance to neutralization escape in a genotype 2a isolate. *PLoS Pathog* 2012; **8**: e1002653 [PMID: [22511875](#) DOI: [10.1371/journal.ppat.1002653](#)]
  - 135 **Kong L**, Giang E, Nieuwma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M. Hepatitis C virus E2 envelope glycoprotein core structure. *Science* 2013; **342**: 1090-1094 [PMID: [24288331](#) DOI: [10.1126/science.1243876](#)]
  - 136 **Khan AG**, Whidby J, Miller MT, Scarborough H, Zatorski AV, Cygan A, Price AA, Yost SA, Bohannon CD, Jacob J, Grakoui A, Marcotrigiano J. Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2. *Nature* 2014; **509**: 381-384 [PMID: [24553139](#) DOI: [10.1038/nature13117](#)]
  - 137 **Keck ZY**, Angus AG, Wang W, Lau P, Wang Y, Gatherer D, Patel AH, Fong SK. Non-random escape pathways from a broadly neutralizing human monoclonal antibody map to a highly conserved region on the hepatitis C virus E2 glycoprotein encompassing amino acids 412-423. *PLoS Pathog* 2014; **10**: e1004297 [PMID: [25122476](#) DOI: [10.1371/journal.ppat.1004297](#)]
  - 138 **Bailey JR**, Wasilewski LN, Snider AE, El-Diwany R, Osburn WO, Keck Z, Fong SK, Ray SC. Naturally selected hepatitis C virus polymorphisms confer broad neutralizing antibody resistance. *J Clin Invest* 2015; **125**: 437-447 [PMID: [25500884](#) DOI: [10.1172/JCI178794](#)]
  - 139 **Fuller MJ**, Shoukry NH, Gushima T, Bowen DG, Callendret B, Campbell KJ, Hasselschwert DL, Hughes AL, Walker CM. Selection-driven immune escape is not a significant factor in the failure of CD4 T cell responses in persistent hepatitis C virus infection. *Hepatology* 2010; **51**: 378-387 [PMID: [19918975](#) DOI: [10.1002/hep.23319](#)]
  - 140 **Cox AL**, Mosbruger T, Lauer GM, Pardoll D, Thomas DL, Ray SC. Comprehensive analyses of CD8+ T cell responses during longitudinal study of acute human hepatitis C. *Hepatology* 2005; **42**: 104-112 [PMID: [15962289](#) DOI: [10.1002/hep.20749](#)]
  - 141 **Neumann-Haefelin C**, Frick DN, Wang JJ, Pybus OG, Salloum S, Narula GS, Eckart A, Biezyński A, Eiermann T, Klennerman P, Viazov S, Roggendorf M, Thimme R, Reiser M, Timm J. Analysis of the evolutionary forces in an immunodominant CD8 epitope in hepatitis C virus at a population level. *J Virol* 2008; **82**: 3438-3451 [PMID: [18216107](#) DOI: [10.1128/JVI.01700-07](#)]
  - 142 **Neumann-Haefelin C**, Oniangue-Ndza C, Kuntzen T, Schmidt J, Nitschke K, Sidney J, Cailliet-Saguy C, Binder M, Kersting N, Kemper MW, Power KA, Ingber S, Reyrol LL, Hills-Evans K, Kim AY, Lauer GM, Lohmann V, Sette A, Henn MR, Bressanelli S, Thimme R, Allen TM. Human leukocyte antigen B27 selects for rare escape mutations that significantly impair hepatitis C virus replication and require compensatory mutations. *Hepatology* 2011; **54**: 1157-1166 [PMID: [22006856](#) DOI: [10.1002/hep.24541](#)]
  - 143 **Söderholm J**, Ahlén G, Kaul A, Frelin L, Alheim M, Barnfield C, Liljeström P, Weiland O, Milich DR, Bartenschlager R, Sällberg M. Relation between viral fitness and immune escape within the hepatitis C virus protease. *Gut* 2006; **55**: 266-274 [PMID: [16105887](#) DOI: [10.1136/gut.2005.072231](#)]
  - 144 **Uebelhoer L**, Han JH, Callendret B, Mateu G, Shoukry NH, Hanson HL, Rice CM, Walker CM, Grakoui A. Stable cytotoxic T cell escape mutation in hepatitis C virus is linked to maintenance of viral fitness. *PLoS Pathog* 2008; **4**: e1000143 [PMID: [18773115](#) DOI: [10.1371/journal.ppat.1000143](#)]
  - 145 **Kelly C**, Swadling L, Capone S, Brown A, Richardson R, Halliday J, von Delft A, Oo Y, Mutimer D, Kurioka A, Hartnell F, Collier J, Ammendola V, Del Sorbo M, Grazioli F, Esposito ML, Di Marco S, Siani L, Traboni C, Hill AV, Colloca S, Nicosia A, Cortese R, Folgori A, Klennerman P, Barnes E. Chronic hepatitis C viral infection subverts vaccine-induced T-cell immunity in humans. *Hepatology* 2016; **63**: 1455-1470 [PMID: [26474390](#) DOI: [10.1002/hep.28294](#)]
  - 146 **Thomas E**, Liang TJ. Experimental models of hepatitis B and C - new insights and progress. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 362-374 [PMID: [27075261](#) DOI: [10.1038/nrgastro.2016.37](#)]
  - 147 **Wakita T**. Cell Culture Systems of HCV Using JFH-1 and Other Strains. *Cold Spring Harb Perspect Med* 2019; **9** [PMID: [31501261](#) DOI: [10.1101/cshperspect.a036806](#)]
  - 148 **Lohmann V**, Körner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 1999; **285**: 110-113 [PMID: [10390360](#) DOI: [10.1126/science.285.5424.110](#)]

- 149 **Kato T**, Furusaka A, Miyamoto M, Date T, Yasui K, Hiramoto J, Nagayama K, Tanaka T, Wakita T. Sequence analysis of hepatitis C virus isolated from a fulminant hepatitis patient. *J Med Virol* 2001; **64**: 334-339 [PMID: [11424123](#) DOI: [10.1002/jmv.1055](#)]
- 150 **Kato T**, Date T, Miyamoto M, Furusaka A, Tokushige K, Mizokami M, Wakita T. Efficient replication of the genotype 2a hepatitis C virus subgenomic replicon. *Gastroenterology* 2003; **125**: 1808-1817 [PMID: [14724833](#) DOI: [10.1053/j.gastro.2003.09.023](#)]
- 151 **Kato T**, Date T, Miyamoto M, Sugiyama M, Tanaka Y, Orito E, Ohno T, Sugihara K, Hasegawa I, Fujiwara K, Ito K, Ozasa A, Mizokami M, Wakita T. Detection of anti-hepatitis C virus effects of interferon and ribavirin by a sensitive replicon system. *J Clin Microbiol* 2005; **43**: 5679-5684 [PMID: [16272504](#) DOI: [10.1128/JCM.43.11.5679-5684.2005](#)]
- 152 **Lohmann V**. Hepatitis C virus cell culture models: an encomium on basic research paving the road to therapy development. *Med Microbiol Immunol* 2019; **208**: 3-24 [PMID: [30298360](#) DOI: [10.1007/s00430-018-0566-x](#)]
- 153 **Ramirez S**, Bukh J. Current status and future development of infectious cell-culture models for the major genotypes of hepatitis C virus: Essential tools in testing of antivirals and emerging vaccine strategies. *Antiviral Res* 2018; **158**: 264-287 [PMID: [30059723](#) DOI: [10.1016/j.antiviral.2018.07.014](#)]
- 154 **Luna JM**, Saeed M, Rice CM. Taming a beast: lessons from the domestication of hepatitis C virus. *Curr Opin Virol* 2019; **35**: 27-34 [PMID: [30875640](#) DOI: [10.1016/j.coviro.2019.02.008](#)]
- 155 **Lindenbach BD**, Evans MJ, Syder AJ, Wölk B, Tellinghuisen TL, Liu CC, Maruyama T, Hynes RO, Burton DR, McKeating JA, Rice CM. Complete replication of hepatitis C virus in cell culture. *Science* 2005; **309**: 623-626 [PMID: [15947137](#) DOI: [10.1126/science.1114016](#)]
- 156 **Zhong J**, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV. Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci* 2005; **102**: 9294-9299 [PMID: [15939869](#) DOI: [10.1073/pnas.0503596102](#)]
- 157 **Desombere I**, Mesalam AA, Urbanowicz RA, Van Houtte F, Verhoye L, Keck ZY, Farhoudi A, Vercauteren K, Weening KE, Baumert TF, Patel AH, Fong SKH, Ball J, Leroux-Roels G, Meuleman P. A novel neutralizing human monoclonal antibody broadly abrogates hepatitis C virus infection in vitro and in vivo. *Antiviral Res* 2017; **148**: 53-64 [PMID: [29074219](#) DOI: [10.1016/j.antiviral.2017.10.015](#)]
- 158 **Pedersen J**, Carlsen TH, Prentoe J, Ramirez S, Jensen TB, Forn X, Alter H, Fong SK, Law M, Gottwein J, Weis N, Bukh J. Neutralization resistance of hepatitis C virus can be overcome by recombinant human monoclonal antibodies. *Hepatology* 2013; **58**: 1587-1597 [PMID: [23729237](#) DOI: [10.1002/hep.26524](#)]
- 159 **Pedersen J**, Jensen TB, Carlsen TH, Schønning K, Christensen PB, Laursen AL, Krarup H, Bukh J, Weis N. Neutralizing antibodies in patients with chronic hepatitis C, genotype 1, against a panel of genotype 1 culture viruses: lack of correlation to treatment outcome. *PLoS One* 2013; **8**: e62674 [PMID: [23667506](#) DOI: [10.1371/journal.pone.0062674](#)]
- 160 **Tarr AW**, Urbanowicz RA, Jayaraj D, Brown RJ, McKeating JA, Irving WL, Ball JK. Naturally occurring antibodies that recognize linear epitopes in the amino terminus of the hepatitis C virus E2 protein confer noninterfering, additive neutralization. *J Virol* 2012; **86**: 2739-2749 [PMID: [22171278](#) DOI: [10.1128/JVI.06492-11](#)]
- 161 **Fauvel C**, Colpitts CC, Keck ZY, Pierce BG, Fong SK, Baumert TF. Hepatitis C virus vaccine candidates inducing protective neutralizing antibodies. *Expert Rev Vaccines* 2016; **15**: 1535-1544 [PMID: [27267297](#) DOI: [10.1080/14760584.2016.1194759](#)]
- 162 **Merz A**, Long G, Hiet MS, Brügger B, Chlanda P, Andre P, Wieland F, Krijnse-Locker J, Bartenschlager R. Biochemical and morphological properties of hepatitis C virus particles and determination of their lipidome. *J Biol Chem* 2011; **286**: 3018-3032 [PMID: [21056986](#) DOI: [10.1074/jbc.M110.175018](#)]
- 163 **Prentoe J**, Bukh J. Hepatitis C virus expressing flag-tagged envelope protein 2 has unaltered infectivity and density, is specifically neutralized by flag antibodies and can be purified by affinity chromatography. *Virology* 2011; **409**: 148-155 [PMID: [21095619](#) DOI: [10.1016/j.virol.2010.10.034](#)]
- 164 **Gottwein JM**, Jensen TB, Mathiesen CK, Meuleman P, Serre SB, Lademann JB, Ghanem L, Scheel TK, Leroux-Roels G, Bukh J. Development and application of hepatitis C reporter viruses with genotype 1 to 7 core-nonstructural protein 2 (NS2) expressing fluorescent proteins or luciferase in modified JFH1 NS5A. *J Virol* 2011; **85**: 8913-8928 [PMID: [21697486](#) DOI: [10.1128/JVI.00049-11](#)]
- 165 **Vietheer PT**, Boo I, Gu J, McCaffrey K, Edwards S, Owczarek C, Hardy MP, Fabri L, Center RJ, Pountourios P, Drummer HE. The core domain of hepatitis C virus glycoprotein E2 generates potent cross-neutralizing antibodies in guinea pigs. *Hepatology* 2017; **65**: 1117-1131 [PMID: [27997681](#) DOI: [10.1002/hep.28989](#)]
- 166 **Gottwein JM**, Scheel TK, Hoegh AM, Lademann JB, Eugen-Olsen J, Lisby G, Bukh J. Robust hepatitis C genotype 3a cell culture releasing adapted intergenotypic 3a/2a (S52/JFH1) viruses. *Gastroenterology* 2007; **133**: 1614-1626 [PMID: [17983807](#) DOI: [10.1053/j.gastro.2007.08.005](#)]
- 167 **Date T**, Morikawa K, Tanaka Y, Tanaka-Kaneko K, Sata T, Mizokami M, Wakita T. Replication and infectivity of a novel genotype 1b hepatitis C virus clone. *Microbiol Immunol* 2012; **56**: 308-317 [PMID: [22320232](#) DOI: [10.1111/j.1348-0421.2012.00437.x](#)]
- 168 **Mori K**, Matsumoto A, Maki N, Ichikawa Y, Tanaka E, Yagi S. Production of infectious HCV genotype 1b virus in cell culture using a novel set of adaptive mutations. *BMC Microbiol* 2016; **16**: 224 [PMID: [27678340](#) DOI: [10.1186/s12866-016-0846-9](#)]

- 169 **Pham LV**, Ramirez S, Gottwein JM, Fahnøe U, Li YP, Pedersen J, Bukh J. HCV Genotype 6a Escape From and Resistance to Velpatasvir, Pibrentasvir, and Sofosbuvir in Robust Infectious Cell Culture Models. *Gastroenterology* 2018; **154**: 2194-2208.e12 [PMID: [29454794](#) DOI: [10.1053/j.gastro.2018.02.017](#)]
- 170 **Gottwein JM**, Jensen SB, Li YP, Ghanem L, Scheel TK, Serre SB, Mikkelsen L, Bukh J. Combination treatment with hepatitis C virus protease and NS5A inhibitors is effective against recombinant genotype 1a, 2a, and 3a viruses. *Antimicrob Agents Chemother* 2013; **57**: 1291-1303 [PMID: [23274664](#) DOI: [10.1128/AAC.02164-12](#)]
- 171 **Bailey JR**, Urbanowicz RA, Ball JK, Law M, Fount SKH. Standardized Method for the Study of Antibody Neutralization of HCV Pseudoparticles (HCVpp). *Methods Mol Biol* 2019; **1911**: 441-450 [PMID: [30593644](#) DOI: [10.1007/978-1-4939-8976-8\\_30](#)]
- 172 **Bartosch B**, Dubuisson J, Cosset FL. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. *J Exp Med* 2003; **197**: 633-642 [PMID: [12615904](#) DOI: [10.1084/jem.20021756](#)]
- 173 **Flint M**, Logvinoff C, Rice CM, McKeating JA. Characterization of infectious retroviral pseudotype particles bearing hepatitis C virus glycoproteins. *J Virol* 2004; **78**: 6875-6882 [PMID: [15194763](#) DOI: [10.1128/JVI.78.13.6875-6882.2004](#)]
- 174 **Urbanowicz RA**, McClure CP, King B, Mason CP, Ball JK, Tarr AW. Novel functional hepatitis C virus glycoprotein isolates identified using an optimized viral pseudotype entry assay. *J Gen Virol* 2016; **97**: 2265-2279 [PMID: [27384448](#) DOI: [10.1099/jgv.0.000537](#)]
- 175 **Underwood AP**, Walker MR, Brasher NA, Eltahla AA, Maher L, Luciani F, Lloyd AR, Bull RA. Understanding the Determinants of BnAb Induction in Acute HCV Infection. *Viruses* 2018; **10**: 659 [PMID: [30469363](#) DOI: [10.3390/v10110659](#)]
- 176 **Soares HR**, Ferreira-Fernandes M, Almeida AI, Marchel M, Alves PM, Coroadinha AS. Enhancing Hepatitis C virus pseudoparticles infectivity through p7NS2 cellular expression. *J Virol Methods* 2019; **274**: 113714 [PMID: [31412271](#) DOI: [10.1016/j.jviromet.2019.113714](#)]
- 177 **Knight A**. The beginning of the end for chimpanzee experiments? *Philos Ethics Humanit Med* 2008; **3**: 16 [PMID: [18518999](#) DOI: [10.1186/1747-5341-3-16](#)]
- 178 **Ploss A**, Kapoor A. Animal Models of Hepatitis C Virus Infection. *Cold Spring Harb Perspect Med* 2020; **10**: a036970 [PMID: [31843875](#) DOI: [10.1101/cshperspect.a036970](#)]
- 179 **Mercer DF**, Schiller DE, Elliott JF, Douglas DN, Hao C, Rinfret A, Addison WR, Fischer KP, Churchill TA, Lakey JR, Tyrrell DL, Kneteman NM. Hepatitis C virus replication in mice with chimeric human livers. *Nat Med* 2001; **7**: 927-933 [PMID: [11479625](#) DOI: [10.1038/90968](#)]
- 180 **Ding Q**, von Schaewen M, Hrebikova G, Heller B, Sandmann L, Plaas M, Ploss A. Mice Expressing Minimally Humanized CD81 and Occludin Genes Support Hepatitis C Virus Uptake In Vivo. *J Virol* 2017; **91**: e01799-16 [PMID: [27928007](#) DOI: [10.1128/JVI.01799-16](#)]
- 181 **Yong KSM**, Her Z, Chen Q. Humanized Mouse Models for the Study of Hepatitis C and Host Interactions. *Cells* 2019; **8**: 604 [PMID: [31213010](#) DOI: [10.3390/cells8060604](#)]
- 182 **Dorner M**, Horwitz JA, Robbins JB, Barry WT, Feng Q, Mu K, Jones CT, Schoggins JW, Catanese MT, Burton DR, Law M, Rice CM, Ploss A. A genetically humanized mouse model for hepatitis C virus infection. *Nature* 2011; **474**: 208-211 [PMID: [21654804](#) DOI: [10.1038/nature10168](#)]
- 183 **Chen J**, Zhao Y, Zhang C, Chen H, Feng J, Chi X, Pan Y, Du J, Guo M, Cao H, Wang Z, Pei R, Wang Q, Pan L, Niu J, Chen X, Tang H. Persistent hepatitis C virus infections and hepatopathological manifestations in immune-competent humanized mice. *Cell Res* 2014; **24**: 1050-1066 [PMID: [25155355](#) DOI: [10.1038/cr.2014.116](#)]
- 184 **Keng CT**, Sze CW, Zheng D, Zheng Z, Yong KS, Tan SQ, Ong JJ, Tan SY, Loh E, Upadya MH, Kuick CH, Hotta H, Lim SG, Tan TC, Chang KT, Hong W, Chen J, Tan YJ, Chen Q. Characterisation of liver pathogenesis, human immune responses and drug testing in a humanised mouse model of HCV infection. *Gut* 2016; **65**: 1744-1753 [PMID: [26149491](#) DOI: [10.1136/gutjnl-2014-307856](#)]
- 185 **Amako Y**, Tsukiyama-Kohara K, Katsume A, Hirata Y, Sekiguchi S, Tobita Y, Hayashi Y, Hishima T, Funata N, Yonekawa H, Kohara M. Pathogenesis of hepatitis C virus infection in Tupaia belangeri. *J Virol* 2010; **84**: 303-311 [PMID: [19846521](#) DOI: [10.1128/JVI.01448-09](#)]
- 186 **Firth C**, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, Conte JM, Ng J, Garcia J, Bhuvana NP, Lee B, Che X, Quan PL, Lipkin WI. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. *mBio* 2014; **5**: e01933-e01914 [PMID: [25316698](#) DOI: [10.1128/mBio.01933-14](#)]
- 187 **Billerbeck E**, Wolfisberg R, Fahnøe U, Xiao JW, Quirk C, Luna JM, Cullen JM, Hartlage AS, Chiriboga L, Ghoshal K, Lipkin WI, Bukh J, Scheel TKH, Kapoor A, Rice CM. Mouse models of acute and chronic hepatitis C virus infection. *Science* 2017; **357**: 204-208 [PMID: [28706073](#) DOI: [10.1126/science.aal1962](#)]
- 188 **Burbelo PD**, Dubovi EJ, Simmonds P, Medina JL, Henriquez JA, Mishra N, Wagner J, Tokarz R, Cullen JM, Iadarola MJ, Rice CM, Lipkin WI, Kapoor A. Serology-enabled discovery of genetically diverse hepatitis C viruses in a new host. *J Virol* 2012; **86**: 6171-6178 [PMID: [22491452](#) DOI: [10.1128/JVI.00250-12](#)]
- 189 **Yu Y**, Scheel TKH, Luna JM, Chung H, Nishiuchi E, Scull MA, Echeverría N, Ricardo-Lax I, Kapoor A, Lipkin WI, Divers TJ, Antezak DF, Tennant BC, Rice CM. miRNA independent hepatitis C virus variants suggest a strong evolutionary pressure to maintain miR-122 dependence. *PLoS Pathog* 2017; **13**: e1006694 [PMID: [29084265](#) DOI: [10.1371/journal.ppat.1006694](#)]



- 190 **Maher L**, White B, Hellard M, Madden A, Prins M, Kerr T, Page K. Candidate hepatitis C vaccine trials and people who inject drugs: challenges and opportunities. *Vaccine* 2010; **28**: 7273-7278 [PMID: 20831914 DOI: 10.1016/j.vaccine.2010.08.085]
- 191 **Cox AL**, Page K, Bruneau J, Shoukry NH, Lauer GM, Kim AY, Rosen HR, Radziewicz H, Grakoui A, Fierer DS, Branch AD, Kaplan DE, Chang KM. Rare birds in North America: acute hepatitis C cohorts. *Gastroenterology* 2009; **136**: 26-31 [PMID: 19059257 DOI: 10.1053/j.gastro.2008.11.049]
- 192 **Garfein RS**, Rondinelli A, Barnes RF, Cuevas J, Metzner M, Velasquez M, Rodriguez D, Reilly M, Xing J, Teshale EH. HCV infection prevalence lower than expected among 18-40-year-old injection drug users in San Diego, CA. *J Urban Health* 2013; **90**: 516-528 [PMID: 22766605 DOI: 10.1007/s11524-012-9728-0]
- 193 **National Institute of Allergy and Infectious Diseases**. Staged Phase I/II Hepatitis C Prophylactic Vaccine (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 10 January 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT01436357>
- 194 **Page K**, Melia MT, Veenhuis RT, Winter M, Rousseau KE, Massaccesi G, Osburn WO, Forman M, Thomas E, Thornton K, Wagner K, Vassilev V, Lin L, Lum PJ, Giudice LC, Stein E, Asher A, Chang S, Gorman R, Ghany MG, Liang TJ, Wierzbicki MR, Scarselli E, Nicosia A, Folgori A, Capone S, Cox AL. Randomized Trial of a Vaccine Regimen to Prevent Chronic HCV Infection. *N Engl J Med* 2021; **384**: 541-549 [PMID: 33567193 DOI: 10.1056/NEJMoa2023345]
- 195 **Lechmann M**, Liang TJ. Vaccine development for hepatitis C. *Semin Liver Dis* 2000; **20**: 211-226 [PMID: 10946425 DOI: 10.1055/s-2000-9947]
- 196 **Sandbrink JB**, Shattock RJ. RNA Vaccines: A Suitable Platform for Tackling Emerging Pandemics? *Front Immunol* 2020; **11**: 608460 [PMID: 33414790 DOI: 10.3389/fimmu.2020.608460]
- 197 **Yokokawa H**, Higashino A, Suzuki S, Moriyama M, Nakamura N, Suzuki T, Suzuki R, Ishii K, Kobiyama K, Ishii KJ, Wakita T, Akari H, Kato T. Induction of humoral and cellular immunity by immunisation with HCV particle vaccine in a non-human primate model. *Gut* 2018; **67**: 372-379 [PMID: 27797937 DOI: 10.1136/gutjnl-2016-312208]
- 198 **Akazawa D**, Moriyama M, Yokokawa H, Omi N, Watanabe N, Date T, Morikawa K, Aizaki H, Ishii K, Kato T, Mochizuki H, Nakamura N, Wakita T. Neutralizing Antibodies Induced by Cell Culture-Derived Hepatitis C Virus Protect Against Infection in Mice. *Gastroenterology* 2013; **145**: 447-455 [PMID: 23673355 DOI: 10.1053/j.gastro.2013.05.007]
- 199 **Mathiesen CK**, Jensen TB, Prentoe J, Krarup H, Nicosia A, Law M, Bukh J, Gottwein JM. Production and characterization of high-titer serum-free cell culture grown hepatitis C virus particles of genotype 1-6. *Virology* 2014; **458-459**: 190-208 [PMID: 24928051 DOI: 10.1016/j.virol.2014.03.021]
- 200 **Lothert K**, Offersgaard AF, Pihl AF, Mathiesen CK, Jensen TB, Alzua GP, Fahnøe U, Bukh J, Gottwein JM, Wolff MW. Development of a downstream process for the production of an inactivated whole hepatitis C virus vaccine. *Sci Rep* 2020; **10**: 16261 [PMID: 33004836 DOI: 10.1038/s41598-020-72328-5]
- 201 **Choo QL**, Kuo G, Ralston R, Weiner A, Chien D, Van Nest G, Han J, Berger K, Thudium K, Kuo C. Vaccination of chimpanzees against infection by the hepatitis C virus. *Proc Natl Acad Sci U S A* 1994; **91**: 1294-1298 [PMID: 7509068 DOI: 10.1073/pnas.91.4.1294]
- 202 **National Institute of Allergy and Infectious Diseases**. Chiron Corp HCV E1/E2 Vaccine (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 10 January 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT00500747>
- 203 **Frey SE**, Houghton M, Coates S, Abrignani S, Chien D, Rosa D, Pileri P, Ray R, Di Bisceglie AM, Rinella P, Hill H, Wolff MC, Schultze V, Han JH, Scharschmidt B, Belshe RB. Safety and immunogenicity of HCV E1E2 vaccine adjuvanted with MF59 administered to healthy adults. *Vaccine* 2010; **28**: 6367-6373 [PMID: 20619382 DOI: 10.1016/j.vaccine.2010.06.084]
- 204 **Chen F**, Nagy K, Chavez D, Willis S, McBride R, Giang E, Honda A, Bukh J, Ordoukhanian P, Zhu J, Frey S, Lanford R, Law M. Antibody Responses to Immunization With HCV Envelope Glycoproteins as a Baseline for B-Cell-Based Vaccine Development. *Gastroenterology* 2020; **158**: 1058-1071.e6 [PMID: 31809725 DOI: 10.1053/j.gastro.2019.11.282]
- 205 **Tauber E**. Phase II Study of Immunization With a Hepatitis C Virus (HCV) Antigen Peptide Vaccine (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 10 January 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT00602784>
- 206 **Klade CS**, Wedemeyer H, Berg T, Hinrichsen H, Cholewinska G, Zeuzem S, Blum H, Buschle M, Jelovcan S, Buerger V, Tauber E, Frisch J, Manns MP. Therapeutic vaccination of chronic hepatitis C nonresponder patients with the peptide vaccine IC41. *Gastroenterology* 2008; **134**: 1385-1395 [PMID: 18471515 DOI: 10.1053/j.gastro.2008.02.058]
- 207 **El Awady MK**. Safety and Efficacy Study of CENV3 Vaccine to Protect Against HCV Infection (CENV3) (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 10 January 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT01718834>
- 208 **Ernsthofer S**. Virological Response Study of the HCV Vaccine IC41 (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 10 January



- 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT00601770>
- 209 **Ikram A**, Anjum S, Tahir M. In Silico Identification and Conservation Analysis of B-cell and T-Cell Epitopes of Hepatitis C Virus 3a Genotype Enveloped Glycoprotein 2 From Pakistan: A Step Towards Heterologous Vaccine Design. *Hepat Mon* 2014; **14**: 1-8 [PMID: [24976845](#) DOI: [10.5812/hepatmon.9832](#)]
- 210 **Meshram RJ**, Gacche RN. Effective epitope identification employing phylogenetic, mutational variability, sequence entropy, and correlated mutation analysis targeting NS5B protein of hepatitis C virus: from bioinformatics to therapeutics. *J Mol Recognit* 2015; **28**: 492-505 [PMID: [25727409](#) DOI: [10.1002/jmr.2466](#)]
- 211 **Hart GR**, Ferguson AL. Empirical fitness models for hepatitis C virus immunogen design. *Phys Biol* 2015; **12**: 066006 [PMID: [26599153](#) DOI: [10.1088/1478-3975/12/6/066006](#)]
- 212 **Ikram A**, Zaheer T, Awan FM, Obaid A, Naz A, Hanif R, Paracha RZ, Ali A, Naveed AK, Janjua HA. Exploring NS3/4A, NS5A and NS5B proteins to design conserved subunit multi-epitope vaccine against HCV utilizing immunoinformatics approaches. *Sci Rep* 2018; **8**: 16107 [PMID: [30382118](#) DOI: [10.1038/s41598-018-34254-5](#)]
- 213 **Guest JD**, Pierce BG. Structure-Based and Rational Design of a Hepatitis C Virus Vaccine. *Viruses* 2021; **13**: 837 [PMID: [34063143](#) DOI: [10.3390/v13050837](#)]
- 214 **Masavuli MG**, Wijesundara DK, Torresi J, Gowans EJ, Grubor-Bauk B. Preclinical Development and Production of Virus-Like Particles As Vaccine Candidates for Hepatitis C. *Front Microbiol* 2017; **8**: article2413 [PMID: [29259601](#) DOI: [10.3389/fmicb.2017.02413](#)]
- 215 **Christiansen D**, Earnest-Silveira L, Grubor-Bauk B, Wijesundara DK, Boo I, Ramsland PA, Vincan E, Drummer HE, Gowans EJ, Torresi J. Pre-clinical evaluation of a quadrivalent HCV VLP vaccine in pigs following microneedle delivery. *Sci Rep* 2019; **9**: 9251 [PMID: [31239471](#) DOI: [10.1038/s41598-019-45461-z](#)]
- 216 **Torresi J**. The Rationale for a Preventative HCV Virus-Like Particle (VLP) Vaccine. *Front Microbiol* 2017; **8**: article2163 [PMID: [29163442](#) DOI: [10.3389/fmicb.2017.02163](#)]
- 217 **Ura T**, Okuda K, Shimada M. Developments in Viral Vector-Based Vaccines. *Vaccines* 2014; **2**: 624-41 [PMID: [26344749](#) DOI: [10.3390/vaccines2030624](#)]
- 218 **Tatsis N**, Ertl HCJ. Adenoviruses as vaccine vectors. *Mol Ther* 2004; **10**: 616-629 [PMID: [15451446](#) DOI: [10.1016/j.ymthe.2004.07.013](#)]
- 219 **Bhatta M**, Nandi S, Dutta S, Saha MK. Coronavirus (SARS-CoV-2): a systematic review for potential vaccines. *Hum Vaccin Immunother* 2021; 1-18 [PMID: [33545014](#) DOI: [10.1080/21645515.2020.1865774](#)]
- 220 **US National Library of Medicine**. ClinicalTrials.gov [Internet]. [cited 18 February 2021]. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/>
- 221 **Folgori A**, Capone S, Ruggeri L, Meola A, Sporeno E, Ercole BB, Pezzanera M, Tafi R, Arcuri M, Fattori E, Lahm A, Luzzago A, Vitelli A, Colloca S, Cortese R, Nicosia A. A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees. *Nat Med* 2006; **12**: 190-197 [PMID: [16462801](#) DOI: [10.1038/nm1353](#)]
- 222 **Penaloza-MacMaster P**, Provine NM, Ra J, Borducchi EN, McNally A, Simmons NL, Iampietro MJ, Barouch DH. Alternative serotype adenovirus vaccine vectors elicit memory T cells with enhanced anamnestic capacity compared to Ad5 vectors. *J Virol* 2013; **87**: 1373-1384 [PMID: [23152535](#) DOI: [10.1128/JVI.02058-12](#)]
- 223 **Bos R**, Rutten L, van der Lubbe JEM, Bakkers MJG, Hardenberg G, Wegmann F, Zuijgeest D, de Wilde AH, Koornneef A, Verwilligen A, van Manen D, Kwaks T, Vogels R, Dalebout TJ, Myeni SK, Kikkert M, Snijder EJ, Li Z, Barouch DH, Vellinga J, Langedijk JPM, Zahn RC, Custers J, Schuitemaker H. Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses. *NPJ Vaccines* 2020; **5**: 91 [PMID: [33083026](#) DOI: [10.1038/s41541-020-00243-x](#)]
- 224 **Mutimer D**. Study of a Novel Therapeutic Vaccine for Hepatitis C Virus (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT01094873>
- 225 **Adams D**. A Study of a New Candidate Vaccine Against Hepatitis C Virus (HCV) (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT01070407>
- 226 **Blanchard TJ**, Alcamí A, Andrea P, Smith GL. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. *J Gen Virol* 1998; **79** ( Pt 5): 1159-1167 [PMID: [9603331](#) DOI: [10.1099/0022-1317-79-5-1159](#)]
- 227 **Brunetto M**. Study of a Novel Therapeutic Vaccine Against Hepatitis C Using Ad6NSmut and MVA-NSmut in Chronically Infected Patients (HCV004) (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT01701336>
- 228 **Klenerman P**, Gorard D. Study of a New MVA Vaccine for Hepatitis C Virus (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT01296451>
- 229 **Swadling L**, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, Halliday J, Kelly C, Bowen D, Fergusson J, Kurioka A, Ammendola V, Del Sorbo M, Grazioli F, Esposito ML, Siani L, Traboni C, Hill A, Colloca S, Davis M, Nicosia A, Cortese R, Folgori A, Klenerman P, Barnes E. A

- human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. *Sci Transl Med* 2014; **6**: 261ra153 [PMID: 25378645 DOI: 10.1126/scitranslmed.3009185]
- 230 **Cox AL**, Page K, Melia M, Veenhuis R, Massaccesi G, Osburn W, Wagner K, Giudice L, Stein E, Asher AK, Vassilev V, Lin L, Nicosia A, Capone S, Scarselli E, Folgori A, Gorman R, Chang S, Wolff P, Liang TJ, Ghany M, Wierzbicki M, Lum P. A Randomized, Double-Blind, Placebo-Controlled Efficacy Trial of a Vaccine to Prevent Chronic Hepatitis C Virus Infection in an at-Risk Population. Abstract LB10. *Open Forum Infect Dis* 2019; **6**: S997-S997 [DOI: 10.1093/ofid/ofz415.2493]
- 231 **Sepulveda-Crespo D**, Resino S, Martinez I. Innate immune response against hepatitis c virus: Targets for vaccine adjuvants. *Vaccines* 2020; **8**: 1-24 [PMID: 32560440 DOI: 10.3390/vaccines8020313]
- 232 **Capone S**, Naddeo M, D'Alise AM, Abbate A, Grazioli F, Del Gaudio A, Del Sorbo M, Esposito ML, Ammendola V, Perretta G, Taglioni A, Colloca S, Nicosia A, Cortese R, Folgori A. Fusion of HCV nonstructural antigen to MHC class II-associated invariant chain enhances T-cell responses induced by vectored vaccines in nonhuman primates. *Mol Ther* 2014; **22**: 1039-1047 [PMID: 24476798 DOI: 10.1038/mt.2014.15]
- 233 **Dorrell L**. Class II Invariant Chain HCV Vaccine Study (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT03688061>
- 234 **Esposito I**, Cicconi P, D'Alise AM, Brown A, Esposito M, Swadling L, Holst PJ, Bassi MR, Stornaiuolo M, Mori F, Vassilev V, Li W, Donnison T, Gentile C, Turner B, von Delft A, Del Sorbo M, Barra F, Contino AM, Abbate A, Novellino E, Thomsen AR, Christensen JP, Lahm A, Grazioli F, Ammendola V, Siani L, Colloca S, Klenerman P, Nicosia A, Dorrell L, Folgori A, Capone S, Barnes E; PEACHI Consortium. MHC class II invariant chain-adjuvanted viral vectored vaccines enhances T cell responses in humans. *Sci Transl Med* 2020; **12**: eaaz7715 [PMID: 32554708 DOI: 10.1126/scitranslmed.aaz7715]
- 235 **Barger G**, Sheikh A. Therapeutic Hepatitis C Virus Vaccine (accessed 2021 Feb 18). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT04318379>
- 236 **Martínez-Molina E**, Chocarro-Wrona C, Martínez-Moreno D, Marchal JA, Boulaiz H. Large-Scale Production of Lentiviral Vectors: Current Perspectives and Challenges. *Pharmaceutics* 2020; **12**: 1051 [PMID: 33153183 DOI: 10.3390/pharmaceutics12111051]
- 237 **Ebrahim GJ**. DNA vaccines. *J Trop Pediatr* 1998; **44**: 64-65 [PMID: 9604589 DOI: 10.1093/tropej/44.2.64]
- 238 **Ho W**, Gao M, Li F, Li Z, Zhang X, Xu X. Next-Generation Vaccines: Nanoparticle-Mediated DNA and mRNA Delivery. *Adv Healthc Mater* 2021; **10**: e2001812 [PMID: 33458958 DOI: 10.1002/adhm.202001812]
- 239 **Malik VS**. Recombinant DNA Technology. *Adv App Micro* 1981; 1-84 [DOI: 10.1007/978-1-4615-7096-7\_5]
- 240 **Strong TV**, Hampton TA, Louro I, Bilbao G, Conry RM, Curiel DT. Incorporation of beta-globin untranslated regions into a Sindbis virus vector for augmentation of heterologous mRNA expression. *Gene Ther* 1997; **4**: 624-627 [PMID: 9231080 DOI: 10.1038/sj.gt.3300423]
- 241 **Boczkowski D**, Nair SK, Snyder D, Gilboa E. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. *J Exp Med* 1996; **184**: 465-472 [PMID: 8760800 DOI: 10.1084/jem.184.2.465]
- 242 **Kreiter S**, Diken M, Selmi A, Türeci Ö, Sahin U. Tumor vaccination using messenger RNA: prospects of a future therapy. *Curr Opin Immunol* 2011; **23**: 399-406 [PMID: 21497074 DOI: 10.1016/j.coi.2011.03.007]
- 243 **Moderna biotech Spain SL**. Annex I-Summary of product characteristics-COVID-19 mRNA vaccine (nucleoside modified) [Internet]. 2021. [cited 18 February 2021]. Available from: [https://www.ema.europa.eu/en/documents/product-information/covid-19-vaccine-moderna-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/covid-19-vaccine-moderna-epar-product-information_en.pdf)
- 244 **Tokushige K**, Wakita T, Pachuk C, Moradpour D, Weiner DB, Zurawski VR Jr, Wands JR. Expression and immune response to hepatitis C virus core DNA-based vaccine constructs. *Hepatology* 1996; **24**: 14-20 [PMID: 8707253 DOI: 10.1053/jhep.1996.v24.pm0008707253]
- 245 **Vidalin O**, Tanaka E, Spengler U, Trépo C, Inchauspé G. Targeting of hepatitis C virus core protein for MHC I or MHC II presentation does not enhance induction of immune responses to DNA vaccination. *DNA Cell Biol* 1999; **18**: 611-621 [PMID: 10463057 DOI: 10.1089/104454999315024]
- 246 **Hu GJ**, Wang RY, Han DS, Alter HJ, Shih JW. Characterization of the humoral and cellular immune responses against hepatitis C virus core induced by DNA-based immunization. *Vaccine* 1999; **17**: 3160-3170 [PMID: 10462252 DOI: 10.1016/S0264-410X(99)00130-9]
- 247 **Acosta-Rivero N**, Dueñas-Carrera S, Alvarez-Lajonchere L, Morales-Grillo J. HCV core protein-expressing DNA vaccine induces a strong class I-binding peptide DTH response in mice. *Biochem Biophys Res Commun* 2004; **314**: 781-786 [PMID: 14741703 DOI: 10.1016/j.bbrc.2003.12.168]
- 248 **Encke J**, Geissler M, Stremmel W, Wands JR. DNA-based immunization breaks tolerance in a hepatitis C virus transgenic mouse model. *Hum Vaccin* 2006; **2**: 78-83 [PMID: 17012868 DOI: 10.4161/hv.2.2.2699]
- 249 **Major ME**, Vitvitski L, Mink MA, Schleef M, Whalen RG, Trépo C, Inchauspé G. DNA-based

- immunization with chimeric vectors for the induction of immune responses against the hepatitis C virus nucleocapsid. *J Virol* 1995; **69**: 5798-5805 [PMID: [7637024](#) DOI: [10.1128/jvi.69.9.5798-5805.1995](#)]
- 250 **Inchauspé G**, Major ME, Nakano I, Vitvitski L, Trépo C. DNA vaccination for the induction of immune responses against hepatitis C virus proteins. *Vaccine* 1997; **15**: 853-856 [PMID: [9234532](#) DOI: [10.1016/s0264-410x\(96\)00275-7](#)]
- 251 **Lee SW**, Cho JH, Lee KJ, Sung YC. Hepatitis C virus envelope DNA-based immunization elicits humoral and cellular immune responses. *Mol Cells* 1998; **8**: 444-451 [PMID: [9749532](#)]
- 252 **Ma X**, Forns X, Gutierrez R, Mushahwar IK, Wu T, Payette PJ, Bukh J, Purcell RH, Davis HL. DNA-based vaccination against hepatitis C virus (HCV): effect of expressing different forms of HCV E2 protein and use of CpG-optimized vectors in mice. *Vaccine* 2002; **20**: 3263-3271 [PMID: [12213395](#) DOI: [10.1016/S0264-410X\(02\)00304-3](#)]
- 253 **Gehring S**, Gregory SH, Kuzushita N, Wands JR. Type 1 interferon augments DNA-based vaccination against hepatitis C virus core protein. *J Med Virol* 2005; **75**: 249-257 [PMID: [15602727](#) DOI: [10.1002/jmv.20264](#)]
- 254 **Kim Y**, Vaughan K, Greenbaum J, Peters B, Law M, Sette A. A meta-analysis of the existing knowledge of immunoreactivity against hepatitis C virus (HCV). *PLoS One* 2012; **7**: e38028 [PMID: [22675428](#) DOI: [10.1371/journal.pone.0038028](#)]
- 255 **Frelin L**, Alheim M, Chen A, Söderholm J, Rozell B, Barnfield C, Liljeström P, Sällberg M. Low dose and gene gun immunization with a hepatitis C virus nonstructural (NS) 3 DNA-based vaccine containing NS4A inhibit NS3/4A-expressing tumors in vivo. *Gene Ther* 2003; **10**: 686-699 [PMID: [12692597](#) DOI: [10.1038/sj.gt.3301933](#)]
- 256 **Ahlén G**, Holmström F, Gibbs A, Alheim M, Frelin L. Long-term functional duration of immune responses to HCV NS3/4A induced by DNA vaccination. *Gene Ther* 2014; **21**: 739-750 [PMID: [24871581](#) DOI: [10.1038/gt.2014.48](#)]
- 257 **Ahlén G**, Nyström J, Pult I, Frelin L, Hultgren C, Sällberg M. In Vivo Clearance of Hepatitis C Virus Nonstructural 3/4A—Expressing Hepatocytes by DNA Vaccine—Primed Cytotoxic T Lymphocytes. *J Infect Dis* 2005; **192**: 2112-2116 [PMID: [16288375](#) DOI: [10.1086/498218](#)]
- 258 **Naderi M**, Saeedi A, Moradi A, Kleshadi M, Zolfaghari MR, Gorji A, Ghaemi A. Interleukin-12 as a genetic adjuvant enhances hepatitis C virus NS3 DNA vaccine immunogenicity. *Virol Sin* 2013; **28**: 167-173 [PMID: [23709057](#) DOI: [10.1007/s12250-013-3291-z](#)]
- 259 **Holmström F**, Pasetto A, Nähr V, Brass A, Kriegs M, Hilt E, Broderick KE, Chen M, Ahlén G, Frelin L. A synthetic codon-optimized hepatitis C virus nonstructural 5A DNA vaccine primes polyfunctional CD8<sup>+</sup> T cell responses in wild-type and NS5A-transgenic mice. *J Immunol* 2013; **190**: 1113-1124 [PMID: [23284053](#) DOI: [10.4049/jimmunol.1201497](#)]
- 260 **Weiland OR**. Phase I/IIa Dose Ranging CHRONVAC-C® Study in Chronic HCV Patients (accessed 2021 Feb 19). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT00563173>
- 261 **Weiland OR**. CHRONVAC-C Study Followed by Standard of Care in Chronic Hepatitis C Virus (HCV) Subjects (accessed 2021 Feb 19). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT01335711>
- 262 **Weiland O**, Ahlén G, Diepolder H, Jung MC, Levander S, Fons M, Mathiesen I, Sardesai NY, Vahlne A, Frelin L, Sällberg M. Therapeutic DNA vaccination using in vivo electroporation followed by standard of care therapy in patients with genotype 1 chronic hepatitis C. *Mol Ther* 2013; **21**: 1796-1805 [PMID: [23752314](#) DOI: [10.1038/mt.2013.119](#)]
- 263 **Morikawa K**, Lange CM, Gouttenoire J, Meylan E, Brass V, Penin F, Moradpour D. Nonstructural protein 3-4A: the Swiss army knife of hepatitis C virus. *J Viral Hepat* 2011; **18**: 305-315 [PMID: [21470343](#) DOI: [10.1111/j.1365-2893.2011.01451.x](#)]
- 264 **Ratnoglik SL**, Jiang DP, Aoki C, Sudarmono P, Shoji I, Deng L, Hotta H. Induction of cell-mediated immune responses in mice by DNA vaccines that express hepatitis C virus NS3 mutants lacking serine protease and NTPase/RNA helicase activities. *PLoS One* 2014; **9**: e98877 [PMID: [24901478](#) DOI: [10.1371/journal.pone.0098877](#)]
- 265 **Pouriyaveali MH**, Bamdad T, Aghasadeghi MR, Sadat SM, Sabahi F. Construction and immunogenicity analysis of hepatitis C virus (HCV) truncated non-structural protein 3 (NS3) plasmid vaccine. *Jundishapur J Microbiol* 2016; **9**: e33909 [PMID: [27226878](#) DOI: [10.5812/jjm.33909](#)]
- 266 **Latimer B**, Toporovski R, Yan J, Pankhong P, Morrow MP, Khan AS, Sardesai NY, Welles SL, Jacobson JM, Weiner DB, Kutzler MA. Strong HCV NS3/4a, NS4b, NS5a, NS5b-specific cellular immune responses induced in Rhesus macaques by a novel HCV genotype 1a/1b consensus DNA vaccine. *Hum Vaccin Immunother* 2014; **10**: 2357-2365 [PMID: [25424943](#) DOI: [10.4161/hv.29590](#)]
- 267 **Capone S**, Zampaglione I, Vitelli A, Pezzanera M, Kierstead L, Burns J, Ruggeri L, Arcuri M, Cappelletti M, Meola A, Ercole BB, Tafi R, Santini C, Luzzago A, Fu T, Colloca S, Ciliberto G, Cortese R, Nicosia A, Fattori E, Folgori A. Modulation of the Immune Response Induced by Gene Electrottransfer of a Hepatitis C Virus DNA Vaccine in Nonhuman Primates. *J Immunol* 2006; **177**: 7462-7471 [PMID: [17082666](#) DOI: [10.4049/jimmunol.177.10.7462](#)]
- 268 **Lee H**, Jeong M, Oh J, Cho Y, Shen X, Stone J, Yan J, Rothkopf Z, Khan AS, Cho BM, Park YK, Weiner DB, Son WC, Maslow JN. Preclinical evaluation of multi antigenic HCV DNA vaccine for

- the prevention of Hepatitis C virus infection. *Sci Rep* 2017; 7: 43531 [PMID: 28266565 DOI: 10.1038/srep43531]
- 269 **Hoon S, Heo J.** Phase I Trial to Evaluate the Safety, Tolerability and Immunogenicity of VGX-6150 for Second-line Therapy of Chronic Hepatitis C Infection (VGX-6150-01) (accessed 2021 Feb 19). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT02027116>
  - 270 **Han JW, Sung PS, Hong SH, Lee H, Koh JY, White S, Maslow JN, Weiner DB, Park SH, Jeong M, Heo J, Ahn SH, Shin EC.** IFN $\lambda$ 3-adjuvanted HCV DNA vaccine reduces regulatory T cell frequency and increases virus-specific T cell responses. *J Hepatol* 2020; 73: 72-83 [PMID: 32088322 DOI: 10.1016/j.jhep.2020.02.009]
  - 271 **GeneOne Life Science Inc.** Evaluation of Safety, Tolerability, and Immunogenicity Study of GLS-6150 in Healthy Volunteers and in Persons Previously Treated for Hepatitis C Virus Infection (accessed 2021 Feb 19). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT03674125>
  - 272 **Jacobson JM.** DNA Vaccine Therapy in Treating Patients With Chronic Hepatitis C Virus Infection (accessed 2021 Feb 19). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 18 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT02772003>
  - 273 **Fournillier A, Frelin L, Jacquier E, Ahlén G, Brass A, Gerossier E, Holmström F, Broderick KE, Sardesai NY, Bonnefoy JY, Inchauspé G, Sällberg M.** A heterologous prime/boost vaccination strategy enhances the immunogenicity of therapeutic vaccines for hepatitis C virus. *J Infect Dis* 2013; 208: 1008-1019 [PMID: 23776192 DOI: 10.1093/infdis/jit267]
  - 274 **World Health Organization (WHO).** Status of COVID-19 Vaccines within WHO EUL/PQ evaluation process [Internet]. 2021. [cited 24 February 2021]. Available from: [https://extranet.who.int/pqweb/sites/default/files/documents/Status\\_COVID\\_VAX\\_24Feb2021.pdf](https://extranet.who.int/pqweb/sites/default/files/documents/Status_COVID_VAX_24Feb2021.pdf)
  - 275 **Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW Jr, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group.** Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020; 383: 2603-2615 [PMID: 33301246 DOI: 10.1056/NEJMoa2034577]
  - 276 **Holzgreve H.** COVID-19: Nobelpreiswürdiger Erfolg in der Impfstoff-Forschung. *MMW Fortschr Med* 2021; 163: 24-25 [PMID: 33527275 DOI: 10.1007/s15006-021-9577-4]
  - 277 **Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Raabe V, Bailey R, Swanson KA, Li P, Koury K, Kalina W, Cooper D, Fontes-Garfias C, Shi PY, Türeci Ö, Tompkins KR, Walsh EE, Frenck R, Falsey AR, Dormitzer PR, Gruber WC, Şahin U, Jansen KU.** Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 2020; 586: 589-593 [PMID: 32785213 DOI: 10.1038/s41586-020-2639-4]
  - 278 **BioNTech SE.** Study to Describe the Safety, Tolerability, Immunogenicity, and Efficacy of RNA Vaccine Candidates Against COVID-19 in Healthy Individuals (accessed 2021 Feb 20). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 24 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT04368728>
  - 279 **BioNTech SE.** A Phase 3 Study to Evaluate the Safety, Tolerability, and Immunogenicity of Multiple Production Lots and Dose Levels of BNT162b2 Against COVID-19 in Healthy Participants (accessed 2021 Feb 20). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 24 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT04713553>
  - 280 **Moderna Tx Inc.** A Study to Evaluate Efficacy, Safety, and Immunogenicity of mRNA-1273 Vaccine in Adults Aged 18 Years and Older to Prevent COVID-19 (accessed 2021 Feb 20). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 24 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT04470427>
  - 281 **Moderna Tx Inc.** A Study to Evaluate the Safety, Reactogenicity, and Effectiveness of mRNA-1273 Vaccine in Adolescents 12 to <18 Years Old to Prevent COVID-19 (TeenCove) (accessed 2021 Feb 20). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 24 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT04649151>
  - 282 **Sharifnia Z, Bandehpour M, Kazemi B, Zarghami N.** Design and Development of Modified mRNA Encoding Core Antigen of Hepatitis C Virus: a Possible Application in Vaccine Production. *Iran Biomed J* 2019; 23: 57-67 [PMID: 30056690 DOI: 10.29252/23.1.57]
  - 283 **Fu C, Zhou L, Mi Q-S, Jiang A.** DC-Based Vaccines for Cancer Immunotherapy. *Vaccines* 2020; 8: 706 [PMID: 33255895 DOI: 10.3390/vaccines8040706]
  - 284 **Zhou Y, Zhang Y, Yao Z, Moorman JP, Jia Z.** Dendritic cell-based immunity and vaccination against hepatitis C virus infection. *Immunology* 2012; 136: 385-396 [PMID: 22486354 DOI: 10.1111/j.1365-2567.2012.03590.x]
  - 285 **Wintermeyer P, Gehring S, Eken A, Wands JR.** Generation of cellular immune responses to HCV NS5 protein through in vivo activation of dendritic cells. *J Viral Hepat* 2010; 17: 705-713 [PMID: 20002303 DOI: 10.1111/j.1365-2893.2009.01228.x]
  - 286 **Chernykh E, Leplina O, Oleynik E, Tikhonova M, Tyrinova T, Starostina N, Ostanin A.** Immunotherapy with interferon- $\alpha$ -induced dendritic cells for chronic HCV infection (the results of



- pilot clinical trial). *Immunol Res* 2018; **66**: 31-43 [PMID: 29164490 DOI: 10.1007/s12026-017-8967-2]
- 287 **Weigand K**, Voigt F, Encke J, Hoyler B, Stremmel W, Eisenbach C. Vaccination with dendritic cells pulsed with hepatitis C pseudo particles induces specific immune responses in mice. *World J Gastroenterol* 2012; **18**: 785-793 [PMID: 22371638 DOI: 10.3748/wjg.v18.i8.785]
- 288 **Mishra S**, Lavelle BJ, Desrosiers J, Ardito MT, Terry F, Martin WD, De Groot AS, Gregory SH. Dendritic cell-mediated, DNA-based vaccination against hepatitis C induces the multi-epitope-specific response of humanized, HLA transgenic mice. *PLoS One* 2014; **9**: e104606 [PMID: 25111185 DOI: 10.1371/journal.pone.0104606]
- 289 **Yu H**, Babiuk LA, van Drunen Littel-van den Hurk S. Strategies for loading dendritic cells with hepatitis C NS5a antigen and inducing protective immunity. *J Viral Hepat* 2008; **15**: 459-70 [PMID: 18221298 DOI: 10.1111/j.1365-2893.2008.00959.x]
- 290 **Tian Y**, Zhang HH, Wei L, Du SC, Chen HS, Fei R, Liu F. The functional evaluation of dendritic cell vaccines based on different hepatitis C virus nonstructural genes. *Viral Immunol* 2007; **20**: 553-561 [PMID: 18158729 DOI: 10.1089/vim.2007.0064]
- 291 **Echeverría I**, Pereboev A, Silva L, Zabaleta A, Riezu-Boj JI, Bes M, Cubero M, Borrás-Cuesta F, Lasarte JJ, Esteban JI, Prieto J, Sarobe P. Enhanced T cell responses against hepatitis C virus by ex vivo targeting of adenoviral particles to dendritic cells. *Hepatology* 2011; **54**: 28-37 [PMID: 21452282 DOI: 10.1002/hep.24325]
- 292 **Zhou Y**, Zhao F, Chen L, Ma L, Wang Y, He Y, Ma Z, Liu H, Guo Y, Zhang Y, Yao Z, Hao C, Jia Z. Development of a dendritic cell vaccine encoding multiple cytotoxic T lymphocyte epitopes targeting hepatitis C virus. *Int J Mol Med* 2013; **32**: 901-909 [PMID: 23934073 DOI: 10.3892/ijmm.2013.1466]
- 293 **Zabaleta A**, D'Avola D, Echeverría I, Llopiz D, Silva L, Villanueva L, Riezu-Boj JI, Larrea E, Pereboev A, Lasarte JJ, Rodríguez-Lago I, Iñarrairaegui M, Sangro B, Prieto J, Sarobe P. Clinical testing of a dendritic cell targeted therapeutic vaccine in patients with chronic hepatitis C virus infection. *Mol Ther - Methods Clin Dev* 2015; **2**: 15006 [PMID: 26029717 DOI: 10.1038/mtm.2015.6]
- 294 **Ostanin AA**. Autologous Dendritic Cell Vaccine for Treatment of Patients With Chronic HCV-Infection. In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 24 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT03119025>
- 295 **Clinica Universidad de Navarra**. Phase I-II Vaccination of Autologous Dendritic Cells Transduced With Adenoviral Vector Encoding NS3 in Hepatitis C Encoding NS3 in Hepatitis C (accessed 2021 Feb 21). In: ClinicalTrials.gov [internet]. Bethesda (MD): U.S. National Library of Medicine. [cited 24 February 2021]. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT02309086>
- 296 **World Health Organization (WHO)**. Vaccines & Diseases [Internet]. [cited 18 February 2021]. Available from: <https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases>
- 297 **Pestova TV**, Shatsky IN, Fletcher SP, Jackson RJ, Hellen CU. A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. *Genes Dev* 1998; **12**: 67-83 [PMID: 9420332 DOI: 10.1101/gad.12.1.67]
- 298 **Khawaja A**, Vopalensky V, Pospisek M. Understanding the potential of hepatitis C virus internal ribosome entry site domains to modulate translation initiation via their structure and function. *Wiley Interdiscip Rev RNA* 2015; **6**: 211-224 [PMID: 25352252 DOI: 10.1002/wrna.1268]
- 299 **Angulo J**, Ulryck N, Deforges J, Chamond N, Lopez-Lastra M, Masquida B, Sargueil B. LOOP IIIId of the HCV IRES is essential for the structural rearrangement of the 40S-HCV IRES complex. *Nucleic Acids Res* 2016; **44**: 1309-1325 [PMID: 26626152 DOI: 10.1093/nar/gkv1325]
- 300 **Echeverría N**. Variabilidad genética, resistencia al tratamiento y eficiencia traduccional del Virus de la Hepatitis C: Caracterización de factores virales y del hospedero. PhD Thesis. Universidad de la República, Uruguay. 2019. [cited 18 February 2021]. Available from: [https://biur.edu.uy/F/SLYLNHRAXQD9KU34M9XC3N2E55S2CQV55UJJSTBBUNPH68XPGJ-18147?func=service&doc\\_library=URE01&doc\\_number=000508554&line\\_number=0001&func\\_code=WEB-BRIEF&service\\_type=MEDIA](https://biur.edu.uy/F/SLYLNHRAXQD9KU34M9XC3N2E55S2CQV55UJJSTBBUNPH68XPGJ-18147?func=service&doc_library=URE01&doc_number=000508554&line_number=0001&func_code=WEB-BRIEF&service_type=MEDIA)
- 301 **Sabin AB**, Boulger LR. History of Sabin attenuated poliovirus oral live vaccine strains. *J Biol Stand* 1973; **1**: 115-118 [DOI: 10.1016/0092-1157(73)90048-6]
- 302 **Macadam AJ**, Pollard SR, Ferguson G, Dunn G, Skuce R, Almond JW, Minor PD. The 5' noncoding region of the type 2 poliovirus vaccine strain contains determinants of attenuation and temperature sensitivity. *Virology* 1991; **181**: 451-458 [PMID: 1707566]
- 303 **Kew OM**, Sutter RW, de Gourville EM, Dowdle WR, Pallansch MA. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. *Annu Rev Microbiol* 2005; **59**: 587-635 [PMID: 16153180 DOI: 10.1146/annurev.micro.58.030603.123625]
- 304 **Frese M**, Lee E, Larena M, Lim PS, Rao S, Matthaei KI, Khromykh A, Ramshaw I, Lobigs M. Internal ribosome entry site-based attenuation of a flavivirus candidate vaccine and evaluation of the effect of beta interferon coexpression on vaccine properties. *J Virol* 2014; **88**: 2056-2070 [PMID: 24307589 DOI: 10.1128/JVI.03051-13]
- 305 **Moratorio G**, Henningsson R, Barbezange C, Carrau L, Bordería AV, Blanc H, Beaucourt S, Poirier EZ, Vallet T, Boussier J, Mounce BC, Fontes M, Vignuzzi M. Attenuation of RNA viruses by redirecting their evolution in sequence space. *Nat Microbiol* 2017; **2**: 17088 [PMID: 28581455 DOI: 10.1038/natmicro.2017.17088]



- 10.1038/nmicrobiol.2017.88]
- 306 **Zhou P**, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270-273 [PMID: 32015507 DOI: 10.1038/s41586-020-2012-7]
- 307 **Defendi HGT**, da Silva Madeira L, Borschiver S. Analysis of the COVID-19 Vaccine Development Process: an Exploratory Study of Accelerating Factors and Innovative Environments. *J Pharm Innov* 2021; 1-17 [PMID: 33552310 DOI: 10.1007/s12247-021-09535-8]
- 308 **Pardi N**, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov* 2018; **17**: 261-279 [PMID: 29326426 DOI: 10.1038/nrd.2017.243]
- 309 **Dong Y**, Dai T, Wei Y, Zhang L, Zheng M, Zhou F. A systematic review of SARS-CoV-2 vaccine candidates. *Signal Transduct Target Ther* 2020; **5**: 237 [PMID: 33051445 DOI: 10.1038/s41392-020-00352-y]
- 310 **Cao Y**, Gao GF. mRNA vaccines: A matter of delivery. *EClinicalMedicine* 2021; **32**: 100746 [PMID: 33644722 DOI: 10.1016/j.eclinm.2021.100746]
- 311 **Ledford H**. Could mixing COVID vaccines boost immune response? *Nature* 2021; **590**: 375-376 [PMID: 33547431 DOI: 10.1038/d41586-021-00315-5]
- 312 **Spencer AJ**, McKay PF, Belij-Rammerstorfer S, Ulaszewska M, Bissett CD, Hu K, Samnuan K, Wright D, Sharpe HR, Gilbride C, Truby A, Allen ER, Gilbert SC, Shattock RJ, Lambe T. Heterologous vaccination regimens with self-amplifying RNA and Adenoviral COVID vaccines induce robust immune responses in mice. *Nat Commun* 2021; **12**: 2893 [PMID: 34001897 DOI: 10.1038/s41467-021-23173-1]
- 313 **Prüß BM**. Current State of the First COVID-19 Vaccines. *Vaccines (Basel)* 2021; **9**: 30 [PMID: 33429880 DOI: 10.3390/vaccines9010030]



## Pediatric non-cirrhotic portal hypertension: Endoscopic outcome and perspectives from developing nations

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### Abstract

Non-cirrhotic portal hypertension (NCPH) forms an important subset of portal hypertension in children. Variceal bleed and splenomegaly are their predominant presentation. Laboratory features show cytopenias (hypersplenism) and preserved hepatic synthetic functions. Repeated sessions of endoscopic variceal ligation or endoscopic sclerotherapy eradicate esophageal varices in almost all cases. After variceal eradication, there is an increased risk of other complications like secondary gastric varices, cholangiopathy, colopathy, growth failure, especially in extra-hepatic portal vein obstruction (EHPVO). Massive splenomegaly-related pain and early satiety cause poor quality of life (QoL). Meso-Rex bypass is the definitive therapy when the procedure is anatomically feasible in EHPVO. Other portosystemic shunt surgeries with splenectomy are indicated when patients present late and spleen-related issues predominate. Shunt surgeries prevent rebleed, improve growth and QoL. Non-cirrhotic portal fibrosis (NCPF) is a less common cause of portal hypertension in children in developing nations. Presentation in the second decade, massive splenomegaly and patent portal vein are discriminating features of NCPF. Shunt surgery is required in severe cases when endotherapy is insufficient for the varices. Congenital hepatic fibrosis (CHF) presents with firm palpable liver and splenomegaly. Ductal plate malformation forms the histological hallmark of CHF. CHF is commonly associated with Caroli's disease, renal cysts, and syndromes associated with neurological defects. Isolated CHF has a favourable prognosis requiring endotherapy. Liver transplantation is required when there is decompensation or recurrent cholangitis, especially in Caroli's syndrome. Combined liver-kidney transplantation is indicated when both liver and renal issues are present.

**Key Words:** Extrahepatic portal vein obstruction; Non-cirrhotic portal fibrosis; Portosystemic shunt surgery; Congenital hepatic fibrosis

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**Core Tip:** The review discusses the natural history, endoscopic outcome, and management of non-cirrhotic causes of portal hypertension in children, especially in resource constraint developing nations. Extrahepatic portal vein obstruction is the most common cause of portal hypertension in developing countries. Endoscopic variceal ligation and sclerotherapy effectively eradicate the esophageal varices. Other complications require shunt surgery that ultimately reverses portal hypertension. Non-cirrhotic portal fibrosis has favourable outcomes in terms of variceal bleeding and mortality. Isolated congenital hepatic fibrosis (CHF) has a relatively good outcome. Liver transplantation is required when CHF is associated with Caroli's disease, recurrent cholangitis, and decompensation. The presence of significant renal disease requires combined liver and kidney transplantation.

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## INTRODUCTION

Portal hypertension refers to a pathological increase in portal pressure. Direct measurement of portal pressure is clinically impractical and cumbersome. The indirect way of estimating of portal pressure is by the measurement of the hepatic venous pressure gradient (HVPG), which is the difference between hepatic venous wedge pressure and free hepatic venous pressure[1]. When the blood flow in the hepatic venous channels is obstructed by a catheter, the proximal static column of blood in the hepatic veins communicates with the hepatic sinusoids reflecting sinusoidal pressure. Normal HVPG is between 1 to 5 mmHg[2]. HVPG  $\geq$  10 mmHg is defined as clinically significant portal hypertension[3]. HVPG  $>$  12 mmHg predisposes to variceal rupture. Non-cirrhotic portal hypertension (NCPH) refers to the conditions where causes other than liver cirrhosis are responsible for portal hypertension. Causes of NCPH are extrahepatic portal vein obstruction (EHPVO), non-cirrhotic portal fibrosis (NCPF) and congenital hepatic fibrosis (CHF). NCPH is different from cirrhosis in various aspects. Unlike cirrhosis, NCPH has normal synthetic functions (hypoalbuminemia, coagulopathy), but mostly presents as variceal bleed and splenomegaly[1,4]. The incidence of decompensation and mortality following a variceal bleed is much lower in NCPH as compared to cirrhosis[5]. NCPH is overall uncommon in the West. Issues in developing countries are unique. This review discusses the endoscopic and outcome perspectives of NCPH in children.

## EHPVO

### Pathophysiological implications

Acute portal vein thrombosis in children is an event that is usually unrecognized and on most occasions, the etiology is unknown. It is perceived that an innocuous insult to the portal vein takes place in infancy or early in childhood. A preceding febrile illness, intra-abdominal infection, or dehydrating illness is usually followed by subtle abdominal pain or transient ascites which may have been forgotten or undetected. In retrospect, a search into the child's past history is often unyielding and perplexing for the physician. Following this event of portal vein thrombosis, the thrombus begins to organize. To bypass the obstruction, multiple hepatopetal collaterals form in 6-20 d to compensate for the high-volume flow from the splanchnic system draining into the liver. A well-established portal cavernoma forms in 3 wk[1,4]. This "temporary adjustment" by the body is however insufficient to decompress the high portal pressures. As a result, varices, hemorrhoids, collaterals, and spontaneous shunts form between the portal and systemic circulation. As evident from the series by Orloff *et al* [6], EHPVO involves portal vein alone in 70%, portal vein and splenic vein in 20%, portal vein and superior mesenteric vein (SMV) in 5%, and all three veins in 10%[6]. A liver biopsy will show mild periportal fibrosis with no signs of hepatocyte injury[7].

### Clinical features

In developed nations, the mean age of presentation is around three years even before the variceal bleed[8]. However, in developing nations, EHPVO predominantly presents as variceal hemorrhage mostly from esophageal varices (77%-84%). The rest present as non-bleeders with isolated splenomegaly (16%-23%)[9-11]. The reason for presentation as variceal bleed in third world countries is due to delay in diagnosis and poor referral systems. The age of presentation is 6.3-9.3 years with a mean number of 1.8-3.1 bleeding episodes *per* child at the time of presentation[11,12]. Antecedent febrile illness and respiratory tract infection (Valsalva maneuver) tends to rupture the varices. Bleeding is worsened by ingestion of non-steroidal anti-inflammatory drugs (*e.g.*, ibuprofen, diclofenac). Long-standing gastroesophageal reflux also predisposes to erosions over the varices. Episodes of variceal bleeding are recurrent and tend to increase in frequency and severity with age. The presence of postural signs (dizziness, syncope, prostration) and hypotension indicates significant blood loss[11]. Clinical examination reveals isolated splenomegaly without any stigmata of chronic liver disease. The liver may be palpable if the patient is in cardiac failure due to anemia (post-bleeding). Splenic size may acutely decrease just after a massive hemorrhage (to compensate for the volume loss) and resume pre-bleeding size soon after blood transfusion.

Massive splenomegaly causes a dragging sensation, left upper quadrant pain, and early satiety[1]. Though hypersplenism is common, symptoms related to the same (symptomatic anemia, spontaneous skin bleeds) are less common in adults and rare in children (5%)[13]. Chronic dragging sensation and apprehensions of rupture of a massive spleen may preclude them from contact sports. Massive bleeding may be accompanied by diuretic-responsive transient transudative ascites in 4%-18% cases[12, 14]. Jaundice is seen in advanced EHPVO due to symptomatic portal cholangiopathy (5%-19%) resulting from obstruction of extrahepatic bile ducts (compression by collaterals or ischemic biliary strictures) but it is extremely rare in children[15-17]. Unscreened blood transfusion in the past may cause chronic hepatitis B or C infection manifesting later with frank liver disease. Growth retardation (stunting and wasting) occurs in up to 33%-54% children[18,19]. Portal colopathy is a complication that presents with bleeding *per* rectum from anorectal varices and mucosal changes in the colon but is less commonly seen in children[20]. Small bowel ectopic varices are rare yet cause a considerable diagnostic dilemma.

### Growth failure and quality of life

Duration and severity of portal hypertension determine the growth of the child. A pediatric series on EHPVO showed that growth retardation (stunting and wasting) occurs in 54% of children[19]. The theories proposed for the same are (1) Malabsorption due to portal enteropathy; (2) Deprivation of hepatotropic factors due to poor portal supply to the liver; (3) Chronic anemia; and (4) Growth hormone resistance as shown by increased levels of growth hormone and decreased levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3. Menon *et al*[21] had observed that after shunt surgery there was an improvement in height velocity in 76% of EHPVO children[21]. The study supported the portal enteropathy hypothesis as a reason for growth retardation. In a prospective study in which adequate nutritional intake was ensured, anthropometry, fasting growth hormone, and insulin-like growth factor 1 were compared between 22 well-nourished patients with EHPVO with growth retardation and 35 age-matched well-nourished controls. Insulin-like growth factor scores were significantly lower in patients ( $-1.48 \pm 0.88$ ) than in controls ( $-0.49 \pm 1.09$ ,  $P < 0.001$ ), whereas basal growth hormone was significantly higher in patients ( $4.60 \pm 3.70$  mIU/L) compared to controls ( $2.66 \pm 0.82$ ,  $P < 0.01$ )[18]. Improvement in growth parameters seen at 12 and 24 mo after meso-Rex bypass, is possibly due to restoration of blood supply to the liver[22].

Poor health-related quality of life (QoL) and school performance is contributed by anemia and various social stigmata. EHPVO children have growth retardation and protuberant abdomen as compared to their peers in school. They also have minimal hepatic encephalopathy causing behavioural issues. QoL scores do not show much improvement on variceal eradication but may improve after shunt surgery[21,23].

### Endoscopic outcome of esophageal varices

The majority of EHPVO patients present as variceal bleed. Unlike cirrhosis, adequately tackling the variceal bleed by endoscopic therapy ensures < 5% mortality. The rate of variceal growth in EHPVO varies among different individuals[9,10]. The 1-year, 3-year, and 5-year probability of development of esophageal varices is 2%, 22%, and 22%

respectively and growth from small to large size is 13%, 40%, and 54% respectively [24]. Endoscopic therapy of the esophageal varices consists of endoscopic variceal ligation (EVL) and endoscopic sclerotherapy (EST). Both are preferred endoscopic therapies for acute variceal bleeding (Figure 1). The eradication rate of esophageal varices with EST is 88%-100%. However, complications like esophageal ulcers (8%-30%) and strictures (6%-20%) are commonly seen with EST[25-27]. Though EVL has the advantages of rapid eradication of varices requiring fewer sessions and lesser incidence of complications, the studies of EVL are limited in children. EST is preferred for smaller children as there is difficulty in inserting the banding cylinder during EVL. Children lesser than 2 years have a physiologically narrow cricopharynx. Smaller band cylinders are compatible with thinner endoscopes but may not generate adequate pressure suction on the esophageal varices for banding. In developing countries, EST is possibly more cost-effective compared to EVL. In a randomized controlled trial of EST *vs* EVL in children by Zargar *et al*[28], the efficacy of controlling bleeding and rate of variceal eradication was similar in both groups (100% in both and 96% *vs* 91.7% respectively), but overall EVL was better as it required lesser number of sessions (3.9 *vs* 6.1), had lower re-bleeding (4% *vs* 26%) and complication rates (4% *vs* 25%)[28]. A study from the authors' center has shown that sequential EVL followed by EST (Group I, *n* = 101) is superior to EST alone (Group II, *n* = 60) in a 3 wkl endoscopy regimen till eradication. Group I required significantly fewer sessions ( $5.2 \pm 1.8$  *vs*  $6.8 \pm 2.8$ ,  $P < 0.005$ ), less sclerosant ( $13 \pm 8.2$  mL *vs*  $30 \pm 20$  mL,  $P < 0.001$ ) and had fewer complications (7% *vs* 28%,  $P < 0.001$ ) as compared with group II[29]. Many pediatric hepatology centers in Asia consider a 3-weekly protocol of sequential downgrading of large esophageal varices by EVL followed by EST injection into the smaller varices till eradication. While EVL rapidly reduces the size of varices, EST effectively blocks the paraesophageal perforators which ultimately lowers the risk of recurrence. This is advantageous as the cumulative dose of sclerosants and risk of complications are much lower in sequential therapy as compared to the EST alone[30]. Long-term sequelae of esophageal dysmotility is a concern with cumulative sclerotherapy.

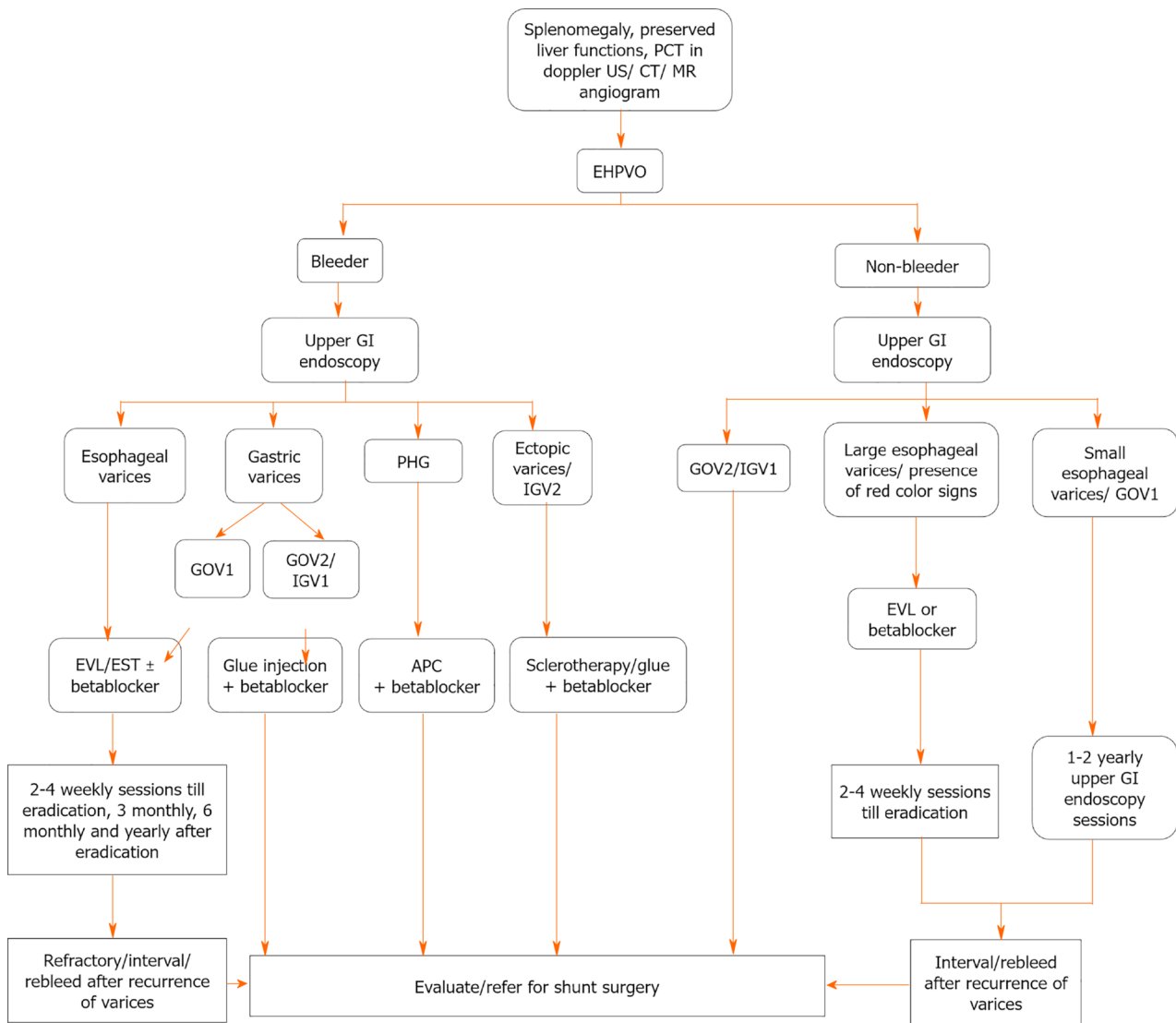
### **Management and outcome of gastric varices**

Gastric varices bleed less frequently but more profusely as compared to esophageal varices[31]. In a study with 274 children with EHPVO, 70% had primary gastric varices at presentation, of which 97% had gastroesophageal varices (GOV) and 3% had isolated gastric varices (IGV)[32]. After esophageal variceal eradication with EST, gastric varices may disappear or persist or develop afresh (secondary gastric varices). Disappearance is seen more often along the lesser curvature of the stomach (GOV1) than the greater curvature (GOV2). In a study from the author's center, GOV1 decreased from 45% to 30% and GOV2 increased from 8% to 13% during esophageal variceal eradication. Secondary gastric varices develop in 28%. Of these, 87% are constituted by isolated gastric varices in the fundus (IGV1) and the rest in the body and antrum (IGV2)[33]. The reduction of GOV1 is attributable to the fact that GOV1 arises from deep submucosal veins from the left gastric vein into which there has been a flow of sclerosant from the esophageal varices. GOV2 varices are formed by the collaterals from the left gastric and short gastric veins. IGV1 is formed exclusively by the short gastric veins. Short gastric veins do not receive any sclerosant as they do not communicate with the esophageal varices. As the esophageal varices and GOV1 shrink during endoscopic therapy, the blood is diverted through IGV1 and GOV2 to accommodate the persistent portal pressure and blood volume in the portal system. Following eradication of esophageal varices, IGV1 incidence increases significantly from 1% to 14% ( $P < 0.001$ ), and the incidence of bleeding from gastric varices increases from 0% to 20%[32]. Acute gastric variceal bleeding is managed by 1-2 mL of glue (N-acetyl-2-butyl-cyanoacrylate) injection[3] (Figure 1). Repeated sessions of glue injection have the risk of glue cast fundal ulcers, obliteration of splenic vein for future portosystemic shunt surgery (PSS), and difficulties in the mobilization of the spleen during surgery. Hence, whenever large fundal varices are noticed, it is better to perform PSS if the anatomy is feasible. Antral varices (IGV2) rarely bleed even after eradication of esophageal varices and hence prophylactic endotherapy is not usually required[31].

### **Management and outcome of portal hypertensive gastropathy**

Frequency, extent, and severity of portal hypertensive gastropathy (PHG) increase after esophageal variceal obliteration by endoscopic therapy. This results from increasing gastric mucosal venous congestion that occurs along with the decreasing collateral blood flow through the varices. In a study from our center, pre-EST PHG was documented in 40% of cases, all were mild. After eradication of esophageal





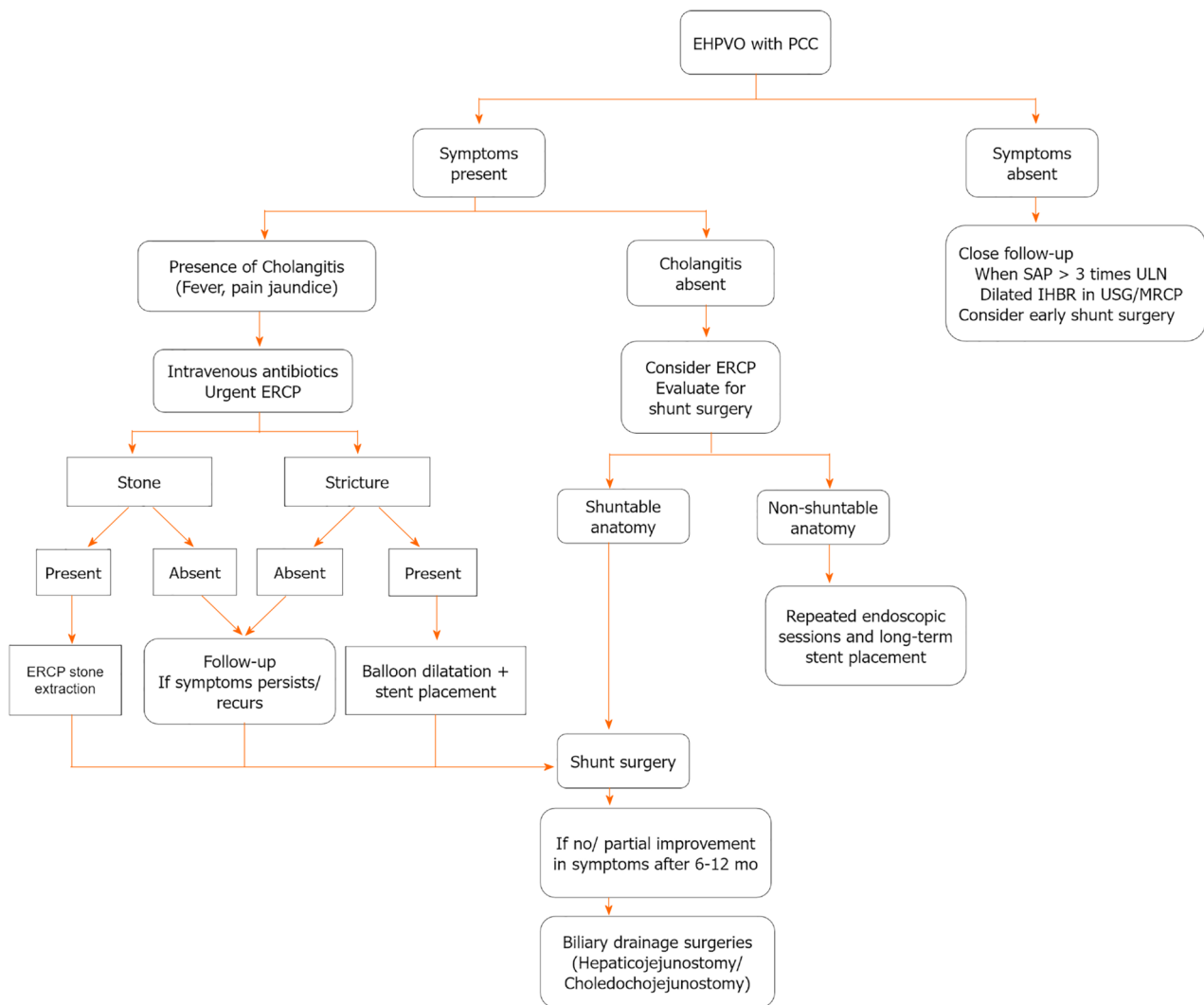
**Figure 1** Algorithm for management of esophageal varices and gastric varices in extra-hepatic portal vein obstruction. EHPVO: Extra-hepatic portal vein obstruction; PHG: Portal hypertensive gastropathy; GOV: Gastroesophageal varices; IGV: Isolated gastric varices; EVL: Endoscopic variceal ligation; EST: Endoscopic sclerotherapy; APC: argon plasma coagulation.

varices, PHG increased to 80%, half were mild and the rest were severe[33]. In another study, the prevalence of mild and severe PHG increased from 25% to 52% and 3.2% to 16% respectively with statistical significance following esophageal variceal eradication. Bleeding from PHG is uncommon in EHPVO children[32]. Repeated sessions of argon plasma coagulation is a promising modality of management for symptomatic gastric antral vascular ectasia.

### **Natural history and outcome of portal cavernoma cholangiopathy**

Portal cavernoma cholangiopathy (PCC) denotes the cholangiographic abnormalities involving both intra-hepatic and extra-hepatic bile ducts including gall bladder wall abnormalities in patients with portal hypertension. It is seen as biliary radical dilatation, filling defects, indentations, angulations, filling defects or a tumor mass (pseudocholangiocarcinoma sign)[34]. They occur due to compression of peri and para choledochal varices. Intracholedochal varices appear as filling defects within the lumen seen on endosonography and choledochoscopy. PCC is most commonly seen in EHPVO (80%-100%) as compared to cirrhosis (0%-33%)[1]. The prevalence of PCC is almost 100% in adults, however, the data is limited in children[15,35,36]. A prospective study conducted in the authors' center in 72 EHPVO children showed the prevalence of PCC as 92% of which 7% were symptomatic. In this study, the age at presentation and the duration of disease in asymptomatic PCC were  $13.9 \pm 2.3$  and  $6.9 \pm 4.0$  years respectively. This was significantly lower than the symptomatic group where age and duration were  $16.1 \pm 0.9$  and  $11.0 \pm 1.4$  years respectively. Age at presentation and

duration of disease had a significant linear correlation[37]. It has been observed symptoms of PCC are more commonly seen in adults as compared to children, implying that the duration of portal hypertension in EHPVO is responsible for progressive bile duct disease to cause symptoms[35]. In a study of adults with symptomatic PCC, the median age of presentation with symptoms of PCC was 41 years[38]. The mean interval between the first presentation with variceal bleed and jaundice was 7.4 years in another adult study[39]. In a study by Llop *et al*[40] in adults, it was shown that the 5-year and 10-year actuarial probability of developing symptomatic PCC after diagnosis of chronic portal vein thrombosis was 9% and 13%, respectively[40]. Zargar *et al*[41] followed 69 EHPVO children for 15 years and 4% developed biliary obstruction[41]. Symptoms arise due to obstruction of bile flow and result in cholestatic jaundice, pruritus, cholangitis, and gall stones. The implication of finding symptomatic PCC in children is grave. This would possibly mean tenacious strictures or stones that would entail multiple therapeutic endoscopies. A series of complications are anticipated. The endoscopic biliary interventions have technical limitations in younger children. Biliary drainage is associated with a risk of hemobilia from rupture of intracholedochal varices. Endoscopic intervention is easier for lower biliary strictures than higher strictures, more so in children. Refractory strictures may necessitate bilio-enteric anastomosis. Long-standing disease results in secondary biliary cirrhosis. In EHPVO, secondary biliary cirrhosis is an unfortunate consequence of a problem where a primary liver disease never existed in the first place. Considering the longevity of a child, QoL in the growing years, and gainful living, it is imperative to actively search for asymptomatic biliary changes with serial imaging. There are two hypotheses for biliary changes in EHPVO, extrinsic compression by portal collaterals and ischemic stricture due to bile duct injury or a combination of both[15,35,42]. In a study by Dhiman *et al*[42], endoscopic retrograde pancreato-cholangiography (ERCP) done in five cases post shunt surgery showed total disappearance of changes in two, partial response in one, and no improvement in two, indicating the relief of compression alone was not the reason for biliary changes[42]. The definitive diagnosis of PCC is by ERCP but due to its invasive nature, magnetic resonance cholangiopancreatography with gadolinium injection to delineate the cavernoma is preferred in children[43]. Symptomatic PCC should be managed but the requirement of management in asymptomatic PCC is doubtful. Also, steps for management of PCC are not clear. Should shunt surgery be offered in all symptomatic PCC followed by bilioenteric anastomosis (hepaticojejunostomy) or should endoscopic drainage be primarily performed before PSS[39,44]? Prior shunt surgery effectively decompresses the cavernoma in 6-12 mo and makes it easier for subsequent biliary drainage surgeries (Figure 2). Second stage hepaticojejunostomy is required in 28%-50% in adult studies following shunt surgery[39,44,45]. Issues with endoscopic biliary drainage are its invasive nature, need for technical expertise, and lack of smaller-sized endoscopes and biliary metallic stents in children. Meso-Rex shunt restores the blood flow to the liver to decompress the cavernoma adequately. In the study by Gauthier-Villars *et al* [46], 2/8 children with symptomatic PCC underwent Rex shunt, and liver biochemistry completely normalized post shunt surgery[46]. Meso-Rex shunt is not possible in most children due to unfavourable vascular anatomy where the left branch of the portal vein or SMV is blocked. Meso-Rex bypass is also ineffective if there is a large spleen at the time of presentation. In the study from the authors' center, 25 children with EHPVO underwent central end to side splenorenal shunt. Despite the patency of shunt 18 mo post-surgery, asymptomatic PCC did not improve in the majority. All the children who had progressive PCC after shunt surgery had concomitant SMV block. SMV block not only makes meso-Rex shunt non-feasible but also causes severe PCC[47]. The venous plexuses on the common bile duct drain into the portal vein and SMV territories. When the portal vein is occluded, the choked peribiliary collaterals compress upon the bile duct. In such a scenario, SMV is the only pathway for decompression. When the SMV is occluded too, the choking effect of the biliary venous plexuses is near total. Peribiliary collaterals enlarge further and compress the already narrowed common bile duct. A central end to side PSS does not effectively relieve the peribiliary portal hypertension since the connection is between the splenic and left renal vein. Future studies are required to address whether PSS is required in an asymptomatic PCC in children to prevent the burden of complicated PCC and the development of SMV block as they enter adulthood. The management of PCC poses great dilemmas in children. Issues such as choice of shunt surgery, adequate decompression of biliary varices, the appropriate time for bilioenteric anastomosis, and prophylactic biliary dilatation for strictures are well debated. Despite active screening for PCC in all children, we must understand that symptoms arise as a result of procrastination in treating asymptomatic PCC. Symptomatic PCC definitely



**Figure 2 Algorithmic approach for management of portal cavernoma cholangiopathy in extra-hepatic portal vein obstruction.** EHPVO: Extra-hepatic portal vein obstruction; PCC: Portal cavernoma cholangiopathy; ERCP: Endoscopic retrograde pancreato-cholangiography; MRCP: Magnetic resonance cholangiopancreatography; SAP: serum alkaline phosphatase; IHBR: intrahepatic biliary radicle; USG: ultrasonography; ULN: upper limit of normal.

requires biliary drainage but the requirement of biliary decompression in asymptomatic PCC is a dilemma not only in children but also in adults. After detecting PCC, the logical step forward has to be decompression of portal hypertension with PSS. In those with symptomatic PCC, endoscopic therapy may be required before the shunt surgery. Endoscopic therapy is reserved for selected cases of cholangitis and choledocholithiasis. Limitations such as lack of appropriate sized endoscopes and biliary metallic stents (not approved yet) are unique issues in children. The experience of meso-Rex bypass for relieving PCC is limited. Non-selective shunt surgeries may not have a wholesome outcome in PCC.

### Natural history and management of portal colopathy

Portal colopathy is most commonly seen with EHPVO as compared to cirrhosis probably due to selective redistribution of portal pressure with time along the inferior mesenteric vein consequent to thrombosis at the junction of the splenic vein and SMV [48,49]. Similar to PCC, the prevalence of portal colopathy is lower in children compared to adults emphasizing the importance of the duration of portal hypertension. Unlike PCC, PSS effectively reverses colopathy. Portal colopathy is defined as the presence of colitis-like abnormalities (edema, erythema, ulcers), vascular lesions (cherry-red spots, ectasia, and spider angiomas) with or without the presence of colorectal varices (3-5 mm) by endoscopy and/or endosonography. Rectal endosonography is superior to sigmoidoscopy for identifying rectal varices [20,50]. Prevalence of rectal varices in adults is 63%-94% [20,49,51]. In a study from the authors' center, rectal varices were seen in 36% of 25 EHPVO children by sigmoidoscopy and 76% by rectal endosonography [50]. Rectal varices occur in 80%-90% of adults with

EHPVO but the overt bleeding frequency is low (3%-8%). In another study from our center, only 16.6% of EHPVO were symptomatic for colopathy/rectal varices. 94% showed rectal varices and 75% showed colitis-like changes on routine colonoscopy. Colopathy and colitis-like lesions were more common than vascular lesions (36/40 *vs* 23/40;  $P = 0.001$ ). Colopathy changes were pancolonic in 52.5%, left-sided in 42.5%, and right-sided in 5% cases. 16% also had ileal changes. Children with colopathy had more often (90% *vs* 57%;  $P = 0.01$ ) PHG, more endotherapy sessions (6[4-8] *vs* 2[1-4];  $P = 0.03$ ), and less often large esophageal varices (12.5% *vs* 43%;  $P = 0.02$ ) than those without colopathy[52]. Mucosal changes like erythema, friability, and superficial ulcerations should not make the endoscopist suspect inflammatory bowel diseases, especially in the setting of portal hypertension as the shunt surgery effectively reverses the colitis like changes in these cases[53]. Bleeding rectal varices can be managed with sclerotherapy or band ligation[20]. PSS is preferred for large rectal varices and symptomatic colopathy. When PSS is anatomically not feasible, beta-blockers should be considered. Laser photocoagulation and Argon plasma coagulation are tried in adults in severe cases, but the studies in children are limited[54].

### **Rare complications in EHPVO**

Minimal hepatic encephalopathy (MHE) in EHPVO without shunt surgery has been observed in 32% of cases using neuropsychological testing and 57% by critical flicker frequency techniques[55,56]. EHPVO is an example of type B hepatic encephalopathy where there is a portosystemic bypass in the absence of intrinsic liver disease. The other reasons attributed are chronic deprivation in hepatic blood flow leading to parenchymal extinction, increased brain glutamine, and increased proinflammatory cytokines[57]. Following shunt surgery, as the toxic substances bypass the liver into the systemic circulation, MHE is more prevalent in non-selective shunts as compared to selective shunts. The reversal of MHE following shunt surgery in EHPVO is not well established.

Ascites is an uncommon complication of EHPVO. In a study from the authors' center, 307 EHPVO children were analyzed, of which 26% developed ascites. 84% of ascites were following variceal bleeding. Younger age of onset, baseline malnutrition, hypoproteinemia are predictors of post-bleed ascites. The time interval between the first bout of bleed to the onset of ascites and hospital admission was 7 (3-20) and 12 (5-45) d respectively. 17% of patients had features of ascitic fluid infection requiring antibiotics. For the resolution of ascites, 32% required only salt restriction, 39% required the addition of diuretics, and 29% required single-time large-volume paracentesis. The overall resolution of ascites was seen in 46%, 76%, 88%, and 100% by days 7, 14, 30, and 60 respectively. In this study, 17 patients re-bled, of which 11 had a recurrence of post-bleeding ascites. None of the patients had any evidence of chronic liver disease on follow-up of 56 (9-112) mo[58]. The mechanism of de novo ascites is not well understood. Secondary causes and hepatic dysfunction are possible responsible factors. Rangari *et al*[14] analyzed 9 chronic EHPVO adults with ascites who had not bled in the last 3 mo. These patients had raised alanine transaminase, hypoalbuminemia, and deranged coagulation. Ascites in this study were attributable to increased age, longer duration of disease, and PCC. They postulated that the underlying liver dysfunction was caused due to a reduced parenchymal liver mass [14].

Hepato-pulmonary syndrome (HPS), though common in advanced cirrhosis, is rarely seen in EHPVO also. The prevalence of HPS in EHPVO is 2%-10%[59,60]. The incidence of HPS in EHPVO shows that apart from hepatic dysfunction, portal hypertension *per se* is responsible for HPS. Hepatic dysfunction is not unseen in EHPVO. It occurs more in the older age due to parenchymal extinction and is more seen with prolonged portal hypertension. PCC is commonly associated with hepatic dysfunction[14]. Portal hypertensive enteropathy is not unusual in children, seen in both cirrhotics and non-cirrhotics. In the study from the authors' center, children with EHPVO showed features of enteropathy as evident by duodenal morphometric features (60%). The features were lower villous to crypt ratio, dilated capillaries, increased thickness of muscularis mucosae and increased small intestinal permeability (lactulose excretion test) as compared to healthy controls[61]. Portal hypertensive enteropathy is one of the most important causes of growth failure in children.

### **Outcome of shunt surgery**

Endotherapy significantly improved mortality due to variceal bleeding as compared to the pre-endoscopic era. Endotherapy (EVL/EST) causes eradication of esophageal varices in 90%-95% EHPVO cases[62]. As endotherapy obliterates portosystemic collaterals in the esophageal region, the persistently elevated portal pressure causes

rebleed in 7%-41% of cases following endotherapy[33,41,62,63]. There is also a significant risk of developing other complications related to high portal pressures such as ectopic varices, gastropathy, colopathy, cholangiopathy, growth failure, and hypersplenism. A randomized trial comparing endotherapy and shunt surgery showed the risk of rebleeding is significantly higher in the endotherapy group[64]. The study by Krishna *et al*[23] showed the QoL remained poor even after variceal eradication on endotherapy due to various reasons like growth retardation, cholangiopathy, ectopic varices, massive spleen related pain, early satiety, and infarction[23].

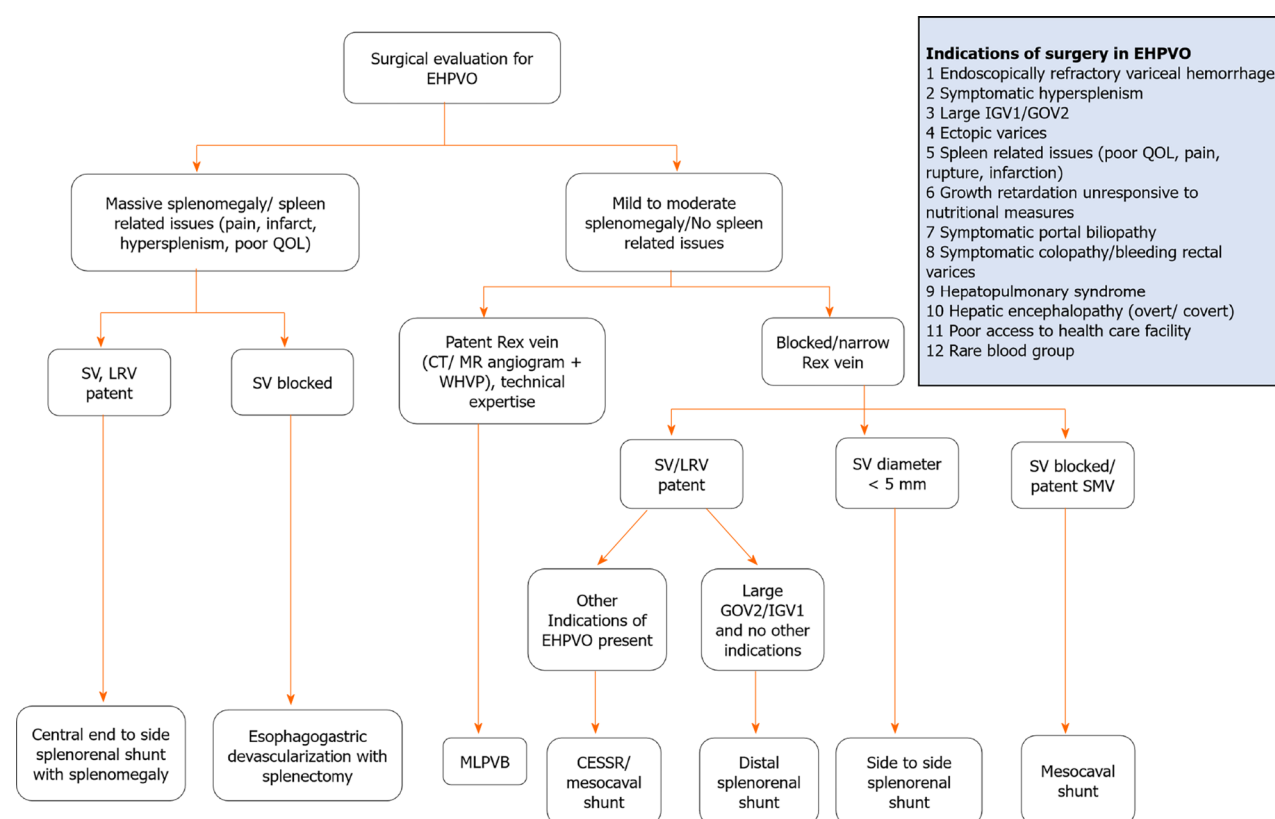
Shunt surgery is indicated in EHPVO whenever feasible. However, there are various approaches in the surgical management of EHPVO (Figure 3). Baveno VI guidelines recommend that meso-Rex Bypass should be offered for primary, pre-primary, and secondary prophylaxis for all cases of EHPVO[3]. However various factors preclude meso-Rex bypass in all children with EHPVO such as anatomic non-feasibility, the need for technical expertise. Another feasible intervention is PSS, a procedure ideally performed after tackling the first episode of variceal bleed endoscopically. However, in developing nations, the bulk of the disease outweighs the number of centers that have expertise in conventional and physiological shunt surgeries. PSS is a popular shunt surgery for EHPVO as it not alone prevents rebleeding but also improves other complications like colopathy, cholangiopathy, growth, QoL, *etc.* PSS consists of selective (distal splenorenal shunt) and non-selective shunt (proximal or central-end to side splenorenal shunt with splenomegaly, side to side splenorenal shunt, and mesocaval shunt)[65,66]. Each of the above-mentioned PSS has its own merits and demerits and hence, the choice of surgical procedure is tailored as *per* the indication for surgery and the anatomy of the splenoportal axis (the patency and diameter of the veins)[67,68]. Broadly, if massive splenomegaly affects QoL adversely, then splenectomy with a central end-to-side splenorenal shunt is indicated. Splenectomy is required when issues related to massive splenomegaly and significant hypersplenism predominate. However, a spleen-preserving shunt is preferred if splenomegaly is not of concern. Side-to-side splenorenal shunts permit a large diameter vascular anastomosis if the splenic vein is of a small diameter (< 5 mm) calibre[68]. The mortality following PSS is 0%-1.9%. Shunt thrombosis occurs in 2.5%-13% following PSS[6,67,69-71]. On a few occasions, other surgical interventions may be required for selected indications when PSS is not feasible due to non-shuntable anatomy. Hepaticojejunostomy is required in symptomatic portal biliopathy, especially related to ischemic strictures. Emergency devascularization procedure is required when endotherapy fails to control acute variceal bleed, interval bleed, or recurrence of bleed following eradication. In the author's center, 110 children underwent surgical intervention for delayed sequelae post-variceal eradication. PSS was performed in 83% whereas esophagogastric devascularization was performed in 17%. 91% showed shunt patency after a median follow-up duration of 28 mo following shunt surgery. Growth parameters, colopathy, issues related to splenomegaly improved in all[72].

Meso-Rex bypass requires placement of autologous vein graft between SMV and left branch of the portal vein and it is an ideal curative procedure conceptually. However, there are various limitations of the meso-Rex bypass. Complete patency of intrahepatic portal veins, including the recess of Rex, is required for performing this procedure. In a pediatric EHPVO study, 62% had favourable anatomy before surgery, and eventually, only 37% culminated into a successful meso-Rex bypass[73]. Wedge hepatic venous portography is the gold standard for imaging of intrahepatic portal veins. 15% of all successful meso-Rex bypass need interventional radiological procedures like thrombectomy, shunt dilatation, or stenting to maintain shunt patency [74]. The shunt blockage following meso-Rex bypass is 4%-19%[74-77]. Meso-Rex bypass is not the procedure of choice when there is gross splenomegaly and hypersplenism.

### Issues in developing countries

In the author's understanding, the issues in developing nations are uniquely different from those in developed countries. Due to poor referral systems, the patients are referred to tertiary care centers in an advanced state where one or more of the above complications would have ensued. Meso-Rex bypass is favourable in the early stages where the left branch of portal vein and confluence are patent. In advanced disease, the anatomy is no longer favourable as the entire portal vein and its branches are affected by stasis and progressive local thrombosis. 64% of EHPVO children also have additional thrombosis of SMV or splenic vein which limits the choice of PSS[37]. This possibly occurs at onset or due to local progression of thrombosis at the trijunction confluence. Proximal splenorenal shunt lowers portal pressure but does not ameliorate





**Figure 3** Indications of surgery in extra-hepatic portal vein obstruction and algorithmic approach for surgical management in extra-hepatic portal vein obstruction in developing countries. EHPVO: Extra-hepatic portal vein obstruction; GOV: Gastroesophageal varices; IGV: Isolated gastric varices; EVL: Endoscopic variceal ligation; SV: splenic vein; SMV: superior mesenteric vein; EST: Endoscopic sclerotherapy; CT: Computed tomography, LRV: left renal vein; MR: magnetic resonance. CESSR: central end-to-side splenorenal shunt, QOL: quality of life; WHVP: wedge hepatic venous pressure; MLPVB: mesenterico left portal vein bypass (meso-Rex).

the PCC. Distal splenorenal shunt and meso-Rex bypass do not ameliorate issues of a large spleen. Those with entire splenoportal axis thrombosis are subjected to esophagogastric devascularization which diverts the blood away from the life-threatening variceal territory but fails to lower the portal pressure. Hence the long-term choice of definitive therapy is that of a compromised one. Keeping in mind the logistic issues in developing countries, it is the authors' opinion that repeated endoscopic sessions should be performed till variceal eradication and an opportune time must be sought for a PSS if the disease is in an advanced state or if a meso-Rex bypass is not feasible. PSS has low post-operative mortality and good long-term shunt patency. Despite the compromise, PSS may be the only available option for amelioration of the disease.

## NCPF

NCPF is also called idiopathic portal hypertension, hepatoportal sclerosis or obliterative venopathy. This is a disorder of no specified etiology characterized by massive splenomegaly, preserved liver function, and patent portal vein[1].

### Pathophysiological implications

Etiopathogenesis of NCPF is not well understood and there are various theories for the same. Infections (*Escherichia coli*), prothrombotic states, immunological disorders, toxins (arsenic), and genetic factors are possible causative factors[75-78]. Human immunodeficiency virus and hepatic schistosomiasis also are responsible for liver fibrosis similar to NCPF[79-83]. Various theories explain the pathogenesis of NCPF though, none of the theories have been effectively proven. The unifying hypothesis suggested by Sarin and Kumar[84], suggests a major thrombotic event in a younger age is responsible for EHPVO but, a micro thrombotic event later in life is responsible for the obliteration of small and medium branches of portal veins resulting in NCPF

[84]. Schouten *et al* [85] proposed a dual theory of splenic vein dilatation (due to high levels of nitric oxide synthase in splenic endothelial cells) and intrahepatic portal vein obliteration as the main pathogenesis in the development of NCPF [85]. Sato and Nakanuma [86], suggested endothelial-mesenchymal transformation theory, according to which endothelial cells in portal vein branches acquire features of myofibroblast due to stress and ischemia thereby causing deposition of collagen in vessel walls causing obliteration [86]. The histological hallmark of NCPF is oblitative portal venopathy. Other prominent features include aberrant vessels in the portal tract (portal angiomatosis), portal tract fibrosis and inflammation, and absence of significant hepatocellular injury. Incomplete nodules and scattered regenerative nodules are seen on a few occasions [87].

### **Clinical features**

The incidence of idiopathic portal hypertension has reduced in Japan in the past two decades. Though no national registries are available, the incidence in India also seems to have decreased along with EHPVO [87]. The change in the scenario could be due to the reduction in the incidence of umbilical sepsis, reduced diarrheal episodes in infancy due to better sanitation and vaccination programs [88,89]. Studies from India show that NCPF accounts for 3.3%-4.6% of all pediatric portal hypertension [90,91]. NCPF is commonly seen in the third to fourth decade in adults. Various pediatric series suggest that NCPF is not an uncommon entity in children [86]. Variceal bleeding is the most common presentation in adults (72%) with a relatively small proportion presenting as a lump in the left upper quadrant (12%) [92,93]. The scenario is different in children. In the author's experience, the median age at presentation of NCPF was 14.5 (6-18) years where 49% and 47% presented as variceal bleed and unbled isolated splenomegaly respectively [90]. Another pediatric series from India showed that only 16% presented as variceal bleed and the remaining 84% presented with isolated splenomegaly. Predominant presentation of variceal bleeding in adults is possibly due to a progressive increase in disease severity as age progresses [91]. However, the overall natural history in adults is not different from pediatric series. 87% of NCPF in the authors' study had hypersplenism with median spleen size 10.5 (1-17.5) cm on examination. Transient ascites and hypoalbuminemia were seen in 20% and 11% patients respectively, mostly after variceal bleeding. A small proportion of patients develop end-stage liver disease requiring liver transplantation [90].

### **Endoscopic outcome**

The analysis of the NCPF cohort in the authors' experience showed the predominant presence of esophageal varices (96%) and portal hypertensive gastropathy (89%) followed by primary gastric varices (56%) at presentation. The majority of the children showed eradication of esophageal varices and GOV1 after 5 (2-12) sessions. 36% showed recurrence of esophageal varices in about 1 year of follow-up and 12% developed secondary gastric varices (GOV2 and IGV1). Most of the PHG was mild in severity and PHG was significantly higher in bleeders as compared to non-bleeders probably due to higher portal pressures [90] (Figure 4). Prevalence of esophageal varices in adult NCPF is similar in children (85%-95%) but the gastric varices at presentation were more common in adults compared to children [91]. In a study by Chawla *et al* [94], endoscopic sessions in 72 adult NCPF patients showed eradication after a mean of 5.7 sessions of EST, and recurrence of varices occur in 9.2% over a follow-up period of 21 mo [94]. Sarin *et al* [95] compared adults with cirrhosis with NCPF, and EHPVO. Cirrhotics had a similar recurrence of variceal bleeding as compared to NCPF. Unlike cirrhosis, none of the EHPVO or NCPF died at follow-up suggesting that despite the progression of portal hypertension in NCPF, the liver parenchyma is preserved like in EHPVO [95].

### **Natural history and surgical outcome**

Pediatric data on long-term follow-up studies are lacking (Table 1). Overall survival of NCPF is favourable. Poor outcomes like death, decompensation, and requirement of surgery were seen in 24% of patients [90]. Adult series by Siramolpiwat *et al* [96] reported native liver survival of 72% at 5 years [96]. Similarly, the Spanish cohort of adults reported 86% native liver survival at 5 years [97]. In a French follow-up study, 46% of patients develop portal vein thrombosis during a follow-up period of 7.6 years [98]. Thus, the development of portal vein thrombosis is a major factor that also contributes to the progression of portal hypertension in NCPF. There is a paucity of published data on surgical management of NCPF both in children and adults. As most of the patients have predominant spleen-related issues, a non-selective PSS like central

**Table 1 Clinical characteristics and outcome of non-cirrhotic portal fibrosis in pediatric studies**

Parameters	Prasad <i>et al</i> [90] (n = 45)	Sood <i>et al</i> [91] (n = 19)	Poddar <i>et al</i> [109] (n = 11)	Franchi-Abella <i>et al</i> [110] (n = 48)
Mean or median (range) age at presentation	14.5 (6-18) yr	13.8 (5.9-17.6) yr	11 (5-14) yr	8.75 (1 mo-16 yr)
<b>At presentation</b>				
Variceal bleed	49%	15.70%	54.60%	18.80%
Lump upper abdomen	47%	84.20%	45.40%	43.80%
Ascites	20%	-	18%	-
Spleen size (mean) cm	10.5	12 (4.75-17.25)	8	-
Variceal recurrence	39%	-	18%	-
<b>Poor outcome</b>				
Decompensation	4%	0	0	12.50%
Hepatopulmonary syndrome	2%	5%	-	4.20%
Follow-up duration (mean)	48 (3-120) mo	18 (2-51) mo	57.5 (12-78) mo	15 (1-26) yr
Survival without transplant	93%	100%	100%	88%

end to side splenorenal shunt with splenectomy would be a favourable compromise. Long-term complications of shunt surgery include hepatic encephalopathy, glomerulonephritis, hepatopulmonary syndrome, and ascites[99]. In the authors' experience, 10% require a central end to side splenorenal shunt with splenectomy[90].

## CHF

CHF is a liver ciliopathy disorder of irregularly shaped proliferating bile ducts and periportal fibrosis. CHF is one of the fibropolycystic diseases, that include Caroli disease/syndrome, autosomal dominant polycystic kidney disease (ADPKD), an autosomal recessive polycystic kidney disease (ARPKD)[100].

### Pathophysiological implications

CHF and related disorders occur as a result of ductal plate malformation (DPM). The ductal plate is the embryonic precursor of intrahepatic bile ducts and it surrounds the portal vein. Remodelling of ductal plate starts at 12 wk of gestation and completes at 20 wk. Defect in the remodelling causes persistence of immature embryonic duct structures called DPM. The persistence of immature ductal elements activates hepatic stellate cells by transforming growth factor-beta secreted by Kupffer cells. The activated stellate cells stimulate the formation of fibrous tissue in the portal tract which is ultimately responsible for recurrent cholangitis and portal hypertension. As embryologically, bile duct development and hepatic vasculature have been closely related, DPM is commonly associated with 'pollard willow' malformation of the portal vein, which predisposes the portal vein to undergo thrombosis and cavernomas transformation[101]. Osteopontin gene mutation and microRNA (miR15a) have also been postulated in the pathogenesis of CHF[102,103].

### Clinical features

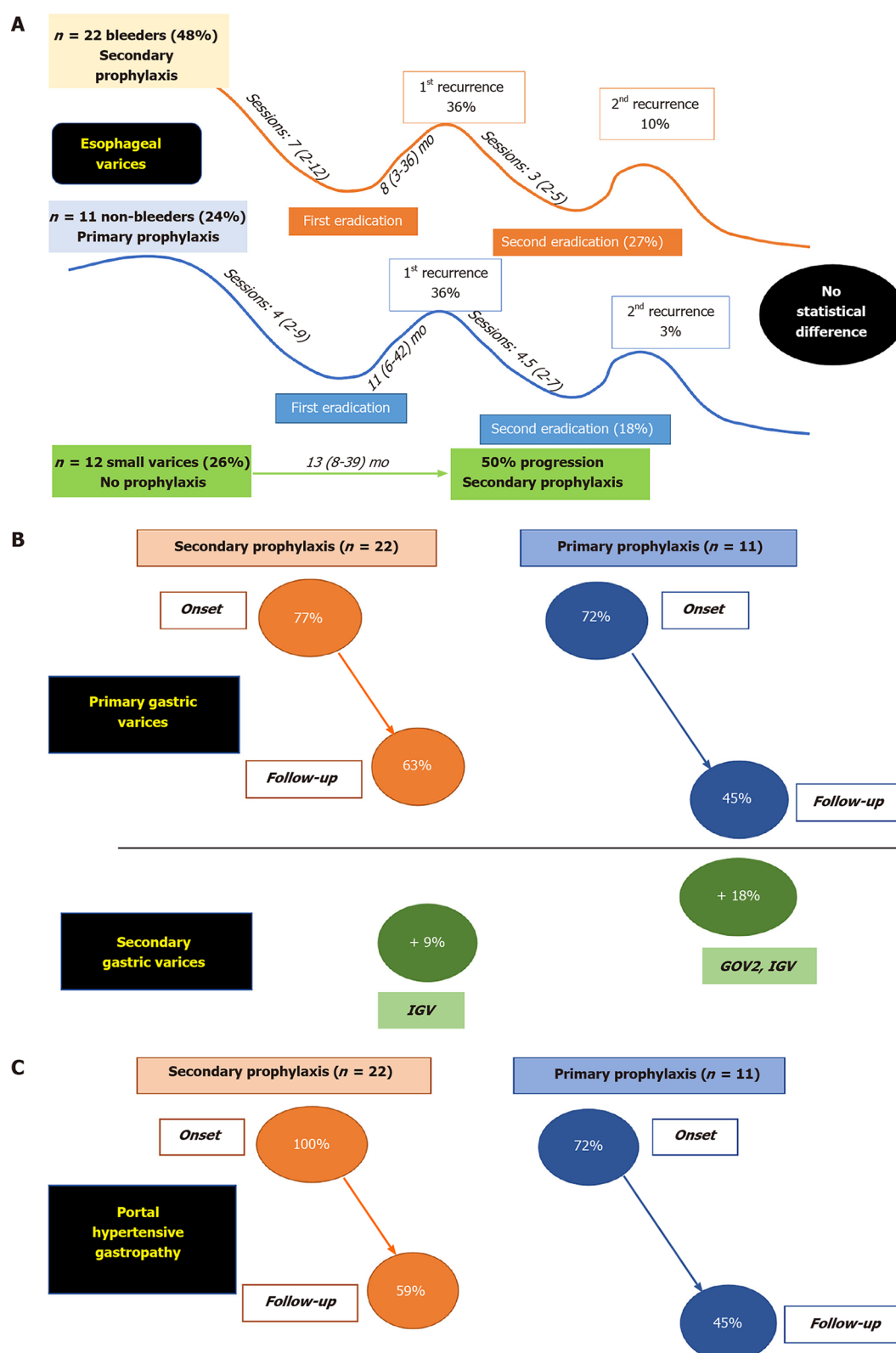
The age of presentation widely varies with CHF diagnosed as early as infancy to late adulthood. A large systematic review of CHF patients showed the mean age of presentation as 11 years[104]. Four forms of CHF have been identified based on the clinical features, most common being portal hypertension followed by cholangitic, mixed, and latent forms. The associations of CHF also widely vary with renal diseases (ARPKD, ADPKD, Jeune syndrome, juvenile nephronophthisis, dysplastic kidney) and Caroli's disease/syndrome commonly seen. However, a few cases of CHF present without any association. Most patients present with features of portal hypertension. Physical examination usually shows firm to hard hepatomegaly with predominant left lobe enlargement, splenomegaly and occasionally nephromegaly. Laboratory workup reveals elevated alkaline phosphatase, gamma-glutamyl transpeptidase, and

**Table 2 Clinical characteristics and outcome of congenital hepatic fibrosis in pediatric studies**

Parameters	Rawat <i>et al</i> [106] (n = 40)	Poddar <i>et al</i> [105] (n = 15)	Parkash <i>et al</i> [111] (n = 25)	Luoto <i>et al</i> [112] (n = 27)
Mean or median age	1.3 yr	8 yr (10 mo-14 yr)	8.5 ± 2.7 yr	2.7 (0-13) yr
<b>Associations</b>				
Caroli's syndrome	52.50%	9%	8%	11%
Renal	92.50%	81.80%	24%	100%
CHF	47.50%	54.50%	92%	37%
<b>Presentations</b>				
Variceal bleeding	27%	54.50%	60%	15%
Cholangitis	25%	9%	0%	7.40%
Recurrent cholangitis	7.50%	9%	0%	3.70%
Decompensation	5%	18.20%	0%	19%
Endotherapy	27%	100%	60%	30%
Shunt surgery	0%	9%	20%	3.70%
<b>Transplant</b>				
Renal transplant	0%	-	-	41%
Liver transplant	5%	-	-	3.70%
Combined liver kidney transplant	45%	-	-	37%
<b>Survival</b>				
Overall survival	90%	100% [41 (1-80) mo]	100%	70% [10.6 (0.6-40) yr]
Survival post-transplant (follow-up duration)	80% [5 (1.2-9)] yr	-	-	73.30%
Survival non-transplant (follow-up duration)	100% [15 (4.5-19)] yr	-	-	100%

CHF: Congenital hepatic fibrosis.

cytopenias. Abnormal renal functions are present in those with significant renal disease[100,105]. There are various syndromes associated with CHF like Caroli's syndrome (intrahepatic bile duct cysts with CHF), Joubert syndrome (cerebellar vermis, retinitis pigmentosa, nystagmus, ataxia), Senior-Loken syndrome (cerebellar ataxia, skeletal abnormalities, nephronophthisis, retinal dystrophy, sensorineural hearing loss), COACH syndrome (cerebellar vermis hypo/aplasia, oligophrenia, ataxia, coloboma, polydactyly), Meckel syndrome (microcephaly, renal cystic disease, hypoplastic or ambiguous genitalia, polydactyly, congenital heart defect, cleft palate, ocular defects) and Bardet- Biedl syndrome (rod-cone dystrophy, postaxial polydactyly, congenital heart defect, cleft palate, mental retardation, hypogonadism). Table 2 describes a few series of pediatric CHF. In a systematic review of 1230 patients, 64% had associated ARPKD, 26% had Caroli's syndrome and 9.5% had isolated CHF. 71% had presented with features of portal hypertension (hepatosplenomegaly, variceal bleeding) however, only a small proportion presented with ascites, hepatopulmonary syndrome, and encephalopathy (< 5%). Features of portal hypertension are commonly seen with ARPKD. Cholangitis is seen in 12% which is commonly seen in Caroli's syndrome[104]. A study from the west (median age at presentation-1.3 years) showed 35% had a neonatal presentation and 78% had associated Caroli's syndrome. Features of portal hypertension are seen in 86% and cholangitis in 25%[106]. Another study from India also showed features of portal hypertension as predominant presentation [105]. In the author's experience (unpublished data) of 33 children, almost 69% presented with features of portal hypertension, and 11% presented with cholangitis. Only 10% developed ascites during follow-up.

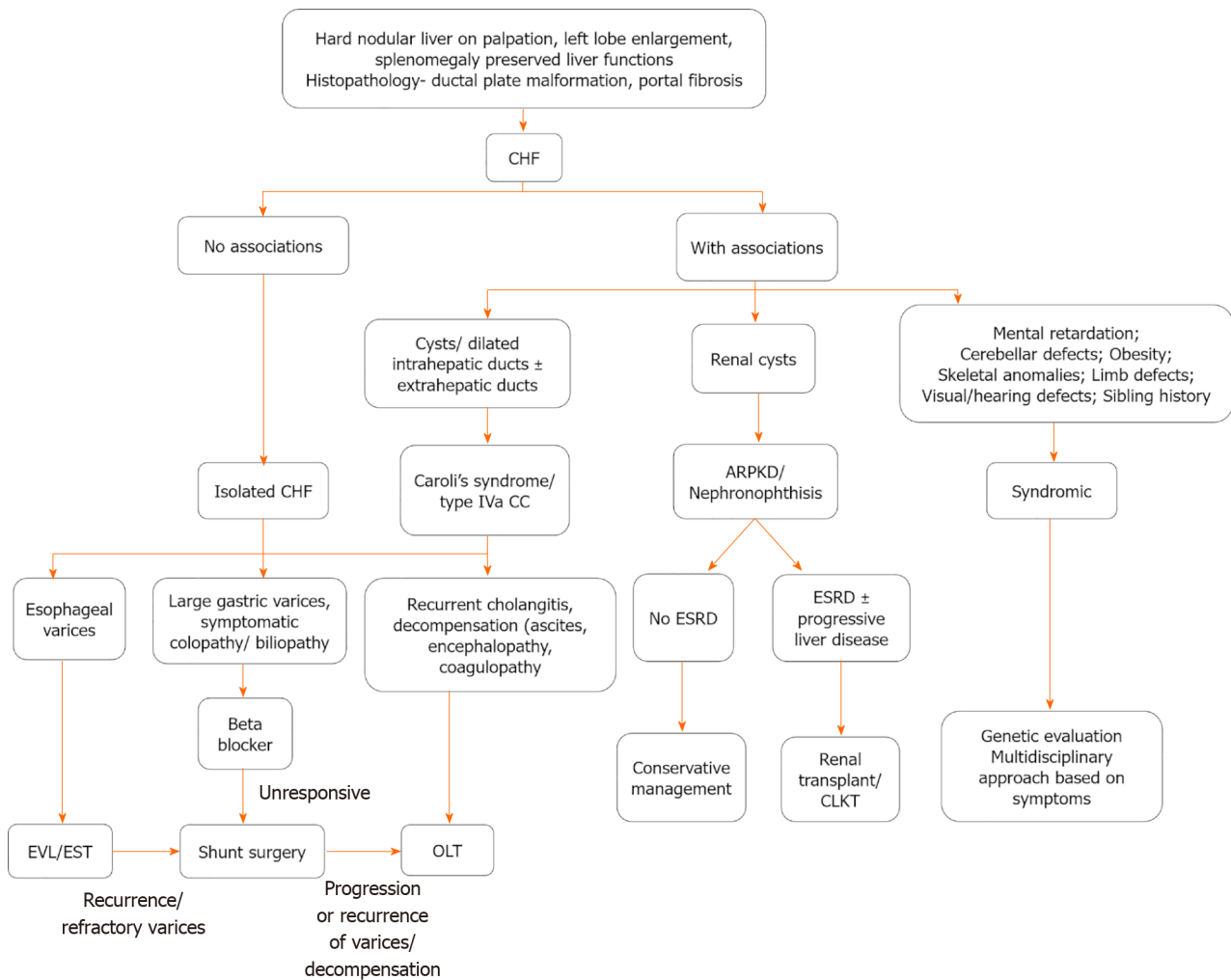


**Figure 4** Natural history and follow-up outcome of esophageal varices, gastric varices and portal hypertensive gastropathy in pediatric non-cirrhotic portal fibrosis in author's experience. A: 22 children presented with variceal bleeding (bleeders), required Endoscopic Variceal Ligation and/or sclerotherapy. 11 children presented with large varices without bleeding (non-bleeders). The incidence of recurrence of varices following eradication is not statistically different between bleeders and non-bleeders. 50% small varices progressed to bleed in the follow-up (median time 13 mo); B: 77% bleeders and 72% non-bleeders had gastric varices (primary) at initial endoscopy. 9% bleeders and 18% non-bleeders develop gastric varices (secondary) in follow-up; C: 100% bleeders and 72% non-bleeders had portal hypertensive gastropathy at initial endoscopy, reduced to 59% and 45% respectively, in follow-up.

### Natural history and outcome

An algorithm for the diagnostic approach and management of CHF is given in Figure 5. Cholangiocarcinoma is seen in 2.5%-16% of Caroli's syndrome but it is less





**Figure 5 Algorithmic approach to diagnosis and management of congenital hepatic fibrosis.** CHF: Congenital hepatic fibrosis; CC: Choledochal cyst; ESRD: End-stage renal disease; EVL: Endoscopic variceal ligation; EST: Endoscopic sclerotherapy; CLKT: Combined liver-kidney transplantation; OLT: Orthotopic liver transplantation, ARPKD: autosomal recessive polycystic kidney disease.

common with isolated CHF[100,107,108]. In the systematic review, 1.5% developed cholangiocarcinoma during median duration of follow-up 7.5 (0-38) years in adults, predominantly in patients with Caroli's syndrome. The incidence of cholangiocarcinoma is extremely uncommon in children. 23% required transplantation (liver, kidney, and combined liver and kidney). Most of the isolated renal transplantation had ARPKD and the majority of the isolated liver transplantation had Caroli's syndrome. 6% died during follow-up most commonly due to sepsis (post-transplant cholangitis) and complications related to cholangiocarcinoma. 2.7% of patients required shunt surgery of which approximately three-quarters showed improvement. A small proportion had shunt block and post-shunt encephalopathy[104]. In another pediatric study, all children with neonatal presentation required renal transplant before the second decade due to underlying ARPKD. In comparison only 23% of those presenting later require required liver/kidney transplantation[106].

## CONCLUSION

In developing countries, NCPH is fraught with challenges of advanced presentation and associated complications related to portal hypertension. Though the management of variceal bleeding is taken care of by endoscopic measures, definitive therapy is often compromised. In a small subset of patients, the disease progresses to end-stage liver disease.

## REFERENCES

- 1 **Khanna R**, Sarin SK. Non-cirrhotic portal hypertension - diagnosis and management. *J Hepatol* 2014; **60**: 421-441 [PMID: [23978714](#) DOI: [10.1016/j.jhep.2013.08.013](#)]
- 2 **Kumar A**, Sharma P, Sarin SK. Hepatic venous pressure gradient measurement: time to learn! *Indian J Gastroenterol* 2008; **27**: 74-80 [PMID: [18695309](#)]
- 3 **de Franchis R**; Baveno VI Faculty. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: [26047908](#) DOI: [10.1016/j.jhep.2015.05.022](#)]
- 4 **Garcia-Pagan JC**, Hernández-Guerra M, Bosch J. Extrahepatic portal vein thrombosis. *Semin Liver Dis* 2008; **28**: 282-292 [PMID: [18814081](#) DOI: [10.1055/s-0028-1085096](#)]
- 5 **D'Amico G**, Pasta L, Morabito A, D'Amico M, Caltagirone M, Malizia G, Tinè F, Giannuoli G, Traina M, Vizzini G, Politi F, Luca A, Virdone R, Licata A, Pagliaro L. Competing risks and prognostic stages of cirrhosis: a 25-year inception cohort study of 494 patients. *Aliment Pharmacol Ther* 2014; **39**: 1180-1193 [PMID: [24654740](#) DOI: [10.1111/apt.12721](#)]
- 6 **Orloff MJ**, Orloff MS, Girard B, Orloff SL. Bleeding esophagogastric varices from extrahepatic portal hypertension: 40 years' experience with portal-systemic shunt. *J Am Coll Surg* 2002; **194**: 717-28; discussion 728 [PMID: [12081062](#) DOI: [10.1016/s1072-7515\(02\)01170-5](#)]
- 7 **Sarin SK**, Agarwal SR. Extrahepatic portal vein obstruction. *Semin Liver Dis* 2002; **22**: 43-58 [PMID: [11928078](#) DOI: [10.1055/s-2002-23206](#)]
- 8 **Abd El-Hamid N**, Taylor RM, Marinello D, Mufti GJ, Patel R, Mieli-Vergani G, Davenport M, Dhawan A. Aetiology and management of extrahepatic portal vein obstruction in children: King's College Hospital experience. *J Pediatr Gastroenterol Nutr* 2008; **47**: 630-634 [PMID: [18955865](#) DOI: [10.1097/MPG.0b013e31817b6eea](#)]
- 9 **Arora NK**, Lodha R, Gulati S, Gupta AK, Mathur P, Joshi MS, Arora N, Mitra DK. Portal hypertension in north Indian children. *Indian J Pediatr* 1998; **65**: 585-591 [PMID: [10773909](#) DOI: [10.1007/BF02730901](#)]
- 10 **Mittal SK**, Kalra KK, Aggarwal V. Diagnostic upper GI endoscopy for hematemesis in children: experience from a pediatric gastroenterology centre in north India. *Indian J Pediatr* 1994; **61**: 651-654 [PMID: [7721369](#) DOI: [10.1007/BF02751973](#)]
- 11 **El-Karakasy HM**, El-Koofy N, Mohsen N, Helmy H, Nabil N, El-Shabrawi M. Extrahepatic portal vein obstruction in Egyptian children. *J Pediatr Gastroenterol Nutr* 2015; **60**: 105-109 [PMID: [25546337](#) DOI: [10.1097/MPG.0000000000000548](#)]
- 12 **Poddar U**, Thapa BR, Rao KL, Singh K. Etiological spectrum of esophageal varices due to portal hypertension in Indian children: is it different from the West? *J Gastroenterol Hepatol* 2008; **23**: 1354-1357 [PMID: [17683492](#) DOI: [10.1111/j.1440-1746.2007.05102.x](#)]
- 13 **Madhu K**, Avinash B, Ramakrishna B, Eapen CE, Shyamkumar NK, Zachariah U, Chandy G, Kurian G. Idiopathic non-cirrhotic intrahepatic portal hypertension: common cause of cryptogenic intrahepatic portal hypertension in a Southern Indian tertiary hospital. *Indian J Gastroenterol* 2009; **28**: 83-87 [PMID: [19907954](#) DOI: [10.1007/s12664-009-0030-3](#)]
- 14 **Rangari M**, Gupta R, Jain M, Malhotra V, Sarin SK. Hepatic dysfunction in patients with extrahepatic portal venous obstruction. *Liver Int* 2003; **23**: 434-439 [PMID: [14986818](#) DOI: [10.1111/j.1478-3231.2003.00879.x](#)]
- 15 **Dilawari JB**, Chawla YK. Pseudosclerosing cholangitis in extrahepatic portal venous obstruction. *Gut* 1992; **33**: 272-276 [PMID: [1541425](#) DOI: [10.1136/gut.33.2.272](#)]
- 16 **Nagi B**, Kochhar R, Bhasin D, Singh K. Cholangiopathy in extrahepatic portal venous obstruction. Radiological appearances. *Acta Radiol* 2000; **41**: 612-615 [PMID: [11092484](#) DOI: [10.1080/028418500127345992](#)]
- 17 **Poddar U**, Thapa BR, Bhasin DK, Prasad A, Nagi B, Singh K. Endoscopic retrograde cholangiopancreatography in the management of pancreaticobiliary disorders in children. *J Gastroenterol Hepatol* 2001; **16**: 927-931 [PMID: [11555109](#) DOI: [10.1046/j.1440-1746.2001.02545.x](#)]
- 18 **Mehrotra RN**, Bhatia V, Dabadghao P, Yachha SK. Extrahepatic portal vein obstruction in children: anthropometry, growth hormone, and insulin-like growth factor I. *J Pediatr Gastroenterol Nutr* 1997; **25**: 520-523 [PMID: [9360206](#) DOI: [10.1097/00005176-199711000-00006](#)]
- 19 **Sarin SK**, Bansal A, Sasan S, Nigam A. Portal-vein obstruction in children leads to growth retardation. *Hepatology* 1992; **15**: 229-233 [PMID: [1735525](#) DOI: [10.1002/hep.1840150210](#)]
- 20 **Heaton ND**, Davenport M, Howard ER. Incidence of haemorrhoids and anorectal varices in children with portal hypertension. *Br J Surg* 1993; **80**: 616-618 [PMID: [8518904](#) DOI: [10.1002/bjs.1800800524](#)]
- 21 **Menon P**, Rao KL, Bhattacharya A, Thapa BR, Chowdhary SK, Mahajan JK, Samujh R. Extrahepatic portal hypertension: quality of life and somatic growth after surgery. *Eur J Pediatr Surg* 2005; **15**: 82-87 [PMID: [15877255](#) DOI: [10.1055/s-2004-830341](#)]
- 22 **Lautz TB**, Sundaram SS, Whittington PF, Keys L, Superina RA. Growth impairment in children with extrahepatic portal vein obstruction is improved by mesenterico-left portal vein bypass. *J Pediatr Surg* 2009; **44**: 2067-2070 [PMID: [19944209](#) DOI: [10.1016/j.jpedsurg.2009.05.016](#)]
- 23 **Krishna YR**, Yachha SK, Srivastava A, Negi D, Lal R, Poddar U. Quality of life in children managed for extrahepatic portal venous obstruction. *J Pediatr Gastroenterol Nutr* 2010; **50**: 531-536 [PMID: [20639711](#) DOI: [10.1097/MPG.0b013e3181b6a55d](#)]

- 24 **Noronha Ferreira C**, Seijo S, Plessier A, Silva-Junior G, Turon F, Rautou PE, Baiges A, Bureau C, Bosch J, Hernández-Gea V, Valla D, García-Pagan JC. Natural history and management of esophagogastric varices in chronic noncirrhotic, nontumoral portal vein thrombosis. *Hepatology* 2016; **63**: 1640-1650 [PMID: [26799606](#) DOI: [10.1002/hep.28466](#)]
- 25 **Poddar U**, Thapa BR, Singh K. Endoscopic sclerotherapy in children: experience with 257 cases of extrahepatic portal venous obstruction. *Gastrointest Endosc* 2003; **57**: 683-686 [PMID: [12709697](#) DOI: [10.1067/mge.2003.194](#)]
- 26 **Thapa BR**, Mehta S. Endoscopic sclerotherapy of esophageal varices in infants and children. *J Pediatr Gastroenterol Nutr* 1990; **10**: 430-434 [PMID: [2358974](#) DOI: [10.1097/00005176-199005000-00003](#)]
- 27 **Howard ER**, Stringer MD, Mowat AP. Assessment of injection sclerotherapy in the management of 152 children with oesophageal varices. *Br J Surg* 1988; **75**: 404-408 [PMID: [3390666](#) DOI: [10.1002/bjs.1800750504](#)]
- 28 **Zargar SA**, Javid G, Khan BA, Yattoo GN, Shah AH, Gulzar GM, Singh J, Rehman BU, Din Z. Endoscopic ligation compared with sclerotherapy for bleeding esophageal varices in children with extrahepatic portal venous obstruction. *Hepatology* 2002; **36**: 666-672 [PMID: [12198659](#) DOI: [10.1053/jhep.2002.35278](#)]
- 29 **Poddar U**, Bhatnagar S, Yachha SK. Endoscopic band ligation followed by sclerotherapy: Is it superior to sclerotherapy in children with extrahepatic portal venous obstruction? *J Gastroenterol Hepatol* 2011; **26**: 255-259 [PMID: [21261713](#) DOI: [10.1111/j.1440-1746.2010.06397.x](#)]
- 30 **Poddar U**, Borkar V. Management of extra hepatic portal venous obstruction (EHPVO): current strategies. *Trop Gastroenterol* 2011; **32**: 94-102 [PMID: [21922871](#)]
- 31 **Sarin SK**, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; **16**: 1343-1349 [PMID: [1446890](#) DOI: [10.1002/hep.1840160607](#)]
- 32 **Poddar U**, Thapa BR, Singh K. Frequency of gastropathy and gastric varices in children with extrahepatic portal venous obstruction treated with sclerotherapy. *J Gastroenterol Hepatol* 2004; **19**: 1253-1256 [PMID: [15482531](#) DOI: [10.1111/j.1440-1746.2004.03470.x](#)]
- 33 **Itha S**, Yachha SK. Endoscopic outcome beyond esophageal variceal eradication in children with extrahepatic portal venous obstruction. *J Pediatr Gastroenterol Nutr* 2006; **42**: 196-200 [PMID: [16456415](#) DOI: [10.1097/01.mpg.0000189351.55666.45](#)]
- 34 **Khuroo MS**, Rather AA, Khuroo NS, Khuroo MS. Portal biliopathy. *World J Gastroenterol* 2016; **22**: 7973-7982 [PMID: [27672292](#) DOI: [10.3748/wjg.v22.i35.7973](#)]
- 35 **Khuroo MS**, Yattoo GN, Zargar SA, Javid G, Dar MY, Khan BA, Boda MI. Biliary abnormalities associated with extrahepatic portal venous obstruction. *Hepatology* 1993; **17**: 807-813 [PMID: [8491448](#)]
- 36 **Malkan GH**, Bhatia SJ, Bashir K, Khemani R, Abraham P, Gandhi MS, Radhakrishnan R. Cholangiopathy associated with portal hypertension: diagnostic evaluation and clinical implications. *Gastrointest Endosc* 1999; **49**: 344-348 [PMID: [10049418](#) DOI: [10.1016/s0016-5107\(99\)70011-8](#)]
- 37 **Sen Sarma M**, Yachha SK, Rai P, Neyaz Z, Srivastava A, Poddar U. Cholangiopathy in children with extrahepatic portal venous obstruction. *J Hepatobiliary Pancreat Sci* 2018; **25**: 440-447 [PMID: [30259697](#) DOI: [10.1002/jhbp.582](#)]
- 38 **Oo YH**, Olliff S, Haydon G, Thorburn D. Symptomatic portal biliopathy: a single centre experience from the UK. *Eur J Gastroenterol Hepatol* 2009; **21**: 206-213 [PMID: [19212209](#) DOI: [10.1097/MEG.0b013e3283060ee8](#)]
- 39 **Khare R**, Sikora SS, Srikanth G, Choudhuri G, Saraswat VA, Kumar A, Saxena R, Kapoor VK. Extrahepatic portal venous obstruction and obstructive jaundice: approach to management. *J Gastroenterol Hepatol* 2005; **20**: 56-61 [PMID: [15610447](#) DOI: [10.1111/j.1440-1746.2004.03528.x](#)]
- 40 **Llop E**, de Juan C, Seijo S, García-Criado A, Abraldes JG, Bosch J, García-Pagán JC. Portal cholangiopathy: radiological classification and natural history. *Gut* 2011; **60**: 853-860 [PMID: [21270119](#) DOI: [10.1136/gut.2010.230201](#)]
- 41 **Zargar SA**, Yattoo GN, Javid G, Khan BA, Shah AH, Shah NA, Gulzar GM, Singh J, Shafi HM. Fifteen-year follow up of endoscopic injection sclerotherapy in children with extrahepatic portal venous obstruction. *J Gastroenterol Hepatol* 2004; **19**: 139-145 [PMID: [14731122](#) DOI: [10.1111/j.1440-1746.2004.03224.x](#)]
- 42 **Dhiman RK**, Puri P, Chawla Y, Minz M, Bapuraj JR, Gupta S, Nagi B, Suri S. Biliary changes in extrahepatic portal venous obstruction: compression by collaterals or ischemic? *Gastrointest Endosc* 1999; **50**: 646-652 [PMID: [10536320](#) DOI: [10.1016/s0016-5107\(99\)80013-3](#)]
- 43 **Condat B**, Vilgrain V, Asselah T, O'Toole D, Rufat P, Zappa M, Moreau R, Valla D. Portal cavernoma-associated cholangiopathy: a clinical and MR cholangiography coupled with MR portography imaging study. *Hepatology* 2003; **37**: 1302-1308 [PMID: [12774008](#) DOI: [10.1053/jhep.2003.50232](#)]
- 44 **Chaudhary A**, Dhar P, Sarin SK, Sachdev A, Agarwal AK, Vij JC, Broor SL. Bile duct obstruction due to portal biliopathy in extrahepatic portal hypertension: surgical management. *Br J Surg* 1998; **85**: 326-329 [PMID: [9529484](#) DOI: [10.1046/j.1365-2168.1998.00591.x](#)]
- 45 **Vibert E**, Azoulay D, Aloia T, Pascal G, Veilhan LA, Adam R, Samuel D, Castaing D. Therapeutic strategies in symptomatic portal biliopathy. *Ann Surg* 2007; **246**: 97-104 [PMID: [17592297](#) DOI: [10.1097/SLA.0b013e318070cada](#)]
- 46 **Gauthier-Villars M**, Franchi S, Gauthier F, Fabre M, Pariente D, Bernard O. Cholestasis in children

- with portal vein obstruction. *J Pediatr* 2005; **146**: 568-573 [PMID: [15812469](#) DOI: [10.1016/j.jpeds.2004.12.025](#)]
- 47 **Ravindranath A**, Sen Sarima M, Yachha SK, Lal R, Singh S, Srivastava A, Poddar U, Neyaz Z, Behari A. Outcome of portosystemic shunt surgery on pre-existing cholangiopathy in children with extrahepatic portal vein obstruction. *J Hepatobiliary Pancreat Sci* 2020; **27**: 141-148 [PMID: [31654467](#) DOI: [10.1002/jhbp.692](#)]
  - 48 **Chawla Y**, Dilawari JB. Anorectal varices--their frequency in cirrhotic and non-cirrhotic portal hypertension. *Gut* 1991; **32**: 309-311 [PMID: [2013427](#) DOI: [10.1136/gut.32.3.309](#)]
  - 49 **Ganguly S**, Sarin SK, Bhatia V, Lahoti D. The prevalence and spectrum of colonic lesions in patients with cirrhotic and noncirrhotic portal hypertension. *Hepatology* 1995; **21**: 1226-1231 [PMID: [7737627](#)]
  - 50 **Yachha SK**, Dhiman RK, Gupta R, Ghoshal UC. Endosonographic evaluation of the rectum in children with extrahepatic portal venous obstruction. *J Pediatr Gastroenterol Nutr* 1996; **23**: 438-441 [PMID: [8956183](#) DOI: [10.1097/00005176-199611000-00014](#)]
  - 51 **Goenka MK**, Kochhar R, Nagi B, Mehta SK. Rectosigmoid varices and other mucosal changes in patients with portal hypertension. *Am J Gastroenterol* 1991; **86**: 1185-1189 [PMID: [1882798](#)]
  - 52 **Ohashi K**, Orihata G, Ohta S, Takamori S, Kojima K, Fukazawa M, Beppu T, Futagawa S. [Portal hypertensive gastropathy and colopathy]. *Nihon Rinsho* 1998; **56**: 2369-2375 [PMID: [9780722](#)]
  - 53 **Ozgediz D**, Devine P, Garcia-Aguilar J, Balibrea JM. Refractory lower gastrointestinal bleeding from portal hypertensive colopathy. *J Am Coll Surg* 2008; **207**: 613 [PMID: [18926469](#) DOI: [10.1016/j.jamcollsurg.2008.02.040](#)]
  - 54 **Rutgeerts P**, Van Gompel F, Geboes K, Vantrappen G, Broeckaert L, Coremans G. Long term results of treatment of vascular malformations of the gastrointestinal tract by neodymium Yag laser photocoagulation. *Gut* 1985; **26**: 586-593 [PMID: [3874122](#) DOI: [10.1136/gut.26.6.586](#)]
  - 55 **Sharma P**, Sharma BC, Puri V, Sarin SK. Minimal hepatic encephalopathy in patients with extrahepatic portal vein obstruction. *Am J Gastroenterol* 2008; **103**: 1406-1412 [PMID: [18510608](#) DOI: [10.1111/j.1572-0241.2008.01830.x](#)]
  - 56 **Yadav SK**, Srivastava A, Thomas MA, Agarwal J, Pandey CM, Lal R, Yachha SK, Saraswat VA, Gupta RK. Encephalopathy assessment in children with extra-hepatic portal vein obstruction with MR, psychometry and critical flicker frequency. *J Hepatol* 2010; **52**: 348-354 [PMID: [20137823](#) DOI: [10.1016/j.jhep.2009.12.012](#)]
  - 57 **Montgomery JY**, Bajaj JS. Advances in the evaluation and management of minimal hepatic encephalopathy. *Curr Gastroenterol Rep* 2011; **13**: 26-33 [PMID: [20924726](#) DOI: [10.1007/s11894-010-0150-z](#)]
  - 58 **Sen Sarima M**, Srivastava A, Yachha SK, Poddar U. Ascites in children with extrahepatic portal venous obstruction: Etiology, risk factors and outcome. *Dig Liver Dis* 2020; **52**: 1480-1485 [PMID: [32907787](#) DOI: [10.1016/j.dld.2020.08.018](#)]
  - 59 **Gupta D**, Vijaya DR, Gupta R, Dhiman RK, Bhargava M, Verma J, Chawla YK. Prevalence of hepatopulmonary syndrome in cirrhosis and extrahepatic portal venous obstruction. *Am J Gastroenterol* 2001; **96**: 3395-3399 [PMID: [11774955](#) DOI: [10.1111/j.1572-0241.2001.05274.x](#)]
  - 60 **Sari S**, Oguz D, Sucak T, Dalgic B, Atasever T. Hepatopulmonary syndrome in children with cirrhotic and non-cirrhotic portal hypertension: a single-center experience. *Dig Dis Sci* 2012; **57**: 175-181 [PMID: [21792618](#) DOI: [10.1007/s10620-011-1832-6](#)]
  - 61 **Borkar VV**, Poddar U, Kumari N, Singh S, Roy R, Yachha SK. Duodenal morphometry and small bowel permeability in children with portal hypertension. *J Pediatr Gastroenterol Nutr* 2015; **60**: 171-176 [PMID: [25250680](#) DOI: [10.1097/MPG.0000000000000578](#)]
  - 62 **Thomas V**, Jose T, Kumar S. Natural history of bleeding after esophageal variceal eradication in patients with extrahepatic portal venous obstruction; a 20-year follow-up. *Indian J Gastroenterol* 2009; **28**: 206-211 [PMID: [20425640](#) DOI: [10.1007/s12664-009-0086-0](#)]
  - 63 **Maksoud-Filho JG**, Gonçalves ME, Cardoso SR, Gibelli NE, Tannuri U. Long-term follow-up of children with extrahepatic portal vein obstruction: impact of an endoscopic sclerotherapy program on bleeding episodes, hepatic function, hypersplenism, and mortality. *J Pediatr Surg* 2009; **44**: 1877-1883 [PMID: [19853741](#) DOI: [10.1016/j.jpedsurg.2009.02.074](#)]
  - 64 **Wani AH**, Shah OJ, Zargar SA. Management of variceal hemorrhage in children with extrahepatic portal venous obstruction-shunt surgery versus endoscopic sclerotherapy. *Indian J Surg* 2011; **73**: 409-413 [PMID: [23204696](#) DOI: [10.1007/s12262-011-0345-z](#)]
  - 65 **Chaudhary A**, Tatke M, Aranya RC. Endoscopic sclerotherapy: the far and near effects. *Br J Surg* 1990; **77**: 963 [PMID: [2207585](#) DOI: [10.1002/bjs.1800770904](#)]
  - 66 **Lal R**, Sarima MS, Gupta MK. Extrahepatic Portal Venous Obstruction: What Should be the Mainstay of Treatment? *Indian J Pediatr* 2017; **84**: 691-699 [PMID: [28612224](#) DOI: [10.1007/s12098-017-2390-5](#)]
  - 67 **Mitra SK**, Rao KL, Narasimhan KL, Dilawari JB, Batra YK, Chawla Y, Thapa BR, Nagi B, Walia BN. Side-to-side lienorenal shunt without splenectomy in noncirrhotic portal hypertension in children. *J Pediatr Surg* 1993; **28**: 398-401; discussion 401 [PMID: [8468654](#) DOI: [10.1016/0022-3468\(93\)90239-h](#)]
  - 68 **Rao KL**, Goyal A, Menon P, Thapa BR, Narasimhan KL, Chowdhary SK, Samujh R, Mahajan JK. Extrahepatic portal hypertension in children: observations on three surgical procedures. *Pediatr Surg Int* 2004; **20**: 679-684 [PMID: [15351894](#) DOI: [10.1007/s00383-004-1272-x](#)]
  - 69 **Bismuth H**, Franco D, Alagille D. Portal diversion for portal hypertension in children. The first



- ninety patients. *Ann Surg* 1980; **192**: 18-24 [PMID: 7406558 DOI: 10.1097/00000658-198007000-00003]
- 70 **Alvarez F**, Bernard O, Brunelle F, Hadchouel P, Odièvre M, Alagille D. Portal obstruction in children. II. Results of surgical portosystemic shunts. *J Pediatr* 1983; **103**: 703-707 [PMID: 6605420 DOI: 10.1016/s0022-3476(83)80461-2]
  - 71 **Prasad AS**, Gupta S, Kohli V, Pande GK, Sahni P, Nundy S. Proximal splenorenal shunts for extrahepatic portal venous obstruction in children. *Ann Surg* 1994; **219**: 193-196 [PMID: 8129490 DOI: 10.1097/00000658-199402000-00011]
  - 72 **Sarma MS**, Lal R, Yachha SK, Behari A, Srivastava A. Portosystemic shunt surgery for extrahepatic portal vein obstruction in children: Shifting indications beyond endoscopic variceal eradication. *Hepatol Int* 2017; **11**: 1091-1093 [DOI: 10.1007/s12072-016-9783-9]
  - 73 **Bertocchini A**, Falappa P, Grimaldi C, Bolla G, Monti L, de Ville de Goyet J. Intrahepatic portal venous systems in children with noncirrhotic prehepatic portal hypertension: anatomy and clinical relevance. *J Pediatr Surg* 2014; **49**: 1268-1275 [PMID: 25092088 DOI: 10.1016/j.jpedsurg.2013.10.029]
  - 74 **Guérin F**, Bidault V, Gonzales E, Franchi-Abella S, De Lambert G, Branchereau S. Meso-Rex bypass for extrahepatic portal vein obstruction in children. *Br J Surg* 2013; **100**: 1606-1613 [PMID: 24264782 DOI: 10.1002/bjs.9287]
  - 75 **Lautz TB**, Keys LA, Melvin JC, Ito J, Superina RA. Advantages of the meso-Rex bypass compared with portosystemic shunts in the management of extrahepatic portal vein obstruction in children. *J Am Coll Surg* 2013; **216**: 83-89 [PMID: 23177370 DOI: 10.1016/j.jamcollsurg.2012.09.013]
  - 76 **Luoto T**, Pakarinen M, Mattila I, Rintala R. Mesoportal bypass using a constructed saphenous vein graft for extrahepatic portal vein obstruction--technique, feasibility, and outcomes. *J Pediatr Surg* 2012; **47**: 688-693 [PMID: 22498382 DOI: 10.1016/j.jpedsurg.2011.10.065]
  - 77 **Sharif K**, McKiernan P, de Ville de Goyet J. Mesoportal bypass for extrahepatic portal vein obstruction in children: close to a cure for most! *J Pediatr Surg* 2010; **45**: 272-276 [PMID: 20105620 DOI: 10.1016/j.jpedsurg.2009.08.019]
  - 78 **Omanwar S**, Rizvi MR, Kathayat R, Sharma BK, Pandey GK, Alam MA, Malhotra V, Sarin SK. A rabbit model of non-cirrhotic portal hypertension by repeated injections of E.coli through indwelling cannulation of the gastrosplenic vein. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 417-422 [PMID: 15313681]
  - 79 **Saito K**, Nakanuma Y, Takegoshi K, Ohta G, Obata Y, Okuda K, Kameda H. Non-specific immunological abnormalities and association of autoimmune diseases in idiopathic portal hypertension. A study by questionnaire. *Hepatogastroenterology* 1993; **40**: 163-166 [PMID: 8509048]
  - 80 **Besmond C**, Valla D, Hubert L, Poirier K, Grosse B, Guettier C, Bernard O, Gonzales E, Jacquemin E. Mutations in the novel gene FOPV are associated with familial autosomal dominant and non-familial obliterative portal venopathy. *Liver Int* 2018; **38**: 358-364 [PMID: 28792652 DOI: 10.1111/liv.13547]
  - 81 **Sarin SK**, Sharma G, Banerjee S, Kathayat R, Malhotra V. Hepatic fibrogenesis using chronic arsenic ingestion: studies in a murine model. *Indian J Exp Biol* 1999; **37**: 147-151 [PMID: 10641134 DOI: 10.1046/j.1440-1746.2002.02882.x]
  - 82 **Gryseels B**. Schistosomiasis. *Infect Dis Clin North Am* 2012; **26**: 383-397 [PMID: 22632645 DOI: 10.1016/j.idc.2012.03.004]
  - 83 **Vispo E**, Moreno A, Maida I, Barreiro P, Cuevas A, Albertos S, Soriano V. Noncirrhotic portal hypertension in HIV-infected patients: unique clinical and pathological findings. *AIDS* 2010; **24**: 1171-1176 [PMID: 20299955 DOI: 10.1097/QAD.0b013e3283389e26]
  - 84 **Sarin SK**, Kumar A. Noncirrhotic portal hypertension. *Clin Liver Dis* 2006; **10**: 627-651, x [PMID: 17162232 DOI: 10.1016/j.cld.2006.08.021]
  - 85 **Schouten JNL**, Garcia-Pagan JC, Valla DC, Janssen HLA. Idiopathic noncirrhotic portal hypertension. *Hepatology* 2011 [DOI: 10.1002/hep.24422]
  - 86 **Sato Y**, Nakanuma Y. Role of endothelial-mesenchymal transition in idiopathic portal hypertension. *Histol Histopathol* 2013; **28**: 145-154 [PMID: 23275299 DOI: 10.14670/HH-28.145]
  - 87 **Sarin SK**, Kumar A, Chawla YK, Baijal SS, Dhiman RK, Jafri W, Lesmana LA, Guha Mazumder D, Omata M, Qureshi H, Raza RM, Sahni P, Sakhuja P, Salih M, Santra A, Sharma BC, Sharma P, Shiha G, Sollano J; Members of the APASL Working Party on Portal Hypertension. Noncirrhotic portal fibrosis/idiopathic portal hypertension: APASL recommendations for diagnosis and treatment. *Hepatol Int* 2007; **1**: 398-413 [PMID: 19669336 DOI: 10.1007/s12072-007-9010-9]
  - 88 **Joshi N**, Yr NK, Kumar A. Age related seroprevalence of antibodies to hepatitis A virus in Hyderabad, India. *Trop Gastroenterol* 2000; **21**: 63-65 [PMID: 10881625]
  - 89 **Dhawan PS**, Shah SS, Alvares JF, Kher A, Shankaran, Kandoth PW, Sheth PN, Kamath H, Kamath A, Koppikar GV, Kalro RH. Seroprevalence of hepatitis A virus in Mumbai, and immunogenicity and safety of hepatitis A vaccine. *Indian J Gastroenterol* 1998; **17**: 16-18 [PMID: 9465507]
  - 90 **Prasad D**, Sen Sarma M, Yachha SK, Srivastava A, Poddar U. Pediatric non-cirrhotic portal fibrosis: role of endoscopic management in determining long-term outcome. *Hepatol Int* 2020; **14**: 281-287 [PMID: 31468295 DOI: 10.1007/s12072-019-09979-8]
  - 91 **Sood V**, Lal BB, Khanna R, Rawat D, Bihari C, Alam S. Noncirrhotic Portal Fibrosis in Pediatric Population. *J Pediatr Gastroenterol Nutr* 2017; **64**: 748-753 [PMID: 28437324 DOI: 10.1097/MPG.0000000000001485]



- 92 **Dhiman RK**, Chawla Y, Vasishta RK, Kakkar N, Dilawari JB, Trehan MS, Puri P, Mitra SK, Suri S. Non-cirrhotic portal fibrosis (idiopathic portal hypertension): experience with 151 patients and a review of the literature. *J Gastroenterol Hepatol* 2002; **17**: 6-16 [PMID: [11895549](#) DOI: [10.1046/j.1440-1746.2002.02596.x](#)]
- 93 **Pande C**, Kumar A, Sarin SK. Non-cirrhotic portal fibrosis: a clinical profile of 366 patients. *Am J Gastroenterol* 2006; **101**: S191 [DOI: [10.14309/00000434-200609001-00439](#)]
- 94 **Chawla YK**, Dilawari JB, Dhiman RK, Goenka MK, Bhasin DK, Kochhar R, Singh K, Kaur U. Sclerotherapy in noncirrhotic portal fibrosis. *Dig Dis Sci* 1997; **42**: 1449-1453 [PMID: [9246045](#) DOI: [10.1023/a:1018858525312](#)]
- 95 **Sarin SK**, Sachdev G, Nanda R. Follow-up of patients after variceal eradication. A comparison of patients with cirrhosis, noncirrhotic portal fibrosis, and extrahepatic obstruction. *Ann Surg* 1986; **204**: 78-82 [PMID: [3729586](#) DOI: [10.1097/00000658-198607000-00011](#)]
- 96 **Siramolpiwat S**, Seijo S, Miquel R, Berzigotti A, Garcia-Criado A, Darnell A, Turon F, Hernandez-Gea V, Bosch J, Garcia-Pagan JC. Idiopathic portal hypertension: natural history and long-term outcome. *Hepatology* 2014; **59**: 2276-2285 [PMID: [24155091](#) DOI: [10.1002/hep.26904](#)]
- 97 **Schouten JN**, Nevens F, Hansen B, Laleman W, van den Born M, Komuta M, Roskams T, Verheij J, Janssen HL. Idiopathic noncirrhotic portal hypertension is associated with poor survival: results of a long-term cohort study. *Aliment Pharmacol Ther* 2012; **35**: 1424-1433 [PMID: [22536808](#) DOI: [10.1111/j.1365-2036.2012.05112.x](#)]
- 98 **Hillaire S**, Bonte E, Denninger MH, Casadevall N, Cadranet JF, Lebrec D, Valla D, Degott C. Idiopathic non-cirrhotic intrahepatic portal hypertension in the West: a re-evaluation in 28 patients. *Gut* 2002; **51**: 275-280 [PMID: [12117894](#) DOI: [10.1136/gut.51.2.275](#)]
- 99 **Wolff M**, Hirner A. Current state of portosystemic shunt surgery. *Langenbecks Arch Surg* 2003; **388**: 141-149 [PMID: [12942328](#) DOI: [10.1007/s00423-003-0367-5](#)]
- 100 **Shorbagi A**, Bayraktar Y. Experience of a single center with congenital hepatic fibrosis: a review of the literature. *World J Gastroenterol* 2010; **16**: 683-690 [PMID: [20135715](#) DOI: [10.3748/wjg.v16.i6.683](#)]
- 101 **Hernandez-Gea V**, Friedman SL. Pathogenesis of liver fibrosis. *Annu Rev Pathol* 2011; **6**: 425-456 [PMID: [21073339](#) DOI: [10.1146/annurev-pathol-011110-130246](#)]
- 102 **Huang L**, Wei MF, Feng JX. Abnormal activation of OPN inflammation pathway in livers of children with biliary atresia and relationship to hepatic fibrosis. *Eur J Pediatr Surg* 2008; **18**: 224-229 [PMID: [18704890](#) DOI: [10.1055/s-2008-1038483](#)]
- 103 **Chu AS**, Friedman JR. A role for microRNA in cystic liver and kidney diseases. *J Clin Invest* 2008; **118**: 3585-3587 [PMID: [18949060](#) DOI: [10.1172/JCI36870](#)]
- 104 **Srinath A**, Shneider BL. Congenital hepatic fibrosis and autosomal recessive polycystic kidney disease. *J Pediatr Gastroenterol Nutr* 2012; **54**: 580-587 [PMID: [22197937](#) DOI: [10.1097/MPG.0b013e31824711b7](#)]
- 105 **Poddar U**, Thapa BR, Vashishta RK, Girish CS, Singh K. Congenital hepatic fibrosis in Indian children. *J Gastroenterol Hepatol* 1999; **14**: 1192-1196 [PMID: [10634156](#) DOI: [10.1046/j.1440-1746.1999.02028.x](#)]
- 106 **Rawat D**, Kelly DA, Milford DV, Sharif K, Lloyd C, McKiernan PJ. Phenotypic variation and long-term outcome in children with congenital hepatic fibrosis. *J Pediatr Gastroenterol Nutr* 2013; **57**: 161-166 [PMID: [23518487](#) DOI: [10.1097/MPG.0b013e318291e72b](#)]
- 107 **Daroca PJ**, Tuthill R, Reed RJ. Cholangiocarcinoma arising in congenital hepatic fibrosis: a case report. *Arch Pathol Lab Med* 1975 [DOI: [10.1016/s0168-8278\(98\)81109-4](#)]
- 108 **Morita D**, Kagata Y, Ogata S, Tsuda H, Hatsuse K, Mochizuki H, Matsubara O. Combined hepatocellular carcinoma and cholangiocarcinoma with components of mucinous carcinoma arising in a cirrhotic liver. *Pathol Int* 2006; **56**: 222-226 [PMID: [16634969](#) DOI: [10.1111/j.1440-1827.2006.01950.x](#)]
- 109 **Poddar U**, Thapa BR, Puri P, Girish CS, Vaiphei K, Vasishta RK, Singh K. Non-cirrhotic portal fibrosis in children. *Indian J Gastroenterol* 2000; **19**: 12-13 [PMID: [10659480](#)]
- 110 **Franchi-Abella S**, Fabre M, Mselati E, De Marsillac ME, Bayari M, Pariente D, Jacquemin E, Bernard O. Obliterative portal venopathy: a study of 48 children. *J Pediatr* 2014; **165**: 190-193.e2 [PMID: [24768253](#) DOI: [10.1016/j.jpeds.2014.03.025](#)]
- 111 **Parkash A**, Cheema HA, Malik HS, Fayyaz Z. Congenital hepatic fibrosis: clinical presentation, laboratory features and management at a tertiary care hospital of Lahore. *J Pak Med Assoc* 2016; **66**: 984-988 [PMID: [27524533](#) DOI: [10.5455/jpma.291186](#)]
- 112 **Luoto TT**, Pakarinen MP, Jahnukainen T, Jalanko H. Liver disease in autosomal recessive polycystic kidney disease: clinical characteristics and management in relation to renal failure. *J Pediatr Gastroenterol Nutr* 2014; **59**: 190-196 [PMID: [24806835](#) DOI: [10.1097/MPG.0000000000000422](#)]



## Acute-on-chronic liver failure in children

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### Abstract

Although various complex definitions of acute-on-chronic liver failure (ACLF) have been suggested in relation to adult patients, there is currently no universal definition of the syndrome in pediatric patients. In simplified terms, ACLF is characterized by the acute deterioration of the liver functions due to the effects of a precipitating factor on the basis of a chronic liver disease. Acute events and underlying liver diseases are very different in children from those seen in adults. Moreover, acute events and underlying chronic liver diseases vary among geographical regions, although it seems that the most common such diseases and acute events are autoimmune hepatitis, Wilson's disease, and their flares. ACLF is associated with a poor prognosis. While no scoring systems have been developed to predict the prognosis for children with ACLF, modified versions of the Asian Pacific Association for the Study of the liver's acute-on-chronic liver failure scoring system and the Chronic Liver Failure-Sequential Organ Failure Assessment criteria can be used in children until specific and validated scoring systems are available. Aside from liver transplantation, there is no proven treatment for ACLF. Thus, the early recognition of ACLF prior to the development of extrahepatic organ failure is important.

**Key Words:** Liver failure; Prognosis; Prevalence; Clinics; Histopathology; Scoring systems; Treatment

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**Core Tip:** Acute-on-chronic liver failure (ACLF) remains poorly defined in pediatric patients. ACLF is associated with acute deterioration in patients with chronic liver disease or cirrhosis due to an underlying precipitating event. In the limited number of pediatric studies conducted to date, the underlying chronic diseases and acute precipitating events have been found to vary among geographical regions, while high rates of short-term mortality have also been reported. This review focuses on ACLF in

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## INTRODUCTION

Acute-on-chronic liver failure (ACLF) remains poorly defined in pediatric patients. Although a few prior pediatric studies have relied on definitions of the syndrome formulated in relation to adult patients[1-9], no study has yet sought to develop a definition of the syndrome in pediatric patients. The simplest definition of ACLF equates it with the development of acute deterioration in patients with chronic liver disease or cirrhosis as a result of an underlying precipitating event[10]. ACLF differs from both acute liver failure (ALF) and acute decompensated cirrhosis. More specifically, ALF is defined as a form of coagulopathy that cannot be corrected with vitamin K when biochemical data indicate the presence of acute liver injury without prior evidence of chronic liver disease[11]. Furthermore, decompensated cirrhosis is defined as the loss of the liver's normal synthetic capacity over time accompanied by the development of jaundice and complications of portal hypertension, including ascites, variceal bleeding, and hepatic encephalopathy (HE)[12].

Many studies have been conducted among adults with ACLF, although such studies have utilized different criteria and etiologies, and they have been conducted in different geographical regions. European, American, and Asian hepatology authorities have devised different definitions of ACLF in light of their specific populations. Despite the use of different definitions and etiologies, the morbidity and mortality rates associated with ACLF have consistently been found to be high in adults[13-15]. In the limited number of pediatric studies conducted to date, the underlying chronic diseases and acute precipitating events in cases of ACLF have been found to vary among geographical regions, while high rates of short-term mortality have also been reported[1-7]. The present review will focus on ACLF in children.

## DEFINING ACLF

Different definitions of ACLF have been suggested in relation to adult patients. For instance, as part of prospective observational studies, the European Association for Liver Studies (EASL)[13], the North American Consortium for End-Stage Liver Disease Studies (NASCELD)[14], and the Asian Pacific Association for the Study of the Liver (APASL)[15] have each suggested different definitions of ACLF in adults, which can sometimes lead to confusion (Table 1). According to both the EASL and the NASCELD, ACLF involves the development of acute hepatic decompensation accompanied by extrahepatic organ failure, which stems from an acute precipitating factor in patients admitted to hospital with cirrhosis. Moreover, the two authorities stress that ACLF is associated with high mortality. With reference to the definition of ACLF suggested by the EASL, in the conducted in the United Kingdom using European (CANONIC) study of cirrhotic patients, acute hepatic decompensation was defined as the development of ascites, HE, gastrointestinal hemorrhage, bacterial infection, or any combination of these disorders. In addition, as a requirement for a diagnosis of ACLF, the NASCELD defines organ failure as shock, HE grade III or IV, renal failure that requires dialysis, and/or respiratory failure that requires mechanical ventilation. Patients with a prior history of decompensated cirrhosis are included within both the EASL and the NASCELD definitions of ACLF. In its definition of ACLF, the APASL includes not only those with cirrhosis, but also those with chronic liver disease. The EASL specifies the time frame for developing ACLF as 4-12 wk, whereas the NASCELD does not specify a time frame[13,14]. The APASL does not include extrahepatic organ failure in its definition of ACLF, although it is recognized as a complication of ACLF. Moreover, patients with decompensated and acutely decompensated cirrhosis are excluded from the APASL definition of ACLF. In fact,

**Table 1 Commonly accepted acute-on-chronic liver failure definitions**

	APASL	EASL	NASCELD
<b>Definition</b>	An acute hepatic insult manifesting as jaundice (serum bilirubin $\geq 5$ mg/dL) and coagulopathy (INR $\geq 1.5$ ) complicated within 4 wk by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis, and is associated with a high 28-d mortality	An acute deterioration of pre-existing chronic liver disease usually related to a precipitating event and associated with increased mortality at 3 mo due to multisystem organ failure	A syndrome characterized by acute deterioration of cirrhosis with two or more extrahepatic organ failure
<b>Included patients</b>	Acute liver deterioration in patients with previously diagnosed or undiagnosed chronic liver disease including cirrhosis. Acute hepatic triggering factors	Cirrhosis (compensated or decompensated)  Renal failure is mandatory  Patients with an acute decompensation of cirrhosis  Patients with prior decompensation of cirrhosis	Cirrhosis (compensated or decompensated)  Two extrahepatic organ failure  Presentation not necessarily to be liver failure  Can be repeated episodes of ACLF
<b>Excluded patients</b>	Patients with bacterial infections  Patients with cirrhosis who develop acute deterioration of their clinical status are considered to have acute decompensation but not ACLF  Prior decompensation. Non-hepatic acute insults (such as sepsis)	HCC  HIV infection  Receiving immunosuppressive treatments	HIV infection  Disseminated malignancies
<b>Pediatric definition</b>	For children less than 3 years, modified HE assessment scale can be used  Clinical and/or radiological ascites can be used for defining ACLF in children	None	None

ACLF: Acute-on-chronic liver failure; APASL: The Asian Pacific Association for the Study of the Liver; EASL: The European Association for Liver Studies; HCC: Hepatocellular cancer, HIV: Human immunodeficiency virus; INR: International normalized ratio; NASCELD: The North American Consortium for End-Stage Liver Disease Studies.

decompensation preceding jaundice and repeated episodes are said to indicate acute decompensation, not ACLF. Another important difference that sets the APASL definition of ACLF apart from the other definitions is the requirement for the diagnosis of jaundice to be followed by the diagnosis of clinical ascites or HE. More specifically, the APASL definition of ACLF states the following:

ACLF is an acute hepatic insult manifesting as jaundice (serum bilirubin  $\geq 5$  mg/dL (85 micromol/L) and coagulopathy [international normalized ratio (INR)  $\geq 1.5$  or prothrombin activity  $< 40\%$ ] complicated within 4 wk by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis, and is associated with a high 28-d mortality[15].

As mentioned above, there is currently no universal definition of ACLF in pediatric patients. Only the APASL has stated, in its latest guidelines, that, with some minor modifications, its definition of ACLF in adults can be used for children. Due to the difficulty associated with identifying clinical ascites and HE in children, those necessary modifications include recognizing ascites as “clinical and/or radiological ascites” and “diagnosing HE in children younger than 3 years using modified HE assessment scale”[15]. However, there are still several major problems with the APASL definition. First, some instances of ALF in children may not be accompanied by a significant increase in the bilirubin level, such as ALF stemming from metabolic liver disease. Second, the cut-off INR for the diagnosis of ACLF is problematic. Indeed, when defining ALF in children, the INR must be  $\geq 1.5$  with HE or  $\geq 2$  regardless of the HE status[11]. The APASL has referred to these two issues, although it has not made any recommendations. In light of this, in a retrospective study conducted in children, we defined ACLF as follows:

The presence of an acute hepatic insult in previously diagnosed or undiagnosed chronic liver disease causing jaundice (total serum bilirubin  $\geq 5$  mg/dL) and coagulopathy (INR  $\geq 2.0$ ) and clinical and/or radiological ascites and/or HE within 4 wk[16].

Finally, in its consensus report, the World Gastroenterology Organization defined ACLF as follows:

ACLF is a syndrome characterised by acute hepatic decompensation resulting in liver failure (jaundice and prolongation of the INR) and one or more extrahepatic organ failures that is associated with increased mortality within a period of 28 d and up to 3 mo from onset[10].

## PREVALENCE

Despite the use of different diagnostic criteria, the prevalence of ACLF has been found to range from 22.6% to 40% in adult patients with cirrhosis[17-19]. Moreover, according to the APASL and EASL criteria, the incidence rate has been determined to be 5.7 and 20.1 cases per 1000 person-years, respectively[20].

We searched the literature published in English and found nine studies concerning ACLF in children[1-9]. Of those nine, six studies were conducted in India. Given that prior studies have relied on different adult definitions and etiologies, and as they have mainly been conducted in a single Asian country, it is difficult to determine the true prevalence of pediatric ACLF. Indeed, the previously reported prevalences are not generalizable. In two centers in India in which the APASL definition of ACLF was used, its prevalence was reported to range from 11.2% to 22.1%[3-5].

## CLINICAL FEATURES OF ACLF

### *Underlying chronic liver disease*

The primary causes of chronic liver disease and cirrhosis in adults are alcohol abuse, hepatitis B (HBV) and C, and non-alcoholic fatty liver disease. While viral hepatitis is the most common cause in Eastern countries, alcohol abuse is the most common cause in Western countries[10,13,14]. In the few studies previously conducted in children, the most common underlying chronic liver diseases were found to be Wilson's disease (WD), autoimmune hepatitis (AIH), and indeterminate chronic liver diseases[4,6]. AIH can present as ACLF, as the exacerbation of a pre-existing chronic liver disease or liver injury caused by an overlapping infectious or toxic agent may lead to ACLF in cases of AIH. There are no definitive data regarding whether or not patients diagnosed with ACLF have a previous history of liver disease. In our prior study, 58.6% of ACLF patients were diagnosed with liver disease for the first time[16].

### *Precipitating acute events*

A precipitating event can trigger the decompensation of liver disease and lead to multiple organ failure. Acute events are known to vary by region in adults. Bacterial infection, sepsis, and alcoholism are the most common acute events in the West, while the reactivation of HBV infection or superinfection, hepatotoxic drugs, and complementary and alternative medicines are the most common acute events in the East[13-15]. The most common acute events in pediatric ACLF were reported in one center in India to be WD (46.5%) and AIH (34.9%) flares[8]. In the other two centers in India, the most common acute events were reported to be hepatitis A virus (HAV) and hepatitis E virus infections[1,2]. In our prior study, the most common acute events were AIH (48.28%) and WD (27.58%) flares. Moreover, the other identified acute events were drug-induced liver injury, Epstein-Barr virus, cytomegalovirus, and HAV infection [16].

## PATHOPHYSIOLOGY

Current knowledge regarding the pathophysiology of ACLF is insufficient. It has been stated that the main trigger of ACLF in adults is increased severe systemic inflammation. Systemic inflammation can cause ACLF through several mechanisms, including: (1) Immune-mediated tissue damage; (2) Mitochondrial dysfunction caused by oxidative stress; and (3) The development of renal hypoperfusion and multiple organ failure due to the effective arteriolar volume decrement caused by vasoactive substances[21,22]. The main causes of systemic inflammation have been reported to be bacterial infection and sepsis originating from the gastrointestinal tract, gastrointestinal bleeding, and severe alcoholic hepatitis[21,23]. It has been suggested



that gastrointestinal hemorrhage causes systemic inflammation through causing ischemia-reperfusion injury secondary to liver ischemia and intestinal bacterial translocation[24]. Excessive alcohol consumption is known to stimulate systemic inflammation by causing both intestinal dysbiosis and bacterial translocation in severe alcoholic hepatitis[23]. The differences in the triggering factors, underlying diseases, and comorbidities seen between children and adults suggest that factors other than those mechanisms also play a role in the pathophysiology of pediatric ACLF.

## LIVER HISTOPATHOLOGY

A diagnosis of chronic liver disease or cirrhosis is typically made on the basis of a physical examination as well as specific laboratory, endoscopic, and/or radiological investigations[12]. A liver biopsy or histopathological examination of the explant liver provides information about necrosis, chronicity, and/or cirrhosis. However, it may not be possible to perform a liver biopsy due to coagulopathy. In such a case, a transjugular liver biopsy or non-invasive modality can be used[11,12,15]. While the histology of ACLF has not yet been thoroughly investigated, it can be predicted that the syndrome has the histopathological features of both ALF and chronic liver disease. Massive necrosis without chronicity is seen in the case of fulminant hepatitis or ALF [11]. Any degree of fibrosis, ductular reaction, or parenchymal collapse in the liver is a sign of ACLF[25]. This issue has not previously been studied in detail in children. In our prior study, massive confluent necrosis and fibrosis with mild to moderate inflammation (neutrophil and eosinophil), as well as evidence of regeneration, were observed in the hepatectomy materials of children who underwent LT. In those who did not undergo LT, the presence of underlying disease (*i.e.*, lymphoplasmacytic cell infiltration in AIH and micro- and macrovesicular steatosis in WD), rare or patchy hepatocellular necrosis, and advanced-stage fibrosis with bridging were all observed[16].

## DIAGNOSIS AND SCORING SYSTEMS

ACLF is associated with a high short-term mortality rate. Data concerning the severity of the syndrome contributes to the selection of an appropriate treatment for it. The validity of a number of scoring models in ACLF has been extensively tested in adults. For instance, the model for end-stage liver disease (MELD), MELD-sodium (MELD-Na), and Child-Pugh-Turcotte scores, which are used in relation to organ allocation, have been found to exhibit low sensitivity because they do not evaluate extrahepatic organ failure, which is important in terms of the prognosis of ACLF[13,14]. Both the Chronic Liver Failure-Sequential Organ Failure Assessment (CLIF-SOFA) (Table 2) and the APASL-ACLF Research Consortium (AARC) (Table 3) scoring systems, which include parameters for evaluating kidney, brain, respiratory, and circulatory functions, have been found to be more reliable with regard to identifying the prognosis of ACLF [13,15]. The APASL has suggested that the AARC system is more sensitive than the CLIF-SOFA when it comes to determining prognoses. ACLF is a dynamic process, which means that the associated scoring systems should be evaluated dynamically. Scoring systems used at the 48<sup>th</sup> hour, after 3–7 d, or after 8–15 d predict the prognosis of ACLF better than a score calculated at the time of admission. An AARC score of < 10, or a score falling below 10 during the first week of admission, indicates a higher likelihood of survival in adults[13–15].

Although there is currently no validated scoring system for pediatric patients with ACLF, a few studies have made use of scoring systems (or their modified versions) designed for use with adults[2,4,8]. The modifications in this regard include the adjustment of the HE assessment, blood pressure, and serum creatinine levels according to the childhood age group[3] (Tables 4 and 5). In one pediatric study[3], the CLIF-SOFA and AARC scores were found to be superior in terms of predicting a poor outcome when compared with the Pediatric End-Stage Liver Disease, Child-Pugh and Pediatric Risk of Mortality-III scores. In the study, AARC and CLIF-SOFA scores of 11 were found to predict a poor prognosis with maximum sensitivity and specificity [area under the receiver operating characteristic curve (AUROC) > 0.9]. In another pediatric study[2] that tested the validity of the CLIF-SOFA system, the maximum sensitivity (100%) and specificity (76%) (AUROC = 0.95) were achieved at a 6.5 cut-off level with regard to predicting mortality. Moreover, in another pediatric study, children with a CLIF-SOFA score ≥ 10 at the time of admission were found to require an urgent referral to an LT center[4]. In our prior study, the AARC and CLIF-SOFA scores were

**Table 2 Chronic liver failure–sequential organ failure assessment score**

Organ/systems	0	1	2	3	4
Liver (bilirubin, mg/dL)	< 1.2	≥ 1.2 to < 2.0	≥ 2.0 to < 6.0	≥ 6.0 to < 12.0	≥ 12.0
Kidney (creatinine, mg/dL)	< 1.2	≥ 1.2 to < 2.0	≥ 2.0 to < 3.5	≥ 3.5 to < 5.0(or RRT)	≥ 5.0(or RRT)
Cerebral (HE grade)	No HE	I	II	III	IV
Coagulation (INR)	< 1.1	≥ 1.1 to < 1.25	≥ 1.25 to < 1.5	≥ 1.5 to < 2.5	≥ 2.5 or platelet < 20 × 10 <sup>9</sup> /L
Circulation (mean arterial pressure, mm Hg)	≥ 70	< 70	Dopamine ≤ 5 or dobutamine or terlipressin(μg/kg/min)	Dopamine > 5 or E ≤ 0.1 or NE ≤ 0.1(μg/kg/min)	Dopamine > 15 or E > 0.1 or NE > 0.1(μg/kg/min)
Lungs					
PaO <sub>2</sub> /FiO <sub>2</sub>	> 400	> 300 to ≤ 400	> 200 to ≤ 300	> 100 to ≤ 200	≤ 100
or SpO <sub>2</sub> /FiO <sub>2</sub>	> 512	> 357 to ≤ 512	> 214 to ≤ 357	< 89 to ≤ 214	≤ 89

BP: Blood pressure; E: Epinephrine; FiO<sub>2</sub>: Fraction of inspired oxygen; HE: Hepatic encephalopathy; INR: International normalized ratio; NE: Norepinephrine; PaO<sub>2</sub>: Partial pressure of arterial oxygen; RRT: Renal replacement therapy; SpO<sub>2</sub>: Pulse oximetric saturation.

**Table 3 Asian Pacific Association for the Study of the Liver Acute-on-Chronic Liver Failure score**

Points	Total bilirubin (mg/dL)	HE grade	INR	Lactate (mmol/L)	Creatinine (mg/dL)
1	< 15	0	< 1.8	< 1.5	< 0.7
2	15-25	I-II	1.8-2.5	1.5-2.5	0.7-1.5
3	> 25	III-IV	> 2.5	> 2.5	> 1.5

HE: Hepatic encephalopathy; INR: International normalized ratio.

**Table 4 Modified chronic liver failure–sequential organ failure assessment score**

Organ/systems	0	1	2	3	4
Liver (bilirubin, mg/dL)	< 1.2	≥ 1.2 to < 2.0	≥ 2.0 to < 6.0	≥ 6.0 to < 12.0	≥ 12.0
Kidney (creatinine, rise from baseline)	< 1.5 ×	1.5 to ≤ 2.0 ×	> 2.0 to ≤ 3 ×	> 3 ×	Need for RRT
Cerebral (HE grade)	0	I	II	III	IV
Coagulation (INR)	< 1.1	≥ 1.1 to ≤ 1.25	> 1.25 to < 1.5	≥ 1.5 to ≤ 2.5	> 2.5
Circulation (systolic BP)	Normal for age	< 5 <sup>th</sup> centile for age	NE < 0.5 μg/kg/min	NE > 0.5 μg/kg/min	NE > 0.5 μg/kg/min and 2 <sup>nd</sup> inotrope
Lungs					
PaO <sub>2</sub> /FiO <sub>2</sub>	> 400	> 300 to ≤ 400	> 200 to ≤ 300	> 100 to ≤ 200	≤ 100

BP: Blood pressure; HE: Hepatic encephalopathy; INR: International normalized ratio; NE: Norepinephrine; PaO<sub>2</sub>: Partial pressure of arterial oxygen; FiO<sub>2</sub>: Fraction of inspired oxygen; RRT: Renal replacement therapy.

found to have high LT-predictive specificity and sensitivity. The CLIF-SOFA system focuses on extrahepatic organ failure, but there were no patients with multiorgan failure in our study. Furthermore, we found that the total bilirubin level ranges were high in the AARC system. Based on these findings, we concluded that the CLIF-SOFA and AARC scoring systems need to be modified for use in children[16].

A previous study found acute kidney injury to occur in 22.6% of children with ACLF and to be associated with a poor prognosis[8]. In a study CANONIC criteria,

**Table 5 Modified Asian Pacific Association for the Study of the Liver Acute-on-Chronic Liver Failure score**

Points	Total bilirubin (mg/dL)	HE grade	INR	Lactate (mmol/L)	Creatinine (rise from baseline)
1	< 15	0	< 1.8	< 1.5	< 1.5 ×
2	15-25	I-II	1.8-2.5	1.5-2.5	1.5 to ≤ 3 ×
3	> 25	III-IV	> 2.5	> 2.5	> 3 × or need RRT

HE: Hepatic encephalopathy; INR: International normalized ratio; RRT: Renal replacement therapy.

20% of 99 patients with biliary atresia were determined to have developed ACLF. Sepsis and gastrointestinal bleeding were identified as the most common precipitants of ACLF. Moreover, the ACLF mortality rate was found to be 20% [9]. In a study conducted among pediatric ACLF patients in the United States, most of the included patients were found to have biliary atresia, while the mortality rate was calculated to be 22% in patients who required hospitalization. In addition, the creatinine and aspartate transaminase levels, the INR, and a positive blood culture on admission were all shown to be associated with the development of ACLF. In this study, the triggers of the underlying decompensation were identified as bleeding, ascites, and an altered mental status in a significant portion of patients [6]. Cholangitis is known to be the most common cause of hepatic decompensation in patients with biliary atresia. Due to it not being a primary parenchymal disease, experts from the APASL study group excluded biliary atresia from among the diseases said to cause ACLF. Additionally, the APASL does not consider extrahepatic causes to be trigger factors in relation to ACLF [15].

## BIOMARKERS

The treatment strategies for ACLF are mainly supportive. Biomarkers have previously been the subject of research concerned with predicting the prognosis of ACLF. These biomarkers aim to predict organ dysfunction at an early stage. Oxidative stress factors (*e.g.*, S100A12 and sRAGE), markers of cell death such as the caspase pathway proteins (which reflect the death of hepatocytes), and immune functions have been investigated in adults patients with ACLF. Unfortunately, the validity of such markers remains unknown [26]. Due to the role of infections in the etiopathogenesis of adult ACLF, the use of certain biomarkers, such as galactomannan or beta-d-glucan for invasive fungal infections and C-reactive protein and procalcitonin for bacterial infections, has been recommended by the APASL in relation to early diagnosis [15]. Renal complications are common in cases of ACLF. While hepatorenal syndrome improves following LT, acute tubular necrosis and structural acute kidney injury, which may cause permanent renal damage, require both LT and kidney transplantation. Thus, the use of new biomarkers of acute tubular necrosis (*e.g.*, N-GAL, Kim-1, IL-18, and 1-FABP) in ACLF may prove beneficial in terms of identifying an appropriate treatment approach [15, 27]. Finally, non-invasive tools and biomarkers developed to measure liver fibrosis may provide useful information when it comes to predicting the prognosis of ACLF.

## PROGNOSIS

Overall, patients with ACLF have a poor prognosis. The APASL emphasizes that ACLF should be recognized during the “golden therapeutic window” prior to the development of extrahepatic organ failure, which is associated with mortality [15]. Studies conducted in adults have reported ACLF mortality rates ranging from 33% to 50% [15, 21]. Pediatric cases of ACLF can be predicted to have better prognoses than adult cases for three main reasons: (1) There are specific treatments for the two most common causes of pediatric ACLF (WD and AIH); (2) Children are likely to have greater liver reserves; and (3) Children exhibit fewer comorbidities [15]. In two studies involving pediatric ACLF cases in two non-transplant centers, the 28-d and three-month mortality rates were reported to be 19.4% and 59%, respectively [1, 2]. In another study, the 28-d mortality and LT rates were reported to be 25% and 8.3%, respectively [3]. In a study conducted in the same center, the three-month mortality and LT rates were reported to be 30.4% and 8.9%, respectively [4]. In a study that used the Pediatric

Acute Liver Failure (PALF) study group's ALF criteria (rather than ACLF criteria), which only included children with the etiologies of AIH, WD, Budd-Chiari syndrome, inborn errors of metabolism affecting the liver, and HBV reactivation, some 59% of patients survived without LT[28]. In a prior study we conducted among 29 pediatric ACLF patients, 24.14% of patients required LT and no patients died[16]. Interestingly, the presence of acute kidney injury increases the likelihood of death or LT by 7.7 times when compared with those who do not develop acute kidney injury[8]. When comparing the mortality rate of ACLF with that of ALF, more than 50% of children with non-acetaminophen-induced ALF died or underwent LT in the PALF study[29].

## TREATMENT OF ACLF

There is no proven treatment for ACLF other than LT. The early recognition of ACLF and its precipitating events during the "golden therapeutic window period" prior to the development of extrahepatic organ failure is important in relation to the success of treatment[15]. ACLF treatments mainly include supportive treatments for hepatic and extrahepatic organ failure (if present). Extracorporeal liver support systems (e.g., the molecular adsorbent recirculating system and plasmapheresis) have long been used as a bridge to LT in both adults and children with liver failure. The purpose behind using such modalities is to improve the clinical situation (especially neurologically) and biochemical parameters. However, the efficacy of extracorporeal liver support systems in adult and childhood liver failure remains unclear[11,15,30]. Optimizing the extracorporeal liver support modalities in children may improve outcomes. A few adult studies have assessed the treatment of ACLF using granulocyte colony-stimulating factor (GCSF)[31,32]. It has been suggested that GCSF may reduce mortality through promoting hepatic regeneration by mobilizing the bone-marrow-derived CD34+ cells and reducing sepsis. In a pediatric study conducted in India, 5 mcg/kg/d GCSF therapy for five days was found to be ineffective in terms of improving survival outcomes[5].

## LT

Although ACLF is associated with high short-term mortality, a significant number of patients recover due to the use of medical and extracorporeal liver support systems[1-4]. The final treatment option is LT in patients who do not otherwise recover. There is no conclusive evidence concerning the efficacy of LT in children, although LT in adults with advanced ACLF has been found to result in good outcomes[13-15]. However, deciding on the timing of LT can be difficult. There are no data available concerning who would benefit from early LT, although the procedure should be performed prior to the development of multiple organ failure and advanced-stage encephalopathy. It may prove useful to use the scoring systems mentioned above when assessing the need for LT.

## CONCLUSION

A definition of ACLF in children has not yet been developed. The etiology of ACLF varies among geographical regions. Moreover, organ dysfunctions are seen less frequently in pediatric ACLF patients than in adult patients. However, the mortality rate associated with ACLF remains high. Although there is no proven scoring system for predicting the prognosis of ACLF, if the AARC system score is > 8-10, a poor prognosis is indicated.

## REFERENCES

- 1 **Jagadisan B**, Srivastava A, Yachha SK, Poddar U. Acute on chronic liver disease in children from the developing world: recognition and prognosis. *J Pediatr Gastroenterol Nutr* 2012; **54**: 77-82 [PMID: 21691224 DOI: 10.1097/MPG.0b013e318228d7da]
- 2 **Lal J**, Thapa BR, Rawal P, Ratho RK, Singh K. Predictors of outcome in acute-on-chronic liver failure in children. *Hepatol Int* 2011; **5**: 693-697 [PMID: 21484110 DOI: 10.1007/s12072-010-9217-z]

- 3 **Lal BB**, Sood V, Khanna R, Alam S. How to identify the need for liver transplantation in pediatric acute-on-chronic liver failure? *Hepatol Int* 2018; **12**: 552-559 [PMID: [30341639](#) DOI: [10.1007/s12072-018-9901-y](#)]
- 4 **Alam S**, Lal BB, Sood V, Rawat D. Pediatric Acute-on-Chronic Liver Failure in a Specialized Liver Unit: Prevalence, Profile, Outcome, and Predictive Factors. *J Pediatr Gastroenterol Nutr* 2016; **63**: 400-405 [PMID: [26967824](#) DOI: [10.1097/MPG.0000000000001179](#)]
- 5 **Sharma S**, Lal SB, Sachdeva M, Bhatia A, Varma N. Role of Granulocyte Colony Stimulating Factor on the Short-Term Outcome of Children with Acute on Chronic Liver Failure. *J Clin Exp Hepatol* 2020; **10**: 201-210 [PMID: [32405176](#) DOI: [10.1016/j.jceh.2019.10.001](#)]
- 6 **Banc-Husu AM**, Neighbors K, Rychlik K, Mohammad S, Harris ZL, Alonso EM. Admission Characteristics Identify Risk of Pediatric Acute-on-Chronic Liver Failure. *J Pediatr Gastroenterol Nutr* 2020; **70**: 783-788 [PMID: [32141996](#) DOI: [10.1097/MPG.0000000000002695](#)]
- 7 **Godfrey E**, Desai M, Lam F, Goss J, Rana A, Miloh T. Higher Waitlist Mortality in Pediatric Acute-on-chronic Liver Failure in the UNOS Database. *J Pediatr Gastroenterol Nutr* 2021; **72**: 80-87 [PMID: [32796428](#) DOI: [10.1097/MPG.0000000000002891](#)]
- 8 **Lal BB**, Alam S, Sood V, Rawat D, Khanna R. Profile, risk factors and outcome of acute kidney injury in paediatric acute-on-chronic liver failure. *Liver Int* 2018; **38**: 1777-1784 [PMID: [29325220](#) DOI: [10.1111/Liv.13693](#)]
- 9 **D'Souza R**, Grammatikopoulos T, Pradhan A, Sutton H, Douiri A, Davenport M, Verma A, Dhawan A. Acute-on-chronic liver failure in children with biliary atresia awaiting liver transplantation. *Pediatr Transplant* 2019; **23**: e13339 [PMID: [30597734](#) DOI: [10.1111/ptr.13339](#)]
- 10 **Jalan R**, Yurdaydin C, Bajaj JS, Acharya SK, Arroyo V, Lin HC, Gines P, Kim WR, Kamath PS; World Gastroenterology Organization Working Party. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology* 2014; **147**: 4-10 [PMID: [24853409](#) DOI: [10.1053/j.gastro.2014.05.005](#)]
- 11 **Squires RH**, Murray KF. Acute liver failure in children In: *Liver Disease in Children* 4th ed. Suchy FJ, Sokol RJ, WF B, editor. Cambridge: Cambridge University Press, 2014: 32-50
- 12 **Hsu EK**, Murray KF. Cirrhosis and chronic liver failure. In: *Liver Disease in Children* 4th ed. Suchy FJ, Sokol RJ, WF B, editor. Cambridge: Cambridge University Press, 2014: 51-67
- 13 **Moreau R**, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V; CANONIC Study Investigators of the EASL-CLIF Consortium. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437, 1437.e1 [PMID: [23474284](#) DOI: [10.1053/j.gastro.2013.02.042](#)]
- 14 **O'Leary JG**, Reddy KR, Garcia-Tsao G, Biggins SW, Wong F, Fallon MB, Subramanian RM, Kamath PS, Thuluvath P, Vargas HE, Maliakkal B, Tandon P, Lai J, Thacker LR, Bajaj JS. NACSELD acute-on-chronic liver failure (NACSELD-ACLF) score predicts 30-day survival in hospitalized patients with cirrhosis. *Hepatology* 2018; **67**: 2367-2374 [PMID: [29315693](#) DOI: [10.1002/hep.29773](#)]
- 15 **Sarin SK**, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, Saigal S, Saraf N, Soin AS, Devarbhavi H, Kim DJ, Dhiman RK, Duseja A, Taneja S, Eapen CE, Goel A, Ning Q, Chen T, Ma K, Duan Z, Yu C, Treeprasertsuk S, Hamid SS, Butt AS, Jafri W, Shukla A, Saraswat V, Tan SS, Sood A, Midha V, Goyal O, Ghazinyan H, Arora A, Hu J, Sahu M, Rao PN, Lee GH, Lim SG, Lesmana LA, Lesmana CR, Shah S, Prasad VGM, Payawal DA, Abbas Z, Dokmeci AK, Sollano JD, Carpio G, Shrestha A, Lau GK, Fazal Karim M, Shiha G, Gani R, Kalista KF, Yuen MF, Alam S, Khanna R, Sood V, Lal BB, Pamecha V, Jindal A, Rajan V, Arora V, Yokosuka O, Niriella MA, Li H, Qi X, Tanaka A, Mochida S, Chaudhuri DR, Gane E, Win KM, Chen WT, Rela M, Kapoor D, Rastogi A, Kale P, Sharma CB, Bajpai M, Singh V, Premkumar M, Maharashi S, Olithselvan A, Philips CA, Srivastava A, Yachha SK, Wani ZA, Thapa BR, Saraya A, Shalimar, Kumar A, Wadhawan M, Gupta S, Madan K, Sakhuja P, Vij V, Sharma BC, Garg H, Garg V, Kalal C, Anand L, Vyas T, Mathur RP, Kumar G, Jain P, Pasupuleti SSR, Chawla YK, Chowdhury A, Song DS, Yang JM, Yoon EL; APASL ACLF Research Consortium (AARC) for APASL ACLF working Party. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. *Hepatol Int* 2019; **13**: 353-390 [PMID: [31172417](#) DOI: [10.1007/s12072-019-09946-3](#)]
- 16 **Islek A**, Keskin H, Aksungur N, Ozturk G. Acute-on-Chronic Liver Failure in Children: A Single-Center Experience. *Exp Clin Transplant* 2021; **19**: 686-692 [PMID: [33663358](#) DOI: [10.6002/ect.2020.0264](#)]
- 17 **Sargenti K**, Prytz H, Nilsson E, Kalaitzakis E. Predictors of mortality among patients with compensated and decompensated liver cirrhosis: the role of bacterial infections and infection-related acute-on-chronic liver failure. *Scand J Gastroenterol* 2015; **50**: 875-883 [PMID: [25697824](#) DOI: [10.3109/00365521.2015.1017834](#)]
- 18 **Shi Y**, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, Zhang S, Xu Z, Wu Y, Yan H, Chen Z. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. *Hepatology* 2015; **62**: 232-242 [PMID: [25800029](#) DOI: [10.1002/hep.27795](#)]
- 19 **Li H**, Chen LY, Zhang NN, Li ST, Zeng B, Pavesi M, Amorós Á, Mookerjee RP, Xia Q, Xue F, Ma X, Hua J, Sheng L, Qiu DK, Xie Q, Foster GR, Dusheiko G, Moreau R, Gines P, Arroyo V, Jalan R. Characteristics, Diagnosis and Prognosis of Acute-on-Chronic Liver Failure in Cirrhosis Associated



- to Hepatitis B. *Sci Rep* 2016; **6**: 25487 [PMID: [27146801](#) DOI: [10.1038/srep25487](#)]
- 20 **Mahmud N**, Kaplan DE, Taddei TH, Goldberg DS. Incidence and Mortality of Acute-on-Chronic Liver Failure Using Two Definitions in Patients with Compensated Cirrhosis. *Hepatology* 2019; **69**: 2150-2163 [PMID: [30615211](#) DOI: [10.1002/hep.30494](#)]
  - 21 **Arroyo V**, Moreau R, Jalan R. Acute-on-Chronic Liver Failure. *N Engl J Med* 2020; **382**: 2137-2145 [PMID: [32459924](#) DOI: [10.1056/NEJMra1914900](#)]
  - 22 **Clària J**, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, Amorós À, Titos E, Alcaraz-Quiles J, Oetli K, Morales-Ruiz M, Angeli P, Domenicali M, Alessandria C, Gerbes A, Wendon J, Nevens F, Trebicka J, Laleman W, Saliba F, Welzel TM, Albillos A, Gustot T, Benten D, Durand F, Ginès P, Bernardi M, Arroyo V; CANONIC Study Investigators of the EASL-CLIF Consortium and the European Foundation for the Study of Chronic Liver Failure (EF-CLIF). Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. *Hepatology* 2016; **64**: 1249-1264 [PMID: [27483394](#) DOI: [10.1002/hep.28740](#)]
  - 23 **Lucey MR**, Mathurin P, Morgan TR. Alcoholic hepatitis. *N Engl J Med* 2009; **360**: 2758-2769 [PMID: [19553649](#) DOI: [10.1056/NEJMra0805786](#)]
  - 24 **Cárdenas A**, Ginès P, Uriz J, Bessa X, Salmerón JM, Mas A, Ortega R, Calahorra B, De Las Heras D, Bosch J, Arroyo V, Rodés J. Renal failure after upper gastrointestinal bleeding in cirrhosis: incidence, clinical course, predictive factors, and short-term prognosis. *Hepatology* 2001; **34**: 671-676 [PMID: [11584362](#) DOI: [10.1053/jhep.2001.27830](#)]
  - 25 **Anand L**, Choudhury A, Bihari C, Sharma BC, Kumar M, Maiwall R, Siam Tan S, Shah SR, Hamid S, Butt AS, Jafri W, Chawla YK, Taneja S, Duseja A, Dhiman RK, Mahtab MA, Ghazinyan H, Duan Z, Chen Y, Shukla A, Hu J, Abbas Z, Treeprasertsuk S, Lesmana LA, Lesmana CR, Sollano JD, Carpio G, Sahu MK, Kumar G, Sarin SK; APASL ACLF (APASL ACLF Research Consortium) Working Party. Flare of Autoimmune Hepatitis Causing Acute on Chronic Liver Failure: Diagnosis and Response to Corticosteroid Therapy. *Hepatology* 2019; **70**: 587-596 [PMID: [30113706](#) DOI: [10.1002/hep.30205](#)]
  - 26 **Mookerjee RP**. Prognosis and Biomarkers in Acute-on-Chronic Liver Failure. *Semin Liver Dis* 2016; **36**: 127-132 [PMID: [27172354](#) DOI: [10.1055/s-0036-1583200](#)]
  - 27 **Wan ZH**, Wang JJ, You SL, Liu HL, Zhu B, Zang H, Li C, Chen J, Xin SJ. Cystatin C is a biomarker for predicting acute kidney injury in patients with acute-on-chronic liver failure. *World J Gastroenterol* 2013; **19**: 9432-9438 [PMID: [24409073](#) DOI: [10.3748/wjg.v19.i48.9432](#)]
  - 28 **Di Giorgio A**, Nicastro E, Dalla Rosa D, Nebbia G, Sonzogni A, D'Antiga L. Transplant-free Survival in Chronic Liver Disease Presenting as Acute Liver Failure in Childhood. *Transplantation* 2019; **103**: 544-551 [PMID: [30028785](#) DOI: [10.1097/TP.0000000000002367](#)]
  - 29 **Squires RH Jr**, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, Dhawan A, Rosenthal P, Rodriguez-Baez N, Murray KF, Horslen S, Martin MG, Lopez MJ, Soriano H, McGuire BM, Jonas MM, Yazigi N, Shepherd RW, Schwarz K, Lobritto S, Thomas DW, Lavine JE, Karpen S, Ng V, Kelly D, Simonds N, Hynan LS. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. *J Pediatr* 2006; **148**: 652-658 [PMID: [16737880](#) DOI: [10.1016/j.jpeds.2005.12.051](#)]
  - 30 **Kjaergard LL**, Liu J, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. *JAMA* 2003; **289**: 217-222 [PMID: [12517233](#) DOI: [10.1001/jama.289.2.217](#)]
  - 31 **Garg H**, Kumar A, Garg V, Sharma P, Sharma BC, Sarin SK. Clinical profile and predictors of mortality in patients of acute-on-chronic liver failure. *Dig Liver Dis* 2012; **44**: 166-171 [PMID: [21978580](#) DOI: [10.1016/j.dld.2011.08.029](#)]
  - 32 **Duan XZ**, Liu FF, Tong JJ, Yang HZ, Chen J, Liu XY, Mao YL, Xin SJ, Hu JH. Granulocyte-colony stimulating factor therapy improves survival in patients with hepatitis B virus-associated acute-on-chronic liver failure. *World J Gastroenterol* 2013; **19**: 1104-1110 [PMID: [23467275](#) DOI: [10.3748/wjg.v19.i7.1104](#)]

## Coronavirus disease 2019 in liver transplant patients: Clinical and therapeutic aspects

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### Abstract

The coronavirus disease 2019 (COVID-19) pandemic has profoundly impacted liver transplant (LT) activity across the world, with notable decreases in the number of donations and procedures in most Western countries, in particular throughout the first wave. The cumulative incidence of COVID-19 in LT recipients (with estimates ranging from 0.34% to 1.56%) appears to be at least comparable to that observed for the general population. Clinical and radiological features at presentation are also similar to non-transplant patients. The risk of death among LT recipients requiring hospital admission is high (from 12% to 19%), although some authors have suggested that overall mortality may be actually lower compared to the general non-transplant population. It is likely that these poor outcomes may be mainly influenced by the older age and higher comorbidity burden of LT recipients, rather than by the transplant status itself. In fact, it has been hypothesized that post-transplant immunosuppression would exert a protective role, with special focus on tacrolimus-containing regimens. There is scarce evidence to guide the optimal management of post-transplant COVID-19 and the use of antiviral or immunomodulatory therapies, although both clinical practice and guidelines support the dose reduction or withdrawal of anti-proliferative agents such as mofetil mycophenolate. Preliminary reports suggest that the antibody response to messenger RNA vaccines is significantly impaired as compared to non-immunocompromised individuals, in line with other transplant populations. Finally, it is foreseeable that the future will be conditioned by the emerging variants of severe acute respiratory syndrome coronavirus 2 with

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increased transmissibility among LT recipients.

**Key Words:** COVID-19; Liver transplantation; Clinical features; Therapy; Immunosuppression; SARS-CoV-2

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**Core Tip:** Coronavirus disease 2019 incidence and clinical and radiological features are similar in liver transplant recipients and the general population. Reported mortality in hospitalized patients is 12%-19%. Risk factors are older age and comorbidity. Tacrolimus could be protective, but anti-proliferative agents such as mycophenolate mofetil should be avoided.

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## INTRODUCTION

The coronavirus disease 2019 (COVID-19) produced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China in December 2019[1,2]. The initial outbreak rapidly spread all over the world, being declared a pandemic by the World Health Organization by March 11, 2020, with 118000 cases declared in 114 countries and 4291 deaths at that time[3]. The pandemic has now affected more than 172 million people and has reached a death toll exceeding 3.7 million[4,5].

Liver transplant (LT) recipients are considered susceptible to infectious complications due to their long-term immunosuppression (IS)[6]. At the time COVID-19 was first described, the potential impact of this emerging condition on this patient population was unpredictable. Previous experiences with related coronaviruses, such as SARS-CoV or Middle East respiratory syndrome coronavirus (MERS-CoV), did not clearly show an increased incidence or case-fatality rate among immunocompromised patients[7,8]. A systematic review and meta-analysis that summarized the literature available between January and April 2020 identified hypertension, diabetes, cardiovascular disease, chronic obstructive pulmonary disease, malignancy, cerebrovascular disease, and human immunodeficiency virus infection as risk factors for severe COVID-19 in the Chinese population. Of note, chronic liver disease was not identified in this preliminary study[9]. Nevertheless, a systematic review focused on solid organ transplantation (SOT), which pooled 60 studies from January to October 2020 and 2772 unique patients, including 505 LT recipients, revealed high rates of both hospitalization (81.0%) and all-cause mortality (18.6%)[10].

In the present review, we summarize the current experience regarding COVID-19 in LT recipients, with particular focus on clinical and therapeutic aspects. Early experiences from different locations all over the world led to the scientific societies to develop guidelines for the management of these patients. This pandemic has exerted a deep impact on the transplant activity. There remain concerns about the medium- and long-term outcomes of infected recipients as well as on the optimal management of IS.

## EARLY EXPERIENCES

On April 19, 2020 it was reported from Wuhan a 50-year-old male patient that had undergone LT in 2017 and developed SARS-CoV-2 pneumonia with mild respiratory failure by the end of January. Tacrolimus was restarted 4 wk later, with normal liver function. The authors suggested that reduction or temporary withdrawal of IS may be beneficial for the reconstitution of the immune response[11]. Huang *et al*[12] subsequently reported a second 59-year-old LT recipient that died on 45 d of

admission due to multiorgan failure in the setting of suspected chronic rejection and septic shock.

During March 2020, three long-term (> 10 years) LT recipients that were receiving low-dose IS and rapidly developed acute respiratory distress syndrome (ARDS) requiring mechanical ventilation died at the Istituto Nazionale dei Tumori di Milano between 3 and 12 d after the onset of symptoms. Three other recipients that developed COVID-19 less than 2 years from transplantation had an uneventful disease. This led the authors to suggest that post-transplant IS might be protective, whereas metabolic-related comorbidities would be associated with an increased risk of severe infection [13].

Six LT recipients from our institution had been admitted by March 23, 2020. Two of them died due to ARDS associated to renal failure and refractory shock, respectively. Both patients were receiving mycophenolate mofetil (MMF) at admission, associated to everolimus in the first case. Two further LT recipients were treated as outpatients. Two patients were temporarily converted to tacrolimus, MMF was halted in one patient, and no modifications were made in the remaining three [14].

Some of the earliest cases of post-transplant COVID-19 from the United States were reported on March 22, 2020. These 4 cases included a 67-year-old man that had undergone LT 19 years before. The patient was initially admitted to the intensive care unit (ICU), cyclosporine therapy was continued without adjustment, and he was discharged home after 6 d [15]. A report from New York City described the initial experience at two centers during the first weeks of the outbreak, including 13 LT recipients, four of them with severe disease. Sixteen out of 90 SOT recipients died, resulting in an overall case-fatality rate of 18%, 24% for hospitalized patients and 52% for those admitted to the ICU [16].

Shortly after the outbreak of the pandemic, first experiences with recent transplant recipients started to be reported. For instance, a 69-year-old patient admitted for LT on January 28, 2020 in Iran became febrile on post-transplant day 4, being diagnosed with hospital-acquired pneumonia. He developed respiratory failure and loss of consciousness on day 9. A brain computerized tomography (CT) scan revealed a hypodensity in the right parietal lobe suggestive of middle cerebral artery ischemic stroke. The patient died on day 23 after transplantation, with SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) being reported positive on the next day [17]. Qin *et al* [18] reported a 37-year-old male patient that underwent LT on January 21, 2020. He started with persistent fever on post-transplant day 9, and a thoracic CT scan revealed minor changes. A second scan performed 9 d later showed multiple ground glass opacification in the left lobes. Tacrolimus and steroids were maintained though titrated to lower doses, and supplemental oxygen therapy through high-flow nasal cannula maintained oxygen saturation ranging from 95% to 99%. The patient was successfully discharged 51 d after transplantation [18].

## COVID-19 AND THE LIVER

Although COVID-19 is primarily a respiratory disease, SARS-CoV-2 may also infect the digestive system through its viral receptor angiotensin-converting enzyme 2 (ACE2). The ACE2 cell surface receptor is more strongly expressed in cholangiocytes, at a similar level in fact than type 2 alveolar cells in the lungs, than hepatocytes (59.7% *vs* 2.6%, respectively) [19]. Increased transaminases is a common laboratory finding in COVID-19, and liver injury has been associated to drug-induced liver toxicity, systemic hyperinflammatory response, or hypoxia-ischemia reperfusion injury [20], rather than direct viral cytopathic effect [21]. Coagulopathy and liver endotheliopathy have been suggested to be at least partially driven by interleukin (IL)-6 trans-signaling, which would lead to the expression of procoagulant (such as factor VIII or von Willebrand factor) and proinflammatory factors as well as increased platelet attachment in liver sinusoidal endothelial cells. Interestingly, these effects were blocked by soluble gp130, which acts as an IL-6 trans-signaling inhibitor, and the janus kinase inhibitor ruxolitinib, providing support for these therapeutic approaches [22]. Histopathologic features suggestive of some level of cytopathic injury, however, have been also observed in liver biopsies [23].

Cai *et al* [24] reported in a large cohort that individuals with abnormal liver tests were at a higher risk of progression to severe COVID-19. Abnormal liver function was observed in 76.3% of patients, with 21.5% of them developing liver injury. The detrimental effect on liver function was mainly related to therapies used during hospitalization, which should be closely monitored and evaluated.

In a retrospective study from Wuhan, 1282 out of 2073 patients (61.8%) had abnormal liver function test during hospitalization, and 14.3% experienced some degree of liver injury. Increased aspartate aminotransferase (AST) and direct bilirubin levels at admission were independent predictors of all-cause mortality, whereas the presence of hepatitis B virus infection did not increase the risk of poor outcome[25].

In a retrospective cohort comprising 234 patients hospitalized in two referral hospitals in France, the rate of abnormal liver function tests at admission was as high as 66.6% and was associated with in-hospital aggravation [odds ratio (OR): 4.1; 95% confidence interval (CI): 1.5-10.8;  $P = 0.004$ ] and mortality (OR: 3.3; 95%CI: 1.04-10.5;  $P = 0.04$ ). A minority of patients (3.8%) had underlying liver disease, and there were no significant differences in the prevalence of alcohol consumption or metabolic syndrome between patients with or without abnormal liver tests on admission, suggesting that this finding may be COVID-19-related and not due to pre-existing liver disease[26].

In a retrospective cohort from New York that included 2273 patients, acute liver injury was common and categorized as mild [alanine transaminase (ALT) levels < 2 times the upper limit of normal (ULN)] in 45% of the cases, moderate (ALT levels two to five times the ULN) in 21%, and severe (ALT levels > 5 times the ULN) in 6.4%. In the multivariate analysis adjusted for age, body mass index, comorbidities, and requirement of invasive mechanical ventilation (IMV) and renal replacement therapy, peak ALT levels were significantly associated with death or discharge to hospice (OR: 1.14;  $P = 0.044$ )[27].

Underlying cirrhosis has been identified as a risk factor for increased severity of COVID-19, with mortality rates ranging from 12% to 43%[28]. Indeed, SARS-CoV-2 may produce acute-on-chronic liver failure (ACLF) among cirrhotic patients[29]. The mortality in 20 patients with ACLF reported from India reached 30%, as compared to 5% among cirrhotic patients without ACLF[30]. Metabolic dysfunction-associated fatty liver disease has been also associated with the severity of SARS-CoV-2 infection in patients below 60 years (OR: 4.07; 95%CI: 1.20-13.79;  $P = 0.02$ )[31].

In the earlier post-mortem examinations, Xu *et al*[32] found moderate microvesicular steatosis and mild inflammatory infiltrates in the hepatic lobule and portal tract. Mild sinusoidal dilatation, focal macrovesicular steatosis, and mild lobular lymphocytic infiltration has been also reported[33]. Fiel *et al*[23] described the biopsies of two patients that successfully recovered from COVID-19, showing a mixed inflammatory infiltrate with prominent bile duct damage, endothelitis, and numerous apoptotic bodies. *In situ* hybridization and electron microscopy suggested the intrahepatic presence of SARS-CoV-2, thus supporting the possibility of a direct cell injury.

Macrovesicular steatosis was the most common finding (75%) in 40 liver biopsies from patients that died due to a complicated COVID-19 course. Mild lobular necroinflammation and portal inflammation were present in 20 cases each (50%), whereas viral RNA was detected by RT-PCR on liver tissue in 55% of patients tested[34].

Both the diagnosis and treatment of cancer have been negatively affected by the COVID-19 pandemic and the resulting pressure on the health care services worldwide. Patients with hepatocellular carcinoma (HCC) represent a vulnerable population with a significant treatment delay. In a multicenter, retrospective study performed in Paris, Amaddeo *et al*[35] found a significant decrease in the number of patients with HCC presented to the multidisciplinary tumor committee. The proportion of patients that experienced a treatment delay longer than 1 mo increased between 2019 and 2020 from 9.5% to 21.5%.

## IMPACT OF COVID-19 ON LT ACTIVITY

The effect of the pandemic has been heterogeneous in terms of donation and transplant activity. Nevertheless, a notable reduction has been reported from most institutions across Europe and North America during the peak of COVID-19 incidence, mainly related to the burden of patients admitted to the ICU and the associated effects on candidate referral and perioperative care[36]. Such a decrease in LT activity was particularly profound in March and April 2020, during the first wave that affected many Western countries. De Simone described the reorganization of LT units carried out in so many centers worldwide during the first wave: Cancellation of routine patient follow-up, outpatient care limited to recent LT recipients, pre-transplant referral limited to priority patients after telephone triage, follow-up by means of phone calls on the waiting list, and implementation of health care worker (HCW) safety



policies[36]. This rapid reorganization allowed for maintaining the activity of a high-volume center in Pisa during the Italian national lockdown (February 18 to May 4, 2020), despite the marked drop observed between March 16 and April 5. This was achieved due to the increase in ICU bed capacity, systematic screening for SARS-CoV-2, creation of COVID-19-dedicated ICUs, recruitment of additional medical and nurse staff, rescheduling of elective surgery to priority cases, and continuation of LT activities in COVID-19-free areas[37].

A preliminary analysis of the impact on Italian LT programs was done by means of a survey issued on March 16, 2020 and completed by 22 centers[38]. There were two major geographical areas with different incidence of SARS-CoV-2 infection, north-central Italy and south-central Italy. Between February 15 and March 15, all transplant programs reduced their outpatient activity by 68% in terms of pre-transplant evaluation and 100% in the post-transplant face-to-face follow-up. A reduction in transplant activity was also seen in northern-central Italy during the first 2 wk of March, but not in the southern-central area. Recovered donors dropped by 46% during the first peak (the 4-wk period after February 23) as compared to the preceding 8-wk period[39].

In Spain, according to data provided by the Spanish National Transplant Organization [Organización Nacional de Trasplantes (ONT)], the mean number of donors declined since the national state of alarm was declared on March 13 from 7.2 to 1.2 per day, and the mean number of transplants from 16.1 to 2.1 per day[40]. There was a saturation of the health care system and ICU capacities (although most hospitals had increased the number of ICU beds), and many HCWs became infected (15.5% of the infected population at that time) or forced to quarantine. The number of potential donors declined due to the decrease in neurocritical patients or due to a positive result in SARS-CoV-2 screening. In addition, logistical problems arose as a consequence of the restricted mobility and declining organ offers following a risk assessment that included the clinical situation of the recipients, and even human resources were reduced due to cases of COVID-19 among HCWs. Finally, in the pandemic scenario, some candidates refused transplantation after informed consent[40].

The impact of the first wave on the LT activity in France resulted in an overall 28% decrease in the number of donations when comparing the first 4 mo of 2019 with the corresponding period of 2020, whereas the number of LT effectively performed dropped by 22%. The north-eastern region of the country (with the highest incidence rate of COVID-19) experienced reductions in multiorgan procurement and LT activity of 33% and 26%, respectively[41].

A national state of emergency was declared in the United States by March 13, 2020. A retrospective analysis of data collected from January 5 to September 5, 2020 by the Organ Procurement and Transplantation Network revealed a decrease of 37% in the number of LT procedures performed between March 8 and April 5[42]. Since mid-March, many waitlist patients were placed in temporarily inactive status due to COVID-19 concerns. This practice affected over 2000 waitlist registrations during the week of March 22. LiveOnNY, the organ procurement organization for the greater New York metropolitan area, suffered a drop to 10 donors in April 2020 from 26 in March, although this figure recovered to 18 donors in May[43].

A multinational study performed in India, the United Kingdom, and the United States compared the weekly organ donation and LT numbers over a 3-mo period (February 17 to May 17, 2020) and the LT activity in six centers with varying local COVID-19 caseload[44]. Peak reduction ranged from 25% in the United States to more than 80% in the United Kingdom and India.

On the contrary, the impact of COVID-19 on LT activity has been reported to be almost negligible in other countries. Lee concluded that establishing safe processes and procedures can be beneficial in reducing the negative effects of the national lockdown and saving patients' lives, as he analyzed LT procedures performed in South Korea[45]. He compared the MERS outbreak, the COVID-19 pandemic, and the average number of LT performed throughout the prior 5 years. There was a significant decrease of 11% in the LT activity during the MERS outbreak, although the number of procedures was maintained from January to March 2020. In addition, none of the 401 patients undergoing LT during the COVID-19 outbreak were confirmed to be infected with SARS-CoV-2. Some Italian centers located in medium- or high-incidence areas were also able to maintain a stable LT activity by means of appropriate screening and isolation practices, dedicated COVID-19-free routes, and reorganization of ICU resources[36,46,47].

A great variability in the adaptation of LT practices in response to the COVID-19 pandemic has been observed within the same country and even the same region[48]. On the other hand, the detrimental impact on LT activity seems to have been not

restricted to those areas facing the highest COVID-19 burden. According to Agopian *et al*[48] such differences across centers likely reflect variations in the allocation and prioritization of hospital resources, local capacities to timely screen for SARS-CoV-2 infection among SOT candidates and recipients, and concerns with respect to donors (*e.g.*, accuracy of testing), recipients (*e.g.*, role of baseline IS), and transplant team members (*e.g.*, risk of hospital-acquired COVID-19).

The effect on the LT waiting list in the United States has been studied by Strauss *et al*[49] using data from the Scientific Registry of Transplant Recipients. From March 15 to April 30, new listings were 11% lower than expected, and deceased donor LTs (DDLTs) decreased by 9%. In May, new listings were 21% lower and living donor LTs were 42% lower, whereas DDLTs increased by 13%. In states with the highest incidence of COVID-19, the number of deaths in the waiting list increased by 59%. By August, waitlist outcomes were occurring at expected rates except for DDLT. According to the authors, these results reflect the adaptability of the transplant community in addressing the COVID-19 pandemic and applying new knowledge to patient care.

Putzer *et al*[50] found a 29% decrease in the number of LT procedures performed in the Eurotransplant area between mid-March and mid-June 2020, with regards to the corresponding periods from 2015 to 2019. Of note, the activity in Germany continued at the same pace during the initial phase of the crisis, likely thanks to the higher number of ICU beds in that country. However, the number of LTs increased slowly compared to the first month of observation.

## INCIDENCE OF COVID-19 IN LT RECIPIENTS

According to the survey performed by the European Liver and Intestine Transplantation Association (ELITA) and the European Liver Transplant Registry (ELTR), the crude incidence of SARS-CoV-2 infection among LT candidates and recipients during the first wave in Europe has been overall estimated in 1.05% (range: 0.5%-20%) and 0.34% (range: 0.1%-4.8%), respectively[51]. One hundred nine out of 149 (73.2%) ELTR centers located in 28 European countries responded to the survey. Eighty-eight centers reported the diagnosis of COVID-19 in 57 LT candidates and 272 recipients. The highest numbers of infected recipients were reported from Spain (77), Italy (66), and France (59). Crude case fatality rates in candidates and recipients were 18% and 15%, respectively. The authors concluded that both LT candidates and recipients are at high risk of COVID-19 and highlighted the need for an early and proactive screening for SARS-CoV-2 infection in these populations.

Cumulative incidence of COVID-19 has been highly variable across European countries. The King's College group only reported 5 cases out of about 4500 LT recipients (0.1%) followed-up in their institutional cohort during the first wave[52]. In fact, LT recipients appeared to have a lower incidence of COVID-19, with less severe symptoms, as compared to the general population or other SOT populations, likely due to the better individual adherence to self-isolation recommendations or the optimal level of IS, which would favorably modulate the response against SARS-CoV-2.

A nationwide study promoted by the Spanish Liver Transplantation Society (SETH) recruited 111 LT patients from February 28 to April, 7 2020 and revealed a higher incidence of COVID-19 compared to the general population, almost doubling the expected number of cases[53]. A preliminary experience from our institution showed a cumulative incidence from March 15 to May 5 of 1.6% (19 out of 1200) among LT recipients compared to 0.95% in the general population of Madrid, although potential underreporting due to limited diagnostic capacities at that time could not be ruled out [54].

A detailed study carried out in the United Kingdom comprised SOT recipients diagnosed with SARS-CoV-2 infection in England up to May 20, 2020 and showed a cumulative incidence of 1.3% and 0.7% (64 out of 8734) for the specific group of LT recipients[55].

As the pandemic evolved during 2020, different institutions and groups have provided updated epidemiological data. On the basis of data collected by the Italian Information Transplant System until June 22, Trapani *et al*[56] found a cumulative incidence of 1.02% among SOT recipients as compared to 0.4% in the non-transplant population ( $P < 0.05$ ). This figure was lower (0.63%) for LT recipients. Authors from the Shiraz University of Medical Sciences in Iran, one of the largest transplant centers in the world, published their results by mid-July[57]. They found 85 cases of COVID-

19 among abdominal transplant recipients (66 in LT recipients). As of July 2020, 0.32% of the population of the country was infected, with a mortality rate of 5.1%. Among 6969 SOT recipients followed-up at their center, 85 (1.21%) had been diagnosed with COVID-19, and 17 (20%) had died. Their conclusion was that LT and kidney transplant recipients face a poorer outcome due to COVID-19.

Not surprisingly, cumulative incidence has steadily increased over the last months, reflecting variations in the epidemiology of COVID-19 in the general population. In our institution, we have registered 67 cases of SARS-CoV-2 infection by the end of January 2021, accounting for more than 5% of followed-up LT recipients (data not published).

## RISK FACTORS FOR SEVERE COVID-19 IN LT RECIPIENTS

In an early retrospective, multicenter cohort study, Zhou *et al*[58] reported detailed clinical course and risk factors for mortality in 191 non-transplant patients with COVID-19 from Wuhan that had been discharged or died by January 31, 2020. Hypertension (30%), diabetes (19%) and coronary heart disease (8%) were the most common comorbidities in the general population. The authors found that older age, higher Sequential Organ Failure Assessment score and D-dimer levels above 1 µg/mL on admission were associated with in-hospital death at multivariable regression.

Mainly reflecting the risk factors identified in the general population, older age, the presence of chronic comorbidities (congestive heart failure, chronic obstructive pulmonary disease, or obesity), lymphopenia (absolute lymphocyte count  $< 0.5 \times 10^9$  cells/L), and abnormal chest imaging at admission were independently associated with mortality (20.5%) in a cohort study comprising 482 SOT recipients (73 LT recipients) from more than 50 United States centers[59].

Preliminary data from the ELITA/ELTR registry on 103 LT recipients diagnosed with COVID-19 between March 1 and April 24, 2020 revealed the following comorbidities: Overweight (56%), hypertension (51%), diabetes (41%), chronic renal impairment (serum creatinine level  $> 2$  mg/dL) (15%), smoking history (13%), and coronary artery disease (7%). After a median follow-up of 18 d, overall all-cause mortality rate was 16%, but it reached 22% among patients  $\geq 60$  years and 44% in those requiring IMV[60]. Although the difference did not achieve statistical significance, mortality was found to be lower among patients that had undergone LT within the previous 2 years as compared to those with longer intervals since transplantation (5% vs 18%). Of note, all deaths occurred among patients aged 60 years or older.

In the SETH study the most common comorbidity was hypertension (57.7%), whereas risk factors for severe COVID-19 among hospitalized patients included Charlson comorbidity index, male gender, dyspnea at diagnosis, and baseline immunosuppression containing MMF, particularly at doses higher than 1000 mg/d [53].

The assessment of SARS-CoV-2-attributable mortality after LT must take into account the impact of baseline conditions. A multicenter study from the COVID-Hep and SECURE-Cirrhosis international registries performed between March 25 and June 26, 2020 compared the outcomes of 151 adult LT recipients and 627 patients with SARS-CoV-2 infection who had not undergone transplantation. Older age, serum creatinine levels, and non-liver cancer were associated with mortality. In a propensity score-matched analysis (adjusted for age, sex, major comorbidities, and ethnicity), LT did not significantly increase the risk of death in patients (absolute risk difference: 14%; 95%CI: -7.7-10.4)[61]. Similar findings have been also reported for kidney transplant recipients[62].

## COVID-19 PRESENTATION IN THE SETTING OF LT

There is a male predominance across different series of LT recipients with COVID-19, from 68%[61] to 78.8%[57]. Median age in adult patients ranges from 60[59] to 65 years [53,60]. Low-grade fever was the most frequent symptom in the earlier reports from Wuhan, followed by cough, fatigue, myalgia, and digestive symptoms (diarrhea, nausea, or vomiting)[58]. Among LT recipients with COVID-19, the presence of fever is also reported in 62.7%[57] to 79%[62] of cases. Cough (with rates ranging from 40.9% [57] to 70.3%[52]), myalgia (37%[60] to 45.5%[57]), fatigue (40.9%[57] to 56%[62]), dyspnea (30.3%[57] to 46%[63]), gastrointestinal symptoms (22.6%[60] to 39.4%[57]), and smell and taste disorders (7%[63]) are also common at presentation.

Becchetti *et al*[63] observed a higher prevalence of fever and dyspnea in long-term LT recipients (more than 10 years from the procedure), whereas the presence of fever and cough was significantly less likely among very short-term recipients ( $\leq 1$  year) [63]. Asymptomatic patients are scarce. In the SETH series they accounted for 6.3% of cases only, whereas most of the patients admitted to the hospital (66%) required some type of respiratory support[52].

Chest X-ray or computed tomography scan showed typical features of COVID-19 in 62% of patients in the series by Belli *et al*[60] and 78.4% in the SETH cohort (unilateral in 19.8% and bilateral in 58.6%)[52]. Becchetti *et al*[63] reported typical radiological features (bilateral, peripheral, consolidation, or ground glass opacities) in 43% of computed tomography scans and 40% of X-ray examinations performed[63].

Only 8% of the patients reported by Becchetti *et al*[63] had a significant increase in transaminases (AST and/or ALT  $> 2$  times the ULN), whereas this figure reached 14.7% in the series by Colmenero *et al*[53]. Mean lymphocyte and platelet counts were decreased in patients with severe disease. Lymphopenia was present in 68.8% of the patients reported by Malekhosseini *et al*[57] and 76% of those reported by Becchetti *et al*[63]. The nadir of absolute lymphocyte count during hospital stay was  $0.31 \times 10^9$  cells/L among severe cases (versus  $0.5 \times 10^9$  cells/L in the non-severe forms of infection;  $P = 0.013$ ). Other markers as D-dimers or ferritin levels were significantly higher in severe cases[53], although data were not available for most patients[63].

## OUTCOME IN LT RECIPIENTS WITH COVID-19

The percentage of mild cases managed as outpatients varied in different series from 13.5%[53] to 42.4%[57]. Most of the published cohorts reported rates of hospitalization in the range of 66% to 82%[60,61,63], with a mean hospital stay of 9-10 d[57,63]. Notable variation was observed in the proportion of ICU admission (from 10%[63] to 31.6%[57] of hospitalized patients), which likely reflect regional differences in the availability of critical care resources. Regarding respiratory support, invasive or non-invasive mechanical ventilation was used in 10%[63] to 20%[61] of recipients, including extracorporeal membrane oxygenation in 10.6% of the patients in one series [57].

Reported mortality rates ranged between 12%[61] and 19%[61], close to those observed in large series in the general population (15-21%)[1,64]. Colmenero *et al*[53] showed that, after adjusting for age and gender, the number of observed deaths among LT patients was slightly lower than expected in the general population, resulting in a standardized mortality ratio of 95.55 (95%CI: 94.25-96.85).

Four out of 5 patients that contracted COVID-19 within the first month after transplantation in Shiraz died[57]. The authors attributed this dismal outcome to the higher amount of IS given during the very early post-transplant period. On the other hand, there are several reports on successful recovery in patients diagnosed with SARS-CoV-2 infection very shortly after LT[65-69].

Bhoori *et al*[13] were the first to suggest that long-term LT survivors on minimal IS therapy would face a greater risk of death following COVID-19 infection, thus proposing that a higher IS level could play a protective role. A systematic review pooling outcomes of 223 LT recipients from case-series and cohorts published up to June 15, 2020, however, revealed no significant differences in mortality rates between recent ( $< 2$  years) and remote ( $\geq 2$  years) LT recipients (16.7% *vs* 21.9%, respectively;  $P = 0.5$ )[70].

## THERAPEUTIC APPROACHES IN LT RECIPIENTS WITH COVID-19

### Antiviral therapies

Most LT recipients included in the series reported during the first pandemic wave were treated with repurposed drugs with *in vitro* activity against SARS-CoV-2, despite the lack of supporting clinical evidence at that time. For instance, the use of hydroxy-chloroquine (HCQ) (66%), azythromycin (33%), and lopinavir/ritonavir (LPV/r) (17%) was common among LT recipients recruited in the ELITA/ELTR registry between March 1 and April 24, 2020[60]. These rates were even higher in the SETH registry, with as many as 88% and 40% of patients receiving HCQ and LPV/r, respectively[53]. Of note, no differences in the use of these agents were observed according to the severity of COVID-19. In addition, the multicenter registry collected by the ONT in Spain showed that the proportion of recipients treated with protease inhibitors



(mainly LPV/r), HCQ, and azithromycin was similar across different SOT populations, suggesting that the therapeutic approach in LT recipients did not substantially differ from that used in patients usually exposed to a higher level of IS, such as heart or lung transplant recipients[71]. As expected, the management of drug-to-drug interactions between LPV/r, a potent cytochrome P450 3A4 inhibitor, and calcineurin or mammalian target of rapamycin (mTOR) inhibitors was particularly challenging[16,72]. In our experience, two LT recipients under everolimus were converted to low-dose prolonged-release tacrolimus (0.5 mg/wk) in order to facilitate the adjustment of IS during hospitalization[54].

No outcome benefit has been demonstrated from the use of LPV/r, HCQ, or subcutaneous interferon- $\beta$  in the setting of randomized controlled trials (RCTs) conducted over the past months[73-75]. The RNA-dependent RNA polymerase inhibitor remdesivir is the only antiviral agent currently approved for the treatment of COVID-19, in view of the shorter time to clinical recovery obtained with this agent as compared to placebo[76]. The clinical experience with remdesivir in LT recipients, nevertheless, is scarce, with only a few treated patients in large multicenter cohorts[53, 71]. Since remdesivir and its main active metabolite GS-441524 are mainly excreted by the kidney, no major drug-to-drug interactions with tacrolimus, MMF, or mTOR inhibitors are to be expected, whereas limited experience with cirrhotic patients has revealed no new safety signals[28]. Abnormal liver function test was not reported as a common adverse event in the ACTT-1 trial, although exclusion criteria included the presence of ALT or AST levels > 5 times the ULN[76].

### **Immunomodulatory therapies**

The clinical course of severe forms of COVID-19 is characterized by the presence of an excessive inflammatory response triggered by SARS-CoV-2 and orchestrated by the host immune system, which contributes to the development of tissue damage, multiorgan failure, and ARDS[77]. Such a pathogenic mechanism has led to the widespread use of various immunomodulatory strategies aimed at blocking this “cytokine storm”, including corticosteroids[78], anti-IL-6 (such as tocilizumab or sarilumab)[79] and anti-IL-1 $\beta$  (canakinumab or anakinra)[80] agents, or janus kinase inhibitors (baricitinib)[81]. With the exception of low-to-intermediate-dose systemic corticosteroids (*i.e.* dexamethasone 6 mg daily for 10 d), which have been shown to decrease 28-d mortality in patients requiring respiratory support[82], there remains controversy regarding the clinical benefit to be expected from these agents in the general population with COVID-19, with conflicting results from observational studies and RCTs.

The available evidence supporting the use of immunomodulatory therapies in SOT recipients is even more limited[83]. Nevertheless, multicenter registries revealed that anti-IL-6 agents were commonly administered during the first pandemic wave (with overall rates ranging from 13%[59] to 21%[71]). In the specific group of LT recipients, 5% and 1% of patients included in the ONT registry as of July 2020 had received tocilizumab and anakinra, respectively[71]. The off label use of tocilizumab in other cohorts ranged from 6.2% in the ELITA/ELTR registry[84] to 15.6% in the SETH registry[53]. As previously stated, no RCTs have assessed to date the role of therapeutic IL-6 blockade in the setting of post-transplant COVID-19 with cytokine release syndrome. A small retrospective study compared 29 SOT recipients treated with tocilizumab for severe COVID-19 (including one single LT recipient) with a matched control group of recipients who did not receive this agent. No significant differences were observed in terms of in-hospital mortality (41% *vs* 28%, respectively;  $P = 0.27$ ), hospital discharge (52% *vs* 72%;  $P = 0.26$ ), or secondary infections (34% *vs* 24%;  $P = 0.55$ ), although the higher rates of IMV and renal replacement therapy observed in the tocilizumab group suggest some degree of confounding by indication not completely controlled by the matching process[85].

### **Management of immunosuppression**

As commented above, some preliminary reports showing a worse outcome among long-term LT recipients on minimal immunosuppressive regimen (as compared to recently transplanted, fully immunosuppressed patients)[15] led to propose during the first weeks of the pandemic that post-transplant IS might be actually protective in severe COVID-19[86]. Clinical experience accumulated over the past months, however, does not seem to confirm this hypothesis. Indeed, the SETH registry demonstrated the deleterious impact of baseline MMF-containing regimens (particularly when given at doses higher than 1000 mg/d). This negative effect was not observed for calcineurin or mTOR inhibitors. Complete MMF withdrawal during hospitalization showed a trend towards a reduced risk of progression to severe COVID-19 (41.7% *vs* 69.2%;  $P = 0.16$ )



[53].

The most common adjustment of baseline IS among more than 600 SOT recipients enrolled within the ONT registry was the withdrawal of the anti-metabolite drug (MMF or azathioprine), whereas calcineurin inhibitors were generally managed with dose reduction[71]. It is likely that the impact of baseline IS on the outcome of SARS-CoV-2 infection differ according to individual drugs. Belli *et al*[84] have recently shown that the use of tacrolimus was independently associated with a reduced mortality risk in the ELITA/ELTR registry (hazard ratio: 0.55; 95%CI: 0.31–0.99). The authors propose that tacrolimus could exert a direct antiviral effect through the immunophilin FK506-binding proteins[87].

In accordance with the survival benefit demonstrated for dexamethasone in the RECOVERY trial[82], baseline corticosteroid dose was usually maintained or increased in most LT recipients hospitalized due to COVID-19. In addition, corticosteroids boluses were given in 12.5% of patients in the SETH registry (4.9% and 25.7% of those with non-severe or severe COVID-19, respectively)[53].

## SARS-COV-2 VACCINATION IN LT RECIPIENTS

Whereas messenger RNA SARS-CoV-2 vaccines provide excellent rates of seroconversion and clinical effectiveness in the general population[88,89], immunogenicity in the setting of SOT appears to be severely compromised. Most available reports, however, are focused on kidney[90–92] or lung transplant recipients[93]. In addition, only a few studies have assessed the development of SARS-CoV-2-specific T-cell-mediated immunity in addition to antibody responses[94,95]. Rabinowich *et al*[96] tested for SARS-CoV-2 immunoglobulin G antibodies against the SARS-CoV-2 spike glycoprotein 10–20 d after the administration of the second BNT162b2 vaccine dose in 80 LT recipients. Detectable humoral response was demonstrated in 47.5% of patients only (as compared to 100% of HCWs used as control group). In addition, the mean antibody titer was significantly lower in LT recipients (95.41 AU/mL *vs* 200.5 AU/mL, respectively). Older age, lower estimated glomerular filtration rate, and treatment with MMF or high dose steroids were associated with the lack of vaccine response, with no apparent impact of the time since transplantation. The vaccine was well tolerated, and there were no episodes of suspected or confirmed graft rejection during the follow-up [96]. This disappointing immunogenicity is, however, in line with the rates reported for other SOT populations. The deleterious effect of the anti-metabolite drug has been also shown for kidney and lung transplant recipients[90,93].

## GUIDELINES FOR THE MANAGEMENT OF LT DURING THE COVID-19 PANDEMIC

On November 9, 2020, the American Association for the Study of Liver Diseases (AASLD) issued updated guidelines for LT providers in the current pandemic scenario [97]. Regarding the management of the waiting list, the document recommends to continue to prioritize the initial evaluation of patients with HCC or those with severe disease and high Model for End-stage Liver Disease (MELD) scores who are more likely to benefit from immediate LT listing. Some listed patients should be still seen in person according on the local incidence of SARS-CoV-2 infection and individual patient factors (such as their Model for End-stage Liver Disease score). Telemedicine alternatives may be considered for the remaining candidates. In addition, the AASLD guidelines recommend to develop hospital-specific policies for organ acceptance, taking into account the availability of ICU beds and other hospital resources. Potential donors and recipients must be screened for SARS-CoV-2 exposure and clinical symptoms compatible with COVID-19 (regardless of test results or availability). In addition, all donors and recipients should be screened for SARS-CoV-2, by means of nasopharyngeal swab, bronchoalveolar lavage, or both, taking into account the risk of false negative results, disease prevalence, and testing turnaround time in your area. Alternatives to RT-PCR-based testing such as chest X-ray may also be also considered. Ideally, LT in SARS-CoV-2-positive candidates should be delayed for at least 14–21 d after symptom resolution and one or two negative SARS-CoV-2 diagnostic tests. Of note, the decision to ultimately proceed with LT in a candidate recovering from COVID-19 must be individualized based on several factors (such as the urgency of transplantation, the presence of respiratory symptoms, and the risk of exposing HCWs

to SARS-CoV-2).

Regarding the approach to LT recipients diagnosed with COVID-19 in the AASLD guidelines, it should be considered lowering the overall level of IS (particularly anti-metabolite doses) based on general principles for managing post-transplant infections and in order to decrease the risk of secondary infection. The risk of COVID-19-associated kidney injury should be also taken into account and calcineurin inhibitor levels must be closely monitored. Likely due to the lack of supporting evidence, no clear recommendations are provided regarding the optimal regimen and timing for antiviral and immunomodulatory therapies.

In addition, the AASLD expert panel advises against making anticipatory adjustments in current immunosuppressive regimens in LT recipients with no diagnosis of SARS-CoV-2 infection. Prevention measures (*e.g.*, hand washing, cleaning frequently touched surfaces, staying away from large crowds, *etc.*) should be emphasized in this at-risk population[97].

Finally, although specific guidelines on the optimal vaccination strategy are scarce and based on low-level evidence, the Italian Association for the Study of the Liver recommends that LT candidates should be prioritized due to the high risk of mortality in the waiting list. Vaccination of the partners and caregivers of cirrhotic patients and LT recipients should be also encouraged[98].

## CONCLUSION

Although with geographical differences across countries, COVID-19 has exerted a negative impact on LT transplant activity (both in the number of donors and procedures) during the first months of the pandemic, with decreases ranging from 28% to 46%[38,40,42,43]. The cumulative incidence of SARS-CoV-2 infection in LT recipients has been estimated between 0.34%[50] to 1.56%[52]. These figures appear to be comparable to that observed for the general population, although some studies suggest that the incidence of COVID-19 after LT would be lower as compared to other types of SOT[54]. The clinical and radiological characteristics of COVID-19 at presentation are overall similar to non-transplant patients, including predictive factors of poor outcomes. All-cause mortality among hospitalized recipients is high (from 12% [61] to 19%[59]), and great heterogeneity in the rates of ICU admission is observed across different series (10%[61] to 31.6%[55]). It has been also proposed that the risk of death may be actually lower compared to the non-transplant population[51]. The outcome of post-transplant COVID-19 seems to depend mainly on the age of the recipient and the number of chronic comorbidities, rather than by the transplant status itself[59]. Some studies have suggested that post-transplant IS – in particular tacrolimus-containing regimens – may play a protective role by abrogating the deleterious effect of the cytokine release syndrome occurring during the course of SARS-CoV-2 infection or through a direct antiviral activity[83]. To date, there is scarce evidence to guide the use of antiviral or immunomodulatory therapies for COVID-19 after LT, including the potential effectiveness and safety of remdesivir or anti-IL-6 agents[82]. Both clinical experience and guidelines recommend the dose reduction of IS or withdrawal of MMF and other anti-proliferative agents[51,87]. Although specific studies are still scarce, messenger RNA vaccines seem to be safe in LT recipients in terms of serious adverse events or risk of alloimmunity, although the magnitude of SARS-CoV-2-specific immunoglobulin G antibody response is severely decreased as compared to non-immunocompromised individuals[97].

## REFERENCES

- 1 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]
- 2 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]
- 3 **World Health Organization**. WHO Director-General's opening remarks at the media briefing on COVID-19, 11 March 2020. [cited 17 February 2021]. Available from: <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19--->

11-march-2020

- 4 **World Health Organization.** WHO Coronavirus disease (COVID-19) Weekly Epidemiological Update and Weekly Operational Update. [cited 10 June 2021]. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>
- 5 COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. [cited 10 June 2021]. Available from: <https://coronavirus.jhu.edu/map.html>
- 6 **Fishman JA.** Infection in Organ Transplantation. *Am J Transplant* 2017; **17**: 856-879 [PMID: 28117944 DOI: 10.1111/ajt.14208]
- 7 **Chan JW,** Ng CK, Chan YH, Mok TY, Lee S, Chu SY, Law WL, Lee MP, Li PC. Short term outcome and risk factors for adverse clinical outcomes in adults with severe acute respiratory syndrome (SARS). *Thorax* 2003; **58**: 686-689 [PMID: 12885985 DOI: 10.1136/thorax.58.8.686]
- 8 **Park JE,** Jung S, Kim A, Park JE. MERS transmission and risk factors: a systematic review. *BMC Public Health* 2018; **18**: 574 [PMID: 29716568 DOI: 10.1186/s12889-018-5484-8]
- 9 **Zhang T,** Huang WS, Guan W, Hong Z, Gao J, Gao G, Wu G, Qin YY. Risk factors and predictors associated with the severity of COVID-19 in China: a systematic review, meta-analysis, and meta-regression. *J Thorac Dis* 2020; **12**: 7429-7441 [PMID: 33447431 DOI: 10.21037/jtd-20-1743]
- 10 **Raja MA,** Mendoza MA, Villavicencio A, Anjan S, Reynolds JM, Kittipibul V, Fernandez A, Guerra G, Camargo JF, Simkins J, Morris ML, Abbo LA, Natori Y. COVID-19 in solid organ transplant recipients: A systematic review and meta-analysis of current literature. *Transplant Rev (Orlando)* 2021; **35**: 100588 [PMID: 33246166 DOI: 10.1016/j.ttre.2020.100588]
- 11 **Liu B,** Wang Y, Zhao Y, Shi H, Zeng F, Chen Z. Successful treatment of severe COVID-19 pneumonia in a liver transplant recipient. *Am J Transplant* 2020; **20**: 1891-1895 [PMID: 32243673 DOI: 10.1111/ajt.15901]
- 12 **Huang JF,** Zheng KI, George J, Gao HN, Wei RN, Yan HD, Zheng MH. Fatal outcome in a liver transplant recipient with COVID-19. *Am J Transplant* 2020; **20**: 1907-1910 [PMID: 32277591 DOI: 10.1111/ajt.15909]
- 13 **Bhoori S,** Rossi RE, Citterio D, Mazzaferro V. COVID-19 in long-term liver transplant patients: preliminary experience from an Italian transplant centre in Lombardy. *Lancet Gastroenterol Hepatol* 2020; **5**: 532-533 [PMID: 32278366 DOI: 10.1016/S2468-1253(20)30116-3]
- 14 **Fernández-Ruiz M,** Andrés A, Loinaz C, Delgado JF, López-Medrano F, San Juan R, González E, Polanco N, Folgueira MD, Lalueza A, Lumberras C, Aguado JM. COVID-19 in solid organ transplant recipients: A single-center case series from Spain. *Am J Transplant* 2020; **20**: 1849-1858 [PMID: 32301155 DOI: 10.1111/ajt.15929]
- 15 **Kates OS,** Fisher CE, Stankiewicz-Karita HC, Shepherd AK, Church EC, Kapnadak SG, Lease ED, Riedo FX, Rakita RM, Limaye AP. Earliest cases of coronavirus disease 2019 (COVID-19) identified in solid organ transplant recipients in the United States. *Am J Transplant* 2020; **20**: 1885-1890 [PMID: 32330356 DOI: 10.1111/ajt.15944]
- 16 **Pereira MR,** Mohan S, Cohen DJ, Husain SA, Dube GK, Ratner LE, Arcasoy S, Aversa MM, Benvenuto LJ, Dadhania DM, Kapur S, Dove LM, Brown RS Jr, Rosenblatt RE, Samstein B, Uriel N, Farr MA, Satlin M, Small CB, Walsh TJ, Kodiyanplakkal RP, Miko BA, Aaron JG, Tsapepas DS, Emond JC, Verna EC. COVID-19 in solid organ transplant recipients: Initial report from the US epicenter. *Am J Transplant* 2020; **20**: 1800-1808 [PMID: 32330343 DOI: 10.1111/ajt.15941]
- 17 **Eslami P,** Moradi M, Dooghaie Moghadam A, Pirsalehi A, Abdul Lateef S, Hadaegh A, Rezaei B, Sadeghi A, Asadzadeh Aghdaei H, Zali MR. Lethal outcome of Covid-19 pneumonia in a new liver recipient with neurological manifestation. *Gastroenterol Hepatol Bed Bench* 2020; **13**: 405-409 [PMID: 33244386]
- 18 **Qin J,** Wang H, Qin X, Zhang P, Zhu L, Cai J, Yuan Y, Li H. Perioperative Presentation of COVID-19 Disease in a Liver Transplant Recipient. *Hepatology* 2020; **72**: 1491-1493 [PMID: 32220017 DOI: 10.1002/hep.31257]
- 19 **Jothimani D,** Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. *J Hepatol* 2020; **73**: 1231-1240 [PMID: 32553666 DOI: 10.1016/j.jhep.2020.06.006]
- 20 **Zhong P,** Xu J, Yang D, Shen Y, Wang L, Feng Y, Du C, Song Y, Wu C, Hu X, Sun Y. COVID-19-associated gastrointestinal and liver injury: clinical features and potential mechanisms. *Signal Transduct Target Ther* 2020; **5**: 256 [PMID: 33139693 DOI: 10.1038/s41392-020-00373-7]
- 21 **Napodano C,** Pocino K, Stefanile A, Marino M, Miele L, Gulli F, Basile V, Pandolfi F, Gasbarrini A, Rapaccini GL, Basile U. COVID-19 and hepatic involvement: The liver as a main actor of the pandemic novel. *Scand J Immunol* 2021; **93**: e12977 [PMID: 32931622 DOI: 10.1111/sji.12977]
- 22 **McConnell MJ,** Kawaguchi N, Kondo R, Sonzogni A, Licini L, Valle C, Bonaffini PA, Sironi S, Alessio MG, Previtali G, Seghezzi M, Zhang X, Lee AI, Pine AB, Chun HJ, Fernandez-Hernando C, Qing H, Wang A, Price C, Sun Z, Utsumi T, Hwa J, Strazzabosco M, Iwakiri Y. Liver injury in COVID-19 and IL-6 trans-signaling-induced endotheliopathy. *J Hepatol* 2021; **75**: 647-658 [PMID: 33991637 DOI: 10.1016/j.jhep.2021.04.050]
- 23 **Fiel MI,** El Jamal SM, Paniz-Mondolfi A, Gordon RE, Reidy J, Bandovic J, Advani R, Kilaru S, Pourmand K, Ward S, Thung SN, Schiano T. Findings of Hepatic Severe Acute Respiratory Syndrome Coronavirus-2 Infection. *Cell Mol Gastroenterol Hepatol* 2021; **11**: 763-770 [PMID: 32992052 DOI: 10.1016/j.jcmgh.2020.09.015]
- 24 **Cai Q,** Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; **73**: 566-574 [PMID: 32298767 DOI: 10.1016/j.jhep.2020.04.006]

- 25 **Ding ZY**, Li GX, Chen L, Shu C, Song J, Wang W, Wang YW, Chen Q, Jin GN, Liu TT, Liang JN, Zhu P, Zhu W, Li Y, Zhang BH, Feng H, Zhang WG, Yin ZY, Yu WK, Yang Y, Zhang HQ, Tang ZP, Wang H, Hu JB, Liu JH, Yin P, Chen XP, Zhang B; Tongji Multidisciplinary Team for Treating COVID-19 (TTTC). Association of liver abnormalities with in-hospital mortality in patients with COVID-19. *J Hepatol* 2021; **74**: 1295-1302 [PMID: [33347952](#) DOI: [10.1016/j.jhep.2020.12.012](#)]
- 26 **Meszaros M**, Meunier L, Morquin D, Klouche K, Fesler P, Malezieux E, Makinson A, Le Moing V, Reynes J, Pageaux GP. Abnormal liver tests in patients hospitalized with Coronavirus disease 2019: Should we worry? *Liver Int* 2020; **40**: 1860-1864 [PMID: [32495496](#) DOI: [10.1111/liv.14557](#)]
- 27 **Phipps MM**, Barraza LH, LaSota ED, Sobieszczyk ME, Pereira MR, Zheng EX, Fox AN, Zucker J, Verna EC. Acute Liver Injury in COVID-19: Prevalence and Association with Clinical Outcomes in a Large U.S. Cohort. *Hepatology* 2020; **72**: 807-817 [PMID: [32473607](#) DOI: [10.1002/hep.31404](#)]
- 28 **Sharma P**, Kumar A, Anikhindi S, Bansal N, Singla V, Shivam K, Arora A. Effect of COVID-19 on Pre-existing Liver disease: What Hepatologist Should Know? *J Clin Exp Hepatol* 2021; **11**: 484-493 [PMID: [33398223](#) DOI: [10.1016/j.jceh.2020.12.006](#)]
- 29 **Rela M**, Patil V, Narasimhan G, Jothamani D. COVID-19 in decompensated cirrhosis. *Hepatol Int* 2020; **14**: 1125-1127 [PMID: [32990918](#) DOI: [10.1007/s12072-020-10092-4](#)]
- 30 **Kumar P**, Sharma M, Sulthana SF, Kulkarni A, Rao PN, Reddy DN. Severe Acute Respiratory Syndrome Coronavirus 2-related Acute-on-chronic Liver Failure. *J Clin Exp Hepatol* 2021; **11**: 404-406 [PMID: [33398222](#) DOI: [10.1016/j.jceh.2020.12.007](#)]
- 31 **Zhou YJ**, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, George J, Zheng MH. Metabolic-associated fatty liver disease is associated with severity of COVID-19. *Liver Int* 2020; **40**: 2160-2163 [PMID: [32573883](#) DOI: [10.1111/liv.14575](#)]
- 32 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: [32085846](#) DOI: [10.1016/S2213-2600\(20\)30076-X](#)]
- 33 **Tian S**, Xiong Y, Liu H, Niu L, Guo J, Liao M, Xiao SY. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Mod Pathol* 2020; **33**: 1007-1014 [PMID: [32291399](#) DOI: [10.1038/s41379-020-0536-x](#)]
- 34 **Lagana SM**, Kudose S, Iuga AC, Lee MJ, Fazlollahi L, Remotti HE, Del Portillo A, De Michele S, de Gonzalez AK, Saqi A, Khairallah P, Chong AM, Park H, Uhlemann AC, Lefkowitz JH, Verna EC. Hepatic pathology in patients dying of COVID-19: a series of 40 cases including clinical, histologic, and virologic data. *Mod Pathol* 2020; **33**: 2147-2155 [PMID: [32792598](#) DOI: [10.1038/s41379-020-00649-x](#)]
- 35 **Amadeo G**, Brustia R, Allaire M, Lequoy M, Hollande C, Regnault H, Blaise L, Ganne-Carrié N, Sèror O, Larrey E, Lim C, Scatton O, El Mouhadi S, Ozenne V, Paye F, Balladur P, Dohan A, Massault PP, Pol S, Dioguardi Burgio M, Vilgrain V, Sepulveda A, Cauchy F, Luciani A, Sommacale D, Leroy V, Roudot-Thoraval F, Bouattour M, Nault JC; Paris Liver Cancer Group. Impact of COVID-19 on the management of hepatocellular carcinoma in a high-prevalence area. *JHEP Rep* 2021; **3**: 100199 [PMID: [33163949](#) DOI: [10.1016/j.jhepr.2020.100199](#)]
- 36 **De Simone P**, Melandro F, Balzano E, Tincani G, Catalano G, Ghinolfi D, Ducci J, Carrai P, Petrucci S, Biancofiore G. Coronavirus Disease 2019 Infection Requires Strengthening of the Chronic Care Model: The Impact on Liver Transplant Practice at a High-Volume Center in Italy. *Liver Transpl* 2020; **26**: 1351-1353 [PMID: [32542881](#) DOI: [10.1002/lt.25821](#)]
- 37 **Melandro F**, Ghinolfi D, Balzano E, Tincani G, Catalano G, Biancofiore G, Peris A, De Simone P. Light at the end of the tunnel: COVID-19 lockdown has not halted liver transplantation at a high volume center in Italy. *Transpl Int* 2020; **33**: 1845-1847 [PMID: [33034081](#) DOI: [10.1111/tri.13764](#)]
- 38 **Gruttadauria S**; Italian Board of Experts in Liver Transplantation (I-BELT) Study Group, The Italian Society of Organ Transplantation (SITO). Preliminary Analysis of the Impact of the Coronavirus Disease 2019 Outbreak on Italian Liver Transplant Programs. *Liver Transpl* 2020; **26**: 941-944 [PMID: [32378325](#) DOI: [10.1002/lt.25790](#)]
- 39 **Maggi U**, De Carlis L, Yiu D, Colledan M, Regalia E, Rossi G, Angrisani M, Consonni D, Fornoni G, Piccolo G, DeFeo TM. The impact of the COVID-19 outbreak on liver transplantation programs in Northern Italy. *Am J Transplant* 2020; **20**: 1840-1848 [PMID: [32330351](#) DOI: [10.1111/ajt.15948](#)]
- 40 **Domínguez-Gil B**, Coll E, Fernández-Ruiz M, Corral E, Del Río F, Zaragoza R, Rubio JJ, Hernández D. COVID-19 in Spain: Transplantation in the midst of the pandemic. *Am J Transplant* 2020; **20**: 2593-2598 [PMID: [32359194](#) DOI: [10.1111/ajt.15983](#)]
- 41 **Turco C**, Lim C, Soubrane O, Malaquin G, Kerbaul F, Bastien O, Conti F, Scatton O. Impact of the first Covid-19 outbreak on liver transplantation activity in France: A snapshot. *Clin Res Hepatol Gastroenterol* 2021; **45**: 101560 [PMID: [33176991](#) DOI: [10.1016/j.clinre.2020.10.005](#)]
- 42 **Goff RR**, Wilk AR, Toll AE, McBride MA, Klassen DK. Navigating the COVID-19 pandemic: Initial impacts and responses of the Organ Procurement and Transplantation Network in the United States. *Am J Transplant* 2021; **21**: 2100-2112 [PMID: [33244847](#) DOI: [10.1111/ajt.16411](#)]
- 43 **Friedman AL**, Delli Carpini KW, Ezzell C, Irving H. There are no best practices in a pandemic: Organ donation within the COVID-19 epicenter. *Am J Transplant* 2020; **20**: 3089-3093 [PMID: [32568471](#) DOI: [10.1111/ajt.16157](#)]
- 44 **Reddy MS**, Hakeem AR, Klair T, Marcon F, Mathur A, Samstein B, Mohanka R, Mathur SK, Prachalias A, Menon KV, Muiesan P, Rela M, Emond JC. Trinitational Study Exploring the Early Impact of the COVID-19 Pandemic on Organ Donation and Liver Transplantation at National and



- Unit Levels. *Transplantation* 2020; **104**: 2234-2243 [PMID: [32804803](#) DOI: [10.1097/TP.00000000000003416](#)]
- 45 **Lee JM**. Effect of COVID-19 on liver transplantation in Korea. *Transpl Infect Dis* 2020; **22**: e13384 [PMID: [32574408](#) DOI: [10.1111/tid.13384](#)]
- 46 **Montalbano M**, Levi Sandri GB, Visco Comandini U, Lionetti R, Vincenzi L, Berardi G, Guglielmo N, Pellicelli A, Ettorre GM, D'Offizi G. The impact of the coronavirus disease 2019 pandemic on a central Italy transplant center. *Medicine (Baltimore)* 2020; **99**: e22174 [PMID: [33031261](#) DOI: [10.1097/MD.00000000000022174](#)]
- 47 **Siniscalchi A**, Vitale G, Morelli MC, Ravaioli M, Laici C, Bianchini A, Del Gaudio M, Conti F, Vizioli L, Cescon M. Liver transplantation in Italy in the era of COVID 19: reorganizing critical care of recipients. *Intern Emerg Med* 2020; **15**: 1507-1515 [PMID: [32979193](#) DOI: [10.1007/s11739-020-02511-z](#)]
- 48 **Agopian V**, Verna E, Goldberg D. Changes in Liver Transplant Center Practice in Response to Coronavirus Disease 2019: Unmasking Dramatic Center-Level Variability. *Liver Transpl* 2020; **26**: 1052-1055 [PMID: [32369251](#) DOI: [10.1002/lt.25789](#)]
- 49 **Strauss AT**, Boyarsky BJ, Garonzik-Wang JM, Werbel W, Durand CM, Avery RK, Jackson KR, Kernodle AB, Baker T, Snyder J, Segev DL, Massie AB. Liver transplantation in the United States during the COVID-19 pandemic: National and center-level responses. *Am J Transplant* 2021; **21**: 1838-1847 [PMID: [33107180](#) DOI: [10.1111/ajt.16373](#)]
- 50 **Putzer G**, Martini J, Gasteiger L, Mathis S, Breikopf R, Hell T, van Enckevort A, Oberhuber R, Öfner D, Schneeberger S. Liver Transplantation Activity in the Eurotransplant Area Is Recovering Slowly During the COVID-19 Crisis. *Transplant Direct* 2020; **6**: e611 [PMID: [33134487](#) DOI: [10.1097/TXD.0000000000001064](#)]
- 51 **Polak WG**, Fondevila C, Karam V, Adam R, Baumann U, Germani G, Nadalin S, Taimr P, Toso C, Troisi RI, Zieniewicz K, Belli LS, Duvoux C. Impact of COVID-19 on liver transplantation in Europe: alert from an early survey of European Liver and Intestine Transplantation Association and European Liver Transplant Registry. *Transpl Int* 2020; **33**: 1244-1252 [PMID: [32609908](#) DOI: [10.1111/tri.13680](#)]
- 52 **Verma A**, Khorsandi SE, Dolcet A, Prachalias A, Suddle A, Heaton N, Jassem W. Low prevalence and disease severity of COVID-19 in post-liver transplant recipients-A single centre experience. *Liver Int* 2020; **40**: 1972-1976 [PMID: [32471013](#) DOI: [10.1111/liv.14552](#)]
- 53 **Colmenero J**, Rodríguez-Perálvarez M, Salcedo M, Arias-Milla A, Muñoz-Serrano A, Graus J, Nuño J, Gastaca M, Bustamante-Schneider J, Cachero A, Lladó L, Caballero A, Fernández-Yunquera A, Loinaz C, Fernández I, Fondevila C, Navasa M, Iñarrairaegui M, Castells L, Pascual S, Ramírez P, Vinaixa C, González-Díez ML, González-Grande R, Hierro L, Nogueras F, Otero A, Álamo JM, Blanco-Fernández G, Fábrega E, García-Pajares F, Montero JL, Tomé S, De la Rosa G, Pons JA. Epidemiological pattern, incidence, and outcomes of COVID-19 in liver transplant patients. *J Hepatol* 2021; **74**: 148-155 [PMID: [32750442](#) DOI: [10.1016/j.jhep.2020.07.040](#)]
- 54 **Loinaz C**, Marcacuzco A, Fernández-Ruiz M, Caso O, Cambra F, San Juan R, Justo I, Calvo J, García-Sesma A, Manrique A, Pérez-Jacoiste Asín MA, Folgueira MD, Aguado JM, Lumberras C. Varied clinical presentation and outcome of SARS-CoV-2 infection in liver transplant recipients: Initial experience at a single center in Madrid, Spain. *Transpl Infect Dis* 2020; **22**: e13372 [PMID: [32562561](#) DOI: [10.1111/tid.13372](#)]
- 55 **Ravanan R**, Callaghan CJ, Mumford L, Ushiro-Lumb I, Thorburn D, Casey J, Friend P, Parameshwar J, Currie I, Burnapp L, Baker R, Dudley J, Oniscu GC, Berman M, Asher J, Harvey D, Manara A, Manas D, Gardiner D, Forsythe JLR. SARS-CoV-2 infection and early mortality of waitlisted and solid organ transplant recipients in England: A national cohort study. *Am J Transplant* 2020; **20**: 3008-3018 [PMID: [32780493](#) DOI: [10.1111/ajt.16247](#)]
- 56 **Trapani S**, Masiero L, Puoti F, Rota MC, Del Manso M, Lombardini L, Riccardo F, Amoroso A, Pezzotti P, Grossi PA, Brusaferrro S, Cardillo M; Italian Network of Regional Transplant Coordinating Centers Collaborating group; Italian Surveillance System of Covid-19, Italian Society for Organ Transplantation (SITO), The Italian Board of Experts in Liver Transplantation (I-BELT) Study Group, Italian Association for the Study of the Liver (AISF), Italian Society of Nephrology (SIN), SIN-SITO Study Group. Incidence and outcome of SARS-CoV-2 infection on solid organ transplantation recipients: A nationwide population-based study. *Am J Transplant* 2021; **21**: 2509-2521 [PMID: [33278850](#) DOI: [10.1111/ajt.16428](#)]
- 57 **Ali Malekhsosini S**, Nikoupour H, Gholami S, Shamsaeefar A, Arasteh P, Kazemi K, Dehghani M, Eghlimi H, Raeisi Shahraki H, Roozbeh J, Rezaianzadeh A, Nikeghbalian S. A Report of 85 Cases of COVID-19 and Abdominal Transplantation From a Single Center: What Are the Associated Factors With Death Among Organ Transplantation Patients. *Transplantation* 2021; **105**: 90-99 [PMID: [32976364](#) DOI: [10.1097/TP.00000000000003470](#)]
- 58 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: [32171076](#) DOI: [10.1016/S0140-6736\(20\)30566-3](#)]
- 59 **Kates OS**, Haydel BM, Florman SS, Rana MM, Chaudhry ZS, Ramesh MS, Safa K, Kotton CN, Blumberg EA, Besharatian BD, Tanna SD, Ison MG, Malinis M, Azar MM, Rakita RM, Morillas JA, Majeed A, Sait AS, Spaggiari M, Hemmige V, Mehta SA, Neumann H, Badami A, Goldman JD, Lala A, Hemmersbach-Miller M, McCort ME, Bajrovic V, Ortiz-Bautista C, Friedman-Moraco R, Sehgal



- S, Lease ED, Fisher CE, Limaye AP; UW COVID-19 SOT Study Team. COVID-19 in solid organ transplant: A multi-center cohort study. *Clin Infect Dis* 2020 [PMID: [32766815](#) DOI: [10.1093/cid/ciaa1097](#)]
- 60 **Belli LS**, Duvoux C, Karam V, Adam R, Cuervas-Mons V, Pasulo L, Loinaz C, Invernizzi F, Patrono D, Bhoori S, Ciccarelli O, Morelli MC, Castells L, Lopez-Lopez V, Conti S, Fondevila C, Polak W. COVID-19 in liver transplant recipients: preliminary data from the ELITA/ELTR registry. *Lancet Gastroenterol Hepatol* 2020; **5**: 724-725 [PMID: [32505228](#) DOI: [10.1016/S2468-1253\(20\)30183-7](#)]
  - 61 **Webb GJ**, Marjot T, Cook JA, Aloman C, Armstrong MJ, Brenner EJ, Catana MA, Cargill T, Dhanasekaran R, García-Juárez I, Hagström H, Kennedy JM, Marshall A, Masson S, Mercer CJ, Perumalswami PV, Ruiz I, Thaker S, Ufere NN, Barnes E, Barritt AS 4th, Moon AM. Outcomes following SARS-CoV-2 infection in liver transplant recipients: an international registry study. *Lancet Gastroenterol Hepatol* 2020; **5**: 1008-1016 [PMID: [32866433](#) DOI: [10.1016/S2468-1253\(20\)30271-5](#)]
  - 62 **Caillard S**, Chavarot N, Francois H, Matignon M, Greze C, Kamar N, Gatault P, Thauinat O, Legris T, Frimat L, Westeel PF, Goutaudier V, Jdidou M, Snanoudj R, Colosio C, Sicard A, Bertrand D, Mousson C, Bamoulid J, Masset C, Thierry A, Couzi L, Chemouny JM, Duveau A, Moal V, Blancho G, Grimbert P, Durrbach A, Moulin B, Anglicheau D, Ruch Y, Kaeuffer C, Benotmane I, Solis M, LeMeur Y, Hazzan M, Danion F; French SOT COVID Registry. Is COVID-19 infection more severe in kidney transplant recipients? *Am J Transplant* 2021; **21**: 1295-1303 [PMID: [33259686](#) DOI: [10.1111/ajt.16424](#)]
  - 63 **Becchetti C**, Zambelli MF, Pasulo L, Donato MF, Invernizzi F, Detry O, Dahlqvist G, Ciccarelli O, Morelli MC, Fraga M, Svegliati-Baroni G, van Vlierberghe H, Coenraad MJ, Romero MC, de Gottardi A, Toniutto P, Del Prete L, Abbati C, Samuel D, Pirenne J, Nevens F, Dufour JF; COVID-LT group. COVID-19 in an international European liver transplant recipient cohort. *Gut* 2020; **69**: 1832-1840 [PMID: [32571972](#) DOI: [10.1136/gutjnl-2020-321923](#)]
  - 64 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020; **323**: 2052-2059 [PMID: [32320003](#) DOI: [10.1001/jama.2020.6775](#)]
  - 65 **Prieto M**, Gastaca M, Ruiz P, Ventoso A, Palomares I, Rodríguez-Álvarez RJ, Salvador P, Bustamante J, Valdivieso A. A case of COVID-19 immediately after liver transplantation: Not only bad news. *Ann Hepatobiliary Pancreat Surg* 2020; **24**: 314-318 [PMID: [32843598](#) DOI: [10.14701/ahbps.2020.24.3.314](#)]
  - 66 **Rouphael C**, D'Amico G, Ricci K, Cywinski J, Miranda C, Koval C, Duggal A, Quintini C, Menon KVN, Miller C, Modaresi Esfeh J. Successful orthotopic liver transplantation in a patient with a positive SARS-CoV2 test and acute liver failure secondary to acetaminophen overdose. *Am J Transplant* 2021; **21**: 1312-1316 [PMID: [33017864](#) DOI: [10.1111/ajt.16330](#)]
  - 67 **Sanghavi DK**, Lowman PE, Harnois DM, Keaveny AP, Nguyen JH, Canabal JM, Moreno Franco P. Changes in Liver Transplant Center Practice in Response to Coronavirus Disease 2019: Unmasking Dramatic Center-Level Variability. *Liver Transpl* 2020; **26**: 1672-1673 [PMID: [32858781](#) DOI: [10.1002/lt.25877](#)]
  - 68 **Raut V**, Sonavane A, Shah K, Raj C A, Thorat A, Sawant A, Chaksota H, Mandot A, Vasanth S, Nagral A, Mirza D. Successful liver transplantation immediately after recovery from COVID-19 in a highly endemic area. *Transpl Int* 2021; **34**: 376-377 [PMID: [33253449](#) DOI: [10.1111/tri.13790](#)]
  - 69 **Martini S**, Patrono D, Pittaluga F, Brunetto MR, Lupo F, Amoroso A, Cavallo R, Balagna R, Romagnoli R. Urgent liver transplantation soon after recovery from COVID-19 in a patient with decompensated liver cirrhosis. *Hepatol Commun* 2020 [PMID: [32838105](#) DOI: [10.1002/hep4.1580](#)]
  - 70 **Fraser J**, Mousley J, Testro A, Smibert OC, Koshy AN. Clinical Presentation, Treatment, and Mortality Rate in Liver Transplant Recipients With Coronavirus Disease 2019: A Systematic Review and Quantitative Analysis. *Transplant Proc* 2020; **52**: 2676-2683 [PMID: [32891405](#) DOI: [10.1016/j.transproceed.2020.07.012](#)]
  - 71 **Coll E**, Fernández-Ruiz M, Sánchez-Álvarez JE, Martínez-Fernández JR, Crespo M, Gayoso J, Bada-Bosch T, Oppenheimer F, Moreso F, López-Oliva MO, Melilli E, Rodríguez-Ferrero ML, Bravo C, Burgos E, Facundo C, Lorenzo I, Yañez Í, Galeano C, Roca A, Cabello M, Gómez-Bueno M, García-Cosío M, Graus J, Lladó L, de Pablo A, Loinaz C, Aguado B, Hernández D, Domínguez-Gil B; Spanish Group for the Study of COVID-19 in Transplant Recipients. COVID-19 in transplant recipients: The Spanish experience. *Am J Transplant* 2021; **21**: 1825-1837 [PMID: [33098200](#) DOI: [10.1111/ajt.16369](#)]
  - 72 **Rezaee H**, Pourkarim F, Pourtaghi-Anvarian S, Entezari-Maleki T, Asvadi-Kermani T, Nouri-Vaskeh M. Drug-drug interactions with candidate medications used for COVID-19 treatment: An overview. *Pharmacol Res Perspect* 2021; **9**: e00705 [PMID: [33421347](#) DOI: [10.1002/prp2.705](#)]
  - 73 **Cao B**, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C.

- A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 2020; **382**: 1787-1799 [PMID: [32187464](#) DOI: [10.1056/NEJMoa2001282](#)]
- 74 **Cavalcanti AB**, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A, Damiani LP, Marcadenti A, Kawano-Dourado L, Lisboa T, Junqueira DLM, de Barros E Silva PGM, Tramuja L, Abreu-Silva EO, Laranjeira LN, Soares AT, Echenique LS, Pereira AJ, Freitas FGR, Gebara OCE, Dantas VCS, Furtado RHM, Milan EP, Golin NA, Cardoso FF, Maia IS, Hoffmann Filho CR, Kormann APM, Amazonas RB, Bocchi de Oliveira MF, Serpa-Neto A, Falavigna M, Lopes RD, Machado FR, Berwanger O; Coalition Covid-19 Brazil I Investigators. Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. *N Engl J Med* 2020; **383**: 2041-2052 [PMID: [32706953](#) DOI: [10.1056/NEJMoa2019014](#)]
  - 75 **WHO Solidarity Trial Consortium**, Pan H, Peto R, Henao-Restrepo AM, Preziosi MP, Sathiyamoorthy V, Abdool Karim Q, Alejandria MM, Hernández García C, Kieny MP, Malekzadeh R, Murthy S, Reddy KS, Roses Periago M, Abi Hanna P, Ader F, Al-Bader AM, Alhasawi A, Allum E, Alotaibi A, Alvarez-Moreno CA, Appadoo S, Asiri A, Aukrust P, Barratt-Due A, Bellani S, Branca M, Cappel-Porter HBC, Cerrato N, Chow TS, Como N, Eustace J, García PJ, Godbole S, Gotuzzo E, Griskevicius L, Hamra R, Hassan M, Hassany M, Hutton D, Irmansyah I, Jancoriene L, Kirwan J, Kumar S, Lennon P, Lopardo G, Lydon P, Magrini N, Maguire T, Manevska S, Manuel O, McGinty S, Medina MT, Mesa Rubio ML, Miranda-Montoya MC, Nel J, Nunes EP, Perola M, Portolés A, Rasmin MR, Raza A, Rees H, Reges PPS, Rogers CA, Salami K, Salvadori MI, Sinani N, Sterne JAC, Stevanovikj M, Tacconelli E, Tikkinen KAO, Trelle S, Zaid H, Rottingen JA, Swaminathan S. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. *N Engl J Med* 2021; **384**: 497-511 [PMID: [33264556](#) DOI: [10.1056/NEJMoa2023184](#)]
  - 76 **Beigel JH**, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC; ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Final Report. *N Engl J Med* 2020; **383**: 1813-1826 [PMID: [32445440](#) DOI: [10.1056/NEJMoa2007764](#)]
  - 77 **Fajgenbaum DC**, June CH. Cytokine Storm. *N Engl J Med* 2020; **383**: 2255-2273 [PMID: [33264547](#) DOI: [10.1056/NEJMra2026131](#)]
  - 78 **van Paassen J**, Vos JS, Hoekstra EM, Neumann KMI, Boot PC, Arbous SM. Corticosteroid use in COVID-19 patients: a systematic review and meta-analysis on clinical outcomes. *Crit Care* 2020; **24**: 696 [PMID: [33317589](#) DOI: [10.1186/s13054-020-03400-9](#)]
  - 79 **Fernández-Ruiz M**, López-Medrano F, Aguado JM. Tocilizumab for the treatment of COVID-19. *Expert Opin Biol Ther* 2021; **21**: 431-434 [PMID: [33504238](#) DOI: [10.1080/14712598.2021.1880563](#)]
  - 80 **Generali D**, Bosio G, Malberti F, Cuzzoli A, Testa S, Romanini L, Fioravanti A, Morandini A, Pianta L, Giannotti G, Viola EM, Giorgi-Pierfranceschi M, Foramitti M, Tira RA, Zangrandi I, Chiodelli G, Machiavelli A, Cappelletti MR, Giossi A, De Giulio V, Costanzi C, Campana C, Bernocchi O, Sirico M, Zoncada A, Molteni A, Venturini S, Giudici F, Scaltriti M, Pan A. Canakinumab as treatment for COVID-19-related pneumonia: A prospective case-control study. *Int J Infect Dis* 2021; **104**: 433-440 [PMID: [33385581](#) DOI: [10.1016/j.ijid.2020.12.073](#)]
  - 81 **Kalil AC**, Patterson TF, Mehta AK, Tomashek KM, Wolfe CR, Ghazaryan V, Marconi VC, Ruiz-Palacios GM, Hsieh L, Kline S, Tapson V, Iovine NM, Jain MK, Sweeney DA, El Sahly HM, Branche AR, Regalado Pineda J, Lye DC, Sandkovsky U, Luetkemeyer AF, Cohen SH, Finberg RW, Jackson PEH, Taiwo B, Paules CI, Arguinchona H, Erdmann N, Ahuja N, Frank M, Oh MD, Kim ES, Tan SY, Mularski RA, Nielsen H, Ponce PO, Taylor BS, Larson L, Roupheal NG, Saklawi Y, Cantos VD, Ko ER, Engemann JJ, Amin AN, Watanabe M, Billings J, Elie MC, Davey RT, Burgess TH, Ferreira J, Green M, Makowski M, Cardoso A, de Bono S, Bonnett T, Proschan M, Deye GA, Dempsey W, Nayak SU, Dodd LE, Beigel JH; ACTT-2 Study Group Members. Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. *N Engl J Med* 2021; **384**: 795-807 [PMID: [33306283](#) DOI: [10.1056/NEJMoa2031994](#)]
  - 82 **RECOVERY Collaborative Group**, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021; **384**: 693-704 [PMID: [32678530](#) DOI: [10.1056/NEJMoa2021436](#)]
  - 83 **Fernández-Ruiz M**, Aguado JM. Immunomodulatory Therapies for COVID-19 in Solid Organ Transplant Recipients. *Curr Transplant Rep* 2020; 1-11 [PMID: [33110739](#) DOI: [10.1007/s40472-020-00306-x](#)]
  - 84 **Belli LS**, Fondevila C, Cortesi PA, Conti S, Karam V, Adam R, Coilly A, Ericzon BG, Loinaz C, Cuervas-Mons V, Zambelli M, Llado L, Diaz-Fontenla F, Invernizzi F, Patrono D, Faitot F, Bhooori S, Pirenne J, Perricone G, Magini G, Castells L, Detry O, Cruchaga PM, Colmenero J, Berrevoet F, Rodriguez G, Ysebaert D, Radenne S, Metselaar H, Morelli C, De Carlis LG, Polak WG, Duvoux C; ELITA-ELTR COVID-19 Registry. Protective Role of Tacrolimus, Deleterious Role of Age and Comorbidities in Liver Transplant Recipients With Covid-19: Results From the ELITA/ELTR Multi-center European Study. *Gastroenterology* 2021; **160**: 1151-1163.e3 [PMID: [33307029](#) DOI: [10.1053/j.gastro.2020.11.045](#)]
  - 85 **Pereira MR**, Aversa MM, Farr MA, Miko BA, Aaron JG, Mohan S, Cohen DJ, Husain SA, Ratner

- LE, Arcasoy S, Uriel N, Zheng EX, Fox AN, Tsapepas DS, Emond JC, Verna EC. Tocilizumab for severe COVID-19 in solid organ transplant recipients: a matched cohort study. *Am J Transplant* 2020; **20**: 3198-3205 [PMID: [32946668](#) DOI: [10.1111/ajt.16314](#)]
- 86 **Romanelli A**, Mascolo S. Immunosuppression drug-related and clinical manifestation of Coronavirus disease 2019: A therapeutical hypothesis. *Am J Transplant* 2020; **20**: 1947-1948 [PMID: [32243698](#) DOI: [10.1111/ajt.15905](#)]
- 87 **Carbajo-Lozoya J**, Müller MA, Kallies S, Thiel V, Drosten C, von Brunn A. Replication of human coronaviruses SARS-CoV, HCoV-NL63 and HCoV-229E is inhibited by the drug FK506. *Virus Res* 2012; **165**: 112-117 [PMID: [22349148](#) DOI: [10.1016/j.virusres.2012.02.002](#)]
- 88 **Polack FP**, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW Jr, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020; **383**: 2603-2615 [PMID: [33301246](#) DOI: [10.1056/NEJMoa2034577](#)]
- 89 **Baden LR**, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGettigan J, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T; COVE Study Group. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* 2021; **384**: 403-416 [PMID: [33378609](#) DOI: [10.1056/NEJMoa2035389](#)]
- 90 **Boyarsky BJ**, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, Garonzik-Wang JM. Antibody Response to 2-Dose SARS-CoV-2 mRNA Vaccine Series in Solid Organ Transplant Recipients. *JAMA* 2021; **325**: 2204-2206 [PMID: [33950155](#) DOI: [10.1001/jama.2021.7489](#)]
- 91 **Grupe A**, Rabinowich L, Schwartz D, Schwartz IF, Ben-Yehoyada M, Shashar M, Katchman E, Halperin T, Turner D, Goykhman Y, Shibolet O, Levy S, Houry I, Baruch R, Katchman H. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. *Am J Transplant* 2021; **21**: 2719-2726 [PMID: [33866672](#) DOI: [10.1111/ajt.16615](#)]
- 92 **Rozen-Zvi B**, Yahav D, Agur T, Zingerman B, Ben-Zvi H, Atamna A, Tau N, Mashraki T, Neshet E, Rahamimov R. Antibody response to SARS-CoV-2 mRNA vaccine among kidney transplant recipients: a prospective cohort study. *Clin Microbiol Infect* 2021; **27**: 1173.e1-1173.e4 [PMID: [33957273](#) DOI: [10.1016/j.cmi.2021.04.028](#)]
- 93 **Shostak Y**, Shafran N, Heching M, Rosengarten D, Shtraichman O, Shitenberg D, Amor SM, Yahav D, Ben Zvi H, Pertzov B, Kramer MR. Early humoral response among lung transplant recipients vaccinated with BNT162b2 vaccine. *Lancet Respir Med* 2021; **9**: e52-e53 [PMID: [33964244](#) DOI: [10.1016/S2213-2600\(21\)00184-3](#)]
- 94 **Miele M**, Busà R, Russell G, Sorrentino MC, Di Bella M, Timoneri F, Mularoni A, Panarello G, Vitulo P, Conaldi PG, Bulati M. Impaired anti-SARS-CoV-2 humoral and cellular immune response induced by Pfizer-BioNTech BNT162b2 mRNA vaccine in solid organ transplanted patients. *Am J Transplant* 2021; **21**: 2919-2921 [PMID: [34058052](#) DOI: [10.1111/ajt.16702](#)]
- 95 **Cucchiari D**, Egri N, Bodro M, Herrera S, Del Risco-Zevallos J, Casals-Urquiza J, Cofan F, Moreno A, Rovira J, Banon-Maneus E, Ramirez-Bajo MJ, Ventura-Aguir P, Pérez-Olmos A, Garcia-Pascual M, Pascal M, Vilella A, Trilla A, Ríos J, Palou E, Juan M, Bayés B, Diekmann F. Cellular and humoral response after mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients. *Am J Transplant* 2021; **21**: 2727-2739 [PMID: [34036720](#) DOI: [10.1111/ajt.16701](#)]
- 96 **Rabinowich L**, Grupe A, Baruch R, Ben-Yehoyada M, Halperin T, Turner D, Katchman E, Levi S, Houry I, Lubezky N, Shibolet O, Katchman H. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. *J Hepatol* 2021; **75**: 435-438 [PMID: [33892006](#) DOI: [10.1016/j.jhep.2021.04.020](#)]
- 97 **American Association for the Study of Liver Diseases (AASLD)**. Clinical best practice advice for hepatology and liver transplant providers during the COVID-19 pandemic: AASLD expert panel consensus statement (updated November 9, 2020). [cited 18 February 2021]. Available from: <https://www.aasld.org/sites/default/files/2020-11/AASLD-COVID19-ExpertPanelConsensusStatement-November092020.pdf>
- 98 **Russo FP**, Piano S, Bruno R, Burra P, Puoti M, Masarone M, Montagnese S, Ponziani FR, Petta S, Aghemo A; Italian Association for the Study of the Liver. Italian association for the study of the liver position statement on SARS-CoV2 vaccination. *Dig Liver Dis* 2021; **53**: 677-681 [PMID: [33941488](#) DOI: [10.1016/j.dld.2021.03.013](#)]



## Pediatric vascular tumors of the liver: Review from the pathologist's point of view

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### Abstract

Differential diagnosis of pediatric vascular liver tumors can be challenging due to inconsistent nomenclature, histologic overlap and the rarity of some entities. Here we give an up-to-date overview of the most important entities. We discuss the clinic, histology and pathophysiology of hepatic congenital and infantile hemangioma, hepatic epithelioid hemangioendothelioma and hepatic angiosarcoma.

**Key Words:** Hepatic congenital hemangioma; Hepatic infantile hemangioma; Hepatic epithelioid hemangioendothelioma; Hepatic angiosarcoma; Hepatic vascular tumors of infancy; Hepatic hemangiomas

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**Core Tip:** Overview of the most important pediatric hepatic vascular tumors from the point of view of the pathologist, including hepatic hemangiomas, hepatic epithelioid hemangioendothelioma and hepatic angiosarcoma.

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### INTRODUCTION

Through the years the classification of vascular anomalies in the liver has evolved due to better biological understanding with substantial contribution of molecular genetics



Belgium

**Peer-review report's scientific quality classification**

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 Grade B (Very good): B, B  
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and immunohistochemical correlates. However, terminology can be difficult due to the existence of multiple (general and organ specific) classifications and inconsistent nomenclature through the years. In 1997, vascular tumors were differentiated from vascular malformations for the first time[1]. In brief, the main difference between the above entities is that vascular tumors are considered as cellular vascular neoplastic proliferations and vascular malformations as errors in the morphogenesis lined by mature endothelium[2,3]. In 2014, The *International Society for the Study of Vascular Anomalies* (ISSVA) divided vascular tumors further in benign, locally aggressive or borderline and malignant entities[4]. Here, we give an overview of the most important pediatric hepatic vascular tumors, including hepatic hemangiomas, hepatic epithelioid hemangioendothelioma and hepatic angiosarcoma.

## HEPATIC HEMANGIOMA

Hepatic hemangiomas belong to the group of benign vascular tumors[4]. The term “*hemangioma*” has been used through the years for a variety of vascular malformations of the liver. In 2018, the ISSVA reserved this term for vascular lesions that match the definition of congenital or infantile hemangiomas[5]. These benign endothelial neoplasms can occur in the liver and belong to the histologic group of “*hepatic hemangioendothelioma, type 1*” (Figure 1). However, the term ‘*hemangioendothelioma*’ has to be used with caution, due to the terminology overlap with epithelioid hemangioendothelioma (which is considered as a malignant vascular entity) and should be avoided in absence of histologic evaluation[5,6]. Further, histologic confirmation of hemangiomas is often not required, since the diagnosis can easily be made with physical examination, imaging and review of patient’s history. Still, a biopsy can be performed when the history or clinical/radiological features are atypical[5]. Hemangiomas are characterized by a proliferation, plateau and involution phase. They occur due to an imbalance in angiogenesis, resulting in an uncontrolled proliferation of vascular elements. Involution of the lesions is characterized by a decrease in angiogenic factors, endothelial cell apoptosis and high levels of angiogenic inhibitors, replacing the endothelial cells by loose stromal tissue [2,6].

### Hepatic congenital hemangioma

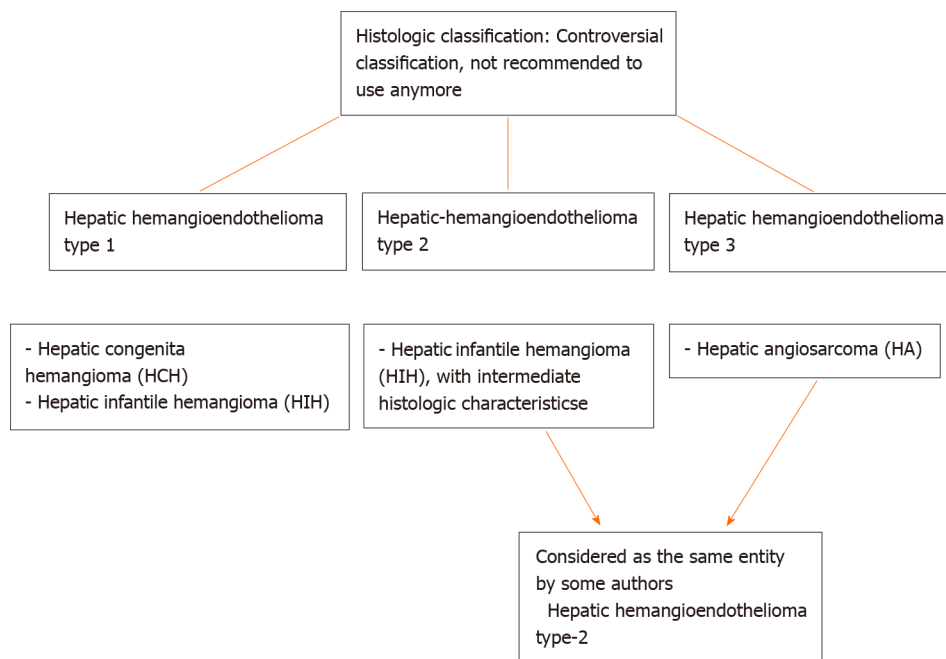
Hepatic congenital hemangiomas (HCH) are benign high-flow vascular tumors that proliferate in utero and are fully grown at birth with no postnatal increase in size. They are less common than hepatic infantile hemangioma (HIH) and present mostly as a solitary lesion[5,7,8]. Diagnosis can be made on prenatal imaging showing a large mass with extensive central infarction, hemorrhage, calcifications and sometimes large abnormal vessels, suggestive for arteriovenous malformation[5,9]. They can be asymptomatic or can cause intratumoral bleeding, thrombocytopenia, hypofibrinogenemia (Kasabach-Merritt syndrome, occasionally associated with large hepatic hemangiomas) and high-output cardiac failure[5,10].

The most important clinical differential diagnoses of a liver mass in infants include hepatic infantile hemangioma (HIH), epithelioid hemangioendothelioma, hepatoblastoma, germ cell tumors, (metastatic) neuroblastoma, mesenchymal hamartoma, cysts and abscesses[10,11].

There are 3 clinical subtypes depending on the pattern of evolution: *rapidly involuting congenital hemangioma* (RICH), *partially involuting congenital hemangioma* (PICH) and *noninvoluting congenital hemangioma* (NICH)[4,5,10]. These subtypes share common histopathologic features and have to be seen as part of a single entity with differences in their clinical behavior[12,13].

Histologically (Figure 2), HCHs are usually well-demarcated vascular lesions which can show entrapment of hepatocytes and bile ducts in interface areas[9]. RICH is composed of lobules of variable sized, mostly small thin-walled vessels lined by plump endothelium without cytonuclear atypia[7,10]. There may be evidence of thrombosis and the central part (*i.e.*, the first area of involution) may contain necrotic and hemorrhagic areas, fibrosis and focal dystrophic calcifications. Extramedullary hematopoiesis can also be observed. At the periphery of the lesion abundant larger vessels occur, sometimes associated with aneurysmal changes[7]. In contrast to RICH, NICH shows lobules of small vessels with interlobular fibrosis but without signs of involution. Arteriovenous microfistulae with large irregular vessels in the center can occur[10]. PICH shows histologic overlap between RICH and NICH and cannot be distinguished histologically[12,13]. Endothelial cells show immunoreactivity for Wilms’ Tumor 1 (WT-1), CD34, CD31, factor VIII and Erythroblast transformation-





**Figure 1** Histologic classification of hepatic hemangioendothelioma.

specific [ETS]-related gene (ERG)[13-15]. Triana *et al*[16] showed there was no expression of podoplanin (D2-40) in HCH. However, El Zein *et al* showed focal positivity for podoplanin in congenital hemangiomas of the skin, mainly in abnormal extralobular lymphatic vessels or in patients with concomitant thrombocytopenia (with decrease of intensity when platelet count normalized)[13]. The endothelial cells of HCH do not stain for glucose transporter-1 (GLUT-1), which is an important hallmark in the differentiation of HCH with HIH (Figure 3)[5,10].

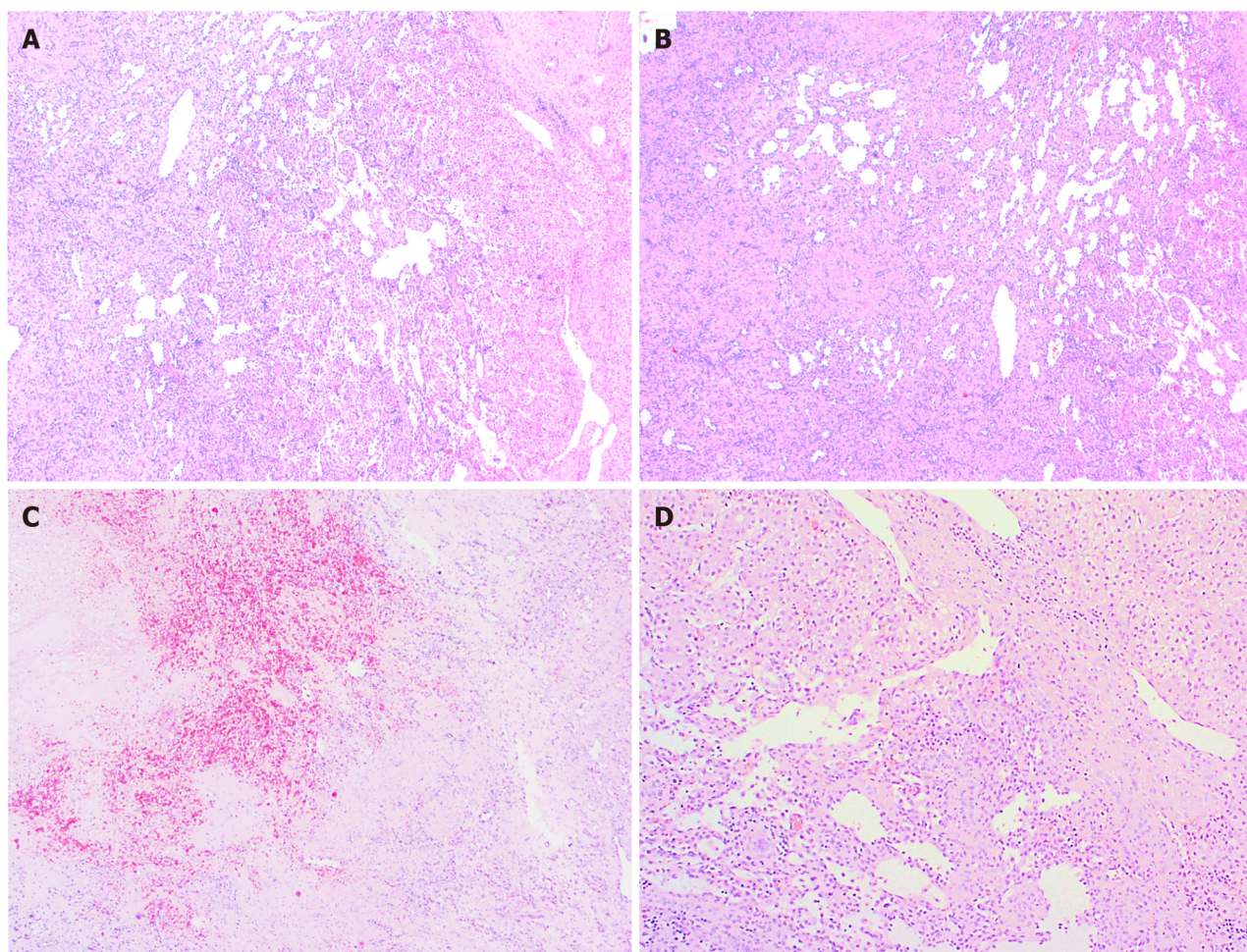
Genetic studies revealed that almost all HCHs have mutually exclusive, missense mutations that alter glutamine at amino acid 209 (Gln209) in the alleles which code for guanine nucleotide-binding protein G(q)alpha (*GNAQ*) and guanine nucleotide-binding protein subunit alpha-11 (*GNA11*), regardless of subtype. This implies that also other genetic, epigenetic and/or environmental factors may influence the behaviour of these lesions[10,17]. A subset shows missense mutations in *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* (*PIK3CA*) (c.3140A > T; p.His1047Leu)[16].

### **Hepatic infantile hemangioma**

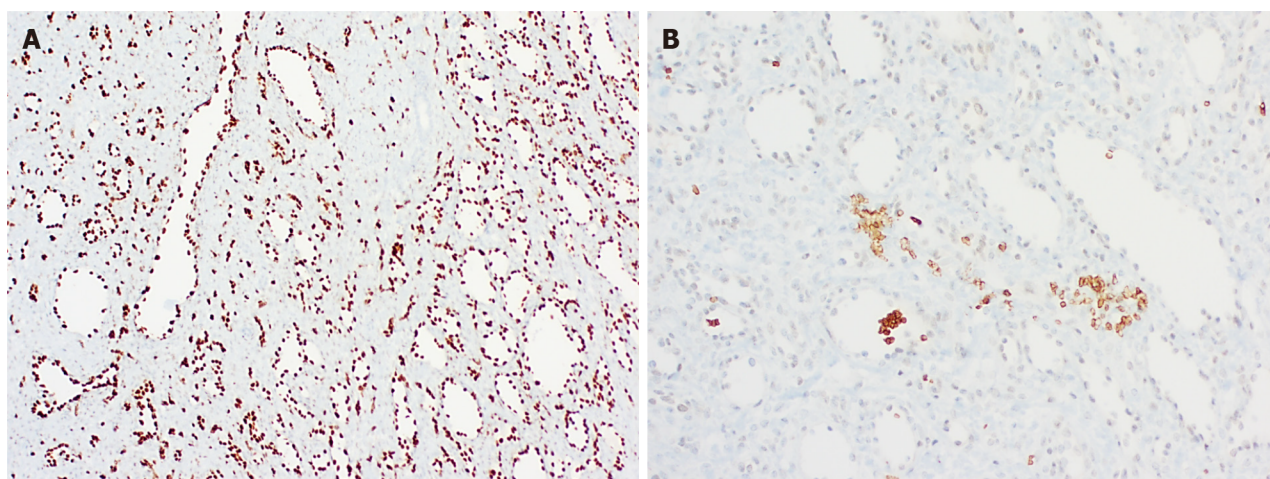
HIH is the most common benign hepatic tumor in infancy, with female predominance [7]. It proliferates rapid after birth, reaching a maximal size at 6 to 12 mo, and then it gradually involutes until 3 to 9 years[5,6]. Most hemangiomas are asymptomatic and remain undetected or are incidental findings on postnatal imaging. Still, a subset can be symptomatic due to their size, location or hemodynamic effects[8]. The high flow within the tumor or presence of shunts can cause cardiac failure. Also, thrombocytopenia and anemia can be observed when intralesional thrombosis occurs[5,8,18,19]. Due to high expression of type 3 iodothyronine deiodinase in these vascular lesions, which inactivates thyroid hormone, acquired consumptive hypothyroidism occurs. All of these complications are detected after birth during the proliferation phase and can be missed initially on newborn screening[5]. Further, HIH can occur in association with Beckwith-Wiedemann syndrome[6].

The clinical differential diagnosis of HIH is broad and includes arteriovenous malformations, arterioportal fistula, mesenchymal hamartoma, hepatoblastoma, angiosarcoma and (metastatic) neuroblastoma[8].

HIH presents clinically/macroscopically as white-tan nodules with occasionally degenerative changes in the centre[9]. They can be divided into 3 categories based on degree of unaffected liver parenchyma: focal, multifocal or diffuse disease. Focal HIH shows overlap with RICH, as it does not express GLUT-1 and can be found on prenatal imaging[8,18,19]. Therefore, focal HIH is not considered as a true HIH[8]. Multifocal HIH presents as areas of hemangioma with intervening segments of normal hepatic parenchyma, whereas a diffuse pattern is defined as innumerable tumors with nearly



**Figure 2 Hepatic congenital hemangioma.** A: A relatively well-demarcated vascular lesion; B: Lobules of variable sized, mostly small thin-walled vascular spaces and more abundant larger vessels at the periphery; C: Necrotic and hemorrhagic areas in the central part (area of involution); D: Entrapment of hepatocytes and bile ducts in interface areas.



**Figure 3 Hepatic congenital hemangioma.** A: Erythroblast transformation-specific-related gene expression of endothelial cells; B: No GLUT1 expression of endothelial cells.

complete hepatic parenchymal replacement[5]. Diffuse HIH shows a higher risk of complications, *e.g.*, abdominal compartment syndrome, heart failure, profound hypothyroidism, and even mortality[5,8]. Associated cutaneous infantile hemangioma is often present in patients with multifocal or diffuse HIH and increases with prematurity. Screening for HIH is therefore advised when multiple cutaneous infantile



hepatic hemangiomas occur (mostly 5 or more), as the liver is the most common visceral site [8,18].

Histologically (Figure 4), HH are well-demarcated, non-encapsulated vascular lesions composed of lobular, mostly small-sized vessels (capillary-like) with a pericytic cuff, highlighted by the immunohistochemical staining smooth muscle actin (SMA) (Figure 4) [6,9,11,20]. The periphery of these vascular lesions is cellular and mitotically active, with plump endothelial cells (suggesting active growth). Involution is particularly prominent in the center of the lesion and is characterized by reduced cellularity and enlarged vascular spaces lined by flat, mitotically inactive endothelium. The interstitium is fibrotic or fibromyxoid [9]. Bile ducts and hepatocytes are often entrapped within the advancing edge of the tumor. Areas of extra-medullary hematopoiesis may be present [11]. Central infarction, hemorrhage, calcification and abnormally enlarged vessels or arteriovenous malformation (AVM) can be observed [9]. Rarely, these vascular lesions show irregular anastomosing vascular spaces with prominent papillary formation lined by plump, pleomorphic endothelial cells with hyperchromatic nuclei [also known as *hepatic hemangioendothelioma type 2* with intermediate histologic characteristics, by some reports considered as a low-grade angiosarcoma (Figure 1)] [5,9,11,21]. The interstitium of these lesions can contain nests of epithelioid endothelial cells, entrapped nests of liver cells, and bile duct epithelium [9]. When these atypical features are seen or when a lesion is persistent or present in an older child, follow-up is indicated, because of the potential of malignant transformation [6,9].

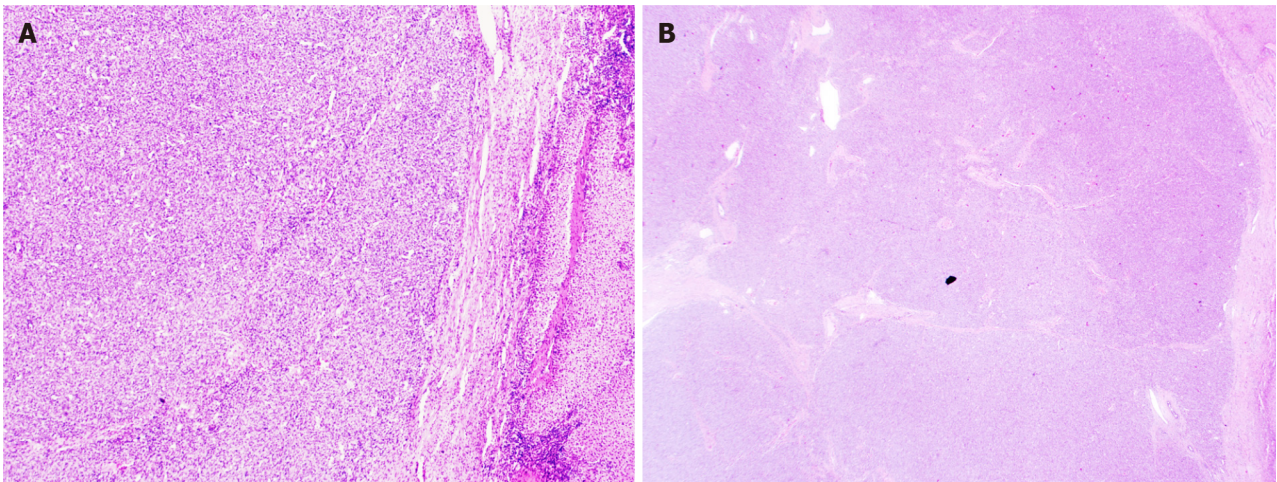
Multifocal and diffuse HH show positive staining for GLUT-1, which correlates with a high cell-proliferation and distinguish them from other types of vascular liver tumors (Figure 5) [5,10,22]. The endothelial cells are also positive for ERG, CD31, CD34 and factor VIII but do not express the lymphatic marker podoplanin [D2-40 (Figure 5)] [5,15,21].

There are several hypotheses for the pathophysiology of HH and its cutaneous counterpart. Clinical observations suggested hypoxia as a trigger for infantile hemangioma (IH). Hypoxia may be due to maternal events as well as the infant's own hypoxia-induced factors and is associated with GLUT-1, as GLUT-1 is a downstream target of hypoxia-inducible factor-1- $\alpha$  (HIF-1 $\alpha$ ), along with vascular endothelial growth factor A (VEGF-A) and insulin-like growth factor 2 (IGF-2). Also, the renin-angiotensin system (RAS) may play a role because high concentrations of angiotensin II (ATII), due to local expression of angiotensin-converting enzyme (ACE) in IH, stimulate cell proliferation. Further, IH expresses GLUT-1 and vascular antigens like Fc-gamma-receptor II, merosin, and Lewis Y antigen, which are also expressed in placental tissue. Another study found that IH endothelial cells share a similar immunophenotype (CD34 and CD133 positive) with embryonic veins, suggesting IH endothelial cells are arrested in an early stage of vascular differentiation [23]. Further, Takahashi *et al* observed an imbalance of vasculogenic factors in IH. During the proliferating phase, IH shows a high expression of type IV collagenase and vascular endothelial growth factor (VEGF) and when involuting there is an increase in tissue metalloproteinases, inhibiting new vessel formation [24]. Moreover, Walter *et al* showed allelic loss after methylation-based and transcription-based polymerase chain reaction clonality assays, suggesting a nonrandom X-inactivation pattern and, thus, a monoclonal origin of IH. In addition, they found 2 cases of IH with a missense mutation, one in the kinase domain of the *vascular endothelial growth factor receptor* (*VEGFR2*) gene and one in the kinase insert of the *VEGFR3* gene. These observations all suggest an alteration in the VEGF signaling pathway in IH [25].

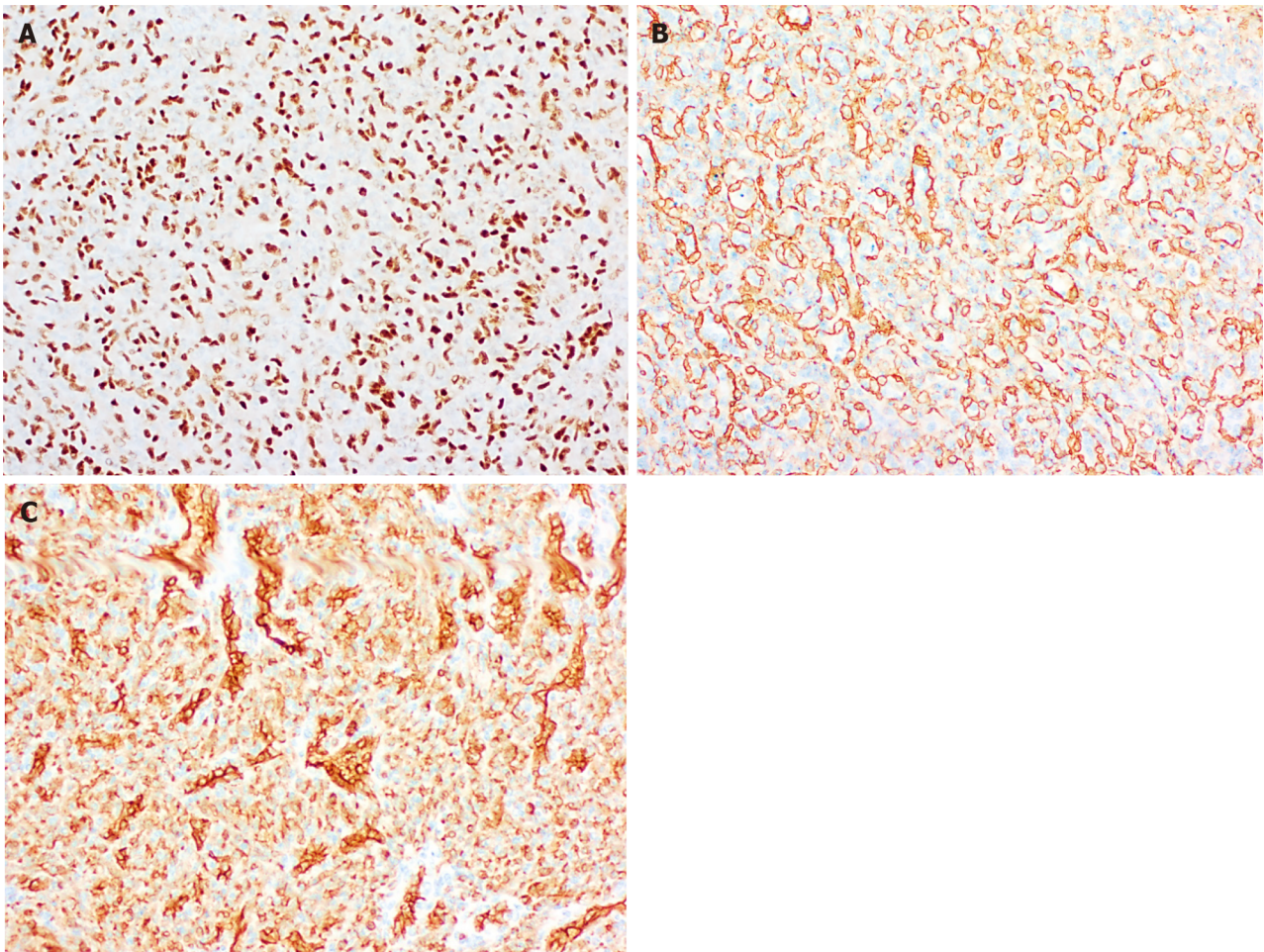
## EPITHELIOID HEMANGIOENDOTHELIOMA

Epithelioid hemangioendothelioma (EHE) is a rare malignant vascular tumor, which can occur anywhere in the body but typically arises in liver and lung [4,26]. It is mostly seen in adults, but can be diagnosed in children (estimated prevalence of 1/1000000, mean age 13.8 years) [27]. Hepatic EHEs show a more aggressive course than when arising in bone/soft tissue and are mostly multifocal. Hepatic EHE presents in most cases as a tumoral mass and has an unpredictable clinical course. It may be indolent, stable or aggressive [26,27]. Size > 3 cm and high mitotic index (> 3 mitoses/50 HPF) are poor prognostic factors in elderly [26].

EHEs appear macroscopically as solid, white lesions with some hemorrhagic changes [20]. Histologically (Figure 6), EHEs are relatively distinctive from the normal liver parenchyma and are composed of nests, cords, strands or single infiltrative epithelioid cells set in a myxohyaline stroma. The cells in EHE are epithelioid with



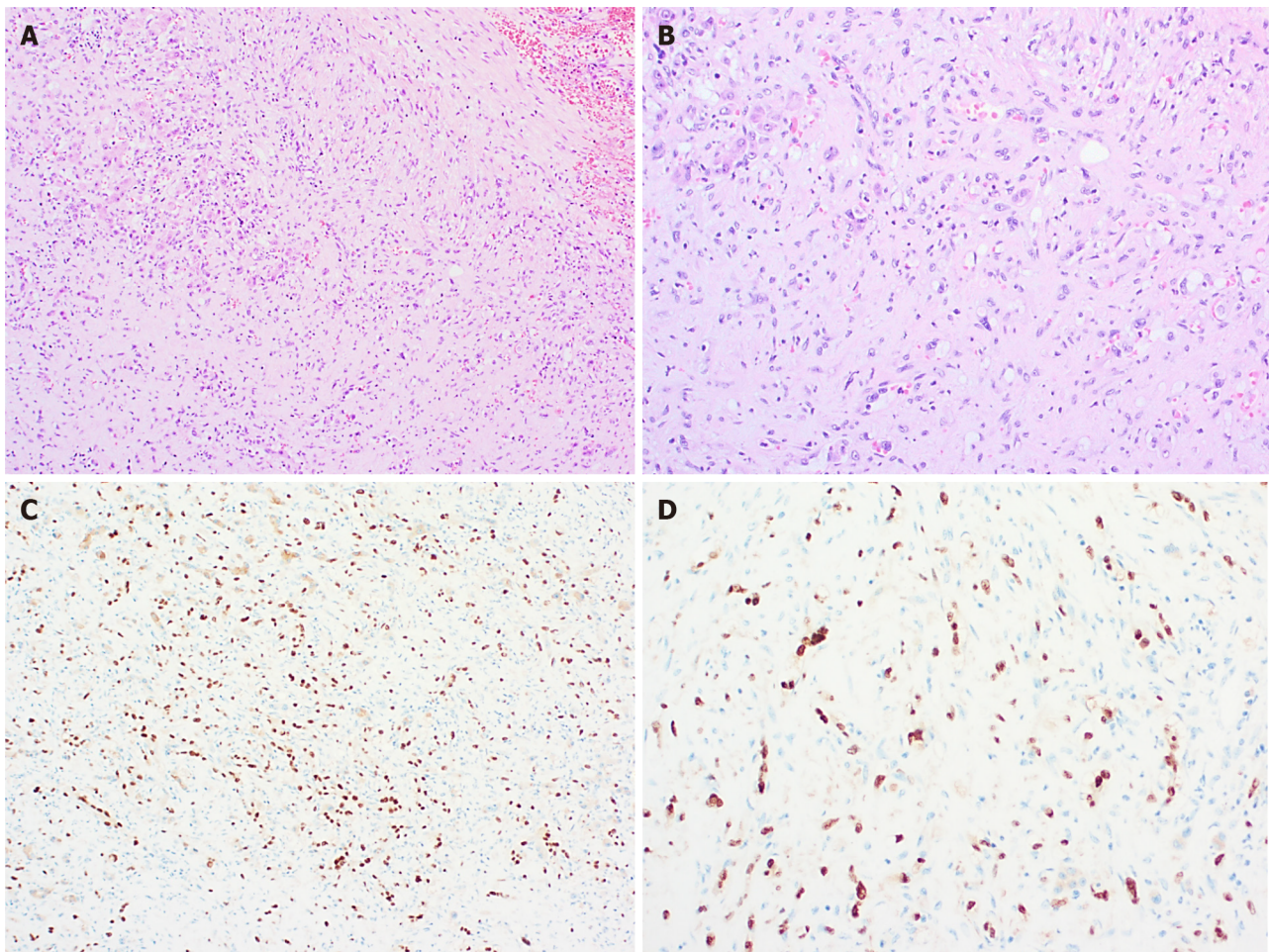
**Figure 4 Hepatic infantile hemangioma.** A: A well-demarcated vascular lesion; B: Lobular, small-sized vascular spaces.



**Figure 5 Hepatic infantile hemangioma.** A: Erythroblast transformation-specific-related gene expression of endothelial cells; B: Smooth muscle actin expression of the pericytic cells; C: GLUT1 expression of endothelial cells.

eosinophilic cytoplasm and frequently show intracytoplasmic vacuoles (so-called “blister cells”)[20,28]. Occasionally, there are tufts or papillary projections into the vessels. A subset of EHE shows histologic overlap with hepatic angiosarcoma (HA) containing necrosis or moderate to severe cytonuclear atypia (with large hyperchromatic cells), without the typical myxoid stromal component. In this setting, the distinction between EHE and HA can be difficult for a pathologist, especially in small liver biopsies. Usually EHE shows nuclear calmodulin-binding transcription activator1





**Figure 6 Hepatic epithelioid hemangioendothelioma.** A: Nests, cords, strands and single infiltrative epithelioid cells with intracytoplasmic vacuoles; B: Nests, cords, strands and single infiltrative epithelioid cells with intracytoplasmic vacuoles; C: Nuclear calmodulin-binding transcription activator1 (CAMTA1) expression; D: Nuclear CAMTA 1 expression.

(CAMTA1) expression, which can be very helpful in the differential diagnosis with HA since this is a highly specific and sensitive marker for EHE with a CAMTA1 rearrangement (Figure 6)[28]. EHE also stains for ERG, CD31, CD34, factor VIII and podoplanin (D2-40)[15,20,28]. Nuclear positivity for transcription factor E3 (TFE3) is seen in most cases of EHE, irrespective of an underlying *TFE3* rearrangement[20,28]. A small subset of EHE expresses pan-cytokeratin or cytokeratin 8/18 (CK8/18)[20].

Most of the EHEs are characterized by chromosomal translocations involving 1p36.3 and 3q25 resulting in WW domain-containing transcription regulator1 (*WWTR1*, also known as *TAZ*) – *CAMTA1* fusion genes. A small subset shows Yes-associated protein 1(*YAP1*)-*TFE3* gene fusions[26,28]. *TAZ* and *YAP* are transcriptional coactivators and effectors, which are downregulated by the Hippo tumor suppressor pathway. *WWTR1* -*CAMTA1* fusion genes therefore induce oncogenic transformation due to constitutive nuclear localization and activation of *TAZ* independent of the Hippo pathway[26].

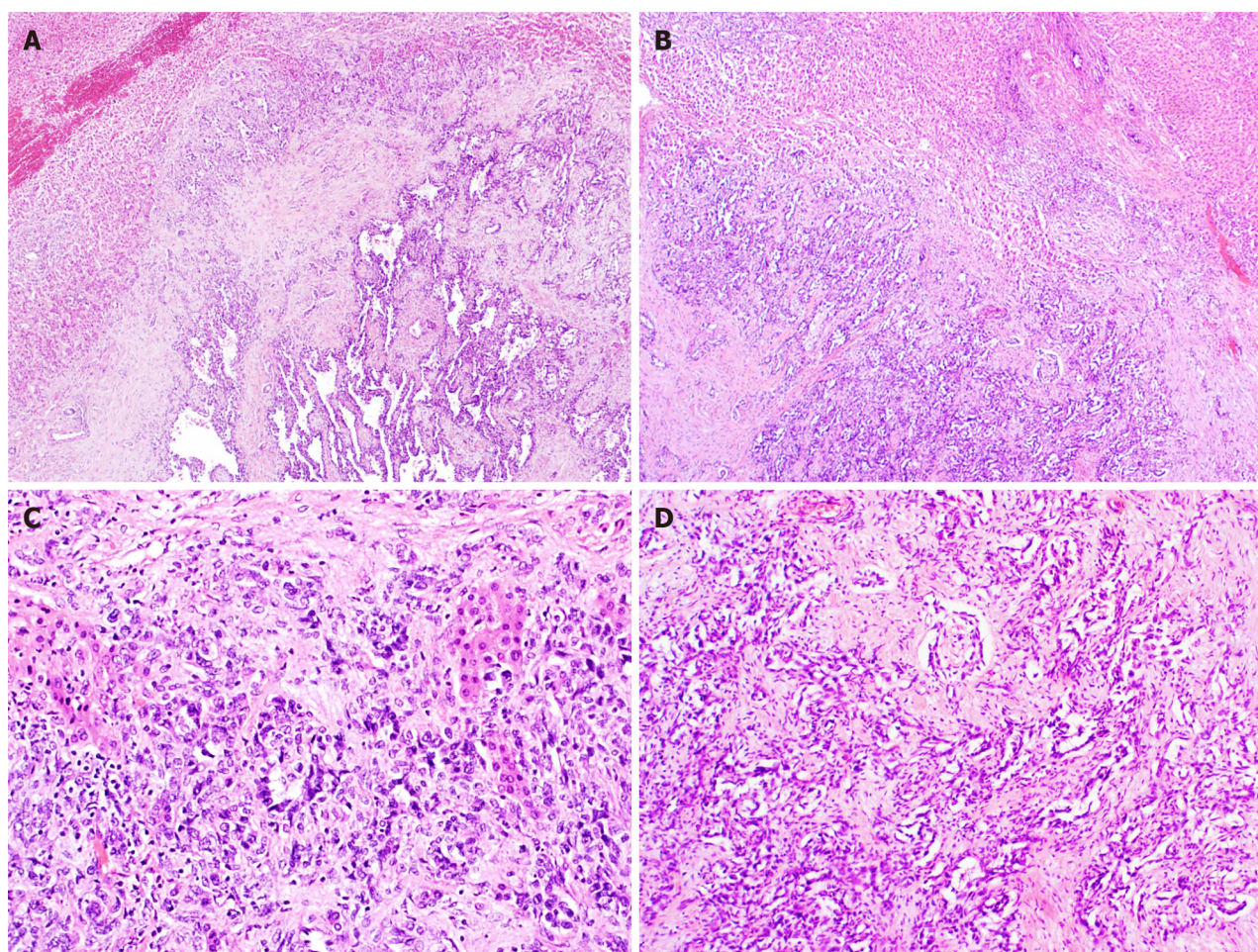
## HEPATIC ANGIOSARCOMA

Hepatic angiosarcoma (HA) is a rare high-grade malignant vascular tumor that occurs mostly in elderly[5,29,30]. Seldom they occur in children and the majority of pediatric angiosarcoma cases arises in the heart/pericardium and mediastinum[29]. When occurring in the liver angiosarcoma presents as a rapid enlargement of the liver associated with jaundice, abdominal pain, vomiting, fever, tachypnea, dyspnea and anemia[30]. Consumptive coagulopathy, disseminated intravascular coagulation and congestive heart failure are known complications[31]. In children HA has a female predominance and occurs mostly around 40 mo. It represents 1%-2% of all pediatric liver tumors and has the potential to metastasize, even at the onset of the disease. Metastasis is commonly found in the lungs[30,32]. HA can occur in the background of





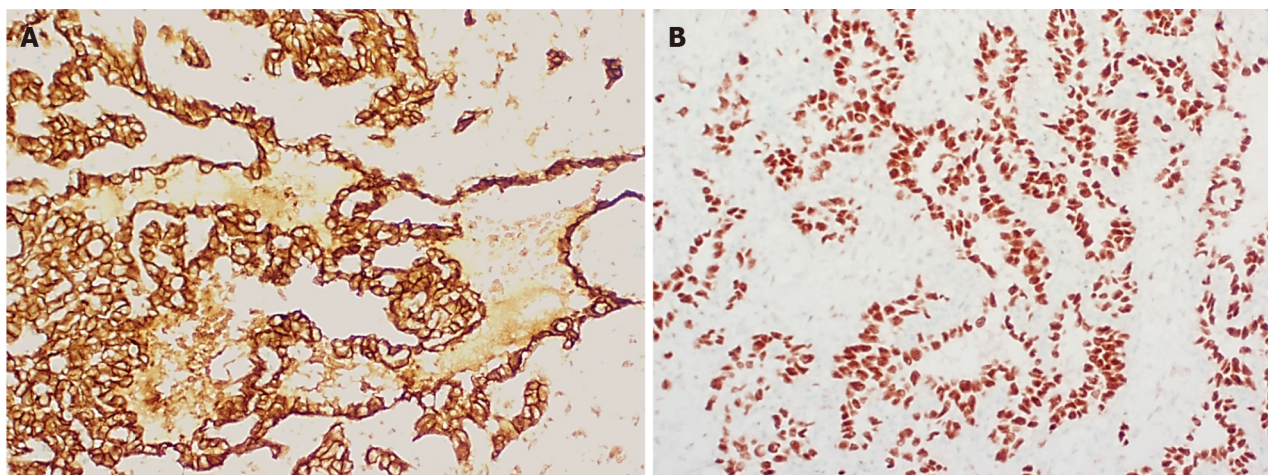
**Figure 7** Hepatic angiosarcoma, macroscopical features.



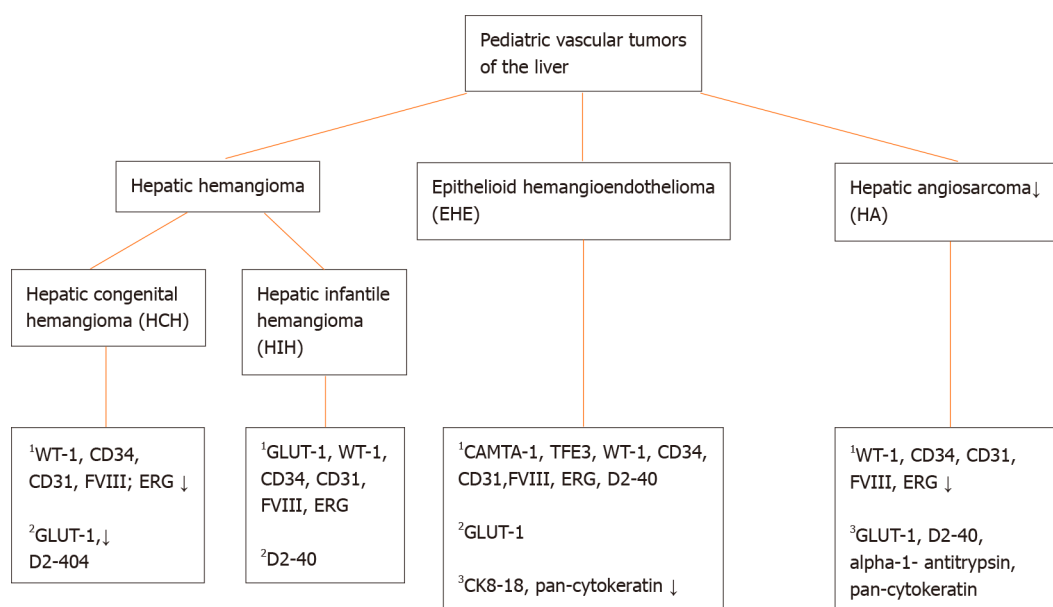
**Figure 8** Hepatic angiosarcoma. A: Unencapsulated vascular lesion; B: Infiltrative growth pattern; C: Anastomosing vascular spaces lined by endothelial cells with marked cytological atypia and multilayering. D: Anastomosing vascular spaces lined by endothelial cells with marked cytological atypia and multilayering.

a HIIH or can develop 4 to 5 years after primary diagnosis of HIIH. Therefore, HIIH in patients older than 1 year, should be followed carefully[30]. Also, in the past, several chemical carcinogens, including vinyl chloride monomer (VCM), thorotrast, radium and arsenic, have been associated with HA formation[33,34]. Pediatric HA has a poor





**Figure 9** Hepatic angiosarcoma. A: CD31 expression of the endothelial cells; B: Erythroblast transformation-specific-related gene expression of the endothelial cells.



**Figure 10** Overview pediatric vascular tumors of the liver and their immunohistochemistry. <sup>1</sup>Positive immunohistochemical staining; <sup>2</sup>Negative immunohistochemical staining; <sup>3</sup>Occasionally positive immunohistochemical staining. WT-1: Wilms' tumor 1; FVIII: factor VIII; ERG: Erythroblast transformation-specific-related gene; GLUT-1: glucose transporter-1; D2-40: podoplanin; CAMTA1: calmodulin-binding transcription activator1; TFE3: transcription factor E3; CK8-18: cytokeratin 8-18.

prognosis with an average survival of 16 mo and a 5-year overall survival of 20%-35% [30,32].

Diagnosis of a HA can be really challenging, as it is an extremely rare tumor and there are no specific radiographic characteristics that differentiate malignant vascular hepatic tumors from benign ones [33,35]. Histologic diagnosis can only be obtained by adequate and representative tissue biopsies, received by laparotomy [35].

Macroscopically, HA presents as a large solitary mass, or as multiple or diffuse nodules in the centre and periphery of the liver. Often sponge-like hemorrhagic areas alternate with solid gray-white nodules, surrounded by normal liver parenchyma (Figure 7) [31,36]. Commonly, both liver lobes are affected [35,36]. Histologically (Figures 8 and 9), HA shows an unencapsulated vascular tumoral lesion composed of anastomosing vascular spaces and sinusoids lined by endothelial cells with marked cytological atypia and multilayering [29,31,33,35]. The cells are plump, pleomorphic with hyperchromatic nuclei and show brisk mitotic activity [33]. Focally infiltrative whorls or glomeruloid foci of sarcomatoid cells or kaposiform spindle cells with intracytoplasmic PAS positive eosinophilic globules can be seen [30,32,33,35]. Tumor necrosis can be observed [29]. Histologically, HA is classified as *hepatic hemangioen-*

dothelioma, type 3 (Figure 10)[5]. HA shows immunoreactivity for ERG, CD31, CD34 and factor VIII[15,28,33]. A small percentage expresses pan-cytokeratin[33]. Ki-67 shows a proliferation of more than 10%[36]. HAs are occasionally positive for GLUT-1 and podoplanin (D2-40)[15,22,32]. The spindle cell component may show cytoplasmic immunopositivity for alpha-1-antitrypsin[30].

Uptil now, little is known about the genetics of HA, due to examination of small cohorts with a selected gene panel[34]. *KRAS* mutations have been described in sporadic and thorotrast-induced HA, and *TP53* mutations in VCM-related HA[37,38]. Also alterations in the *RAS*-*RAF*-*MAPK* pathway, *CDKN2A/p16* and *PTEN* gene have been found[34,39]. Recently a *ROS1-GOPC/FIG* (Fused In Glioblastome) fusion has been found in 1 case[34,37]. This fusion gene can act as a potential target for therapy. Further, upregulation of VEGF-receptor and consistent increased expression of VEGF are commonly seen[34].

## CONCLUSION

Diagnosis of a pediatric hepatic vascular tumor can be challenging, not only for the clinici/radiologist, but for the pathologist as well. Throughout the years immunohistochemical markers[10] and molecular genetics have been proven very helpful in the differential diagnosis of vascular tumors. Here we gave an overview of the most important pediatric hepatic vascular tumors and their histology and pathophysiology. Still there is a lot to discover about these vascular lesions.

## REFERENCES

- 1 Enjolras O. Classification and management of the various superficial vascular anomalies: hemangiomas and vascular malformations. *J Dermatol* 1997; **24**: 701-710 [PMID: 9433027 DOI: 10.1111/j.1346-8138.1997.tb02522.x]
- 2 Gampper TJ, Morgan RF. Vascular anomalies: hemangiomas. *Plast Reconstr Surg* 2002; **110**: 572-85; quiz 586; discussion 587 [PMID: 12142679 DOI: 10.1097/00006534-200208000-00032]
- 3 Yang B, Li L, Zhang LX, Sun YJ, Ma L. Clinical Characteristics and Treatment Options of Infantile Vascular Anomalies. *Medicine (Baltimore)* 2015; **94**: e1717 [PMID: 26448027 DOI: 10.1097/MD.0000000000001717]
- 4 Wassef M, Blei F, Adams D, Alomari A, Baselga E, Berenstein A, Burrows P, Frieden IJ, Garzon MC, Lopez-Gutierrez JC, Lord DJ, Mitchel S, Powell J, Prendiville J, Vikkula M; ISSVA Board and Scientific Committee. Vascular Anomalies Classification: Recommendations From the International Society for the Study of Vascular Anomalies. *Pediatrics* 2015; **136**: e203-e214 [PMID: 26055853 DOI: 10.1542/peds.2014-3673]
- 5 Iacobas I, Phung TL, Adams DM, Trenor CC 3rd, Blei F, Fishman DS, Hammill A, Masand PM, Fishman SJ. Guidance Document for Hepatic Hemangioma (Infantile and Congenital) Evaluation and Monitoring. *J Pediatr* 2018; **203**: 294-300.e2 [PMID: 30244993 DOI: 10.1016/j.jpeds.2018.08.012]
- 6 Zavras N, Dimopoulou A, Machairas N, Paspala A, Vaos G. Infantile hepatic hemangioma: current state of the art, controversies, and perspectives. *Eur J Pediatr* 2020; **179**: 1-8 [PMID: 31758313 DOI: 10.1007/s00431-019-03504-7]
- 7 Roebuck D, Sebire N, Lehmann E, Barnacle A. Rapidly involuting congenital haemangioma (RICH) of the liver. *Pediatr Radiol* 2012; **42**: 308-314 [PMID: 22302317 DOI: 10.1007/s00247-011-2268-z]
- 8 Kulungowski AM, Alomari AI, Chawla A, Christison-Lagay ER, Fishman SJ. Lessons from a liver hemangioma registry: subtype classification. *J Pediatr Surg* 2012; **47**: 165-170 [PMID: 22244411 DOI: 10.1016/j.jpedsurg.2011.10.037]
- 9 Mo JQ, Dimashkieh HH, Bove KE. GLUT1 endothelial reactivity distinguishes hepatic infantile hemangioma from congenital hepatic vascular malformation with associated capillary proliferation. *Hum Pathol* 2004; **35**: 200-209 [PMID: 14991538 DOI: 10.1016/j.humpath.2003.09.017]
- 10 Lewis D, Hachey K, Fitzgerald S, Vaidya R. Rapidly involuting congenital haemangioma of the liver. *BMJ Case Rep* 2018; **2018** [PMID: 29871961 DOI: 10.1136/bcr-2018-224337]
- 11 Regier TS, Ramji FG. Pediatric hepatic hemangioma. *Radiographics* 2004; **24**: 1719-1724 [PMID: 15537980 DOI: 10.1148/rg.246035188]
- 12 Nasser E, Piram M, McCuaig CC, Kokta V, Dubois J, Powell J. Partially involuting congenital hemangiomas: a report of 8 cases and review of the literature. *J Am Acad Dermatol* 2014; **70**: 75-79 [PMID: 24176519 DOI: 10.1016/j.jaad.2013.09.018]
- 13 El Zein S, Boccara O, Soupre V, Vieira AF, Bodemer C, Coulomb A, Wassef M, Fraitag S. The histopathology of congenital haemangioma and its clinical correlations: a long-term follow-up study of 55 cases. *Histopathology* 2020; **77**: 275-283 [PMID: 32281140 DOI: 10.1111/his.14114]
- 14 Trindade F, Tellechea O, Torrelo A, Requena L, Colmenero I. Wilms tumor 1 expression in vascular neoplasms and vascular malformations. *Am J Dermatopathol* 2011; **33**: 569-572 [PMID: 21697701]

- DOI: [10.1097/DAD.0b013e3182092527](https://doi.org/10.1097/DAD.0b013e3182092527)]
- 15 **Fujii T**, Zen Y, Sato Y, Sasaki M, Enomae M, Minato H, Masuda S, Uehara T, Katsuyama T, Nakanuma Y. Podoplanin is a useful diagnostic marker for epithelioid hemangioendothelioma of the liver. *Mod Pathol* 2008; **21**: 125-130 [PMID: [18084256](https://pubmed.ncbi.nlm.nih.gov/18084256/) DOI: [10.1038/modpathol.3800986](https://doi.org/10.1038/modpathol.3800986)]
  - 16 **Triana P**, Rodríguez-Laguna L, Giacaman A, Salinas-Sanz JA, Martín-Santiago A, López-Santamaría M, Palacios E, Beato MJ, Martínez-González V, López-Gutiérrez JC. Congenital hepatic hemangiomas: Clinical, histologic, and genetic correlation. *J Pediatr Surg* 2020; **55**: 2170-2176 [PMID: [32115227](https://pubmed.ncbi.nlm.nih.gov/32115227/) DOI: [10.1016/j.jpedsurg.2020.02.008](https://doi.org/10.1016/j.jpedsurg.2020.02.008)]
  - 17 **Ayturk UM**, Couto JA, Hann S, Mulliken JB, Williams KL, Huang AY, Fishman SJ, Boyd TK, Kozakewich HPW, Bischoff J, Greene AK, Warman ML. Somatic Activating Mutations in GNAQ and GNA11 Are Associated with Congenital Hemangioma. *Am J Hum Genet* 2016; **98**: 1271 [PMID: [27259057](https://pubmed.ncbi.nlm.nih.gov/27259057/) DOI: [10.1016/j.ajhg.2016.05.010](https://doi.org/10.1016/j.ajhg.2016.05.010)]
  - 18 **Gnarra M**, Behr G, Kitajewski A, Wu JK, Anupindi SA, Shawber CJ, Zavras N, Schizas D, Salakos C, Economopoulos KP. History of the infantile hepatic hemangioma: From imaging to generating a differential diagnosis. *World J Clin Pediatr* 2016; **5**: 273-280 [PMID: [27610342](https://pubmed.ncbi.nlm.nih.gov/27610342/) DOI: [10.5409/wjcp.v5.i3.273](https://doi.org/10.5409/wjcp.v5.i3.273)]
  - 19 **Christison-Lagay ER**, Burrows PE, Alomari A, Dubois J, Kozakewich HP, Lane TS, Paltiel HJ, Klement G, Mulliken JB, Fishman SJ. Hepatic hemangiomas: subtype classification and development of a clinical practice algorithm and registry. *J Pediatr Surg* 2007; **42**: 62-7; discussion 67 [PMID: [17208542](https://pubmed.ncbi.nlm.nih.gov/17208542/) DOI: [10.1016/j.jpedsurg.2006.09.041](https://doi.org/10.1016/j.jpedsurg.2006.09.041)]
  - 20 **Flucke U**, Vogels RJ, de Saint Aubain Somerhausen N, Creyten DH, Riedl RG, van Gorp JM, Milne AN, Huysentruyt CJ, Verdijk MA, van Asseldonk MM, Suurmeijer AJ, Bras J, Palmedo G, Groenen PJ, Mentzel T. Epithelioid Hemangioendothelioma: clinicopathologic, immunohistochemical, and molecular genetic analysis of 39 cases. *Diagn Pathol* 2014; **9**: 131 [PMID: [24986479](https://pubmed.ncbi.nlm.nih.gov/24986479/) DOI: [10.1186/1746-1596-9-131](https://doi.org/10.1186/1746-1596-9-131)]
  - 21 **Zhang Z**, Chen HJ, Yang WJ, Bu H, Wei B, Long XY, Fu J, Zhang R, Ni YB, Zhang HY. Infantile hepatic hemangioendothelioma: a clinicopathologic study in a Chinese population. *World J Gastroenterol* 2010; **16**: 4549-4557 [PMID: [20857525](https://pubmed.ncbi.nlm.nih.gov/20857525/) DOI: [10.3748/wjg.v16.i36.4549](https://doi.org/10.3748/wjg.v16.i36.4549)]
  - 22 **Hernández F**, Navarro M, Encinas JL, López Gutiérrez JC, López Santamaría M, Leal N, Martínez L, Patrón M, Tovar JA. The role of GLUT1 immunostaining in the diagnosis and classification of liver vascular tumors in children. *J Pediatr Surg* 2005; **40**: 801-804 [PMID: [15937818](https://pubmed.ncbi.nlm.nih.gov/15937818/) DOI: [10.1016/j.jpedsurg.2005.01.046](https://doi.org/10.1016/j.jpedsurg.2005.01.046)]
  - 23 **Smith CJF**, Friedlander SF, Guma M, Kavanaugh A, Chambers CD. Infantile Hemangiomas: An Updated Review on Risk Factors, Pathogenesis, and Treatment. *Birth Defects Res* 2017; **109**: 809-815 [PMID: [28402073](https://pubmed.ncbi.nlm.nih.gov/28402073/) DOI: [10.1002/bdr2.1023](https://doi.org/10.1002/bdr2.1023)]
  - 24 **Takahashi K**, Mulliken JB, Kozakewich HP, Rogers RA, Folkman J, Ezekowitz RA. Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 1994; **93**: 2357-2364 [PMID: [7911127](https://pubmed.ncbi.nlm.nih.gov/7911127/) DOI: [10.1172/JCI117241](https://doi.org/10.1172/JCI117241)]
  - 25 **Walter JW**, North PE, Waner M, Mizeracki A, Blei F, Walker JW, Reinisch JF, Marchuk DA. Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma. *Genes Chromosomes Cancer* 2002; **33**: 295-303 [PMID: [11807987](https://pubmed.ncbi.nlm.nih.gov/11807987/) DOI: [10.1002/gcc.10028](https://doi.org/10.1002/gcc.10028)]
  - 26 **Hettmer S**, Andrieux G, Hochrein J, Kurz P, Rössler J, Lassmann S, Werner M, von Bubnoff N, Peters C, Koscielniak E, Sparber-Sauer M, Niemeyer C, Mentzel T, Busch H, Boerries M. Epithelioid hemangioendotheliomas of the liver and lung in children and adolescents. *Pediatr Blood Cancer* 2017; **64** [PMID: [28598585](https://pubmed.ncbi.nlm.nih.gov/28598585/) DOI: [10.1002/pbc.26675](https://doi.org/10.1002/pbc.26675)]
  - 27 **Cournoyer E**, Al-Ibraheemi A, Engel E, Chaudry G, Stapleton S, Adams DM. Clinical characterization and long-term outcomes in pediatric epithelioid hemangioendothelioma. *Pediatr Blood Cancer* 2020; **67**: e28045 [PMID: [31724797](https://pubmed.ncbi.nlm.nih.gov/31724797/) DOI: [10.1002/pbc.28045](https://doi.org/10.1002/pbc.28045)]
  - 28 **Jung H**, Kim HN, Jang Y, Park CK, Ha SY. CAMTA-1 Expression in 24 Cases of Hepatic Epithelioid Hemangioendothelioma in a Single Institute: Diagnostic Utility for Differential Diagnosis from Hepatic Angiosarcoma. *In Vivo* 2019; **33**: 2293-2297 [PMID: [31662570](https://pubmed.ncbi.nlm.nih.gov/31662570/) DOI: [10.21873/in vivo.11736](https://doi.org/10.21873/in vivo.11736)]
  - 29 **Deyrup AT**, Miettinen M, North PE, Khoury JD, Tighiouart M, Spunt SL, Parham D, Weiss SW, Shehata BM. Angiosarcomas arising in the viscera and soft tissue of children and young adults: a clinicopathologic study of 15 cases. *Am J Surg Pathol* 2009; **33**: 264-269 [PMID: [18987547](https://pubmed.ncbi.nlm.nih.gov/18987547/) DOI: [10.1097/PAS.0b013e3181875a5f](https://doi.org/10.1097/PAS.0b013e3181875a5f)]
  - 30 **Dimashkieh HH**, Mo JQ, Wyatt-Ashmead J, Collins MH. Pediatric hepatic angiosarcoma: case report and review of the literature. *Pediatr Dev Pathol* 2004; **7**: 527-532 [PMID: [15547777](https://pubmed.ncbi.nlm.nih.gov/15547777/) DOI: [10.1007/s10024-004-4041-x](https://doi.org/10.1007/s10024-004-4041-x)]
  - 31 **Chavhan GB**, Siddiqui I, Ingley KM, Gupta AA. Rare malignant liver tumors in children. *Pediatr Radiol* 2019; **49**: 1404-1421 [PMID: [31620842](https://pubmed.ncbi.nlm.nih.gov/31620842/) DOI: [10.1007/s00247-019-04402-8](https://doi.org/10.1007/s00247-019-04402-8)]
  - 32 **Grassia KL**, Peterman CM, Iacobas I, Margolin JF, Bien E, Padhye B, Meyers RL, Adams DM. Clinical case series of pediatric hepatic angiosarcoma. *Pediatr Blood Cancer* 2017; **64** [PMID: [28521077](https://pubmed.ncbi.nlm.nih.gov/28521077/) DOI: [10.1002/pbc.26627](https://doi.org/10.1002/pbc.26627)]
  - 33 **Marletta S**, Cavallo E, Ammendola S, Stefanizzi L, Mastrosimini MG, D'Onofrio M, Brunelli M, Calìò A, Pecori S, Dalbeni A, Ruzzenente A, Capelli P. Multifocal Hepatic Angiosarcoma with Atypical Presentation: Case Report and Literature Review. *J Gastrointest Cancer* 2021; **52**: 771-775 [PMID: [32894473](https://pubmed.ncbi.nlm.nih.gov/32894473/) DOI: [10.1007/s12029-020-00504-x](https://doi.org/10.1007/s12029-020-00504-x)]
  - 34 **Marks EI**, Pamarthy S, Dizon D, Birnbaum A, Yakirevich E, Safran H, Carneiro BA. *ROSI-*



- GOPC/FIG*: a novel gene fusion in hepatic angiosarcoma. *Oncotarget* 2019; **10**: 245-251 [PMID: 30719217 DOI: 10.18632/oncotarget.26521]
- 35 **Awan S**, Davenport M, Portmann B, Howard ER. Angiosarcoma of the liver in children. *J Pediatr Surg* 1996; **31**: 1729-1732 [PMID: 8987004 DOI: 10.1016/s0022-3468(96)90065-2]
  - 36 **Wang ZB**, Wei LX. [Primary hepatic angiosarcoma: a clinical and pathological analysis]. *Zhonghua Bing Li Xue Za Zhi* 2013; **42**: 376-380 [PMID: 24060070 DOI: 10.3760/cma.j.issn.0529-5807.2013.06.005]
  - 37 **Gigante E**, Paradis V, Ronot M, Cauchy F, Soubrane O, Ganne-Carrié N, Nault JC. New insights into the pathophysiology and clinical care of rare primary liver cancers. *JHEP Rep* 2021; **3**: 100174 [PMID: 33205035 DOI: 10.1016/j.jhepr.2020.100174]
  - 38 **Przygodzki RM**, Finkelstein SD, Keohavong P, Zhu D, Bakker A, Swalsky PA, Soini Y, Ishak KG, Bennett WP. Sporadic and Thorotrast-induced angiosarcomas of the liver manifest frequent and multiple point mutations in K-ras-2. *Lab Invest* 1997; **76**: 153-159 [PMID: 9010458]
  - 39 **Tate G**, Suzuki T, Mitsuya T. Mutation of the PTEN gene in a human hepatic angiosarcoma. *Cancer Genet Cytogenet* 2007; **178**: 160-162 [PMID: 17954274 DOI: 10.1016/j.cancergencyto.2007.07.017]



## Autoimmune hepatitis in genetic syndromes: A literature review

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### Abstract

Genetic syndromes represent relevant and rare diseases. These conditions include a large amount of epidemiological, pathogenetic and clinical features. However, a systematic approach to genetic syndromes is often prevented by the rareness of these diseases. So, although clinical features are usually precisely defined, nowadays more uncommon associations between genetic syndromes and internal medicine related diseases have been insufficiently studied. Autoimmune hepatitis (AIH) is a chronic liver disease caused by loss of tolerance to hepatocyte-specific auto-antigens. Conversely, a better knowledge about specific genetic syndromes in which AIH is more frequent could be important in the clinical management of patients, both for an early diagnosis and for a prompt therapy. Furthermore, a systematic approach could explain if onset, clinical course, and response to treatment of AIH are typical for specific genetic syndromes. We took in consideration all the scientific articles reported in PubMed in the last 10 years, from 2010 to 2020. The purpose of this review is to explore the prevalence of AIH in genetic syndrome, but also to suggest new classification, that could be useful for pathogenetic hypothesis and clinical approach to genetic syndrome. From the 139 publications selected using keywords "autoimmune hepatitis" and "genetic syndrome", 30 papers (21.6%) respected the chosen inclusion criteria, reporting the association between AIH in patients with a genetic syndrome. We have collected in all 47 patients with AIH and genetic syndrome, and with median age of 12.6-year-old. We suggest that when a patient presents a clinical picture of cryptogenic chronic hepatitis, that is unexplained, it is useful to explore differential diagnosis of AIH associated with genetic syndrome. Given the clinical relevance of this topic, further reports are needed to demonstrate our hypothesis and collect new evidence in this field.

**Key Words:** Autoimmunity; Hepatitis; Gene; Syndrome; Liver; Disease; Immunity

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**quality classification**

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 Grade B (Very good): B  
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**Core Tip:** Autoimmunity is a relevant health problem, burdened by delay in diagnosis and difficult therapeutic approach. Genetic syndromes often include autoimmune diseases in their typically complex clinical picture. This review explores the association between genetic syndromes and a specific autoimmune disease, autoimmune hepatitis in order to understand if there are pathogenetic mechanisms based on specific mutations, but also how much autoimmune hepatitis is frequent in genetic syndromes. This systematic approach showed an interesting correlation between these two important groups of diseases.

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## INTRODUCTION

Rare genetic diseases are a topic of relevant importance for multi-organ complications and complex clinical pictures. These conditions include a large amount of epidemiological, pathogenetic and clinical features. The most of them have defined DNA mutations, typical phenotypes and characteristic clinical courses. Auto-inflammatory and autoimmune complications are described in several genetic syndromes. This occurs more often when immunoregulatory genes are involved in the pathogenesis of the disease.

The autoimmune hepatitis (AIH) is a complex immune-mediated and chronic liver disease, caused by loss of tolerance to hepatocyte-specific autoantigens.

It is an autoimmune disease of unknown etiology. There is no clear evidence for a hereditary etiology of this disease. Association studies of major histocompatibility complex and other genes demonstrate an influence of immunogenetics[1].

The AIH have annual incidence ranges from 0.67 cases to 2.0 cases per 100000 and annual prevalence ranges from 4.0 to 24.5 per 100000 people depending on the geographical location[2]. Familial cases of AIH are reported to occur in only 1% of AIH cases[3]. This observation suggests role of genetic predisposition. The pathophysiologic mechanisms of AIH are not fully understood. Both genetic predisposition and an imbalance between effector and regulatory immunity are key pathologic factors for disease development[1,2]. Due to an aggressive course of the disease, the diagnosis must be made early and therapy with steroids and immunosuppressant drugs started [1,4].

In 2015, we described a 6-year-old girl with Noonan syndrome (NS) and AIH type 1 [5]. Molecular analysis of *PTPN11* gene showed heterozygous mutation c.923A>G (Asn308Ser) in exon 8. This was the second case described in literature of association between NS and AIH type 1. We supposed that it was not a causality and we thought that autoimmunity represents a characteristic of NS, even if the etiopathogenesis is still unknown.

Then in 2018, we published with Le Coz *et al*[6] two cases with *ctla-4* haploinsufficiency, due to heterozygous microdeletions of chromosome 2q, complicated by autoimmune manifestations. One of these patients had AIH. It is known that about 15% to 20% of patients with the autoimmune polyglandular syndrome type 1 (APS1), also referred to as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a rare disease with prevalence of 1-9:1000000, suffer from an autoantibody-positive AIH, linked to mutations in the autoimmune regulator gene (AIRE)[1,7].

In this review we report literature data of association between AIH and genetic syndromes. Through a detailed and systematic analysis of the literature, we aim to evaluate AIH as a possible complication in patients affected by a genetic syndrome.

We do a systematic review through the choice of the best current works and which refer to the association between AIH and patients with genetic syndrome diagnosis.

The purpose of this work is to evaluate how many reports of genetic syndromes have AIH as a complication and to suppose pathogenetic mechanisms related to the causative mutation of the syndrome and the autoimmune or autoinflammatory processes that may have the liver as a target organ. The correlation between AIH and

genetic syndromes is still controversial and the cause and effect relationship is under investigation in order to understand if it is a simple coincidence/co-occurrence.

When a genetic syndrome has the possibility of developing AIH, the monitoring of this risk is a non-negligible aspect during the follow-up of these patients. AIH is a severe complication, which can have an unfavorable outcome, even with the death of the patient. Indeed, the untreated AIH has a very poor prognosis, with reported survival rates of 50% and 10% at 5 and 10-years respectively[4]. We also investigate the etiopathogenetic hypotheses related to the underlying genetic conditions. Besides, as more is becoming understood, it is also clear that in some cases, there is important overlap between genetic disease causation and the development of AIH.

Any classification is arbitrary and should be considered as a new proposal, as an evolving classification. Here, we try to distinguish the influence of genetic factors in causing AIH complication in a specific population, like patients with a genetic syndrome. We present the state of the art, by reporting all the well described cases, reported in literature.

The collection of clinical evidence could increase the knowledge in this field, improving the management of rare syndromes and AIH, as possible complication with high morbidity and mortality.

## METHODOLOGY

We conducted a standard systematic literature review on PubMed, using the combination of keywords: “autoimmune hepatitis”, “liver disease”, “genetic syndrome”.

The application of these search terms aimed to cover most of the publication regarding the description of the association of AIH and genetic syndromes.

We consider only those studies in which the above-mentioned terms are present, alone or variously combined together, in the main text, in the title, in the abstract and in MeSH terms. Since genetic syndromes are rare diseases, we have chosen both previous reviews and case reports. We took in consideration all the scientific articles reported in PubMed in the last 10 years, from 2010 to 2020. The search performed on February 17<sup>th</sup>, 2021 retrieved 8094, if we use combination of “liver disease” and “genetic syndrome” as keywords, while there are 139, if the combination used is “autoimmune hepatitis” and “genetic syndrome”. The inclusion criteria include a clear clinical diagnosis of AIH and genetic syndrome. We checked in each article the congruence of the diagnosis of AIH with the recognized criteria and the confirmation of the diagnosis of specific genetic syndrome with a proper genetic test. Of 139 articles, 30 are accessible, compatible with our inclusion criteria and are included in the analysis. The exclusion criteria for the remaining 109 articles are in a language different from English, regarding familiar but not syndromic cases and a not specific diagnosis of AIH.

It has been paid attention to diagnostic criteria in diagnosis of AIH[1]. According to the Ab profile, AIH can be divided into three subtypes: AIH type 1 by the presence of antinuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA); AIH type 2 by anti-liver-kidney microsomal autoantibodies (LKM-1) directed against cytochrome P450 (CYP) 2D6; AIH type 3 by autoantibodies against a soluble liver antigen (SLA/LP)[1,2].

The established specific diagnostic criteria and scoring systems of AIH include analysis of autoantibodies (ANA, SMA, anti-LKM1, and anti SLA), immunoglobulin (Ig) G, viral markers (IgM anti-HAV, HBsAg, HBV DNA, and HCV RNA) and histological findings[1,2,8]. The diagnosis of syndromes condition is confirmed through genetic tests, using a cytogenetic, cytogenomic or molecular approach.

## RESULTS

From the 139 publications selected using keywords “autoimmune hepatitis” and “genetic syndrome”, 30 papers (21.6%) respected the chosen inclusion criteria, reporting the association between AIH in patients with a genetic syndrome.

From 2010 to 2020, the articles which have reported AIH as complication of a genetic syndrome have a median of 1.7% of all scientific production on liver disease in genetic syndromes, with a peak between 2014 and 2015 years of publication.



There are many case reports (24/30) and some reviews (2/30) and few original or research articles, cohort studies or clinical trials. Here, we considered the review which described case reports, because of the rarity of diseases.

Most of the syndromes found are forms of immunodeficiency or immunodysregulation, such as APS1, Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome (IPEX), Immunodeficiency-centromeric instability-facial anomalies syndrome, spondilocondrodisplasia (SPENCDI), X-linked agammaglobulinemia (XLA), Shwachman-Diamond syndrome (SDS) and severe combined immunodeficiency (SCID).

A new findings are the unbalanced genomic diseases, like Down syndrome, Smith-Magenis syndrome (SMS), 22q13.3 deletion syndrome and 2q deletion syndrome.

Interesting is the presence of 2 articles about Wilson disease (WD), that is a disease with primary hepatic involvement, describing 2 patients in which a form of autoimmune liver disease is hypothesized.

Moreover, we found some very different syndromes in association with AIH: NS, cutaneous amyloidosis, H syndrome, familial hemophagocytic lymphohistiocytosis (FHL) with STXBP2 mutations, progressive familial intrahepatic cholestasis type 3 (PFIC3) and sclerosis tuberosus syndrome (TSC).

We have collected in all 47 patients, with variable age of AIH onset. We observed median age of patients of 12.6-year-old and a high incidence (70.2%) of patients with age < 12-year-old. The ratio of males to females is 40.4% to 55.3% respectively, with female prevalence. The 30% of patients were died. We found also some publication that includes pathogenetic hypothesis, which are reported and commented in the discussion.

The articles and case reports are described in Tables 1-3.

## DISCUSSION

AIH is a relatively rare progressive chronic liver disease that mainly affects women and is usually characterized by increased IgG levels, circulating autoantibodies and a favorable response to immunosuppressive treatment[1,2,4]. The etiology of AIH is still unknown and all the causes of chronic liver disease must be excluded in advance before diagnosing AIH. The literature data exhibit that AIH can show up in any age of both sexes and all ethnic groups, with peaks around puberty and between 4<sup>th</sup> and 6<sup>th</sup> decades. The onset of AIH may be insidious, acute or chronic, and one third of patients have already developed cirrhosis at the moment of diagnosis, suggesting a delay in diagnosis[8]. The presence of other autoimmune or immune-mediated diseases is frequent and an unusual form of AIH has been reported in 10%-18% of patients with APECED, also known as APS1[7-9]. AIH develops in genetically predisposed individuals, after exposure to triggering factors like microbes, viruses or drugs. When the autoimmune attack against the liver starts, it continues through “molecular mimicry” mechanisms, and is promoted by the diminished control of regulatory T-cells[8].

The evidence of an hepatic CD4 and CD8 T cell and B cell infiltration confirms the immune-mediated pathogenesis, related to defective regulatory mechanisms, antigen-specific immunization, pro-inflammatory CD4 T cell and their cytokines profile. The dysregulation of adaptive immune response has a pathogenetic role, due to the production of autoantibodies and the persistence in the liver of autoreactive CD4 T cells that maintain inflammation with a predominant secretion of tumor necrosis factor (TNF), interferon- $\gamma$  (IFN- $\gamma$ ), interleukin (IL)-21. Furthermore, T-reg cell are not able to stop inflammation[10].

AIH is principally divided in type 1 (AIH-1) and type 2 (AIH-2), based on autoantibodies. The authors confirm that there are many differences between two types. AIH-2 is more frequent in children and young adults, has an acute or severe course and treatment failure, with relapse after stopping treatment and need for long-term treatment, compared to AIH-1[8,11,12]. A panel of experts, namely International AIH Group (IAIHG), reported the descriptive criteria of AIH, updated periodically [13]. Some AIH patients has clinical cholestatic presentation, that is known as primary biliary cholangitis or primary sclerosing cholangitis (PSC). In 2001, Gregorio *et al*[14] introduced the term “autoimmune sclerosing cholangitis” for the patients characterized by lesions of both AIH and sclerosing cholangitis. This presentation was named “overlap syndromes or variants of AIH” and its appearance was more frequent in children. The authors suggested an investigation of the biliary tree in all children with a diagnosis of AIH[8,15]. The IAIHG do not support the concept of “overlap

Table 1 Group-1: Disease gene is one of immunoregulatory genes

Genetic syndrome	Inheritance	Gene	Ref.	Number of AIH cases	Sex	Age at diagnosis	Nucleotide variant	Protein variant	Outcome
APECED/APS1	AD, AR	AIRE	Meloni <i>et al</i> [17], 2017	6	F; F; F; F; M	3 yr; 6 yr; 11 yr; 5 yr; 8 yr; 12 yr	c.[415C>T];[415C>T]	p.[(R139X)];[(R139X)]	Alive; Alive; Death; Death; Alive; Alive
			Huibregtse <i>et al</i> [7], 2014	1	F	10 yr	c.[20_115de196];[967_979del13]	p.[(?)];	Alive
			Zaidi <i>et al</i> [18], 2017	2	M; M	3 yr; 5 yr	NR	NR	Alive; Death
IPEX	XLR	FOXP3	López <i>et al</i> [21], 2011	1	M	4 yr	c.[748-750delAAG];[0]	p.[(250Kdel)];[(0)]	Alive
			Baris <i>et al</i> [22], 2014	1	M	3 yr	c.[816+5G>A];[0]	p.[(?)];	Death
			Magg <i>et al</i> [23], 2018	1	M	3 yr	c.[816+2T>A];[0]	p.[(?)];	Death
			Duclaux-Loras <i>et al</i> [20], 2018	3	M; M; M	4 wk; 4 wk; 3 wk	c.[751_753delGAG];[0]; c.[1157G>A];[0]; c.[227delT];[0]	p.[(E251del)];[(0)]; p.[(R386H)];[0]; p.[(L76Qfs*53)];[(0)]	Death; Death; Alive
ICF2	AR	ZBTB24	von Bernuth <i>et al</i> [25], 2014	1	F	3 yr	c.[1222T>G];[1222T>G]	p.[(C408G)];[(C408G)]	Alive (not responding to therapy)
ICF1	AR	DNMT3B	Sterlin <i>et al</i> [24], 2016	1	M	5 yr	c.[2324C>T];[2324C>T]	NR	Alive
SPENCDI	AR	APC5	Briggs <i>et al</i> [26], 2016	3	F; F; F	9 yr; 3 yr; 6 mo	c.[725A>G];[725A>G]; c.[389+1G>A];[389+1G>A]; c.[131C>T];[712T>C]	p.[(H242R)];[(H242R)]; p.[(?)];	Alive; Alive; Alive
SDS	AR	SBDS	Veropalumbo <i>et al</i> [28], 2015	2	NR; NR	9 mo; 12 mo	c.[258+2T];[183-184A>CT]; c.[258+2T>C];[183-184TA>CT]	p.[(?)];	Alive; Alive
SCID	AR	CD3γ	Tokgoz <i>et al</i> [30], 2013	1	F	12 yr	c.[IVS2-1G>C];[IVS2-1G>C]	p.[(?)];	Alive

AIH: Autoimmune hepatitis; AD: Autosomal dominant; AR: Autosomal recessive; XLR: X-linked recessive; F: Female; M: Male; NR: Not reported; SDS: Shwachman-Diamond syndrome; SCID: Severe combined immunodeficiency; SPENCDI: Spondylocondrodysplasia; ICF: Immunodeficiency, centromeric instability and facial dysmorphism; IPEX: Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome; APECED: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; APS1: Autoimmune polyglandular syndrome type 1.

syndromes” as new and distinct disorders[13].

We suspect that genetic syndromes with particular imbalance of immune response, could represent a genetic predisposition to develop autoimmune disease, especially AIH. Some genetic syndromes are known to have autoimmune complications, for examples APS, IPEX syndrome and Down syndrome. Also in rare genomic imbalance diseases could appear autoimmune complications.

We have found some case reports of patients with genetic syndrome complicated by AIH. The main found syndromes are APS/APECED, IPEX syndrome, unbalanced genomic syndromes, RASopathies.

We propose a classification system for genetic syndromes associated with AIH due to genetics and etiopathogenesis aspects. There are three possible groups: group-1, that includes genetic syndromes whose disease gene is one of immunoregulatory genes, directly involved in AIH pathogenesis; group-2, that includes those syndromes in which there is a polygenic involvement of immune-mediated risk and of AIH pathogenesis; group-3, that includes those in which there is a possible association related to the disease causative mutation, seems to be not directly involved in AIH pathogenesis. For the last group, we try to propose some possible pathogenesis mechanism in AIH development.

Table 2 Group-2: Polygenic involvement of immune-mediated risk (unbalanced genomic disease)

Genetic syndrome	Inheritance	Chromosomal region	Ref.	Number of AIH cases	Sex	Age at diagnosis	Deletion breakpoints [build GRCh37/hg19]	Outcome
Down syndrome	IC	-	Ravel <i>et al</i> [32], 2020	1	M	29 yr	-	Death
SMS	AD, IC	del17p11.2	Yang <i>et al</i> [36], 2014	1	F	24 yr	chr17: 16,660,721-20,417,975 <i>dn</i>	Alive
PHMDS	AD	del22q13.31-qter	Bartsch <i>et al</i> [37], 2010	1	F	3 yr	-	Alive
del2q	IC	del2q33.1-q34	Le Coz <i>et al</i> [6], 2018	1	F	12 yr	chr2:197,942,576–209,522,220 <i>dn</i>	Alive

AIH: Autoimmune hepatitis; IC: Isolated cases; AD: Autosomal dominant; F: Female; M: Male; SMS: Smith-Magenis syndrome.

### Group-1 genetic syndromes includes

**Autoimmune polyendocrinopathy syndromes:** The term APS refers to a group of rare endocrine diseases characterized by autoimmune activity against more than one endocrine organ, with possible additional involvement of non-endocrine organs. Autoimmunity is typically directed against different target antigens in different tissues. The two more common autoimmune polyendocrine syndromes, APS type 1 and type 2, have a strong genetic background and have Addison's disease as a major feature. The group furthermore includes APS type 3 and type 4.

The APS type 1 is a rare recessive autosomal disease, also named APECED syndrome (OMIM 240300), and related to *AIRE* gene mutations. Because of a founder effect, APECED is particularly prevalent in Finland (1:25000) but is observed worldwide with variable prevalence[15]. Diagnosis is classically based on presence of at least two out of three "majors" criteria of Whitaker's triad (chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism and adrenal insufficiency or Addison disease). *AIRE* gene (21q22.3), coding for the *AIRE* transcription factor, is involved in immune tolerance mechanisms and contributes to the negative selection of autoreactive T lymphocytes in the thymus, lymph nodes and spleen. AIH and hepatitis as an APECED component may be distinguished on the basis of a different autoantibody profile. The anti-LM antibodies are specific of AIH, which develops in individuals with APECED.

The major target autoantigen of anti-LM antibodies has been documented as the CYP1A2[8,12,14]. In the considered period, we have found four papers reporting in all six patients with APECED syndrome and AIH, that is non-endocrine complication[7, 16-18].

The girl described by Huibregtse *et al*[7] had homozygous 967-979del13bp mutation. Meloni *et al*[17] described a longitudinal cohort study in which AIH was seen in 27% of their APS1 Sardinian patients. There are five female patients with a median age of 6.5-year-old and one male of 12-year-old. The course of AIH varied from chronic moderate/severe hepatitis to fatal forms (in two Sardinian and one Indian children) [17,18].

They noted predominance in females, presence in all AIH patients of R139X homozygotes and *HLA-DRB1\*0301-DQB1\*0201* combination plus LKM autoantibodies (anti-CYP1A2), onset in infancy/childhood, a hitherto unreported predilection for hepatitis and that AIH can be the initial manifestation of APS1. Then they concluded that the role of HLA, in addition to the R139X *AIRE* variant, could influence the APS1 phenotype. Therapy for severe AIH consisted of oral prednisone, tapered off in about 6 mo, and azathioprine, that was continued for years.

In the review of Gatselis *et al*[8], published in 2015, the AIH associated with APECED is considered a component of this syndrome, that the authors described as a third type of AIH, because of the presence of characteristic autoantibodies, such as ANA, anti-LC, anti-LKM, anti-LM.

This review is not included in our listed papers, because of the lack of the established inclusion criteria, but it was interesting for improvement of information about this syndrome. In 2016, Sorkina *et al*[19] described an interesting 4-year-old patient with *AIRE* mutation and AIH, but their diagnosed criteria are not reported; for this reason we exclude the paper in this review. The authors concluded that regular screening for autoantibodies can help identify higher risk for development of AIH.

Table 3 Group-3: Association not directly related to the disease causative mutation

Genetic syndrome	Inheritance	Gene	Ref.	Number of AIH cases	Sex	Age at diagnosis	Nucleotide variant	Protein variant	Outcome
NS	AD	PTPN11	Quaio <i>et al</i> [38], 2012	1	M	19 yr	c.[836A>G];[=]	p.[(Y279C)];[(=)]	Alive
			Loddo <i>et al</i> [5], 2015	1	F	6 yr	c.[923A>G];[=]	p.[(N308S)];[(=)]	Alive
WD	AR	ATP7B	Ganesh <i>et al</i> [40], 2017	1	M	6 yr	c.[2906G>A];[2906G>A]	p.[(R969Q)];[(R969Q)]	Alive
			Santos <i>et al</i> [41], 2019	1	F	25 yr	N.R.	N.R.	Alive
H syndrome	AR	SLC29A3	Bloom <i>et al</i> [42], 2017	1	M	17 mo	c.[1087C>T];[1087C>T]	p.[(R363W)];[(R363W)]	Alive
FHL5	AR	STXBP2	Esmailzadeh <i>et al</i> [43], 2015	1	M	7 yr	c.[1247-1G>C];[1247-1G>C]	p.[(?)];[(?)]	Death
TSC	AD	TSC1	Di Marco <i>et al</i> [44], 2017	1	F	47 yr	c.[682C>T];[=]	p.[(R228*)];[(=)]	Alive
SCD	AR	HBB	Jitraruch <i>et al</i> [45], 2017	7	F; M; M; F; F; F; F	5 yr; 16 yr; 13 yr; 13 yr; 8 yr; 8 yr; 3 yr	c.[20A>T];[20A>T]	p.[(E7V)];[(E7V)]	Alive; Alive; Death; Alive; Alive; Alive; Death
			Zellos <i>et al</i> [46], 2010	1	F	25 yr	c.[20A>T];[20A>T]	p.[(E7V)];[(E7V)]	Death
			Hurtova <i>et al</i> [47], 2011	1	F	54 yr	c.[20A>T];[20A>T]	p.[(E7V)];[(E7V)]	Death
GD	AR	GBA	Ayto <i>et al</i> [48], 2010	1	F	51 yr	c.[1226A>G];[115+1G>A]	p.[(N409S)];[(?)]	Death
PLCA	AD	-	González-Moreno <i>et al</i> [50], 2015	1	M	36 yr	NR	NR	Alive
			Yan and Jin [49]	1	F	50 yr	NR	N.R.	Alive
PFIC3	AR	ABCB4	Oliveira <i>et al</i> [51], 2017	1	M	22 yr	c.[874A>T];[3680T>C]	p.[(K292*)];[(I1227T)]	Alive

AIH: Autoimmune hepatitis; AD: Autosomal dominant; AR: Autosomal recessive; F: Female; M: Male; NR: Not reported; NS: Noonan syndrome; WD: Wilson disease; FHL: Familial hemophagocytic lymphohistiocytosis; TSC: Tuberous syndrome; SCD: Sickle cell disease; GD: Gaucher disease; PLCA: Primary cutaneous amyloidosis; PFIC3: Progressive familial intrahepatic cholestasis type 3.

**IPEX syndrome:** The IPEX syndrome (OMIM 304790) is a rare X-linked recessive life-threatening disorder characterized by autoimmunity and early death. The causative gene is *FOXP3*. We report four papers and six patients with IPEX syndrome and AIH [20-23]. These patients were hemizygote males of median age of 1.7-year-old. In 2018, Duclaux-Loras R *et al*[20] reported 14% of AIH in a cohort of French IPEX patients. Among these, three patients had AIH with early onset in the first months of life and two died at 8 and 7 mo. In IPEX syndrome the course of AIH is very severe.

**Immunodeficiency, centromeric instability and facial dysmorphism syndromes:** The immunodeficiency, centromeric instability and facial dysmorphism (ICF) syndrome (OMIM 242860) is a rare autosomal recessive immunodeficiency, that involves agammaglobulinemia or hypoglobulinemia with B cells, centromere-adjacent instability of chromosomes 1 and/or 16 (and sometimes 9) in mitogen-stimulated lymphocytes, with facial anomalies and psychomotor delay. Approximately 50 patients have been reported.

It is distinguished in ICF1 correlate to *DNMT3B* gene mutations and ICF2 due to *ZBTB24* gene, ICF3 caused by mutation in the *CDCA7* gene and ICF4 caused by



mutation in the *HELLS* gene. There are two papers which described two patients, one male and one female, with 5 and 3-year-old respectively, affected by ICF1 and ICF2 with AIH[24,25].

**Spondyloenchondrodysplasia with immune dysregulation:** SPENCDI (OMIM 607944) is a very rare autosomal recessive genetic skeletal dysplasia, that may have a heterogeneous clinical spectrum with neurological involvement or autoimmune manifestations. The prevalence is < 1.1000000 and onset is in childhood. In all, we found four patients who have AIH and SPENCDI. In the original article of Briggs *et al* [26], three female patients of 9-year-old, 3-year-old and 6-mo-old have been AIH and SPENCDI, confirmed by homozygous variants in APC5 gene.

In an abstract in Chinese language, for this not included in Table 1, the authors reported a case of a 12-year-old girl with type IIAIH, associated with systemic lupus erythematosus (SLE), treated with methylprednisolone and immunosuppressants, with improvement. Gene sequencing was performed, revealing a compound heterozygous mutations in ACP5 gene. The same paper showed a review of 25 articles (1 Chinese, 24 English) with 74 SPENCDI patients (92%) with autoimmune diseases. They concluded for a strong predisposition to these complications in SPENCDI[27].

**SDS:** SDS (OMIM 260400) is a rare autosomal recessive multisystemic syndrome characterized by chronic and usually mild neutropenia, pancreatic exocrine insufficiency, caused by mutations in the *SBDS* gene. It might be hepatomegaly and liver abnormalities. We found an article which described two patients with SDS and AIH [28].

**Immunodeficiency:** The primary immunodeficiency disorders are a rare heterogeneous group of inherited defects characterized by poor or absent function in one or more components of the immune system. The estimated prevalence of these disorders in the United States is approximately 1:1200 live births[29]. The clinical presentation involves increased susceptibility to infection, chronic diarrhea, failure to thrive, severe and recurrent infections with opportunistic pathogens.

In SCID there is a lack of functional T cells and immune function. We found an article reporting one of two siblings, 12 year-old girl, with SCID, due to homozygous splicing mutation (IVS2-1G>C) in the *CD3 $\gamma$*  gene and AIH[30]. About immunodeficiency syndromes, we want to cite one article, excluded for language, which describe a very rare case of a girl of 18-month-old with chronic granulomatous disease and AIH [31].

### Group-2 includes

**Down syndrome:** Trisomy of chromosome 21 (OMIM 190685) is characterized by cognitive impairment, cardiac and gastrointestinal abnormalities and immunodeficiency.

Relevant is also the incidence of autoimmune diseases. Our research found a review in which only two cases with Down syndrome were associated to autoimmune chronic active hepatitis and autoimmune PSC[32]. Because the case reported have been excluded for publication over the years, we evaluated the aforementioned review, which is the only publication in the period considered, that referred to cases of AIH and Down syndrome. The first case was a 29-year-old male, reported by McCulloch *et al*[33] in 1982 while the second was a 21-year-old male with autoimmune PSC by Mehta *et al*[34], in 1995. In 1990, another case of a 12-year-old child is described with Down syndrome and AIH[35]. Considering the known risk of autoimmune complications in Down syndrome, we thought we would find more cases of AIH. On the contrary, literature data showed many cases of viral hepatitis occurring in Down syndrome, due to immunodeficiency condition.

**Other unbalanced genomic diseases:** They are rare genetic syndromes caused by deletion and/or duplication of chromosomes. The correlation of symptoms is variable of cognitive deficit and multiorgan involvement. Monosomy and trisomy for different regions in chromosomes account for about 1% of cases of developmental delay and intellectual disability. Some of them are noted to have immunodeficiency and immune-mediated complications. In our review, we found description of a 24-year-old woman with AIH and SMS (OMIM 182290), due to a 17p11.2 deletions (16,660,721-20,417,975, GRCh37/hg19)[36], another 3-year-old girl patient with 22q13.3 deletion syndrome (Phelan-McDermid syndrome) (OMIM 606232)[37], finally a 12-year-old girl with de novo heterozygous 11.6 Mb chromosome 2q33.1-q34 deletion (197,942,576-209,522,220, GRCh37/hg19)[37].

We think that AIH is due to haploinsufficiency of key genes located in the deleted region. Lymphocyte-specific member of the TNF receptor superfamily (*TACI* gene) located within the SMS region, plays a crucial role in humoral immunity. So we might speculate that *TACI* haploinsufficiency, in this condition, could cause hyperactive B cells and increased capacity for antigen-specific antibody production. In similar manner, the loss of one copy in one or more of the 55 genes, from *NUP50* to *RABL2B*, in 22q13.3 region in Phelan-McDermid syndrome; and of the *CD28/CTLA4/ICOS* gene cluster in 2q33.1-q34 deletion, similar to *ALPS5* due to *CTLA4* haploinsufficiency, would be predisposing AIH. In this case, probably the deletion of the *CD28/CTLA4/ICOS* gene cluster induced a multi-organ inflammation and exhibited a Treg suppressive defect.

### Group-3 includes

**NS/RASopathies:** NS (OMIM 163950) is characterized by short stature, typical facial dysmorphology and congenital heart defects. The incidence of NS is estimated to be between 1:1000 and 1:2500 live births. The syndrome is transmitted as an autosomal dominant trait. In more than 50% of patients with NS, mutations in the Protein Tyrosine Phosphatase Non-Receptor Type 11 (*PTPN11*) gene are identified.

We found two patients with the association of NS and AIH. In 2012, Quaio *et al*[38] published the first case of patient with AIH and NS. Another case is a 6 year-old girl, that we reported in 2015, with heterozygous mutation c.923A>G (Asn308Ser) in exon 8 of *PTPN11* gene[5]. Autoimmune diseases and autoantibodies were frequently present in patients with RASopathies, even if the etiopathogenesis is still unknown.

The *PTPN11* are clustered in the interacting portions of the amino N-SH2 (Src homology 2) domain and the phosphotyrosine phosphatase (PTP) domains, which are involved in switching the protein between its inactive and active conformations. Missense mutation causes a gain-of-function changes resulting in excessive *SHP2* activity, that underlie the pathogenesis of NS. We hypothesize that *SHP2* modulates *ERK/MAPK* pathway and its involvement in cytokine/inflammatory signaling. In an interesting article published in 2016, it was highlighted that inhibition of *SHP2* activity blocks T cell proliferation, leading to decreased IFN- $\gamma$  and IL-17 Levels, ultimately normalizing SLE associated pathogenicity in target tissues. These data suggest *SHP2* activity is integrally involved in SLE and that its normalization may be a potent and targeted therapy for treatment of patients with SLE[39].

**WD:** In our research on PubMed, we found two articles about AIH and WD[40,41], that is a disorder of copper metabolism (OMIM 277900). The diagnosis is established by a combination of low serum copper and ceruloplasmin concentrations, increased urinary copper excretion and detection of biallelic *ATP7B* pathogenic variants by molecular genetic testing. The manifestations include neurologic, psychiatric or liver diseases. These include recurrent jaundice, simple acute self-limited hepatitis-like illness, autoimmune-type hepatitis, fulminant hepatic failure, or chronic liver disease. The AIH in WD patients responds well to chelation therapy with D-penicillamine. There were reported a 6-year-old boy and a 25-year-old female patients, presented with clinical symptoms suggestive of AIH, with a mutation in *ATP7B* gene, confirming the diagnosis of WD. In patients who showed chronic hepatopathy resembling AIH, the differential diagnosis with WD is mandatory, because resolving the dilemma allows the clinician to prescribe the appropriate therapy.

**H syndrome:** H Syndrome (OMIM 612391) is an autosomal recessive disorder characterized by cutaneous hyperpigmentation, hypertrichosis and induration with numerous systemic manifestations. The syndrome is caused by homozygous or compound heterozygous mutations in *SLC29A3* a gene on chromosome 10q22 that encodes a nucleoside transporter (hENT3). There is one case report that described a 17 mo-old male with mild to moderate autoimmune chronic active hepatitis, confirmed with biopsy and treated with prednisone and immunosuppressor[42].

**FHL:** In 2015, Esmaeilzadeh *et al*[43] described a patient with FHL5 (OMIM 613101) caused by *STXBP2* gene mutation presenting with AIH. This syndrome is a rare disorder characterized by immune dysregulation, defective function of natural killer cell, proliferation and infiltration of hyperactivated macrophages and T-lymphocytes, cytopenia and hepatosplenomegaly. It was the first description of AIH.

**Tuberous sclerosis complex:** TSC (OMIM 191100) is a rare autosomal-dominant neurocutaneous disorder, with prevalence of 1:6000, characterized by multisystem hamartomas and benign tumors developing. This condition is caused by heterozygous loss-of-function mutations in the *TSC1* or *TSC2* tumor suppressor genes coding for hamartin and tuberlin, respectively.

We found an article about a 47 year-old woman, affected by TSC, with a mutation identified in the *TSC1* gene [c.682C>T (p.Arg228\*)] and lymphangioleiomyomatosis, sarcoidosis, primary biliary cirrhosis and AIH[44]. This was the first report of this coexistence, and we might speculate that this is related with the dysregulation of the pathway involving *mTOR* and *MAPK* and their interaction.

In literature, *PI3K/AKT/mTOR* signaling has been implicated in SLE pathogenesis. Its activity is increased in SLE mice models as well as in human lupus patients. The expression of this signaling pathway exists broadly in immune cells, including T cells, B cells, monocytes, macrophages, neutrophils and dendritic cells[39].

**Sickle cell disease:** It is a chronic hemolytic disease (OMIM 603903) that may induce acute accidents, like severe anemia, bacterial infections, and ischemic vaso-occlusive accidents caused by sickle-shaped red blood cells obstructing small blood vessels and capillaries. The patients have beta globin variant (Hb S). Our PubMed research found three articles.

In 2017, a retrospective review reported 7 patients of median age of 9 years with sickle cell disease (SCD) and AIH. The patients were treated with standard immunosuppressive therapy[45]. Previous case reports described two patients with SCD and AIH[46,47].

The occurrence of AIH may be due to a complex interaction with the underlying liver disease in altered immunoregulatory mechanisms. AIH is common in patients with SCD and they respond satisfactorily to immunosuppressive treatment. The authors reported how liver biopsy may be helpful in confirming the diagnosis and to exclude acute vaso-occlusive sickling episodes[45].

**Gaucher disease type 1:** It is the chronic non-neurological form of Gaucher disease autosomal recessive (OMIM 230800), characterized by prevalence of 1:100000 organomegaly, bone involvement and cytopenia, caused by a mutation in the *GBA* gene. The hepatomegaly (80% of cases) in rare cases can progress towards fibrosis followed by cirrhosis. We found an article, who described one gaucher disease type 1 patient with autoimmune chronic active hepatitis[48].

**Primary cutaneous amyloidosis:** It refers to a variety of skin diseases characterized by the extracellular accumulation of amyloid. They have genetic heterogeneity and may be caused: Primary cutaneous amyloidosis (PLCA)-1 by heterozygous mutation in the gene encoding oncostatin-M-receptor-beta (*OSMR*) (OMIM 105250), PLCA-2 by heterozygous mutation in the *IL31RA* gene (OMIM 613955), PLCA-3 by mutation in the *GPNMB* gene (OMIM 617920). There were two case reports which described one patient each other, a 36 year-old male and a 50 year-old female, with PLCA and AIH [49,50]. These reports in the literature have been associated to autoimmune disorders, which suggests the possibility of a common underlying immune-mediated mechanism.

**PFIC3:** The PFIC3 is a heterogeneous group of autosomal recessive liver disorders (OMIM 602347), with childhood predominance, which causes cholestasis of hepatocellular, caused by a genetic defect in the *ABCB4* gene. In literature there is the first interesting association of PFIC3 and AIH type 1[51]. It regards a 22 year-old patient with diagnosis of PFIC3 caused by an allele with a previously described mutation and a new genetic variant (c.3680T>C; p.Ile1227Thr), transmitted by his mother, which is associated with AIH. The authors reported the importance of genetic testing of the *ABCB4* gene in patients with autoimmune liver disease with incomplete response to immunosuppressive treatment.

## CONCLUSION

In this review, we performed a research of literature, during the last 10 years, from 2010 to 2020, to collect all clinical cases reporting the association between AIH and genetic syndromes. We observed that AIH is a frequent complication of group-1 syndrome, that includes disease whose causative gene have a role in immunoregulation. AIH is more rarely present in other group of genetic syndromes. If we consider a single disease, the number of articles is very limited, but we suppose that this could be related to rarity of genetic syndrome.

We hypothesize that AIH and genetic syndromes are combination of rare manifestation. Over the last decade, the attention of AIH diagnosis is increased and there is evidence that many triggers are involved for AIH pathogenesis, such as

familiarity, genetic predisposition, drug and infections. This paper suggests that genetic syndromes, as observed in the reported clinical cases, are a trigger for AIH, whose pathogenetic mechanism could be specific for each other, also related to genetic factors.

Genetic syndromes could contribute to the risk of developing AIH with a primitive gene mutation that compromises an immune response. For examples, it is demonstrated role of some gene products such as, *FOXP3*, *ICOS*, *TIGIT*, *CTLA4*, in pro-inflammatory/pro-B helper profile[10].

We suggest that the association between AIH and genetic syndrome might be not casual and claim that there might be an etiopathogenetic correlation between the causative genetic mutation and the immune imbalance, that is expressed as AIH. Considering that we have dealt with rare diseases and sometimes very rare, having found 34 articles in 10 years, we think there are not a few. On the other hand, it is fair to observe that when the clinical cases described are few, it is difficult to exclude that it is a coincidence. Much attention should be paid by clinicians to AIH diagnosis, with periodical autoantibody detection and identification of AIH manifestations and interpretation of liver autoimmune serology, to minimize the problem of underestimation of AIH diagnosis. Moreover, we underly the severity of AIH complication and in these cases the time of diagnosis should be crucial in order to start, as soon as possible, an appropriate therapy.

We suggest that when a patient presents a clinical picture of cryptogenic chronic hepatitis, that is unexplained, it is useful to explore differential diagnosis of AIH associated with genetic syndrome. Given the clinical relevance of this topic, further reports are needed to demonstrate our hypothesis and collect new evidence in this field.

## REFERENCES

- 1 **Strassburg CP.** Autoimmune hepatitis. *Best Pract Res Clin Gastroenterol* 2010; **24**: 667-682 [PMID: 20955969 DOI: 10.1016/j.bpg.2010.07.011]
- 2 **Sucher E, Sucher R, Gradistanac T, Brandacher G, Schneeberger S, Berg T.** Autoimmune Hepatitis-Immunologically Triggered Liver Pathogenesis-Diagnostic and Therapeutic Strategies. *J Immunol Res* 2019; **2019**: 9437043 [PMID: 31886312 DOI: 10.1155/2019/9437043]
- 3 **Omori K, Yoshida K, Yokota M, Daa T, Kan M.** Familial occurrence of autoimmune liver disease with overlapping features of primary biliary cholangitis and autoimmune hepatitis in a mother and her daughter. *Clin J Gastroenterol* 2016; **9**: 312-318 [PMID: 27503128 DOI: 10.1007/s12328-016-0676-1]
- 4 **Strassburg CP, Manns MP.** Therapy of autoimmune hepatitis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 673-687 [PMID: 22117634 DOI: 10.1016/j.bpg.2011.08.003]
- 5 **Loddo I, Romano C, Cutrupi MC, Sciveres M, Riva S, Salpietro A, Ferraù V, Gallizzi R, Briuglia S.** Autoimmune liver disease in Noonan Syndrome. *Eur J Med Genet* 2015; **58**: 188-190 [PMID: 25595571 DOI: 10.1016/j.ejmg.2014.12.013]
- 6 **Le Coz C, Nolan BE, Trofa M, Kamsheh AM, Khokha MK, Lakhani SA, Novelli A, Zackai EH, Sullivan KE, Briuglia S, Bhatti TR, Romberg N.** Cytotoxic T-Lymphocyte-Associated Protein 4 Haploinsufficiency-Associated Inflammation Can Occur Independently of T-Cell Hyperproliferation. *Front Immunol* 2018; **9**: 1715 [PMID: 30087679 DOI: 10.3389/fimmu.2018.01715]
- 7 **Huibregtse KE, Wolfram P, Winer KK, Connor EL.** Polyglandular autoimmune syndrome type I - a novel AIRE mutation in a North American patient. *J Pediatr Endocrinol Metab* 2014; **27**: 1257-1260 [PMID: 24945421 DOI: 10.1515/jpem-2013-0328]
- 8 **Gatselis NK, Zachou K, Koukoulis GK, Dalekos GN.** Autoimmune hepatitis, one disease with many faces: etiopathogenetic, clinico-laboratory and histological characteristics. *World J Gastroenterol* 2015; **21**: 60-83 [PMID: 25574080 DOI: 10.3748/wjg.v21.i1.60]
- 9 **Obermayer-Straub P, Perheentupa J, Braun S, Kayser A, Barut A, Loges S, Harms A, Dalekos G, Strassburg CP, Manns MP.** Hepatic autoantigens in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Gastroenterology* 2001; **121**: 668-677 [PMID: 11522751 DOI: 10.1053/gast.2001.27103]
- 10 **Cardon A, Conchon S, Renand A.** Mechanisms of autoimmune hepatitis. *Curr Opin Gastroenterol* 2021; **37**: 79-85 [PMID: 33315790 DOI: 10.1097/MOG.0000000000000704]
- 11 **Zachou K, Muratori P, Koukoulis GK, Granito A, Gatselis N, Fabbri A, Dalekos GN, Muratori L.** Review article: autoimmune hepatitis -- current management and challenges. *Aliment Pharmacol Ther* 2013; **38**: 887-913 [PMID: 24010812 DOI: 10.1111/apt.12470]
- 12 **Liberal R, Grant CR, Mieli-Vergani G, Vergani D.** Autoimmune hepatitis: a comprehensive review. *J Autoimmun* 2013; **41**: 126-139 [PMID: 23218932 DOI: 10.1016/j.jaut.2012.11.002]
- 13 **Boberg KM, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrupf E; International Autoimmune Hepatitis Group.** Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. *J Hepatol* 2011; **54**: 374-385 [PMID: 21067838]



- DOI: [10.1016/j.jhep.2010.09.002](https://doi.org/10.1016/j.jhep.2010.09.002)]
- 14 **Gregorio GV**, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, Mieli-Vergani G. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. *Hepatology* 2001; **33**: 544-553 [PMID: [11230733](https://pubmed.ncbi.nlm.nih.gov/11230733/) DOI: [10.1053/jhep.2001.22131](https://doi.org/10.1053/jhep.2001.22131)]
  - 15 **Vogel A**, Liermann H, Harms A, Strassburg CP, Manns MP, Obermayer-Straub P. Autoimmune regulator AIRE: evidence for genetic differences between autoimmune hepatitis and hepatitis as part of the autoimmune polyglandular syndrome type 1. *Hepatology* 2001; **33**: 1047-1052 [PMID: [11343230](https://pubmed.ncbi.nlm.nih.gov/11343230/) DOI: [10.1053/jhep.2001.24031](https://doi.org/10.1053/jhep.2001.24031)]
  - 16 **Kluger N**, Jokinen M, Krohn K, Ranki A. Gastrointestinal manifestations in APECED syndrome. *J Clin Gastroenterol* 2013; **47**: 112-120 [PMID: [23314667](https://pubmed.ncbi.nlm.nih.gov/23314667/) DOI: [10.1097/MCG.0b013e31827356e1](https://doi.org/10.1097/MCG.0b013e31827356e1)]
  - 17 **Meloni A**, Willcox N, Meager A, Atzeni M, Wolff AS, Husebye ES, Furcas M, Rosatelli MC, Cao A, Congia M. Autoimmune polyendocrine syndrome type 1: an extensive longitudinal study in Sardinian patients. *J Clin Endocrinol Metab* 2012; **97**: 1114-1124 [PMID: [22344197](https://pubmed.ncbi.nlm.nih.gov/22344197/) DOI: [10.1210/jc.2011-2461](https://doi.org/10.1210/jc.2011-2461)]
  - 18 **Zaidi G**, Bhatia V, Sahoo SK, Sarangi AN, Bharti N, Zhang L, Yu L, Eriksson D, Bensing S, Kämpe O, Bharani N, Yachha SK, Bhansali A, Sachan A, Jain V, Shah N, Aggarwal R, Aggarwal A, Srinivasan M, Agarwal S, Bhatia E. Autoimmune polyendocrine syndrome type 1 in an Indian cohort: a longitudinal study. *Endocr Connect* 2017; **6**: 289-296 [PMID: [28446514](https://pubmed.ncbi.nlm.nih.gov/28446514/) DOI: [10.1530/EC-17-0022](https://doi.org/10.1530/EC-17-0022)]
  - 19 **Sorkina E**, Frolova E, Rusinova D, Polyakova S, Roslavtseva E, Vasilyev E, Petrov V, Tiulpakov A. Progressive Generalized Lipodystrophy as a Manifestation of Autoimmune Polyglandular Syndrome Type 1. *J Clin Endocrinol Metab* 2016; **101**: 1344-1347 [PMID: [26891119](https://pubmed.ncbi.nlm.nih.gov/26891119/) DOI: [10.1210/jc.2015-3722](https://doi.org/10.1210/jc.2015-3722)]
  - 20 **Duclaux-Loras R**, Charbit-Henrion F, Neven B, Nowak J, Collardeau-Frachon S, Malcus C, Ray PF, Moshous D, Beltrand J, Goulet O, Cerf-Bensussan N, Lachaux A, Rieux-Laucat F, Ruemmele FM. Clinical Heterogeneity of Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked Syndrome: A French Multicenter Retrospective Study. *Clin Transl Gastroenterol* 2018; **9**: 201 [PMID: [30385752](https://pubmed.ncbi.nlm.nih.gov/30385752/) DOI: [10.1038/s41424-018-0064-x](https://doi.org/10.1038/s41424-018-0064-x)]
  - 21 **López SI**, Ciocca M, Oleastro M, Cuarterolo ML, Rocca A, de Dávila MT, Roy A, Fernández MC, Nievas E, Bosaleh A, Torgerson TR, Ruiz JA. Autoimmune hepatitis type 2 in a child with IPEX syndrome. *J Pediatr Gastroenterol Nutr* 2011; **53**: 690-693 [PMID: [21629128](https://pubmed.ncbi.nlm.nih.gov/21629128/) DOI: [10.1097/MPG.0b013e3182250651](https://doi.org/10.1097/MPG.0b013e3182250651)]
  - 22 **Baris S**, Schulze I, Ozen A, Karakoç Aydinler E, Altuncu E, Karasu GT, Ozturk N, Lorenz M, Schwarz K, Vraetz T, Ehl S, Barlan IB. Clinical heterogeneity of immunodysregulation, polyendocrinopathy, enteropathy, X-linked: pulmonary involvement as a non-classical disease manifestation. *J Clin Immunol* 2014; **34**: 601-606 [PMID: [24916357](https://pubmed.ncbi.nlm.nih.gov/24916357/) DOI: [10.1007/s10875-014-0059-7](https://doi.org/10.1007/s10875-014-0059-7)]
  - 23 **Magg T**, Wiebking V, Conca R, Krebs S, Arens S, Schmid I, Klein C, Albert MH, Hauck F. IPEX due to an exon 7 skipping FOXP3 mutation with autoimmune diabetes mellitus cured by selective T<sub>Reg</sub> cell engraftment. *Clin Immunol* 2018; **191**: 52-58 [PMID: [29567430](https://pubmed.ncbi.nlm.nih.gov/29567430/) DOI: [10.1016/j.clim.2018.03.008](https://doi.org/10.1016/j.clim.2018.03.008)]
  - 24 **Sterlin D**, Velasco G, Moshous D, Touzot F, Mahlaoui N, Fischer A, Suarez F, Francastel C, Picard C. Genetic, Cellular and Clinical Features of ICF Syndrome: a French National Survey. *J Clin Immunol* 2016; **36**: 149-159 [PMID: [26851945](https://pubmed.ncbi.nlm.nih.gov/26851945/) DOI: [10.1007/s10875-016-0240-2](https://doi.org/10.1007/s10875-016-0240-2)]
  - 25 **von Bernuth H**, Ravindran E, Du H, Fröhler S, Strehl K, Krämer N, Issa-Jahns L, Amulic B, Ninnemann O, Xiao MS, Eirich K, Kölsch U, Hauptmann K, John R, Schindler D, Wahn V, Chen W, Kaundl AM. Combined immunodeficiency develops with age in Immunodeficiency-centromeric instability-facial anomalies syndrome 2 (ICF2). *Orphanet J Rare Dis* 2014; **9**: 116 [PMID: [25330735](https://pubmed.ncbi.nlm.nih.gov/25330735/) DOI: [10.1186/s13023-014-0116-6](https://doi.org/10.1186/s13023-014-0116-6)]
  - 26 **Briggs TA**, Rice GI, Adib N, Ades L, Barete S, Baskar K, Baudouin V, Cebeci AN, Clapuyt P, Coman D, De Somer L, Finezilber Y, Frydman M, Guven A, Heritier S, Karall D, Kulkarni ML, Lebon P, Levitt D, Le Merrer M, Linglart A, Livingston JH, Navarro V, Okenfuss E, Puel A, Revencu N, Scholl-Bürgi S, Vivarelli M, Wouters C, Bader-Meunier B, Crow YJ. Spondyloenchondrodysplasia Due to Mutations in ACP5: A Comprehensive Survey. *J Clin Immunol* 2016; **36**: 220-234 [PMID: [26951490](https://pubmed.ncbi.nlm.nih.gov/26951490/) DOI: [10.1007/s10875-016-0252-y](https://doi.org/10.1007/s10875-016-0252-y)]
  - 27 **Zhong LQ**, Wang L, Song HM, Wang W, Wei M, He YY. [Spondyloenchondrodysplasia with immune dysregulation: a case report and literature review]. *Zhonghua Er Ke Za Zhi* 2018; **56**: 611-616 [PMID: [30078244](https://pubmed.ncbi.nlm.nih.gov/30078244/) DOI: [10.3760/cma.j.issn.0578-1310.2018.08.011](https://doi.org/10.3760/cma.j.issn.0578-1310.2018.08.011)]
  - 28 **Veropalumbo C**, Campanozzi A, De Gregorio F, Corraia A, Raia V, Vajro P. Shwachman-Diamond syndrome with autoimmune-like liver disease and enteropathy mimicking celiac disease. *Clin Res Hepatol Gastroenterol* 2015; **39**: e1-e4 [PMID: [25129842](https://pubmed.ncbi.nlm.nih.gov/25129842/) DOI: [10.1016/j.clinre.2014.06.017](https://doi.org/10.1016/j.clinre.2014.06.017)]
  - 29 **McCusker C**, Warrington R. Primary immunodeficiency. *Allergy Asthma Clin Immunol* 2011; **7** Suppl 1: S11 [PMID: [22165913](https://pubmed.ncbi.nlm.nih.gov/22165913/) DOI: [10.1186/1710-1492-7-S1-S11](https://doi.org/10.1186/1710-1492-7-S1-S11)]
  - 30 **Tokgoz H**, Caliskan U, Keles S, Reisli I, Guin IS, Morgan NV. Variable presentation of primary immune deficiency: two cases with CD3 gamma deficiency presenting with only autoimmunity. *Pediatr Allergy Immunol* 2013; **24**: 257-262 [PMID: [23590417](https://pubmed.ncbi.nlm.nih.gov/23590417/) DOI: [10.1111/pai.12063](https://doi.org/10.1111/pai.12063)]
  - 31 **Gargouri L**, Safi F, Mejdoub I, Maalej B, Mekki N, Mnif H, Ben Mustapha I, Barbouche MR, Boudawara T, Mahfoudh A. [Auto-immune hepatitis in chronic granulomatous disease in a 2-year-old girl]. *Arch Pediatr* 2015; **22**: 518-522 [PMID: [25800633](https://pubmed.ncbi.nlm.nih.gov/25800633/) DOI: [10.1016/j.arcped.2015.02.003](https://doi.org/10.1016/j.arcped.2015.02.003)]

- 32 **Ravel A**, Mircher C, Rebillat AS, Cieuta-Walti C, Megarbane A. Feeding problems and gastrointestinal diseases in Down syndrome. *Arch Pediatr* 2020; **27**: 53-60 [PMID: [31784293](#) DOI: [10.1016/j.arcped.2019.11.008](#)]
- 33 **McCulloch AJ**, Ince PG, Kendall-Taylor P. Autoimmune chronic active hepatitis in Down's syndrome. *J Med Genet* 1982; **19**: 232-234 [PMID: [6213775](#) DOI: [10.1136/jmg.19.3.232](#)]
- 34 **Mehta DI**, Hill ID, Singer-Granick C, Balloch Z, Blecker U. Primary sclerosing cholangitis and multiple autoimmune disorders in a patient with Down syndrome. *Clin Pediatr (Phila)* 1995; **34**: 502-505 [PMID: [7586925](#) DOI: [10.1177/000992289503400910](#)]
- 35 **O'Mahony D**, Whelton MJ, Hogan J. Down syndrome and autoimmune chronic active hepatitis: satisfactory outcome with therapy. *Ir J Med Sci* 1990; **159**: 21-22 [PMID: [2138593](#) DOI: [10.1007/BF02937210](#)]
- 36 **Yang J**, Chandrasekharappa SC, Vilboux T, Smith AC, Peterson EJ. Immune complex-mediated autoimmunity in a patient With Smith-Magenis syndrome (del 17p11.2). *J Clin Rheumatol* 2014; **20**: 291-293 [PMID: [25036569](#) DOI: [10.1097/RHU.0000000000000118](#)]
- 37 **Bartsch O**, Schneider E, Damatova N, Weis R, Tufano M, Iorio R, Ahmed A, Beyer V, Zechner U, Haaf T. Fulminant hepatic failure requiring liver transplantation in 22q13.3 deletion syndrome. *Am J Med Genet A* 2010; **152A**: 2099-2102 [PMID: [20635403](#) DOI: [10.1002/ajmg.a.33542](#)]
- 38 **Quaio CR**, Carvalho JF, da Silva CA, Bueno C, Brasil AS, Pereira AC, Jorge AA, Malaquias AC, Kim CA, Bertola DR. Autoimmune disease and multiple autoantibodies in 42 patients with RASopathies. *Am J Med Genet A* 2012; **158A**: 1077-1082 [PMID: [22488759](#) DOI: [10.1002/ajmg.a.35290](#)]
- 39 **Wang J**, Mizui M, Zeng LF, Bronson R, Finnell M, Terhorst C, Kyttaris VC, Tsokos GC, Zhang ZY, Kontaridis MI. Inhibition of SHP2 ameliorates the pathogenesis of systemic lupus erythematosus. *J Clin Invest* 2016; **126**: 2077-2092 [PMID: [27183387](#) DOI: [10.1172/JCI87037](#)]
- 40 **Ganesh R**, Suresh N, Vasanthi T, Sathiyasekaran M, Thulasiraman R. A 6-year-old boy with Wilson disease-A diagnostic dilemma. *Indian J Gastroenterol* 2017; **36**: 149-154 [PMID: [28435998](#) DOI: [10.1007/s12664-017-0746-4](#)]
- 41 **Santos BC**, Guedes LR, Faria LC, Couto CA. Wilson's disease presentation resembling autoimmune hepatitis. *BMJ Case Rep* 2019; **12** [PMID: [31653624](#) DOI: [10.1136/bcr-2019-230721](#)]
- 42 **Bloom JL**, Lin C, Imundo L, Guthery S, Stepenaskie S, Galambos C, Lowichik A, Bohnsack JF. H syndrome: 5 new cases from the United States with novel features and responses to therapy. *Pediatr Rheumatol Online J* 2017; **15**: 76 [PMID: [29041934](#) DOI: [10.1186/s12969-017-0204-y](#)]
- 43 **Esmailzadeh H**, Bermanian MH, Nabavi M, Arshi S, Fallahpour M, Fuchs I, zur Stadt U, Warnatz K, Ammann S, Ehl S, Lehmborg K, Rezaei N. Novel Patient with Late-Onset Familial Hemophagocytic Lymphohistiocytosis with STXPB2 Mutations Presenting with Autoimmune Hepatitis, Neurological Manifestations and Infections Associated with Hypogammaglobulinemia. *J Clin Immunol* 2015; **35**: 22-25 [PMID: [25491289](#) DOI: [10.1007/s10875-014-0119-z](#)]
- 44 **Di Marco F**, Palumbo G, Terraneo S, Imeri G, Lesma E, Sverzellati N, Peron A, Gualandri L, Canevini MP, Centanni S. Lymphangioleiomyomatosis, multifocal micronodular pneumocyte hyperplasia, and sarcoidosis: more pathological findings in the same chest CT, or a single pathological pathway? *BMC Pulm Med* 2017; **17**: 107 [PMID: [28754097](#) DOI: [10.1186/s12890-017-0447-x](#)]
- 45 **Jitraruch S**, Fitzpatrick E, Deheragoda M, Deganello A, Mieli-Vergani G, Height S, Rees D, Hadzic N, Samyn M. Autoimmune Liver Disease in Children with Sickle Cell Disease. *J Pediatr* 2017; **189**: 79-85.e2 [PMID: [28735981](#) DOI: [10.1016/j.jpeds.2017.06.035](#)]
- 46 **Zellos A**, Boitnott JK, Schwarz KB. New-onset autoimmune hepatitis in young patients with preexisting liver disease. *Dig Liver Dis* 2010; **42**: 657-660 [PMID: [20227931](#) DOI: [10.1016/j.dld.2010.01.022](#)]
- 47 **Hurtova M**, Bachir D, Lee K, Calderaro J, Decaens T, Kluger MD, Zafrani ES, Cherqui D, Mallat A, Galactéros F, Duvoux C. Transplantation for liver failure in patients with sickle cell disease: challenging but feasible. *Liver Transpl* 2011; **17**: 381-392 [PMID: [21445921](#) DOI: [10.1002/lt.22257](#)]
- 48 **Ayto RM**, Hughes DA, Jeevaratnam P, Rolles K, Burroughs AK, Mistry PK, Mehta AB, Pastores GM. Long-term outcomes of liver transplantation in type 1 Gaucher disease. *Am J Transplant* 2010; **10**: 1934-1939 [PMID: [20659098](#) DOI: [10.1111/j.1600-6143.2010.03168.x](#)]
- 49 **Yan X**, Jin J. Primary cutaneous amyloidosis associated with autoimmune hepatitis-primary biliary cirrhosis overlap syndrome and Sjögren syndrome: A case report. *Medicine (Baltimore)* 2018; **97**: e0004 [PMID: [29465536](#) DOI: [10.1097/MD.00000000000010004](#)]
- 50 **González-Moreno EI**, Cámara-Lemarroy CR, Borjas-Almaguer DO, Martínez-Cabriales SA, Paz-Delgadillo J, Gutiérrez-Udave R, Ayala-Cortés AS, Ocampo-Candiani J, Cortés-Hernández CA, Maldonado-Garza HJ. Cutaneous amyloidosis associated with autoimmune hepatitis-primary biliary cirrhosis overlap syndrome. *Ann Hepatol* 2015; **14**: 416-419 [PMID: [25864224](#)]
- 51 **Oliveira HM**, Pereira C, Santos-Silva E, Pinto-Basto J, Vizcaíno JR, Pessegueiro-Miranda H. A New Mutation Causing Progressive Familial Intrahepatic Cholestasis Type 3 in Association with Autoimmune Hepatitis. *Eur J Case Rep Intern Med* 2017; **4**: 000537 [PMID: [30755924](#) DOI: [10.12890/2016\\_000537](#)]

## Assessing the prognosis of cirrhotic patients in the intensive care unit: What we know and what we need to know better

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### Abstract

Critically ill cirrhotic patients have high in-hospital mortality and utilize significant health care resources as a consequence of the need for multiorgan support. Despite this fact, their mortality has decreased in recent decades due to improved care of critically ill patients. Acute-on-chronic liver failure (ACLF), sepsis and elevated hepatic scores are associated with increased mortality in this population, especially among those not eligible for liver transplantation. No score is superior to another in the prognostic assessment of these patients, and both liver-specific and intensive care unit-specific scores have satisfactory predictive accuracy. The sequential assessment of the scores, especially the Sequential Organ Failure Assessment (SOFA) and Chronic Liver Failure Consortium (CLIF)-SOFA scores, may be useful as an auxiliary tool in the decision-making process regarding the benefits of maintaining supportive therapies in this population. A CLIF-ACLF > 70 at admission or at day 3 was associated with a poor prognosis, as well as SOFA score > 19 at baseline or increasing SOFA score > 72. Additional studies addressing the prognostic assessment of these patients are necessary.

**Key Words:** Cirrhosis; Extrahepatic organ failure; Organ replacement therapy; Mortality; Prognostic scores; Chronic Liver Failure Consortium-Sequential Organ Failure Assess-

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**Core Tip:** Assessing the potential benefits of maintaining or suspending supportive therapies for cirrhotic patients who are not eligible for liver transplantation is a major challenge at the bedside, especially in those admitted to general intensive care units (ICUs). In this article, we identify the main causes of ICU admission, analyze the main factors associated with prognosis, and provide a tool to assist the decision-making process.

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## INTRODUCTION

Liver cirrhosis (LC) accounts for more than 7000 deaths per year in France and more than 25000 deaths per year in the United States[1]. The World Health Organization recently estimated that cirrhosis is the 12<sup>th</sup> leading cause of mortality in the world, with alcohol, hepatitis B virus and hepatitis C virus being the main causes of cirrhosis [2,3]. Cirrhotic patients account for 2.3% and 4.5% of all intensive care units (ICUs) admissions[1], and their mortality is traditionally high-approximately 34% to 69% depending on the reason for admission[2]. The increased effectiveness of supportive treatments and the spread of liver transplantation programs have improved the prognosis of these patients[1,4-6]. Nonetheless, the prognosis of cirrhotic patients admitted to the ICU remains poor[7], especially among those admitted to the general ICU who are ineligible for transplantation. The prognosis is determined by the extent of hepatic and extrahepatic organ dysfunction[8]. The occurrence of three or more organ failures in cirrhotic patients has an almost certain fatal outcome[6,9]. For ethical reasons and due to limited resources, physicians need to be able to quickly identify cases that benefit from aggressive treatment and ICU admission, discriminating good candidates for ICUs from those for whom the prognosis is poor despite strong therapeutic interventions.

## CIRRHOTIC PATIENTS ADMITTED TO THE ICU – AN OVERVIEW

Hemodynamic changes in patients with cirrhosis, linked to sodium retention, the development of ascites, and alterations in systemic and splanchnic hemodynamics and coagulation, are linked to systemic impairments in organ function, especially cardiomyopathy and renal dysfunction in this population[10]. A systemic inflammatory response has been observed in these patients, with complex immune dysfunction that increases the complexity of treatment and mortality in comparison with the general population[6,11]. High-grade hepatic encephalopathy (HE), septic shock, acute-on-chronic liver failure (ACLF), variceal bleeding, the need for mechanical ventilation and acute kidney injury (AKI) are clinical decompensations that most commonly motivate admission to the ICU[6].

### Sepsis and septic shock

Infections are among the main reasons for admission of these patients to the ICU, as 30%-50% of patients with cirrhosis either present with infection during admission or develop infection during hospitalization[2,12]. Sepsis is a consequence of the host response to infection[13] and it is characterized by the release of pro- and anti-inflammatory cytokines and pro- and anti-coagulant substances in response to pathogens [14]. Several studies have highlighted the major influence of cirrhosis on the susceptibility to severe bacterial infections, with higher in-hospital mortality rates as a result



of septic shock in cirrhotic relative to noncirrhotic patients (71% *vs* 49%, respectively) [15,16]. Cirrhotic patients have an altered defense against bacteria associated with reduced bacterial clearance. This immune defect facilitates bacterial translocation induced by the increased intestinal permeability and gut bacterial overgrowth observed in cirrhosis[17]. Sepsis leads to the production of various inflammatory mediators that are increased in cirrhotic patients compared to noncirrhotic septic patients[6]. This state leads to complex organ alterations that often lead to the development of extrahepatic organ dysfunction, including HE and renal, respiratory, and circulatory failure during sepsis, a syndrome referred to as ACLF, which is also associated with a deterioration in hepatic function[18]. Commonly encountered infections in cirrhosis include spontaneous bacterial peritonitis, pneumonia, urinary tract infection, and cellulitis[19]. Sepsis is more common in cirrhotic than in noncirrhotic ICU patients, and it is also associated with a higher mortality rate[15]. Variables associated with mortality in septic cirrhotic patients are the presence of more than one site of infection, Child C status and elevated Model for End-stage Liver Disease (MELD) score[12].

### **Variceal bleeding**

Cirrhotic patients with variceal bleeding are usually transferred to the ICU for hemodynamic stabilization. The fate of variceal bleeding in cirrhotic patients has changed over the last two decades[14]. Overall hospital mortality decreased from 42% in 1980 to 14% in 2000[20]. ICU admissions for variceal bleeding fell significantly in the last decade and were associated with a decrease in mortality over time[21]. Although overall mortality rates have decreased in cirrhotic patients with variceal bleeding, it is still high in the first 6 wk after the initial episode, and could exceed 30% in those with more severe disease and in those with multiorgan failure[5,6,22]. Rebleeding occurs in up to 20% of patients during the first 6 wk, and in this case, the mortality rate can exceed 50%. Patients with Child C or MELD  $\geq$  18, portal vein thrombosis, bacterial infections, and renal failure have a high likelihood of recurrence or death[6].

### **AKI**

Cirrhosis-associated AKI is usually multifactorial and commonly involves bacterial infections, hypovolemia (secondary to overdiuresis, hemorrhagic shock, large-volume paracentesis or diarrhea), drug-induced nephrotoxicity, parenchymal renal disease and, in the absence of these causes, hepatorenal syndrome (HRS)[5,23]. With a yearly rate of 8%–12%, HRS-AKI is quite common in decompensated cirrhosis with ascites[10, 23]. In hospitalized patients, it is approximately 25% and it increases up to 40%–60% in those admitted to the ICU[14,24]. AKI is associated with a poor prognosis and represents an important predictor for short-term mortality in patients with cirrhosis [25].

### **Encephalopathy**

HE is a brain dysfunction caused by liver failure and/or portosystemic shunts and it manifests as a wide spectrum of neurological and/or psychiatric abnormalities[26]. Approximately 30%–40% of patients with cirrhosis present with an episode of HE at some time of their illness, with a poor prognosis and a mortality increase of 50% within 1 year after the episode of HE[6]. Patients with more severe grades (grade III–IV) could require admission to the ICU and orotracheal intubation and eventually prolonged MV, variables that are associated with increased mortality in this scenario [27,28].

### **Short and long-term mortality in ICU-cirrhotic patients**

Short-term mortality in ICU-cirrhotic patients ranges from 42% in the ICU to 54% during hospitalization[29]. There is variability between different studies due to different selection criteria for patient admission between centers, differences between therapeutic strategies (including liver transplantation) and the low number of patients studied in each cohort in this short period of time[30]. During the ICU stay, prolonged MV is an important prognostic marker for ICU mortality[28]. Among the long-term mortality data for cirrhotic patients, there is high in-hospital mortality with reduced survival rates at 6 mo and 1 year. Thus, the one-year survival rate was 32% among patients alive at discharge from the ICU[9]. In another large study of short- and long-term survival, we found a comparable reduction in survival, with 8%–21% patients dying shortly after ICU discharge. In the ICU, 28-d, 3-, 6-mo, and 1-year mortality rates were 47%, 53% (116/218), 66%, 74%, and 77%, respectively[7]. The Glasgow coma scale, mean arterial pressure, bilirubin, and albumin determined on admission to the ICU have independent prognostic significance for assessing 6-month mortality. Severe

sepsis had the strongest association with increased 6-month mortality among the primary ICU admission reasons[29].

## PROGNOSTIC SCORES IN CIRRHOTIC PATIENTS ADMITTED TO THE ICU

Liver cirrhosis is characterized by a long phase of compensated disease until the first episode of decompensation occurs. The time elapsed until such an event is variable and unpredictable; however, it marks a change in the progression of the liver disease [30]. Upon acute decompensation, some of these patients develop organ failure and need to be admitted to the ICU for optimal treatment. Historically, the in-hospital mortality rates of these patients are very high, promoting the idea that admitting them to the ICU would be a futile measure[22]. More current series show that the hospital mortality of these patients is quite heterogeneous, reflecting the varying degrees of hepatic involvement that these patients may present on admission to the ICU, as well as their different reasons for admission to the ICU[31].

Even so, the nonnegligible mortality rates of critically ill patients with liver cirrhosis, associated with scarce and expensive intensive care resources, make the indication of ICU admission of this population a matter of debate. Prognostic scores are helpful in this decision-making, aiming at therapeutic proportionality at the individual level and an adequate allocation of resources at the institutional level. The prognostic scores can be specific to each pathology. In the case of liver cirrhosis, we can mention Child-Pugh (CP), the MELD, and the Chronic Liver Failure-Consortium ACLF (CLIF-ACLF) score, for example, or assessments common to all patients admitted to the ICU, such as the Sequential Organ Failure Assessment (SOFA) score and the Acute Physiology and Chronic Health Evaluation II (APACHE II) score. These scores can be performed immediately upon admission to the ICU (first 24 h) or during the first days of hospitalization, leading to an evolutionary assessment over this short period of time. We can also evaluate the prognosis of decompensated liver cirrhosis taking into account the number of organic disorders at its presentation. The most relevant studies regarding prognostic scores are summarized in Table 1.

General ICU scores have been frequently used in the evaluation of cirrhotic patients. However, these scores do not include the complexity of chronic liver disease, including the heterogeneity of its clinical stages and possible etiologies, thus imposing caution in the use of these tools. On the other hand, CP and MELD incorporate limited information about extrahepatic organic dysfunction. Next, the main scores will be discussed, as well as comparisons of their performances.

## HEPATIC-SPECIFIC SCORES

### CP and MELD

The chronic liver disease severity score described by Child in 1964 and modified by Pugh in 1973 was used to describe the prognosis of patients undergoing surgical ligation of esophageal varices, demonstrating that patients with less perioperative liver dysfunction had lower mortality in six months[32]. It is currently used to assess the severity of chronic liver disease. The MELD score was described to predict mortality at 3 mo in patients electively submitted to the placement of portosystemic shunts[33] and later used to prioritize patients listed for liver transplantation because it proved to be a reliable mortality risk index[34].

Specific scores for cirrhosis, such as CP and MELD, seem ideal for prognosis in cirrhotic patients with slow decompensation but do not perform well in those with acute decompensation accompanied by multiple organ and system dysfunction (DMOS). DMOS is a clinical condition where there are multiple acute systemic failures (renal, circulatory, neurologic, hematological, pulmonary, hepatic) associated with an initial injury, most commonly sepsis, trauma or shock[35,36]. They show moderate results[37], with the MELD score showing slightly better results than the CP[3]. The MELD score has reasonable discriminatory power (AUROC = 0.81) in predicting mortality in cirrhotic patients admitted to the ICU, approaching the SOFA score (AUROC = 0.83)[31].

### Variations of MELD: MELD-sodium

Dilutional hyponatremia is common in patients with advanced cirrhosis, and the inclusion of natremia in the MELD score has been suggested to increase its prognostic

**Table 1 Accuracy of prognostic scores in intensive care units cirrhotic patients**

Ref.	Year	n	ICU/hospital mortality	APACHE II	SAPS II	SOFA	CP	MELD	MELD-Na	RFH	CLIF-SOFA
Cholongitas <i>et al</i> [31], 2006	2006	312	65%	0.78		0.83	0.72	0.81		0.83	
Das <i>et al</i> [41], 2010	2010	138	54%		0.78	0.84	0.76	0.77	0.75		
Levesque <i>et al</i> [42], 2012	2012	377	43%		0.89	0.92	0.79	0.82	0.79		
Cholongitas <i>et al</i> [44], 2008	2012	412	61%	0.74		0.85	0.67	0.80	0.75		
Emerson <i>et al</i> [45], 2014	2014	59	48%	0.72		0.76	0.70	0.74			0.75
Campbell <i>et al</i> [46], 2015	2015	115	46%	0.71		0.71	0.68	0.70		0.77	0.74
McPhail <i>et al</i> [51], 2015	2015	971	52%	0.76	0.78	0.79		0.78			0.81

ICU: Intensive care units.

capacity for mortality, with greater importance when the MELD scores are lower[38, 39]. The MELD-Na score was better than the MELD score for predicting mortality in some studies[40] but less accurate than the SOFA score[41-43].

### CP variation: CP + L

More recent data suggest that lactate, a component of the prognostic model of fulminant hepatitis, is an independent marker of mortality in patients with cirrhosis admitted to the ICU[44] and it seems to significantly improve the CP score's ability to predict ICU mortality[45]. Serum lactate and ascites are independent predictors of ICU mortality, as proposed by the CTP + L score. This score incorporates serum lactate levels into CP, increasing its discriminatory ability as a prognostic stratification tool. Subsequently, a retrospective cohort study with a total of 199 cirrhotic patients admitted to a general ICU at two different centers validated the CP + L score as a predictor of mortality, showing results superior to the original CP: AUC CP + L 0.75 and AUC CP 0.68. In this work, the MELD and SOFA scores had AUCs of 0.7 and 0.71, respectively[2].

### Royal free hospital score

Studies have suggested that an alternative approach to predict mortality in patients with decompensated cirrhosis could be the number of organ dysfunctions at its presentation, ranging from 4% in patients without DMOS to 90% in those with three or more organ dysfunctions and thus in a DMOS scenario[31]. In this context, a specific score for cirrhosis was developed and subsequently modified[43], taking into account possible organic failures involved during acute decompensation, the Royal Free Hospital Score (RFH). This score was shown to have a performance similar to the SOFA score and superior to APACHE II, MELD, and CP.

A retrospective cohort study by Campbell *et al*[46], with a total of 199 cirrhotic patients admitted to the ICU, validated the RFH score as a predictor of mortality in the ICU with an accuracy of 0.77, which was higher than the other scores evaluated: CP, CP-L, MELD, SOFA and CLIF-SOFA. The RFH score is the first liver-specific score to be matched, in terms of mortality predictive ability, to the general ICU scores used in these patients. In addition to the fact that it includes hepatic and extrahepatic parameters of organ dysfunction associated with higher mortality in this subset of patients, the inclusion of lactate levels in this score should be highlighted. Despite the well-known relationship between serum lactate levels and worse outcomes[2], no other hepatic-specific score proposed thus far has included this parameter.

### ICU mortality and morbidity scores (dysfunction)

ICU-specific mortality scores were created to assist the intensive care physician in predicting the outcome of patients admitted to the ICU. Among these scores, the most important are the APACHE II and SOFA scores. APACHE II uses the worst physiological variables of the patient in the first 24 h of ICU stay for its elaboration, in addition to previous comorbidities and age[47]. The SOFA score assesses the severity of patients admitted to the ICU according to the number of organ dysfunctions. The score is graded in five levels (from 0 to 4 points) for six organ systems: neurological, hemodynamic, respiratory, renal, hematological and hepatic, with a score greater than or equal to 3 in any organ system constituting organ failure[48]. Unlike the APACHE II

score, which is performed at a specific time in the ICU (24 h of admission), the morbidity scores allow for an evolutionary assessment throughout the days of ICU admission[48].

These scores have already been evaluated in specific populations of cirrhosis[15]. When compared to each other and with specific scores for cirrhosis, the SOFA score shows moderate to high accuracy, higher than the other scores, even for long-term mortality[3,45,49]. Lindvig *et al*[3], in their systematic review, found that the SOFA score has better accuracy for death prediction, with an AUROC between 0.81% and 0.95%, a value higher than the APACHE II score (AUROC 0.66-0.83), MELD (AUROC 0.77-0.93) and CP (AUROC 0.71-0.87).

## ACLF

ACLF is a clinical syndrome characterized by acute liver cirrhosis decompensation associated with one or more organic disorders and a high short-term mortality rate. The European Association for Study of Liver/CLIF (EASL-CLIF Consortium) has established diagnostic criteria for ACLF with a view, above all, to identify patients at greater risk of death in the short term. For the establishment of the ACLF diagnostic criteria, the presence of organic dysfunction and a high mortality rate at 28 d (> 15%) in cirrhotic patients with acute decompensation were considered. The assessment of organ dysfunction, in turn, was based on the SOFA score, but with modifications taking into account the pathophysiological and clinical characteristics of cirrhosis, giving rise to the CLIF-SOFA score[50].

CLIF-SOFA improves the hematological, neurological, cardiovascular, and renal domains by considering commemoratives usually present in chronic liver disease patients, as well as the peculiarities of the clinical manifestations and therapy used during acute decompensation. Objectively, the hematological parameter is no longer the platelet count giving rise to the measurement of INR. The neurological parameter now includes the presence of HE stratified under West Haven criteria, and in the cardiovascular and renal domains, it takes into account the use of terlipressin and renal replacement therapy, respectively. There is also a change in the hepatic domain with elevation of the total bilirubin threshold to characterize this organ dysfunction.

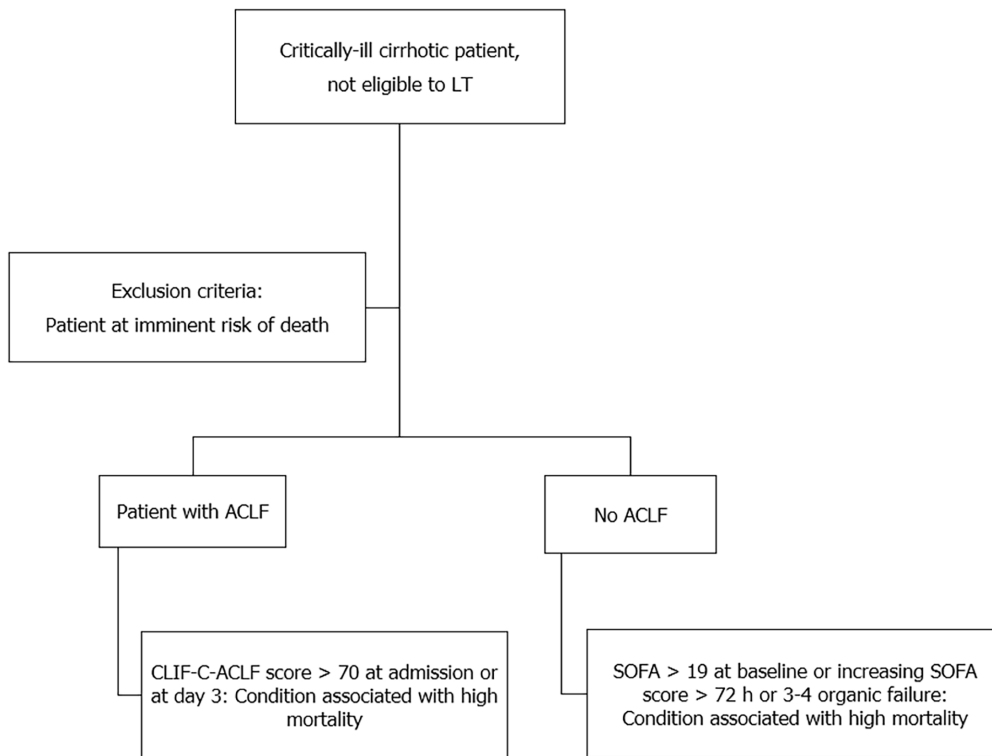
McPhail *et al*[51] demonstrated the validity of the CLIF-SOFA score in terms of its ability to predict mortality with a slight improvement over the SOFA score and other prognostic scores. Aiming at a better performance than CLIF-SOFA, the CLIF-C ACLF score was developed based on CLIF organ failure score scores, the latter also a derivation of SOFA and CLIF-SOFA[52]. However, the CLIF-C ACLF showed a slightly higher prognostic accuracy for 28-d mortality than the CLIF-SOFA scores and it was moderately higher than MELD, MELD-Na and Child-Pugh: agreement index of 0.76; 0.72; 0.68; 0.68; 0.66, respectively[52].

## Evolutionary assessment of scores-what we need to know better?

Most prognostic scores in critically ill populations are constructed with data collected over the first 24 h of ICU admission. However, multiorgan failure seems to be related to a worse prognosis among patients with acute cirrhosis decompensation[1,4,22]. Seeking to increase the accuracy of prognostic scores in cirrhotic patients admitted to the ICU, a baseline assessment of the score followed by its reanalysis in a short period of time seems to be more accurate in predicting hospital mortality. The SOFA score seems to be the score with the best discrimination power when compared to the CTP, MELD, APACHE II scores, both at the initial time and when reassessed at 48 h: AUC for mortality, after 48 h of 0.88; 0.78; 0.86 and 0.78, respectively[44]. The modified SOFA score (removing the hepatic component from the score) was also shown to be highly accurate and with better discriminative power when compared to CP, MELD, and APACHE II scores both on the first day of ICU admission (AUC 0.84) and on the third day (AUC 0.83)[41]. It is interesting to note that the presence of 3 to 4 organ dysfunctions after 72 h of admission to the ICU is related to an important increase in mortality during hospitalization[41].

A limitation of the prognostic scores evaluated on admission to the ICU is to neglect the continuum of physiological changes in critical patients with decompensated cirrhosis[53]. The serial assessment of the SOFA score throughout the ICU stay contemplates the dynamics of the occurrence of organic dysfunctions, including the effects of the offered therapy[44,54]. Both the analysis of the variation in the SOFA score ( $\Delta$ -SOFA) and access to the mean and maximum SOFA values during ICU admission are good prognostic indicators, regardless of the value of the score accessed





**Figure 1** Proposed algorithm for prognostic scores in critically-ill cirrhotic patients. ACLF: Acute-on-chronic liver failure; CLIF: Chronic Liver Failure Consortium; SOFA: Sequential Organ Failure Assessment.

at the time of admission[54]. In a retrospective cohort study comprised of 971 patients, the CLIF-SOFA score seemed to have a slightly higher accuracy than the SOFA score for mortality (AUC 0.81 *vs* 0.79) when evaluated during the first day of hospitalization and an improvement in death prediction at 48 h after ICU admission. However, the results seem overlapping when evaluated on the seventh day of ICU stay, with both showing good discriminatory power[51]. Dynamic prognostication seems to be the most promising strategy when establishing the prognosis of this population, especially in those with ACLF, septic shock and multiorgan failure[55]. A proposed algorithm is summarized in Figure 1. A trial of unrestricted intensive care for a few days could be proposed as a reasonable strategy in this population[41]. There are also opportunities for novel biomarkers of ACLF to improve existing models and potentially reflect information not currently captured in the conventional clinical and biochemical data [56].

An important limitation of prognostic studies in this field is that the interpretation of ROC curves is necessary because the criteria for therapeutic limitations or even the removal of supports are not reported in these studies, which leads to falsely high areas under the curves. Another limitation of prognostic scores is that they were not designed to predict outcomes beyond mortality, such as cost-effective treatment, recovery of physical activity or the quality of life after the ICU stay. In addition, some organ dysfunction scores may give similar weights for organ dysfunction with very different prognoses[57]. Alteration of the level of consciousness due to HE after bleeding from esophageal varices and even chronic thrombocytopenia, common in advanced cirrhosis, has a better prognosis than that of vasopressor or acute loss of renal function. Figure 1 outlines a structured assessment model based on prognostic scores in this population. A condition associated with high mortality, based on these scores, does not necessarily mean that therapeutic efforts should be stopped but that patients, family members and staff can have a better understanding of the prognosis, in light of current knowledge. Knowledge of the patients' wishes, beliefs and desires is fundamental to establish future therapeutic strategies.

## CONCLUSION

In critically ill cirrhotic patients who are not awaiting liver transplantation, there is no

“gold standard” for predicting their short- and long-term prognosis. Several variables are associated with a worse prognosis, such as the presence of sepsis, the number and intensity of associated organ failures, and the duration of MV. Baseline severity scores, as well as the sequential assessment of organ failure scores, provide more certainty regarding the impact of critical illness on the prognosis of this population.

## REFERENCES

- 1 **Galbois A**, Trompette ML, Das V, Boëlle PY, Carbonell N, Thabut D, Housset C, Ait-Oufella H, Offenstadt G, Maury E, Guidet B. Improvement in the prognosis of cirrhotic patients admitted to an intensive care unit, a retrospective study. *Eur J Gastroenterol Hepatol* 2012; **24**: 897-904 [PMID: 22569082 DOI: 10.1097/MEG.0b013e3283544816]
- 2 **Baudry T**, Hernu R, Valleix B, Jahandiez V, Faucher E, Simon M, Cour M, Argaud L. Cirrhotic Patients Admitted to the ICU With Septic Shock: Factors Predicting Short and Long-Term Outcome. *Shock* 2019; **52**: 408-413 [PMID: 30395082 DOI: 10.1097/SHK.0000000000001282]
- 3 **Lindvig KP**, Teisner AS, Kjeldsen J, Strøm T, Toft P, Furhmann V, Krag A. Allocation of patients with liver cirrhosis and organ failure to intensive care: Systematic review and a proposal for clinical practice. *World J Gastroenterol* 2015; **21**: 8964-8973 [PMID: 26269687 DOI: 10.3748/wjg.v21.i29.8964]
- 4 **Galbois A**, Aegerter P, Martel-Samb P, Housset C, Thabut D, Offenstadt G, Ait-Oufella H, Maury E, Guidet B; Collège des Utilisateurs des Bases des données en Réanimation (CUB-Réa) Group. Improved prognosis of septic shock in patients with cirrhosis: a multicenter study. *Crit Care Med* 2014; **42**: 1666-1675 [PMID: 24732239 DOI: 10.1097/CCM.0000000000000321]
- 5 **Karvellas CJ**, Bagshaw SM. Advances in management and prognostication in critically ill cirrhotic patients. *Curr Opin Crit Care* 2014; **20**: 210-217 [PMID: 24514101 DOI: 10.1097/MCC.0000000000000067]
- 6 **Fernández J**, Aracil C, Solà E, Soriano G, Cinta Cardona M, Coll S, Genescà J, Hombrados M, Morillas R, Martín-Llahí M, Pardo A, Sánchez J, Vargas V, Xiol X, Ginès P. Evaluación y tratamiento del paciente cirrótico crítico. *Gastroenterología y Hepatología* 2016; **39**: 607-626 [DOI: 10.1016/j.gastrohep.2015.09.019]
- 7 **Piton G**, Chagnat C, Giabiconi M, Cervoni JP, Tamion F, Weiss E, Paugam-Burtz C, Capellier G, Di Martino V. Prognosis of cirrhotic patients admitted to the general ICU. *Ann Intensive Care* 2016; **6**: 94 [PMID: 27709556 DOI: 10.1186/s13613-016-0194-9]
- 8 **Ho YP**, Chen YC, Yang C, Lien JM, Chu YY, Fang JT, Chiu CT, Chen PC, Tsai MH. Outcome prediction for critically ill cirrhotic patients: a comparison of APACHE II and Child-Pugh scoring systems. *J Intensive Care Med* 2004; **19**: 105-110 [PMID: 15070520 DOI: 10.1177/0885066603261991]
- 9 **Levesque E**, Saliba F, Ichai P, Samuel D. Outcome of patients with cirrhosis requiring mechanical ventilation in ICU. *J Hepatol* 2014; **60**: 570-578 [PMID: 24280294 DOI: 10.1016/j.jhep.2013.11.012]
- 10 **Simonetto DA**, Gines P, Kamath PS. Hepatorenal syndrome: pathophysiology, diagnosis, and management. *BMJ* 2020; **370**: m2687 [PMID: 32928750 DOI: 10.1136/bmj.m2687]
- 11 **Bassetti M**, Peghin M, Carnelutti A, Righi E, Merelli M, Ansaldi F, Trucchi C, Alicino C, Sartor A, Toniutto P, Wauters J, Laleman W, Tascini C, Menichetti F, Luzzati R, Brugnaro P, Mesini A, Raviolo S, De Rosa FG, Lagunes L, Rello J, Dimopoulos G, Colombo AL, Nucci M, Vena A, Bouza E, Muñoz P, Tumbarello M, Losito R, Martin-Loeches I, Viscoli C. Clinical characteristics and predictors of mortality in cirrhotic patients with candidemia and intra-abdominal candidiasis: a multicenter study. *Intensive Care Med* 2017; **43**: 509-518 [PMID: 28271321 DOI: 10.1007/s00134-017-4717-0]
- 12 **Lim LG**, Tan XX, Woo SJ, Dan YY, Lee YM, Lai V, Lim SG. Risk factors for mortality in cirrhotic patients with sepsis. *Hepatol Int* 2011; **5**: 800-807 [PMID: 21484126 DOI: 10.1007/s12072-011-9258-y]
- 13 **Levy MM**, Evans LE, Rhodes A. The Surviving Sepsis Campaign Bundle: 2018 update. *Intensive Care Med* 2018; **44**: 925-928 [PMID: 29675566 DOI: 10.1097/CCM.0000000000003119]
- 14 **Ginès P**, Fernández J, Durand F, Saliba F. Management of critically-ill cirrhotic patients. *J Hepatol* 2012; **56** Suppl 1: S13-S24 [PMID: 22300462 DOI: 10.1016/S0168-8278(12)60003-8]
- 15 **Gustot T**, Felleiter P, Pickkers P, Sakr Y, Rello J, Velissaris D, Pierrakos C, Taccone FS, Sevcik P, Moreno C, Vincent JL; EPIC II Group of Investigators. Impact of infection on the prognosis of critically ill cirrhotic patients: results from a large worldwide study. *Liver Int* 2014; **34**: 1496-1503 [PMID: 24606193 DOI: 10.1111/Liv.12520]
- 16 **Sauneuf B**, Champigneulle B, Soummer A, Mongardon N, Charpentier J, Cariou A, Chiche JD, Mallet V, Mira JP, Pène F. Increased survival of cirrhotic patients with septic shock. *Crit Care* 2013; **17**: R78 [PMID: 23601847 DOI: 10.1186/cc12687]
- 17 **Wiest R**, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005; **41**: 422-433 [PMID: 15723320 DOI: 10.1002/hep.20632]
- 18 **Thomson SJ**, Berry PA, Rahman TM. The impact of organ dysfunction in cirrhosis: survival at a cost? *J Hepatol* 2012; **57**: 707-708 [PMID: 22704199 DOI: 10.1016/j.jhep.2012.03.032]
- 19 **Fernández J**, Gustot T. Management of bacterial infections in cirrhosis. *J Hepatol* 2012; **56** Suppl 1:

- S1-12 [PMID: 22300459 DOI: 10.1016/S0168-8278(12)60002-6]
- 20 **Carbonell N**, Pauwels A, Serfaty L, Fourdan O, Lévy VG, Poupon R. Improved survival after variceal bleeding in patients with cirrhosis over the past two decades. *Hepatology* 2004; **40**: 652-659 [PMID: 15349904 DOI: 10.1002/hep.20339]
  - 21 **Majeed A**, Majumdar A, Bailey M, Kemp W, Bellomo R, Pilcher D, Roberts SK. Declining Mortality of Cirrhotic Variceal Bleeding Requiring Admission to Intensive Care: A Binational Cohort Study. *Crit Care Med* 2019; **47**: 1317-1323 [PMID: 31306178 DOI: 10.1097/CCM.0000000000003902]
  - 22 **Austin MJ**, Shawcross DL. Outcome of patients with cirrhosis admitted to intensive care. *Curr Opin Crit Care* 2008; **14**: 202-207 [PMID: 18388684 DOI: 10.1097/MCC.0b013e3282f6a40d]
  - 23 **Bucsics T**, Krones E. Renal dysfunction in cirrhosis: acute kidney injury and the hepatorenal syndrome. *Gastroenterol Rep (Oxf)* 2017; **5**: 127-137 [PMID: 28533910 DOI: 10.1093/gastro/gox009]
  - 24 **Cholongitas E**, Senzolo M, Patch D, Shaw S, O'Beirne J, Burroughs AK. Cirrhotics admitted to intensive care unit: the impact of acute renal failure on mortality. *Eur J Gastroenterol Hepatol* 2009; **21**: 744-750 [PMID: 20160527 DOI: 10.1097/MEG.0b013e328308bb9c]
  - 25 **Russ KB**, Stevens TM, Singal AK. Acute Kidney Injury in Patients with Cirrhosis. *J Clin Transl Hepatol* 2015; **3**: 195-204 [PMID: 26623266 DOI: 10.14218/JCTH.2015.00015]
  - 26 **Weiss N**, Jalan R, Thabut D. Understanding hepatic encephalopathy. *Intensive Care Med* 2018; **44**: 231-234 [PMID: 28547522 DOI: 10.1007/s00134-017-4845-6]
  - 27 **Juneja D**, Gopal PB, Kapoor D, Raya R, Sathyanarayanan M. Profile and outcome of patients with liver cirrhosis requiring mechanical ventilation. *J Intensive Care Med* 2012; **27**: 373-378 [PMID: 21436171 DOI: 10.1177/0885066611400277]
  - 28 **Lai CC**, Tseng KL, Ho CH, Chiang SR, Chan KS, Chao CM, Hsing SC, Cheng KC, Chen CM. Outcome of liver cirrhosis patients requiring prolonged mechanical ventilation. *Sci Rep* 2020; **10**: 4980 [PMID: 32188892 DOI: 10.1038/s41598-020-61601-2]
  - 29 **Weil D**, Levesque E, McPhail M, Cavallazzi R, Theocharidou E, Cholongitas E, Galbois A, Pan HC, Karvellas CJ, Sauneuf B, Robert R, Fichet J, Piton G, Thevenot T, Capellier G, Di Martino V; METAREACIR Group. Prognosis of cirrhotic patients admitted to intensive care unit: a meta-analysis. *Ann Intensive Care* 2017; **7**: 33 [PMID: 28321803 DOI: 10.1186/s13613-017-0249-6]
  - 30 **Saliba F**, Ichai P, Levesque E, Samuel D. Cirrhotic patients in the ICU: prognostic markers and outcome. *Curr Opin Crit Care* 2013; **19**: 154-160 [PMID: 23426137 DOI: 10.1097/MCC.0b013e32835f0e17]
  - 31 **Cholongitas E**, Senzolo M, Patch D, Shaw S, Hui C, Burroughs AK. Review article: scoring systems for assessing prognosis in critically ill adult cirrhotics. *Aliment Pharmacol Ther* 2006; **24**: 453-464 [PMID: 16886911 DOI: 10.1111/j.1365-2036.2006.02998.x]
  - 32 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913 DOI: 10.1002/bjs.1800600817]
  - 33 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]
  - 34 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]
  - 35 **Pool R**, Gomez H, Kellum JA. Mechanisms of Organ Dysfunction in Sepsis. *Crit Care Clin* 2018; **34**: 63-80 [PMID: 29149942 DOI: 10.1016/j.ccc.2017.08.003]
  - 36 **Sauaia A**, Moore FA, Moore EE. Postinjury Inflammation and Organ Dysfunction. *Crit Care Clin* 2017; **33**: 167-191 [PMID: 27894496 DOI: 10.1016/j.ccc.2016.08.006]
  - 37 **Cavallazzi R**, Awe OO, Vasu TS, Hirani A, Vaid U, Leiby BE, Kraft WK, Kane GC. Model for End-Stage Liver Disease score for predicting outcome in critically ill medical patients with liver cirrhosis. *J Crit Care* 2012; **27**: 424.e1-424.e6 [PMID: 22227088 DOI: 10.1016/j.jcrc.2011.11.014]
  - 38 **Ruf AE**, Kremers WK, Chavez LL, Descalzi VI, Podesta LG, Villamil FG. Addition of serum sodium into the MELD score predicts waiting list mortality better than MELD alone. *Liver Transpl* 2005; **11**: 336-343 [PMID: 15719386 DOI: 10.1002/Lt.20329]
  - 39 **Mazumder NR**, Atiemo K, Kappus M, Cullaro G, Harinstein ME, Ladner D, Verna E, Lai J, Levitsky J. A Comprehensive Review of Outcome Predictors in Low MELD Patients. *Transplantation* 2020; **104**: 242-250 [PMID: 31517785 DOI: 10.1097/TP.0000000000002956]
  - 40 **Luca A**, Angermayr B, Bertolini G, Koenig F, Vizzini G, Ploner M, Peck-Radosavljevic M, Gridelli B, Bosch J. An integrated MELD model including serum sodium and age improves the prediction of early mortality in patients with cirrhosis. *Liver Transpl* 2007; **13**: 1174-1180 [PMID: 17663415 DOI: 10.1002/Lt.21197]
  - 41 **Das V**, Boelle PY, Galbois A, Guidet B, Maury E, Carbonell N, Moreau R, Offenstadt G. Cirrhotic patients in the medical intensive care unit: early prognosis and long-term survival. *Crit Care Med* 2010; **38**: 2108-2116 [PMID: 20802324 DOI: 10.1097/CCM.0b013e3283181f3dea9]
  - 42 **Levesque E**, Hoti E, Azoulay D, Ichai P, Habouchi H, Castaing D, Samuel D, Saliba F. Prospective evaluation of the prognostic scores for cirrhotic patients admitted to an intensive care unit. *J Hepatol* 2012; **56**: 95-102 [PMID: 21835136 DOI: 10.1016/j.jhep.2011.06.024]
  - 43 **Theocharidou E**, Pieri G, Mohammad AO, Cheung M, Cholongitas E, Agarwal B, Burroughs AK. The Royal Free Hospital score: a calibrated prognostic model for patients with cirrhosis admitted to

- intensive care unit. Comparison with current models and CLIF-SOFA score. *Am J Gastroenterol* 2014; **109**: 554-562 [PMID: [24492755](#) DOI: [10.1038/ajg.2013.466](#)]
- 44 **Cholongitas E**, Betrosian A, Senzolo M, Shaw S, Patch D, Manousou P, O'Beirne J, Burroughs AK. Prognostic models in cirrhotics admitted to intensive care units better predict outcome when assessed at 48 h after admission. *J Gastroenterol Hepatol* 2008; **23**: 1223-1227 [PMID: [18086109](#) DOI: [10.1111/j.1440-1746.2007.05269.x](#)]
  - 45 **Emerson P**, McPeake J, O'Neill A, Gilmour H, Forrest E, Puxty A, Kinsella J, Shaw M. The utility of scoring systems in critically ill cirrhotic patients admitted to a general intensive care unit. *J Crit Care* 2014; **29**: 1131.e1-1131.e6 [PMID: [25175945](#) DOI: [10.1016/j.jcrc.2014.06.027](#)]
  - 46 **Campbell J**, McPeake J, Shaw M, Puxty A, Forrest E, Soulsby C, Emerson P, Thomson SJ, Rahman TM, Quasim T, Kinsella J. Validation and analysis of prognostic scoring systems for critically ill patients with cirrhosis admitted to ICU. *Crit Care* 2015; **19**: 364 [PMID: [26462911](#) DOI: [10.1186/s13054-015-1070-y](#)]
  - 47 **Knaus WA**, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818-829 [PMID: [3928249](#)]
  - 48 **Vincent JL**, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996; **22**: 707-710 [PMID: [8844239](#) DOI: [10.1007/BF01709751](#)]
  - 49 **Boone MD**, Celi LA, Ho BG, Pencina M, Curry MP, Lior Y, Talmor D, Novack V. Model for End-Stage Liver Disease score predicts mortality in critically ill cirrhotic patients. *J Crit Care* 2014; **29**: 881.e7-881.13 [PMID: [24974049](#) DOI: [10.1016/j.jcrc.2014.05.013](#)]
  - 50 **Moreau R**, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V; CANONIC Study Investigators of the EASL-CLIF Consortium. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437 [PMID: [23474284](#) DOI: [10.1053/j.gastro.2013.02.042](#)]
  - 51 **McPhail MJ**, Shawcross DL, Abeles RD, Chang A, Patel V, Lee GH, Abdulla M, Sizer E, Willars C, Auzinger G, Bernal W, Wendon JA. Increased Survival for Patients With Cirrhosis and Organ Failure in Liver Intensive Care and Validation of the Chronic Liver Failure-Sequential Organ Failure Scoring System. *Clin Gastroenterol Hepatol* 2015; **13**: 1353-1360.e8 [PMID: [25240417](#) DOI: [10.1016/j.cgh.2014.08.041](#)]
  - 52 **Jalan R**, Saliba F, Pavesi M, Amoros A, Moreau R, Ginès P, Levesque E, Durand F, Angeli P, Caraceni P, Hopf C, Alessandria C, Rodriguez E, Solis-Muñoz P, Laleman W, Trebicka J, Zeuzem S, Gustot T, Mookerjee R, Elkrif L, Soriano G, Cordoba J, Morando F, Gerbes A, Agarwal B, Samuel D, Bernardi M, Arroyo V; CANONIC study investigators of the EASL-CLIF Consortium. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol* 2014; **61**: 1038-1047 [PMID: [24950482](#) DOI: [10.1016/j.jhep.2014.06.012](#)]
  - 53 **Feltracco P**, Brezzi M, Barbieri S, Milevoj M, Galligioni H, Cillo U, Zanusi G, Vitale A, Ori C. Intensive care unit admission of decompensated cirrhotic patients: prognostic scoring systems. *Transplant Proc* 2011; **43**: 1079-1084 [PMID: [21620058](#) DOI: [10.1016/j.transproceed.2011.01.153](#)]
  - 54 **Ferreira FL**, Bota DP, Bross A, Melot C, Vincent JL. Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA* 2001; **286**: 1754-1758 [PMID: [11594901](#) DOI: [10.1001/jama.286.14.1754](#)]
  - 55 **Karvellas CJ**, Garcia-Lopez E, Fernandez J, Saliba F, Sy E, Jalan R, Pavesi M, Gustot T, Ronco JJ, Arroyo V; Chronic Liver Failure Consortium and European Foundation for the Study of Chronic Liver Failure. Dynamic Prognostication in Critically Ill Cirrhotic Patients With Multiorgan Failure in ICUs in Europe and North America: A Multicenter Analysis. *Crit Care Med* 2018; **46**: 1783-1791 [PMID: [30106759](#) DOI: [10.1097/CCM.0000000000003369](#)]
  - 56 **Mookerjee RP**. Prognosis and Biomarkers in Acute-on-Chronic Liver Failure. *Semin Liver Dis* 2016; **36**: 127-132 [PMID: [27172354](#) DOI: [10.1055/s-0036-1583200](#)]
  - 57 **O'Brien AJ**, Welch CA, Singer M, Harrison DA. Prevalence and outcome of cirrhosis patients admitted to UK intensive care: a comparison against dialysis-dependent chronic renal failure patients. *Intensive Care Med* 2012; **38**: 991-1000 [PMID: [22456768](#) DOI: [10.1007/s00134-012-2523-2](#)]



# Liver transplantation for pediatric inherited metabolic liver diseases

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## Abstract

Liver transplantation (LT) remains the gold standard treatment for end stage liver disease in the pediatric population. For liver based metabolic disorders (LBMDs), the decision for LT is predicated on a different set of paradigms. With improved outcomes post-transplantation, LT is no longer merely life saving, but has the potential to also significantly improve quality of life. This review summarizes the clinical presentation, medical treatment and indications for LT for some of the common LBMDs. We also provide a practical update on the dilemmas and controversies surrounding the indications for transplantation, surgical considerations and prognosis and long terms outcomes for pediatric LT in LBMDs. Important progress has been made in understanding these diseases in recent years and with that we outline some of the new therapies that have emerged.

**Key Words:** Pediatric metabolic liver disease; Liver transplantation; Liver based metabolic disorders; Inherited; Cell therapy; Gene therapy

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**Core Tip:** The decision for liver transplantation (LT) in liver based metabolic disorders (LBMDs) is not straightforward. As outcomes from pediatric LT continue to improve, transplantation is no longer merely life saving, but also potentially significantly improves the child's quality of life. We herein discuss the clinical presentation, medical and surgical treatment for some of the common LBMDs. We provide a practical update on the indications, dilemmas and controversies for LT and the long-term outcomes for children with LBMDs.

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## INTRODUCTION

Liver transplantation (LT) remains the standard of care for children with end-stage liver disease. With advances in the perioperative transplant management, the outcomes after pediatric LT continue to improve with better survival rates[1] and quality of life measures[2].

Indications for pediatric LT can be broadly divided into three main groups (Figure 1). (1) Cholestatic diseases, such as biliary atresia and other conditions leading to biliary cirrhosis are the most common indications for LT in the pediatric population [3]; (2) Inherited metabolic liver diseases constitutes a wider group of diseases, in which inborn errors of liver metabolism lead to severe intra- or extra-hepatic manifestations. Within this group of conditions, LT results in a cure in some, whilst others have an improved quality of life after transplantation, without necessarily being cured from their primary illness; and (3) The third group is more varied, with indications of acute liver failure, tumors and re-transplantations.

Some of the more common liver based metabolic disorders (LBMDs) are exemplified below.

## LBMDS CURED BY LT

### Crigler-Najjar syndrome type 1

Crigler-Najjar syndrome (CNS) type 1 is secondary to a total deficiency of the uridine diphosphoglucuronate glucuronosyltransferase activity[4]. This results in a severe indirect hyperbilirubinaemia from birth, with an otherwise normal liver biochemistry. It is an extremely rare familial disease affecting one *per* 600000-1000000 live births worldwide. It has an autosomal recessive inheritance pattern and is caused by biallelic mutations of the *UGT1A1* gene[5].

**Natural history and medical treatment:** The build up of unconjugated bilirubin, which deposits in the brain, eventually leads to kernicterus, which is irreversible in most cases. Exchange transfusion in the neonatal period and plasmapheresis in older children, may be indicated for acute episodes of severe hyperbilirubinaemia.

Intensive phototherapy is the mainstay of treatment for CNS type 1, particularly in the newborn period. It is less effective in older children and adults due to skin thickness, pigmentation and lower body surface area to body mass[6].

Other treatments include bilirubin-binding agents such as orlistat—a lipase inhibitor which works better in tandem with calcium phosphate. Both of these agents help in the excretion of bilirubin through the gut[7,8]. Other pharmacological agents with limited evidence for efficacy include enzyme-inducing agents (phenobarbital), choleretics (ursodiol) and heme-oxygenase inhibitors (tin-protoporphyrin and zinc-protoporphyrin).

**LT:** At present, the only definitive treatment for CNS type 1 is LT. The two main types of LT include orthotopic LT (OLT) and auxiliary partial OLT (APOLT). The host liver is replaced with a whole or partial liver graft in OLT, whilst in APOLT only part of the native liver is removed and replaced with the graft. APOLT has the theoretical advantage for future novel therapies directed at native hepatocytes, such as gene replacement and genome editing[4].

The transplant provides the child with a normal liver with normal *UGT1A1* enzymatic activity, thereby completely normalizing bilirubin levels and providing the child with a normal quality of life. LT is advisable before neurological damage occurs [9]. As the outcomes of transplantation in infants are now similar to children, transplantation is indicated in the first few years of life to prevent prolonged impairment to the child and family.

**Future research implications:** In recent years, allogenic hepatocyte transplantation has become an attractive alternative to LT[10]. Normal hepatocytes are transplanted *via* the portal vein or peritoneal space. Encouraging results have been observed with a reduction in bilirubin levels and reduced need for phototherapy[11]. Issues still exist around the longevity of the transplanted cells—which decreases after a few months, limited supply and cell quality. Mesenchymal stem cell therapy has shown some promise in animal models and may provide a new alternative treatment in the future [12].

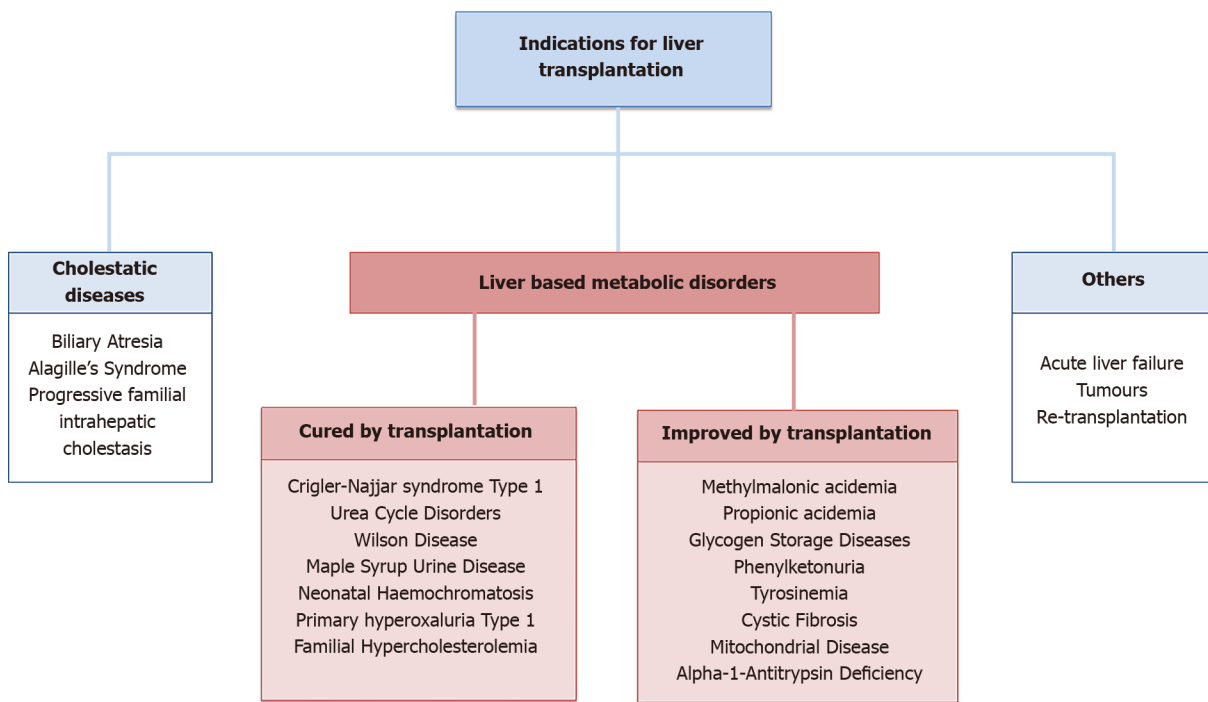


Figure 1 Indications for liver transplantation.

*Ex vivo* and *in vivo* gene therapy is another new avenue for treatment of CNS type 1. Different approaches including infusing autologous liver or induced pluripotent stem cells into the liver and *in vivo* gene replacement using a vector delivery system have been proposed, but there remain little safety and efficacy data[4].

### Urea cycle disorders

Urea cycle disorders (UCDs) are a group of disorders secondary to defects of urea synthesis and related metabolic pathways. UCDs result from a deficiency in either one of the six enzymes [n-acetylglutamine synthetase (NAGS), carbamoylphosphate synthetase I (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthase, argininosuccinate lyase (ASL), and arginase 1] or two mitochondrial transporters of the urea cycle pathway or metabolites of the amino acids related to the urea cycle[13]. The liver is central to these metabolic pathways, and plays a key role in removing waste from protein catabolism. The defect in the pathway leads to life threatening hyperammonaemia[14]. It is the most common IEM based in the liver with an incidence of 1 in 30000–46000 Live births. All UCDs are inherited in an autosomal recessive manner apart from OTC deficiency, which is inherited in an X-linked manner.

**Natural history and medical treatment:** Clinical findings are secondary to hyperammonaemia including seizures, coma, cerebral edema and death, with long-term neurodevelopmental implications in survivors. The severity of symptoms can be variable, with some presenting with fatal hyperammonaemia in infancy to asymptomatic adults. In the neonatal period, symptoms occur within hours to days after birth. Initially, neonates with UCD may present with non-specific features such as poor feeding, vomiting, lethargy and tachypnea, but quickly progress to coma and death secondary to hyperammonaemia. NAGS, CPS1 and OTC deficiencies, have the poorest outcomes with neonatal onset of hyperammonaemia and death within the first year of life[15]. Some children may have a delayed presentation with less severe features such as mild gastrointestinal or neurological symptoms. The long-term outcome is dependent on the number of episodes of hyperammonaemia (due to non-adherence, infections and lack of compliance to diet).

The medical management of UCDs requires multidisciplinary input and is complex. The treatment strategy for acute hyperammonaemia is three-fold[16]: (1) Reduce blood ammonia levels through hemodialysis or hemofiltration; (2) Reversal of the catabolic state through caloric and arginine supplementation; and (3) Elimination of excess nitrogen pharmacologically (*e.g.* benzoate and phenylbutyrate)

In the long term, a diet restrictive of protein, alongside supplementation with essential amino acids is key. Medications to increase waste nitrogen excretion are also important[17]. Despite aggressive and prompt medical treatment, not all episodes of acute hyperammonaemia can be avoided, and the risk of neurological damage remains.

**LT:** LT offers a practical cure for UCDs as the metabolic defect is predominantly or exclusively within the liver. A long waiting list duration is associated with long-term risk of cognitive delay[18]. As such LT should be considered in children with UCD to prevent progressive neurologic injury and improve cognitive outcomes. Post-transplantation, patients are allowed a normal diet without taking nitrogen scavengers [19]. LT should be offered early to patients with severe UCDs, poorly controlled with medical interventions to prevent long term neurological damage. Living related transplantation offers the advantage of optimal timing after confirmation of the donor phenotype[20].

**Future research implications:** Allogenic hepatocyte transplantation has been shown to have a sustained partial correction of the metabolic defect in OTC and ASL deficiency patients[21,22]. Another promising treatment for UCD is gene therapy and has seen many years of preclinical evaluation, but concerns still remain around the safety of the application[23].

### **Maple syrup urine disease**

Maple syrup urine disease (MSUD) is an autosomal recessive disease, secondary to mutations in six gene loci where branched-chain alpha-ketoacid dehydrogenase complex is encoded. This results in the inability of the body to fully breakdown the essential amino acids valine, leucine and isoleucine. It has an estimated incidence of 1 in 185000 live births[24].

**Natural history and medical treatment:** There are five distinct clinical phenotypes of MSUD, without clear correlation of genotype-phenotype. Classic MSUD manifests in the neonatal period with delayed development, feeding difficulties, failure to thrive, opisthotonus, “bicycling” movements and maple syrup odor[25]. Metabolites accumulate and are excreted in the urine, sweat and ear cerumen, leading to the sweet odor of maple syrup. If left untreated, irreversible neurological damage and metabolic crisis occurs.

The most common medical treatment for patients with MSUD is dietary restriction of the affected amino acids, with supplementation[26]. Despite aggressive treatment, many patients will still experience episodes of metabolic decompensation during acute illness or stress, with risk of developing cerebral edema. Acute metabolic decompensation management includes effectively treating the underlying stressor, restricting protein intake, ample caloric support, supplementation with cofactors, elimination of toxic metabolites and correcting metabolic abnormalities[27].

**LT:** In patients with recurrent metabolic crises and high risk of cerebral edema, despite optimal medical treatment, LT should be considered[28]. LT is curative and significantly improves quality of life in children with MSUD. Patients can immediately cease protein-restricted diet and are safe from catabolic crisis[28]. Preexisting neuro-disability does not get reversed but LT offers neurological function stability and risk of cerebral edema is greatly reduced[29].

Domino transplantation where the explanted liver is used for another recipient without the underlying disease, has been used successfully in MSUD[30-32]. The new liver provides the metabolic protection in the MSUD patient, whilst the domino recipient has a normal systemic metabolism of branched amino acids and can counter the effects of an MSUD liver. This helps with organ allocation and diminishes the impact of the original transplant in the overall pool of organs[33].

**Future research implications:** Sodium phenylbutyrate (NaPBA) is commonly used for treatment in patients with UCD. In a cohort of 533 patients with UCD, Burrage *et al*[34] showed a reduction in branched chain amino acids and suggested follow up studies to investigate its utility in MSUD[34]. Studies are currently ongoing to assess its efficacy in MSUD patients.

Animal studies have shown encouraging therapeutic results using hepatocyte transplantation with partial metabolic correction of MSUD in a murine model[35]. Whilst promising, this intervention still warrants further clinical investigation.



### Wilson disease

Wilson disease (WD) is secondary to mutations of the gene *ATP7B* on chromosome 13, which codes for the transmembrane ATP7B transporter, involved in the transport of copper, incorporation of copper to the protein caeruloplasmin and excretion of excessive copper into bile. Excess copper in the liver leads to liver destruction, diffusion in to blood and eventually deposition in the other organs[36]. It is an autosomal recessive condition with a prevalence of 1 in 30000 people. An age-phenotypic presentation has been observed with hepatic presentations seen in the younger age groups (< 10 years: 83%, 10-18 years: 52%, > 18 years: 24%), whilst a neuropsychiatric presentation was more common in the older age groups (> 18 years: 74%, 10-18 years: 48%, < 10 years: 17%). The median age of presentation is 13.2 years (range 3–74 years), but children are rarely symptomatic before the age of 5 years[37].

**Natural history and medical treatment:** The clinical features in the pediatric population depend mainly on the predominant organ involved (liver and brain). The deposition of copper in various site of the body leads to the plethora of clinical presentations.

The majority of children present with liver disease, ranging from an asymptomatic rise in transaminases, acute hepatitis, acute liver failure, acute on chronic liver failure, chronic hepatitis, cirrhosis, fatty liver disease or malignancy[38]. It is important to remember that the finding of another cause of liver dysfunction such as acute viral hepatitis or non-alcoholic steatohepatitis, does not necessarily rule out Wilson's disease[39].

Up to 25% of children and adolescents present with acute or decompensated liver failure[40]. The presentation is similar to that of acute hepatitis, but the condition leads to rapid deterioration, with a high mortality. Symptoms include severe jaundice, Coombs- negative hemolytic anemia, deranged coagulation, ascites, encephalopathy and renal failure. Children present with very high serum bilirubin, rise in liver enzymes, low serum alkaline phosphatase and defective synthetic functions.

By the time children present with neurological symptoms, most already have liver disease, although may not be overtly symptomatic. Subtle signs may start from a young age such as deterioration of school performance or handwriting and dysarthria. Neurological signs tend to be wide-ranging and variable. Behavioral and psychological changes are very common in WD and make up for roughly one-third of presenting symptoms.

Medical therapy is mainly focused around the copper chelation. Main drugs currently in use include D penicillamine, trientine, zinc and ammonium tetrathiomolybdate. Treatment should be commenced as soon as the child is diagnosed, as untreated WD can be fatal. In patients with acute liver failure or advanced liver disease, LT is the only effective therapy.

**LT:** The liver disease is cured by LT and extra-hepatic symptoms generally improve after LT, particularly neurological signs. LT is the only option for patients with acute liver failure with encephalopathy secondary to WD. In children with liver dysfunction without encephalopathy, but are unresponsive to medical treatment, the indications are less clear. The Wilson Index is helpful in identifying children with decompensated liver failure, with a 93% sensitivity and 98% specificity[41].

**Future research implications:** Animal models have shown that restoration of 30%-50% of metabolic function may protect the rest of liver cells. This raises the possibility of gene therapy and hepatocyte transplantation as a potential therapeutic option in children with WD[42]. For patients with acute liver failure secondary to WD, hepatocyte transplantation may be used as transient support until chelation treatment shows its effect or as a definitive cure through repopulation of the liver by healthy donor cells as seen in animal models of WD[43].

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## LBMS IMPROVED BY LT

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### Methylmalonic acidemia and propionic acidemia

Methylmalonic acidemia (MMA) and propionic acidemia (PA) are the commonest forms of organic acidemias resulting from defective catabolism of the amino acids[44]. MMA is an autosomal recessive disorder secondary to the complete or partial deficiency of methylmalonyl-CoA mutase. MMA is also caused by several inborn errors of cobalamin or B12 metabolism. It is rare with an incidence of 1 in 80000 live births[45]. PA is also an autosomal recessive disorder due to a defect in the enzyme

propionyl-CoA carboxylase[46].

**Natural history and medical treatment:** The presentation can be divided into three categories: (1) Neonatal presentation with signs of sleepiness, encephalopathy, coma, hypotonia and hepatomegaly; (2) Infantile form with recurrent metabolic crisis and neurological changes; and (3) Chronic presentation with developmental delay, failure to thrive and recurrent infections[44]. Neurological signs include epilepsy, developmental delay and dystonia resulting from lesions in the basal ganglia. Methylmalonate is also nephrotoxic and can lead to progressive renal disease and end-stage renal failure by adolescence. Investigations in these patients show ketoacidosis, hyperammonaemia and hyperglycinemia. Urine organic acids will reveal propionyl-CoA derivative or methylmalonate.

Each metabolic crisis requires correction and maintenance therapy is dependent on dietary restriction of protein (low protein and high caloric diet with continuous overnight feeding), supplementation with amino acids, carnitine, metronidazole (reduce production of propionate in the gut) and cobalamin[47]. Intensive clinical management with aggressive treatment with dialysis and haemofiltration is often needed to minimize neurological sequelae. Despite early detection and maximal medical therapy, many children develop significant neurological, renal and cardiac complications.

**LT:** LT provides the deficient enzyme in MMA, but the overall biochemical defect is not entirely corrected as the enzyme is expressed in most cells in the body. Neurological and renal function may deteriorate further after LT in some MMA patients[48]. Kidney transplantation has had long-term success in reducing MMA levels and avoiding metabolic crisis in moderate forms of the disease[49]. In more severe forms, combined liver and kidney transplantation may reduce frequency of metabolic crises, severity of illness and probably decreases risk of further neurological deterioration, but does not abolish it entirely[45]. With that in mind, the indications for liver and/or kidney transplantation are still unclear in MMA.

The idea behind transplantation is to provide the deficient enzyme through the graft and correct renal failure if present. It is important to remember that transplantation does not cure MMA but may reduce the frequency of crisis and improve the child's quality of life. Pre-transplant assessment should include a thorough neurological assessment as transplantation does potentially have the risk of further neurological deterioration. Dietary restriction of protein should be continued as a precaution against future metabolic decompensation and late complications after transplantation [45].

In PA, LT also only partially corrects the metabolic defect[50]. However, the improvement seen appears to be more significant in PA compared to MMA—diet can usually be normalized, no further metabolic crises and neurocognitive function remains as it is pre-transplantation[51]. LT should be indicated in patients with recurrent metabolic crises despite optimal medical therapy, with a view of preventing further neurological deterioration and cardiac complications[50].

**Future therapies:** The role of new and novel treatments such as genetic modification, hepatocyte transplantation and chronic medical therapies remains uncertain[52].

### **Glycogen storage diseases**

Glycogen storage diseases (GSD) constitutes a group of mainly autosomal recessive metabolic disorders, caused by the accumulation of either an abnormal amount or type of glycogen. It has an incidence of 1 in 20000 to 40000 live births. Various enzymes of glycogen metabolism are potentially involved, with 12 types of GSD recognized – seven of which have an enzymatic defect in the liver. Types I, III, IV, VI and IX are associated with severe liver disease[53–55].

**Natural history and medical treatment:** Typically, it presents with fasting hypoglycemia, hepatomegaly and growth retardation. In the GSD type I, hepatocellular adenomas with risk of transformation to hepatocellular carcinoma has been found[55, 56], particularly in those with pre-existing adenomatous nodules. In GSD type III, some patients may progress to liver cirrhosis, whilst some develop hepatocellular carcinoma[54]. GSD IV patients have a variable phenotype and some develop liver cirrhosis and hepatocellular carcinoma early on. Extrahepatic manifestations such as renal dysfunction in GSD type 1, myopathy in GSD type III and IV may also be present. It is important to distinguish between subtypes for optimal management. Diagnosis is through enzyme assays in the liver other tissues and mutation analysis. Presence of PAS-positive glycogen staining in liver biopsy samples is useful in

confirming the diagnosis.

Treatment for liver GSD includes dietary changes and medical treatment when symptoms are not corrected by diet. In GSD type I, continuous overnight enteral drip-feeding is used to avoid fasting hypoglycemia and regular oral cornstarch intake is used for prolonged glucose release and have significantly improved metabolic control [57]. Other pharmacotherapy may be needed such as allopurinol for hyperuricaemia, angiotensin converting enzyme inhibitors for proteinuria and granulocyte-colony stimulating factor for neutropenia in GSD type Ib [58]. In patients with GSD types III, VI and IX, a high protein diet alongside uncooked cornstarch is standard therapy. Whilst metabolic control is generally successful with medical therapy, long-term complications still occur [15]. Adherence is also a common issue in children and adolescents and may not be tolerated in many, results in a higher rate of complications.

**LT:** In patients with very poor metabolic control despite optimal medical therapy, those with multiple recurrent adenomas with increasing size, progressive liver cirrhosis and/ or hepatic failure, LT should be considered. In children with GSD type IV, LT is generally the best option for treatment, particularly in those that develop liver cirrhosis [59]. Children with GSD are also living longer and despite medical treatment, many develop long-term complications. With the outcomes of LT improving, including better biochemical and clinical parameters, LT offers the potential to be both preventative and curative for patients with GSD.

Indications for LT in GSD can be summarized as: (1) Correction of LBMD when medical therapy is unsuccessful or impairs quality of life; (2) Cirrhosis and complications; and (3) Liver tumors such as adenoma and hepatocellular carcinoma.

LT corrects the enzymatic defect, but the extrahepatic manifestations often complicate post-transplantation management [59].

**Future therapies:** There has been limited experience with hepatocyte transplantation, but initial reports are positive [60,61]. Gene therapy has been developed in animal models, but there remains insufficient data for clinical trials [62].

### **Phenylketonuria**

Phenylketonuria (PKU) is a rare autosomal recessive condition secondary to mutations in the phenylalanine hydroxylase gene (PAH). This results in a deficiency of PAH, an enzyme in the liver that converts phenylalanine (Phe) to tyrosine. The incidence is roughly 1 in 10000 and does vary by ethnic group, being higher in Caucasians.

**Natural history and medical treatment:** The lack of this enzyme results in abnormally high levels of phenylalanine in the brain, causing intellectual problems, developmental delay and psychiatric issues. Universal newborn screening in most developed nations has led to early detection and significantly reduced the number of children with intellectual disability secondary to PKU. Despite ongoing and early treatment of patients with PKU, majority of patients will still have a lower intellectual ability compared to family members and suffer from mental health issues [63,64].

Medical therapy consists of restriction of phenylalanine intake and supplementation with phenylalanine-free amino acid mixtures to ensure adequate protein intake [65]. The diet needed is extremely restrictive and include mainly fruits, vegetables and low protein modified foods such as bread, rice and pasta [66]. Dietary treatment, when maintained in childhood and well into adulthood has been shown to result in markedly improved outcomes at a cognitive and psychiatric level for patients. However, adherence to this strict regime is not ideal, particularly in adolescents and in adulthood.

Dietary modification has evolved with the introduction of glycomacropeptides (GMP), which are proteins contained in “whey”. These contain very little phenylalanine, which makes them suitable for replacing amino acid substitutes. Compliance has been shown to be improved with GMP compared to traditional amino acid foods [67]. The medication sapropterin, a form of tetrahydrobiopterin cofactor of phenylalanine hydroxylase has a success rate of up to 55% in PKU patients [68]. Patients with milder form of disease are more likely to respond to this drug. Another recent pharmaceutical drug known as pegylated phenylalanine ammonia lyase or pegvaliase, an enzyme substitute therapy has been assessed in Phase 2 and Phase 3 clinical trials [69]. Over 24 mo, patients showed a 69% decrease in Phe levels from baseline but almost all patients had mild to severe adverse events [70].

**LT:** Whole liver LT is not thought to be acceptable in majority of patients and physicians due to the availability of non-surgical treatment options.

**Future therapies:** Gene therapy has been shown to be successful in mouse models but no studies have reported trials in patients yet[71]. A variation of gene therapy is gene-editing techniques (Crispr/Cas9 or TALENS) to repair common mutations or insert active gene into “safe” areas of the gene. The development of an expressive synthetic RNA for the *PAH* gene is in development, but not with human subjects[72].

Cell-based therapies including hepatocyte and stem cell transplantation have been considered viable alternatives[73]. One patient has received hepatocyte transplantation with temporary improvement in Phe levels[74].

## DILEMMAS AND CONTROVERSIES

The decision for transplantation in LBMDs remains a complex one. Whilst the distinctions between each group of LBMDs is relatively arbitrary and may overlap, the indication for LT is one that must be carefully considered.

In a disease process such as biliary atresia, the risk-benefit decision for LT may be relatively simple. In a child with failed portoenterostomy, with progressive liver disease and poor survival beyond 36 mo of life, LT offers long-term survival of over 80% in biliary atresia patients[75]. Therefore the risk/benefit decision is based on quantitative improved survival outcomes.

Indications for LT for LBMDs however, are based on a different set of paradigms. Some LBMDs result in progressive liver disease, leading to cirrhosis and liver failure, therefore making LT a life-saving procedure, whilst some LBMDs do not cause liver injury, but the toxic intermediary metabolites have significant extra-hepatic effects.

LT remains the mainstay of treatment for LBMDs causing life-threatening illness such as the neonatal form of the UCD OTC deficiency, primary hyperoxaluria and CNS type 1[6,76,77]. The enzymatic defects in these conditions are well documented and present with severe clinical phenotypes manifesting in life-threatening complications. LT offers a replacement for the hepatic enzymes, therefore providing a life-saving metabolic cure.

With improvement in the outcomes and reduced risks associated with LT, LT has become an attractive treatment strategy for a significant number of other LBMDs with a considerably more complicated phenotype and risk/benefit profile. The utility of LT as a life improving *vs* life saving treatment modality raises a number of important questions. This paradigm shift of improving quality of life as opposed to saving lives has dramatically changed the plethora of diseases for which LT may be considered appropriate therapy. The blurring of lines between standard medical therapy and more aggressive surgical intervention, increasingly poses complex decisions for the transplant community[78].

Furthermore, LBMDs are relatively rare, and a detailed understanding in to the natural progression/history is still lacking. There is also a diverse genotype and phenotype correlation for many of these rare disorders. The risk/benefit consideration is made even more complicated for a given individual as the inherent risks of a condition are not always well-defined.

## SURGICAL CONSIDERATIONS

As more children receive transplants for LBMDs, organ allocation is an important consideration. In the United States, the Pediatric end-stage liver disease score and Model for End Stage Liver Disease score are used to prioritize candidates for LT. These scoring systems are centered mainly on worsening biochemical parameters which progress with advancing liver failure. In many LBMDs, there is typically no evidence of progressive liver disease and as such predicting risk of which candidate is most likely to benefit for LT can be challenging. As we expand the indication for LT for metabolic conditions, the issue of organ allocation must also be addressed.

The issue of scarcity of donor organs has led to optimization of the available grafts through various surgical techniques such as reduction of an adult donor graft in children, particularly through split liver grafts[79], auxiliary transplantation and the use of heterozygous donors.



Auxiliary transplantation[80]—where the whole or partial left lobe of a living or deceased donor is transplanted in an orthotopic site whilst preserving the right lobe of the recipient[81] is increasingly being used (Figure 2). Whilst technically challenging, advantages are two fold; (1) It allows the native liver to continue functioning normally, aside from the enzymatic defect, serving as a safety net should the graft fail; and (2) It may serve as a bridge to gene therapy, a new and novel developing area of metabolic medicine. Despite the initial discouraging results with higher mortality and morbidity, more recent studies[82,83] from experienced centers have shown comparable outcomes to whole LT and successful weaning of immunosuppression with native liver regeneration[84]. Study by Sze *et al*[85], showed that of the 96 paediatric LT patients with LBMDs, 14 (13%) children had auxiliary transplantation. Of these, 11 children had noncirrhotic LBMDs (CNS type 1, OTC, familial hypercholesterolemia, propionic acidemia). Long term patient and graft survival was not statistically different to standard orthotopic LT at 1 and 10-years post-transplantation[85]. Cautious selection of patients for auxiliary transplantation is vital as LBMDs that lead to cirrhosis or produce abnormal enzymes or proteins such as primary hyperoxaluria should not be treated with auxiliary transplantation as the underlying abnormality results in disease progression[86].

Living related living transplant using relatives as donors has emerged as a solution to the scarcity of donor organs. In Japan, where there are no deceased donors, living related donor LT for metabolic disorders is a key option[87]. As described above, most metabolic disorders have an autosomal recessive inheritance pattern. Parents, who are obligate carriers of the recipient's disorders, become potentially heterozygous donors. Kasahara *et al*[20], conducted an extensive review from a Japanese multicenter registry of living related LT[20]. Among the patients transplanted for metabolic conditions, 95% of donors were parents who were carriers of the recipients' disorders. Indications for transplantation were WD in 30%, UCD in 29%, MMA in 10% and GSD 7.7%[88]. The outcome reported after using heterozygous donors was excellent with better long-term survival rate, especially in WD and UCD. Other studies have also demonstrated the safety of heterozygous donors for LT in LBMDs with excellent metabolic correction [89,90].

As previously discussed, LT for organic acidurias is not curative, but may improve quality of life. Combined liver and kidney transplantation can be considered in patients with MMA and PA with frequent metabolic decompensation episodes in spite of rigorous medical therapy, based on highly individualized criteria[47]. The experience with combined liver and kidney transplantation in this cohort of patients remains limited. In MMA patients specifically, it has become an effective treatment modality with favorable graft survival and short-term outcomes, and good survival rates[45,91,92]. Combined liver and kidney transplantation does not cure the disease, but leads to partial correction of the metabolic derangement and improvement in clinical features. Medical therapy is generally continued, although less stringent than pre-transplantation, in order to lower the risk of renal and neurological worsening [47]. Choice of immunosuppressive therapy that is renal-sparing is encouraged and neurological side effects from medication need to be carefully monitored[93].

## OUTCOMES AND PROGNOSIS

With LBMDs constituting roughly 15%-25% of LT in the pediatric population, it is important to consider the outcomes of these children. Single and multicenter studies have suggested that their outcomes are comparable if not better than those transplanted for decompensated cirrhosis or other forms of chronic liver disease with excellent survival rate of > 82% at 10 years[85,94] (Figure 3) (Graph data from King's College Hospital, 2009).

Some studies, however, have shown that chronic rejection is a common problem in LT for LBMDs, often leading to re-transplantation[85,95]. Re-transplantation is associated with higher morbidity and mortality. Immunosuppression regimes are important in maintaining long-term allograft health, but may also contribute to potentially serious complications over time.

Optimization of immunosuppression can be challenging and is not standardized[85, 96]. In children receiving LT for LBMDs, the optimal use of immunosuppressive agents is to achieve a balance between minimizing risks of allograft rejection and secondary toxicity[97]. Renal impairment specifically is frequently seen in these children. Thus, choosing an immunosuppressive agent with minimal nephrotoxic potential is important. The use of basiliximab, a chimeric anti-IL2 receptor antibody,

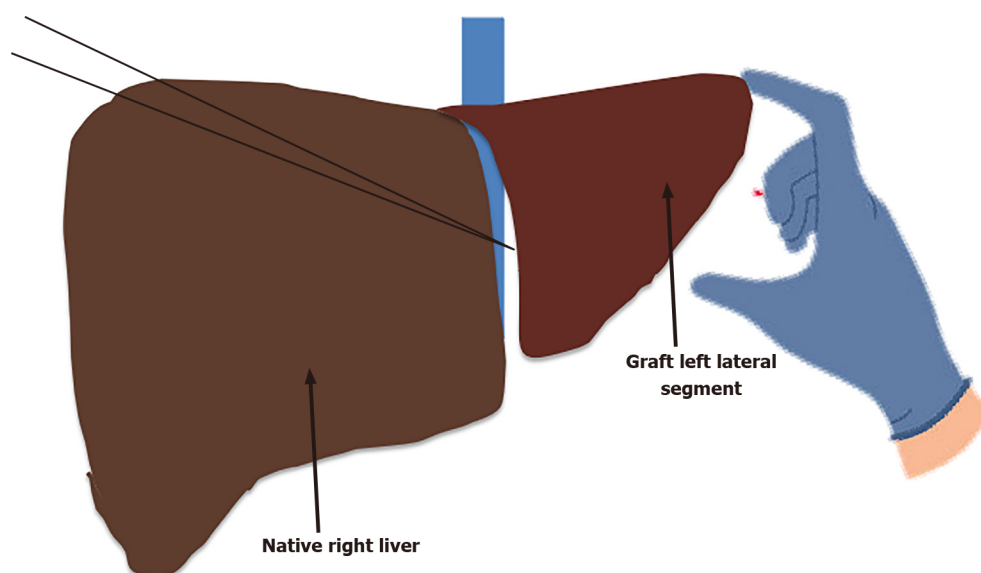


Figure 2 Auxiliary liver transplantation for select liver based metabolic disorders.

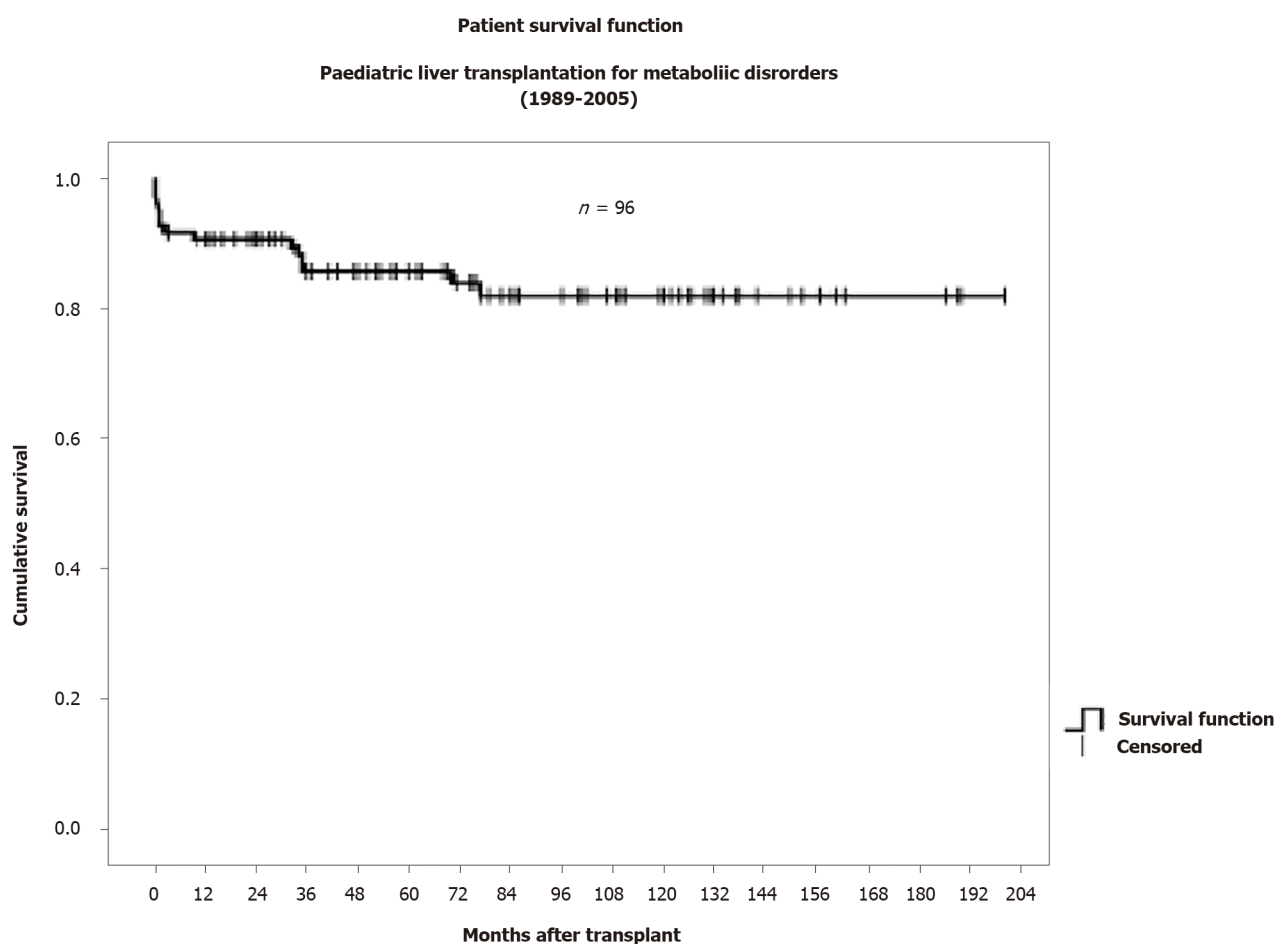


Figure 3 Patient survival for pediatric liver transplantation for metabolic disorders (data from King's College Hospital, London).

has been shown to be an effective renal-sparing agent with delayed entry and lower early target trough levels of calcineurin inhibitors (CNI) in children with renal impairment[98]. Mycophenolate mofetil (MMF) is also a CNI sparing agent, useful in children with CNI toxicity. Induction with monoclonal antibodies such as Basilixumab as an induction, followed by the use of MMF may be a helpful renal-sparing strategy in children with renal dysfunction.

The overall prognosis for children receiving transplantation for LBMDs must account for both allograft and extrahepatic complications. Meaningful survival in all pediatric LT recipients should be a state of complete physical, mental and social wellbeing[99]. Long-term management of children transplanted for LBMDs must include aspects such as growth and nutrition, neurological outcomes and psychosocial well-being.

## CONCLUSION

Pediatric LT has come leaps and bounds in the treatment of children with LBMDs. Where it has been previously viewed only as life-saving for some LBMDs, there is good reason to consider a shift in the utility of LT beyond metabolic rescue. It remains the gold standard for children with end stage liver disease. The success rate in most LBMDs is promising but the clinician plays a vital role in determining which patients are most suited for LT. The care pre- and post-transplantation is especially important. Pre-transplantation, identifying the most appropriate candidate for transplant will involve assessment of the severity of the primary disease, neurological status, and comorbidities which may affect transplant survival and ensuring that all alternative treatment modalities have been explored. It is important to remember that good metabolic control including ongoing dietary management and medical therapy supplements often results in better post-transplantation outcomes. A multidisciplinary network of professionals is key in the management of these children post LT, to ensure all aspects including growth and development, psychosocial well-being and nutrition are considered.

## REFERENCES

- 1 Venick RS, Farmer DG, Soto JR, Vargas J, Yersiz H, Kaldas FM, Agopian VG, Hiatt JR, McDiarmid SV, Busuttil RW. One Thousand Pediatric Liver Transplants During Thirty Years: Lessons Learned. *J Am Coll Surg* 2018; **226**: 355-366 [PMID: 29410290 DOI: 10.1016/j.jamcollsurg.2017.12.042]
- 2 Duffy JP, Kao K, Ko CY, Farmer DG, McDiarmid SV, Hong JC, Venick RS, Feist S, Goldstein L, Saab S, Hiatt JR, Busuttil RW. Long-term patient outcome and quality of life after liver transplantation: analysis of 20-year survivors. *Ann Surg* 2010; **252**: 652-661 [PMID: 20881772 DOI: 10.1097/SLA.0b013e3181f5f23a]
- 3 Arnon R, Annunziato RA, D'Amelio G, Chu J, Shneider BL. Liver Transplantation for Biliary Atresia: Is There a Difference in Outcome for Infants? *J Pediatr Gastroenterol Nutr* 2016; **62**: 220-225 [PMID: 26418214 DOI: 10.1097/MPG.0000000000000986]
- 4 Dhawan A, Lawlor MW, Mazariegos GV, McKiernan P, Squires JE, Strauss KA, Gupta D, James E, Prasad S. Disease burden of Crigler-Najjar syndrome: Systematic review and future perspectives. *J Gastroenterol Hepatol* 2020; **35**: 530-543 [PMID: 31495946 DOI: 10.1111/jgh.14853]
- 5 Ebrahimi A, Rahim F. Crigler-Najjar Syndrome: Current Perspectives and the Application of Clinical Genetics. *Endocr Metab Immune Disord Drug Targets* 2018; **18**: 201-211 [PMID: 29237388 DOI: 10.2174/1871530318666171213153130]
- 6 van der Veere CN, Sinaasappel M, McDonagh AF, Rosenthal P, Labruno P, Odièvre M, Fevery J, Otte JB, McClean P, Bürk G, Masakowski V, Sperl W, Mowat AP, Vergani GM, Heller K, Wilson JP, Shepherd R, Jansen PL. Current therapy for Crigler-Najjar syndrome type 1: report of a world registry. *Hepatology* 1996; **24**: 311-315 [PMID: 8690398 DOI: 10.1002/hep.510240205]
- 7 Van Der Veere CN, Schoemaker B, Bakker C, Van Der Meer R, Jansen PL, Elferink RP. Influence of dietary calcium phosphate on the disposition of bilirubin in rats with unconjugated hyperbilirubinemia. *Hepatology* 1996; **24**: 620-626 [PMID: 8781334 DOI: 10.1002/hep.510240326]
- 8 Nishioka T, Hafkamp AM, Havinga R, van Lierop PP, Velvis H, Verkade HJ. Orlistat treatment increases fecal bilirubin excretion and decreases plasma bilirubin concentrations in hyperbilirubinemic Gunn rats. *J Pediatr* 2003; **143**: 327-334 [PMID: 14517515 DOI: 10.1067/s0022-3476(03)00298-1]
- 9 Schauer R, Stangl M, Lang T, Zimmermann A, Chouker A, Gerbes AL, Schildberg FW, Rau HG. Treatment of Crigler-Najjar type 1 disease: relevance of early liver transplantation. *J Pediatr Surg* 2003; **38**: 1227-1231 [PMID: 12891498 DOI: 10.1016/s0022-3468(03)00273-2]
- 10 Ambrosino G, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, Caenazzo L, Basso S, Carraro P, Valente ML, D'Amico D, Zancan L, D'Antiga L. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. *Cell Transplant* 2005; **14**: 151-157 [PMID: 15881424 DOI: 10.3727/000000005783983250]
- 11 Jorns C, Nowak G, Nemeth A, Zemack H, Mörk LM, Johansson H, Gramignoli R, Watanabe M, Karadagi A, Alheim M, Hauzenberger D, van Dijk R, Bosma PJ, Ebbesen F, Szakos A, Fischler B, Strom S, Ellis E, Ericzon BG. De Novo Donor-Specific HLA Antibody Formation in Two Patients

- With Crigler-Najjar Syndrome Type I Following Human Hepatocyte Transplantation With Partial Hepatectomy Preconditioning. *Am J Transplant* 2016; **16**: 1021-1030 [PMID: [26523372](#) DOI: [10.1111/ajt.13487](#)]
- 12 **Spitzhorn LS**, Kordes C, Megges M, Sawitza I, Götze S, Reichert D, Schulze-Matz P, Graffmann N, Bohndorf M, Wruck W, Köhler JP, Herebian D, Mayatepek E, Oreffo ROC, Häussinger D, Adjaye J. Transplanted Human Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Support Liver Regeneration in Gunn Rats. *Stem Cells Dev* 2018; **27**: 1702-1714 [PMID: [30280963](#) DOI: [10.1089/scd.2018.0010](#)]
  - 13 **Seminara J**, Tuchman M, Krivitzy L, Krischer J, Lee HS, Lemons C, Baumgartner M, Cederbaum S, Diaz GA, Feigenbaum A, Gallagher RC, Harding CO, Kerr DS, Lanpher B, Lee B, Lichter-Konecki U, McCandless SE, Merritt JL, Oster-Granite ML, Seashore MR, Stricker T, Summar M, Waisbren S, Yudkoff M, Batshaw ML. Establishing a consortium for the study of rare diseases: The Urea Cycle Disorders Consortium. *Mol Genet Metab* 2010; **100** Suppl 1: S97-105 [PMID: [20188616](#) DOI: [10.1016/j.ymgme.2010.01.014](#)]
  - 14 **Stone WL**, Basit H, Jaishankar GB. Urea Cycle Disorders. 2021 Aug 11. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan– [PMID: [29493985](#)]
  - 15 **Mazariegos G**, Shneider B, Burton B, Fox IJ, Hadzic N, Kishnani P, Morton DH, McIntire S, Sokol RJ, Summar M, White D, Chavanon V, Vockley J. Liver transplantation for pediatric metabolic disease. *Mol Genet Metab* 2014; **111**: 418-427 [PMID: [24495602](#) DOI: [10.1016/j.ymgme.2014.01.006](#)]
  - 16 **Matsumoto S**, Häberle J, Kido J, Mitsubuchi H, Endo F, Nakamura K. Urea cycle disorders-update. *J Hum Genet* 2019; **64**: 833-847 [PMID: [31110235](#) DOI: [10.1038/s10038-019-0614-4](#)]
  - 17 **Häberle J**, Burlina A, Chakrapani A, Dixon M, Karall D, Lindner M, Mandel H, Martinelli D, Pintos-Morell G, Santer R, Skouma A, Servais A, Tal G, Rubio V, Huemer M, Dionisi-Vici C. Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision. *J Inher Metab Dis* 2019; **42**: 1192-1230 [PMID: [30982989](#) DOI: [10.1002/jimd.12100](#)]
  - 18 **Ziogas IA**, Wu WK, Matsuoka LK, Pai AK, Hafberg ET, Gillis LA, Morgan TM, Alexopoulos SP. Liver Transplantation in Children with Urea Cycle Disorders: The Importance of Minimizing Waiting Time. *Liver Transpl* 2021 [PMID: [34058057](#) DOI: [10.1002/lt.26186](#)]
  - 19 **Kim IK**, Niemi AK, Krueger C, Bonham CA, Concepcion W, Cowan TM, Enns GM, Esquivel CO. Liver transplantation for urea cycle disorders in pediatric patients: a single-center experience. *Pediatr Transplant* 2013; **17**: 158-167 [PMID: [23347504](#) DOI: [10.1111/petr.12041](#)]
  - 20 **Kasahara M**, Sakamoto S, Horikawa R, Koji U, Mizuta K, Shinkai M, Takahito Y, Taguchi T, Inomata Y, Uemoto S, Tatsuo K, Kato S. Living donor liver transplantation for pediatric patients with metabolic disorders: the Japanese multicenter registry. *Pediatr Transplant* 2014; **18**: 6-15 [PMID: [24283623](#) DOI: [10.1111/petr.12196](#)]
  - 21 **Stéphenne X**, Najimi M, Sibille C, Nassogne MC, Smets F, Sokal EM. Sustained engraftment and tissue enzyme activity after liver cell transplantation for argininosuccinate lyase deficiency. *Gastroenterology* 2006; **130**: 1317-1323 [PMID: [16618422](#) DOI: [10.1053/j.gastro.2006.01.008](#)]
  - 22 **Stéphenne X**, Najimi M, Smets F, Reding R, de Ville de Goyet J, Sokal EM. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. *Am J Transplant* 2005; **5**: 2058-2061 [PMID: [15996260](#) DOI: [10.1111/j.1600-6143.2005.00935.x](#)]
  - 23 **Raper SE**, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP, Wilson JM, Batshaw ML. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab* 2003; **80**: 148-158 [PMID: [14567964](#) DOI: [10.1016/j.ymgme.2003.08.016](#)]
  - 24 **Edelmann L**, Wasserstein MP, Kornreich R, Sansaricq C, Snyderman SE, Diaz GA. Maple syrup urine disease: identification and carrier-frequency determination of a novel founder mutation in the Ashkenazi Jewish population. *Am J Hum Genet* 2001; **69**: 863-868 [PMID: [11509994](#) DOI: [10.1086/323677](#)]
  - 25 **Blackburn PR**, Gass JM, Vairo FPE, Farnham KM, Atwal HK, Macklin S, Klee EW, Atwal PS. Maple syrup urine disease: mechanisms and management. *Appl Clin Genet* 2017; **10**: 57-66 [PMID: [28919799](#) DOI: [10.2147/TACG.S125962](#)]
  - 26 **Morton DH**, Strauss KA, Robinson DL, Puffenberger EG, Kelley RI. Diagnosis and treatment of maple syrup disease: a study of 36 patients. *Pediatrics* 2002; **109**: 999-1008 [PMID: [12042535](#) DOI: [10.1542/peds.109.6.999](#)]
  - 27 **Hassan SA**, Gupta V. Maple Syrup Urine Disease. 2021 Feb 6. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan– [PMID: [32491705](#)]
  - 28 **Strauss KA**, Mazariegos GV, Sindhi R, Squires R, Finegold DN, Vockley G, Robinson DL, Hendrickson C, Virji M, Cropcho L, Puffenberger EG, McGhee W, Seward LM, Morton DH. Elective liver transplantation for the treatment of classical maple syrup urine disease. *Am J Transplant* 2006; **6**: 557-564 [PMID: [16468966](#) DOI: [10.1111/j.1600-6143.2005.01209.x](#)]
  - 29 **Mazariegos GV**, Morton DH, Sindhi R, Soltys K, Nayyar N, Bond G, Shellmer D, Shneider B, Vockley J, Strauss KA. Liver transplantation for classical maple syrup urine disease: long-term follow-up in 37 patients and comparative United Network for Organ Sharing experience. *J Pediatr* 2012; **160**: 116-21.e1 [PMID: [21839471](#) DOI: [10.1016/j.jpeds.2011.06.033](#)]
  - 30 **Khanna A**, Hart M, Nyhan WL, Hassanein T, Panyard-Davis J, Barshop BA. Domino liver transplantation in maple syrup urine disease. *Liver Transpl* 2006; **12**: 876-882 [PMID: [16628687](#)]



- DOI: [10.1002/lt.20744](https://doi.org/10.1002/lt.20744)]
- 31 **Badell IR**, Hanish SI, Hughes CB, Hewitt WR, Chung RT, Spivey JR, Knechtle SJ. Domino liver transplantation in maple syrup urine disease: a case report and review of the literature. *Transplant Proc* 2013; **45**: 806-809 [PMID: [23267808](https://pubmed.ncbi.nlm.nih.gov/23267808/) DOI: [10.1016/j.transproceed.2012.04.031](https://doi.org/10.1016/j.transproceed.2012.04.031)]
  - 32 **Herden U**, Li J, Fischer L, Brinkert F, Blohm M, Santer R, Nashan B, Grabhorn E. The first case of domino-split-liver transplantation in maple syrup urine disease. *Pediatr Transplant* 2017; **21** [PMID: [28580726](https://pubmed.ncbi.nlm.nih.gov/28580726/) DOI: [10.1111/ptr.12993](https://doi.org/10.1111/ptr.12993)]
  - 33 **Celik N**, Squires JE, Soltys K, Vockley J, Shellmer DA, Chang W, Strauss K, McKiernan P, Ganoza A, Sindhi R, Bond G, Mazariegos G, Khanna A. Domino liver transplantation for select metabolic disorders: Expanding the living donor pool. *JIMD Rep* 2019; **48**: 83-89 [PMID: [31392117](https://pubmed.ncbi.nlm.nih.gov/31392117/) DOI: [10.1002/jmd2.12053](https://doi.org/10.1002/jmd2.12053)]
  - 34 **Burrage LC**, Jain M, Gandolfo L, Lee BH; Members of the Urea Cycle Disorders Consortium, Nagamani SC. Sodium phenylbutyrate decreases plasma branched-chain amino acids in patients with urea cycle disorders. *Mol Genet Metab* 2014; **113**: 131-135 [PMID: [25042691](https://pubmed.ncbi.nlm.nih.gov/25042691/) DOI: [10.1016/j.ymgme.2014.06.005](https://doi.org/10.1016/j.ymgme.2014.06.005)]
  - 35 **Skvorak KJ**, Paul HS, Dorko K, Marongiu F, Ellis E, Chace D, Ferguson C, Gibson KM, Homanics GE, Strom SC. Hepatocyte transplantation improves phenotype and extends survival in a murine model of intermediate maple syrup urine disease. *Mol Ther* 2009; **17**: 1266-1273 [PMID: [19436271](https://pubmed.ncbi.nlm.nih.gov/19436271/) DOI: [10.1038/mt.2009.99](https://doi.org/10.1038/mt.2009.99)]
  - 36 **Fernando M**, van Mourik I, Wassmer E, Kelly D. Wilson disease in children and adolescents. *Arch Dis Child* 2020; **105**: 499-505 [PMID: [31974298](https://pubmed.ncbi.nlm.nih.gov/31974298/) DOI: [10.1136/archdischild-2018-315705](https://doi.org/10.1136/archdischild-2018-315705)]
  - 37 **Lin LJ**, Wang DX, Ding NN, Lin Y, Jin Y, Zheng CQ. Comprehensive analysis on clinical features of Wilson's disease: an experience over 28 years with 133 cases. *Neurol Res* 2014; **36**: 157-163 [PMID: [24107488](https://pubmed.ncbi.nlm.nih.gov/24107488/) DOI: [10.1179/1743132813Y.0000000262](https://doi.org/10.1179/1743132813Y.0000000262)]
  - 38 **Sternlieb I**. Wilson's disease: indications for liver transplants. *Hepatology* 1984; **4**: 15S-17S [PMID: [6363255](https://pubmed.ncbi.nlm.nih.gov/6363255/) DOI: [10.1002/hep.1840040706](https://doi.org/10.1002/hep.1840040706)]
  - 39 **Roberts EA**, Yap J. Nonalcoholic Fatty Liver Disease (NAFLD): Approach in the Adolescent Patient. *Curr Treat Options Gastroenterol* 2006; **9**: 423-431 [PMID: [16942668](https://pubmed.ncbi.nlm.nih.gov/16942668/) DOI: [10.1007/BF02738532](https://doi.org/10.1007/BF02738532)]
  - 40 **Pandit A**, Bavdekar A, Bhawe S. Wilson's disease. *Indian J Pediatr* 2002; **69**: 785-791 [PMID: [12420912](https://pubmed.ncbi.nlm.nih.gov/12420912/) DOI: [10.1007/BF02723693](https://doi.org/10.1007/BF02723693)]
  - 41 **Dhawan A**, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. *Liver Transpl* 2005; **11**: 441-448 [PMID: [15776453](https://pubmed.ncbi.nlm.nih.gov/15776453/) DOI: [10.1002/lt.20352](https://doi.org/10.1002/lt.20352)]
  - 42 **Irani AN**, Malhi H, Slehria S, Gorla GR, Vollenberg I, Schilsky ML, Gupta S. Correction of liver disease following transplantation of normal rat hepatocytes into Long-Evans Cinnamon rats modeling Wilson's disease. *Mol Ther* 2001; **3**: 302-309 [PMID: [11273771](https://pubmed.ncbi.nlm.nih.gov/11273771/) DOI: [10.1006/mthe.2001.0271](https://doi.org/10.1006/mthe.2001.0271)]
  - 43 **Filippi C**, Dhawan A. Current status of human hepatocyte transplantation and its potential for Wilson's disease. *Ann N Y Acad Sci* 2014; **1315**: 50-55 [PMID: [24605914](https://pubmed.ncbi.nlm.nih.gov/24605914/) DOI: [10.1111/nyas.12386](https://doi.org/10.1111/nyas.12386)]
  - 44 **Deodato F**, Boenzi S, Santorelli FM, Dionisi-Vici C. Methylmalonic and propionic aciduria. *Am J Med Genet C Semin Med Genet* 2006; **142C**: 104-112 [PMID: [16602092](https://pubmed.ncbi.nlm.nih.gov/16602092/) DOI: [10.1002/ajmg.c.30090](https://doi.org/10.1002/ajmg.c.30090)]
  - 45 **Kasahara M**, Horikawa R, Tagawa M, Uemoto S, Yokoyama S, Shibata Y, Kawano T, Kuroda T, Honna T, Tanaka K, Saeki M. Current role of liver transplantation for methylmalonic acidemia: a review of the literature. *Pediatr Transplant* 2006; **10**: 943-947 [PMID: [17096763](https://pubmed.ncbi.nlm.nih.gov/17096763/)]
  - 46 **Sass JO**, Hofmann M, Skladal D, Mayatepek E, Schwahn B, Sperl W. Propionic acidemia revisited: a workshop report. *Clin Pediatr (Phila)* 2004; **43**: 837-843 [PMID: [15583780](https://pubmed.ncbi.nlm.nih.gov/15583780/) DOI: [10.1177/000992280404300908](https://doi.org/10.1177/000992280404300908)]
  - 47 **Baumgartner MR**, Hörster F, Dionisi-Vici C, Haliloglu G, Karall D, Chapman KA, Huemer M, Hochuli M, Assoun M, Ballhausen D, Burlina A, Fowler B, Grünert SC, Grünewald S, Honzik T, Merinero B, Pérez-Cerdá C, Scholl-Bürgi S, Skovby F, Wijburg F, MacDonald A, Martinelli D, Sass JO, Valayannopoulos V, Chakrapani A. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. *Orphanet J Rare Dis* 2014; **9**: 130 [PMID: [25205257](https://pubmed.ncbi.nlm.nih.gov/25205257/) DOI: [10.1186/s13023-014-0130-8](https://doi.org/10.1186/s13023-014-0130-8)]
  - 48 **Leonard JV**, Walter JH, McKiernan PJ. The management of organic acidemias: the role of transplantation. *J Inherit Metab Dis* 2001; **24**: 309-311 [PMID: [11405351](https://pubmed.ncbi.nlm.nih.gov/11405351/) DOI: [10.1023/a:1010395724012](https://doi.org/10.1023/a:1010395724012)]
  - 49 **Lubrano R**, Elli M, Rossi M, Travasso E, Raggi C, Barsotti P, Carducci C, Berloco P. Renal transplant in methylmalonic acidemia: could it be the best option? *Pediatr Nephrol* 2007; **22**: 1209-1214 [PMID: [17401587](https://pubmed.ncbi.nlm.nih.gov/17401587/) DOI: [10.1007/s00467-007-0460-z](https://doi.org/10.1007/s00467-007-0460-z)]
  - 50 **Barshes NR**, Vanatta JM, Patel AJ, Carter BA, O'Mahony CA, Karpen SJ, Goss JA. Evaluation and management of patients with propionic acidemia undergoing liver transplantation: a comprehensive review. *Pediatr Transplant* 2006; **10**: 773-781 [PMID: [17032422](https://pubmed.ncbi.nlm.nih.gov/17032422/) DOI: [10.1111/j.1399-3046.2006.00569.x](https://doi.org/10.1111/j.1399-3046.2006.00569.x)]
  - 51 **Schlenzig JS**, Poggi-Travert F, Laurent J, Rabier D, Jan D, Wendel U, Sewell AC, Revillon Y, Kamoun P, Saudubray JM. Liver transplantation in two cases of propionic acidemia. *J Inherit Metab Dis* 1995; **18**: 448-461 [PMID: [7494403](https://pubmed.ncbi.nlm.nih.gov/7494403/) DOI: [10.1007/BF00710056](https://doi.org/10.1007/BF00710056)]
  - 52 **Chandler RJ**, Chandrasekaran S, Carrillo-Carrasco N, Senac JS, Hofherr SE, Barry MA, Venditti CP. Adeno-associated virus serotype 8 gene transfer rescues a neonatal lethal murine model of propionic acidemia. *Hum Gene Ther* 2011; **22**: 477-481 [PMID: [20950151](https://pubmed.ncbi.nlm.nih.gov/20950151/) DOI: [10.1007/s12017-011-9151-1](https://doi.org/10.1007/s12017-011-9151-1)]

- 10.1089/hum.2010.164]
- 53 **Manzia TM**, Angelico R, Toti L, Cillis A, Ciano P, Orlando G, Anselmo A, Angelico M, Tisone G. Glycogen storage disease type Ia and VI associated with hepatocellular carcinoma: two case reports. *Transplant Proc* 2011; **43**: 1181-1183 [PMID: [21620082](#) DOI: [10.1016/j.transproceed.2011.01.129](#)]
  - 54 **Kishnani PS**, Austin SL, Arn P, Bali DS, Boney A, Case LE, Chung WK, Desai DM, El-Gharbawy A, Haller R, Smit GP, Smith AD, Hobson-Webb LD, Wechsler SB, Weinstein DA, Watson MS; ACMG. Glycogen storage disease type III diagnosis and management guidelines. *Genet Med* 2010; **12**: 446-463 [PMID: [20631546](#) DOI: [10.1097/GIM.0b013e3181e655b6](#)]
  - 55 **Koeberl DD**, Kishnani PS, Chen YT. Glycogen storage disease types I and II: treatment updates. *J Inherit Metab Dis* 2007; **30**: 159-164 [PMID: [17308886](#) DOI: [10.1007/s10545-007-0519-9](#)]
  - 56 **Yuen WY**, Quak SH, Aw MM, Karthik SV. Long-term outcome after liver transplantation in children with type I glycogen storage disease. *Pediatr Transplant* 2021; **25**: e13872 [PMID: [33044776](#) DOI: [10.1111/ptr.13872](#)]
  - 57 **Parikh NS**, Ahlawat R. Glycogen Storage Disease Type I. 2020 Aug 12. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. [PMID: [30480935](#)]
  - 58 **Melis D**, Fulceri R, Parenti G, Marcolongo P, Gatti R, Parini R, Riva E, Della Casa R, Zammarchi E, Andria G, Benedetti A. Genotype/phenotype correlation in glycogen storage disease type 1b: a multicentre study and review of the literature. *Eur J Pediatr* 2005; **164**: 501-508 [PMID: [15906092](#) DOI: [10.1007/s00431-005-1657-4](#)]
  - 59 **Davis MK**, Weinstein DA. Liver transplantation in children with glycogen storage disease: controversies and evaluation of the risk/benefit of this procedure. *Pediatr Transplant* 2008; **12**: 137-145 [PMID: [18307661](#) DOI: [10.1111/j.1399-3046.2007.00803.x](#)]
  - 60 **Muraca M**, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, Meroni M, Giron G, Burlina AB. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. *Lancet* 2002; **359**: 317-318 [PMID: [11830200](#) DOI: [10.1016/S0140-6736\(02\)07529-3](#)]
  - 61 **Lee KW**, Lee JH, Shin SW, Kim SJ, Joh JW, Lee DH, Kim JW, Park HY, Lee SY, Lee HH, Park JW, Kim SY, Yoon HH, Jung DH, Choe YH, Lee SK. Hepatocyte transplantation for glycogen storage disease type 1b. *Cell Transplant* 2007; **16**: 629-637 [PMID: [17912954](#) DOI: [10.3727/000000007783465019](#)]
  - 62 **Salabarria SM**, Nair J, Clement N, Smith BK, Raben N, Fuller DD, Byrne BJ, Corti M. Advancements in AAV-mediated Gene Therapy for Pompe Disease. *J Neuromuscul Dis* 2020; **7**: 15-31 [PMID: [31796685](#) DOI: [10.3233/JND-190426](#)]
  - 63 **Waisbren SE**, Azen C. Cognitive and behavioral development in maternal phenylketonuria offspring. *Pediatrics* 2003; **112**: 1544-1547 [PMID: [14654662](#)]
  - 64 **Waisbren SE**, Noel K, Fahrback K, Cella C, Frame D, Dorenbaum A, Levy H. Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. *Mol Genet Metab* 2007; **92**: 63-70 [PMID: [17591452](#) DOI: [10.1016/j.ymgme.2007.05.006](#)]
  - 65 **MacDonald A**, Rocha JC, van Rijn M, Feillet F. Nutrition in phenylketonuria. *Mol Genet Metab* 2011; **104** Suppl: S10-S18 [PMID: [21944460](#) DOI: [10.1016/j.ymgme.2011.08.023](#)]
  - 66 **Jameson E**, Remington T. Dietary interventions for phenylketonuria. *Cochrane Database Syst Rev* 2020; **7**: CD001304 [PMID: [32672365](#) DOI: [10.1002/14651858.CD001304.pub3](#)]
  - 67 **Ney DM**, Stroup BM, Clayton MK, Murali SG, Rice GM, Rohr F, Levy HL. Glycomacropeptide for nutritional management of phenylketonuria: a randomized, controlled, crossover trial. *Am J Clin Nutr* 2016; **104**: 334-345 [PMID: [27413125](#) DOI: [10.3945/ajcn.116.135293](#)]
  - 68 **Qu J**, Yang T, Wang E, Li M, Chen C, Ma L, Zhou Y, Cui Y. Efficacy and safety of sapropterin dihydrochloride in patients with phenylketonuria: A meta-analysis of randomized controlled trials. *Br J Clin Pharmacol* 2019; **85**: 893-899 [PMID: [30720885](#) DOI: [10.1111/bcp.13886](#)]
  - 69 **Thomas J**, Levy H, Amato S, Vockley J, Zori R, Dimmock D, Harding CO, Bilder DA, Weng HH, Olbertz J, Merilainen M, Jiang J, Larimore K, Gupta S, Gu Z, Northrup H; PRISM investigators. Pegvaliase for the treatment of phenylketonuria: Results of a long-term phase 3 clinical trial program (PRISM). *Mol Genet Metab* 2018; **124**: 27-38 [PMID: [29653686](#) DOI: [10.1016/j.ymgme.2018.03.006](#)]
  - 70 **Longo N**, Zori R, Wasserstein MP, Vockley J, Burton BK, Decker C, Li M, Lau K, Jiang J, Larimore K, Thomas JA. Long-term safety and efficacy of pegvaliase for the treatment of phenylketonuria in adults: combined phase 2 outcomes through PAL-003 extension study. *Orphanet J Rare Dis* 2018; **13**: 108 [PMID: [29973227](#) DOI: [10.1186/s13023-018-0858-7](#)]
  - 71 **Harding CO**, Gillingham MB, Hamman K, Clark H, Goebel-Daghighi E, Bird A, Koeberl DD. Complete correction of hyperphenylalaninemia following liver-directed, recombinant AAV2/8 vector-mediated gene therapy in murine phenylketonuria. *Gene Ther* 2006; **13**: 457-462 [PMID: [16319949](#) DOI: [10.1038/sj.gt.3302678](#)]
  - 72 **Lichter-Konecki U**, Vockley J. Phenylketonuria: Current Treatments and Future Developments. *Drugs* 2019; **79**: 495-500 [PMID: [30864096](#) DOI: [10.1007/s40265-019-01079-z](#)]
  - 73 **Harding C**. Progress toward cell-directed therapy for phenylketonuria. *Clin Genet* 2008; **74**: 97-104 [PMID: [18498375](#) DOI: [10.1111/j.1399-0004.2008.01027.x](#)]
  - 74 **Stéphenne X**, Debray FG, Smets F, Jazouli N, Sana G, Tondreau T, Menten R, Goffette P, Boemer F, Schoos R, Gersting SW, Najimi M, Muntau AC, Goyens P, Sokal EM. Hepatocyte transplantation using the domino concept in a child with tetrahydropterin nonresponsive phenylketonuria. *Cell Transplant* 2012; **21**: 2765-2770 [PMID: [22889463](#) DOI: [10.3727/096368912X653255](#)]
  - 75 **Diem HV**, Evrard V, Vinh HT, Sokal EM, Janssen M, Otte JB, Reding R. Pediatric liver

- transplantation for biliary atresia: results of primary grafts in 328 recipients. *Transplantation* 2003; **75**: 1692-1697 [PMID: [12777858](#) DOI: [10.1097/01.TP.0000062570.83203.A3](#)]
- 76 **Morioka D**, Kasahara M, Takada Y, Shirouzu Y, Taira K, Sakamoto S, Uryuhara K, Egawa H, Shimada H, Tanaka K. Current role of liver transplantation for the treatment of urea cycle disorders: a review of the worldwide English literature and 13 cases at Kyoto University. *Liver Transpl* 2005; **11**: 1332-1342 [PMID: [16237708](#) DOI: [10.1002/lt.20587](#)]
- 77 **Bergstrahl EJ**, Monico CG, Lieske JC, Herges RM, Langman CB, Hoppe B, Milliner DS; IPHR Investigators. Transplantation outcomes in primary hyperoxaluria. *Am J Transplant* 2010; **10**: 2493-2501 [PMID: [20849551](#) DOI: [10.1111/j.1600-6143.2010.03271.x](#)]
- 78 **Shneider BL**, Vockley J, Mazariegos GV. Trading places: liver transplantation as a treatment, not a cure, for metabolic liver disease. *Liver Transpl* 2011; **17**: 628-630 [PMID: [21384526](#) DOI: [10.1002/lt.22293](#)]
- 79 **Cherukuru R**, Reddy MS, Shanmugam NP, Rajalingam R, Kota V, Gunasekaran V, Narasimhan G, Kaliamoorthy I, Rela M. Feasibility and Safety of Split-Liver Transplantation in a Nascent Framework of Deceased Donation. *Liver Transpl* 2019; **25**: 450-458 [PMID: [30586233](#) DOI: [10.1002/lt.25405](#)]
- 80 **Rammohan A**, Reddy MS, Narasimhan G, Rajalingam R, Kaliamoorthy I, Shanmugam N, Rela M. Auxiliary Partial Orthotopic Liver Transplantation for Selected Noncirrhotic Metabolic Liver Disease. *Liver Transpl* 2019; **25**: 111-118 [PMID: [30317682](#) DOI: [10.1002/lt.25352](#)]
- 81 **Terpstra OT**, Schalm SW, Reuvers CB, Baumgartner D, Groenland TH, ten Kate FJ, Stibbe J, Terpstra JL, Weimar W, Willemse PJ. The role of auxiliary liver transplantation. *Transplant Proc* 1987; **19**: 4370-4372 [PMID: [3314045](#)]
- 82 **Faraj W**, Dar F, Bartlett A, Melendez HV, Marangoni G, Mukherji D, Vergani GM, Dhawan A, Heaton N, Rela M. Auxiliary liver transplantation for acute liver failure in children. *Ann Surg* 2010; **251**: 351-356 [PMID: [20054274](#) DOI: [10.1097/SLA.0b013e3181bdfef6](#)]
- 83 **Weiner J**, Griesemer A, Island E, Lobritto S, Martinez M, Selvaggi G, Lefkowitz J, Velasco M, Tryphonopoulos P, Emond J, Tzakis A, Kato T. Longterm outcomes of auxiliary partial orthotopic liver transplantation in preadolescent children with fulminant hepatic failure. *Liver Transpl* 2016; **22**: 485-494 [PMID: [26479577](#) DOI: [10.1002/lt.24361](#)]
- 84 **Kato T**, Selvaggi G, Levi D, Hernandez E, Takahashi H, Velasco M, Moon J, Nishida S, Thompson J, Ruiz P, Sfakianakis G, Tzakis A. Routine use of auxiliary partial orthotopic liver transplantation for children with fulminant hepatic failure: Preliminary report. *Transplant Proc* 2006; **38**: 3607-3608 [PMID: [17175345](#) DOI: [10.1016/j.transproceed.2006.10.038](#)]
- 85 **Sze YK**, Dhawan A, Taylor RM, Bansal S, Mieli-Vergani G, Rela M, Heaton N. Pediatric liver transplantation for metabolic liver disease: experience at King's College Hospital. *Transplantation* 2009; **87**: 87-93 [PMID: [19136896](#) DOI: [10.1097/TP.0b013e31818bc0c4](#)]
- 86 **Ciria R**, Davila D, Heaton N. Auxiliary liver transplantation in children. *Curr Opin Organ Transplant* 2011; **16**: 489-493 [PMID: [21897245](#) DOI: [10.1097/MOT.0b013e32834a94cf](#)]
- 87 **Oishi K**, Arnon R, Wasserstein MP, Diaz GA. Liver transplantation for pediatric inherited metabolic disorders: Considerations for indications, complications, and perioperative management. *Pediatr Transplant* 2016; **20**: 756-769 [PMID: [27329540](#) DOI: [10.1111/petr.12741](#)]
- 88 **Morioka D**, Kasahara M, Takada Y, Corrales JP, Yoshizawa A, Sakamoto S, Taira K, Yoshitoshi EY, Egawa H, Shimada H, Tanaka K. Living donor liver transplantation for pediatric patients with inheritable metabolic disorders. *Am J Transplant* 2005; **5**: 2754-2763 [PMID: [16212637](#) DOI: [10.1111/j.1600-6143.2005.01084.x](#)]
- 89 **Kim JS**, Kim KM, Oh SH, Kim HJ, Cho JM, Yoo HW, Namgoong JM, Kim DY, Kim KH, Hwang S, Lee SG. Liver transplantation for metabolic liver disease: experience at a living donor dominant liver transplantation center. *Pediatr Gastroenterol Hepatol Nutr* 2015; **18**: 48-54 [PMID: [25866733](#) DOI: [10.5223/pghn.2015.18.1.48](#)]
- 90 **Sood V**, Squires JE, Mazariegos GV, Vockley J, McKiernan PJ. Living Related Liver Transplantation for Metabolic Liver Diseases in Children. *J Pediatr Gastroenterol Nutr* 2021; **72**: 11-17 [PMID: [32969959](#) DOI: [10.1097/MPG.0000000000002952](#)]
- 91 **Leal R**, Costa J, Santos T, Galvão A, Santos L, Romãozinho C, Macário F, Alves R, Campos M, Furtado E, Mota A. Combined liver and kidney transplantation in two women with primary hyperoxaluria: Different roads led to different outcomes. *Nefrologia* 2017; **37**: 433-434 [PMID: [28209444](#) DOI: [10.1016/j.nefro.2016.10.006](#)]
- 92 **Mc Guire PJ**, Lim-Melia E, Diaz GA, Raymond K, Larkin A, Wasserstein MP, Sansaricq C. Combined liver-kidney transplant for the management of methylmalonic aciduria: a case report and review of the literature. *Mol Genet Metab* 2008; **93**: 22-29 [PMID: [17964841](#) DOI: [10.1016/j.ymgme.2007.08.119](#)]
- 93 **Molema F**, Williams M, Langendonk J, Darwish-Murad S, van de Wetering J, Jacobs E, Onkenhout W, Brusse E, van der Eerden A, Wagenmakers M. Neurotoxicity including posterior reversible encephalopathy syndrome after initiation of calcineurin inhibitors in transplanted methylmalonic acidemia patients: Two case reports and review of the literature. *JIMD Rep* 2020; **51**: 89-104 [PMID: [32071844](#) DOI: [10.1002/jmd2.12088](#)]
- 94 **Arnon R**, Kerkar N, Davis MK, Anand R, Yin W, González-Peralta RP; SPLIT Research Group. Liver transplantation in children with metabolic diseases: the studies of pediatric liver transplantation experience. *Pediatr Transplant* 2010; **14**: 796-805 [PMID: [20557477](#) DOI: [10.1111/j.1399-3046.2010.01339.x](#)]

- 95 **Jain A**, Mazariegos G, Kashyap R, Kosmach-Park B, Starzl TE, Fung J, Reyes J. Pediatric liver transplantation. A single center experience spanning 20 years. *Transplantation* 2002; **73**: 941-947 [PMID: [11923697](#) DOI: [10.1097/00007890-200203270-00020](#)]
- 96 **Jain A**, Mazariegos G, Kashyap R, Green M, Gronsby C, Starzl TE, Fung J, Reyes J. Comparative long-term evaluation of tacrolimus and cyclosporine in pediatric liver transplantation. *Transplantation* 2000; **70**: 617-625 [PMID: [10972220](#) DOI: [10.1097/00007890-200008270-00015](#)]
- 97 **Miloh T**, Barton A, Wheeler J, Pham Y, Hewitt W, Keegan T, Sanchez C, Bulut P, Goss J. Immunosuppression in pediatric liver transplant recipients: Unique aspects. *Liver Transpl* 2017; **23**: 244-256 [PMID: [27874250](#) DOI: [10.1002/lt.24677](#)]
- 98 **Mouzaki M**, Yap J, Avinashi V, Babu A, Fu A, Deangelis M, Van Roestel K, Ghanekar A, Kamath B, Avitzur Y, Fecteau A, Jones N, Ling S, Grant D, Ng V. Basiliximab with delayed introduction of calcineurin inhibitors as a renal-sparing protocol following liver transplantation in children with renal impairment. *Pediatr Transplant* 2013; **17**: 751-756 [PMID: [24118898](#) DOI: [10.1111/petr.12158](#)]
- 99 **Williams R**, Aithal G, Alexander GJ, Allison M, Armstrong I, Aspinall R, Baker A, Batterham R, Brown K, Burton R, Cramp ME, Day N, Dhawan A, Drummond C, Ferguson J, Foster G, Gilmore I, Greenberg J, Henn C, Jarvis H, Kelly D, Mathews M, McCloud A, MacGilchrist A, McKee M, Moriarty K, Morling J, Newsome P, Rice P, Roberts S, Rutter H, Samyn M, Severi K, Sheron N, Thorburn D, Verne J, Vohra J, Williams J, Yeoman A. Unacceptable failures: the final report of the Lancet Commission into liver disease in the UK. *Lancet* 2020; **395**: 226-239 [PMID: [31791690](#) DOI: [10.1016/S0140-6736\(19\)32908-3](#)]



# Liver and COVID-19: From care of patients with liver diseases to liver injury

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## Abstract

The global pandemic of coronavirus disease 2019 (COVID-19) changed dramatically all priorities on medical society and created several challenges for clinicians caring for patients with liver diseases. We performed a comprehensive review about how COVID-19 can affect the liver, the influence of liver diseases on the risk of developing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and COVID-19 severity and also some strategies to overcome all the challenges clinicians have to face in the management of patients with liver diseases in a period of time when all the focus turned on COVID-19. We analyze the relationship between COVID-19 and non-alcoholic fatty liver disease, alcoholic liver disease, viral hepatitis, autoimmune liver disease, cirrhosis, hepatocellular carcinoma and liver transplantation, as well as the approach to SARS-CoV-2 vaccination.

**Key Words:** COVID-19; Liver diseases; Vaccination; SARS-CoV-2

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**Core Tip:** Coronavirus disease 2019 (COVID-19) has become a major health problem worldwide in the last few months, affecting the health system dramatically. Apart from the respiratory system, associated liver injury is one of the main concerns in severe acute respiratory syndrome coronavirus 2 infection and several mechanisms could explain liver abnormalities. In this mini-review, and different from other papers, we not only analyze liver injury by COVID-19, the effect of COVID-19 in liver diseases, its pathophysiology and strategies to keep an adequate care of liver patients, but also

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highlight the potential higher risk of severe disease or risk of infection in patients with different etiologies of liver disease. We also analyze the recent recommendations and prioritization regarding vaccination in patients with liver disease.

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## INTRODUCTION

Coronaviruses are single-stranded RNA viruses that mainly cause upper respiratory tract infections in humans. Two coronaviruses were previously described, severe acute respiratory syndrome coronavirus (SARS-CoV), causing an epidemic in 2003, and middle eastern respiratory syndrome coronavirus (MERS-CoV), causing an epidemic in 2012[1].

The new SARS-CoV-2 is responsible for one of the most important and devastating pandemic in the human history - the first case of severe pneumonia caused by SARS-CoV-2 was described on 3rd January 2020 in Wuhan, China, the first epicenter of the disease[2]. Since then, SARS-CoV-2 have widespread across the world, causing a global pandemic - in the beginning of May 2021, World Health Organization reported more than 150000000 infected patients and more than 3000000 deaths[3].

Coronavirus disease 2019 (COVID-19) has a variety of clinical presentations, with the majority of patients remaining asymptomatic or with mild symptoms, such as cough, anosmia, fatigue, diarrhea, headache or fever. However, 10%-15% will present acute hypoxemia or respiratory distress syndrome that might progress to multi-organ failure and death[4-7].

The respiratory tract is the main target of SARS-CoV-2 but several reports revealed a systemic involvement of the disease, including liver and the gastrointestinal tract[8].

In this review, we will highlight the relationship between COVID-19 and the liver.

## LIVER INJURY IN COVID-19

It is well established that the respiratory tract is involved in the majority cases of SARS-CoV-2 infections but several studies reported COVID-19 associated liver injury, defined as liver damage during disease progression or treatment[9].

Elevated serum liver biochemistries in patients with COVID-19 was first described by Chen *et al*[10] in Wuhan where 43.9% of patients had elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Overall, the incidence of liver injury ranged from 14.8% to 78% and the most common changes are mild elevations of AST and/or ALT (mainly within 3 times the upper limit of normal)[11-13]. The wide range of incidence could be explained by the different cut-off values of upper limit of normal and geographical variability in prevalence and type of underlying chronic liver disease[7,14].

It was also described a possible relationship between liver injury and severity of the disease: Abnormalities in liver function were significantly higher in critically ill patients and associated with poorer outcome. One large Chinese study showed that 18% of non-severe COVID-19 patients had elevated ALT *vs* 56% in the group of severe COVID-19[1,15,16].

Liver biopsies in COVID-19 patients did not show any typical pattern of hepatic lesions and liver injury is probably associated with multiple mechanisms (Table 1)[1,7,9,17-26]: (1) Direct cytotoxicity by active replication of SARS-CoV-2 in hepatic cells due to abundance of its receptor in cholangiocytes - however, the major COVID-19 induced liver function abnormalities are in aminotransferases that might be explained by others factors such as mitochondrial dysfunction, SARS-CoV-2 induced hepatic steatosis, transaminase release due to breakdown of skeletal and cardiac muscle and venous and arterial thromboses; (2) Hyper-inflammatory reaction to COVID-19: Substantial elevations in serum ALT are usually associated with high levels of C reactive protein, D-dimer, ferritin and interleucine-6 and result from the development of the cytokine

**Table 1 Mechanisms of coronavirus disease 2019 liver injury**

Mechanisms	Pathophysiology
Direct cytotoxicity	Active replication of SARS-CoV-2 in hepatic cells
Hyper-inflammatory reaction	Cytokine storm and activation of immune system
Systemic hypoxia	COVID-19 cardiomyopathy
Drug-induced liver injury	Liver toxicity to medication used to treat COVID-19

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019.

storm and activation of the innate and adaptive immune system; (3) Systemic hypoxia and hepatic congestion related to cardiomyopathy (hypoxia hepatitis is frequent in the severe cases); and (4) Drug-induced liver injury: Mainly with lopinavir-ritonavir, tocilizumab and remdesivir.

## COVID-19 AND LIVER DISEASES

The presence of previous liver disease could influence the prognosis of COVID-19 and SARS-CoV-2 could also pose some difficult challenges in care of liver diseases' patients (Table 2).

### **Non-alcoholic fatty liver disease and metabolic associated fatty liver disease**

Non-alcoholic fatty liver disease (NAFLD) is one of the most common etiologies of liver disease in the world and the most emerging cause in developed countries, being expected to become the leading cause of liver transplantation worldwide[27,28]. Recently, a new concept has merged, metabolic associated fatty liver disease (MAFLD), diagnosed in the presence of hepatic steatosis and any of the following metabolic conditions: Diabetes mellitus, obesity/overweight or evidence of metabolic dysregulation in lean patients[29].

Several studies investigated the possible relationship between NAFLD/MAFLD and the outcome of COVID-19. Ji *et al*[30] reported liver abnormalities in 50% at admission of COVID-19 and NAFLD patients and in 75% during hospitalization and NAFLD was an independent risk factor for COVID-19 progression[27]. Another study, a meta-analysis by Pan *et al*[31], showed that NAFLD increased the risk of disease progression among patients with COVID-19.

NAFLD patients may also suffer from comorbidities known to be important risk factors for severity of COVID-19 and that could negatively influence prognosis, such as hypertension, obesity or diabetes[27]. However, Zhou *et al*[32] established a synergic effect of NAFLD for severe COVID-19 in patients less than 60 years-old and independent of other comorbidities, showing that NAFLD alone could be an important prognostic factor. This might be explained by metabolically active fat, which is associated with[17,33]: (1) Chronic inflammatory changes and higher cytokine levels, making NAFLD patients more vulnerable to cytokine storm in COVID-19; and (2) Imbalance in host inflammatory and tolerance response to SARS-CoV-2. On the other hand, it was also demonstrated that COVID-19 patients exhibited higher levels of monocyte chemoattractant protein-1 that is associated with steatohepatitis exacerbation, increasing the risk of NAFLD progression[34].

Therefore, it is of paramount importance to carefully follow NAFLD and COVID-19 patients due to the higher risk of poorer outcomes in both diseases.

**Alcoholic liver disease:** Alcoholic liver disease is one of the main causes of liver disease and its patients were considered one of the most affected groups during the pandemic as they present[35-37]: (1) Higher risk of developing SARS-CoV-2 infection due to reduced immunity to bacterial and viral infection (due to heavy alcohol consumption) and also willingness to adopt prevention measures; (2) Worse COVID-19 outcomes with a study reporting to be the only liver disease with a significant odds ratio for death; and (3) Higher alcohol consumption during the time of social isolation, increasing the risk of decompensation.

Strategies to overcome all these difficulties should be implemented and include social and psychological support (locally or *via* telemedicine), educational sessions to deal with the risk of COVID-19 as well as regular appointments with hepatologists.

**Table 2 Influence of liver diseases in risk of infection or outcome of coronavirus disease 2019**

Higher risk of infection or severe outcome of COVID-19	Apparently non-higher risk of infection or severe outcome of COVID-19
Non-alcoholic fatty liver disease	Hemochromatosis
Alcoholic liver disease	Wilson's disease
Alpha-1 antitrypsin deficiency	Autoimmune liver disease
Cirrhosis	Hepatitis B infection
Hepatocellular carcinoma	

COVID-19: Coronavirus disease 2019.

A debatable question is the use of corticosteroids in alcoholic hepatitis: There are some recommendations suggesting to avoid steroids in this situation as it may delay viral clearance but benefits must be weighed against risks and there are some reports showing that prednisolone might be an effective and safe treatment in patients with SARS-CoV-2 infection and alcoholic hepatitis[38,39].

**Other metabolic liver diseases:** There is no data on the risk of infection and severity of COVID-19 in patients with hemochromatosis and Wilson's disease. It is always important to search for iron overload in patients with SARS-CoV-2 and abnormal liver tests as elevated ferritin levels could be associated to viral infection and mask an underlying hemochromatosis[40].

Alpha-1 antitrypsin might inhibit infection by SARS-CoV-2, has anticoagulation effects and protect against inflammation[41]. Therefore, patients with alpha-1 antitrypsin deficiency seems to have increased risk of infection and COVID-19 severity, mainly Pi\*ZZ and/or low alpha-1 antitrypsin levels.

**Autoimmune liver diseases:** Autoimmune liver diseases are a group of diseases that include autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC).

The management of autoimmune liver diseases was one of the main concerns of hepatologists during COVID-19 pandemic due to the use of immunosuppressive therapy. Previous reports with other coronaviruses (SARS-CoV or MERS-CoV) did not show worse outcomes in patients who were undergoing transplantation, chemotherapy or other immunosuppressive treatments and there was also some evidence that immunosuppressive therapy might have a protective effect against severe COVID-19[42,43]. Therefore, the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) were against stopping immunosuppressive therapy as it may lead to disease flares that will need high doses of steroids, which will increase the susceptibility for SARS-CoV-2 infection. More recently, a multicenter study evaluated the outcomes of COVID-19 in patients with AIH and showed that the overall outcome of SARS-CoV-2 disease was favorable in patients without cirrhosis and that ongoing immunosuppression was not associated with increased risk of severe COVID-19[44]. Efe *et al*[44] also described that the risk of AIH relapse may be related with hyperstimulation of the immune system by COVID-19[45]. There is scarce information about COVID-19 and PBC or PSC - an Italian study found an incidence of SARS-CoV-2 infection of 5.6% in AIH patients but only 1.5% in PBC patients - the higher incidence in AIH might be related with the use of immunosuppressive therapy (not used in PBC)[46]. Another important finding, also described in other autoimmune and inflammatory conditions, is the development of new-onset PBC after COVID-19, where SARS-CoV-2 triggered the development of PBC in a genetically predisposed individual[45,47,48].

**Viral hepatitis:** COVID-19 did not seem to influence the course of hepatitis C virus (HCV) or hepatitis B virus (HBV) infection. A meta-analysis by Mantovani *et al*[49] reported an incidence in COVID-19 patients of only less than 0.1% HCV infection and 0.1% of HBV infection. In fact, the major effect of COVID-19 is the impact in HCV elimination efforts. A Spanish study showed that the interruption of HCV screening, linkage to care and harm reduction programs, would substantially decrease HCV diagnosis and treatment, consequently, increase the number of HCV liver-related deaths, hepatocellular carcinoma (HCC) and decompensated cirrhosis[50]. Thus, it is of paramount importance to keep HCV elimination a major health priority through



innovative programs as telehealth or home-delivery HCV drugs[50].

Akin to HBV infection, one of the largest cohorts of patients with COVID-19 and past or current HBV infection did not show an association with acute liver injury. Patients that fulfill the criteria for HBV treatment or under corticosteroid therapy should receive antiviral treatment but it may not be necessary in all patients with COVID-19 and current or past HBV infection[51]. A study by Liu *et al*[52] also reported that HBV infection did not predispose COVID-19 patients to more severe outcomes. There is also a report of COVID-19 accompanied by HBV infection causing a fulminant hepatitis[53].

**Cirrhosis:** Cirrhosis is one of the major causes of morbidity and mortality in the entire world and the second leading cause of digestive disease mortality[54].

Patients with cirrhosis have multiple mechanisms of immune dysfunction and are more susceptible to infection, not only to severe bacterial infection but also to viral and fungal-related disease[7,22,55]. However, data about risk of COVID-19 in this population is controversial, with Richardson *et al*[56] not suggesting a higher prevalence of cirrhotic patients in COVID-19 population while Kushner *et al*[57] reporting higher risk of infection, severity of the disease and hepatic decompensation. In cirrhotic patients, there is also a relationship between severity of liver disease and SARS-CoV-2 morbidity and mortality, with Child-Pugh C patients presenting higher frequency of Intensive Care Unit admission, renal replacement therapy and mortality [58].

Bajaj *et al*[59] showed that cirrhotic patients hospitalized with COVID-19 had similar mortality rates of patients admitted with cirrhosis alone but higher than patients with COVID-19 alone. An Italian study also demonstrated that cirrhotic patients that develop COVID-19 present a worse prognosis due to respiratory complications but also worsening of liver function leading to end-stage liver disease. They also found that the 30-d mortality in non-cirrhotic patients was significantly lower[60].

A very interesting finding in a multicenter cohort is that COVID-19 is associated with hepatic decompensation and, in this study, 24.3% had no respiratory symptoms at the time of diagnosis[4]. As so, testing to SARS-CoV-2 infection is advisable in patients with hepatic decompensation and early admission should be considered due to high rates of mortality.

SARS-CoV-2 infection can also cause acute-on-chronic liver failure characterized by hepatic decompensation events, extrahepatic organ failure and high rates of mortality.

EASL and World Gastroenterology Organization recommend that care should be maintained as this fragile population have a very high risk of decompensation. Prophylaxis of spontaneous bacterial peritonitis and encephalopathy, therapeutic paracentesis and variceal banding in high risk patients should be always performed in a COVID-19 free environment and following all the protective measures, as this will reduce the risk of further decompensation and hospitalization[37,61].

**Cancer and hepatocellular carcinoma:** Patients with COVID-19 and cancer are at increased risk of infection and worse outcomes[62]. A nationwide Chinese study that included 1590 patients (18% with history of cancer) reported higher risk of adverse events in patients with active or past history of cancer. This might be explained as cancer patients are more susceptible to infection (due to their systemic immunosuppressive state associated with malignancy but also with its treatment) and have increased risk of COVID-19 related serious events[63,64].

HCC is the sixth most commonly diagnosed and the fourth leading cause of cancer-related death in the world, being one of the major health challenges in liver clinic[65, 66]. There is scarce information on the impact of COVID-19 in patients with HCC - in a small study, Zhang *et al*[67] reported poorer outcomes in patients with HCC but also with other malignancies when compared to the general population.

The major impact of COVID-19 on HCC is related to the delay on the proper management of HCC. A French multicenter study reported a significant decrease in the rate of HCC patients referred for first diagnosis or treatment[68]. Several interpretations could be made but may be related to the increase delay of referral by other professionals, patients' fear to search for healthcare services, delay in the Hepatology appointments and limited assessment to diagnostic and therapeutic tools. They also found a higher rate of treatment delay longer than one month when compared 2019 to 2020[68].

Currently, AASLD and EASL recommend to continue HCC surveillance and treatment with an acceptable delay of a maximum of two months to reduce the number of patients presenting with HCC not amenable to curative treatment[43,69]. Whenever possible, telemedicine could replace clinic visits and multidisciplinary team

meetings, and all diagnostic and therapeutic procedures should be performed according to the COVID-19 prophylactic measures to avoid nosocomial spread on infection[60].

The real effect of COVID-19 on HCC management is still undetermined and only the middle-term follow-up will clarify the pandemic impact on HCC morbidity and mortality.

**Liver transplantation:** The risk and severity of COVID-19 in liver transplant patients is still unclear[70]. A multinational cohort reported a similar risk to the general population of contracting infection with SARS-CoV-2[71]. The proportion of liver transplant recipients hospitalized with COVID-19 was 82% and 19% died and advanced age, presence of non-liver cancer and elevated baseline creatinine were associated with higher mortality rates, while the type of immunosuppression and time since transplantation were not associated[71]. However, the European Liver and Intestine Transplantation Association established a registry and suggested that longer time of transplantation might have higher rates of mortality[72].

Liver transplantation programmes were heavily affected by COVID-19 pandemic by several reasons: Limited access to intensive care unit (ICU) due to the number of COVID-19 patients needing ventilation support, reduced number of organs because all major guidelines recommended against using organs from donors with SARS-CoV-2 infection and also limited access of patients to liver transplant centers[58].

It is crucial to maintain liver transplant programs to reduce liver diseases mortality, facing all the new challenges through innovative tools, in which telemedicine might play a key role.

The postoperative period is also a challenge and should follow a SARS-CoV-2 free pathway, with proper free-SARS-CoV-2 ICU to ensure high transplant success rates and preventing nosocomial infection[5]. In the perioperative period, patients' follow-up should be preferably through telemedicine and, in case of symptoms, the threshold for testing for SARS-CoV-2 infection should be low[5]. In case of COVID-19, patients should always present to the hospital for medical evaluation[5].

Regarding immunosuppression after liver transplants, all liver associations recommend to maintain medication as there is no data suggesting a higher risk of COVID-19 severity, while stopping will increase the risk of graft rejection[43,61,73]. However, in case of COVID-19, immunosuppression should be reduced, particularly antimetabolite dosages[43].

**Vaccination:** The development of SARS-CoV-2 vaccine is one of the major advances to mitigate all the health and economic issues. This development started in January 2020 and progressed very rapidly, being now available more than 5 vaccines. The process of vaccination is moving forward worldwide in order to achieve herd immunity as soon as possible.

Despite some concerns about vaccines' adverse events, the safety profile is excellent and, based on current knowledge, there is no contra-indication for vaccination of liver disease patients, as the potential benefits are higher than the risks[74]. However, there is a report of auto-immune hepatitis developing post-COVID-19 vaccination[75].

Vaccination should also be prioritized in[74]: (1) Cirrhotic patients or with liver decompensation; (2) Hepatobiliary malignancies patients; (3) Chronic liver disease patients and risk factors for severe COVID-19; (4) Liver transplant recipients (prior to liver transplant whenever possible or 3-6 mo after transplantation); and (5) Healthcare professionals caring for these patients.

## CONCLUSION

Liver abnormalities in COVID-19 patients are common and may result from direct cytotoxicity, hyper-inflammatory status or DILI. In addition, a direct relationship between grade of liver injury and severity of the disease was also established.

The existence of previous liver disease could influence the prognosis, with patients with NAFLD, cirrhosis and HCC presenting higher risk of severe COVID-19 and death (Table 2). In this population, vaccination should be considered a priority. On the other hand, the focus on SARS-CoV-2 infection lead to reduced access to care for patients with liver disease that must be reestablished to improve the outcome of these diseases.

In conclusion, the consequences of COVID-19 on liver ranges from its direct liver injury to the profound negative effect on liver disease patients' care which might increase liver disease burden and negatively influence prognosis.

## REFERENCES

- 1 **Jothimani D**, Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. *J Hepatol* 2020; **73**: 1231-1240 [PMID: 32553666 DOI: 10.1016/j.jhep.2020.06.006]
- 2 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]
- 3 **World Organization Health**. Weekly operational update on COVID-19 - 3 May 2021. [cited 20 May 2021]. Available from: <https://www.who.int/publications/m/item/weekly-operational-update-on-covid-19---3-may-2021>
- 4 **Moon AM**, Webb GJ, Aloman C, Armstrong MJ, Cargill T, Dhanasekaran R, Genescà J, Gill US, James TW, Jones PD, Marshall A, Mells G, Perumalswami PV, Qi X, Su F, Ufere NN, Barnes E, Barritt AS, Marjot T. High mortality rates for SARS-CoV-2 infection in patients with pre-existing chronic liver disease and cirrhosis: Preliminary results from an international registry. *J Hepatol* 2020; **73**: 705-708 [PMID: 32446714 DOI: 10.1016/j.jhep.2020.05.013]
- 5 **Mohammed A**, Paranj N, Chen PH, Niu B. COVID-19 in Chronic Liver Disease and Liver Transplantation: A Clinical Review. *J Clin Gastroenterol* 2021; **55**: 187-194 [PMID: 33394628 DOI: 10.1097/MCG.0000000000001481]
- 6 **Berlin DA**, Gulick RM, Martinez FJ. Severe Covid-19. *N Engl J Med* 2020; **383**: 2451-2460 [PMID: 32412710 DOI: 10.1056/NEJMcp2009575]
- 7 **Marjot T**, Webb GJ, Barritt AS 4th, Moon AM, Stamataki Z, Wong VW, Barnes E. COVID-19 and liver disease: mechanistic and clinical perspectives. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 348-364 [PMID: 33692570 DOI: 10.1038/s41575-021-00426-4]
- 8 **Nardo AD**, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. *Liver Int* 2021; **41**: 20-32 [PMID: 33190346 DOI: 10.1111/liv.14730]
- 9 **Sun J**, Aghemo A, Forner A, Valenti L. COVID-19 and liver disease. *Liver Int* 2020; **40**: 1278-1281 [PMID: 32251539 DOI: 10.1111/liv.14470]
- 10 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]
- 11 **Zhang C**, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020; **5**: 428-430 [PMID: 32145190 DOI: 10.1016/S2468-1253(20)30057-1]
- 12 **Musa S**. Hepatic and gastrointestinal involvement in coronavirus disease 2019 (COVID-19): What do we know till now? *Arab J Gastroenterol* 2020; **21**: 3-8 [PMID: 32253172 DOI: 10.1016/j.ajg.2020.03.002]
- 13 **Li J**, Fan JG. Characteristics and Mechanism of Liver Injury in 2019 Coronavirus Disease. *J Clin Transl Hepatol* 2020; **8**: 13-17 [PMID: 32274341 DOI: 10.14218/JCTH.2020.00019]
- 14 **Mao R**, Qiu Y, He JS, Tan JY, Li XH, Liang J, Shen J, Zhu LR, Chen Y, Iacucci M, Ng SC, Ghosh S, Chen MH. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 667-678 [PMID: 32405603 DOI: 10.1016/S2468-1253(20)30126-6]
- 15 **Liu C**, Jiang ZC, Shao CX, Zhang HG, Yue HM, Chen ZH, Ma BY, Liu WY, Huang HH, Yang J, Wang Y, Liu HY, Xu D, Wang JT, Yang JY, Pan HQ, Zou SQ, Li FJ, Lei JQ, Li X, He Q, Gu Y, Qi XL. [Preliminary study of the relationship between novel coronavirus pneumonia and liver function damage: a multicenter study]. *Zhonghua Gan Zang Bing Za Zhi* 2020; **28**: 107-111 [PMID: 32077660 DOI: 10.3760/cma.j.issn.1007-3418.2020.02.003]
- 16 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]
- 17 **Metawea MI**, Yousif WI, Moheb I. COVID 19 and liver: An A-Z literature review. *Dig Liver Dis* 2021; **53**: 146-152 [PMID: 32988758 DOI: 10.1016/j.dld.2020.09.010]
- 18 **Amin M**. COVID-19 and the liver: overview. *Eur J Gastroenterol Hepatol* 2021; **33**: 309-311 [PMID: 32558697 DOI: 10.1097/MEG.0000000000001808]
- 19 **Li Y**, Xiao SY. Hepatic involvement in COVID-19 patients: Pathology, pathogenesis, and clinical implications. *J Med Virol* 2020; **92**: 1491-1494 [PMID: 32369204 DOI: 10.1002/jmv.25973]
- 20 **Mehta P**, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; **395**: 1033-1034 [PMID: 32192578 DOI: 10.1016/S0140-6736(20)30628-0]
- 21 **Da BL**, Kushner T, El Halabi M, Paka P, Khalid M, Uberoi A, Lee BT, Perumalswami PV, Rutledge SM, Schiano TD, Friedman S, Saberi B. Liver Injury in Hospitalized Patients with COVID-19 Correlates with Hyper Inflammatory Response and Elevated IL-6. *Hepatol Commun* 2020 [PMID: 33230491 DOI: 10.1002/hep4.1631]
- 22 **Sonzogni A**, Previtali G, Seghezzi M, Grazia Alessio M, Gianatti A, Licini L, Morotti D, Zerbi P,

- Carsana L, Rossi R, Lauri E, Pellegrinelli A, Nebuloni M. Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations. *Liver Int* 2020; **40**: 2110-2116 [PMID: 32654359 DOI: 10.1111/liv.14601]
- 23 **Gordon DE**, Jang GM, Bouhaddou M, Xu J, Obernier K, O'Meara MJ, Guo JZ, Swaney DL, Tummino TA, Hüttenhain R, Kaake RM, Richards AL, Tutuncuoglu B, Foussard H, Batra J, Haas K, Modak M, Kim M, Haas P, Polacco BJ, Braberg H, Fabius JM, Eckhardt M, Soucheray M, Bennett MJ, Cakir M, McGregor MJ, Li Q, Naing ZZC, Zhou Y, Peng S, Kirby IT, Melnyk JE, Chorba JS, Lou K, Dai SA, Shen W, Shi Y, Zhang Z, Barrio-Hernandez I, Memon D, Hernandez-Armenta C, Mathy CJP, Perica T, Pilla KB, Ganesan SJ, Saltzberg DJ, Ramachandran R, Liu X, Rosenthal SB, Calviello L, Venkataramanan S, Lin Y, Wankowicz SA, Bohn M, Trenker R, Young JM, Cavero D, Hiatt J, Roth T, Rathore U, Subramanian A, Noack J, Hubert M, Roesch F, Vallet T, Meyer B, White KM, Miorin L, Agard D, Emerman M, Ruggero D, Garcia-Sastre A, Jura N, von Zastrow M, Taunton J, Schwartz O, Vignuzzi M, d'Enfert C, Mukherjee S, Jacobson M, Malik HS, Fujimori DG, Ideker T, Craik CS, Floor S, Fraser JS, Gross J, Sali A, Kortemme T, Beltrao P, Shokat K, Shoichet BK, Krogan NJ. A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. *bioRxiv* 2020 [PMID: 32511329 DOI: 10.1101/2020.03.22.002386]
- 24 **Cao B**, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 2020; **382**: 1787-1799 [PMID: 32187464 DOI: 10.1056/NEJMoa2001282]
- 25 **Muhović D**, Bojović J, Bulatović A, Vukčević B, Ratković M, Lazović R, Smolović B. First case of drug-induced liver injury associated with the use of tocilizumab in a patient with COVID-19. *Liver Int* 2020; **40**: 1901-1905 [PMID: 32478465 DOI: 10.1111/liv.14516]
- 26 **Beigel JH**, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC; ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Final Report. *N Engl J Med* 2020; **383**: 1813-1826 [PMID: 32445440 DOI: 10.1056/NEJMoa2007764]
- 27 **Portincasa P**, Krawczyk M, Smyk W, Lammert F, Di Ciaula A. COVID-19 and non-alcoholic fatty liver disease: Two intersecting pandemics. *Eur J Clin Invest* 2020; **50**: e13338 [PMID: 32589264 DOI: 10.1111/eci.13338]
- 28 **Younossi ZM**, Marchesini G, Pinto-Cortez H, Petta S. Epidemiology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis: Implications for Liver Transplantation. *Transplantation* 2019; **103**: 22-27 [PMID: 30335697 DOI: 10.1097/TP.0000000000002484]
- 29 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbaek H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]
- 30 **Ji D**, Qin E, Xu J, Zhang D, Cheng G, Wang Y, Lau G. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020; **73**: 451-453 [PMID: 32278005 DOI: 10.1016/j.jhep.2020.03.044]
- 31 **Pan L**, Huang P, Xie X, Xu J, Guo D, Jiang Y. Metabolic associated fatty liver disease increases the severity of COVID-19: A meta-analysis. *Dig Liver Dis* 2021; **53**: 153-157 [PMID: 33011088 DOI: 10.1016/j.dld.2020.09.007]
- 32 **Zhou YJ**, Zheng KI, Wang XB, Yan HD, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, George J, Zheng MH. Younger patients with MAFLD are at increased risk of severe COVID-19 illness: A multicenter preliminary analysis. *J Hepatol* 2020; **73**: 719-721 [PMID: 32348790 DOI: 10.1016/j.jhep.2020.04.027]
- 33 **Lefere S**, Tacke F. Macrophages in obesity and non-alcoholic fatty liver disease: Crosstalk with metabolism. *JHEP Rep* 2019; **1**: 30-43 [PMID: 32149275 DOI: 10.1016/j.jhepr.2019.02.004]
- 34 **Boeckmans J**, Rodrigues RM, Demuyser T, Piérard D, Vanhaecke T, Rogiers V. COVID-19 and drug-induced liver injury: a problem of plenty or a petty point? *Arch Toxicol* 2020; **94**: 1367-1369 [PMID: 32266419 DOI: 10.1007/s00204-020-02734-1]
- 35 **Chick J**. Alcohol and COVID-19. *Alcohol Alcohol* 2020; **55**: 341-342 [PMID: 32400878 DOI: 10.1093/alcalc/agaa039]
- 36 **Rutledge SM**, Schiano TD, Florman S, Im GY. COVID-19 Aftershocks on Alcohol-associated Liver Disease: An Early Cross-Sectional Report from the U.S. Epicenter (Manuscript HEP4-20-0491). *Hepatol Commun* 2021 [PMID: 33821225 DOI: 10.1002/hep4.1706]
- 37 **Hamid S**, Alvares da Silva MR, Burak KW, Chen T, Drenth JPH, Esmat G, Gaspar R, LaBrecque D, Lee A, Macedo G, McMahon B, Ning Q, Reau N, Sonderup M, van Leeuwen DJ, Armstrong D, Yurdaydin C. WGO Guidance for the Care of Patients With COVID-19 and Liver Disease. *J Clin*



- Gastroenterol* 2021; **55**: 1-11 [PMID: [33230011](#) DOI: [10.1097/MCG.0000000000001459](#)]
- 38 **Sanders JM**, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* 2020; **323**: 1824-1836 [PMID: [32282022](#) DOI: [10.1001/jama.2020.6019](#)]
  - 39 **Zelman S**, Holzwanger E, Malik R, Dickstein A, Aron MD. Alcoholic Hepatitis and COVID-19: The Question of Steroids. *ACG Case Rep J* 2020; **7**: e00504 [DOI: [10.14309/crj.0000000000000504](#)]
  - 40 **Aamar**, A. O., Jose; Aloreidi, Khalil; Bandaru, Praneeth; Reddy, Madhavi; Etienne, Denzil, Hemochromatosis in a Patient With Coronavirus Disease 2019. *Amer J Gastroenterol* 2020; **115**: S1326-S1326
  - 41 **Yang C**, Keshavjee S, Liu M. Alpha-1 Antitrypsin for COVID-19 Treatment: Dual Role in Antiviral Infection and Anti-Inflammation. *Front Pharmacol* 2020; **11**: 615398 [PMID: [33362565](#) DOI: [10.3389/fphar.2020.615398](#)]
  - 42 **Di Giorgio A**, Nicastro E, Speziani C, De Giorgio M, Pasulo L, Magro B, Fagioli S, D' Antiga L. Health status of patients with autoimmune liver disease during SARS-CoV-2 outbreak in northern Italy. *J Hepatol* 2020; **73**: 702-705 [PMID: [32413378](#) DOI: [10.1016/j.jhep.2020.05.008](#)]
  - 43 **Fix OK**, Hameed B, Fontana RJ, Kwok RM, McGuire BM, Mulligan DC, Pratt DS, Russo MW, Schilsky ML, Verna EC, Loomba R, Cohen DE, Bezerra JA, Reddy KR, Chung RT. Clinical Best Practice Advice for Hepatology and Liver Transplant Providers During the COVID-19 Pandemic: AASLD Expert Panel Consensus Statement. *Hepatology* 2020; **72**: 287-304 [PMID: [32298473](#) DOI: [10.1002/hep.31281](#)]
  - 44 **Efe C**, Dhanasekaran R, Lammert C, Ebik B, Higuera-de la Tijera F, Aloman C, Rıza Calışkan A, Peralta M, Gerussi A, Massoumi H, Catana AM, Torgutalp M, Purnak T, Rigamonti C, Gomez Aldana AJ, Khakoo N, Kacmaz H, Nazal L, Frager S, Demir N, Irak K, Ellik ZM, Balaban Y, Atay K, Eren F, Cristoferi L, Batbay E, Urzua A, Snijders R, Kıyıcı M, Akyıldız M, Ekin N, Carr RM, Harputluoğlu M, Hatemi I, Mendizabal M, Silva M, Idilman R, Silveira M, Drenth JPH, Assis DN, Björnsson E, Boyer JL, Invernizzi P, Levy C, Schiano TD, Ridruejo E, Wahlin S. Outcome of COVID-19 in Patients With Autoimmune Hepatitis: An International Multicenter Study. *Hepatology* 2021; **73**: 2099-2109 [PMID: [33713486](#) DOI: [10.1002/hep.31797](#)]
  - 45 **Rodriguez Y**, Novelli L, Rojas M, De Santis M, Acosta-Ampudia Y, Monsalve DM, Ramírez-Santana C, Costanzo A, Ridgway WM, Ansari AA, Gershwin ME, Selmi C, Anaya JM. Autoinflammatory and autoimmune conditions at the crossroad of COVID-19. *J Autoimmun* 2020; **114**: 102506 [PMID: [32563547](#) DOI: [10.1016/j.jaut.2020.102506](#)]
  - 46 **Rigamonti C**, Cittone MG, De Benedittis C, Rizzi E, Casciaro GF, Bellan M, Sainaghi PP, Pirisi M. Rates of Symptomatic SARS-CoV-2 Infection in Patients With Autoimmune Liver Diseases in Northern Italy: A Telemedicine Study. *Clin Gastroenterol Hepatol* 2020; **18**: 2369-2371.e1 [PMID: [32480009](#) DOI: [10.1016/j.cgh.2020.05.047](#)]
  - 47 **Marabotto E**, Ziola S, Shejani AD, Giannini EG. COVID-19 and liver disease: Not all evil comes to harm. *Liver Int* 2021; **41**: 237-238 [PMID: [33159405](#) DOI: [10.1111/liv.14721](#)]
  - 48 **Bartoli A**, Gitto S, Sighinolfi P, Cursaro C, Andreone P. Primary biliary cholangitis associated with SARS-CoV-2 infection. *J Hepatol* 2021; **74**: 1245-1246 [PMID: [33610679](#) DOI: [10.1016/j.jhep.2021.02.006](#)]
  - 49 **Mantovani A**, Beatrice G, Dalbeni A. Coronavirus disease 2019 and prevalence of chronic liver disease: A meta-analysis. *Liver Int* 2020; **40**: 1316-1320 [PMID: [32329563](#) DOI: [10.1111/liv.14465](#)]
  - 50 **Buti M**, Domínguez-Hernández R, Casado MA. Impact of the COVID-19 pandemic on HCV elimination in Spain. *J Hepatol* 2021; **74**: 1246-1248 [PMID: [33358780](#) DOI: [10.1016/j.jhep.2020.12.018](#)]
  - 51 **Yip TC**, Wong VW, Lui GC, Chow VC, Tse YK, Hui VW, Liang LY, Chan HL, Hui DS, Wong GL. Current and Past Infections of HBV Do Not Increase Mortality in Patients With COVID-19. *Hepatology* 2021; **74**: 1750-1765 [PMID: [33961298](#) DOI: [10.1002/hep.31890](#)]
  - 52 **Liu R**, Zhao L, Cheng X, Han H, Li C, Li D, Liu A, Gao G, Zhou F, Liu F, Jiang Y, Zhu C, Xia Y. Clinical characteristics of COVID-19 patients with hepatitis B virus infection - a retrospective study. *Liver Int* 2021; **41**: 720-730 [PMID: [33351265](#) DOI: [10.1111/liv.14774](#)]
  - 53 **Yigit Y**, Haddad M, Elmoheen A, Shogaa MR, Tawel R, Mohamed YK, Salem W, Fawzy Eltawagny M. Can COVID-19 Cause Flare-Ups of Acute Hepatitis B? *Case Rep Infect Dis* 2021; **2021**: 8818678 [PMID: [33564481](#) DOI: [10.1155/2021/8818678](#)]
  - 54 **Johnson KB**, Campbell EJ, Chi H, Zheng H, King LY, Wu Y, Delemos A, Hurairah A, Corey K, Richter JM, Chung RT. Advanced disease, diuretic use, and marital status predict hospital admissions in an ambulatory cirrhosis cohort. *Dig Dis Sci* 2014; **59**: 174-182 [PMID: [23990035](#) DOI: [10.1007/s10620-013-2832-5](#)]
  - 55 **Qian Q**, Fan L, Liu W, Li J, Yue J, Wang M, Ke X, Yin Y, Chen Q, Jiang C. Direct Evidence of Active SARS-CoV-2 Replication in the Intestine. *Clin Infect Dis* 2021; **73**: 361-366 [PMID: [32638022](#) DOI: [10.1093/cid/ciaa925](#)]
  - 56 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefe J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020; **323**: 2052-2059 [PMID: [32320003](#) DOI: [10.1001/jama.2020.6775](#)]

- 57 **Kushner T**, Cafardi J. Chronic Liver Disease and COVID-19: Alcohol Use Disorder/Alcohol-Associated Liver Disease, Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis, Autoimmune Liver Disease, and Compensated Cirrhosis. *Clin Liver Dis (Hoboken)* 2020; **15**: 195-199 [PMID: [32537135](#) DOI: [10.1002/clid.974](#)]
- 58 **Marjot T**, Moon AM, Cook JA, Abd-El Salam S, Aloman C, Armstrong MJ, Pose E, Brenner EJ, Cargill T, Catana MA, Dhanasekaran R, Eshraghian A, García-Juárez I, Gill US, Jones PD, Kennedy J, Marshall A, Matthews C, Mells G, Mercer C, Perumalswami PV, Avitabile E, Qi X, Su F, Ufere NN, Wong YJ, Zheng MH, Barnes E, Barritt AS 4th, Webb GJ. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: An international registry study. *J Hepatol* 2021; **74**: 567-577 [PMID: [33035628](#) DOI: [10.1016/j.jhep.2020.09.024](#)]
- 59 **Bajaj JS**, Garcia-Tsao G, Biggins SW, Kamath PS, Wong F, McGeorge S, Shaw J, Pearson M, Chew M, Fagan A, de la Rosa Rodriguez R, Worthington J, Olofson A, Weir V, Trisolini C, Dwyer S, Reddy KR. 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**: 531-536 [PMID: [32660964](#) DOI: [10.1136/gutjnl-2020-322118](#)]
- 60 **Iavarone M**, D'Ambrosio R, Soria A, Triolo M, Pugliese N, Del Poggio P, Perricone G, Massironi S, Spinetti A, Buscarini E, Viganò M, Carriero C, Fagioli S, Aghemo A, Belli LS, Lucà M, Pedaci M, Rimondi A, Rumi MG, Invernizzi P, Bonfanti P, Lampertico P. High rates of 30-day mortality in patients with cirrhosis and COVID-19. *J Hepatol* 2020; **73**: 1063-1071 [PMID: [32526252](#) DOI: [10.1016/j.jhep.2020.06.001](#)]
- 61 **Boettler T**, Newsome PN, Mondelli MU, Maticic M, Cordero E, Cornberg M, Berg T. Care of patients with liver disease during the COVID-19 pandemic: EASL-ESCMID position paper. *JHEP Rep* 2020; **2**: 100113 [PMID: [32289115](#) DOI: [10.1016/j.jhepr.2020.100113](#)]
- 62 **Tsamakis K**, Gavriatopoulou M, Schizas D, Stravodimou A, Mougkou A, Tsiptsios D, Sioulas V, Spartalis E, Sioulas AD, Tsamakis C, Charalampakis N, Mueller C, Arya D, Zarogoulidis P, Spandidos DA, Dimopoulos MA, Papageorgiou C, Rizos E. Oncology during the COVID-19 pandemic: challenges, dilemmas and the psychosocial impact on cancer patients. *Oncol Lett* 2020; **20**: 441-447 [PMID: [32565968](#) DOI: [10.3892/ol.2020.11599](#)]
- 63 **Liang W**, Guan W, Chen R, Wang W, Li J, Xu K, Li C, Ai Q, Lu W, Liang H, Li S, He J. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *Lancet Oncol* 2020; **21**: 335-337 [PMID: [32066541](#) DOI: [10.1016/S1470-2045\(20\)30096-6](#)]
- 64 **Gosain R**, Abdou Y, Singh A, Rana N, Puzanov I, Ernstoff MS. COVID-19 and Cancer: a Comprehensive Review. *Curr Oncol Rep* 2020; **22**: 53 [PMID: [32385672](#) DOI: [10.1007/s11912-020-00934-7](#)]
- 65 **Pascual S**, Miralles C, Bernabé JM, Irurzun J, Planells M. Surveillance and diagnosis of hepatocellular carcinoma: A systematic review. *World J Clin Cases* 2019; **7**: 2269-2286 [PMID: [31531321](#) DOI: [10.12998/wjcc.v7.i16.2269](#)]
- 66 **Harris PS**, Hansen RM, Gray ME, Massoud OI, McGuire BM, Shoreibah MG. Hepatocellular carcinoma surveillance: An evidence-based approach. *World J Gastroenterol* 2019; **25**: 1550-1559 [PMID: [30983815](#) DOI: [10.3748/wjg.v25.i13.1550](#)]
- 67 **Zhang L**, Zhu F, Xie L, Wang C, Wang J, Chen R, Jia P, Guan HQ, Peng L, Chen Y, Peng P, Zhang P, Chu Q, Shen Q, Wang Y, Xu SY, Zhao JP, Zhou M. Clinical characteristics of COVID-19-infected cancer patients: a retrospective case study in three hospitals within Wuhan, China. *Ann Oncol* 2020; **31**: 894-901 [PMID: [32224151](#) DOI: [10.1016/j.annonc.2020.03.296](#)]
- 68 **Amadeo G**, Brustia R, Allaire M, Lequoy M, Hollande C, Regnault H, Blaise L, Ganne-Carrié N, Séror O, Larrey E, Lim C, Scatton O, El Mouhadi S, Ozenne V, Paye F, Balladur P, Dohan A, Massault PP, Pol S, Dioguardi Burgio M, Vilgrain V, Sepulveda A, Cauchy F, Luciani A, Sommacale D, Leroy V, Roudot-Thoraval F, Bouattour M, Nault JC; Paris Liver Cancer Group. Impact of COVID-19 on the management of hepatocellular carcinoma in a high-prevalence area. *JHEP Rep* 2021; **3**: 100199 [PMID: [33163949](#) DOI: [10.1016/j.jhepr.2020.100199](#)]
- 69 **Boettler T**, Marjot T, Newsome PN, Mondelli MU, Maticic M, Cordero E, Jalan R, Moreau R, Cornberg M, Berg T. Impact of COVID-19 on the care of patients with liver disease: EASL-ESCMID position paper after 6 months of the pandemic. *JHEP Rep* 2020; **2**: 100169 [PMID: [32835190](#) DOI: [10.1016/j.jhepr.2020.100169](#)]
- 70 **D'Antiga L**. Coronaviruses and Immunosuppressed Patients: The Facts During the Third Epidemic. *Liver Transpl* 2020; **26**: 832-834 [PMID: [32196933](#) DOI: [10.1002/lt.25756](#)]
- 71 **Webb GJ**, Marjot T, Cook JA, Aloman C, Armstrong MJ, Brenner EJ, Catana MA, Cargill T, Dhanasekaran R, García-Juárez I, Hagström H, Kennedy JM, Marshall A, Masson S, Mercer CJ, Perumalswami PV, Ruiz I, Thaker S, Ufere NN, Barnes E, Barritt AS 4th, Moon AM. Outcomes following SARS-CoV-2 infection in liver transplant recipients: an international registry study. *Lancet Gastroenterol Hepatol* 2020; **5**: 1008-1016 [PMID: [32866433](#) DOI: [10.1016/S2468-1253\(20\)30271-5](#)]
- 72 **Belli LS**, Duvoux C, Karam V, Adam R, Cuervas-Mons V, Pasulo L, Loinaz C, Invernizzi F, Patrono D, Bhoori S, Ciccarelli O, Morelli MC, Castells L, Lopez-Lopez V, Conti S, Fondevila C, Polak W. COVID-19 in liver transplant recipients: preliminary data from the ELITA/ELTR registry. *Lancet Gastroenterol Hepatol* 2020; **5**: 724-725 [PMID: [32505228](#) DOI: [10.1016/S2468-1253\(20\)30183-7](#)]
- 73 **APASL Covid-19 Task Force**. Lau G, Sharma M. Clinical practice guidance for hepatology and liver transplant providers during the COVID-19 pandemic: APASL expert panel consensus recommendations. *Hepatol Int* 2020; **14**: 415-428 [PMID: [32447721](#) DOI: [10.1007/s12072-020-00934-7](#)]

- [10.1007/s12072-020-10054-w](https://doi.org/10.1007/s12072-020-10054-w)]
- 74 **Cornberg M**, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. *J Hepatol* 2021; **74**: 944-951 [PMID: [33563499](https://pubmed.ncbi.nlm.nih.gov/33563499/) DOI: [10.1016/j.jhep.2021.01.032](https://doi.org/10.1016/j.jhep.2021.01.032)]
  - 75 **Bril F**, Al Diffalha S, Dean M, Fettig DM. Autoimmune hepatitis developing after coronavirus disease 2019 (COVID-19) vaccine: Causality or casualty? *J Hepatol* 2021; **75**: 222-224 [PMID: [33862041](https://pubmed.ncbi.nlm.nih.gov/33862041/) DOI: [10.1016/j.jhep.2021.04.003](https://doi.org/10.1016/j.jhep.2021.04.003)]

## Basic Study

## Direct modulation of hepatocyte hepcidin signaling by iron

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## Abstract

## BACKGROUND

Liver-secreted hepcidin is the systemic master switch of iron homeostasis and decreased levels of hepcidin are considered to cause iron overload not only in hereditary hemochromatosis but also in hemolytic anemia and chronic liver diseases. The regulation of hepcidin is complex and its response to iron is still not completely understood.

## AIM

To study the direct effect of iron on various established hepcidin signaling pathways in hepatoma cells or primary hepatocytes.

## METHODS

Hepcidin mRNA expression was studied by quantitative real-time (qRT)-PCR in the presence of various forms of iron including ferric ammonium citrate (FAC) in hepatoma cells (Huh7), murine primary hepatocytes and an established co-culture model of phorbol myristate acetate-differentiated THP-1 monocytes and Huh7 cells. To analyze hepcidin signaling, the response to bone morphogenetic protein 6 (BMP6), interleukin (IL)-6, IL-1 $\beta$ , hypoxia and lipopolysaccharide (LPS) were studied. Hepcidin and small mothers against decapentaplegic 6 (SMAD6) mRNA levels were assessed by qRT-PCR and the expression of phosphorylated signal transducer and activator of transcription 3 (phospho-STAT3), STAT3, phospho-SMAD1/5/8 and SMAD1 proteins were analyzed by western blot.

## RESULTS

All iron III forms including FAC efficiently blocked hepcidin mRNA expression at non-toxic dosages in Huh7 cells or primary hepatocytes in a time and dose-



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dependent manner ( $P < 0.001$ ;  $P < 0.05$ ). Hepcidin blockage could be efficiently blunted by iron chelators salicylaldehyde isonicotinoyl hydrazone (SIH) and Desferal ( $P < 0.001$ ). FAC also inhibited BMP6, hypoxia, IL-1 $\beta$  and IL-6-mediated hepcidin induction ( $P < 0.001$ ;  $P < 0.001$ ;  $P < 0.05$ ;  $P < 0.001$ ), and FAC also inhibited LPS-mediated hepatic hepcidin induction in co-culture model ( $P < 0.001$ ). Moreover, FAC reduced SMAD6 mRNA and p-SMAD1/5/8 protein expression at basal or upon stimulation by BMP6 ( $P < 0.05$ ;  $P < 0.01$ ), and FAC also reduced SMAD6 and p-SMAD1/5/8 expression under hypoxia ( $P < 0.01$ ;  $P < 0.05$ ). However, FAC has no significant effect on p-STAT3 protein expression at basal or upon stimulation by various stimuli. Notably, in the presence of the BMP/SMAD signaling pathway inhibitor LDN193189 Hydrochloride (LDN), FAC was unable to further decrease hepcidin, SMAD6 and p-SMAD1/5/8 expression compared with LDN alone.

## CONCLUSION

Iron directly blocks hepatocellular hepcidin signaling through the BMP/SMAD pathway but independent of STAT3. This mechanism may contribute to continued iron overload in many pathophysiological conditions ultimately causing a vicious cycle of continued hepcidin suppression.

**Key Words:** Hepcidin/iron metabolism; Iron overload; Inflammation; Hypoxia; BMP/SMAD; STAT3

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**Core Tip:** Hepcidin is paradoxically and strongly suppressed during hemolytic iron overload. Although various upstream regulators of hepcidin have been discovered, the direct iron sensing mechanisms by hepcidin remain obscure. This study investigated the direct effect of iron on hepcidin signaling and for the first time to show that iron directly blocks hepcidin transcription *via* bone morphogenetic protein/small mothers against decapentaplegic but not the STAT3 signaling in various established *in vitro* models of hepcidin signaling.

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## INTRODUCTION

Excess iron causes cancer and severe tissue damage and chronic iron overload is not only driving the rather rare hereditary iron overload diseases but also secondary iron overload diseases due to hemolysis or common chronic liver diseases such as alcoholic liver disease or hepatitis C[1]. In most of these diseases, suppression of hepcidin, the systemic master switch of iron homeostasis in mammals, has been identified to play a key role. Hepcidin is primarily expressed in hepatocytes as a precursor pro-peptide and to a lesser extent in macrophages or cardiomyocytes[2-4]. It is regulated at the transcription side, and its mRNA levels correspond well with concentrations of the peptide[5]. By binding to and degrading the iron exporter ferroportin 1 (Fpn1) which is localized at the basolateral membranes of duodenal enterocytes, macrophages and hepatocytes[6], circulating hepcidin efficiently blocks iron absorption, iron recycling and iron storage[7,8]. Consequently, its overexpression leads to hypoferrremia and anemia[9], while the reduction of hepcidin levels causes iron overload[10,11].

The regulation of hepcidin is complex and the direct mechanisms of iron sensing are still not completely understood. Bone morphogenetic protein 6 (BMP6) released from endothelial cells (ECs) can efficiently induce hepcidin transcription *via* the SMAD pathway[12]. BMP6 binds to the BMP receptor on the liver cell membrane and its co-receptor hemojuvelin to promote the phosphorylation of the receptor-associated proteins small mothers against decapentaplegic (SMAD) 1/5/8. The latter interacts

with SMAD4 to form the SMAD complex, translocates into the nucleus and binds to the hepcidin promoter[13]. In addition, inflammation mediators (*e.g.*, IL-6, IL-1 $\beta$ , hypoxia or ROS/H<sub>2</sub>O<sub>2</sub>) can also induce hepcidin transcription by promoting the phosphorylation of STAT3 to initiate STAT3-mediated hepcidin signaling[14]. Cytokines namely IL-6 and microbial molecules such as lipopolysaccharide (LPS) represent an important evolutionary conserved mechanism during infection/inflammation to strongly induce hepatic hepcidin secretion leading to a rapid decrease of serum iron, which is thought to function as anti-bacterial defense mechanism[15]. More recently, the central redox signaling molecule H<sub>2</sub>O<sub>2</sub> has been also identified as a potent inducer of hepcidin[16] with hypoxia further enhancing hepcidin-expression *via* the STAT3 signaling pathway[17]. Further data suggest that intracellular oxidases such as NOX4 may play an important upstream role in controlling hepcidin *via* the STAT3 pathway[17].

C/EBP $\alpha$ , BMP6, SMAD 1, 5, 8 and 4, Tmprss6, IL-6, CREBH, CHOP and TLR4), an overall and conclusive regulatory network regarding the control of iron is not yet fully understood. This includes the experimental and clinical finding that hepcidin responds differentially to iron overload *in vitro* and *in vivo*[18-20]. Although recent data suggest important intercellular crosstalks *e.g.*, between hepatocytes and endothelial cells or macrophages[14,21-23], the direct iron sensing mechanisms by hepcidin remain obscure. It has been reported that Tfr1, ERFE or GDF15 overexpression contributes to iron overload by suppressing hepcidin *in vivo*[24-28]. However, there are examples that the seemingly paradox direct negative impact of iron on hepcidin, identified *in vitro*[19], may have direct clinical implications. For instance, in the most common human liver disease, alcoholic liver disease[29], hepatic iron overload is one of the key factors that drive the diseases and determine survival[30] with alcohol directly suppressing hepcidin[31]. In thalassemia, hepcidin is also strongly suppressed during hemolysis. While repetitive blood transfusions have been long thought to cause iron overload[32], a recently established thalassemia mouse model could demonstrate that hepatic iron overload occurs without additional blood supply through suppressed hepcidin levels[33].

These considerations prompted us to study the direct effect of iron in an *in vitro* setting on various established hepcidin signaling pathways including the BMP/SMAD signaling pathway and STAT3-mediated hepcidin signaling *via* cytokines, hypoxia, and LPS using a recently established macrophage-hepatocyte co-culture model[14]. Our data show that iron inhibits primarily the BMP/SMAD pathway but does not affect the STAT3 pathway. In conclusion, direct exposure of hepatocytes to pathophysiological iron deposits is a strong suppressor of BMP-mediated hepcidin signaling that could initiate a vicious cycle of continued hepcidin suppression.

## MATERIALS AND METHODS

### Cell culture

Huh7 cells from the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan) were grown under standard conditions using Dulbecco's modified Eagle medium (Sigma-Aldrich, Taufkirchen, Germany), 25 mmol/L glucose and 10% fetal calf serum under 210 mL/L O<sub>2</sub> (21% O<sub>2</sub>) and 50 mL/L CO<sub>2</sub> (5% CO<sub>2</sub>)[16]. Murine primary hepatocytes kindly provided by Dr. Sai Wang (University of Heidelberg, Germany) were grown under standard conditions using Williams' medium (Sigma-Aldrich, Taufkirchen, Germany), 10% fetal bovine serum, 1% P/S (Penicillin and Streptomycin), 1% L-Glutamine, 0.5% ITS (Insulin-Transferrin-Selenium), 0.1% Dexamethasone, and were seeded at a cell density of 2 x 10<sup>5</sup> cell/well in 12-well plates for experiment. The immortalized human monocyte THP-1 cells from the American Type Culture Collection (ATCC, Manassas, VA, United States) were grown in RPMI-1640 medium with 25 mmol/L glucose (Gibco, Thermo Fisher Scientific, Waltham, MA, United States) supplemented with 10% fetal bovine serum. THP-1 cells were seeded in 12-well plates and treated with phorbol myristate acetate (PMA) at 100 ng/mL for 24 h to induce differentiation. After differentiation, cells were washed and incubated in fresh media for 24 h before experiment[14].

### Chemicals and reagents

PMA, LPS, LDN, FAC, FeCl<sub>3</sub>, FC, FeSO<sub>4</sub>, Hemin, Desferal, human recombinant IL-6 were all purchased from Sigma-Aldrich. Ferrlecit (sodium ferric gluconate) was obtained from a commercial pharmacy in its retail packaging. Human recombinant IL-1 $\beta$  was purchased from Enzo Lifesciences (Lörrach, Germany) and human

recombinant BMP6 was purchased from R&D, Germany. SIH was a gift of Dr. P. Ponka (McGill University, Montreal, Canada).

### Macrophage differentiation and co-culture

THP-1 monocytes were differentiated to macrophages and co-cultured as described recently[14]. Briefly, THP-1 cells were seeded for differentiation with PMA (100 ng/mL) at a density of  $0.25 \times 10^5$  cells/well in 12-well plates. After 48 h of differentiation, Huh7 cells were seeded on the top of macrophages at a density of  $0.7 \times 10^5$  cells/well and incubated overnight for attachment. The co-culture was conditioned to LPS (0.5 µg/mL) and/or FAC (50 µmol/L) under 21% O<sub>2</sub> and 5% CO<sub>2</sub> for 24 h. Aiming at studying the effects of macrophage-conditioned medium, differentiated THP-1 macrophages were conditioned to LPS and/or to FAC for 24 h. Huh7 cells were exposed to the macrophage-conditioned medium for 24 h. In the co-culture experiments, a pathophysiological hepatocytes-to-macrophages ratio of 4 to 1 was used as described previously[14].

### Hypoxia experiments

Huh7 cells were seeded at a cell density of  $0.7 \times 10^5$  cell/well in 12-well plates. Huh7 cells were treated with or without FAC. Hypoxia was induced as described recently using a hypoxia chamber[14]. Briefly, cell culture plates were placed in the hypoxia chamber and flushed with a gas mixture of 1% O<sub>2</sub>, 5% CO<sub>2</sub> and 940 mL/L N<sub>2</sub> (94% N<sub>2</sub>) for 3 min and incubated at 37 °C for 24 h[16].

### RNA isolation, cDNA synthesis and quantitative real-time PCR analysis

Total RNA was isolated with Trifast (Peqlab biotechnology GmbH, Erlangen, Germany) according to the manufacturer specifications. Reverse transcription and quantitative real-time PCR (qRT-PCR) reactions were performed as previously described[16]. Primers and probes were designed using the Profinder software (Roche, Mannheim, Germany) and the sequences are shown in Table 1. Primarily, levels of hepcidin mRNA were assessed since they correspond well to the levels of the propeptide. The levels of secreted peptide are only used in clinical studies where liver biopsies are not available[5].

### Immunoblotting

Cells were washed in ice-cold 1xPBS and harvested in RIPA buffer plus 1 × Complete® protease inhibitor with EDTA (Roche Applied Sciences, Penzberg, Germany) on ice. Western Blotting was performed as described previously[16]. Following the transfer, the proteins immobilized on nitrocellulose membranes were incubated overnight with the antibodies anti-pSTAT3, anti-STAT3 (1:1000 dilution; Cell Signaling Technology, Frankfurt am Main, Germany); anti-pSMAD1/5/8, anti-SMAD1 (1:1000 dilution; Cell Signaling Technology, Frankfurt am Main, Germany) or anti-GAPDH (1:2000 dilution; Cell Signaling Technology, Frankfurt am Main, Germany). After incubation with the IRDye-conjugated 680 anti-mouse or 800 anti-rabbit antibodies (1:10000 dilutions; LI-COR, Inc., Lincoln, NE, United States), the membranes were scanned using an infrared imaging system (Odyssey CLx; LI-COR, Inc., Lincoln, NE, United States).

### Statistical analysis

All the data were expressed as mean ± SD. Significant differences ( $P < 0.05$ ) between means of data sets were assessed by one-way ANOVA with Tukey's test or two-way ANOVA with Sidak's test using GraphPad Prism 6 software.

## RESULTS

### Efficient suppression of hepatocellular hepcidin by higher iron levels

Although iron injection *in vivo* causes strong induction of hepcidin[34,35], direct exposure of isolated hepatoma cells or murine primary hepatocytes to various forms of iron causes an efficient suppression of hepcidin mRNA expression (Figure 1A and B;  $P < 0.001$  and  $P < 0.05$  vs control). The inhibiting effect of iron was observed over a wide concentration range (Supplementary Figure 1) and could be efficiently blocked by two iron chelators (SIH and Desferal) (Figure 1C;  $P < 0.001$  vs FAC group). While this “paradox” response towards iron may be explained by the absence of co-factors or other neighboring cells *in vitro*, the direct inhibition of hepcidin by iron may have important pathophysiological implications for hepatic iron overload in the context of

**Table 1 Primer list of the genes analyzed by quantitative real-time polymerase chain reaction**

Gene	Primer sequence
<i>human <math>\beta</math>2-mg</i>	forward: 5'-tga ctt tgt cac agc cca aga ta-3' reverse: 5'-aat cca aat gcg gca tct tc-3' probe: FAM-tga tgc tgc tta cat gtc tcg atc cca-TAM
<i>human GAPDH</i>	forward: 5'-gaa ggt gaa ggt cgg agt-3' reverse: 5'-gaa gat ggt gat ggg att tc-3' probe: FAM-caa gct tcc cgt tct cag cc-TAM
<i>human hepcidin</i>	forward 5'-cag gac aga gct gga gcc a-3' reverse: 5'-gca gca cat ccc aca ctt tg-3' probe: FAM-ctg ctg cgg ctg ctg tca tcg a-TAM
<i>human SMAD6</i>	forward: 5'-tgc aac ccc tac cac ttc a-3' reverse: 5'-cga gga gac agc cga gag t-3' probe UPL # 10 (Roche)
<i>mouse HPRT</i>	forward: 5'-ggt cca ttc cta tga ctg tag att tt-3' reverse: 5'-caa tca aga cgt tct ttc cag tt-3' probe UPL # 22 (Roche)

chronic liver diseases or due to hemolysis. We further demonstrate that the suppression of hepcidin mRNA expression is not due to toxic or subtoxic effects as even five times higher FAC concentration did not affect growth or cell division (see [Supplementary Figure 2A](#)). Moreover, a significant suppression of hepcidin mRNA expression by FAC was observed at 6 h and continued over the observed time interval of 24 h ([Supplementary Figure 2B](#);  $P < 0.001$  *vs* control). In summary, *in vitro* exposure of hepatocytes to high levels of iron suppresses hepcidin, which may have important pathophysiological implications by initiating a vicious iron overloading cycle. Further experiments were carried out with FAC as a standard model for iron exposure.

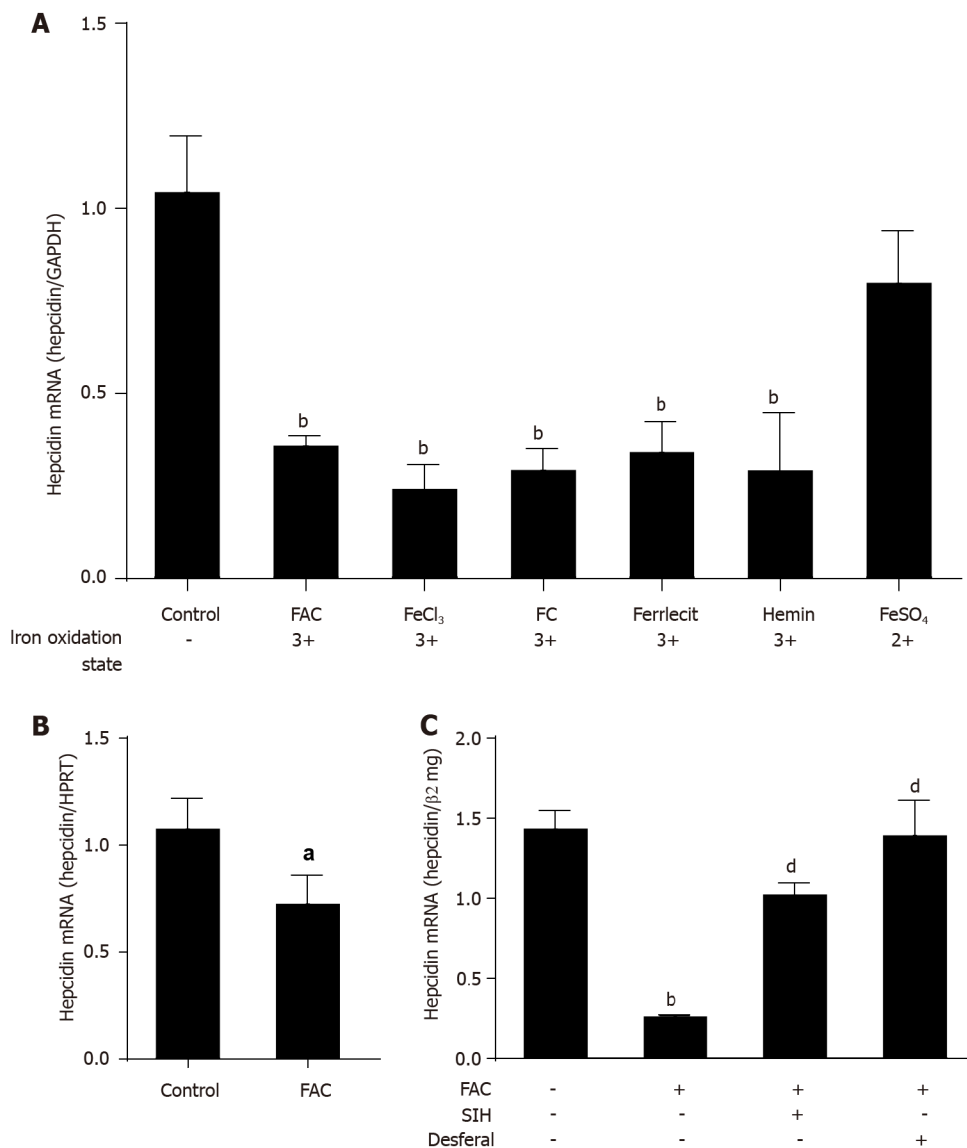
### **Iron efficiently blocks BMP6 to induce hepatocellular hepcidin**

We next studied the influence of iron (FAC) on BMP6-mediated hepcidin signaling, one of the major pathways in basal and iron-responsive expression of hepcidin. As shown in [Figure 2A](#), recombinant BMP6 efficiently increased hepcidin mRNA levels by almost four times ( $P < 0.001$  *vs* control). However, the presence of iron FAC not only blocked basal hepcidin expression under control conditions but completely inhibited BMP6-mediated hepcidin induction ([Figure 2A](#);  $P < 0.001$  *vs* BMP6 group). In fact, even in the presence of BMP6, FAC inhibited hepcidin mRNA levels by ca. 50% ([Figure 2A](#);  $P < 0.05$  *vs* control). Notably, BMP6 was unable to induce SMAD6 mRNA and p-SMAD1/5/8 protein expression under FAC conditions ([Figure 2B, C and D](#);  $P < 0.01$  *vs* BMP6 group), while no effect on p-STAT3 protein expression was seen ([Figure 2E and F](#)). In conclusion, *in vitro*, external iron has a profound inhibitory effect of basal hepcidin expression and completely abolished BMP6-mediated hepcidin signaling through SMAD but not the STAT3 pathway.

### **FAC inhibits hypoxia-mediated hepcidin induction in a STAT3-independent manner**

Recently, hypoxia and hydrogen peroxide have been identified as important modulators of hepcidin expression predominantly through the STAT3 pathway and involving oxidase enzymes of the NOX family[16,17]. To avoid direct interactions between iron and *e.g.*, peroxide, we therefore next focused on hypoxia to study the role of FAC in a STAT3-mediated hepcidin signaling. In confirmation of previous experiments[14], [Figure 3A](#) demonstrates that hypoxia is able to significantly increase hepcidin mRNA levels ( $P < 0.05$  *vs* normoxia control). However, hypoxia was unable to induce hepcidin mRNA expression under FAC conditions ([Figure 3A](#);  $P < 0.01$  *vs* normoxia control and  $P < 0.001$  *vs* hypoxia control). Expectedly, hypoxia did not have any significant effect on SMAD6 mRNA and p-SMAD1/5/8 protein expression ([Figure 3B, C and D](#)), but efficiently upregulated p-STAT3 protein expression as shown previously ([Figure 3E and F](#);  $P < 0.05$  *vs* normoxia control). In contrast, FAC



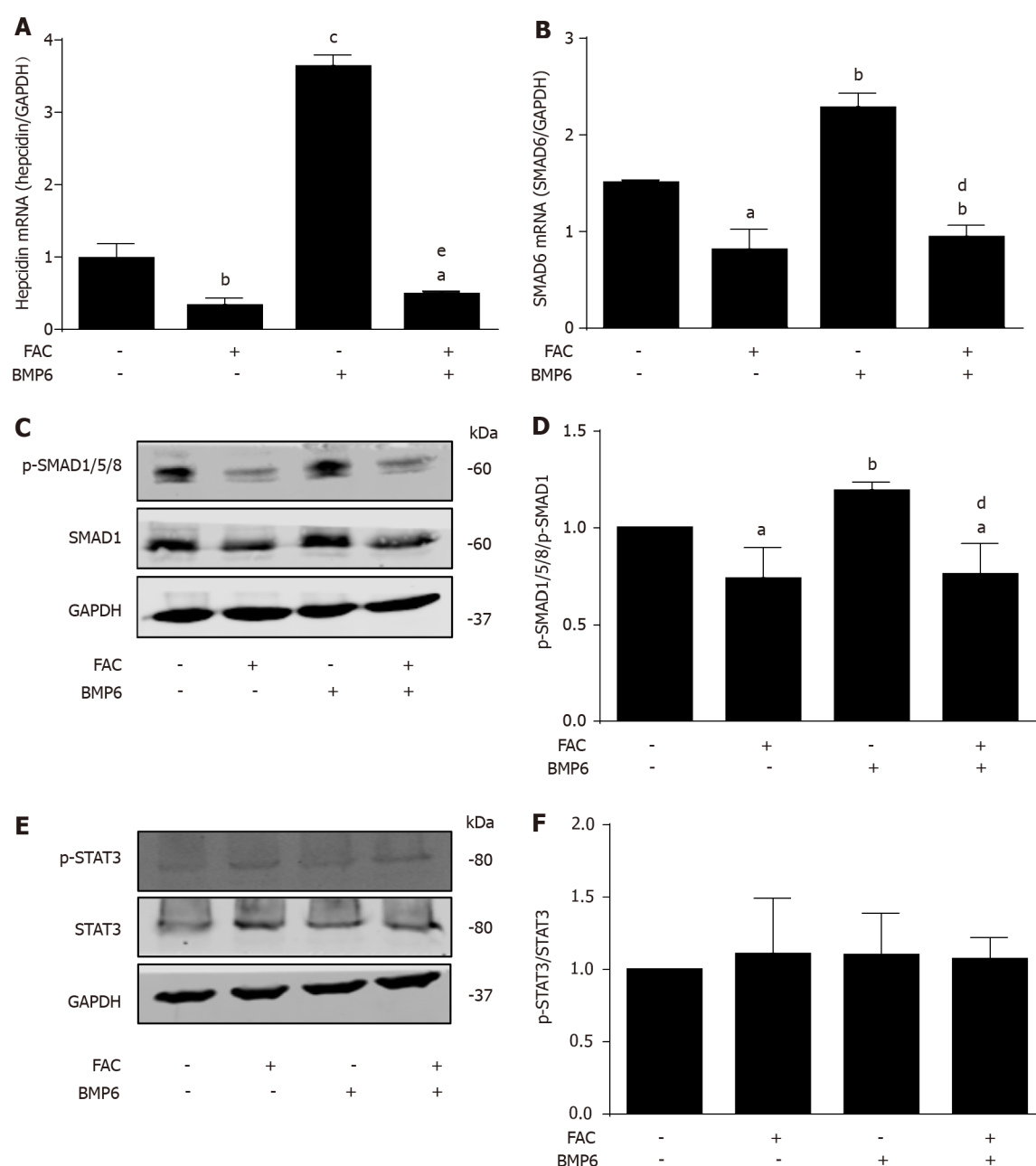


**Figure 1 Efficient suppression of hepcidin by higher iron levels.** A: Huh7 cells were treated with 50  $\mu$ mol/L of FAC, FeCl<sub>3</sub>, FC, ferrlecit, hemin or FeSO<sub>4</sub> for 24 h; B: Murine primary hepatocytes were treated with FAC (50  $\mu$ mol/L) for 24 h; C: Huh7 cells were treated with FAC (50  $\mu$ mol/L) in the presence or absence of SIH (100  $\mu$ mol/L) or Desferal (50  $\mu$ mol/L) for 24 h. Total RNA was extracted from Huh7 cells or murine primary hepatocytes. Hepcidin mRNA levels were determined by quantitative real-time PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase or hypoxanthine phosphoribosyltransferase or  $\beta$ 2mg. Data are presented as mean  $\pm$  SD. <sup>a</sup> $P$  < 0.05, <sup>b</sup> $P$  < 0.001 vs control; <sup>c</sup> $P$  < 0.001 vs FAC group. FAC: Ferric ammonium citrate; FeCl<sub>3</sub>: Ferric chloride; FC: Ferric citrate; FeSO<sub>4</sub>: Ferrous sulfate; SIH: Salicylaldehyde isonicotinoyl hydrazine; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase;  $\beta$ 2mg,  $\beta$ 2-microglobulin; HPRT: Hypoxanthine phosphoribosyltransferase.

still decreased SMAD6 mRNA and p-SMAD1/5/8 protein expression under hypoxia (Figure 3B, C and D;  $P$  < 0.01 and  $P$  < 0.05 vs hypoxia control), but had no effect on p-STAT3 protein expression even under hypoxia (Figure 3E and F). These results demonstrate that FAC also and primarily affects hepcidin even in a typical STAT3-signaling setting through basal modulation of the SMAD pathway.

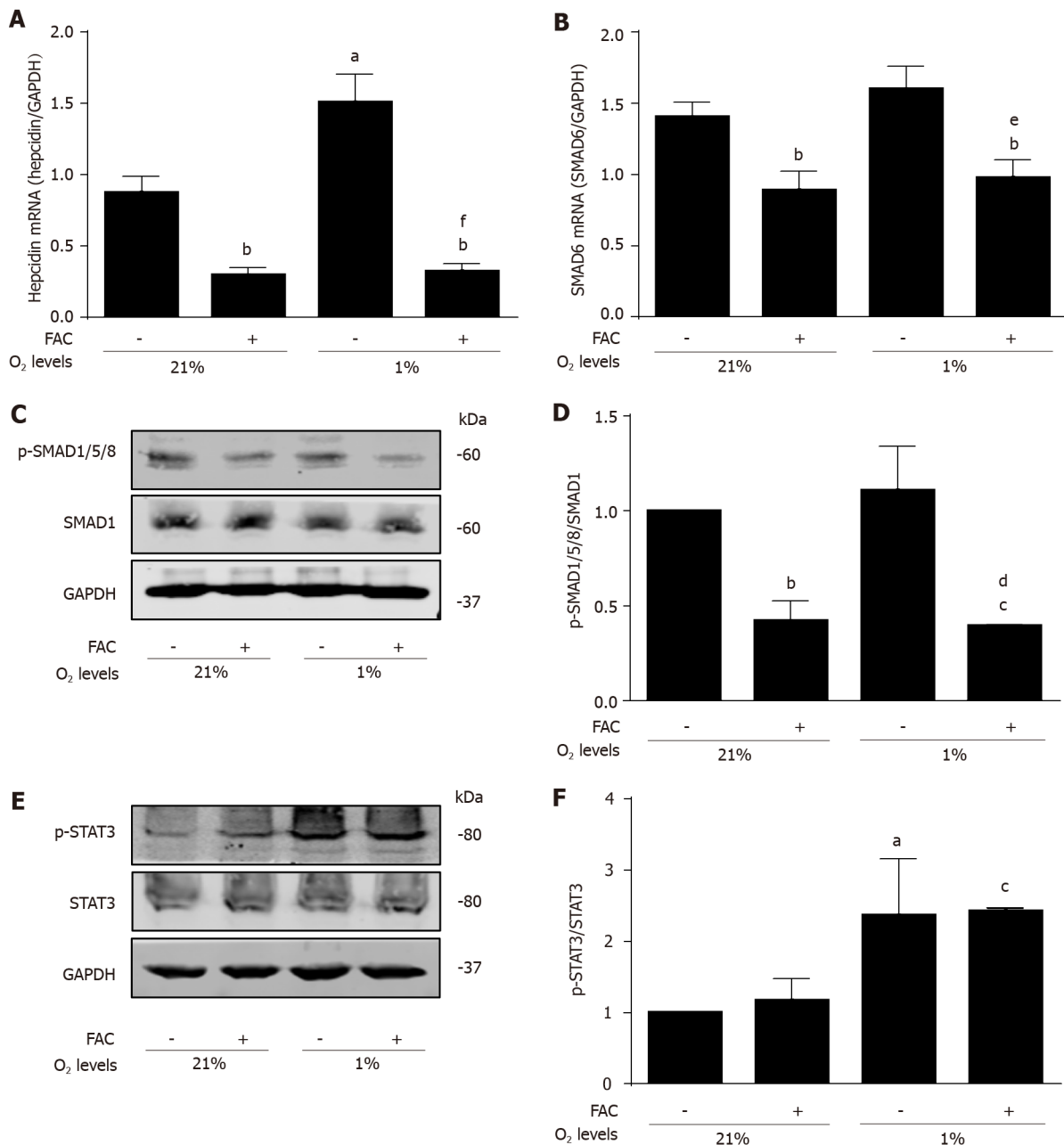
#### FAC efficiently blocks cytokine-mediated hepcidin expression

Cytokines such as IL-6 and IL-1 $\beta$  are important upstream regulators of hepcidin playing an important role in the so-called anemia of chronic disease response[36]. For instance, they are primarily responsible for the general hypoferrremia observed during infections[37,38]. To study the effect of iron on cytokine signaling, hepatoma cells were exposed to FAC and/or IL-1 $\beta$  or IL-6 *in vitro* for 24 h and hepcidin mRNA was assessed by qRT-PCR. As shown in Figure 4A and B, both cytokines efficiently increased hepcidin mRNA levels while FAC blocked IL-1 $\beta$ -mediated induction by about 50% and IL-6-mediated induction completely ( $P$  < 0.05 vs IL-1 $\beta$  group and  $P$  < 0.001 vs IL-6 group). FAC not only decreased the basal but also the SMAD6 mRNA and p-SMAD1/5/8 protein expression induced by IL-1 $\beta$  (see Supplemen-



**Figure 2 Ferric ammonium citrate profoundly blocks bone morphogenetic protein 6-mediated hepcidin signaling.** Huh7 cells were treated with or without bone morphogenetic protein 6 (BMP6) (40 ng/mL) in the presence or absence of ferric ammonium citrate (FAC) (50  $\mu$ mol/L) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC decreased the hepcidin mRNA expression in the presence or absence of BMP6; B: FAC decreased small mothers against decapentaplegic 6 (SMAD6) mRNA expression in the presence or absence of BMP6; C, D: FAC decreased p-SMAD1/5/8 protein expression in the presence or absence of BMP6; E, F: Both BMP6 and FAC have no significant effect on phosphorylated signal transducer and activator of transcription 3 (p-STAT3) protein expression. SMAD1, p-SMAD1/5/8, STAT3, p-STAT3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. Hepcidin and SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western Blots are representatives of three independent experiments. Data are presented as mean  $\pm$  SD. <sup>a</sup> $P$  < 0.05, <sup>b</sup> $P$  < 0.01, <sup>c</sup> $P$  < 0.001 vs control; <sup>d</sup> $P$  < 0.01, <sup>e</sup> $P$  < 0.001 vs BMP6 group. FAC: Ferric ammonium citrate; BMP6: Bone morphogenetic protein 6; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

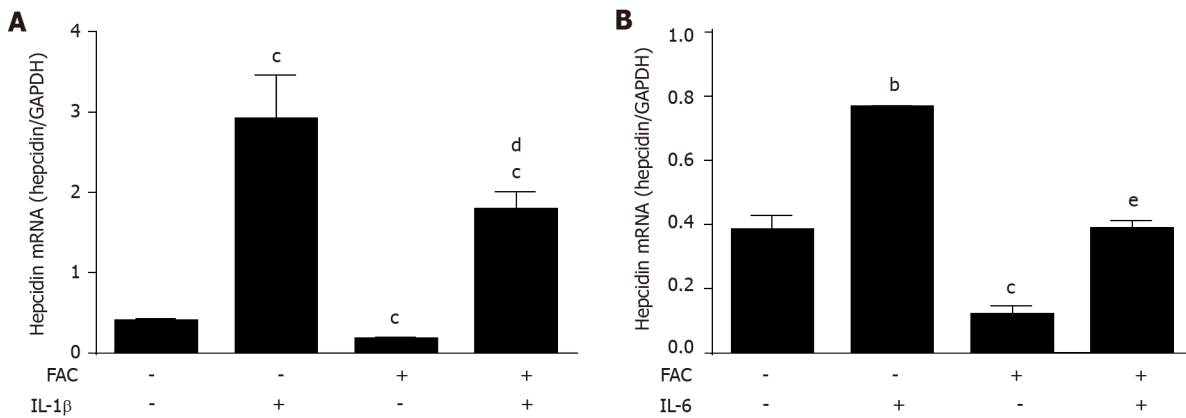
tary Figure 3A). FAC still decreased the SMAD6 mRNA and p-SMAD1/5/8 protein expression in the presence of IL-6 (see Supplementary Figure 4A). In addition, while both cytokines induced p-STAT3 protein expression (see Supplementary Figure 3B or Supplementary Figure 4B;  $P$  < 0.01 vs IL-1 $\beta$  group or IL-6 group), FAC had significant effect on p-STAT3 protein expression neither in the presence nor absence of IL-1 $\beta$  or IL-6. Notably, IL-6 was still able to induce hepcidin under FAC conditions (See Figure 4B). Taken together, these findings suggest that the presence of FAC significantly attenuates hepcidin response to cytokines, which is SMAD dependent but does not involve STAT3.



**Figure 3 Ferric ammonium citrate efficiently inhibits hypoxia-mediated hepcidin response independent of signal transducer and activator of transcription 3.** Huh7 cells were exposed to normoxia (210 mL/L O<sub>2</sub>, 21% O<sub>2</sub>) or hypoxia (10 mL/L O<sub>2</sub>, 1% O<sub>2</sub>) in the presence or absence of ferric ammonium citrate (FAC) (50 μmol/L) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC decreased the basal and hypoxia-induced hepcidin mRNA expression; B: Hypoxia has no obvious effect on small mothers against decapentaplegic 6 (SMAD6) mRNA expression, but FAC decreased SMAD6 mRNA expression in the presence or absence of hypoxia; C, D: Hypoxia has no significant effect on p-SMAD1/5/8 protein expression, while FAC decreased p-SMAD1/5/8 protein expression in the presence or absence of hypoxia; E, F: Hypoxia increased phosphorylated signal transducer and activator of transcription 3 (p-STAT3) protein expression, while FAC has no significant effect on p-STAT3 protein expression in the presence or absence of hypoxia. SMAD1, p-SMAD1/5/8, STAT3, p-STAT3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. Hepcidin and SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western Blots are representatives of three independent experiments. Data are presented as mean ± SD. <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, <sup>c</sup>*P* < 0.001 vs control (21% O<sub>2</sub>); <sup>d</sup>*P* < 0.05, <sup>e</sup>*P* < 0.01, <sup>f</sup>*P* < 0.001 vs control (1% O<sub>2</sub>). FAC: Ferric ammonium citrate; O<sub>2</sub>: oxygen; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

### Inhibition of hepatocellular hepcidin by FAC requires BMP/SMAD signaling

We next studied the role of BMP/SMAD signaling in the modulation of hepatocellular hepcidin by FAC using a BMP/SMAD signaling inhibitor LDN193189 (LDN)[39]. LDN suppressed the basal hepcidin mRNA expression (Figure 5A; *P* < 0.001 vs control), while FAC in combination with LDN could not further suppress hepcidin mRNA expression compared with LDN alone (Figure 5A). FAC in combination with LDN could not further suppress SMAD6 mRNA and p-SMAD1/5/8 protein expression compared with LDN alone (Figure 5B, C and D). Neither FAC nor LDN had a



**Figure 4 Ferric ammonium citrate efficiently blocks cytokine-mediated hepcidin expression.** Huh7 cells were treated with or without IL-1 $\beta$  (10 ng/mL) or IL-6 (10 ng/mL) in the presence or absence of ferric ammonium citrate (FAC) (50  $\mu$ mol/L) for 24 h. Total RNA was extracted from Huh7 cells. A: FAC significantly decreased IL-1 $\beta$ -induced hepcidin mRNA expression; B: FAC efficiently blocks IL-6-induced hepcidin mRNA expression. Hepcidin mRNA levels were determined by qRT-PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase. Data are presented as mean  $\pm$  SD. <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$  vs control; <sup>d</sup> $P < 0.05$ , <sup>e</sup> $P < 0.001$  vs IL-6 group. IL-1 $\beta$ : Interleukin 1 $\beta$ ; IL-6: Interleukin 6; FAC: Ferric ammonium citrate; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

significant effect on p-STAT3 protein expression (Figure 5E and F). In conclusion, these data suggest that the BMP/SMAD signaling is necessary for FAC to inhibit hepcidin expression.

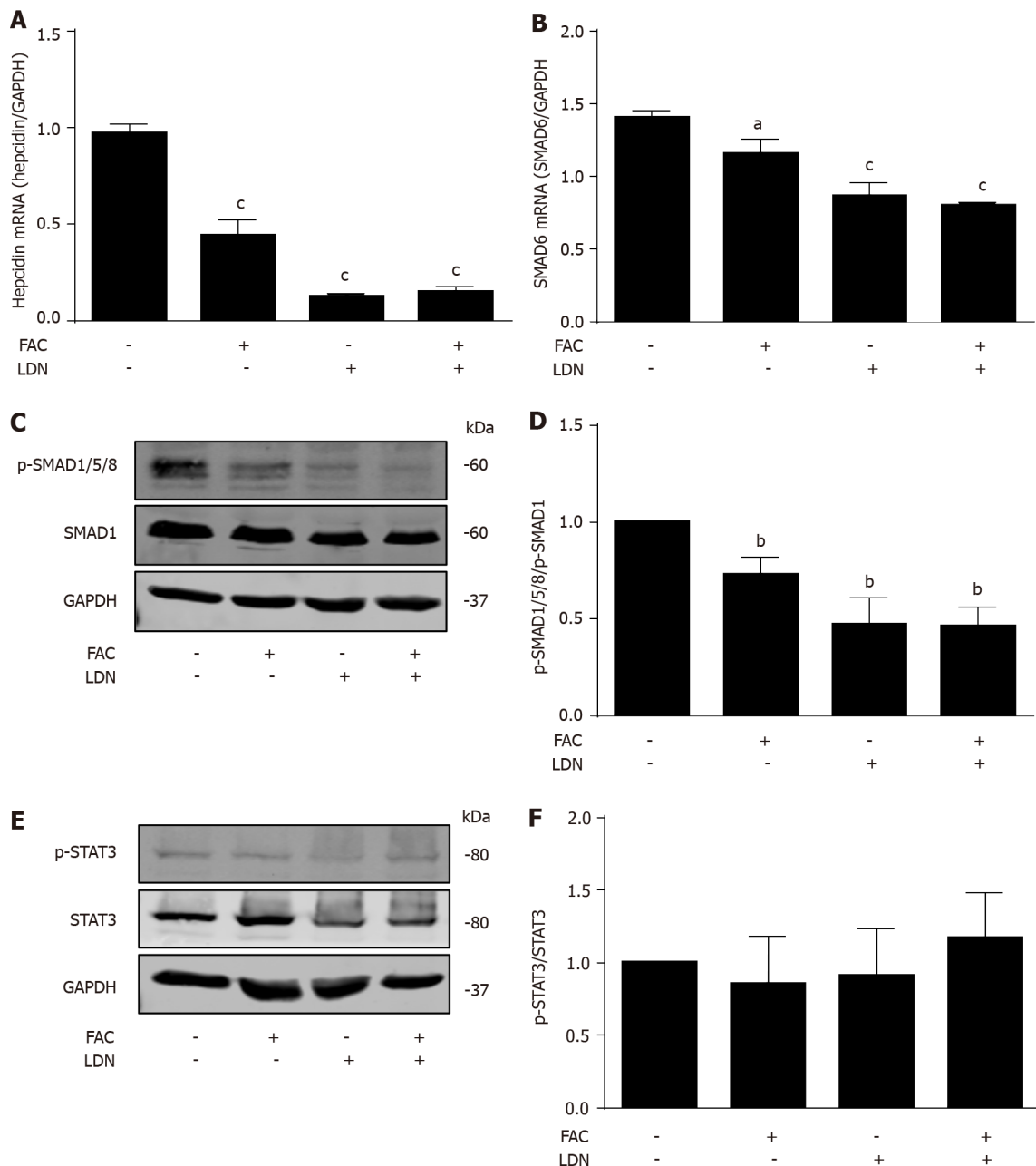
#### **FAC decreases hepatic hepcidin expression induced by LPS in a macrophage-hepatocyte co-culture model**

We finally studied the effect of FAC on a more complex and recently established co-culture model of macrophages and hepatocytes to mimic an inflammatory bacterial response by LPS under crosstalk conditions of both cell lines. Human THP-1 monocytes were differentiated into macrophages using PMA as described recently [40]. We examined the effect of LPS on hepatocellular hepcidin mRNA expression in the presence or absence of macrophages. A co-culture model of macrophages and hepatocytes was established according to the cell ratio of 4 to 1 of hepatocytes to macrophages in order to mimic pathophysiological cell ratios in the liver microenvironment[14]. In a normal experimental setting, THP-1 monocytes were differentiated with PMA for 24 h, washed with PBS, and then cultured in fresh medium for another 24h followed by co-cultivation for another 24h with huh7 cells. Huh7 cells were treated by LPS for 24h, and Huh7 cells were co-cultured with THP-1 macrophages in the presence of LPS or exposed to LPS-conditioned macrophage medium for 24 h. LPS slightly induced hepcidin mRNA expression in Huh7 cell monoculture. Co-culture with macrophages induced hepcidin mRNA expression (Figure 6A;  $P < 0.001$  vs Huh7 control), which was further enhanced by LPS (Figure 6A;  $P < 0.001$  vs co-culture control) in line with recent studies[14,41]. Notably, the effects of macrophages on hepcidin mRNA expression are even stronger than direct LPS-stimulation (Figure 6A;  $P < 0.001$  vs Huh7 LPS group). FAC also significantly decreased hepatic hepcidin mRNA expression in our co-culture model (see Figure 6B;  $P < 0.05$  vs control), and the presence of FAC also significantly attenuated the LPS-mediated expression of hepatic hepcidin mRNA in our co-culture model (see Figure 6B;  $P < 0.001$  vs LPS group). As demonstrated in Supplementary Figure 5A, FAC decreased the LPS-induced SMAD6 mRNA and p-SMAD1/5/8 protein expression ( $P < 0.05$  vs LPS group). Moreover, LPS induced p-STAT3 protein expression (see Supplementary Figure 5B;  $P < 0.05$  vs control), while FAC had no significant effect on p-STAT3 (see Supplementary Figure 5B). Similar results to the directly co-culture model were also observed by using the macrophage-conditioned medium (data not shown). In conclusion, iron also significantly blocks hepcidin expression in a more complex macrophage-hepatocyte co-culture model upon LPS stimulation in SMAD but not STAT3 dependent fashion.

## **DISCUSSION**

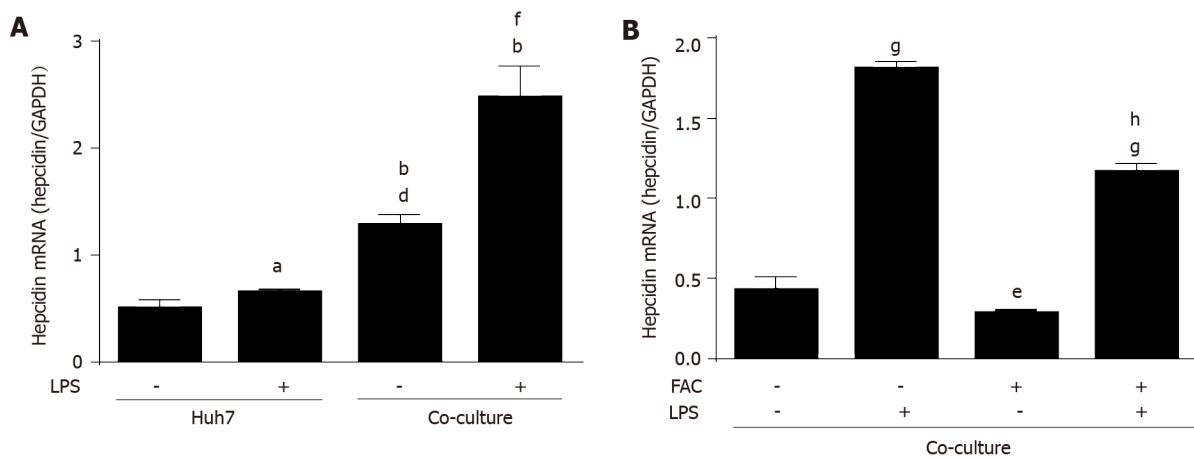
We here show that iron suppresses hepatocellular hepcidin signaling directly under *in*





**Figure 5 Inhibition of hepatocellular hepcidin by ferric ammonium citrate requires bone morphogenetic protein/small mothers against decapentaplegic signaling.** Huh7 cells were treated with or without ferric ammonium citrate (FAC) (50  $\mu\text{mol/L}$ ) in the presence or absence of LDN193189 Hydrochloride (LDN) (20 nmol/L) for 24 h. Total RNA and protein were extracted from Huh7 cells. A: FAC or LDN decreased the basal hepcidin mRNA expression, but FAC in combination with LDN did not further suppress hepcidin mRNA expression compared with LDN alone; B-D: FAC or LDN decreased the basal small mothers against decapentaplegic (SMAD)6 mRNA and p-SMAD1/5/8 protein expression, but FAC in combination with LDN did not further suppress SMAD6 and p-SMAD1/5/8 expression compared with LDN alone; E, F: Both FAC and LDN had no significant effect on phosphorylated signal transducer and activator of transcription 3 (p-STAT3) protein expression. SMAD1, p-SMAD1/5/8, STAT3, p-STAT3 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein levels were determined by Western blotting. Hepcidin and SMAD6 mRNA levels were determined by qRT-PCR, normalized to GAPDH. Western Blots are representatives of three independent experiments. Data are presented as mean  $\pm$  SD. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$  vs control. FAC: Ferric ammonium citrate; LDN: LDN193189 Hydrochloride; p-: Phospho-; SMAD: Small mothers against decapentaplegic; STAT3: Signal transducer and activator of transcription 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

*vitro* conditions. By exploring several established *in vitro* models of hepcidin signaling, we further demonstrate that this direct inhibitory effect of iron on hepcidin transcription unanimously affects the BMP-SMAD pathway but not the STAT3 pathway. Since iron-mediated blockage of hepcidin mRNA expression is also observed in primary hepatocytes at higher iron dosages and can be prevented by iron chelators, we suggest that this mechanism could contribute to hepcidin suppression in various iron overload diseases including hemolytic iron overload.



**Figure 6 Ferric ammonium citrate decreases hepatic hepcidin expression induced by lipopolysaccharide in a macrophage-hepatocyte co-culture model.**

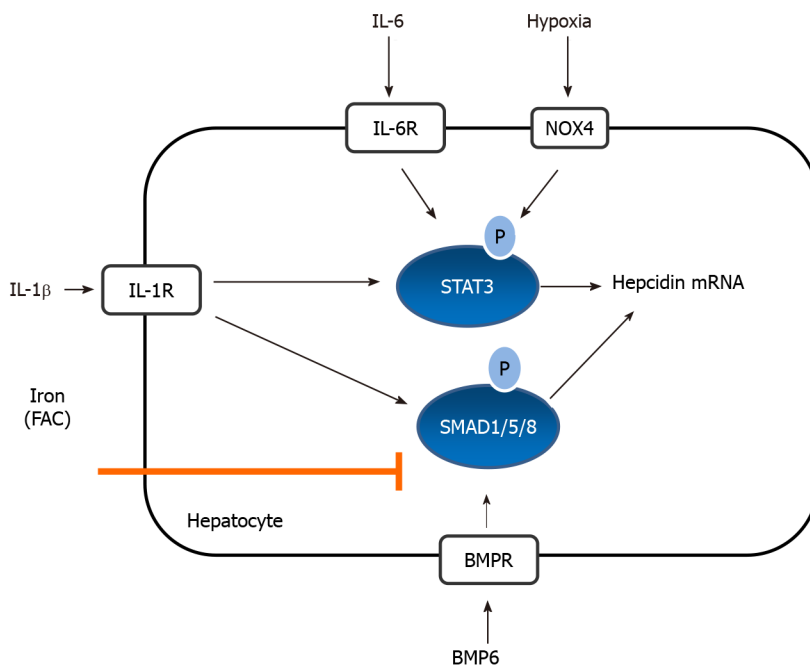
Huh7 cells were treated with or without lipopolysaccharide (LPS) (500 ng/mL) for 24 h. Huh7 cells were directly co-cultured with THP-1 macrophages according to pathophysiological macrophage/hepatocyte cell ratio (1:4) and then treated with or without LPS (500 ng/mL) for 24 h in the presence or absence of ferric ammonium citrate (FAC) (50  $\mu$ mol/L). Total RNA was extracted from Huh7 cells or Huh7 cells and THP-1 macrophages. A: Hepcidin mRNA levels were slightly increased by LPS in monoculture of Huh7 cells, and macrophages increased hepcidin mRNA levels compared with monoculture control and the presence of LPS further markedly increased hepcidin mRNA levels; B: FAC decreased the basal and LPS-induced hepcidin mRNA levels in the co-culture model. Hepcidin mRNA levels were determined by qRT-PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase. Data are presented as mean  $\pm$  SD. <sup>a</sup> $P$  < 0.05, <sup>b</sup> $P$  < 0.001 vs Huh7 control; <sup>d</sup> $P$  < 0.001 vs Huh7 LPS group; <sup>e</sup> $P$  < 0.05, <sup>f</sup> $P$  < 0.01, <sup>g</sup> $P$  < 0.001 vs co-culture control; <sup>h</sup> $P$  < 0.001 vs co-culture LPS group. LPS: Lipopolysaccharide; FAC: Ferric ammonium citrate.

Although not widely gained attention, it has already been known for many years that hepatocellular hepcidin rapidly loses its responsiveness to iron under cultured conditions[19,41]. While this could be due to the loss of serum factors, the “*in vivo* liver microenvironment”, altered oxygen conditions or loss of metabolic demand *ex vivo*, the absence of an essential intercellular crosstalk could be another explanation. Namely with the identification of the BMP6-SMAD pathway, the role of endothelial released BMP6 has been identified as a major upstream event of the hepcidin response [23,26]. Indeed, and also shown here, exposure of cultured hepatocytes to recombinant BMP6 is able to efficiently recover the hepcidin response.

On the other hand, such paradox responses of hepcidin towards iron levels have been also well documented in patients with severe thalassemia. These patients show pronounced hemolytic anemia and require repeated blood transfusion[32]. Patients with severe disease typically show progressive liver damage and cirrhosis due to serious iron toxicity[42]. The recent establishment of a murine thalassemia model clearly demonstrates that hepatic iron overload occurs also in the absence of additional blood supply under continued hemolysis-mediated suppression of hepcidin[33].

The mechanisms behind this hepcidin suppression in hemolytic diseases are still controversially discussed. Erythropoietin (EPO) has been proposed as an important factor although the underlying mechanisms are not completely understood and cannot be recapitulated by direct exposure of hepatocytes to EPO[43]. The recent identification of bone marrow-derived erythroferrone (ERFE) and Growth Differentiation Factor-15 (GDF15) in response to EPO stimulation suggests that these factors at least partly contribute to hepcidin suppression during hemolysis[28,44-46]. However, our data on the direct inhibiting effect of iron on hepcidin signaling *in vitro* suggest that iron *per se* could also contribute to hepcidin suppression.

Chronic liver diseases represent another important model of chronic iron overload and ca. 50% of chronic liver diseases show hepatic iron overload with an inadequate hepcidin response[30]. While primary liver damage either through alcohol damage or viral replication could account for the total loss of hepcidin response[47-49], iron itself could also play a regulatory role. In our various *in vitro* models of hepcidin signaling, we here demonstrate that iron efficiently blocks hepcidin response primarily through the SMAD pathway. Although this seems rather counteractive towards the iron-mediated BMP-hepcidin response, this experiment deserves serious consideration especially during pathophysiological conditions such as severe hemolysis or damage to the liver sinus-endothelial layer. It may explain why continued hepatic iron overload would initiate a vicious cycle of hepcidin suppression and further iron uptake through the duodenal brush border[50]. It would also implicate that besides pharmacological approaches to re-introduce hepcidin or increase hepcidin peptide



**Figure 7 Scheme of iron-mediated blockage of hepcidin transcription via bone morphogenetic protein/small mothers against decapentaplegic but independent of signal transducer and activator of transcription 3 signaling.** Iron (ferric ammonium citrate) primarily blocks hepcidin transcription via the bone morphogenetic protein (BMP)/small mothers against decapentaplegic pathway while no effect on signal transducer and activator of transcription 3 signaling was observed. The scheme also shows all studied hepcidin signaling pathways including BMP6, interleukin (IL)-6, IL-1 $\beta$ , hypoxia or a complex co-culture model with macrophages. IL-1 $\beta$ : Interleukin 1 $\beta$ ; IL-6: Interleukin 6; BMP6: Bone morphogenetic protein 6; FAC: Ferric ammonium citrate; IL-1R: IL-1 receptor; IL-6R: IL-6 receptor; NOX4: NADPH Oxidase 4; BMPR: BMP receptor; p-STAT3: Phosphorylated signal transducer and activator of transcription 3; p-SMAD1/5/8: Phosphorylated small mothers against decapentaplegic 1/5/8.

levels (e.g., mini hepcidins), removal of iron remains the cornerstone of the treatment. Not only would it remove the primary toxic agent iron but it would interrupt the suppressing effect of hepcidin on iron. It may also stimulate a mechanistic discussion on the therapeutic usage of iron chelators *vs* phlebotomy.

Although our data clearly show an exclusive effect of *in vitro* iron on the SMAD signaling cascade, the direct molecular mechanisms still remain elusive. Notably, hepcidin signaling was inhibited by iron in all explored models including the co-culture model with macrophages. Even in primary STAT3-mediated processes such as cytokines, hypoxia or LPS, iron efficiently blocked hepcidin transcription underlining the important role of the SMAD pathway for basal hepcidin expression. In line with this is the observation that efficient SMAD blockage by the SMAD inhibitor LDN could not be further enhanced by iron. Second, experiments with membrane permeable or non-permeable iron chelators (SIH or Desferal) show that iron chelators efficiently counteract the inhibitory effect of iron on hepcidin. Although do not provide definite answers to the underlying mechanisms of the iron-mediated hepcidin inhibition, the almost immediate effect restricted to the SMAD pathway and the fact that only oxidized forms of iron are effective suggests to us that iron may directly act through the BMP receptor or associated molecules such as Tfr1 or Tfr2[30].

On a final note, we were surprised not to see any interaction of iron with the STAT3 pathway. Since STAT3 is responsive to peroxide and iron and H<sub>2</sub>O<sub>2</sub> are known for decades to chemically interfere *via* the Fenton chemistry[30], it would have been no surprise to see direct effects on hepcidin transcription. However, it remains open whether compensating mechanisms exist to counteract decreased peroxide levels e.g. by upregulating oxidases *etc*.

In summary, to our knowledge, this work is the first to show that iron directly blocks hepcidin transcription, at baseline or upon stimulation by different stimuli, through the BMP/SMAD but not STAT3 signaling *in vitro*. A summarizing scheme is shown in Figure 7. We think that in addition to potential hepcidin suppressing factors such as GDF15 or ERF, iron could directly block hepcidin transcription under conditions of either excess iron or a liver endothelial fenestration with larger access to the hepatocellular membrane. Specifically under pathological conditions such as severe hemolysis or chronic iron overload as observed in alcoholic liver disease, this novel mechanism may contribute to further iron overload and initiate a vicious cycle.

To interrupt this cycle, the removal of iron should be the most efficient therapeutic goal. It will not be an easy task to validate this concept in *in vivo* models since iron levels in the direct environment of hepatocytes are not easy to quantitate.

## CONCLUSION

In conclusion, iron including FAC per se, directly blocks hepcidin transcription and the inhibitory effect could be observed over a large concentration range involving all forms of iron-III, which was not caused by toxicity or inhibition of cell growth. FAC has a profound inhibitory effect on hepcidin expression at baseline or upon stimulation by stimuli in various cell models, which was controlled through the BMP/SMAD pathway but independent of STAT3. We suggest that this mechanism may contribute to continued iron overload in many pathophysiological conditions ultimately causing a vicious cycle of continued hepcidin suppression. Anyway, this study provides a new idea for in-depth exploration of iron overload diseases and provides an experimental basis for the underlying therapeutic goal.

## ARTICLE HIGHLIGHTS

### Research background

Excess iron causes cancer and severe tissue damage and chronic iron overload is not only driving the rather rare hereditary iron overload diseases but also secondary iron overload diseases due to hemolysis or common chronic liver diseases such as alcoholic liver disease or hepatitis C. In most of these diseases, suppression of hepcidin, the systemic master switch of iron homeostasis in mammals, has been identified to play a key role. Hepcidin is primarily expressed in hepatocytes as a precursor pro-peptide and to a lesser extent in macrophages or cardiomyocytes. Elevated hepcidin causes hypoferremia and anemia by efficiently blocking iron absorption, iron recycling and iron storage by binding to and degrading the major iron export pump ferroportin 1.

### Research motivation

The direct iron sensing mechanisms by hepcidin remain obscure and seemingly paradox response of hepcidin have been observed in various clinical scenarios. Thus, direct intravenous injection of iron causes rapid induction of hepcidin, iron release in the context of hemolytic diseases such as thalassemia efficiently block hepcidin expression and cause further detrimental iron accumulation. Moreover, it still remains largely unexplained why hepatocellular hepcidin is downregulated under *in vitro* conditions. These observations prompted us to study in detail the direct effect of iron in cultured hepatocytes.

### Research objectives

The authors here aimed to study the direct effect of iron on various established hepcidin signaling pathways including the bone morphogenetic protein (BMP)/small mothers against decapentaplegic (SMAD) signaling pathway and signal transducer and activator of transcription 3 (STAT3)-mediated hepcidin signaling *via* cytokines, hypoxia, and lipopolysaccharide (LPS) using a recently established macrophage-hepatocyte co-culture model.

### Research methods

Hepcidin mRNA expression in presence of various forms of iron was studied, using hepatoma cells (Huh7), murine primary hepatocyte and a co-culture model of phorbol myristate acetate-differentiated THP-1 monocytes and hepatoma cells. The response to BMP6, interleukin (IL)-6, IL-1 $\beta$ , hypoxia and LPS were studied in order to analyze hepcidin signaling. Hepcidin and SMAD6 mRNA levels were assessed and the expression of phospho-STAT3, STAT3, phospho-SMAD1/5/8 and SMAD1 proteins were analyzed.

### Research results

All iron III forms including ferric ammonium citrate efficiently blocked hepcidin mRNA expression at non-toxic dosages in hepatoma cells or primary hepatocytes. Using iron chelators, the blockage of hepcidin by iron could be efficiently blunted. Iron



also had a profound inhibitory effect of basal hepcidin expression and completely abolished BMP6-mediated hepcidin signaling through SMAD but not the STAT3 pathway. Iron also and primarily affected hepcidin even in a typical STAT3-signaling setting through basal modulation of the SMAD pathway and iron significantly attenuated hepcidin response to cytokines, which is SMAD dependent but does not involve STAT3. In the co-culture model, iron inhibited LPS-mediated hepcidin induction.

### Research conclusions

In conclusion, iron directly blocks hepatocellular hepcidin transcription involving all forms of iron III and the effect was not caused by toxicity or reduced cell growth. Iron also inhibits hepcidin upregulation in various models of hepcidin stimulation primarily through the BMP/SMAD pathway but independent of STAT3 signaling. We propose that this mechanism may contribute to continued iron overload at least under pathophysiological conditions of iron release ultimately causing a vicious cycle of continued hepcidin suppression and further iron overload.

### Research perspectives

This study provides a new concept for better understanding the seemingly paradox response of hepcidin in *in vivo* and *in vitro* settings. Moreover, understanding the direct inhibitory effects of iron on hepcidin signaling at the hepatocellular side could help to identify novel molecular targets for future therapies.

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## REFERENCES

- 1 Abergel A, Ruivard M, Bonny C. [Iron overload and chronic liver diseases]. *Rev Prat* 2006; **56**: 2130-2134 [PMID: 17416050]
- 2 Sow FB, Florence WC, Satoskar AR, Schlesinger LS, Zwilling BS, Lafuse WP. Expression and localization of hepcidin in macrophages: a role in host defense against tuberculosis. *J Leukoc Biol* 2007; **82**: 934-945 [PMID: 17609338 DOI: 10.1189/jlb.0407216]
- 3 Papanikolaou G, Pantopoulos K. Systemic iron homeostasis and erythropoiesis. *IUBMB Life* 2017; **69**: 399-413 [PMID: 28387022 DOI: 10.1002/iub.1629]
- 4 Lakhal-Littleton S. Cardiomyocyte hepcidin: From intracellular iron homeostasis to physiological function. *Vitam Horm* 2019; **110**: 189-200 [PMID: 30798812 DOI: 10.1016/bs.vh.2019.01.009]
- 5 Détivaud L, Nemeth E, Boudjema K, Turlin B, Troadec MB, Leroyer P, Ropert M, Jacquelinet S, Courselaud B, Ganz T, Brissot P, Loral O. Hepcidin levels in humans are correlated with hepatic iron stores, hemoglobin levels, and hepatic function. *Blood* 2005; **106**: 746-748 [PMID: 15797999 DOI: 10.1182/blood-2004-12-4855]
- 6 Ganz T. Systemic iron homeostasis. *Physiol Rev* 2013; **93**: 1721-1741 [PMID: 24137020 DOI: 10.1152/physrev.00008.2013]
- 7 Ward DM, Kaplan J. Ferroportin-mediated iron transport: expression and regulation. *Biochim Biophys Acta* 2012; **1823**: 1426-1433 [PMID: 22440327 DOI: 10.1016/j.bbamcr.2012.03.004]
- 8 Ross SL, Tran L, Winters A, Lee KJ, Plewa C, Foltz I, King C, Miranda LP, Allen J, Beckman H, Cooke KS, Moody G, Sasu BJ, Nemeth E, Ganz T, Molineux G, Arvedson TL. Molecular mechanism of hepcidin-mediated ferroportin internalization requires ferroportin lysines, not tyrosines or JAK-STAT. *Cell Metab* 2012; **15**: 905-917 [PMID: 22682226 DOI: 10.1016/j.cmet.2012.03.017]
- 9 Nicolas G, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, Sirito M, Sawadogo M, Kahn A, Vaulont S. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *Proc Natl Acad Sci U S A* 2002; **99**: 4596-4601 [PMID: 11930010 DOI: 10.1073/pnas.072632499]
- 10 Nicolas G, Bennoun M, Devaux I, Beaumont C, Grandchamp B, Kahn A, Vaulont S. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proc Natl Acad Sci U S A* 2001; **98**: 8780-8785 [PMID: 11447267 DOI: 10.1073/pnas.151179498]
- 11 Roetto A, Papanikolaou G, Politou M, Alberti F, Girelli D, Christakis J, Loukopoulos D, Camaschella C. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nat Genet* 2003; **33**: 21-22 [PMID: 12469120 DOI: 10.1038/ng1053]
- 12 Andriopoulos B Jr, Corradini E, Xia Y, Faasse SA, Chen S, Grgurevic L, Knutson MD, Pietrangolo A, Vukicevic S, Lin HY, Babitt JL. BMP6 is a key endogenous regulator of hepcidin expression and

- iron metabolism. *Nat Genet* 2009; **41**: 482-487 [PMID: 19252486 DOI: 10.1038/ng.335]
- 13 **Core AB**, Canali S, Babitt JL. Hemojuvelin and bone morphogenetic protein (BMP) signaling in iron homeostasis. *Front Pharmacol* 2014; **5**: 104 [PMID: 24860505 DOI: 10.3389/fphar.2014.00104]
- 14 **Silva I**, Peccerella T, Mueller S, Rausch V. IL-1 beta-mediated macrophage-hepatocyte crosstalk upregulates hepcidin under physiological low oxygen levels. *Redox Biol* 2019; **24**: 101209 [PMID: 31108461 DOI: 10.1016/j.redox.2019.101209]
- 15 **Weiss G**, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; **352**: 1011-1023 [PMID: 15758012]
- 16 **Millonig G**, Ganzleben I, Peccerella T, Casanovas G, Brodziak-Jarosz L, Breitkopf-Heinlein K, Dick TP, Seitz HK, Muckenthaler MU, Mueller S. Sustained submicromolar H<sub>2</sub>O<sub>2</sub> Levels induce hepcidin via signal transducer and activator of transcription 3 (STAT3). *J Biol Chem* 2012; **287**: 37472-37482 [PMID: 22932892 DOI: 10.1074/jbc.M112.358911]
- 17 **Silva I**, Rausch V, Peccerella T, Millonig G, Seitz HK, Mueller S. Hypoxia enhances H<sub>2</sub>O<sub>2</sub>-mediated upregulation of hepcidin: Evidence for NOX4-mediated iron regulation. *Redox Biol* 2018; **16**: 1-10 [PMID: 29459227 DOI: 10.1016/j.redox.2018.02.005]
- 18 **Ramos E**, Kautz L, Rodriguez R, Hansen M, Gabayan V, Ginzburg Y, Roth MP, Nemeth E, Ganz T. Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. *Hepatology* 2011; **53**: 1333-1341 [PMID: 21480335 DOI: 10.1002/hep.24178]
- 19 **Nemeth E**, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood* 2003; **101**: 2461-2463 [PMID: 12433676 DOI: 10.1182/blood-2002-10-3235]
- 20 **Trombini P**, Paolini V, Pelucchi S, Mariani R, Nemeth E, Ganz T, Piperno A. Hepcidin response to acute iron intake and chronic iron loading in dysmetabolic iron overload syndrome. *Liver Int* 2011; **31**: 994-1000 [PMID: 21733088 DOI: 10.1111/j.1478-3231.2011.02520.x]
- 21 **Takayama G**, Taniguchi A, Okano T. Identification of differentially expressed genes in hepatocyte/endothelial cell co-culture system. *Tissue Eng* 2007; **13**: 159-166 [PMID: 17518589 DOI: 10.1089/ten.2006.0143]
- 22 **Canali S**, Zumbrennen-Bullough KB, Core AB, Wang CY, Nairz M, Bouley R, Swirski FK, Babitt JL. Endothelial cells produce bone morphogenetic protein 6 required for iron homeostasis in mice. *Blood* 2017; **129**: 405-414 [PMID: 27864295 DOI: 10.1182/blood-2016-06-721571]
- 23 **Silvestri L**, Nai A, Dulja A, Pagani A. Hepcidin and the BMP-SMAD pathway: An unexpected liaison. *Vitam Horm* 2019; **110**: 71-99 [PMID: 30798817 DOI: 10.1016/bs.vh.2019.01.004]
- 24 **Li H**, Choesang T, Bao W, Chen H, Feola M, Garcia-Santos D, Li J, Sun S, Follenzi A, Pham P, Liu J, Zhang J, Ponka P, An X, Mohandas N, Fleming RE, Rivella S, Li G, Ginzburg YZ. Decreasing TfR1 expression reverses anemia and hepcidin suppression in  $\beta$ -thalassemic mice. *Blood* 2017; **129**: 1514-1526 [PMID: 28151426 DOI: 10.1182/blood-2016-09-742387]
- 25 **El-Gamal RAE**, Abdel-Messih IY, Habashy DM, Zaiema SEG, Pessar SA. Erythroferrone, the new iron regulator: evaluation of its levels in Egyptian patients with beta thalassemia. *Ann Hematol* 2020; **99**: 31-39 [PMID: 31834456 DOI: 10.1007/s00277-019-03882-w]
- 26 **Arezes J**, Foy N, McHugh K, Sawant A, Quinkert D, Terraube V, Brintha A, Tam M, LaVallie ER, Taylor S, Armitage AE, Pasricha SR, Cunningham O, Lambert M, Draper SJ, Jasuja R, Drakesmith H. Erythroferrone inhibits the induction of hepcidin by BMP6. *Blood* 2018; **132**: 1473-1477 [PMID: 30097509 DOI: 10.1182/blood-2018-06-857995]
- 27 **Kim A**, Nemeth E. New insights into iron regulation and erythropoiesis. *Curr Opin Hematol* 2015; **22**: 199-205 [PMID: 25710710 DOI: 10.1097/MOH.0000000000000132]
- 28 **Tanno T**, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, Moroney JW, Reed CH, Luban NL, Wang RH, Eling TE, Childs R, Ganz T, Leitman SF, Fucharoen S, Miller JL. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. *Nat Med* 2007; **13**: 1096-1101 [PMID: 17721544 DOI: 10.1038/nm1629]
- 29 World Health Organization. Global status report on alcohol and health. Available from [https://www.who.int/substance\\_abuse/publications/alcohol\\_2014/en](https://www.who.int/substance_abuse/publications/alcohol_2014/en)
- 30 **Mueller S**, Rausch V. The role of iron in alcohol-mediated hepatocarcinogenesis. *Adv Exp Med Biol* 2015; **815**: 89-112 [PMID: 25427903 DOI: 10.1007/978-3-319-09614-8\_6]
- 31 **Harrison-Findik DD**, Klein E, Evans J, Gollan J. Regulation of liver hepcidin expression by alcohol *in vivo* does not involve Kupffer cell activation or TNF-alpha signaling. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G112-G118 [PMID: 19008338 DOI: 10.1152/ajpgi.90550.2008]
- 32 **Wahidiyat PA**, Iskandar SD, Sekarsari D. Evaluation of Iron Overload Between Age Groups Using Magnetic Resonance Imaging and Its Correlation with Iron Profile in Transfusion-dependent Thalassemia. *Acta Med Indones* 2018; **50**: 230-236 [PMID: 30333273]
- 33 **Liu J**, Liu W, Liu Y, Miao Y, Guo Y, Song H, Wang F, Zhou H, Ganz T, Yan B, Liu S. New thiazolidinones reduce iron overload in mouse models of hereditary hemochromatosis and  $\beta$ -thalassemia. *Haematologica* 2019; **104**: 1768-1781 [PMID: 30792208 DOI: 10.3324/haematol.2018.209874]
- 34 **Burden RJ**, Pollock N, Whyte GP, Richards T, Moore B, Busbridge M, Srai SK, Otto J, Pedlar CR. Effect of Intravenous Iron on Aerobic Capacity and Iron Metabolism in Elite Athletes. *Med Sci Sports Exerc* 2015; **47**: 1399-1407 [PMID: 25386711 DOI: 10.1249/MSS.0000000000000568]
- 35 **Wang JW**, Xu LH, Yao X, Fang JP. [Expression Changes of Hepcidin and Ferroportin 1 in Murine Model of Iron Overload]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2017; **25**: 936-940 [PMID: 28641663 DOI: 10.7534/j.issn.1009-2137.2017.03.054]

- 36 **Matsumura I**, Kanakura Y. [Pathogenesis of anemia of chronic disease]. *Nihon Rinsho* 2008; **66**: 535-539 [PMID: [18326322](#)]
- 37 **Moran-Lev H**, Weisman Y, Cohen S, Deutsch V, Cipok M, Bondar E, Lubetzky R, Mandel D. The interrelationship between hepcidin, vitamin D, and anemia in children with acute infectious disease. *Pediatr Res* 2018; **84**: 62-65 [PMID: [29795199](#) DOI: [10.1038/s41390-018-0005-0](#)]
- 38 **Queiroz DM**, Rocha AM, Melo FF, Rocha GA, Teixeira KN, Carvalho SD, Bittencourt PF, Castro LP, Crabtree JE. Increased gastric IL-1 $\beta$  concentration and iron deficiency parameters in *H. pylori* infected children. *PLoS One* 2013; **8**: e57420 [PMID: [23451225](#) DOI: [10.1371/journal.pone.0057420](#)]
- 39 **Cuny GD**, Yu PB, Laha JK, Xing X, Liu JF, Lai CS, Deng DY, Sachidanandan C, Bloch KD, Peterson RT. Structure-activity relationship study of bone morphogenetic protein (BMP) signaling inhibitors. *Bioorg Med Chem Lett* 2008; **18**: 4388-4392 [PMID: [18621530](#) DOI: [10.1016/j.bmcl.2008.06.052](#)]
- 40 **Matak P**, Chaston TB, Chung B, Srai SK, McKie AT, Sharp PA. Activated macrophages induce hepcidin expression in HuH7 hepatoma cells. *Haematologica* 2009; **94**: 773-780 [PMID: [19454498](#) DOI: [10.3324/haematol.2008.003400](#)]
- 41 **Montosi G**, Corradini E, Garuti C, Barelli S, Recalcati S, Cairo G, Valli L, Pignatti E, Vecchi C, Ferrara F, Pietrangeli A. Kupffer cells and macrophages are not required for hepatic hepcidin activation during iron overload. *Hepatology* 2005; **41**: 545-552 [PMID: [15726660](#) DOI: [10.1002/hep.20620](#)]
- 42 **Hershko C**. Pathogenesis and management of iron toxicity in thalassemia. *Ann N Y Acad Sci* 2010; **1202**: 1-9 [PMID: [20712765](#) DOI: [10.1111/j.1749-6632.2010.05544.x](#)]
- 43 **Pasricha SR**, McHugh K, Drakesmith H. Regulation of Hepcidin by Erythropoiesis: The Story So Far. *Annu Rev Nutr* 2016; **36**: 417-434 [PMID: [27146013](#) DOI: [10.1146/annurev-nutr-071715-050731](#)]
- 44 **Ganz T**. Erythropoietic regulators of iron metabolism. *Free Radic Biol Med* 2019; **133**: 69-74 [PMID: [29981834](#) DOI: [10.1016/j.freeradbiomed.2018.07.003](#)]
- 45 **Sasaki Y**, Noguchi-Sasaki M, Yasuno H, Yorozu K, Shimonaka Y. Erythropoietin stimulation decreases hepcidin expression through hematopoietic activity on bone marrow cells in mice. *Int J Hematol* 2012; **96**: 692-700 [PMID: [23160767](#) DOI: [10.1007/s12185-012-1217-4](#)]
- 46 **Kautz L**, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet* 2014; **46**: 678-684 [PMID: [24880340](#) DOI: [10.1038/ng.2996](#)]
- 47 **Varghese J**, James JV, Sagi S, Chakraborty S, Sukumaran A, Ramakrishna B, Jacob M. Decreased hepatic iron in response to alcohol may contribute to alcohol-induced suppression of hepcidin. *Br J Nutr* 2016; **115**: 1978-1986 [PMID: [27080262](#) DOI: [10.1017/S0007114516001197](#)]
- 48 **Fujita N**, Sugimoto R, Takeo M, Urawa N, Mifuji R, Tanaka H, Kobayashi Y, Iwasa M, Watanabe S, Adachi Y, Kaito M. Hepcidin expression in the liver: relatively low level in patients with chronic hepatitis C. *Mol Med* 2007; **13**: 97-104 [PMID: [17515961](#) DOI: [10.2119/2006-00057.Fujita](#)]
- 49 **Gao YH**, Wang JY, Liu PY, Sun J, Wang XM, Wu RH, He XT, Tu ZK, Wang CG, Xu HQ, Niu JQ. Iron metabolism disorders in patients with hepatitis B-related liver diseases. *World J Clin Cases* 2018; **6**: 600-610 [PMID: [30430115](#) DOI: [10.12998/wjcc.v6.i13.600](#)]
- 50 **Bergamaschi G**, Di Sabatino A, Pasini A, Ubezio C, Costanzo F, Grataroli D, Masotti M, Alvisi C, Corazza GR. Intestinal expression of genes implicated in iron absorption and their regulation by hepcidin. *Clin Nutr* 2017; **36**: 1427-1433 [PMID: [27729173](#) DOI: [10.1016/j.clnu.2016.09.021](#)]

## Basic Study

## Serum zonulin levels in patients with liver cirrhosis: Prognostic implications

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## Abstract

## BACKGROUND

Increased gut permeability and bacterial translocation play an important role in liver cirrhosis. Zonulin is a recently recognized protein involved in the disintegration of the intestinal barrier.

## AIM

To investigate possible differences in serum zonulin levels among patients with different cirrhosis stages and their potential prognostic implications.

## METHODS

Consecutive cirrhotic patients who attended our liver clinic were included in the study. Serum zonulin levels, clinical, radiological and biochemical data were collected at baseline. Patients who accepted participation in a regular surveillance program were followed-up for at least 12 mo.

## RESULTS

We enrolled 116 cirrhotics [mean Child-Turcotte-Pugh (CTP) score:  $6.2 \pm 1.6$ ; model for end-stage liver disease score:  $11 \pm 3.9$ ]. The causes of cirrhosis were viral hepatitis (39%), alcohol (30%), non-alcoholic fatty liver disease (17%), and other (14%). At baseline, 53% had decompensated cirrhosis, 48% had ascites, and 32% had history of hepatic encephalopathy. Mean zonulin levels were significantly higher in patients with CTP-B class than CTP-A class ( $4.2 \pm 2.4$  ng/dL vs  $3.5 \pm 0.9$  ng/dL,  $P = 0.038$ ), with than without ascites ( $P = 0.006$ ), and



**Institutional review board**

**statement:** The study was reviewed and approved by the Greek Committee for the Protection of Personal Data (approval No. 1990) after the official request of the “Laiko” General Hospital Athens Attiki (Laiko Hospital Institutional Review Board Decision: 712/8/5/2017).

**Conflict-of-interest statement:** The authors declare having no real or potential conflicts to disclose.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [jvlavhog@hotmail.com](mailto:jvlavhog@hotmail.com). Consent was not obtained but the presented data are anonymized and risk of identification is low.

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with than without history of encephalopathy ( $P = 0.011$ ). Baseline serum zonulin levels were independently associated with the probability of decompensation at 1 year ( $P = 0.039$ ), with an area under the receiving operating characteristic of 0.723 for predicting hepatic decompensation. Higher CTP score ( $P = 0.021$ ) and portal vein diameter ( $P = 0.022$ ) were independent predictors of mortality.

**CONCLUSION**

Serum zonulin levels are higher in patients with more advanced chronic liver disease and have significant prognostic value in identifying patients who will develop decompensation.

**Key Words:** Zonulin; Cirrhosis; Intestinal barrier; Bacterial translocation; Decompensation; Permeability

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**Core Tip:** Zonulin is a protein that appears to play a significant role in gut barrier integrity. Increased zonulin levels and deregulation of intestinal permeability have been demonstrated in patients suffering from celiac disease or type 2 diabetes. However, the role of zonulin as a promoting factor of intestinal barrier disruption in patients with liver cirrhosis has not been studied adequately. We evaluated serum zonulin levels in patients with different stages of advanced liver disease. According to our findings, serum zonulin levels are increased in patients with more advanced liver disease and are independently associated with progression to decompensation.

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**INTRODUCTION**

Bacterial translocation (BT) is defined as the passage of viable endogenous bacteria and endotoxins from the intestinal lumen through the mucosa into the mesenteric lymph nodes and other organs[1]. In patients with liver disease, BT has been demonstrated to play a pivotal role on the occurrence or aggravation of serious complications[2]. Bacterial overgrowth, decreased intestinal peristalsis with concomitant increased permeability, as well as immunological alterations that have been found in patients with chronic liver diseases appear to be the main causative factors of BT[3-6]. Among them, the exact pathophysiological mechanism leading to increased intestinal permeability is the most difficult to investigate and remains to be thoroughly explained.

Recently, Fasano[7] identified zonulin, a novel 47-kDa protein precursor of haptoglobin-2 (pre-HP2), which is synthesized by the intestinal and liver cells and may play a significant role in disruption of the gut barrier. Evidence exist to support that small intestine epithelial cells exposed to enteric bacteria, secrete zonulin, which in turn attaches to special receptors located on the membrane of intestinal epithelial cells, leading to a disconnection of occludin from ZO-1. This disrupts the tight junctions and consequently increases the gut permeability[8].

Currently, the connection between increased zonulin levels and the deregulation of intestinal permeability has been observed in patients suffering from celiac disease, type 2 diabetes, obesity and inflammatory bowel disease (IBD)[9-13]. However, the role of zonulin and its possible involvement in the dysfunction of intestinal barrier function in patients with cirrhosis has not been studied thoroughly.

The aim of our study was to assess the serum zonulin levels in patients with cirrhosis and investigate their possible impact on patients' prognosis.

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## MATERIALS AND METHODS

Over a period of 12 mo (February 2017-January 2018), all cirrhotic patients, aged from 18 years to 80 years, who attended our outpatient liver clinics were considered eligible for inclusion in the study, regardless of the etiology and severity of their liver disease. We excluded patients with alcoholic hepatitis, porto-splenic vein thrombosis, non-cirrhotic portal hypertension, hepatocellular carcinoma (HCC), transjugular intrahepatic portosystemic shunt (TIPS), chronic kidney disease, celiac disease, acute infection, IBD, or any other chronic intestinal disease.

The diagnosis of cirrhosis was based on clinical and laboratory findings, imaging studies or liver histology, when available. All patients had liver stiffness measurement (LSM) of  $\geq 14$  kPa (by elastography). At baseline, all patients underwent abdominal ultrasound with spleen and portal diameter measurements and baseline LSM and spleen stiffness measurement (SSM) by shear wave elastography (SWE). In addition, all patients underwent clinical examination and laboratory testing every 3 mo, and abdominal ultrasound every 6 mo.

The study protocol was approved by the Ethics Committee of “Laiko” General Hospital of Athens, Greece. A written consent was obtained from each patient with respect to all ethical guidelines issued by the 2000 revision (Edinburgh) of the 1975 Declaration of Helsinki.

### Clinical and laboratory data

Clinical and laboratory data, routine blood parameters, including platelet count, prothrombin time, serum albumin, serum creatinine, international normalized ratio (INR), serum aspartate aminotransferase, alanine aminotransferase, and bilirubin, were measured at the time of patient enrollment. Likewise, the existence of ascites or hepatic encephalopathy (HE) was noted. The severity of liver disease was determined by Child-Turcotte-Pugh (CTP) scoring, and the model for end-stage liver disease (MELD) score calculated according to the UNOS formula. Study end-points included death, liver transplantation and liver decompensation in patients with compensated cirrhosis at baseline.

### Two-dimensional SWE

All patients underwent LSM and SSM by two-dimensional (2D)-SWE performed by a single experienced operator (> 500-exam experience) in fasting patients. The Aixplorer® ultrasound system (Supersonic Imagine S.A., Aix-en-Provence, France) with an abdominal 3.5 MHz curved array probe was used, as recommended. 2D-SWE measurements were performed at each patient's initial assessment. Ten reliable LSM and ten reliable SSM values were obtained from each patient and the mean values were then calculated respectively. The SD was < 20% of the mean values of LSM and SSM, respectively.

### Sample collection-zonulin measurement

A venous blood sample was collected from each patient, with or without precooled anticoagulant (heparinized/EDTA)-coated tube. The serum or plasma was then separated from the blood by centrifugation at 3000 rpm for 10 min at room temperature. The samples were stored at -80 °C.

Serum levels of zonulin were measured using an enzyme-linked immune-sorbent assay (Immundiagnostik AG, Bensheim, Germany); the sensitivity of the assay was 0.01 ng/mL.

### Statistical analysis

Statistical analysis was performed by SPSS V23 (IBM Corp., Armonk, NY, United States). Data were expressed as frequencies, mean with SD, or median with interquartile range, as appropriate. Quantitative variables were compared with Student's *t*-test or Mann-Whitney test for normally distributed and non-normally distributed variables, respectively. Qualitative variables were compared with chi-squared test or Fisher's exact test, as appropriate. The relationship between parameters was assessed by using Spearman's correlation coefficient. Multivariate logistic regression analysis models were used to identify independent, significant, predictive factors of a poor outcome. Only parameters with a significant or a trend for significant associations ( $P < 0.10$ ) with the dependent variable in the univariate analysis being included in the multivariate analysis models. The area under the receiving operating characteristic (AUROC) curves for zonulin predictability, as well as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were

calculated. The c-statistics of AUROC curves were provided with their 95% confidence intervals (CIs). Diagnostic accuracy was considered to be poor when a c-statistic was 0.65-0.75, good when a c-statistic was 0.76-0.85, and excellent when a c-statistic was > 0.85. The optimal cut-off was selected from the AUROC curves as the point which provided the maximum sum of sensitivity and specificity. All tests were two-sided and *P* values < 0.05 were considered to be significant.

## RESULTS

In total, 127 consecutive cirrhotic patients were initially assessed. Eleven patients were excluded, due to HCC (*n* = 5), acute infection (*n* = 4) or portal vein thrombosis (*n* = 2). Therefore, 116 patients were finally included in the study. Mean age was  $59 \pm 13$  years, and 71 (61.2%) were male. Viral hepatitis was the main cause of liver disease (38.8%). Compensated and decompensated liver disease were marginally equally distributed in our cohort, while a significant proportion of patients had ascites at the time of enrollment. Esophageal or gastric varices were documented in 65 (55.2%) of the patients and 60 (51.7%) were under treatment with b-blockers. Patient characteristics are presented in Table 1.

Compared to patients with compensated liver disease, those with decompensated liver disease had significantly lower platelet counts ( $106 \pm 37 \times 10^9/L$  vs  $137 \pm 55 \times 10^9/L$ , *P* = 0.006), higher INR values ( $1.3 \pm 0.28$  vs  $1.2 \pm 0.2$ , *P* = 0.003) and lower albumin levels ( $3.5 \pm 0.5$  g/dL vs  $4.8 \pm 0.6$  g/dL, *P* < 0.001) as well as higher MELD ( $12.6 \pm 4.1$  vs  $9.2 \pm 1.6$ , *P* < 0.001) and CTP scores ( $7 \pm 1.7$  vs  $5.3 \pm 0.5$ , *P* < 0.001).

### Zonulin levels

Mean serum zonulin levels were  $3.6 \pm 1.5$  ng/dL. Patients with CTP-B had significantly higher serum zonulin levels compared to those with CTP-A cirrhosis ( $4.2 \pm 2.4$  ng/dL vs  $3.5 \pm 0.9$  ng/dL, *P* = 0.038). On the other hand, patients with CTP-C cirrhosis had lower levels of serum zonulin compared to the two other groups. Specifically, CTP-C patients had lower levels of zonulin than CTP-A ( $2.6 \pm 0.7$  ng/dL vs  $3.5 \pm 0.9$  ng/dL, *P* = 0.035) or CTP-B patients, although the latter difference did not reach statistical significance ( $2.6 \pm 0.7$  ng/dL vs  $4.2 \pm 2.4$  ng/dL, *P* = 0.157) (Figure 1).

Serum zonulin levels were higher in patients with than without ascites ( $4.16$  ng/dL vs  $3.26$  ng/dL, *P* = 0.006). Similarly, patients with a history of HE had higher zonulin levels compared to those without history of HE ( $4.17$  ng/dL vs  $3.39$  ng/dL, *P* = 0.011). The presence of varices was also associated with numerically higher levels of zonulin but this difference did not reach statistical significance (Figure 2).

No significant correlation was observed between serum zonulin levels and platelets, serum albumin, bilirubin, INR, MELD score, age or body mass index. Moreover, treatment with b-blockers was not found to affect the levels of zonulin (patients on treatment:  $3.6 \pm 1.5$  ng/dL vs no treatment with b-blockers:  $3.4 \pm 0.9$  ng/dL, *P* = 0.513).

### Follow-up

Sixty-three out of the one-hundred and sixteen patients were followed for at least 12 mo or until death/Liver transplantation, whichever occurred first. Their mean age was  $60 \pm 15$  years and 30 (48%) were male. The majority of patients (*n* = 36, 57%) had compensated cirrhosis at baseline. Forty-four (69.8%) patients had CTP-A and nineteen had CTP-B (30.2%) cirrhosis. Mean MELD score was  $11.3 \pm 3.2$ . Thirty-nine (61.9%) patients had no varices or small varices without red spots and twenty-four (38.1%) patients had high-risk varices (large varices or small varices with red spots). Twenty of the twenty-seven (74%) patients with decompensated cirrhosis had ascites at baseline. No patient with compensated liver disease who was in the follow-up group was under rifaximin treatment, while 8/27 patients with decompensated disease were receiving rifaximin. Patients with decompensated cirrhosis receiving rifaximin on baseline and followed up for at least 12 mo showed numerically higher serum zonulin levels at baseline, though not statistically significant (patients on rifaximin treatment:  $4.49 \pm 2.37$  ng/dL vs no rifaximin treatment:  $3.41 \pm 1.08$  ng/dL, *P* = 0.144).

Specific treatment was received by 32 (50.8%) of the 63 patients. Among them, mean baseline LSM was  $22.9 \pm 9.3$  kPa and mean baseline SSM was  $35.3 \pm 8.6$  kPa.

Twelve (33.3%) of the thirty-six patients with compensated cirrhosis at baseline progressed to decompensated disease [11/36 (30.5%) developed ascites and 1/36 (2.8%) developed variceal bleeding]. Patients who progressed to liver decompensation (*n* = 12) had higher baseline serum zonulin levels at ( $3.98 \pm 0.79$  ng/dL vs  $3.18 \pm 1.02$  ng/dL, *P* = 0.011) and lower albumin levels ( $3.64 \pm 0.53$  g/dL vs  $4.10 \pm 0.51$  g/dL, *P* =

**Table 1** Baseline clinical and laboratory characteristics of the patients

Examined parameter	Baseline value
Sex as M/F, <i>n</i> (%)	71/45 (61.2)
Age in yr <sup>1</sup>	59 ± 13
BMI, kg/m <sup>2</sup>	27.5 ± 5.0
Liver disease etiology, <i>n</i> (%)	
Chronic hepatitis B	25 (21.6)
Chronic hepatitis C	20 (17.2)
Alcohol abuse	35 (30.2)
NAFLD	20 (17.2)
Autoimmune hepatitis	5 (4.3)
Other	11 (9.5)
CTP class, <i>n</i> (%)	
A	78 (67.2)
B	33 (28.4)
C	5 (4.4)
CTP score	6.2 ± 1.6
MELD score	11.0 ± 3.9
Decompensated cirrhosis, <i>n</i> (%)	61 (52.6)
History of HE, <i>n</i> (%)	37 (31.9)
Ascites, <i>n</i> (%)	56 (48.3)
Bilirubin in mg/dL	1.3 ± 0.9
Creatinine in mg/dL	1.0 ± 1.2
Albumin in g/L	41.0 ± 4.0
Platelet count as × 10 <sup>9</sup> /L	121 ± 49
INR	1.3 ± 0.3

<sup>1</sup>Quantitative variables are expressed as mean ± SD.

BMI: Body mass index; CTP: Child-Turcotte-Pugh; F: Female; HE: Hepatic encephalopathy; INR: International normalized ratio; M: Male; MELD: Model for end-stage liver disease; NAFLD: Nonalcoholic fatty liver disease.

0.013) as well as a trend for lower platelet counts ( $104 \times 10^9/L$  vs  $138 \times 10^9/L$ ,  $P = 0.094$ ) and higher SSM ( $36.1 \pm 9.3$  kPa vs  $31.1 \pm 7.4$  kPa,  $P = 0.087$ ) compared to patients who remained compensated during follow-up (Table 2). In multivariate logistic regression analysis, progression to liver decompensation within 12 mo was independently associated with higher serum zonulin [odds ratio (OR): 6.53, 95%CI: 1.08-39.57,  $P = 0.041$ ] and lower albumin at baseline (OR: 0.03, 95%CI: 0.002-0.92,  $P = 0.044$ ). Baseline serum zonulin levels offered an AUROC of 0.723 ( $P = 0.039$ ) for predicting development of decompensation within 1 year (Figure 3). The cut-off point that could better predict progression to decompensation was 3.65 ng/dL, with specificity 73%, sensitivity 73%, NPV 84% and PPV 57%.

In total, 7 (11.3%) patients died (6 due to liver related causes and 1 due to non-liver related malignancy), while 2 patients (2.9%) underwent liver transplantation. Patients who died or underwent liver transplantation ( $n = 9$ ) had lower baseline albumin levels compared to patients ( $n = 54$ ) who survived ( $3.20 \pm 0.62$  g/dL vs  $3.87 \pm 0.62$  g/dL,  $P = 0.010$ ), higher CTP score ( $7.4$  vs  $5.9$ ,  $P < 0.001$ ) and greater portal vein diameter ( $1.55$  cm vs  $1.27$  cm,  $P = 0.002$ ) (Table 3). In multivariate logistic regression analysis, higher CTP score (OR: 2.06, 95%CI: 1.02-4.16,  $P = 0.021$ ) and portal vein diameter (OR: 71.54, 95%CI: 1.56-329.52,  $P = 0.022$ ) were independently associated with mortality.



**Table 2 Univariate analysis of factors associated to liver disease decompensation**

Examined parameter, baseline values	Patients who remained compensated during follow-up, <i>n</i> = 24	Patients who proceed to decompensated disease during follow-up, <i>n</i> = 12	<i>P</i> value
Sex as M/F	13/11	3/9	0.157
Age in yr <sup>1</sup>	59 ± 12	61 ± 14	0.710
Liver-specific treatment, Y/N	13/11	9/3	0.292
High-grade varices, Y/N	6/24	6/12	0.157
Platelet count as × 10 <sup>9</sup> /L	138 ± 54	105 ± 46	0.094
Albumin in g/dL	3.64 ± 0.53	4.13 ± 0.51	0.013
Spleen diameter in cm	13.1 ± 2.5	14.0 ± 2.3	0.325
Portal diameter in cm	1.29 ± 0.22	1.27 ± 0.21	0.768
Liver stiffness in kPa	19.7 ± 6.9	23.9 ± 9.2	0.139
Spleen stiffness in kPa	31.1 ± 7.4	36.1 ± 9.3	0.087
Serum zonulin levels in ng/mL	3.19 ± 1.02	4.15 ± 0.95	0.011

<sup>1</sup>Quantitative variables are expressed as mean ± standard deviation.

F: Female; M: Male; N: No; Y: Yes.

**Table 3 Univariate analysis of factors associated to transplant free survival**

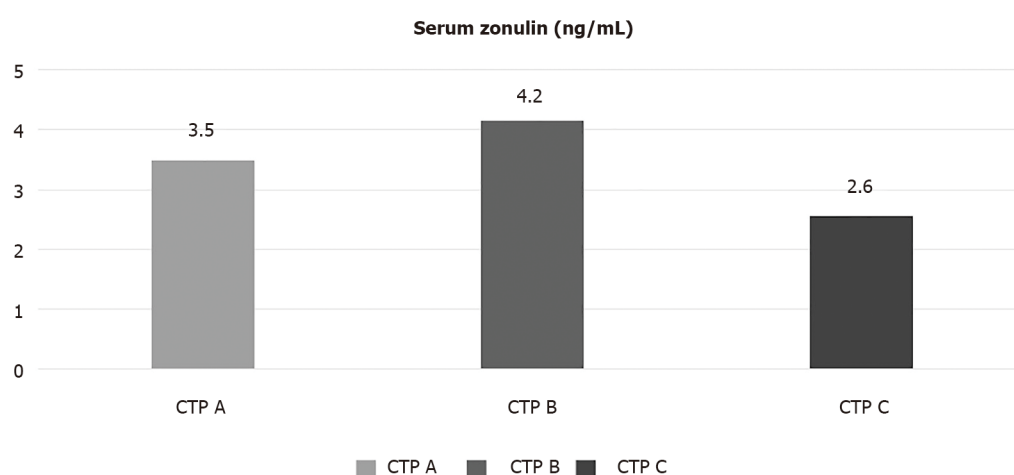
Examined parameter, baseline values	Patients alive/not transplanted at the end of the follow-up, <i>n</i> = 54	Patients transplanted or dead, <i>n</i> = 9	<i>P</i> value
Age in yr <sup>1</sup>	59 ± 15	66 ± 14	0.238
Liver-specific treatment, Y/N	28/26	4/5	0.474
CTP score	5.9 ± 1.0	7.4 ± 1.4	< 0.001
MELD score	11.3 ± 3.0	11.7 ± 3.6	0.772
High-risk varices, Y/N	19/54	5/9	0.241
Platelet count as × 10 <sup>9</sup> /L	120 ± 52	109 ± 40	0.602
Albumin in g/dL	3.87 ± 0.62	3.20 ± 0.62	0.010
Spleen diameter in cm	13.7 ± 2.8	15.2 ± 2.5	0.154
Portal diameter in cm	1.27 ± 0.21	1.55 ± 0.25	0.002
Liver stiffness in kPa	22.5 ± 9.2	27.9 ± 10.3	0.127
Spleen stiffness in kPa	35.0 ± 8.8	37.9 ± 6.9	0.383
Serum zonulin levels in ng/mL	3.70 ± 1.36	3.17 ± 1.21	0.300

<sup>1</sup>Quantitative variables are expressed as mean ± SD.

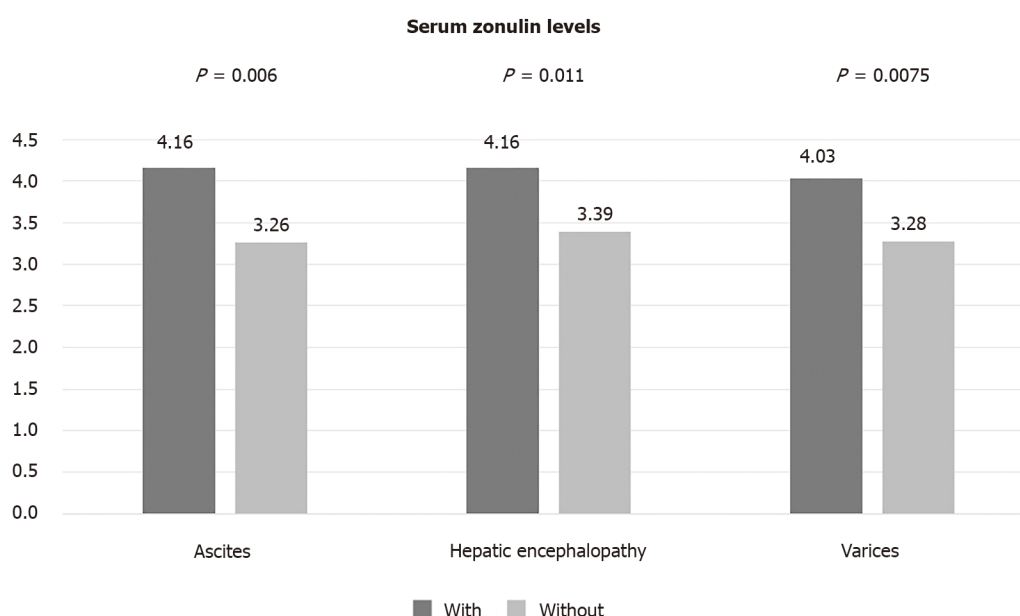
CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; N: No; Y: Yes.

## DISCUSSION

BT is increased in cirrhosis and seems to play a pivotal pathophysiological role in the development of complications related to end-stage liver disease, such as hepatorenal syndrome, HE, spontaneous bacterial peritonitis and acute-on-chronic liver failure[2, 14]. Although many factors have been implicated in the pathophysiology of BT, the exact pathogenic mechanisms leading to gut epithelial dysfunction in liver cirrhosis remain unclear[1,15,16]. To date, the role of zonulin as a promoting factor of the intestinal barrier's disruption has been thoroughly investigated in several diseases, but in patients with cirrhosis there is only limited information.

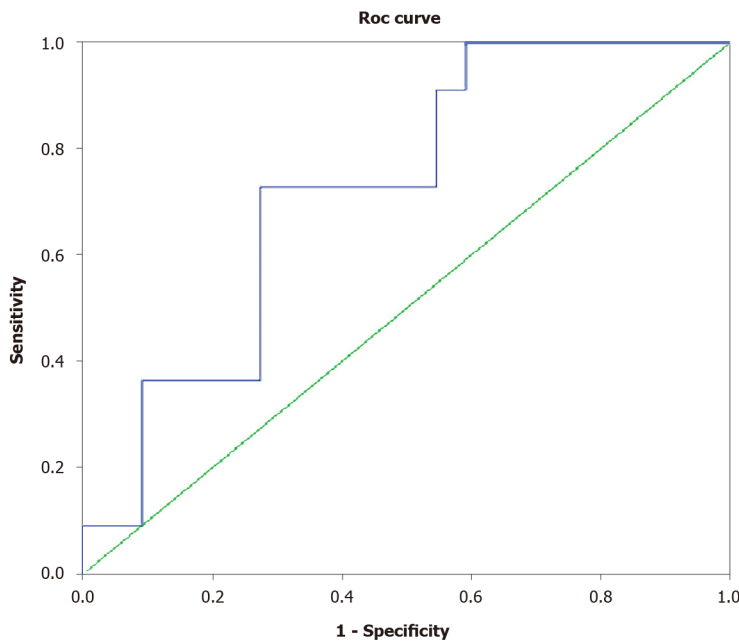


**Figure 1 Mean serum zonulin levels (ng/mL) among different Child-Turcotte-Pugh classes.** Serum zonulin levels (expressed in ng/mL as mean) according to Child-Turcotte-Pugh stage. CTP: Child-Turcotte-Pugh.



**Figure 2 Mean serum zonulin levels (ng/mL) among different manifestations of advanced liver disease.**

In our cohort, we investigated whether serum zonulin levels have any impact on the prognosis of patients with cirrhosis. Initially, we found that mean serum zonulin levels were higher in patients with CTP-B than CTP-A class cirrhosis, supporting its possible contribution in the development of decompensated liver disease (CTP-B stage). Interestingly, serum zonulin levels were lowest in our few cases with CTP-C cirrhosis; although, the small number of CTP-C patients in our study weakens the validity of such a finding, as any type of statistical errors cannot be excluded. The latter finding is in contrast to the results of a recently published study, which reported increasing serum zonulin levels from CTP-A to -B and to -C class. However, only chronic HBV patients were included in the abovementioned study and, more importantly, the study also included patients with HCC, a fact that could have affected the result[17]. The role of zonulin has also been previously investigated by others in small cohorts of patients with chronic liver disease. Serum zonulin levels were reported to decrease progressively, as liver function deteriorated in 9 patients with chronic viral hepatitis [18]. Obviously, such an under-powered study cannot lead to any valuable conclusion. In another study, serum zonulin levels were found to be lower in 40 patients with chronic HBV infection compared to 17 controls, but besides the small sample size of the study, no data for stage of liver disease were provided[19]. A pivotal study in children and adolescents reported increased serum zonulin levels in cases with rather



**Figure 3 Receiver operating characteristic curve displacing baseline serum zonulin levels in predicting liver disease decompensation.** Area under the receiver operating characteristic curve: 0.723,  $P = 0.039$ . ROC: Receiver operating characteristic.

than without NASH[20]. In the latter study, zonulin levels were found to correlate with the severity of liver steatosis and not of liver fibrosis, but cases with cirrhosis were not included. Contrary to the previous studies, we recruited a larger number of cirrhotic patients, irrespective of liver disease etiology, while at the same time we excluded older patients or patients with HCC which could jeopardize our results.

Additionally, in our study, we found that patients with more advanced cirrhosis, as documented by the presence of ascites or history of HE, had higher serum zonulin levels compared to those without these complications. Unexpectedly, in our cohort, we found a numerical but not statistically significant difference in zonulin levels between patients with or without varices. Moreover, there was no correlation between serum zonulin and SSM, which by recent data is suggested to correlate well with hepatic venous pressure gradient levels and the presence of high-risk varices[21,22]. It could be argued that the secretion of zonulin is regulated by mechanisms acting locally in the gut and is not directly affected by changes in portal pressure. However, such a speculation, taking under consideration the complexity of mechanisms implicated in the regulation of gut permeability in liver cirrhosis carries a great level of uncertainty.

Finally, the potential association between serum zonulin levels and the development of liver decompensation is further supported by the predictive role of zonulin for such an outcome within 1 year of follow-up. In particular, baseline serum zonulin levels in our patients with compensated cirrhosis were found to be independently associated with progression to decompensated liver disease within the next year. The predictability of serum zonulin levels to predict progression to decompensated liver disease was significant but suboptimal (AUROC: 0.723). In addition, serum zonulin levels  $< 3.65$  ng/mL at baseline offered a NPV of 84% for progression to liver decompensation within the next year.

Our study has some limitations. A substantial proportion of patients did not participate in the follow-up study and we included a small number of patients with CTP-C stage disease. Furthermore, serum zonulin levels were measured in a single time frame. According to guidelines, in our department, no patient with compensated disease was under rifaximin treatment. Therefore, the effect of rifaximin or other antibiotic treatment (patients with acute infection were excluded from our study) in the transition from compensated towards decompensated disease and their correlation to zonulin levels were not assessed. Undoubtedly, serial measurements of zonulin levels and their fluctuations during the course of the liver disease would enforce its prognostic value.

## CONCLUSION

In conclusion, we have clearly shown that serum zonulin levels are increased in patients with more advanced liver disease and are independently associated with the progression to decompensation. The results of our study may be of particular value as they reveal, for the first time, the adverse effect of a new agent, zonulin, on the deterioration of chronic liver disease. More studies are needed to confirm our findings and to further investigate the pathophysiological mechanisms by which zonulin is involved in alteration of intestinal barrier and gut permeability. Taking into consideration that zonulin antagonists are already being tested in phase IIb studies in diseases characterized by disrupted intestinal permeability, such as celiac disease, confirmation of our results may have significant clinical implications[23].

## ARTICLE HIGHLIGHTS

### Research background

Gut permeability is distorted in patients with liver cirrhosis and the observed deregulation of the intestinal integrity plays a crucial role in the development of bacterial translocation. Bacterial translocation contributes to the occurrence or aggravation of serious complications in patients with liver cirrhosis. Zonulin is a recently recognized protein, synthesized by the intestinal and liver cells, and thought to play an important role in the regulation of tight junctions between intestinal cells.

### Research motivation

Increased zonulin levels have been observed in such diseases as celiac disease and inflammatory bowel disease and have shown correlation to the impairment of intestinal permeability. The exact mechanism that leads to the deregulation of the intestinal integrity in liver cirrhosis is not thoroughly investigated. Zonulin may have a role in the observed alterations of the gut barrier in advanced chronic liver disease.

### Research objectives

We aimed to investigate if serum zonulin levels are altered in patients with different stages of liver cirrhosis and investigate their possible impact on patients' prognosis.

### Research methods

We included 116 cirrhotic patients who attended our outpatient clinic during a 12-month period. Serum zonulin levels were measured, as were epidemiological, laboratory and clinical data, and data from elastography and ultrasonography at baseline. Sixty-three patients were followed up for at least 1 year and data from clinical events (death, liver transplantation and liver disease decompensation) were collected.

### Research results

Our study included mainly Child-Turcotte-Pugh (CTP)-A (67%) and CTP-B patients (28%). We observed that serum zonulin levels are increased in patients with more advanced liver disease, such as patients with CTP-B stage, patients with ascites, or those with history of hepatic encephalopathy. What is more, serum zonulin levels were independently associated with the probability of decompensation within the next year.

### Research conclusions

According to our study results, serum zonulin levels are increased in patients with advanced chronic liver disease. What is more, a new agent, zonulin, is found to be implicated in the progress towards advanced liver disease.

### Research perspectives

Our findings highlight once more the significance of gut barrier deregulation in the setting of liver cirrhosis and emphasize the need of further studies in the field, aiming to reveal the complex pathophysiological interplay which leads to bacterial translocation. Especially, the role of zonulin should be further investigated, due to its possible therapeutic implications, as a zonulin antagonist already exists and is being tested in studies of celiac disease.



## REFERENCES

- Schnabl B**, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 2014; **146**: 1513-1524 [PMID: [24440671](#) DOI: [10.1053/j.gastro.2014.01.020](#)]
- Bosch J**, Groszmann RJ, Shah VH. Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *J Hepatol* 2015; **62**: S121-S130 [PMID: [25920081](#) DOI: [10.1016/j.jhep.2015.01.003](#)]
- Wiest R**, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014; **60**: 197-209 [PMID: [23993913](#) DOI: [10.1016/j.jhep.2013.07.044](#)]
- Du Plessis J**, Vanheel H, Janssen CE, Roos L, Slavik T, Stivaktas PI, Nieuwoudt M, van Wyk SG, Vieira W, Pretorius E, Beukes M, Farré R, Tack J, Laleman W, Fevery J, Nevens F, Roskams T, Van der Merwe SW. Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* 2013; **58**: 1125-1132 [PMID: [23402745](#) DOI: [10.1016/j.jhep.2013.01.038](#)]
- Genescà J**, Martí R, Rojo F, Campos F, Peribáñez V, González A, Castells L, Ruiz-Marcellán C, Margarit C, Esteban R, Guardia J, Segura R. Increased tumour necrosis factor alpha production in mesenteric lymph nodes of cirrhotic patients with ascites. *Gut* 2003; **52**: 1054-1059 [PMID: [12801966](#) DOI: [10.1136/gut.52.7.1054](#)]
- Gunnarsdottir SA**, Sadik R, Shev S, Simrén M, Sjövall H, Stotzer PO, Abrahamsson H, Olsson R, Björnsson ES. Small intestinal motility disturbances and bacterial overgrowth in patients with liver cirrhosis and portal hypertension. *Am J Gastroenterol* 2003; **98**: 1362-1370 [PMID: [12818282](#) DOI: [10.1111/j.1572-0241.2003.07475.x](#)]
- Fasano A**. Zonulin, regulation of tight junctions, and autoimmune diseases. *Ann N Y Acad Sci* 2012; **1258**: 25-33 [PMID: [22731712](#) DOI: [10.1111/j.1749-6632.2012.06538.x](#)]
- El Asmar R**, Panigrahi P, Bamford P, Berti I, Not T, Coppa GV, Catassi C, Fasano A. Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure. *Gastroenterology* 2002; **123**: 1607-1615 [PMID: [12404235](#) DOI: [10.1053/gast.2002.36578](#)]
- Lammers KM**, Lu R, Brownley J, Lu B, Gerard C, Thomas K, Rallabhandi P, Shea-Donohue T, Tamiz A, Alkan S, Netzel-Arnett S, Antalis T, Vogel SN, Fasano A. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. *Gastroenterology* 2008; **135**: 194-204.e3 [PMID: [18485912](#) DOI: [10.1053/j.gastro.2008.03.023](#)]
- Sapone A**, de Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, Lampis R, Kryszak D, Carteni M, Generoso M, Iafusco D, Prisco F, Laghi F, Riegler G, Carratu R, Counts D, Fasano A. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 2006; **55**: 1443-1449 [PMID: [16644703](#) DOI: [10.2337/db05-1593](#)]
- Caviglia GP**, Dughera F, Ribaldone DG, Rosso C, Abate ML, Pellicano R, Bresso F, Smedile A, Saracco GM, Astegiano M. Serum zonulin in patients with inflammatory bowel disease: a pilot study. *Minerva Med* 2019; **110**: 95-100 [PMID: [30160088](#) DOI: [10.23736/S0026-4806.18.05787-7](#)]
- Moreno-Navarrete JM**, Sabater M, Ortega F, Ricart W, Fernández-Real JM. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. *PLoS One* 2012; **7**: e37160 [PMID: [22629362](#) DOI: [10.1371/journal.pone.0037160](#)]
- Tripathi A**, Lammers KM, Goldblum S, Shea-Donohue T, Netzel-Arnett S, Buzza MS, Antalis TM, Vogel SN, Zhao A, Yang S, Arrietta MC, Meddings JB, Fasano A. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. *Proc Natl Acad Sci U S A* 2009; **106**: 16799-16804 [PMID: [19805376](#) DOI: [10.1073/pnas.0906773106](#)]
- Wiest R**, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005; **41**: 422-433 [PMID: [15723320](#) DOI: [10.1002/hep.20632](#)]
- Ghosh G**, Jesudian AB. Small Intestinal Bacterial Overgrowth in Patients With Cirrhosis. *J Clin Exp Hepatol* 2019; **9**: 257-267 [PMID: [31024208](#) DOI: [10.1016/j.jceh.2018.08.006](#)]
- Tsiaoussis GI**, Assimakopoulos SF, Tsamandas AC, Triantos CK, Thomopoulos KC. Intestinal barrier dysfunction in cirrhosis: Current concepts in pathophysiology and clinical implications. *World J Hepatol* 2015; **7**: 2058-2068 [PMID: [26301048](#) DOI: [10.4254/wjch.v7.i17.2058](#)]
- Wang X**, Li MM, Niu Y, Zhang X, Yin JB, Zhao CJ, Wang RT. Serum Zonulin in HBV-Associated Chronic Hepatitis, Liver Cirrhosis, and Hepatocellular Carcinoma. *Dis Markers* 2019; **2019**: 5945721 [PMID: [31485278](#) DOI: [10.1155/2019/5945721](#)]
- Akao T**, Morita A, Onji M, Miyake T, Watanabe R, Uehara T, Kawasaki K, Miyaike J, Oomoto M. Low Serum Levels of Zonulin in Patients with HCV-Infected Chronic Liver Diseases. *Euroasian J Hepatogastroenterol* 2018; **8**: 112-115 [PMID: [30828551](#) DOI: [10.5005/jp-journals-10018-1275](#)]
- Calgin MK**, Cetinkol Y. Decreased levels of serum zonulin and copeptin in chronic Hepatitis-B patients. *Pak J Med Sci* 2019; **35**: 847-851 [PMID: [31258605](#) DOI: [10.12669/pjms.35.3.144](#)]
- Pacifico L**, Bonci E, Marandola L, Romaggioli S, Bascetta S, Chiesa C. Increased circulating zonulin in children with biopsy-proven nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 17107-17114 [PMID: [25493023](#) DOI: [10.3748/wjg.v20.i45.17107](#)]
- Buechter M**, Manka P, Theysohn JM, Reinboldt M, Canbay A, Kahraman A. Spleen stiffness is positively correlated with HVPg and decreases significantly after TIPS implantation. *Dig Liver Dis* 2018; **50**: 54-60 [PMID: [29102174](#) DOI: [10.1016/j.dld.2017.09.138](#)]
- Karagiannakis DS**, Voulgaris T, Koureta E, Chloupi E, Papatheodoridis GV, Vlachogiannakos J.

- Role of Spleen Stiffness Measurement by 2D-Shear Wave Elastography in Ruling Out the Presence of High-Risk Varices in Cirrhotic Patients. *Dig Dis Sci* 2019; **64**: 2653-2660 [PMID: 30989464 DOI: 10.1007/s10620-019-05616-4]
- 23 **Leffler DA**, Kelly CP, Green PH, Fedorak RN, DiMarino A, Perrow W, Rasmussen H, Wang C, Bercik P, Bachir NM, Murray JA. Larazotide acetate for persistent symptoms of celiac disease despite a gluten-free diet: a randomized controlled trial. *Gastroenterology* 2015; **148**: 1311-9.e6 [PMID: 25683116 DOI: 10.1053/j.gastro.2015.02.008]

## Retrospective Cohort Study

## Impact of biliary complications on quality of life in live-donor liver transplant recipients

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## Abstract

## BACKGROUND

Despite significant advancements in liver transplantation (LT) surgical procedures and perioperative care, post-LT biliary complications (BCs) remain a significant source of morbidity, mortality, and graft failure. In addition, data are conflicting regarding the health-related quality of life (HRQoL) of LT recipients. Thus, the success of LT should be considered in terms of both the survival and recovery of HRQoL.

## AIM

To assess the impact of BCs on the HRQoL of live-donor LT recipients (LDLT-Rs).

## METHODS

We retrospectively analysed data for 25 LDLT-Rs who developed BCs post-LT between January 2011 and December 2016 at our institution. The Short Form 12 version 2 (SF 12v2) health survey was used to assess their HRQoL. We also included 25 LDLT-Rs without any post-LT complications as a control group.

## RESULTS

The scores for HRQoL of LDLT-Rs who developed BCs were significantly higher than the norm-based scores in the domains of physical functioning ( $P = 0.003$ ), role-physical ( $P < 0.001$ ), bodily pain ( $P = 0.003$ ), general health ( $P = 0.004$ ), social

**Institutional review board**

**statement:** The study was reviewed and approved by the institutional review board of Faculty of Medicine, Ain Shams University, Cairo, Egypt.

**Informed consent statement:** Was waived due to the retrospective nature of the study

**Conflict-of-interest statement:** All authors have nothing to disclose.

**Data sharing statement:** The statistical code and dataset are available from the corresponding author at [ghadaabdelrahman@med.asu.edu.eg](mailto:ghadaabdelrahman@med.asu.edu.eg). The participants gave informed consent for the data sharing.

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functioning ( $P = 0.005$ ), role-emotional ( $P < 0.001$ ), and mental health ( $P < 0.001$ ). No significant difference between the two groups regarding vitality was detected ( $P = 1.000$ ). The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains ( $P < 0.001$ ) and the mental ( $P < 0.001$ ) and physical ( $P = 0.0002$ ) component summary scores.

**CONCLUSION**

The development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

**Key Words:** Live-donor liver transplantation; Quality of life; The Short Form 12 version 2; Cirrhosis; Biliary complications; Mental health

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**Core Tip:** We retrospectively analysed data for 25 Live-donor liver transplantation recipients (LDLT-Rs) with biliary complications (BCs) and described their health-related quality of life (HRQoL) using the Short Form 12 version 2 health survey. All scores for HRQoL domains of LDLT-Rs with BCs were significantly higher than the norm-based scores except for vitality. The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains ( $P < 0.001$ ) and in the mental ( $P < 0.001$ ) and physical ( $P = 0.0002$ ) component summary scores. We conclude that the development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

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**INTRODUCTION**

Health-related quality of life (HRQoL) is a multidimensional model reflecting the domains of social, mental, emotional, and physical health[1,2]. More than 50 different HRQoL tools have been used in liver transplant (LT) research[3], and no golden standard instrument has existed until now[4]. These tools can be classified into generic and disease-specific tools[3,5]. Generic HRQoL tools, of which the validated Short Form 36 (SF-36) health survey is the most frequently used for evaluating LT recipients, allow assessments across various medical conditions and health states[6,7].

Short Form 12 version 2 (SF-12v2) is a validated concise version of the SF-36 version 2 (SF-36v2) with only 12 questions[8,9]. Similar to the SF-36v2, it evaluates the same eight dimensions of HRQoL covering the previous 4 wk: General health, bodily pain, physical functioning, role physical, vitality, role emotional, mental health, and social functioning. Physical Component Summary (PCS) and Mental Component Summary (MCS) scores were created from patient responses[10]. The sum of scores ranges from 0 to 100, where 0 indicates the worst state of health and 100 indicates the best state of health[10,11].

The data are conflicting regarding the HRQoL of LT recipients. The heterogeneity between studies regarding the type of graft, diversity of included patients, and health survey precludes definitive conclusions[4,12]. In addition, an overlap exists between the primary liver disease and LT process with diverse events during peri- and postoperative management.

The global assessment of HRQoL after LT usually confirms improvement compared with pretransplant status[13]; however, it may remain suboptimal compared to the general population due to post-LT complications, recurrence of primary liver disease, or adverse effects of immunosuppressants[14-17]. In addition, cirrhosis leads to loss of muscle mass, sarcopenia, malnutrition, and physical impairment that manifest as



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physical frailty, increasing the risk of pretransplant mortality[18-20] and delayed improvement of physical functioning post-LT[21-23].

Fatigue affects up to 50% of patients with chronic liver disease; moreover, it demonstrates a significant association with poor HRQoL[24,25]. It also affects up to 60% of LT recipients[26]. It is a complex symptom that may be influenced by physical and mental states, including poor sleep quality, anxiety, and depression[27].

The LT candidates often have impaired HRQoL with a high prevalence of anxiety and depressive symptoms[28,29]. Moreover, LT was considered as post-traumatic stress disorder and was also found to be associated with anxiety and depression, which may further impair the HRQoL of LT recipients[30-33].

In the light of the above, HRQoL should be considered in terms of the outcome after LT[34,35]. Hence, we aimed to assess the impact of biliary complications (BCs) on the HRQoL of live-donor LT recipients (LDLT-Rs).

## MATERIALS AND METHODS

### Study design

We retrospectively analysed all LDLT-Rs at Ain Shams Centre for Organ Transplantation, Ain Shams Specialised Hospital, Cairo, Egypt, between January 2011 and December 2016. During this period, 215 adult patients underwent right-lobe LDLT at our centre. We included LDLT-Rs who developed BCs post-LT. We excluded LDLT-Rs with any of the following situations: cholestatic liver diseases (primary biliary cirrhosis or primary sclerosing cholangitis), vascular complications, acute or chronic rejection, recurrent hepatitis C virus (HCV) infection, graft failure, failure to follow up for at least one year post-LT, or patients who refused to participate in the research. As a result, 25 LDLT-Rs with BCs were included in the final analysis. We enrolled 25 LDLT-Rs who did not develop any post-LT complications as a control group. LT recipients were assessed at least 12 months post-LT, with median follow up duration of 5.5 years (range: 12 mo - 8 years).

This study was performed per the ethical principles of the declaration of Helsinki and was approved by the ethical committee of the Faculty of Medicine, Ain Shams University (No: FMASU MD 187/2016), which waived the requirement of informed consent due to the retrospective nature of the research.

### Quality-of-life assessment

Eligible LDLT-Rs were invited to fulfil the SF-12v2 questionnaire during follow-up visits after obtaining verbal consent. We used anonymous questionnaires to ensure strict confidentiality. The SF-12v2 includes 12 questions: one question on general health perceptions, two questions concerning physical functioning, two questions on role limitations because of physical health problems, one question on bodily pain, one question on vitality, two questions on role limitations, one question on social functioning, and two questions on general mental health.

### Statistical analysis

The data were analysed using IBM SPSS Statistics (v. 23; IBM Corp., Armonk, New York). Nonparametric numerical variables are presented as the median and interquartile range. Nominal variables are presented as the number and percentage. Ordinal data were analysed using the chi-squared test for trends. Two-sided *P* values < 0.05 were considered statistically significant.

## RESULTS

This study included 25 adult right-lobe LDLT-Rs who experienced BCs. At the time of LT, the mean age of the recipients was  $52 \pm 7$  years, and 19 (76%) recipients were male. Cirrhosis due to HCV was the most common indication for LT in 21 patients (84%; Tables 1 and 2).

### Development and management of biliary complications

Among the 25 LDLT-Rs included in this study, minor biliary leakage occurred in 15 recipients (83.3%) and stopped spontaneously without further management. In only three (16.6%) recipients, pigtail insertion and further interventional management were needed. Moreover, 25 recipients developed a biliary infection, mainly occurring early

**Table 1** Descriptive categorical data for live-donor liver transplant recipients with biliary complications

Variable		n (%)
Indication of liver transplantation	HCV	21 (84)
	HBV	1 (4)
	Combined HCV and HBV	1 (4)
	Hepatocellular carcinoma	2 (8)
Donors' gender	Male	17 (68)
	Female	8 (32)
Recipients' gender	Male	19 (76)
	Female	6 (24)
Immunosuppressant	Tacrolimus	22 (88)
	Cyclosporine	3 (12)
Biliary leakage	-	7 (28)
	+	18 (72)
Need of pigtail catheter for biloma (total = 18)	-	15 (83.3)
	+	3 (16.6)
Biliary infection	-	0 (0)
	+	25 (100)
Frequency of biliary infection (total = 25)	1-2 Episodes	16 (64)
	≥ 3 Episodes	9 (36)
Biliary stricture	-	5 (20)
	+	20 (80)
Frequency of biliary stricture (total = 20)	1-2 Episodes	13 (65)
	≥ 3 Episodes	7 (28)
Need for ERCP	-	5 (20)
	+	20 (80)
Frequency of ERCP	1-2 ERCP	13 (65)
	≥ 3 ERCP	7 (28)
Need for PTC	-	22 (88)
	+	3 (12)
Frequency of PTC	1 PTC	2 (66.6)
	2 PTC	1 (33.3)
Surgical intervention for stricture	-	19 (95)
	+	1 (5)
Admission related to biliary complications	-	0 (0)
	+	25 (100)
Early biliary infection (total = 25)	-	2 (8)
	+	23 (92)
Early biliary stricture (total = 20)	-	17 (68)
	+	8 (32)

Data presented in number (*n*) and percentage (%). ERCP: Endoscopic retrograde cholangiopancreatography; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PTC: Percutaneous transhepatic cholangiography.

(23; 92%) and in one to two episodes in 16 (64%) recipients (Table 1). Furth-

**Table 2 Descriptive numerical data for live-donor liver transplant recipients with biliary complications**

Variable	Data
MELD score	15 ± 3
Child score	9 ± 2
Donors' age (yr)	30 ± 4
Donors' BMI (kg/m <sup>2</sup> )	25 ± 4
Recipient's age (yr)	52 ± 7
Recipient's BMI (kg/m <sup>2</sup> )	27 ± 6
Total bilirubin (mg/dL)	2.9 (2-3.9)
Direct bilirubin (mg/dL)	1.6 (0.9-2.3)
Alkaline phosphatase (IU/L)	190 ± 49
Gamma-glutamyl transferase (IU/L)	100 (50-130)
Platelets (10 <sup>9</sup> /L)	75 ± 31
Cold ischemia time (min)	48 ± 25
Warm ischemia time (min)	47 ± 23
Graft arterialization time (min)	145 ± 53
Time to biliary infection (d)	13 (11-36)
Time to biliary stricture (d)	130 (120-190)

Data are presented as mean ± SD or median and range. BMI: Body mass index; MELD: Model for end stage liver disease.

er, 20 (80%) recipients developed biliary stricture, most of which presented in one to two episodes (13; 65%). The development of BCs caused a prolonged hospital stay (median = 46 days; range: 15 - 67 days), with nine (36%) patients needing ≥ three episodes of admission. Concerning the management of BCs, endoscopic retrograde cholangiopancreatography (ERCP) with stenting ± dilatation was done for 20 (80%) recipients, with seven (28%) recipients needing ≥ three ERCP sessions. Percutaneous transhepatic cholangiography was needed for only three (12%) recipients, with one recipient requiring another session. These methods only failed in one recipient who needed surgical reconstruction of the biliary stricture (Table 1).

### Health-related quality of life

The scores of HRQoL of LDLT-Rs with BCs were significantly higher than the norm-based scores in the domains of physical functioning ( $P = 0.003$ ), role-physical ( $P < 0.001$ ), bodily pain ( $P = 0.003$ ), general health ( $P = 0.004$ ), social functioning ( $P = 0.005$ ), role-emotional ( $P < 0.001$ ), and mental health ( $P < 0.001$ ). In contrast, no significant difference was found between the two groups regarding vitality ( $P = 1.000$ ; Table 3 and Figure 1). The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains ( $P < 0.001$ ) and in the mental ( $P < 0.001$ ) and physical ( $P = 0.0002$ ) component summary scores (Tables 4 and 5; Figures 1 and 2).

## DISCUSSION

Despite the considerable advances in LT surgical techniques and perioperative care, post-LT BCs remain a significant source of morbidity, mortality, and graft failure[36]. To our knowledge, no previous study has specifically assessed the impact of BCs on the HRQoL of LDLT-Rs. In our study, LDLT-Rs with BCs had significantly higher HRQoL domain scores except for the vitality domain than norm-based scores; however, those patients gained a significantly lower range of improvement in HRQoL domains with lower MCS and PCS scores than those without BCs. This result can be attributed to more prolonged and frequent hospital admission and expectation reduction with anxiety, stress, and depression[37]. In agreement with the current results, the published literature has observed the positive effects of LT on the

**Table 3 Comparison of the quality-of-life scores for live-donor liver transplant recipients with biliary complications and their corresponding norm-based scores**

HRQoL score	LDLT-R with BC	NBS score	P value <sup>1</sup>
Physical functioning	50 (50-75)	41.3 (41.3-49.2)	0.003
Role physical	50 (31.3-75)	40.5 (34.2-49)	0.001
Bodily pain	50 (50-75)	39.7 (39.7-48.7)	0.003
General health	60 (60-85)	47.8 (47.8-57.7)	0.004
Vitality	50 (25-50)	49.1 (39.2-49.1)	1.000
Social functioning	50 (50-50)	39.1 (39.1-39.1)	0.005
Role emotion	50 (37.5-75)	35.5 (30.3-45.9)	< 0.001
Mental health	50 (50-62.5)	41.3 (41.3-47)	0.001

<sup>1</sup>Wilcoxon signed ranks test.

Data are shown as median and interquartile range. BC: Biliary complications; HRQoL: Health related quality of life; LDLT-R: Live donor liver transplant recipients; NBS: Norm based score.

**Table 4 Comparison of health-related quality-of-life scores between patients and controls**

HRQoL domain	Patients (n = 25)	Controls (n = 25)	P value <sup>1</sup>
Physical functioning	50 (50-75)	100 (100-100)	< 0.001
Role physical	50 (31.3-75)	100 (87.5-100)	< 0.001
Bodily pain	50 (50-75)	100 (100-100)	< 0.001
General health	60 (60-85)	85 (85-85)	< 0.001
Vitality	50 (25-50)	75 (75-87.5)	< 0.001
Social functioning	50 (50-50)	75 (75-100)	< 0.001
Role emotion	50.0 (37.5-75)	87.5 (75-100)	< 0.001
Mental health	50 (50-62.5)	87.5 (75-87.5)	< 0.001
PCS	44.8 (41.7-52.9)	57.8 (55.2-59)	< 0.001
MCS	42 (35.6-45.2)	52.9 (50.2-57.9)	< 0.001

<sup>1</sup>Wilcoxon signed ranks test.

Data are shown as median and interquartile range; Patients: Live donor liver transplant recipients with biliary complications; Controls: Live donor liver transplant recipients without biliary complications; HRQoL: Health-related quality-of-life; MCS: Mental component summary score; PCS: Physical component summary score.

recipients' HRQoL[12,37-40].

Similar to the present study[41], other authors have assessed the LT recipients' HRQoL using the WHOQOLBREF questionnaire[42] and Transplant Effects Questionnaire[43] and concluded that LT recipients, especially those who received LDLT, reported the highest level of HRQoL in all four dimensions of HRQoL in comparison to those with other organ transplantation.

In partial agreement with the current study, a review of 32 studies and 5402 patients found that the overall HRQoL scores of LT recipients remain improved and equivalent to the general population in the long term. However, physical functioning continues to be inferior to the general population despite a noticeable improvement from preoperative physical functioning[4]. Similarly, a review article of 31 publications reported improved overall HRQoL and physical functioning in deceased donor LT (DDLT) adult recipients during the first 2 years, which remains stable in the long term but does not reach the level of the general population[35]. Additionally, Sullivan *et al* [44] assessed the HRQoL two decades after DDLT using the SF-12 survey. In adult survivors, the MCS score (54.6) was equivalent to that of the general population; however, the PCS score (39.3) remained below average. This outcome can be explained by the presence of comorbidities, primary liver disease severity, postoperative

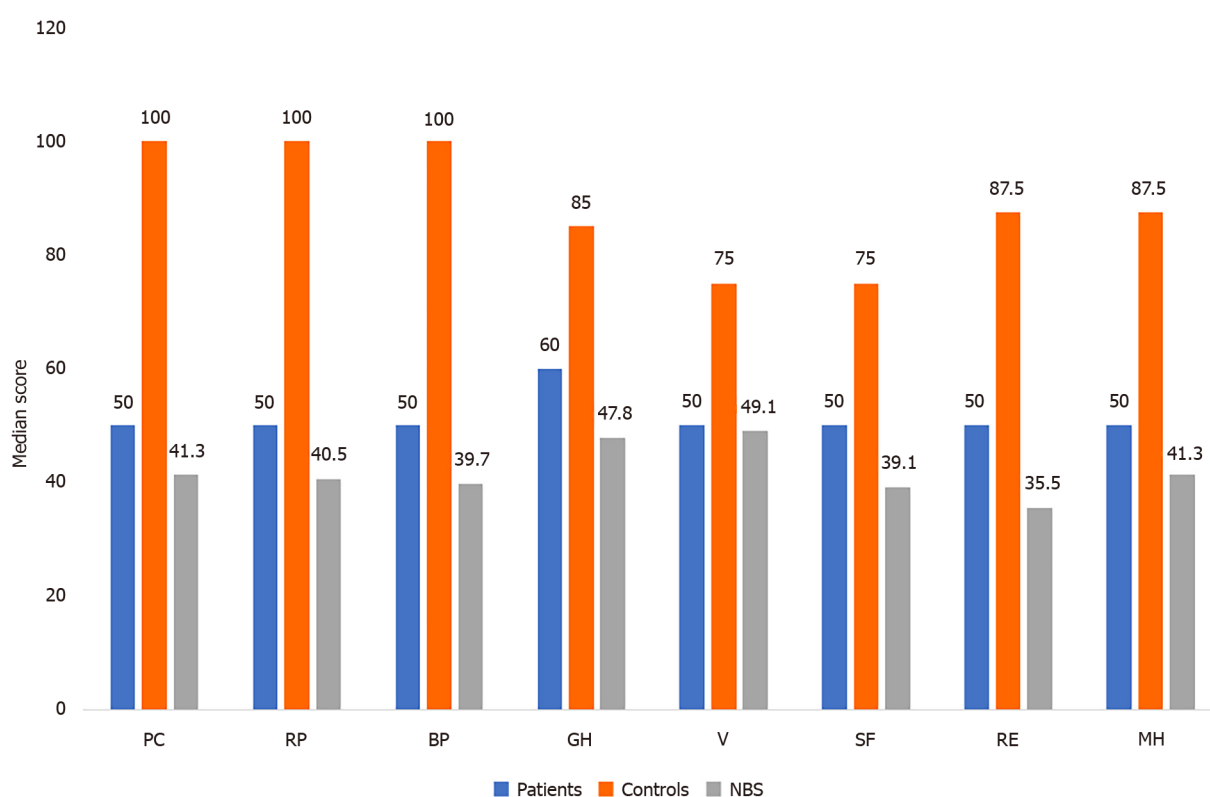


**Table 5 Physical and mental component summary scores in patients and controls compared with norm-based scores**

Variable	NBS	Patients (n = 25), %	Control (n = 25), %	P value <sup>1</sup>
Physical component summary score	At or above	11 (44)	25 (100)	0.0002
	Below	8 (32)	0 (0)	
	Far below	6 (24)	0 (0)	
Mental component summary score	At or above	7 (28)	24 (96)	< 0.0001
	Below	7 (28)	1 (4)	
	Far below	11 (44)	0 (0)	

<sup>1</sup>Chi-squared test for trend.

Data are shown as number and percentage. Patients: Live donor liver transplant recipients with biliary complications; Controls: Live donor liver transplant recipients without biliary complications; NBS: Norm based score.

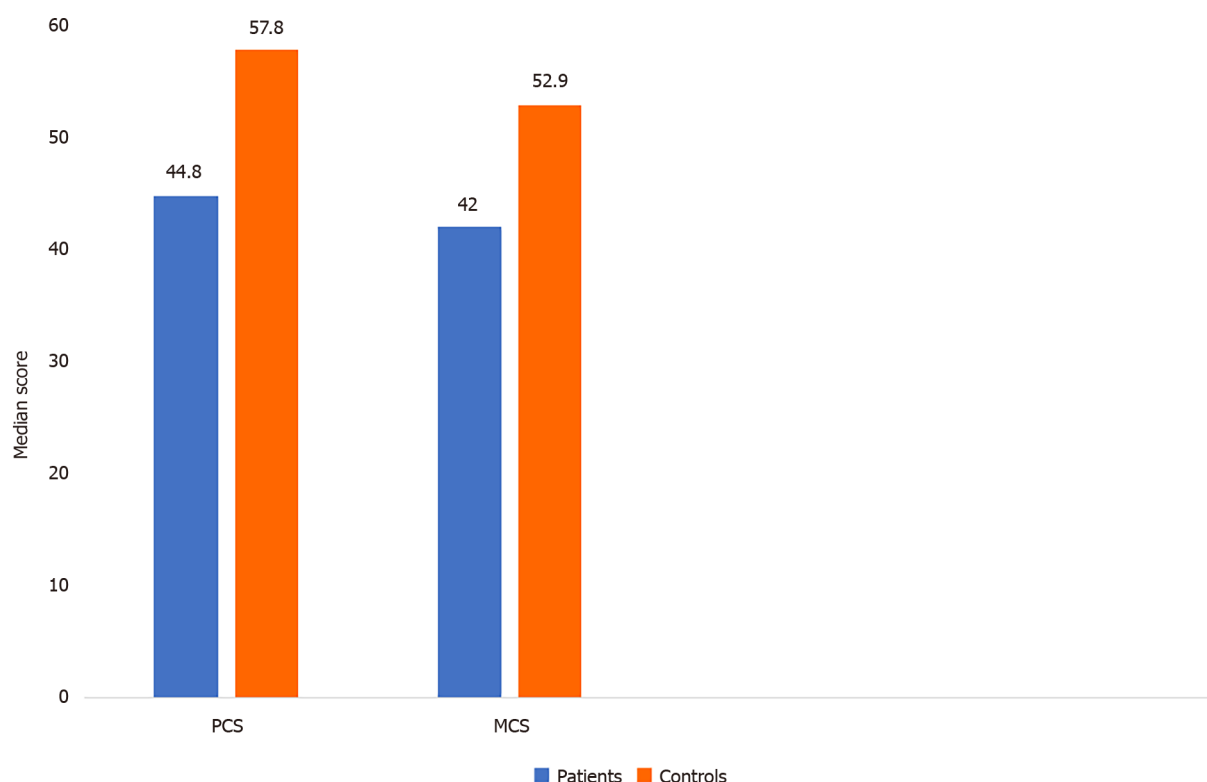


**Figure 1 Short Form 12 (v. 2) domains in patients and controls compared to the norm-based score.** BP: Bodily pain; GH: General health; MH: Mental health; NBS: Norm based score; PF: Physical functioning; RE: Role emotion; RP: Role physical; SF: Social functioning; V: Vitality.

morbidity, and graft type[20,33]. Additionally, Dunn *et al*[45] reported that group exercise activities were correlated with improved physical function, mental health, and HRQoL, independent of comorbidities, for up to 5 years after LT. Therefore, physical activity should be encouraged after LT[46].

In a study by Casanovas *et al*[47], the SF-36 scores of 156 LT candidates were assessed pre- and post-LT. They observed significantly lower patient baseline scores in all HRQoL domains than general population scores, especially in physical health. As early as 3 months till 1-year post-LT, they detected improvement in all SF-36 domains except vitality and social functioning, revealing no significant improvement. Moreover, sleeping problems were observed at the baseline and persisted post-LT. The poor sleep quality frequently noted in cirrhotic patients is known to cause fatigue and impair cognitive and physical functions[48].

In contrast to our results, Domingos *et al*[37] retrospectively assessed the HRQoL of 93 DDLT recipients who survived 10 years post-LT using the SF-36 survey and observed that LT recipients had lower mental health scores than the general



**Figure 2 Physical and mental component summary scores of patients and controls.** Patients: Live donor liver transplant recipients with biliary complications; Controls: Live donor liver transplant recipients without biliary complications; MCS: Mental component summary score; PCS: Physical component summary score.

population. In all other domains, LT recipients had similar (emotional limitations, pain, and general health status) or superior (physical limitations, social aspects, functional capacity, and vitality) scores than the general population. In addition, Dąbrowska-Bender *et al*[15] assessed the SF-36 health survey in 121 DDLT recipients and observed no change in mental health score, whereas significant physical impairment was reported by 18.18% of the recipients.

In a study by Annema *et al*[30], LT had a beneficial effect on the mental health of LT recipients by ameliorating anxiety and depression symptom severity. However, recipients with persistent symptoms of anxiety and depression experienced a negative effect on HRQoL and therapeutic adherence. They also observed that persistent anxiety and depression were correlated with the development of BCs and the duration of the hospital stay. Similarly, in another report[49], the HRQoL of 82 LT recipients was retrospectively assessed, finding 94% reported high mean scores on HRQoL, the McGill Quality of Life Questionnaire, and adherence to medications. Conversely, patients with a low HRQoL reported anxiety, depression, fatigue, slowing pace, and physical limitations, suggesting that LT recipients who fail to adapt to their post-LT state experienced a decreased ability to tolerate physical symptoms and post-LT complications[50]. Other causes for lower mental health scores post-LT are the worry regarding medication side effects, hepatic disease recurrence, and other potential complications[51].

Candidates for LT may have overly optimistic anticipations for post-LT improvement in their HRQoL. Unfulfillment of these expectations may negatively affect their HRQoL, highlighting the need to help patients expect and understand the outcomes of LT. Moreover, LT candidate education positively affects post-LT HRQoL[40]. Education is associated with better outcomes and higher patient adherence[52].

This study is limited by its retrospective nature and small sample size. More research is required to define the predictors of HRQoL and plan multidisciplinary strategies for HRQoL improvement in LT recipients. According to the current literature, HRQoL should be integrated into the clinical care of LT[53].

## CONCLUSION

We conclude that the development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

## ARTICLE HIGHLIGHTS

### Research background

Despite the considerable advances in liver transplantation (LT) surgical techniques and perioperative care, post-LT biliary complications (BCs) remain a significant source of morbidity, mortality, and graft failure. Due to the current high survival rates of LT, the focus has shifted to improving the quality of life of LT recipients.

### Research motivation

The data are conflicting regarding the health-related quality of life (HRQoL) of LT recipients.

### Research objectives

To assess the impact of BCs on the HRQoL of live-donor LT recipients (LDLT-Rs).

### Research methods

We retrospectively analysed data for 25 LDLT-Rs with BCs and described their HRQoL through the Short Form 12 version 2 (SF-12v2) health survey compared to 25 LDLT-Rs without post-LT complications.

### Research results

The scores of HRQoL of LDLT-Rs with BCs were significantly higher than the norm-based scores in all HRQoL domains except vitality. The LDLT-Rs with BCs had significantly lower scores than LDLT-Rs without BCs in all HRQoL domains ( $P < 0.001$ ) and in the mental ( $P < 0.001$ ) and physical ( $P = 0.0002$ ) component summary scores.

### Research conclusions

The development of BCs in LDLT-Rs causes a lower range of improvement in HRQoL.

### Research perspectives

The assessment of HRQoL should be integrated into the clinical care of LT recipients. Identifying the determinants of HRQoL could improve the management plan of these patients through a multidisciplinary approach.

## REFERENCES

- 1 Health-Related Quality of Life and Well-Being | Healthy People 2020. [cited 20 February 2021]. Available online: <https://www.healthypeople.gov/2020/about/foundation-health-measures/Health-Related-Quality-of-Life-and-Well-Being>
- 2 Haraldstad K, Wahl A, Andenæs R, Andersen JR, Andersen MH, Beisland E, Borge CR, Engebretsen E, Eisemann M, Halvorsrud L, Hanssen TA, Haugstvedt A, Haugland T, Johansen VA, Larsen MH, Løvereide L, Løyland B, Kvarme LG, Moons P, Norekvål TM, Ribbu L, Rohde GE, Urstad KH, Helseth S; LIVSFORSK network. A systematic review of quality of life research in medicine and health sciences. *Qual Life Res* 2019; **28**: 2641-2650 [PMID: 31187410 DOI: 10.1007/s11136-019-02214-9]
- 3 Jay CL, Butt Z, Ladner DP, Skaro AI, Abecassis MM. A review of quality of life instruments used in liver transplantation. *J Hepatol* 2009; **51**: 949-959 [PMID: 19775771 DOI: 10.1016/j.jhep.2009.07.010]
- 4 Yang LS, Shan LL, Saxena A, Morris DL. Liver transplantation: a systematic review of long-term quality of life. *Liver Int* 2014; **34**: 1298-1313 [PMID: 24703371 DOI: 10.1111/liv.12553]
- 5 Saab S, Ng V, Landaverde C, Lee SJ, Comulada WS, Arevalo J, Durazo F, Han SH, Younossi Z, Busuttil RW. Development of a disease-specific questionnaire to measure health-related quality of life in liver transplant recipients. *Liver Transpl* 2011; **17**: 567-579 [PMID: 21506245 DOI: 10.1002/lt.22267]
- 6 RAND Corporation. 36-Item Short Form Survey (SF-36) Scoring Instructions. [cited 20 February 2021]. Available from: [https://www.rand.org/health-care/surveys\\_tools/mos/36-item-short-form](https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form)

- form/scoring.html
- 7 **Ware JE Jr**, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; **30**: 473-483 [PMID: [1593914](#)]
- 8 **Ware J Jr**, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Med Care* 1996; **34**: 220-233 [PMID: [8628042](#) DOI: [10.1097/00005650-199603000-00003](#)]
- 9 SF-12 and SF-12v2 Health Survey. [cited 20 February 2021]. Available from: <https://www.qualitymetric.com/health-surveys/the-sf-12v2-health-survey/>
- 10 **Ware JE**, Kosinski M, Turner-Bowker DM, Gandek B. How to Score Version 2 of the SF-12 Health Survey (with a supplement Documenting Version 1). Lincoln, RI: Quality Metric Incorporated, 2002 [DOI: [10.1023/a:1026179517081](#)]
- 11 **Maruish ME**. User's Manual for the SF-12v2 Health Survey. 3rd ed. Lincoln, RI: Quality Metric Incorporated, 2012 [DOI: [10.1007/978-94-007-0753-5\\_103826](#)]
- 12 **McLean KA**, Drake TM, Sgrò A, Camilleri-Brennan J, Knight SR, Ots R, Adair A, Wigmore SJ, Harrison EM. The effect of liver transplantation on patient-centred outcomes: a propensity-score matched analysis. *Transpl Int* 2019; **32**: 808-819 [PMID: [30793373](#) DOI: [10.1111/tri.13416](#)]
- 13 **Burra P**, Ferrarese A, Feltrin G. Quality of life and adherence in liver transplant recipients. *Minerva Gastroenterol Dietol* 2018; **64**: 180-186 [PMID: [29160655](#) DOI: [10.23736/S1121-421X.17.02459-X](#)]
- 14 **Burra P**, Ferrarese A. Health-related quality of life in liver transplantation: another step forward. *Transpl Int* 2019; **32**: 792-793 [PMID: [30924559](#) DOI: [10.1111/tri.13435](#)]
- 15 **Dąbrowska-Bender M**, Kozaczuk A, Pączek L, Milkiewicz P, Słoniewski R, Staniszevska A. Patient Quality of Life After Liver Transplantation in Terms of Emotional Problems and the Impact of Sociodemographic Factors. *Transplant Proc* 2018; **50**: 2031-2038 [PMID: [30177104](#) DOI: [10.1016/j.transproceed.2018.03.113](#)]
- 16 **Rodríguez-Perálvarez M**, Guerrero-Misas M, Thorburn D, Davidson BR, Tsochatzis E, Gurusamy KS. Maintenance immunosuppression for adults undergoing liver transplantation: a network meta-analysis. *Cochrane Database Syst Rev* 2017; **3**: CD011639 [PMID: [28362060](#) DOI: [10.1002/14651858.CD011639.pub2](#)]
- 17 **Amodio P**, Salari L, Montagnese S, Schiff S, Neri D, Bianco T, Minazzato L. Hepatitis C virus infection and health-related quality of life. *World J Gastroenterol* 2012; **18**: 2295-2299 [PMID: [22654420](#) DOI: [10.3748/wjg.v18.i9.2295](#)]
- 18 **Bhanji RA**, Takahashi N, Moynagh MR, Narayanan P, Angirekula M, Mara KC, Dierkhising RA, Watt KD. The evolution and impact of sarcopenia pre- and post-liver transplantation. *Aliment Pharmacol Ther* 2019; **49**: 807-813 [PMID: [30714184](#) DOI: [10.1111/apt.15161](#)]
- 19 **Ooi PH**, Hager A, Mazurak VC, Dajani K, Bhargava R, Gilmour SM, Mager DR. Sarcopenia in Chronic Liver Disease: Impact on Outcomes. *Liver Transpl* 2019; **25**: 1422-1438 [PMID: [31242345](#) DOI: [10.1002/lt.25591](#)]
- 20 **Tandon P**, Montano-Loza AJ eds. Frailty and Sarcopenia in Cirrhosis. The Basics, the Challenges and the Future. Cham, Switzerland: Springer Nature, 2019: 1-279
- 21 **Lai JC**, Segev DL, McCulloch CE, Covinsky KE, Dodge JL, Feng S. Physical frailty after liver transplantation. *Am J Transplant* 2018; **18**: 1986-1994 [PMID: [29380529](#) DOI: [10.1111/ajt.14675](#)]
- 22 **Dasarathy S**, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. *J Hepatol* 2016; **65**: 1232-1244 [PMID: [27515775](#) DOI: [10.1016/j.jhep.2016.07.040](#)]
- 23 **Davuluri G**, Krokowski D, Guan BJ, Kumar A, Thapaliya S, Singh D, Hatzoglou M, Dasarathy S. Metabolic adaptation of skeletal muscle to hyperammonemia drives the beneficial effects of l-leucine in cirrhosis. *J Hepatol* 2016; **65**: 929-937 [PMID: [27318325](#) DOI: [10.1016/j.jhep.2016.06.004](#)]
- 24 **Barboza KC**, Salinas LM, Sahebjam F, Jesudian AB, Weisberg IL, Sigal SH. Impact of depressive symptoms and hepatic encephalopathy on health-related quality of life in cirrhotic hepatitis C patients. *Metab Brain Dis* 2016; **31**: 869-880 [PMID: [27032930](#) DOI: [10.1007/s11011-016-9817-y](#)]
- 25 **Jopson L**, Dyson JK, Jones DE. Understanding and Treating Fatigue in Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis. *Clin Liver Dis* 2016; **20**: 131-142 [PMID: [26593295](#) DOI: [10.1016/j.cld.2015.08.007](#)]
- 26 **Elliott C**, Frith J, Pairman J, Jones DE, Newton JL. Reduction in functional ability is significant postliver transplantation compared with matched liver disease and community dwelling controls. *Transpl Int* 2011; **24**: 588-595 [PMID: [21382100](#) DOI: [10.1111/j.1432-2277.2011.01240.x](#)]
- 27 **Kang SH**, Choi Y, Han HS, Yoon YS, Cho JY, Kim S, Kim KH, Hyun IG, Shehta A. Fatigue and weakness hinder patient social reintegration after liver transplantation. *Clin Mol Hepatol* 2018; **24**: 402-408 [PMID: [30300990](#) DOI: [10.3350/cmh.2018.0028](#)]
- 28 **Paglione HB**, Oliveira PC, Mucci S, Roza BA, Schirmer J. Quality of life, religiosity, and anxiety and depressive symptoms in liver transplantation candidates. *Rev Esc Enferm USP* 2019; **53**: e03459 [PMID: [30942299](#) DOI: [10.1590/S1980-220X2018010203459](#)]
- 29 **Nusrat S**, Khan MS, Fazili J, Madhoun MF. Cirrhosis and its complications: evidence based treatment. *World J Gastroenterol* 2014; **20**: 5442-5460 [PMID: [24833875](#) DOI: [10.3748/wjg.v20.i18.5442](#)]
- 30 **Annema C**, Drent G, Roodbol PF, Stewart RE, Metselaar HJ, van Hoek B, Porte RJ, Ranchor AV. Trajectories of Anxiety and Depression After Liver Transplantation as Related to Outcomes During 2-Year Follow-Up: A Prospective Cohort Study. *Psychosom Med* 2018; **80**: 174-183 [PMID: [29112588](#) DOI: [10.1097/PSY.0000000000000539](#)]
- 31 **Saracino RM**, Jutagir DR, Cunningham A, Foran-Tuller KA, Driscoll MA, Sledge WH, Emre SH,



- Fehon DC. Psychiatric Comorbidity, Health-Related Quality of Life, and Mental Health Service Utilization Among Patients Awaiting Liver Transplant. *J Pain Symptom Manage* 2018; **56**: 44-52 [PMID: 29526612 DOI: 10.1016/j.jpainsymman.2018.03.001]
- 32 **Annema C**, Drent G, Roodbol PF, Metselaar HJ, Van Hoek B, Porte RJ, Schreovers MJ, Ranchor AV. A prospective cohort study on posttraumatic stress disorder in liver transplantation recipients before and after transplantation: Prevalence, symptom occurrence, and intrusive memories. *J Psychosom Res* 2017; **95**: 88-93 [PMID: 28185646 DOI: 10.1016/j.jpsychores.2017.01.012]
- 33 **Chen PX**, Yan LN, Wang WT. Health-related quality of life of 256 recipients after liver transplantation. *World J Gastroenterol* 2012; **18**: 5114-5121 [PMID: 23049223 DOI: 10.3748/wjg.v18.i36.5114]
- 34 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol* 2016; **64**: 433-485 [DOI: 10.1016/j.jhep.2015.10.006]
- 35 **Onghena L**, Develtere W, Poppe C, Geerts A, Troisi R, Vanlander A, Berrevoet F, Rogiers X, Van Vlierberghe H, Verhelst X. Quality of life after liver transplantation: State of the art. *World J Hepatol* 2016; **8**: 749-756 [PMID: 27366301 DOI: 10.4254/wjh.v8.i18.749]
- 36 **Chang JH**, Lee I, Choi MG, Han SW. Current diagnosis and treatment of benign biliary strictures after living donor liver transplantation. *World J Gastroenterol* 2016; **22**: 1593-1606 [PMID: 26819525 DOI: 10.3748/wjg.v22.i4.1593]
- 37 **Domingos MF**, Coelho JCU, Nogueira IR, Parolin MB, Matias JEF, De Freitas ACT, Zeni Neto C, Ramos EJB. Quality of Life after 10 Years of Liver Transplantation. *J Gastrointest Liver Dis* 2020; **29**: 611-616 [PMID: 33118544 DOI: 10.15403/jgld-2829]
- 38 **Jover-Aguilar M**, Martínez-Alarcón L, Ramis G, Gago FA, Ríos A, Febrero B, García CC, Hiciano Guillermo AI, Ramírez P. Self-Esteem Related to Quality of Life in Patients Over 60 Years Old Who Received an Orthotopic Liver Transplantation More Than 10 Years Ago. *Transplant Proc* 2020; **52**: 562-565 [PMID: 32057494 DOI: 10.1016/j.transproceed.2019.12.017]
- 39 **Beekman L**, Berzigotti A, Banz V. Physical Activity in Liver Transplantation: A Patient's and Physicians' Experience. *Adv Ther* 2018; **35**: 1729-1734 [PMID: 30328060 DOI: 10.1007/s12325-018-0797-7]
- 40 **Legendre C**, Viebahn R, Crespo M, Dor F, Gustafsson B, Samuel U, Karam V, Binet I, Aberg F, De Geest S, Moes DJAR, Tonshoff B, Oppenheimer F, Asberg A, Halleck F, Loupy A, Suesal C. Beyond Survival in Solid Organ Transplantation: A Summary of Expert Presentations from the Sandoz 6th Standalone Transplantation Meeting, 2018. *Transplantation* 2019; **103**: S1-S13 [PMID: 31449167 DOI: 10.1097/TP.0000000000002846]
- 41 **Tarabeih M**, Bokek-Cohen Y, Azuri P. Health-related quality of life of transplant recipients: a comparison between lung, kidney, heart, and liver recipients. *Qual Life Res* 2020; **29**: 1631-1639 [PMID: 32016682 DOI: 10.1007/s11136-020-02434-4]
- 42 **WHO**. WHOQOL-BREF: Introduction, administration, scoring and generic version of the assessment; Field trial version. Geneva: World Health Organization; 1996. [cited 20 February 2021]. Available from: [https://www.who.int/mental\\_health/media/en/76.pdf](https://www.who.int/mental_health/media/en/76.pdf)
- 43 **Ziegelmann JP**, Griva K, Hankins M, Harrison M, Davenport A, Thompson D, Newman SP. The Transplant Effects Questionnaire (TxEQ): The development of a questionnaire for assessing the multidimensional outcome of organ transplantation - example of end stage renal disease (ESRD). *Br J Health Psychol* 2002; **7**: 393-408 [PMID: 12614493 DOI: 10.1348/135910702320645381]
- 44 **Sullivan KM**, Radosevich DM, Lake JR. Health-related quality of life: two decades after liver transplantation. *Liver Transpl* 2014; **20**: 649-654 [PMID: 24678028 DOI: 10.1002/lt.23855]
- 45 **Dunn MA**, Rogal SS, Duarte-Rojo A, Lai JC. Physical Function, Physical Activity, and Quality of Life After Liver Transplantation. *Liver Transpl* 2020; **26**: 702-708 [PMID: 32128971 DOI: 10.1002/lt.25742]
- 46 **Neale J**, Smith AC, Bishop NC. Effects of Exercise and Sport in Solid Organ Transplant Recipients: A Review. *Am J Phys Med Rehabil* 2017; **96**: 273-288 [PMID: 27552351 DOI: 10.1097/PHM.0000000000000599]
- 47 **Casanovas T**, Herdman M, Chandía A, Peña MC, Fabregat J, Vilallonga JS. Identifying Improved and Non-improved Aspects of Health-related Quality of Life After Liver Transplantation Based on the Assessment of the Specific Questionnaire Liver Disease Quality of Life. *Transplant Proc* 2016; **48**: 132-137 [PMID: 26915858 DOI: 10.1016/j.transproceed.2015.11.009]
- 48 **Stewart CA**, Auger RR, Enders FT, Felmlee-Devine D, Smith GE. The effects of poor sleep quality on cognitive function of patients with cirrhosis. *J Clin Sleep Med* 2014; **10**: 21-26 [PMID: 24426816 DOI: 10.5664/jcsn.3350]
- 49 **Cohen SR**, Mount BM, Strobel MG, Bui F. The McGill Quality of Life Questionnaire: a measure of quality of life appropriate for people with advanced disease. A preliminary study of validity and acceptability. *Palliat Med* 1995; **9**: 207-219 [PMID: 7582177 DOI: 10.1177/026921639500900306]
- 50 **Girgenti R**, Tropea A, Buttafarro MA, Ragusa R, Ammirata M. Quality of Life in Liver Transplant Recipients: A Retrospective Study. *Int J Environ Res Public Health* 2020; **17** [PMID: 32471157 DOI: 10.3390/ijerph17113809]
- 51 **Burra P**, Germani G. Long-term quality of life for transplant recipients. *Liver Transpl* 2013; **19** Suppl 2: S40-S43 [PMID: 23960031 DOI: 10.1002/lt.23725]
- 52 **Pérez-San-Gregorio MÁ**, Martín-Rodríguez A, Sánchez-Martín M, Borda-Mas M, Avargues-Navarro ML, Gómez-Bravo MÁ, Conrad R. Spanish Adaptation and Validation of the Transplant Effects Questionnaire (TxEQ-Spanish) in Liver Transplant Recipients and Its Relationship to

- Posttraumatic Growth and Quality of Life. *Front Psychiatry* 2018; **9**: 148 [PMID: [29720952](#) DOI: [10.3389/fpsy.2018.00148](#)]
- 53 Åberg F. Quality of life after liver transplantation. *Best Pract Res Clin Gastroenterol* 2020; **46-47**: 101684 [PMID: [33158471](#) DOI: [10.1016/j.bpg.2020.101684](#)]

## Retrospective Study

# Machine learning models for predicting non-alcoholic fatty liver disease in the general United States population: NHANES database

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## Abstract

### BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, affecting over 30% of the United States population. Early patient identification using a simple method is highly desirable.

### AIM

To create machine learning models for predicting NAFLD in the general United States population.

### METHODS

Using the NHANES 1988-1994. Thirty NAFLD-related factors were included. The dataset was divided into the training (70%) and testing (30%) datasets. Twenty-four machine learning algorithms were applied to the training dataset. The best-performing models and another interpretable model (*i.e.*, coarse trees) were tested using the testing dataset.

### RESULTS

There were 3235 participants ( $n = 3235$ ) that met the inclusion criteria. In the training phase, the ensemble of random undersampling (RUS) boosted trees had the highest F1 (0.53). In the testing phase, we compared selective machine learning models and NAFLD indices. Based on F1, the ensemble of RUS boosted trees remained the top performer (accuracy 71.1% and F1 0.56) followed by the fatty liver index (accuracy 68.8% and F1 0.52). A simple model (coarse trees) had

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an accuracy of 74.9% and an F1 of 0.33.

## CONCLUSION

Not every machine learning model is complex. Using a simpler model such as coarse trees, we can create an interpretable model for predicting NAFLD with only two predictors: fasting C-peptide and waist circumference. Although the simpler model does not have the best performance, its simplicity is useful in clinical practice.

**Key Words:** Artificial intelligence; Machine learning; Non-alcoholic fatty liver disease; Fatty liver; United States population; NHANES

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**Core Tip:** A simple method with a good accuracy for identifying patients with non-alcoholic fatty liver disease is highly desirable. Among 24 machine learning models, the ensemble of random undersampling boosted trees was the top performer (accuracy 71.1% and F1 0.56). A simple model (coarse trees) with only two predictors (fasting C-peptide and waist circumference) had an accuracy of 74.9% and an F1 of 0.33. Not every machine learning model is complex. Using a simple model such as coarse trees, physicians can easily integrate machine learning model into their practice without any software implementation.

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common chronic metabolic disease found in 25.5% of the United States population, and it is more common in patients with diabetes (55.5%), leading to a health and economic burden[1-3]. Non-alcoholic steatohepatitis (NASH) can lead to liver-related consequences, such as cirrhosis, hepatocellular carcinoma, and mortality. NASH is the second most common indication for liver transplantation in the United States and is likely to replace hepatitis C infection as the leading cause of liver transplantation in the future[4]. NAFLD is diagnosed primarily with imaging studies, transient elastography, magnetic resonance elastography, or liver biopsy[5]. Some of these diagnostic modalities are not available in every health care facility, require expert interpretation, and are invasive in case of biopsy[5,6]. To prevent adverse outcomes in these patients, early screening and detection based on risk factors are warranted. Healthcare providers and patients are aware of the risk factors of NAFLD, which include diabetes, obesity, dyslipidemia, and metabolic syndrome[5,7,8]. However, there is no well-performing tool for the early prediction of NAFLD; for example, liver enzyme levels can be normal in patients with NAFLD[9,10]. There are existing studies on the risk factors and prediction risk scores; however, their results are controversial[11-15]. Machine learning is a potential approach for the identification of the best predictive model[16].

Machine learning can be used to construct a predictive model by teaching computer algorithms to learn from data without being explicitly programmed. Applications of machine learning in gastroenterology field are steadily increasing[17]. However, there is no machine learning model for predicting NAFLD in the United States. The published models in China, Germany, and Canada focus on NAFLD prediction scores using laboratory parameters and demographic data[11,13-15]. Therefore, we aimed to evaluate the applications of machine learning in NAFLD diagnosis for easy use at clinical setting.

## MATERIALS AND METHODS

### Study population and study design

The Third National Health and Nutrition Examination Survey (NHANES III) was a nationwide probability sample of 39695 persons aged 2 mo and older, conducted from 1988-1994 by the National Center for Health Statistics (NCHS). It aimed to evaluate the health and nutritional status of the general United States population[18]. Multiple datasets were collected in this survey, including demographics, interviews, physical examinations, and laboratory testing of biologic samples. The NHANES protocol was approved by the NCHS Research Ethics Review Board.

### Definitions

Participants aged 20 years or older in NHANES III with gradable ultrasound results were included in this study. The exclusion criteria included: (1) Excessive alcohol consumption; (2) Hepatitis B or C infection; (3) Fasting period outside of 8-24 h; and (4) Incomplete or missing data on physical examination and laboratory testing. The participants were divided into two groups: The NAFLD participants and non-NAFLD participants. Since participants aged above 74 years were not eligible for ultrasonography in NHANES III, participants aged above 74 years were excluded from this study.

'NAFLD participants' was defined based on: (1) Moderate to severe hepatic steatosis on ultrasound; (2) No history of alcohol drinking more than 2 drinks per day for men or 1 drink per day for women in the last 12 mo; and (3) No history of hepatitis B or C infection.

Thirty factors associated with NAFLD were included in this study: demographic (*i.e.*, age, gender, and race/ethnicity), body measurement [*i.e.*, body mass index (BMI) and waist circumference], general biochemistry tests [*i.e.*, iron, total iron-binding capacity, transferrin saturation, ferritin, cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, C-reactive protein, and uric acid], liver chemistry (aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase, total bilirubin, total protein, albumin, and serum globulin), diabetes testing profile [*i.e.*, glycated hemoglobin, fasting plasma glucose, fasting C-peptide, and fasting insulin], and the use of diabetes medication.

### Statistical analysis

Categorical and ordinal factors are presented as frequencies (%). Continuous factors are presented as medians (interquartile ranges). The dataset was divided into the training (70%) and testing (30%) datasets using stratified sampling. Differences between the two datasets were tested using the Mann-Whitney U test. Twenty-four machine learning algorithms were applied to the training dataset. Then, we selected the best performing models determined by accuracy and the F1 score and compared the out-of-sample performance with another interpretable model (coarse trees, a decision tree model with a maximum of four splits) and three NAFLD indices on the testing dataset. The selected NAFLD indices included fatty liver index (FLI), hepatic steatosis index (HSI), and triglyceride and glucose index (TyG)[19-21]. The cut-off levels for NAFLD were  $\geq 60$  for FLI,  $> 36$  for HSI, and  $\geq 8.5$  for TyG. The performance metrics include accuracy, sensitivity or recall, specificity, precision, area under the receiver operating characteristic curve (AUC), and the F1 score. It is worth noting that the F1 score is the harmonic mean of precision and recall. All statistical analyses were performed using MATLAB R2020a (MathWorks, MA, United States).

## RESULTS

The study had 3235 participants ( $n = 3235$ ). The participant selection process is shown in Figure 1. Based on ultrasound findings, 817 (25.26%) participants had NAFLD. The data of 2265 (70%) and 970 (30%) participants made up the training and testing groups, respectively. The baseline characteristics of participants in the training and testing groups are summarized in Table 1. There were no significant differences between the datasets for all factors.

The performances of 24 machine learning algorithms that were applied to the training dataset are illustrated in Table 2. The ensemble of subspace discriminant and ensemble of random undersampling (RUS) boosted trees had the highest accuracy (78.3%) and highest F1 score (0.53), respectively; both models had an AUC of 0.76. The coarse trees, decision trees with a few leaves, had an accuracy of 76%, AUC of 0.68,



**Table 1** Baseline characteristics of participants in training and testing data

	Training data (n = 2265)	Testing data (n = 970)	P value
Demographic			
Age (yr)	43 (29)	43.5 (28)	0.328
Gender (male) (%)	944 (41.68)	428 (44.12)	0.197
Race/ethnicity			
White (non-Hispanic) (%)	959 (42.34)	392 (40.41)	0.308
Black (non-Hispanic) (%)	627 (27.68)	271 (27.94)	0.882
Mexican American (%)	576 (25.43)	254 (26.19)	0.652
Others (%)	103 (4.55)	53 (5.46)	0.265
Body measurement			
Body mass index (kg/m <sup>2</sup> )	26.4 (7.2)	26.7 (7.4)	0.120
Waist circumference (cm)	93 (20.5)	93.5 (20.8)	0.182
Biochemistry tests			
Iron (ug/dL)	73 (39)	74 (39)	0.098
Total iron-binding capacity (ug/dL)	355 (72)	356 (72)	0.450
Transferrin saturation (%)	20.5 (11.1)	20.8 (11.8)	0.329
Ferritin (ng/mL)	87 (125)	84.5 (124)	0.508
Cholesterol (mg/dL)	201 (57)	204 (59)	0.155
Triglyceride (mg/dL)	120 (100.25)	122.5 (102)	0.562
HDL cholesterol (mg/dL)	48 (18)	48.5 (18)	0.585
C-reactive protein (mg/dL)	0.21 (0.29)	0.21 (0.23)	0.686
Uric acid (mg/dL)	5 (1.9)	5.1 (2)	0.427
Liver chemistry			
Aspartate aminotransferase (U/L)	19 (8)	19 (7)	0.908
Alanine aminotransferase (U/L)	14 (10)	14 (10)	0.581
Gamma glutamyl transferase (U/L)	21 (18)	21 (18)	0.787
Alkaline phosphatase (U/L)	83 (33)	81 (32)	0.524
Total bilirubin (mg/dL)	0.5 (0.2)	0.5 (0.2)	0.855
Total protein (g/dL)	7.4 (0.6)	7.4 (0.6)	0.559
Albumin (g/dL)	4.1 (0.5)	4.1 (0.4)	0.543
Serum globulin (g/dL)	3.3 (0.6)	3.3 (0.7)	0.941
Diabetes testing profile			
Glycated hemoglobin (%)	5.4 (0.8)	5.4 (0.7)	0.075
Fasting plasma glucose (mg/dL)	91.6 (12.52)	92.05 (12.2)	0.726
Fasting C-peptide (pmol/mL)	0.65 (0.68)	0.66 (0.69)	0.746
Fasting insulin (uU/mL)	9.36 (9.51)	9.73 (10.04)	0.378
Diabetes medication	165 (7.28%)	68 (7.01%)	0.782

and F1 score of 0.36.

As shown in the first half of [Table 3](#), the ensemble of subspace discriminant, coarse trees, and ensemble of RUS-boosted trees models were selected for testing the process on the testing data. When tested on the testing data, ensemble of subspace discriminant and ensemble of RUS-boosted trees still had a high accuracy (77.7%) and high F1 (0.56), respectively. The coarse tree had an accuracy of 74.9% and an F1 of 0.33. All the machine learning models and datasets are available for public access in the File

**Table 2** The performance comparison of machine learning models on training data

No.	Description	Accuracy (%)	AUC	PPV/precision (%)	NPV (%)	Sensitivity/recall (%)	Specificity (%)	F1
1	Fine tree	71.6	0.64	42.9	79.8	37.8	83.0	0.40
2	Medium tree	74.4	0.70	48.9	79.1	30.1	89.4	0.37
3	Coarse tree	76.0	0.68	55.1	78.9	26.4	92.7	0.36
4	Linear discriminant	78.0	0.75	61.1	80.9	35.5	92.4	0.45
5	Logistic regression	78.1	0.75	62.2	80.6	33.9	93.0	0.44
6	Gaussian naïve Bayes	75.1	0.74	50.8	81.1	40.2	86.8	0.45
7	Kernel naïve Bayes	72.7	0.73	46.8	85.1	60.1	76.9	0.53
8	Linear SVM	77.0	0.74	64.4	78.1	19.9	96.3	0.30
9	Quadratic SVM	77.4	0.70	59.9	80.1	31.8	92.8	0.42
10	Cubic SVM	72.8	0.64	45.1	79.6	35.3	85.5	0.40
11	Fine Gaussian SVM	74.7	0.67		74.7		100.0	
12	Medium Gaussian SVM	77.5	0.74	63.9	79.0	25.3	95.2	0.36
13	Coarse Gaussian SVM	75.7	0.74	66.2	76.0	7.9	98.6	0.14
14	Fine KNN	68.9	0.58	38.0	78.9	36.9	79.7	0.37
15	Medium KNN	76.5	0.71	59.7	78.1	21.0	95.2	0.31
16	Coarse KNN	76.6	0.75	78.1	76.5	10.0	99.1	0.18
17	Cosine KNN	76.6	0.72	57.9	79.2	27.6	93.2	0.37
18	Cubic KNN	77.0	0.72	62.0	78.5	22.6	95.3	0.33
19	Weighted KNN	76.5	0.71	56.7	79.4	28.8	92.6	0.38
20	Ensemble of boosted trees	76.9	0.74	57.3	80.3	33.6	91.6	0.42
21	Ensemble of bagged trees	77.2	0.74	58.9	80.2	32.5	92.3	0.42
22	Ensemble of subspace discriminant	78.3	0.76	66.7	79.7	28.3	95.2	0.40
23	Ensemble of subspace KNN	75.5	0.69	54.7	77.2	16.4	95.4	0.25
24	Ensemble of RUS boosted trees	70.4	0.76	44.2	86.3	66.4	71.7	0.53

AUC: Area under the curve; KNN: K-nearest neighbors; NPV: Negative predictive value; PPV: Positive predictive value; RUS: Random undersampling; SVM: Support vector machine.

Exchange portal of the MATLAB Central File Exchange[22]. The performance of three NAFLD on the testing data are also displayed in the second half of Table 3. FLI was the best performer among the NAFLD indices with the accuracy of 68.6% and F1 score of 0.52. However, the ensemble of RUS boosted trees was superior to FLI in all metrics.

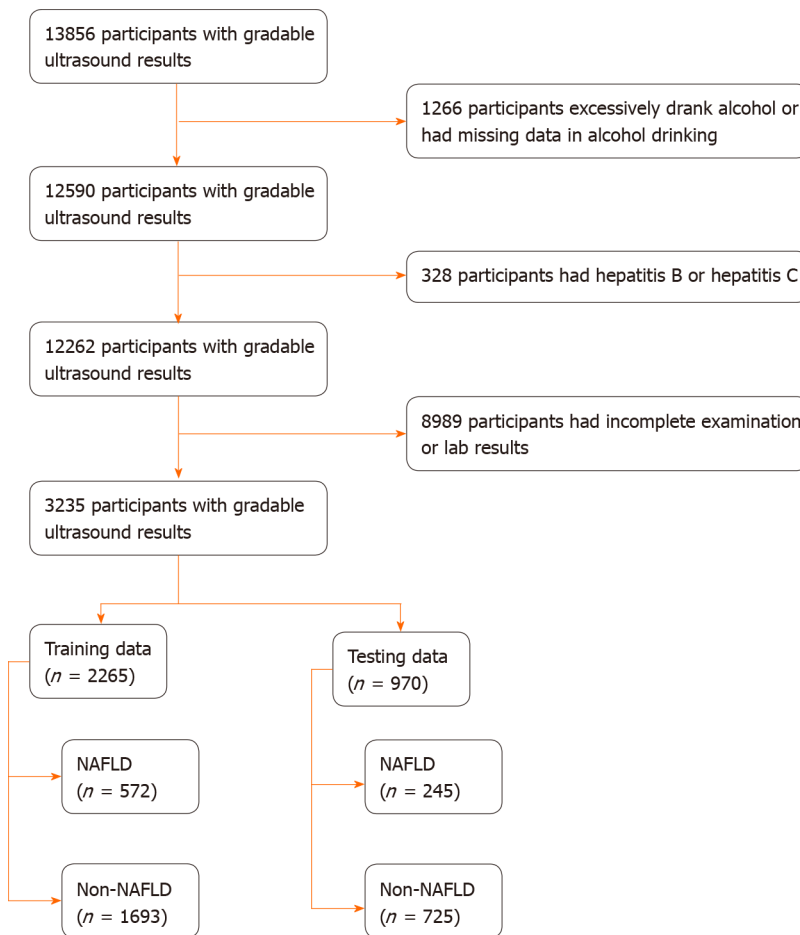
## DISCUSSION

Our study compared 24 different machine learning techniques to determine the optimal clinical predictive model for NAFLD. The accuracy of these models on the training data did not show much variation (range 9.4%), with an average of 75.5% (Table 2). The top two models were ensemble of subspace discriminant and ensemble of RUS boosted trees. The ensemble of subspace discriminant model had a higher accuracy while the ensemble of RUS boosted trees model had a better performance in classifying positive NAFLD, as indicated by the F1 score. Both models were ensemble type, which use multiple diverse models in combination to produce an optimal prediction. They are more complex machine learning models that apparently yield better predictions. Compared to accuracy, the F1 score is regarded as a superior performance metric for a class imbalance problem (often a large number of actual

**Table 3** The performance of machine learning models and other non-alcoholic fatty liver disease indices on testing data

No.	Description	Accuracy (%)	AUC	PPV/precision (%)	NPV (%)	Sensitivity/recall (%)	Specificity (%)	F1
<b>Machine learning models</b>								
1	Ensemble of subspace discriminant	77.7	0.78	66.7	78.8	23.7	96	0.35
2	Coarse trees	74.9	0.72	50.8	78.3	24.5	92	0.33
3	Ensemble of RUS boosted trees	71.1	0.79	45.5	88.4	72.7	70.6	0.56
<b>NAFLD indices</b>								
4	Fatty liver index	68.6	0.74	42.4	86.6	68.6	68.6	0.52
5	Hepatic steatosis index	65.1	0.70	37.9	83.3	60.4	66.6	0.47
6	Triglyceride and glucose index	56.9	0.69	34.8	88.3	80.8	48.8	0.49

AUC: Area under the curve; NAFLD: Non-alcoholic fatty liver disease; NPV: Negative predictive value; PPV: Positive predictive value; RUS: Random undersampling.


**Figure 1** Study design and data partitioning flow chart. NAFLD: Non-alcoholic fatty liver disease.

negatives). In our opinion, the ensemble of RUS boosted trees model was the best performing machine learning model in this study.

Technically, the final prediction of the ensemble method was derived from a combination of multiple predictions from different algorithms. In our case, the predicted outcome of the ensemble of RUS boosted trees model was derived from a weighted average outcome of 30 RUS boosted trees; the sample visualization of these RUS boosted trees can be found in the file uploaded to the MATLAB Central File Exchange[22].

On the other hand, we compared the performance of the previous model with the coarse trees model, simple decision trees with several leaves and splits (Figure 2). The decision logic of the coarse trees model consisted of only two factors: Waist circumference and serum C-peptide. In terms of testing performance, it had a reasonable accuracy (AUC, 0.72; accuracy, 74.9%; and F1 score, 0.33). Since it is simple-to-use and easily interpretable, the coarse trees model can be more practically used in clinical practice.

Waist circumference is directly associated with obesity and metabolic syndrome[23, 24]. They are also the established risk factors of NAFLD. The cut-off of 109.35 cm seems to be slightly higher than the general cut off value for metabolic syndrome (men, 102 cm and women, 80 cm)[25]. It is used to calculate the visceral adiposity index, which provides a good predictive capability[26]. The advantage of incorporating waist circumference into the model is its retrieval ability.

Our results are similar to those of a previous study identifying the risk factors of NAFLD[27]. C-peptide is an indicator of insulin resistance[28,29]. Serum C-peptide is associated with NAFLD, NASH, and fibrosis progression[28-30]. Additionally, serum C-peptide levels increase with NAFLD severity[29,31,32]. In our study, serum C-peptide is more significantly associated with NAFLD prediction than liver function test. This can be explained by the fact that liver enzymes are possibly not specific to NAFLD. They can also be elevated in other liver diseases. On the contrary, serum C-peptide is related to metabolic alterations, which play a direct role in NAFLD development.

We compared the performance of three NAFLD indices (FLI, HSI, and TyG) on the testing data. Among these three NAFLD indices, FLI had the highest performance in terms of accuracy (68.6%) and F1 (0.52). However, performance-wise, the ensemble of RUS boosted trees was superior to FLI in all aspects. In terms of simplicity, FLI is not complex, but it might be impossible for physicians to use it without spreadsheets or computers because it involves many mathematical operations, such as multiplication, logarithm function, and exponential function. Therefore, coarse trees remained the simplest model.

Previously developed machine learning models for NAFLD prediction have used more complex parameters, including laboratory and noninvasive scores. A population-based study in Italy developed a score for NAFLD diagnosis with a moderate accuracy of 68% in the model development phase, but extremely high performance in the testing (prediction) phase using the small sample size of 50. The predictors used in the model were of abdominal volume index, glucose, gamma glutamyl transferase, age, and sex[33]. A Chinese study incorporated three demographic factors and 15 Laboratory tests as predictors for Bayesian network model[8]. The inclusion of simple constituents, liver enzymes, lipid panels, and complete blood count resulting in an accuracy of up to 80% in a 10-fold cross validation; there was no separate data set for external validation or testing. A Taiwanese study revealed that waist circumference was the most influential factor in the model resulting in a high performance with an AUC of 0.925[13]. Similarly, such performance was based on a 10-fold cross validation, not on a separate data set for external validation or testing. In addition, the ethnic Chinese population generally has a lower alcohol consumption; it might not be generalized to other ethnic groups[12,15]. A Canadian study revealed that HDL, BMI, sex, plasma glucose, blood pressure, and age were factors used in the decision criteria of decision trees with an AUC of 0.73[14]. These reports showed different significant factors in their models. This might be explained by the different populations in terms of ethnicity, alcohol consumption, and obesity prevalence. Compared to prior reports, our study involved a general population of the United States, which has less selection bias and contains diverse races. Therefore, the derived models in this study can be applied to diverse ethnic and racial backgrounds. A detailed comparison of the proposed machine learning models in prior reports is summarized in Table 4.

The application of machine learning in regarding NAFLD has evolved from the diagnosis with the noninvasive screening methods to liver biopsy. The new score achieves the reasonable performance with AUC of 0.70, in terms of differentiating between NAFL and NASH[11]. Deep learning model was evaluated for diagnosis NAFLD based on ultrasound images and had a good predictive ability (AUC > 0.7) [34]. Given the advancement in this field, it can also be used to quantify steatosis, inflammation, ballooning, and fibrosis in biopsy histology of patients with NAFLD having excellent results[35].

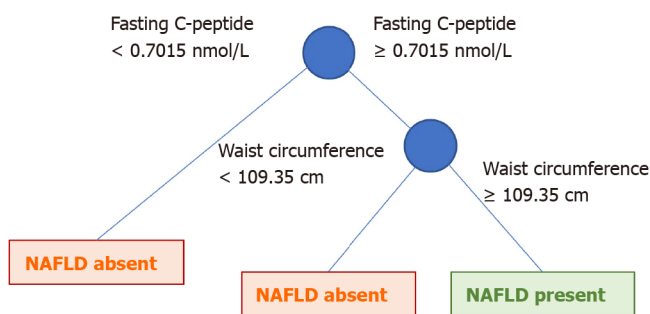
This study had strengths. First, this is the first United States population-based study with more than 3000 individuals from NHANES III. Secondly, we aimed to propose the simple model with a reasonable predictive power for NAFLD. This model will be potentially applied in clinical practice, especially by primary care providers, prior to

**Table 4 The performance comparison of published machine learning models on non-alcoholic fatty liver disease prediction**

Ref.	Type of data/country or territory of data	Number of train/external testing data	Model	Accuracy (%)	AUC	Sensitivity (%)	Specificity (%)	F1
Sorino <i>et al</i> [33], 2020	Population/Italy	2920/50	Support vector machine	68 <sup>1</sup>	N/A	98.5	100	N/A
Wu <i>et al</i> [13], 2019	Hospital/Taiwan	577/NA	Random forest	86.5 <sup>1</sup>	0.925 <sup>1</sup>	87.2 <sup>1</sup>	85.9 <sup>1</sup>	N/A
Islam <i>et al</i> [36], 2018	Hospital/Taiwan	994/NA	Logistic regression	70 <sup>1</sup>	0.763 <sup>1</sup>	74.1 <sup>1</sup>	64.9 <sup>1</sup>	N/A
Ma <i>et al</i> [12], 2018	Hospital/China	10508/NA	Bayesian network	82.92 <sup>1</sup>	N/A	67.5 <sup>1</sup>	87.8 <sup>1</sup>	0.655 <sup>1</sup>
Perveen <i>et al</i> [14], 2018	Primary care network/Canada	64%/34% of 40637	Decision trees	N/A	0.73	73	N/A	0.67
Yip <i>et al</i> [15], 2017	Hospital/Hong Kong	500/442	Ridge regression	87	0.87	92	90	N/A
Birjandi <i>et al</i> [37], 2016	Hospital/Iran	359/1241	Decision trees	75	0.75	73	77	N/A
Our study	Population based/United States	2265/970	Ensemble of RUS boosted trees	71.1	0.79	72.7	70.6	0.56
			Coarse trees	74.9%	0.72	24.5%	92%	0.33

<sup>1</sup>Cross-validation performance (no separate dataset designated for testing the performance).

RUS: Random undersampling; AUC: area under the receiver operating characteristic curve; N/A: Not applicable; NA: Not available.


**Figure 2 The decision logic of coarse trees.** NAFLD: Non-alcoholic fatty liver disease.

referring patients to hepatologists. This study had some limitations. (1) Missing data were inherited from the nature of population dataset from NHANES III; (2) NAFLD was diagnosed with ultrasonography, which is not the gold standard; however, it is the primary imaging modality for NAFLD diagnosis in population-based studies and available in primary care medical facilities; (3) At the time of writing this article, there was no external dataset available that like that of NHANES III for validating the models; and (4) It may be impossible to completely reproduce the machine learning algorithms in this study since randomization was used in the modeling process, such as data partitioning, cross validation, and creation of some machine learning models. This explains why we made the trained models available to the public so that anyone can use the models directly and/or validate our results.

## CONCLUSION

Machine learning algorithms can summarize a large dataset into predictive models. The best performing model measured by the F1 score from our study is the ensemble of RUS boosted trees, which is a complex model that uses all 30 factors and behaves more like a black box to physicians. In contrast, the coarse trees model, which is composed of serum C-peptide and waist circumference, can generate a reasonable predictive performance, and most importantly is the simplest to use. To facilitate



clinical decision-making, complex models should be incorporated into the electronic medical record system. This will lead to proper investigation and treatment selection for specific individuals at risk, helping to maximize healthcare resource utilization. If software deployment is not achievable, a simple model be used directly by physicians. Therefore, the model choice depends on the user objectives and resources. Therefore, the more complex model required more resources and was likely to outperform. The less complex model may not be the most accurate model but can be easily implemented and interpreted in clinical practice.

## ARTICLE HIGHLIGHTS

### Research background

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease that can progress to more severe liver disease.

### Research motivation

Early patient identification using a simple method is highly desirable for preventing the progression of NAFLD.

### Research objectives

To create machine learning models for predicting NAFLD in the general United States population.

### Research methods

This study was designed as a retrospective cohort by using the NHANES 1988-1994. Adults (20 years and above in age) with gradable ultrasound results were included in this study.

### Research results

Based on F1, the ensemble of ensemble of random undersampling boosted trees was the top performer (accuracy 71.1% and F1 0.56) while a simple model (coarse trees) had an accuracy of 74.9% and an F1 of 0.33.

### Research conclusions

Although a simpler model such as coarse trees was not the top performer, it consisted of only two predictors: fasting C-peptide and waist circumference. Its simplicity is useful in clinical practice.

### Research perspectives

The findings from this study can facilitate clinical decision-making for clinicians and also allow researchers to investigate the developed machine learning models. This will lead to proper investigation and treatment selection for specific individuals at risk, helping to maximize healthcare resource utilization.

## REFERENCES

- 1 **Fazel Y**, Koenig AB, Sayiner M, Goodman ZD, Younossi ZM. Epidemiology and natural history of non-alcoholic fatty liver disease. *Metabolism* 2016; **65**: 1017-1025 [PMID: 26997539 DOI: 10.1016/j.metabol.2016.01.012]
- 2 **Younossi ZM**, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, Racila A, Hunt S, Beckerman R. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology* 2016; **64**: 1577-1586 [PMID: 27543837 DOI: 10.1002/hep.28785]
- 3 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
- 4 **Younossi Z**, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, Eguchi Y, Wong VW, Negro F, Yilmaz Y, Romero-Gomez M, George J, Ahmed A, Wong R, Younossi I, Ziayee M, Afendy A; Global Nonalcoholic Steatohepatitis Council. Nonalcoholic Steatohepatitis Is the Fastest Growing Cause of Hepatocellular Carcinoma in Liver Transplant Candidates. *Clin Gastroenterol Hepatol* 2019; **17**: 748-755.e3 [PMID: 29908364 DOI: 10.1016/j.cgh.2018.05.057]
- 5 **Younossi ZM**, Loomba R, Anstee QM, Rinella ME, Bugianesi E, Marchesini G, Neuschwander-Tetri BA, Serfaty L, Negro F, Caldwell SH, Ratziu V, Corey KE, Friedman SL, Abdelmalek MF, Harrison

- SA, Sanyal AJ, Lavine JE, Mathurin P, Charlton MR, Goodman ZD, Chalasani NP, Kowdley KV, George J, Lindor K. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. *Hepatology* 2018; **68**: 349-360 [PMID: 29222917 DOI: 10.1002/hep.29721]
- 6 Arab JP, Dirchwolf M, Álvares-da-Silva MR, Barrera F, Benítez C, Castellanos-Fernandez M, Castro-Narro G, Chavez-Tapia N, Chiodi D, Cotrim H, Cusi K, de Oliveira CPMS, Díaz J, Fassio E, Gerona S, Giralda M, Hernandez N, Marciano S, Masson W, Méndez-Sánchez N, Leite N, Lozano A, Padilla M, Panduro A, Paraná R, Parise E, Perez M, Poniachik J, Restrepo JC, Ruf A, Silva M, Tagle M, Tapias M, Torres K, Vilar-Gomez E, Costa Gil JE, Gadano A, Arrese M. Latin American Association for the study of the liver (ALEH) practice guidance for the diagnosis and treatment of non-alcoholic fatty liver disease. *Ann Hepatol* 2020; **19**: 674-690 [PMID: 33031970 DOI: 10.1016/j.aohp.2020.09.006]
- 7 Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, Subbarayan S, Webb A, Hecht J, Cusi K. High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. *J Clin Endocrinol Metab* 2015; **100**: 2231-2238 [PMID: 25885947 DOI: 10.1210/jc.2015-1966]
- 8 Ma J, Hwang SJ, Pedley A, Massaro JM, Hoffmann U, Chung RT, Benjamin EJ, Levy D, Fox CS, Long MT. Bi-directional analysis between fatty liver and cardiovascular disease risk factors. *J Hepatol* 2017; **66**: 390-397 [PMID: 27729222 DOI: 10.1016/j.jhep.2016.09.022]
- 9 Siddiqui MS, Sterling RK, Luketic VA, Puri P, Stravitz RT, Bouneva I, Boyett S, Fuchs M, Sargeant C, Warnick GR, Grami S, Sanyal AJ. Association between high-normal levels of alanine aminotransferase and risk factors for atherogenesis. *Gastroenterology* 2013; **145**: 1271-9.e1 [PMID: 23973920 DOI: 10.1053/j.gastro.2013.08.036]
- 10 Blais P, Husain N, Kramer JR, Kowalkowski M, El-Serag H, Kanwal F. Nonalcoholic fatty liver disease is underrecognized in the primary care setting. *Am J Gastroenterol* 2015; **110**: 10-14 [PMID: 24890441 DOI: 10.1038/ajg.2014.134]
- 11 Canbay A, Kälisch J, Neumann U, Rau M, Hohenester S, Baba HA, Rust C, Geier A, Heider D, Sowa JP. Non-invasive assessment of NAFLD as systemic disease-A machine learning perspective. *PLoS One* 2019; **14**: e0214436 [PMID: 30913263 DOI: 10.1371/journal.pone.0214436]
- 12 Ma H, Xu CF, Shen Z, Yu CH, Li YM. Application of Machine Learning Techniques for Clinical Predictive Modeling: A Cross-Sectional Study on Nonalcoholic Fatty Liver Disease in China. *Biomed Res Int* 2018; **2018**: 4304376 [PMID: 30402478 DOI: 10.1155/2018/4304376]
- 13 Wu CC, Yeh WC, Hsu WD, Islam MM, Nguyen PAA, Poly TN, Wang YC, Yang HC, Jack Li YC. Prediction of fatty liver disease using machine learning algorithms. *Comput Methods Programs Biomed* 2019; **170**: 23-29 [PMID: 30712601 DOI: 10.1016/j.cmpb.2018.12.032]
- 14 Perveen S, Shahbaz M, Keshavjee K, Guergachi A. A Systematic Machine Learning Based Approach for the Diagnosis of Non-Alcoholic Fatty Liver Disease Risk and Progression. *Sci Rep* 2018; **8**: 2112 [PMID: 29391513 DOI: 10.1038/s41598-018-20166-x]
- 15 Yip TC, Ma AJ, Wong VW, Tse YK, Chan HL, Yuen PC, Wong GL. Laboratory parameter-based machine learning model for excluding non-alcoholic fatty liver disease (NAFLD) in the general population. *Aliment Pharmacol Ther* 2017; **46**: 447-456 [PMID: 28585725 DOI: 10.1111/apt.14172]
- 16 Ahn JC, Connell A, Simonetto DA, Hughes C, Shah VH. Application of Artificial Intelligence for the Diagnosis and Treatment of Liver Diseases. *Hepatology* 2021; **73**: 2546-2563 [PMID: 33098140 DOI: 10.1002/hep.31603]
- 17 Spann A, Yasodhara A, Kang J, Watt K, Wang B, Goldenberg A, Bhat M. Applying Machine Learning in Liver Disease and Transplantation: A Comprehensive Review. *Hepatology* 2020; **71**: 1093-1105 [PMID: 31907954 DOI: 10.1002/hep.31103]
- 18 National Center for Health Statistics. Third National Health and Nutrition Examination Survey Data (NHANES III). [cited 15 Dec 2020]. In: National Center for Health Statistics [Internet]. Available from: <https://www.cdc.gov/nchs/nhanes/nhanes3/>
- 19 Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]
- 20 Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; **42**: 503-508 [PMID: 19766548 DOI: 10.1016/j.dld.2009.08.002]
- 21 Zhang S, Du T, Zhang J, Lu H, Lin X, Xie J, Yang Y, Yu X. The triglyceride and glucose index (TyG) is an effective biomarker to identify nonalcoholic fatty liver disease. *Lipids Health Dis* 2017; **16**: 15 [PMID: 28103934 DOI: 10.1186/s12944-017-0409-6]
- 22 Atsawarungruangkit A. Machine learning models for predicting NAFLD. MATLAB Central File Exchange. [cited 15 Dec 2020]. In: MathWorks [Internet]. Available from: <https://www.mathworks.com/matlabcentral/fileexchange/83953-machine-learning-models-for-predicting-naflD>
- 23 Bitew ZW, Alemu A, Ayele EG, Tenaw Z, Alebel A, Worku T. Metabolic syndrome among children and adolescents in low and middle income countries: a systematic review and meta-analysis. *Diabetol Metab Syndr* 2020; **12**: 93 [PMID: 33117455 DOI: 10.1186/s13098-020-00601-8]
- 24 Staynor JMD, Smith MK, Donnelly CJ, Sallam AE, Ackland TR. DXA reference values and anthropometric screening for visceral obesity in Western Australian adults. *Sci Rep* 2020; **10**: 18731 [PMID: 33127940 DOI: 10.1038/s41598-020-73631-x]

- 25 **Eyvazlou M**, Hosseinpouri M, Mokarami H, Gharibi V, Jahangiri M, Cousins R, Nikbakht HA, Barkhordari A. Prediction of metabolic syndrome based on sleep and work-related risk factors using an artificial neural network. *BMC Endocr Disord* 2020; **20**: 169 [PMID: [33183282](#) DOI: [10.1186/s12902-020-00645-x](#)]
- 26 **Vural Keskinler M**, Mutlu HH, Sirin A, Erkalma Senates B, Colak Y, Tuncer I, Oguz A. Visceral Adiposity Index As a Practical Tool in Patients with Biopsy-Proven Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis. *Metab Syndr Relat Disord* 2021; **19**: 26-31 [PMID: [32898457](#) DOI: [10.1089/met.2020.0054](#)]
- 27 **Atsawarungruangkit A**, Chenbhanich J, Dickstein G. C-peptide as a key risk factor for non-alcoholic fatty liver disease in the United States population. *World J Gastroenterol* 2018; **24**: 3663-3670 [PMID: [30166861](#) DOI: [10.3748/wjg.v24.i32.3663](#)]
- 28 **Yesilova Z**, Ozata M, Oktenli C, Bagci S, Ozcan A, Sanisoglu SY, Uygur A, Yaman H, Karaeren N, Dagalp K. Increased acylation stimulating protein concentrations in nonalcoholic fatty liver disease are associated with insulin resistance. *Am J Gastroenterol* 2005; **100**: 842-849 [PMID: [15784030](#) DOI: [10.1111/j.1572-0241.2005.40838.x](#)]
- 29 **Chitturi S**, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; **35**: 373-379 [PMID: [11826411](#) DOI: [10.1053/jhep.2002.30692](#)]
- 30 **Chalasani N**, Deeg MA, Persohn S, Crabb DW. Metabolic and anthropometric evaluation of insulin resistance in nondiabetic patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003; **98**: 1849-1855 [PMID: [12907343](#) DOI: [10.1111/j.1572-0241.2003.07619.x](#)]
- 31 **Francque SM**, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, Michielsens P, Van Gaal L. Noninvasive assessment of nonalcoholic fatty liver disease in obese or overweight patients. *Clin Gastroenterol Hepatol* 2012; **10**: 1162-1168; quiz e87 [PMID: [22796457](#) DOI: [10.1016/j.cgh.2012.06.019](#)]
- 32 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54 [PMID: [15239085](#) DOI: [10.1002/hep.20280](#)]
- 33 **Sorino P**, Caruso MG, Misciagna G, Bonfiglio C, Campanella A, Mirizzi A, Franco I, Bianco A, Buongiorno C, Liuzzi R, Cisternino AM, Notarnicola M, Chiloio M, Pascoschi G, Osella AR; MICOL Group. Selecting the best machine learning algorithm to support the diagnosis of Non-Alcoholic Fatty Liver Disease: A meta learner study. *PLoS One* 2020; **15**: e0240867 [PMID: [33079971](#) DOI: [10.1371/journal.pone.0240867](#)]
- 34 **Cao W**, An X, Cong L, Lyu C, Zhou Q, Guo R. Application of Deep Learning in Quantitative Analysis of 2-Dimensional Ultrasound Imaging of Nonalcoholic Fatty Liver Disease. *J Ultrasound Med* 2020; **39**: 51-59 [PMID: [31222786](#) DOI: [10.1002/jum.15070](#)]
- 35 **Forlano R**, Mullish BH, Giannakeas N, Maurice JB, Angkathunyakul N, Lloyd J, Tzallas AT, Tsipouras M, Yee M, Thursz MR, Goldin RD, Manousou P. High-Throughput, Machine Learning-Based Quantification of Steatosis, Inflammation, Ballooning, and Fibrosis in Biopsies From Patients With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2020; **18**: 2081-2090.e9 [PMID: [31887451](#) DOI: [10.1016/j.cgh.2019.12.025](#)]
- 36 **Islam MM**, Wu CC, Poly TN, Yang HC, Li YJ. Applications of Machine Learning in Fatty Live Disease Prediction. *Stud Health Technol Inform* 2018; **247**: 166-170 [PMID: [29677944](#)]
- 37 **Birjandi M**, Ayatollahi SM, Pourahmad S, Safarpour AR. Prediction and Diagnosis of Non-Alcoholic Fatty Liver Disease (NAFLD) and Identification of Its Associated Factors Using the Classification Tree Method. *Iran Red Crescent Med J* 2016; **18**: e32858 [PMID: [28191344](#) DOI: [10.5812/ircmj.32858](#)]



Retrospective Study

# Acute liver failure with hemolytic anemia in children with Wilson's disease: Genotype-phenotype correlations?

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## Abstract

### BACKGROUND

Wilson's disease (WD) is a rare autosomal recessive inherited disorder of copper metabolism. Acute liver failure (ALF) and hemolytic anemia represent the most severe presentation of WD in children. No clear genotype-phenotype correlations exist in WD. Protein-truncating nonsense, frame-shift, or splice-site variants may be associated with more severe disease. In contrast, missense variants may be associated with late-onset, less severe disease, and more neurological manifestations. Recently, a gene variant (HSD17B13:TA, rs72613567) with a possible hepatic protective role against toxins was associated with a less severe hepatic phenotype in WD.

### AIM

To analyze the possible genotype-phenotype correlations in children with WD presented with ALF and non-immune hemolytic anemia.

### METHODS

The medical records of children with WD diagnosed and treated in our hospital from January 2006 to December 2020 were retrospectively analyzed. The clinical manifestations (ALF with non-immune hemolytic anemia or other less severe forms), laboratory parameters, copper metabolism, *ATP7B* variants, and the HSD17B13:TA (rs72613567) variant were reviewed to analyze the possible genotype-phenotype correlations.

details that might disclose the identity of the subjects under study were omitted or anonymized.

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## RESULTS

We analyzed the data of 51 patients with WD, 26 females (50.98%), with the mean age at the diagnosis of  $12.36 \pm 3.74$  years. ALF and Coombs-negative hemolytic anemia was present in 8 children (15.67%), all adolescent girls. The Kayser-Fleisher ring was present in 9 children (17.65%). The most frequent variants of the *ATP7B* gene were p.His1069Gln (c.3207A>G) in 38.24% of all alleles, p.Gly1341Asp (c.4021G>A) in 26.47%, p.Trp939Cys (c.2817G>T) in 9.80%, and p.Lys844Ter (c.2530A>T) in 4.90%. In ALF with hemolytic anemia, p.Trp939Cys (c.2817G>T) and p.Lys844Ter (c.2530A>T) variants were more frequent than in other less severe forms, in which p.His1069Gln (c.3207A>G) was more frequent. p.Gly1341Asp (c.4021G>A) has a similar frequency in all hepatic forms. For 33 of the patients, the HSD17B13 genotype was evaluated. The overall HSD17B13:TA allele frequency was 24.24%. Its frequency was higher in patients with less severe liver disease (26.92%) than those with ALF and hemolytic anemia (14.28%).

## CONCLUSION

It remains challenging to prove a genotype-phenotype correlation in WD patients. In children with ALF and hemolytic anemia, the missense variants other than p.His1069Gln (c.3207A>G) and frame-shift variants were the most frequently present in homozygous status or compound heterozygous status with site splice variants. As genetic analysis is usually time-consuming and the results are late, the importance at the onset of the ALF is questionable. If variants proved to be associated with severe forms are found in the pre-symptomatic phase of the disease, this could be essential to predict a possible severe evolution.

**Key Words:** Wilson's disease; Children; Acute liver failure; Hemolytic anemia; *ATP7B* variant; Genotype-phenotype correlation

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**Core Tip:** Acute liver failure (ALF) and hemolytic anemia represent the most severe presentation of Wilson's disease (WD) in children, with a possible fatal evolution. There is no definite genotype-phenotype correlation in WD, but many studies try to solve this puzzle. Our research reports a higher presence of a missense [p.Trp939Cys (c.2817G>T)] and frame-shift variant [p.Lys844Ter (c.2530A>T)] in children with ALF and hemolytic anemia, while in less severe form, p.His1069Gln (c.3207A>G) was more frequent. HSD17B13:TA variant may be associated with less severe liver disease, as it was proved to have a protective role against liver toxins.

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## INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive inherited disorder of copper metabolism caused by homozygous or compound heterozygous variants of the *ATP7B* gene. The prevalence of WD is estimated as 1/30000[1]. The *ATP7B* gene encodes transmembrane copper-transporting ATPase (*ATP7B*) and is located on chromosome 13q14.3, containing 20 introns and 21 exons[2]. According to the Human Gene Mutation Database, more than 800 variants of the *ATP7B* gene have been described. More than half of these variants are single nucleotide missense and nonsense, and the others are insertions/deletions and splice-site variants[3,4].

The clinical forms of WD are very variable due to the copper accumulation in different organs. The age of onset has been reported to be between 2 and 70 years[5,6]. Liver disease is the first clinical manifestation in 40%-60% of WD patients, more often





in the first decade[1,5,7,8]. In children, WD patients present with an incidental finding of high levels of transaminases in an asymptomatic child, acute or chronic hepatitis, or decompensation of cirrhosis (in older children and adolescents)[5,9]. As an initial form of presentation, neurological disease is described in 18%-68% of patients, mainly in young adults (20-30 years). WD's most common neurological features are tremor, dystonia, parkinsonism, associated with dysarthria, gait and posture disturbances, drooling, and dysphagia[1]. Also, psychiatric disease (mainly mood disturbances, depression, or bipolar disease) may be present, mainly in adulthood. A decline in school performances, impulsiveness, and inappropriate behavior was reported in adolescents[1]. Hematologic disease, renal disease, skeletal and cardiac disease may be described in WD patients[1,10,11].

Acute liver failure (ALF) may be the initial presentation or a complication during WD evolution in children and young adults[12]. Approximately 2%-6% of ALF cases may be caused by WD[13,14]. A rare presentation of WD (5%[15]), ALF accompanied by a hemolytic crisis may have a severe evolution, with coagulopathy, encephalopathy, and progressive renal failure, resulting in death without an emergency liver transplantation[1,10,11,16]. This clinical form of WD occurred in 30% of children with ALF requesting liver transplantation and 60% of those with fatal evolution before transplantation[5]. Therefore, early diagnosis and referral to specialized centers are determinants for the prognosis in these patients[12].

There is a continuous interest in genotype-phenotype correlations in WD. Based on the phenotypic classification, studies tried to find a link between the genetic variants and clinical forms or severity of WD disease, important for the prognosis of the disease [2,17]. The *ATP7B* gene variants may have different effects on the presence and function of the ATPase encoded with various consequences on the clinical presentation. Many studies have tried to analyze these correlations regarding the age of the onset, neurological or hepatic form, ceruloplasmin activity, hepatic copper level, or the presence of Kayser-Fleischer (KF) ring[18-20]. Still, there is no definite genotype-phenotype correlation so far, which may be due to the disease's high genetic heterogeneity and rareness[2,21]. Some authors suggest that the severe hepatic phenotype and earlier onset are more likely associated with the nonsense or frame-shift variants. A less severe hepatic or a neurologic phenotype is linked to missense variants [2,22-26]. The clinical presentation in WD may also be influenced by environmental and epigenetic factors or modifier genes[4]. Recently, a gene variant (HSD17B13:TA, rs72613567) with a possible hepatic protective role against toxins was associated with a less severe hepatic phenotype in WD[27].

Our study aimed to analyze the possible genotype-phenotype correlations in children with WD presented with ALF and non-immune hemolytic anemia and to investigate the most common *ATP7B* variants in our patients with this severe form of the disease.

## MATERIALS AND METHODS

The medical records of children with WD diagnosed and treated in our hospital from January 2006 to December 2020 were retrospectively analyzed. The clinical manifestations (acute or chronic liver disease, neurologic disease, ALF with non-immune hemolytic anemia), laboratory parameters, copper metabolism, and *ATP7B* variants were reviewed.

Diagnostic of WD was based on positive family history, clinical symptoms (including the presence of KF ring), and laboratory tests (low serum ceruloplasmin, < 20 mg/dL, elevated 24-h urinary copper excretion, baseline or stimulated by penicillamine) following the current diagnostic and management guidelines[28,29]. ALF with hemolytic anemia was diagnosed on the coagulopathy (prolonged prothrombin time, increased international normalized ratio (INR) > 2 without hepatic encephalopathy or > 1.5 in the presence of encephalopathy), low hemoglobin level, and negative Coombs test. Laboratory tests were performed using standard methods. None of our patients had a liver biopsy to assess the histology, as we could not measure the copper content in our service. The severity of the fibrosis was evaluated at diagnosis and during the follow-up using a non-invasive assessment of liver stiffness by transient elastography (FibroScan, Echosense, France)[9].

The molecular analysis of the *ATP7B* gene was performed using a semi-nested polymerase chain reaction-based restriction fragment length polymorphism assay for p.His1069Gln (c.3207A>G) variant detection as previously described. If negative or heterozygous for p.His1069Gln (c.3207A>G) variant, samples were Sanger sequenced

by the ABI Prism 310 Genetic Analyzer (Perkin Elmer; Norwalk, CT, United States) until 2012, followed by the 3500 Genetic Analyzer (Applied Biosystems; Foster City, CA, United States) using published primers.

The HSD17B13:TA (rs72613567) variant was determined using allelic discrimination real-time polymerase chain reaction and validated by Sanger sequencing in normal controls having different HSD17B13 genotypes. Unfortunately, this evaluation was technically possible only for the second half of the study, and only 33 patients were assessed. We included only the children with WD confirmed by molecular analysis, and we excluded all suspected WD patients without genetic confirmation or with incomplete data.

We analyzed the clinical and laboratory features, including the most frequent variants in children with ALF and hemolytic anemia compared to those with other clinical forms.

Statistical analysis of the data collected was performed using Statistica 13.5 (Tibco Software; Palo Alto, CA, United States). The variables with normal distribution were presented as mean and standard. Comparison of continuous variables was performed using the Student *t*-test. Categorical variables were presented as numbers and percentages; they were compared using the Chi-square test. Two-sided *P* values were analyzed, and the *P* value < 0.05 was considered statistically significant.

## RESULTS

During the last 15 years, 67 patients with WD were diagnosed and treated in our clinic. After reviewing genetic data, we included 51 patients, 26 females (50.98%), with the mean age at WD diagnosis of  $12.36 \pm 3.74$  years (between 5 and 23 years).

Almost all patients included in our study presented liver diseases; only one was with a neurological form, and one was diagnosed following the screening due to WD in the family. Our clinic is the main pediatric hepatology service and center for expertise in pediatric liver rare disorders in Transylvania, Romania. Therefore, the selection of the patients referred to our center would be biased regarding the clinical presentation in our WD patients. ALF and Coombs-negative hemolytic anemia was present in 8 children (15.67%), all adolescent girls. The KF ring was present in 9 children (17.65%). The clinical and laboratory characteristics of the WD patients included in this study, based on the clinical onset, are presented in Table 1.

In two girls, ALF with hemolytic anemia was not the initial presentation that led to the WD diagnostic. Initially, they had only increased transaminases but progressed shortly to this severe clinical evolution.

In our patients, the most frequent variant of the *ATP7B* gene was p.His1069Gln (c.3207A>G), present in 12 children in homozygous status and 17 children in compound heterozygous status (38.24% of all alleles). p.Gly1341Asp (c.4021G>A) variant was present in 10 children in homozygous status and 7 patients in compound heterozygous status (26.47%). The other two frequent variants (mainly in patients with ALF and hemolytic anemia) were p.Trp939Cys (c.2817G>T), in 5 children in homozygous status (9.80%) and p.Lys844Ter (c.2530A>T), in one child as homozygous status and three children as a part of compound heterozygous status (4.90%). In Table 2, we present the most frequent variants grouped by the clinical form of presentation. In ALF with hemolytic anemia, p.Trp939Cys (c.2817G>T) and p.Lys844Ter (c.2530A>T) were more frequent than in other less severe forms, in which p.His1069Gln (c.3207A>G) was more frequent. p.Gly1341Asp (c.4021G>A) variant has a similar frequency in all hepatic forms.

For 33 of the patients included in our study, the HSD17B13 genotype was evaluated. The overall HSD17B13:TA allele frequency in our study was 24.24%. HSD17B13:TA allele frequency was higher in patients with less severe liver disease (26.92%) than ALF and hemolytic anemia (14.28%). Table 3 presents the demographic, clinical, and genotype association in patients investigated for the HSD17B13:TA variant.

Two patients with ALF were transplanted, five survived with the native liver following supportive intensive care, and one girl had a fatal evolution on the second day after admission. Also, another child with cirrhosis died due to severe complications before liver transplantation was possible.

**Table 1 Clinical and laboratory characteristics in Wilson's disease children with acute liver failure and hemolytic anemia**

Parameter	All (n = 51)	ALF and hemolytic anemia (8 pts)	Other clinical forms (43 pts)	P value
Females, n (%)	26 (50.98)	8 (100)	18 (41.86)	0.00252
Mean age (yr)	12.36 ± 3.74	14.59 ± 2.21	11.59 ± 3.79	0.03598
ALT (IU/L)	131.54 ± 119.69	87.38 ± 154.07	142.24 ± 110.04	0.24969
AST (IU/L)	125.05 ± 88.73	78.62 ± 47.44	136.30 ± 93.16	0.09950
TB (mg/dL)	5.41 ± 12.93	22.55 ± 21.95	0.99 ± 0.95	0.000002
DB (mg/dL)	4.20 ± 11.56	18.84 ± 20.40	0.42 ± 0.51	0.000007
GGT (IU/L)	83.54 ± 43.44	97.71 ± 57.14	80.44 ± 40.33	0.34731
WBC (mm <sup>3</sup> )	8568 ± 7360	14838 ± 14185	6666 ± 1799	0.00415
Hb (g/dL)	12.15 ± 2.96	7.4 ± 2.39	13.51 ± 1.22	0.000000
PLT (mm <sup>3</sup> )	253306 ± 125421	164875 ± 74043	278571 ± 126455	0.02142
INR	2.94 ± 6.37	7.53 ± 11.34	1.18 ± 0.27	0.01359
KF ring, n (%)	9 (17.65)	3 (37.50)	6 (13.95)	0.10868
Ceruloplasmin (mg/dL)	9.81 ± 6.118	7.98 ± 5.77	10.11 ± 6.19	0.39976
Urinary copper (µg/24 h)	648.94 ± 1093.90	2 236.33 ± 2 174.46	384.38 ± 455.04	0.000006
Outcome, n (%)				0.00121
Survivors	47 (92.16)	5 (62.50)	42 (97.67)	
Transplanted	2 (3.92)	2 (25.00)	0	
Deceased	2 (3.92)	1 (12.50)	1 (2.33)	

ALF: Acute liver failure; ALT: Alanine-aminotransferase; AST: Aspartate-aminotransferase; TB: Total bilirubin; DB: Direct bilirubin; GGT: Gamma-glutamyl transferase; WBC: White blood cells; Hb: Hemoglobin; PLT: Platelets; INR: International normalized ratio; KF ring: Kayser-Fleisher ring.

**Table 2 Variants of *ATP7B* gene in children with hemolytic anemia and acute liver failure**

Variants		Hemolytic Anemia + ALF (8 patients)	Other clinical forms (44 patients)	P value
p.Trp939Cys (c.2817G>T)	5 homozygotes	6 (42.86%)	4 (4.55%)	0.0000
p.Lys844Ter (c.2530A>T)	1 homozygotes	4 (28.57%)	1 (1.14%)	0.0000
	3 heterozygotes			
p.Gly1341Asp (c.4021G>A)	10 homozygotes	4 (28.57%)	23 (26.14%)	0.8482
	7 heterozygotes			
p.His1069Gln (c.3207A>G)	12 homozygotes	0	39 (44.32%)	0.0015
	15 heterozygotes			
Other variants	1 homozygotes	2 (14.29%)	19 (21.59%)	0.5304
	19 heterozygotes			
Total alleles		14 (100%)	88 (100%)	

ALF: Acute liver failure.

## DISCUSSION

Our study aimed to assess the possible genotype-phenotype correlations regarding WD's most severe clinical form in children and adolescents. This endeavor in patients with WD is challenging, as was proved by many studies already published. So far, the research failed to conclude this issue due to the high heterogeneity of *ATP7B* variants

**Table 3** Data regarding the patients evaluated for the HSD17B13:TA variant

	All	HSD17B13 genotype		
		T/T	TA/T	TA/TA
<i>n</i> (%)	33	19 (57.58)	12 (36.36)	2 (6.06)
Females, <i>n</i> (%)	16	11	5	0
Mean age (yr)	11.67 ± 3.12	11.09 ± 3.45	12.14 ± 2.25	14.70 ± 3.24
<b>Clinical presentation, <i>n</i> (%)</b>				
ALF and/or hemolytic anemia	7 (21.21)	5 (71.43)	2 (28.57)	0
Less severe hepatic forms	26 (78.79)	14 (53.85)	10 (38.46)	2 (7.69)
<b>ATP7B variant</b>				
p.His1069Gln (c.3206A>G)/p.His1069Gln (c.3206A>G)	5	2	3	0
p.His1069Gln (c.3206A>G)/p.Gly1341Asp (c.4021G>A)	6	3	3	0
p.His1069Gln (c.3206A>G)/other	7	4	3	0
p.Gly1341Asp (c.4021G>A)/p.Gly1341Asp (c.4021G>A)	7	3	2	2
p.Trp939Cys (c.2817G>T)/p.Trp939Cys (c.2817G>T)	4	4	0	0
p.Trp939Cys (c.2817G>T)/other	1	1	0	0
p.Lys844Ter (c.2530A>T)/p.Lys844Ter (c.2530A>T)	1	1	0	0
p.Lys844Ter (c.2530A>T)/ other	2	1	1	0

ALF: Acute liver failure.

(more than 1300 described) and the rareness of the disease (small series of patients). Furthermore, the increased number of compound heterozygotes involving different kinds of variants makes this analysis more difficult[18,19,24,30,31]. As phenotypic differences were reported in siblings with the same genotype or monozygotic twins, the involvement of other factors is possible[2,30,32-35]. Environmental factors (nutritional copper intake, infections, drugs, or other toxins), modifier genes, and epigenetic factors' interaction with the genetic variants may explain the different clinical presentations in WD[4,8,18,22].

The introduction of a phenotype classification tried to ease analyzing the clinical forms in WD[17]. Our patients mainly have the hepatic form (the most frequent one in children and adolescents). Some of our older patients also had neurological and psychiatric manifestations. Not all our patients suspected of WD had a genetic confirmation of the *ATP7B* variants. Therefore, only patients with two WD variants in cis were included in our study to analyze the possible genotype-phenotype correlations.

ALF with hemolytic anemia was present in 8 children, all girls. The age of onset was higher than other hepatic presentations (acute or chronic hepatitis, autoimmune features, or cirrhosis, data not shown). The increased frequency of ALF described in females is not fully understood, but it may be explained by hormonal differences or the intervention of epigenetic factors (methyloome and transcriptome differences)[4,13,18,19].

The KF ring was described in 9 children with liver disease (17.65%), three of them with ALF, and 6 in the other forms. Four of those six children with KF ring in other forms of liver disease presented neurological manifestations in their evolution. Other studies proved that ocular involvement is less frequent in hepatic than in neurological involvement[31]. The presence of the KF ring was reported lower in children. In Greek children with WD, the KF ring was present in 48.7% of those with liver disease and 16% of those diagnosed through family screening[7], while in the Italian children, only in 8.6% of those with liver disease[8]. The KF ring was present in more than half of the ALF patients, compared to 37.5% in our small series[10].

Regarding the laboratory results, the differences in children with ALF and hemolytic anemia and the other forms are expected for the bilirubin, hemoglobin, and INR. The number of white blood cells (WBC) is higher in children with ALF, and platelets are lower. There are no significant differences in the serum level of transam-

inases (even lower in ALF patients) and gamma-glutamyl transferase. The number of WBCs is an important risk factor as it was included in the prognostic score to predict mortality and evaluate the need for liver transplantation[5]. Also, the low level of transaminases in children with the ALF form of WD is a well-known feature and would help the diagnostic. High aspartate-aminotransferase (AST) to ALT ratio and low alkaline phosphatase to serum bilirubin ratio may be used to differentiate the WD patients in ALF[13,15]. In our cohort, two children with ALF had an AST/ALT ratio higher than 4 and only one alkaline phosphatase to bilirubin level ratio lower than 2. In children, the ratio between serum alkaline phosphatase and total bilirubin level may not always be helpful due to bone-derived alkaline phosphatase[5]. The transaminase level was lower than in children with acute hepatitis. As the age is higher in patients presenting with ALF, the evolution of the disease without any clinical sign for years explains the severe fibrosis or cirrhosis in these patients. In a large study that aims to analyze the genotype-phenotype correlation, fulminant liver failure or hemolysis were associated with liver cirrhosis in 93.4% and 66.7% of patients, higher than in the other milder presentations of WD[19].

In our children with ALF and hemolytic anemia, the serum ceruloplasmin level was lower than in the other patients but not significantly. In the meantime, the urinary copper excretion was higher, as can be expected, due to the severe necrosis associated with ALF.

No clear genotype-phenotype correlations exist in WD. Protein-truncating nonsense, frame-shift, or splice-site variants have a significant functional and structural impact on the ATP7B protein and may be associated with more severe disease (early-onset, low ceruloplasmin level, high copper content in liver). In contrast, missense variants are associated with late-onset, less severe disease and more neurological manifestations[18,25,26,36]. There are also reports of some missense variants associated with the early onset of disease with various severity in the same family[30]. Previous reports proposed the association of exon 18-20 variants with hepatic and hematological onset but not with neurological disease[37].

The most frequent variants in Central Europe, p.His1069Gln (c.3207A>G), was also the most frequent one in our cohort. It was found in homozygous or heterozygous status in 38.24% of all alleles in our study, compared with 72% in Poland, 35% in Greece, and 38% in a previous study from Romania[17,21,23,26,38]. This variant is more frequent in older patients with the neurological form of WD[3,7,23]. In our cohort, there was no child with ALF and hemolytic anemia with p.His1069Gln (c.3207A>G) variant. This is a missense variant and is probably associated with protein misfolding, abnormal phosphorylation of the P-domain, and altered ATP binding orientation and affinity[13]. R969Q, another missense variant present in our children, is almost exclusively associated with late-onset liver disease[3,23].

Another missense variant, p.Gly1341Asp (c.4021G>A), was the second most frequent one in our children. p.Gly1341Asp (c.4021G>A) is a variant of the transmembrane domain of the ATP7B gene and, in homozygous status, was proved to be associated with more severe and early onset of WD[39]. This variant was associated in homozygous status with ALF and/or hemolytic anemia in two children. In one girl, hemolytic anemia developed after treatment with zinc for a chronic increase of transaminases with questionable compliance. The second girl with this genotype-phenotype association has a younger sister with the same genetic status presenting only an increase of transaminases. The most frequent variant in our patients with ALF and/or hemolytic anemia was p.Trp939Cys (c.2817G>T), described previously in early-onset hepatic disease and with a high risk for liver failure in homozygotes[24]. Three adolescents (girls) with ALF presented this variant in homozygous status; the other two children (males) had the same status but did not have a severe form. The p.Lys844Ter (c.2530A>T) variant is the fourth most frequent in our cohort; it was present more in children with a severe form of WD. One girl was homozygote, and in another two girls, the variant was associated with splice-site variants in a compound heterozygous status. The p.Lys844Ter (c.2530A>T) variant is a frame-shift variant presumed to be associated with severe clinical evolution, as are also splice-site variants. It was previously described in WD patients of Hungarian origin[40] and few patients with late-onset of WD[41].

The early diagnosis of WD in children would probably prevent the evolution and sometimes the onset of the disease with a severe form. As mentioned in other studies, gender would modify the disease presentation due to different hormone balance[18,19]. If we analyze the possible influence of the sex of the patients, the severe form of the disease was present in two of the four girls and none of the boys with p.Gly1341Asp (c.4021G>A) homozygous status. All children with p.Gly1341Asp (c.4021G>A) variant in compound heterozygous status associated with p.His1069Gln



(c.3207A>G) variant experienced a less severe form of WD.

HSD17B13 encodes a protein involved in regulating the biosynthesis of lipids, and by its enzymatic roles, is implicated in lipid-mediated inflammation. Recently, a protein-truncation variant (HSD17B13:TA, rs72613567) was shown to have a protective role against liver toxins, including copper toxicity in WD[27]. In our cohort, the allele frequency of HSD17B13:TA was similar to other results for the Caucasian population, higher in patients with less severe liver disease than those presented with ALF and hemolytic anemia. The age of diagnosis was higher in patients homozygous for this variant than in heterozygous status or without this variant. Even without statistical significance, these results suggest the possible role of the HSD17B13:TA variant in the modulation of the WD severity together with factors, including sex, age, *ATP7B* variant, and other gene variants.

ALF was fatal only in one of our cases included in this study. Two girls underwent emergency liver transplantation on the fourth day after their presentation in our service. The liver transplantation was performed at the Fundeni Institute in Bucharest, Romania. This clinical presentation should be regarded as an emergency[5,42]. The patients should be referred as soon as possible to a center that could provide intensive care, including extrahepatic liver support, until liver transplantation would be possible for severe cases. Unfortunately, one girl died the second day after her admission to our center.

**Strengths and limitations.** This study presents the largest cohort of children with genetically confirmed WD from our country and the neighboring region. It represents the first description of the possible correlation of ALF and hemolytic anemia with p.Trp939Cys (c.2817G>T) and p.Lys844Ter (c.2530A>T) variants in Eastern European children with WD. However, there are some limitations of our study. Firstly, the small number of children with this severe form made the statistical analysis of our findings difficult. Another issue is represented by the selection of patients, as our pediatric hepatology service admits mainly children and adolescents with hepatic disease. A significant limitation was the difficulty of considering and analyzing other possible factors that would lead to an acute, severe clinical form compared to children with the same genotype [p.Gly1341Asp (c.4021G>A)].

In the future, with the onset of a National Registry for patients with WD, including the genetic analyzes, more data on WD patients from Romania would be available. In the severe clinical form of WD, the genetic background would be less critical from the point of view of immediate medical care. The result of the genetic analysis would arrive with the clinician late, after the evolution of the patient would be clear. With the recent progress in screening for WD[43], the genetic analysis in children with an early suspected disease would help predict future evolution. When nonsense, frame-shift, or splicing-site variants are identified in a pre-symptomatic period, the importance of this genotype-phenotype correlation for the prognostic is evident.

## CONCLUSION

It remains challenging to prove a genotype-phenotype correlation in WD patients due to the small number of patients in the reported series and the increased genetic heterogeneity. In children with ALF and non-immune hemolytic anemia, the nonsense variants other than p.His1069Gln [as p.Trp939Cys (c.2817G>T)] and frame-shift variants [p.Lys844Ter (c.2530A>T)] were the most frequently present in homozygous status or compound heterozygous status with site splice variants. As genetic analysis is usually time-consuming and the results are late (except in the screening of the relative of an index patient), the importance for the prognosis at the onset of the ALF is questionable. However, if variants proved to be associated with severe forms are found early in the evolution of the disease, this could be essential to predict a possible severe evolution if the patients would not follow treatment.

## ARTICLE HIGHLIGHTS

### Research background

There is a continuous interest in genotype-phenotype correlations in Wilson's disease (WD).

### Research motivation

The aim is to study the possible genotype-phenotype correlations in children with acute liver failure (ALF) and hemolytic anemia in WD.

### Research objectives

The objectives include the analysis of *ATP7B* variants in children with ALF and hemolytic anemia in WD compared to the other clinical presentations and the possible role of the HSD17B13:TA variant in the modulation of the WD severity.

### Research methods

The retrospective study included 63 children with WD diagnosed and follow-up during 2006-2020. The clinical manifestations (acute or chronic liver disease, neurologic disease, ALF with non-immune hemolytic anemia), laboratory parameters, copper metabolism, *ATP7B* variants, and the HSD17B13:TA (rs72613567) variant were reviewed.

### Research results

In our cohort, in children with ALF and non-immune hemolytic anemia, the nonsense variants other than p.His1069Gln (c.3206A>G), as p.Trp939Cys (c.2817G>T), and frame-shift variants, as p.Lys844Ter (c.2530A>T), were the most frequently present. The allele frequency of HSD17B13:TA was similar to other results for the Caucasian population, higher in patients with the less severe liver disease than those presented with ALF and hemolytic anemia.

### Research conclusions

It remains challenging to prove a genotype-phenotype correlation in WD patients due to the small number of patients in the reported series and the increased genetic heterogeneity. When nonsense, frame-shift, or splicing-site variants are identified in a pre-symptomatic period, the importance of this genotype-phenotype correlation for the prognostic is evident.

### Research perspectives

A more extensive study involving children and adolescents with ALF and hemolytic anemia form of WD should be provided to confirm the findings. New studies are needed to evaluate the role of protective variant, HSD17B13:TA (rs72613567), in association with other factors, in less severe forms of WD in children.

## REFERENCES

- 1 **Czlonkowska A**, Litwin T, Dusek P, Ferenci P, Lutsenko S, Medici V, Rybakowski JK, Weiss KH, Schilsky ML. Wilson disease. *Nat Rev Dis Primers* 2018; **4**: 21 [PMID: 30190489 DOI: 10.1038/s41572-018-0018-3]
- 2 **Chang IJ**, Hahn SH. The genetics of Wilson disease. *Handb Clin Neurol* 2017; **142**: 19-34 [PMID: 28433102 DOI: 10.1016/B978-0-444-63625-6.00003-3]
- 3 **Cooper DN**, Ball EV, Stenson PD, Phillips AD, Evans K, Heywood S, Hayden MJ, Chapman MM, Mort ME, Azevedo L, Millar DS. The Human Gene Mutation Database. Qiagen. [cited 22 April 2021]. Available from: <http://www.hgmd.cf.ac.uk/ac/index.php>
- 4 **Medici V**, LaSalle JM. Genetics and epigenetic factors of Wilson disease. *Ann Transl Med* 2019; **7**: S58 [PMID: 31179295 DOI: 10.21037/atm.2019.01.67]
- 5 **Dhawan A**, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. *Liver Transpl* 2005; **11**: 441-448 [PMID: 15776453 DOI: 10.1002/lt.20352]
- 6 **Schmidt HH**. Role of genotyping in Wilson's disease. *J Hepatol* 2009; **50**: 449-452 [PMID: 19118916 DOI: 10.1016/j.jhep.2008.11.008]
- 7 **Manolaki N**, Nikolopoulou G, Daikos GL, Panagiotakaki E, Tzetis M, Roma E, Kanavakis E, Syriopoulou VP. Wilson disease in children: analysis of 57 cases. *J Pediatr Gastroenterol Nutr* 2009; **48**: 72-77 [PMID: 19172127 DOI: 10.1097/MPG.0b013e31817d80b8]
- 8 **Nicastro E**, Loudianos G, Zancan L, D'Antiga L, Maggiore G, Marcellini M, Barbera C, Marazzi MG, Francavilla R, Pastore M, Vajro P, D'Ambrosi M, Vegnente A, Ranucci G, Iorio R. Genotype-phenotype correlation in Italian children with Wilson's disease. *J Hepatol* 2009; **50**: 555-561 [PMID: 19118915 DOI: 10.1016/j.jhep.2008.09.020]
- 9 **Stefanescu AC**, Pop TL, Stefanescu H, Miu N. Transient elastography of the liver in children with Wilson's disease: Preliminary results. *J Clin Ultrasound* 2016; **44**: 65-71 [PMID: 26177868 DOI: 10.1002/jcu.22281]

- 10 **Shribman S**, Warner TT, Dooley JS. Clinical presentations of Wilson disease. *Ann Transl Med* 2019; **7**: S60 [PMID: [31179297](#) DOI: [10.21037/atm.2019.04.27](#)]
- 11 **Walshe JM**. The acute haemolytic syndrome in Wilson's disease--a review of 22 patients. *QJM* 2013; **106**: 1003-1008 [PMID: [23842488](#) DOI: [10.1093/qjmed/hct137](#)]
- 12 **Mainardi V**, Rando K, Valverde M, Olivari D, Castelli J, Rey G, Gerona S. Acute Liver Failure due to Wilson Disease: Eight Years of the National Liver Transplant Program in Uruguay. *Ann Hepatol* 2019; **18**: 187-192 [PMID: [31113589](#) DOI: [10.5604/01.3001.0012.7911](#)]
- 13 **Camarata MA**, Gottfried M, Rule JA, Ala A, Lee WM, Todd Stravitz R, Schilsky ML. Outcomes of Acute Liver Injury in Adults Due to Wilson's Disease: Is Survival Without Transplant Possible? *Liver Transpl* 2020; **26**: 330-336 [PMID: [31838773](#) DOI: [10.1002/lt.25703](#)]
- 14 **Grama A**, Aldea CO, Burac L, Delean D, Bulata B, Sirbe C, Duca E, Boghitoiu D, Coroleuca A, Pop TL. Etiology and Outcome of Acute Liver Failure in Children-The Experience of a Single Tertiary Care Hospital from Romania. *Children (Basel)* 2020; **7** [PMID: [33317098](#) DOI: [10.3390/children7120282](#)]
- 15 **Eisenbach C**, Sieg O, Stremmel W, Encke J, Merle U. Diagnostic criteria for acute liver failure due to Wilson disease. *World J Gastroenterol* 2007; **13**: 1711-1714 [PMID: [17461475](#) DOI: [10.3748/wjg.v13.i11.1711](#)]
- 16 **Tian Y**, Gong GZ, Yang X, Peng F. Diagnosis and management of fulminant Wilson's disease: a single center's experience. *World J Pediatr* 2016; **12**: 209-214 [PMID: [26041495](#) DOI: [10.1007/s12519-015-0026-2](#)]
- 17 **Ferenci P**, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003; **23**: 139-142 [PMID: [12955875](#) DOI: [10.1034/j.1600-0676.2003.00824.x](#)]
- 18 **Ferenci P**. Phenotype-genotype correlations in patients with Wilson's disease. *Ann N Y Acad Sci* 2014; **1315**: 1-5 [PMID: [24517292](#) DOI: [10.1111/nyas.12340](#)]
- 19 **Ferenci P**, Stremmel W, Czlonkowska A, Szalay F, Viveiros A, Stättermayer AF, Bruha R, Houwen R, Pop TL, Stauber R, Gschwantler M, Pfeifferberger J, Yurdaydin C, Aigner E, Steindl-Munda P, Dienes HP, Zoller H, Weiss KH. Age and Sex but Not ATP7B Genotype Effectively Influence the Clinical Phenotype of Wilson Disease. *Hepatology* 2019; **69**: 1464-1476 [PMID: [30232804](#) DOI: [10.1002/hep.30280](#)]
- 20 **Shah AB**, Chernov I, Zhang HT, Ross BM, Das K, Lutsenko S, Parano E, Pavone L, Evgrafov O, Ivanova-Smolenskaya IA, Annerén G, Westermarck K, Urrutia FH, Penchaszadeh GK, Sternlieb I, Scheinberg IH, Gilliam TC, Petrukhin K. Identification and analysis of mutations in the Wilson disease gene (ATP7B): population frequencies, genotype-phenotype correlation, and functional analyses. *Am J Hum Genet* 1997; **61**: 317-328 [PMID: [9311736](#) DOI: [10.1086/514864](#)]
- 21 **Cocoş R**, Şendroi A, Schipor S, Bohltea LC, Şendroi I, Raicu F. Genotype-phenotype correlations in a mountain population community with high prevalence of Wilson's disease: genetic and clinical homogeneity. *PLoS One* 2014; **9**: e98520 [PMID: [24897373](#) DOI: [10.1371/journal.pone.0098520](#)]
- 22 **Horslen S**, Hahn SH. Genotype-phenotype correlation in Wilson disease. *J Clin Gastroenterol* 2010; **44**: 387-388 [PMID: [20421802](#) DOI: [10.1097/MCG.0b013e3181d96ac4](#)]
- 23 **Panagiotakaki E**, Tzetzis M, Manolaki N, Loudianos G, Papatheodorou A, Manesis E, Nousia-Arvanitakis S, Syriopoulou V, Kanavakis E. Genotype-phenotype correlations for a wide spectrum of mutations in the Wilson disease gene (ATP7B). *Am J Med Genet A* 2004; **131**: 168-173 [PMID: [15523622](#) DOI: [10.1002/ajmg.a.30345](#)]
- 24 **Usta J**, Abu Daya H, Halawi H, Al-Shareef I, El-Rifai O, Malli AH, Sharara AI, Habib RH, Barada K. Homozygosity for Non-H1069Q Missense Mutations in ATP7B Gene and Early Severe Liver Disease: Report of Two Families and a Meta-analysis. *JIMD Rep* 2012; **4**: 129-137 [PMID: [23430908](#) DOI: [10.1007/8904\\_2011\\_91](#)]
- 25 **Merle U**, Weiss KH, Eisenbach C, Tuma S, Ferenci P, Stremmel W. Truncating mutations in the Wilson disease gene ATP7B are associated with very low serum ceruloplasmin oxidase activity and an early onset of Wilson disease. *BMC Gastroenterol* 2010; **10**: 8 [PMID: [20082719](#) DOI: [10.1186/1471-230X-10-8](#)]
- 26 **Gromadzka G**, Schmidt HH, Genschel J, Bochow B, Rodo M, Tarnacka B, Litwin T, Chabik G, Czlonkowska A. Frameshift and nonsense mutations in the gene for ATPase7B are associated with severe impairment of copper metabolism and with an early clinical manifestation of Wilson's disease. *Clin Genet* 2005; **68**: 524-532 [PMID: [16283883](#) DOI: [10.1111/j.1399-0004.2005.00528.x](#)]
- 27 **Ferenci P**, Pfeifferberger J, Stättermayer AF, Stauber RE, Willheim C, Weiss KH, Munda-Steindl P, Trauner M, Schilsky M, Zoller H. HSD17B13 truncated variant is associated with a mild hepatic phenotype in Wilson's Disease. *JHEP Rep* 2019; **1**: 2-8 [PMID: [32039348](#) DOI: [10.1016/j.jhepr.2019.02.007](#)]
- 28 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol* 2012; **56**: 671-685 [PMID: [22340672](#) DOI: [10.1016/j.jhep.2011.11.007](#)]
- 29 **Socha P**, Janczyk W, Dhawan A, Baumann U, D'Antiga L, Tanner S, Iorio R, Vajro P, Houwen R, Fischler B, Dezsofi A, Hadzic N, Hierro L, Jahnel J, McLin V, Nobili V, Smets F, Verkade HJ, Debray D. Wilson's Disease in Children: A Position Paper by the Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2018; **66**: 334-344 [PMID: [29341979](#) DOI: [10.1097/MPG.0000000000001787](#)]
- 30 **Barada K**, Nemer G, ElHajj II, Touma J, Cortas N, Boustany RM, Usta J. Early and severe liver disease associated with homozygosity for an exon 7 mutation, G691R, in Wilson's disease. *Clin Genet*

- 2007; **72**: 264-267 [PMID: [17718866](#) DOI: [10.1111/j.1399-0004.2007.00853.x](#)]
- 31 **Merle U**, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. *Gut* 2007; **56**: 115-120 [PMID: [16709660](#) DOI: [10.1136/gut.2005.087262](#)]
- 32 **Sapuppo A**, Pavone P, Praticò AD, Ruggieri M, Bertino G, Fiumara A. Genotype-phenotype variable correlation in Wilson disease: clinical history of two sisters with the similar genotype. *BMC Med Genet* 2020; **21**: 128 [PMID: [32532207](#) DOI: [10.1186/s12881-020-01062-6](#)]
- 33 **Riordan SM**, Williams R. The Wilson's disease gene and phenotypic diversity. *J Hepatol* 2001; **34**: 165-171 [PMID: [11211896](#) DOI: [10.1016/s0168-8278\(00\)00028-3](#)]
- 34 **Takeshita Y**, Shimizu N, Yamaguchi Y, Nakazono H, Saitou M, Fujikawa Y, Aoki T. Two families with Wilson disease in which siblings showed different phenotypes. *J Hum Genet* 2002; **47**: 543-547 [PMID: [12376745](#) DOI: [10.1007/s100380200082](#)]
- 35 **Kegley KM**, Sellers MA, Ferber MJ, Johnson MW, Joelson DW, Shrestha R. Fulminant Wilson's disease requiring liver transplantation in one monozygotic twin despite identical genetic mutation. *Am J Transplant* 2010; **10**: 1325-1329 [PMID: [20346064](#) DOI: [10.1111/j.1600-6143.2010.03071.x](#)]
- 36 **Lee BH**, Kim JH, Lee SY, Jin HY, Kim KJ, Lee JJ, Park JY, Kim GH, Choi JH, Kim KM, Yoo HW. Distinct clinical courses according to presenting phenotypes and their correlations to ATP7B mutations in a large Wilson's disease cohort. *Liver Int* 2011; **31**: 831-839 [PMID: [21645214](#) DOI: [10.1111/j.1478-3231.2011.02503.x](#)]
- 37 **Leggio L**, Addolorato G, Loudianos G, Abenavoli L, Gasbarrini G. Genotype-phenotype correlation of the Wilson disease ATP7B gene. *Am J Med Genet A* 2006; **140**: 933 [PMID: [16532467](#) DOI: [10.1002/ajmg.a.31191](#)]
- 38 **Iacob R**, Iacob S, Nastase A, Vagu C, Ene AM, Constantinescu A, Anghel D, Banica C, Paslaru L, Coriu D, Dima S, Gheorghe C, Ionica E, Gheorghe L. The His1069Gln mutation in the ATP7B gene in Romanian patients with Wilson's disease referred to a tertiary gastroenterology center. *J Gastrointest Liver Dis* 2012; **21**: 181-185 [PMID: [22720308](#)]
- 39 **Scortova E**. Evaluarea expresiei genei ATP7B în liniile celulare CHO transgenice la nivelul proteinei. [Assesing the ATP7B gene expression in CHO transgenic cell lines at protein levels]. Romanian. Sanatate Publica. *Eco Si Manag Med* 2014; **1**: 54-57
- 40 **Folhoffer A**, Horváth A, Hegedüs D, Firneisz G, Dunkel K, Willheim C, Ferenci P, Szönyi L, Abonyi M, Lakatos PL, Szalay F. [ATP7B gene mutations in Hungarian patients with Wilson disease--case reports to illustrate the diverse clinical presentations]. *Orv Hetil* 2003; **144**: 2509-2515 [PMID: [14974157](#)]
- 41 **Ferenci P**, Członkowska A, Merle U, Ferenc S, Gromadzka G, Yurdaydin C, Vogel W, Bruha R, Schmidt HT, Stremmel W. Late-onset Wilson's disease. *Gastroenterology* 2007; **132**: 1294-1298 [PMID: [17433323](#) DOI: [10.1053/j.gastro.2007.02.057](#)]
- 42 **Nazer H**, Ede RJ, Mowat AP, Williams R. Wilson's disease: clinical presentation and use of prognostic index. *Gut* 1986; **27**: 1377-1381 [PMID: [3792921](#) DOI: [10.1136/gut.27.11.1377](#)]
- 43 **Collins CJ**, Yi F, Dayuha R, Duong P, Horslen S, Camarata M, Coskun AK, Houwen RHJ, Pop TL, Zoller H, Yoo HW, Jung SW, Weiss KH, Schilsky ML, Ferenci P, Hahn SH. Direct Measurement of ATP7B Peptides Is Highly Effective in the Diagnosis of Wilson Disease. *Gastroenterology* 2021; **160**: 2367-2382.e1 [PMID: [33640437](#) DOI: [10.1053/j.gastro.2021.02.052](#)]

## Observational Study

## Clinical outcomes of patients with two small hepatocellular carcinomas

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## Abstract

### BACKGROUND

Management of single small hepatocellular carcinoma (HCC) is straightforward with curative outcomes achieved by locoregional therapy or resection. Liver transplantation is often considered for multiple small or single large HCC. Management of two small HCC whether presenting synchronously or sequentially is less clear.

### AIM

To define the outcomes of patients presenting with two small HCC.

### METHODS

Retrospective review of HCC databases from multiple institutions of patients with either two synchronous or sequential HCC  $\leq 3$  cm between January 2000 and March 2018. Primary outcomes were overall survival (OS) and transplant-free survival (TFS).

### RESULTS

104 patients were identified (male  $n = 89$ ). Median age was 63 years (interquartile range 58–67.75) and the most common aetiology of liver disease was hepatitis C (40.4%). 59 (56.7%) had synchronous HCC and 45 (43.3%) had sequential. 36 patients died (34.6%) and 25 were transplanted (24.0%). 1, 3 and 5-year OS was 93.0%, 66.1% and 62.3% and 5-year post-transplant survival was 95.8%. 1, 3 and 5-year TFS was 82.1%, 45.85% and 37.8%. When synchronous and sequential groups were compared, OS (1,3 and 5 year synchronous 91.3%, 63.8%, 61.1%, sequential 95.3%, 69.5%, 64.6%,  $P = 0.41$ ) was similar but TFS was higher in the sequential group (1,3 and 5 year synchronous 68.5%, 37.3% and 29.7%, sequential 93.2%, 56.6%, 48.5%,  $P = 0.02$ ) though this difference did not remain during multivariate analysis.

### CONCLUSION

TFS in patients presenting with two HCC  $\leq 3$  cm is poor regardless of the timing of the second tumor. All patients presenting with two small HCC should be considered for transplantation.

**Key Words:** Hepatocellular carcinoma; Liver cancer; Prognosis; Transplantation; Transplant-free survival

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**Core Tip:** Transplant-free survival in patients with 2 small hepatocellular carcinomas is poor, whether presenting synchronously or sequentially, and so should be considered for transplantation.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and is the fourth leading cause of cancer-related mortality globally[1]. With uptake of standardized HCC surveillance programs, a greater number of patients are being diagnosed at earlier stages of disease when curative treatment is still possible[2-5]. In patients presenting with small tumors the probability of survival has progressively

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improved over recent decades with 5-year survival rates greater than 50% now frequently reported[6,7].

Curative therapies for HCC include surgical resection, percutaneous thermal ablation and liver transplantation. Within widely adopted eligibility criteria, transplantation may be considered when up to three individual HCC are present[8,9]. For solitary HCC, selection of therapy is based upon tumor size and location, in addition to severity of underlying hepatic dysfunction and portal hypertension. Surgical resection and ablative therapies have comparable survival rates in patients with solitary HCC less than 3 cm in diameter[10-13].

Whilst the guidelines are relatively clear for management of patients presenting with a single HCC  $\leq 3$  cm or three small HCC, there is little data to guide decision-making in patients who present with two small HCC, particularly when a second lesion appears sequentially after the index lesion. In this present study we sought to define the outcome of patients presenting with two HCC each up to 3 cm, in addition to exploring whether outcomes vary depending on whether tumors present either synchronously or sequentially (metachronously).

## MATERIALS AND METHODS

### Study design

Retrospective data of all HCC diagnosed between 1<sup>st</sup> of January 2000 to 31<sup>st</sup> of March 2018 from four tertiary referral centres in Melbourne, Victoria were reviewed. Data were retrieved from site-specific prospectively collected electronic health records. Institutional ethics committee approval was obtained from participating sites prior to commencement at each centre.

### Inclusion criteria

Patients  $\geq 18$  years old with either two synchronous or two sequential HCC each up to 3 cm in size were identified. Patients with and without cirrhosis were included. Cirrhosis was established on standardized clinical, biochemical and radiologic grounds with or without histologic confirmation. In non-cirrhotic patients, HCC diagnosis was established histologically in all cases. HCC diagnoses between 2001 and 2012 were made according to 2001 European Association for the Study of the Liver (EASL) guidelines; all other lesions outside of these criteria required biopsy for diagnosis[14]. Diagnoses made beyond 2012 were in accordance with revised EASL criteria[2].

### Exclusion criteria

Patients who only ever had a single HCC or more than two tumors at diagnosis were excluded. Patients were also excluded if either of their first two HCC exceeded 3 cm or if they had radiologic evidence of vascular invasion or distant metastasis. Patients managed at more than one centre were only included once. After inclusion and exclusion criteria were applied, 104 patients were included in the study for analysis.

### Data collection

Data was collated from patient records into a central database and included demographics (age, gender), aetiology of chronic liver disease, the presence of or absence of cirrhosis, Child-Turcotte-Pugh (CTP) and model for end-stage liver disease (MELD)[15] scores,  $\alpha$ -feto protein (AFP) level and radiologic tumor characteristics (total diameter of both lesions and diameter of largest individual lesion). Date of disease progression, the nature of progression (local recurrence, new disease, portal vein invasion or metastases) and date of death were recorded.

### Treatment

Treatment modalities and number of treatments were recorded. Treatment was administered according to multidisciplinary consensus at each institution. Locoregional therapies included percutaneous ablation (inclusive of microwave and radiofrequency ablation), percutaneous ethanol injection (PEI), transarterial chemoembolization (TACE) and irreversible electroporation. All cases being considered for transplantation were referred to the Victorian Liver Transplantation Unit at Austin Health. Patients with HCC waitlisted for transplantation in Victoria are not granted MELD exception points, with decisions on timing of transplant made at twice-weekly multidisciplinary meetings and priority given to patients with active tumor rather

than cumulative time on the waitlist.

### Outcome measures

For the synchronous group, follow-up time began at the date two HCC were confirmed radiologically (Figure 1). For the sequential group, records of patients presenting with a single lesion were reviewed for occurrence of a second lesion. Follow-up time in the sequential group began at the time the second HCC was diagnosed (the first lesion may have received treatment; response to treatment whether it be partial or complete was not a requirement for inclusion). The primary outcome was overall survival (OS) which was calculated from the date of meeting inclusion criteria until death. Transplant-free survival (TFS) was calculated from the date of meeting inclusion criteria until liver transplantation or death without transplantation. Progression-free survival was from date of meeting inclusion criteria until either disease progression according to mRECIST[16] criteria or death without confirmed radiologic progression.

### Statistical analysis

Demographic and continuous variables were assessed for normality and were accordingly presented as mean  $\pm$  SD or median and interquartile range (IQR). Categorical variables were presented as frequencies with percentages. Baseline characteristics were compared between groups using one-way ANOVA and Mann-Whitney *U* test for normally-distributed and non-normally-distributed continuous variables, respectively. Pearson chi square test was used to compare categorical variables.

Survival was calculated by Kaplan-Meier analysis with all patients alive at the end of the follow-up period or transplanted before confirmed radiological progression being censored from survival analysis. Univariate analysis of prognostic factors was performed by log-rank testing; group comparisons included age  $\leq 70$  *vs*  $> 70$  years, male *vs* female, aetiology of underlying liver disease, CTP class, MELD  $\leq 14$  *vs*  $> 14$ , AFP at diagnosis  $< 10$  or  $\geq 10$   $\mu\text{g/L}$ , presentation with synchronous or sequential lesions both  $\leq 3$  cm and transplanted *vs* non-transplanted. Multivariate Cox proportional hazard analysis of univariate variables with a *P* value  $< 0.10$  was performed and reported as hazard ratios (HR) with 95% CI. Significance tests were two-tailed with a *P* value  $< 0.05$  considered statistically significant. All analyses were performed using SPSS version 22 (Armonk, NY: IBM Corp).

## RESULTS

### Patient characteristics

One hundred and four patients were identified as having two HCC and were followed up for a median of 2.54 years (IQR 2.73 years, range 0.08-13.67); only six patients (5.8%) had less than six months follow-up. Eighty-nine (85.6%) were male and the median age was 63 years (IQR 58-68). The most common cause of liver disease was chronic hepatitis C ( $n = 42$ , 40.4%) followed by chronic hepatitis B ( $n = 15$ , 14.4%). The majority were CTP score A ( $n = 66$ , 63.7%) and median MELD at diagnosis was 9.5 (IQR 7-13).

Baseline characteristics comparing synchronous *vs* sequential tumors are shown in Table 1. Fifty-nine patients (56.7%) had two synchronous HCC at inclusion, whilst forty-five (43.3%) had sequential lesions with the median time between index and sequential lesions 14 mo (IQR 7.5-29.5). There was no difference in follow-up time between the two groups ( $P = 0.54$ ). Mean MELD score at diagnosis was the only statistically significant difference between the two groups, higher in the synchronous cohort ( $11 \pm 7$  *vs*  $8 \pm 5$ ,  $P = 0.01$ ). The median combined diameter of the two tumors in the synchronous group was not significantly different from the sequential group (3.8 cm *vs* 3.4 cm,  $P = 0.28$ ).

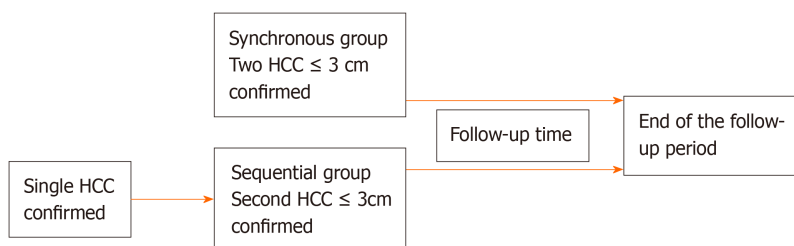
### Treatment

The most common single treatment for patients with synchronous HCC was TACE (32.2%) followed by percutaneous ablation (20.3%), whilst two patients (3.4%) had unsuccessful locoregional therapy due to technical limitations and received transplantation as their primary treatment modality (Supplementary Table 1). Percutaneous ablation was the commonest single treatment for index lesions in the sequential group (57.8%) followed by surgical resection (17.8%). As first line treatment, TACE was more commonly utilized in the synchronous group (32.2% *vs* 8.9%,  $P < 0.01$ ), whilst percutaneous ablation was more common in the sequential group (57.8%

**Table 1** Baseline characteristics of 104 patients with two hepatocellular carcinomas according to synchronous or sequential tumor

	All ( <i>n</i> = 104)	Synchronous group ( <i>n</i> = 59)	Sequential group ( <i>n</i> = 45)	<i>P</i> value
Age, yr, median (IQR)	63 (10)	63 (10)	63 (9)	0.41
Gender, <i>n</i> (%)				0.08
Male	89 (85.6)	51 (86.4)	38 (84.4)	
Female	15 (14.4)	8 (13.6)	7 (15.6)	
Aetiology, <i>n</i> (%)				0.73
Alcohol	12 (11.5)	8 (13.6)	4 (8.9)	
HCV	42 (40.4)	22 (37.3)	20 (44.4)	
HBV	15 (14.4)	8 (13.6)	7 (15.6)	
NASH	5 (4.8)	4 (6.8)	1 (2.2)	
Alcohol and HCV	18 (17.3)	9 (15.3)	9 (20.0)	
Other <sup>1</sup>	12 (11.5)	8 (13.6)	4 (8.9)	
Cirrhosis status, <i>n</i> (%)				0.07
Non-cirrhotic	10 (9.6)	3 (5.1)	7 (15.6)	
Cirrhotic	94 (90.4)	56 (94.9)	38 (84.4)	
CTP class, <i>n</i> (%)				0.1
A	66 (63.5)	35 (59.3)	31 (68.9)	
B	25 (24.0)	13 (22.0)	12 (26.7)	
C	13 (12.5)	11 (18.6)	2 (4.4)	
MELD, median (IQR)	9.6 (6)	11 (7)	8 (5)	0.01
AFP (μg/L), median (IQR)	9.6 (24.0)	8.6 (26.0)	10.4 (22.8)	0.61
Combined tumour diameter (cm), median (IQR)	3.5 (1.7)	3.8 (1.7)	3.4 (1.2)	0.28
Transplanted, <i>n</i> (%)	25 (24)	18 (30.5)	7 (15.6)	0.08
Death, <i>n</i> (%)	36 (34.6)	23 (39.0)	13 (28.9)	0.28

<sup>1</sup>Other refers to aetiologies not listed here and is inclusive of: Mixed aetiologies, autoimmune hepatitis, hereditary haemochromatosis,  $\alpha$ -1-antitrypsin deficiency and cryptogenic liver disease. IQR: Interquartile range; AFP:  $\alpha$ -feto protein; CTP: Child-Turcotte-Pugh; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MELD: Model for End-Stage Liver Disease score; NASH: Non-alcoholic steatohepatitis.

**Figure 1** Study flow diagram. HCC: Hepatocellular carcinoma.

*vs* 20.3%,  $P < 0.01$ ). There was no significant difference in the rate of PEI or resection between the two groups ( $P = 0.25$  and  $P = 0.16$ , respectively). Synchronous lesions were more frequently treated with two modalities upfront (30.5% *vs* 13.3%,  $P = 0.04$ ). The second lesion in the sequential group was most frequently treated by percutaneous ablation (31.1%) followed by TACE (28.9%), with only three patients (6.67%) undergoing transplantation (Supplementary Table 2).

During the follow-up period, 25 patients (24%) were transplanted with median time to transplantation 12 mo (IQR 2.83). The only significant differences between transplanted and non-transplanted patients were CTP and MELD score ( $P < 0.01$  for both) (Supplementary Table 3). Although a higher proportion of patients with synchronous HCC were transplanted compared to the sequential group (30.5% *vs* 15.6%), this did not reach statistical significance ( $P = 0.08$ ).

### Survival analysis

**Overall survival:** Thirty-six (34.6%) patients died during the study period with median time to death 1.45 years (IQR 1.17-2.63) (Supplementary Figure 1). OS at 1-, 3- and 5-years was 93%, 66.1% and 62.3%, respectively (Table 2). There was no difference in OS between the synchronous and sequential groups ( $P = 0.41$ , Figure 2A). On univariate analysis (Supplementary Table 4), only age  $\geq 70$  years was associated with increased risk of mortality (HR 2.19, 95%CI: 1.08-4.45,  $P = 0.03$ ), whilst only transplantation was associated with reduced mortality (HR 0.19, 95%CI: 0.07-0.55,  $P < 0.01$ ). On multivariate analysis, only transplantation remained significant with HR 0.20, 95%CI: 0.07-0.61,  $P < 0.01$  (Supplementary Table 5).

**TFS:** TFS was 77.1%, 45.4% and 37.8% at 1-, 3 and 5-years, respectively (Table 2, Supplementary Figure 2). TFS was significantly different between the synchronous and sequential groups, with five-year transplant-free survival of 29.7% in the synchronous group and 48.5% in the sequential group ( $P = 0.02$ , Figure 2B). Univariate analysis identified CTP C status (HR 5.17, 95%CI: 2.59-10.29,  $P < 0.01$ ) and MELD  $> 14$  (HR 4.07 95%CI: 2.27-7.32,  $P < 0.01$ ) as predictors of mortality (Supplementary Table 6), whilst the sequential tumor was associated with survival (HR 0.53, 95%CI: 0.31-0.92,  $P = 0.03$ ). After multivariate analysis (Table 3), the difference between the sequential and synchronous groups did not remain significant (HR 0.70, 95%CI: 0.38-1.27,  $P = 0.24$ ) and only MELD  $> 14$  remained a significant predictor of death (HR 2.51, 95%CI: 1.15-5.46  $P = 0.02$ ).

**Transplanted patients:** 1-, 3- and 5-year survival in transplanted patients was 100%, 95.8% and 95.8% (Table 2) with median time to death after transplant 6.42 years (IQR 1.33-6.67 years). Four transplanted patients (16%) died; three from recurrent HCC and the fourth from complications of motor neurone disease. All three transplanted patients with recurrent HCC had initially presented with synchronous lesions.

### Disease progression

Progressive disease in the entire cohort was seen in 71 patients (68%) by five years. Median time to progression was 1.58 years (IQR 1-3). Amongst those with disease progression, recurrence with new lesions was the commonest form of progression, occurring in 30 patients (42.2%). Progression-free survival was not significantly different between the synchronous and sequential groups ( $P = 0.19$ ). Subgroup analysis showed that the sequential group had longer progression-free survival without local recurrence ( $P < 0.01$ , Supplementary Figure 3) and without new lesions ( $P < 0.01$ , Supplementary Figure 4). No differences were seen in survival without progression, survival without failure of primary treatment or survival without metastatic spread (data not shown).

## DISCUSSION

This study provides novel data on the clinical outcome of patients who develop two HCC up to 3 cm in diameter and explores the question of whether small HCC behave differently when presenting synchronously compared to sequentially. We found that regardless of whether HCC are diagnosed synchronously or sequentially, transplant-free survival is poor, with 5-year transplant free survival being only 37.8%. This suggests that liver transplantation should be considered earlier amongst the treatment options for patients with two HCC regardless of the timing of the second HCC. This is supported by the excellent five-year survival of transplanted patients in our cohort of 95.8%.

Our five-year OS of 62.3% was similar to that reported elsewhere. A retrospective survival analysis of an international, multi-institution HCC cohort of 814 patients that underwent hepatectomy with curative intent identified a five-year OS of 69% in patients with BCLC stage A disease[17]. Whilst this encompasses patients with two small HCC  $\leq 3$ cm, the target group in our study, their cohort also included patients



**Table 2 One-, three-, and five-year survival analysis of patients with two hepatocellular carcinomas**

	Overall survival (n = 104)	Overall survival	Overall survival	Transplanted survival (n = 25)	Transplant-free survival (n = 104)	Transplant-free survival	Transplant-free survival
		Synchronous group (n = 59)	Sequential group (n = 45)			Synchronous group (n = 59)	Sequential group (n = 45)
1-yr survival (%)	93	91.3	95.3	100	77.1	68.5	93.2
3-yr survival (%)	66.1	63.8	69.5	95.8	45.4	37.3	56.6
5-yr survival (%)	62.3	61.1	64.6	95.8	37.8	29.7	48.5

**Table 3 Multivariate analysis of factors impacting transplant-free survival**

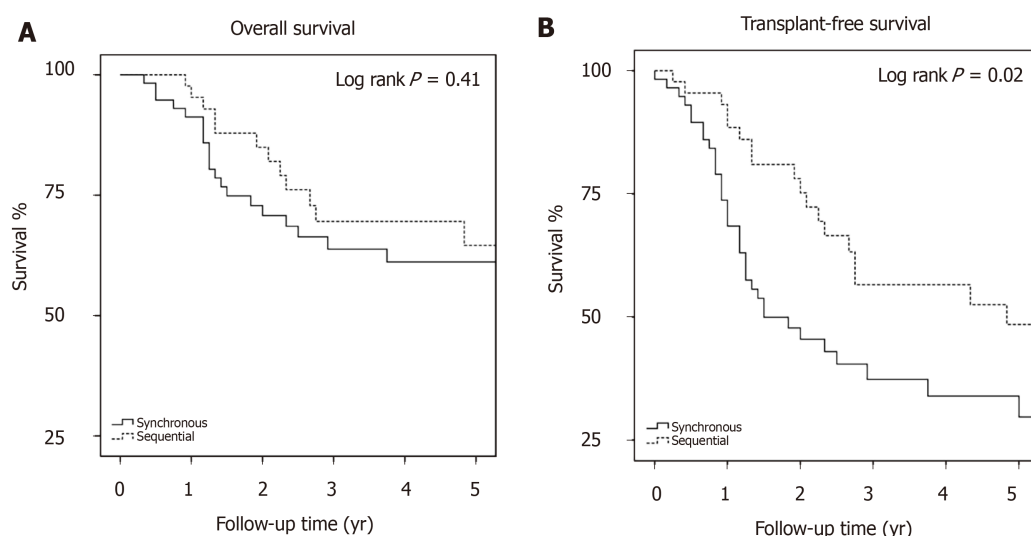
	n (%)	HR	95%CI	P value
CTP class				
A	66 (63.5)	-	-	-
B	25 (24.0)	1.51	0.81-2.82	0.19
C	13 (12.5)	2.26	0.90-5.65	0.8
MELD at diagnosis				
≤ 14	85 (81.7)	-	-	-
> 14	19 (18.3)	2.51	1.15-5.46	0.02
Lesion group				
Synchronous	59 (56.7)	-	-	-
Sequential	45 (43.3)	0.7	0.38-1.27	0.24

HR: Hazard ratios; CI: Confidence interval; CTP: Child-Turcotte-Pugh; MELD: Model for End-Stage Liver Disease score.

with single lesions ranging 2-5 cm in size or 3 lesions  $\leq$  3 cm each and therefore represented a broader range of patients. Additionally, we included patients that received a heterogeneous array of therapies in contrast to this study that looked only at surgical outcomes. The authors identified AFP > 400 ng/mL as being associated with poorer survival, in line with data elsewhere on surgical outcomes in low volume disease[18], yet our study did not find this association at AFP thresholds of 10  $\mu$ g/L nor 400  $\mu$ g/L (latter data not shown). Rather, we identified transplantation as the single independent variable that influenced survival.

We had excellent outcomes in patients who underwent transplantation for two small HCC, with 5-year survival 95.8%. The reported five year survival for transplantation with HCC is in the order of 70%[7]. For early HCC, a recent meta-analysis of low volume disease showed post-transplant survival to be 61.26% at 5 years[19]. Our higher post-transplant survival is likely due to the selection criteria for inclusion in this study, with patients only included if they had two small HCC. Despite excellent survival data, we note that in four deaths amongst transplanted patients, three were from recurrent HCC and all three of these patients had synchronous HCC.

The only independent factor impacting TFS in this study was MELD score. This suggests that in patients with two HCC, the severity of liver disease is an important factor in defining outcome, rather than lesion synchronous or sequential presentation, a similar finding to other series that examined the prognostic value of MELD scores in non-transplant HCC survival[20]. It is noteworthy that the non-transplant outcomes in patients with MELD  $\leq$  14 remained poor in our cohort, with five-year survival of only 45.9%. This indicates that many patients with two small HCC would benefit from



**Figure 2** Kaplan-Meier survival curve for synchronous vs sequential groups. A: Overall survival; B: Transplant-free survival.

consideration of transplantation.

Strengths of this study include robust and comprehensive follow-up data, with only 5.8% of patients having less than 6 mo follow-up, and real-world data from four large tertiary centres. The data in this series was prospectively collected onto HCC databases at treating institutions. Given that all transplants occur in a single centre, we are confident that all transplant records are complete with accurate data and outcome of transplantation. The primary methodological limitation of this study is that it was not randomized, which can lead to inherent biases in the groups transplanted and not transplanted that may have influenced outcomes. Some patients who were deemed not appropriate for transplantation may have had other co-factors that influenced survival, such as severe non-liver comorbidities or ongoing substance abuse. There are also differing treatment algorithms and techniques between institutions involved in our study. The index presentation of a single small HCC tends to be treated by thermal ablative techniques, rather than transarterial chemoembolization, which was the treatment of choice for unresectable synchronous tumors[21].

Our study was also limited by being focused on tumor number and size as surrogate markers for tumor biology. We were not able to evaluate the impact of histology on outcomes as the majority of diagnoses were made according to radiological criteria, in line with international guidelines[2,14]. As reported previously, transplantation according histological tumor grade leads to improved outcomes beyond selection by Milan criteria alone[22]. However, a single-centre series found that pre-transplant liver biopsy did not affect outcomes when selecting patients that are within Milan criteria, as our patients were[23]. Additionally, we recognize that amongst both groups it is not possible to determine which patients experienced intrahepatic metastasis compared to multi-centric hepatocarcinogenesis as both scenarios may lead to presentation with 'two' lesions. However, our study was focused purely on the number of lesions and whether this clinical determinant could guide our multidisciplinary meeting treatment decisions.

Choice of curative *vs* non-curative locoregional therapies may also have affected survival time between the two groups. The synchronous group had a higher rate of TACE as initial therapy compared to the sequential group, which more frequently received ablative therapies as first line treatment. This in part may explain the difference seen in TFS between the two groups.

Our data collection period spanned almost two decades and it is recognized that survival of patients diagnosed at the beginning of the observation period may not be directly comparable to patients diagnosed towards the latter portion. In an analysis of HCC cases from the Australian Cancer Registry, a national database that began in 1982, the median OS of patients doubled from 6.15 mo in those diagnosed between 2000-2004 to 12.07 mo for those diagnosed 2010-2014[6]. These data represent all patients and due to this heterogeneity, identification of the causes of improved survival are difficult but potentially attributable to better patient selection, earlier detection through HCC screening, widespread adoption of multidisciplinary decision-making, evolving locoregional treatments along with emergence of palliative therapies for advanced disease, such as oral multi tyrosine kinase inhibitors.

## CONCLUSION

In conclusion we report for the first-time data specifically pertaining to patients presenting with two small HCC 3 cm in size or smaller. Our results demonstrate that the non-transplant survival of patients presenting with two small HCC is poor. Survival was similarly poor in patients presenting with two synchronous HCC as compared to sequential HCC. We therefore recommend that patients that develop a second small HCC after their first should be considered for early liver transplantation. Further larger-scale studies are required to validate these results in other populations and determine broader implications for liver transplantation waitlist management.

## ARTICLE HIGHLIGHTS

### Research background

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, and is a growing cause for cancer-related mortality globally. Curative therapies include ablation for small tumors, surgical resection, and liver transplantation.

### Research motivation

At present, there is clear evidence underpinning the guidelines for management of small tumors ( $\leq 3$  cm in maximal diameter) and three small tumors (*i.e.*, all  $\leq 3$  cm), however a scarcity of literature surrounding the optimal management of two small tumors. In addition, it is unclear if synchronous (*i.e.*, occurring at the same time) and sequential (*i.e.*, occurring at different points in time) tumors have differing prognoses.

### Research objectives

This study aimed to assess the outcome of two small tumors (*i.e.*,  $\leq 3$  cm in maximal diameter), and whether there was a difference in prognosis between those occurring synchronously and sequentially. This is to help guide future guidelines for management of two small HCCs.

### Research methods

This was a retrospective multicenter study conducted in Victoria, Australia, including all patients diagnosed with two small HCCs between 1<sup>st</sup> January 2000 and 31<sup>st</sup> March 2018. Review of the medical record for patient demographics, liver disease, tumor-specific details, treatment and outcome was collected. Diagnosis of HCC was based on accepted radiographic and/or histologic criteria. Primary outcomes were overall survival (OS) and transplant-free survival (TFS).

### Research results

One-hundred and four patients, majority male ( $n = 89$ , 86%), with a median age of 63 years-old (interquartile range 58-67.75), and predominantly suffering from viral chronic liver disease ( $n = 57$ , 55%) were included in the final analysis and followed up for a median of 2.54 years. There was a slight majority in those presenting synchronously ( $n = 59$ , 57%) compared with those diagnosed sequentially ( $n = 45$ , 43%), with the only difference between these two groups being more severe liver disease on the basis of model for end stage liver disease (MELD) (11 *vs* 8,  $P = 0.01$ ). 1-, 3-, and 5-year OS was similar between the two groups ( $P = 0.41$ ), however TFS was higher in the sequential group (1-, 3- and 5-year TFS 93.2%, 56.6% and 48.5%, compared with 68.5%, 37.3% and 29.7% in the synchronous group,  $P = 0.02$ ). This difference did not persist in multivariate analysis ( $P = 0.24$ ), with only MELD  $> 14$  being predictive of mortality in the model (hazard ratio 2.51, 95%CI: 1.15-5.46,  $P = 0.02$ ).

### Research conclusions

Transplant-free survival in patients with two HCCs  $\leq 3$  cm is poor irrespective if diagnosed synchronously or sequentially, and so all patients with two small tumors should be assessed and considered for liver transplantation.

### Research perspectives

Given limited availability of liver transplantation, future research should aim to define the molecular carcinogenetic signature in multifocal tumors, which can occur from

multi-centric hepatocarcinogenesis or intrahepatic metastases, and whether this impacts recurrence, prognosis, and response to curative therapy.

## REFERENCES

- 1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: [30207593](#) DOI: [10.3322/caac.21492](#)]
- 2 **European Association for the Study of the Liver**, European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: [22424438](#) DOI: [10.1016/j.jhep.2011.12.001](#)]
- 3 **Heimbach JK**, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: [28130846](#) DOI: [10.1002/hep.29086](#)]
- 4 **Fitzmorris P**, Singal AK. Surveillance and Diagnosis of Hepatocellular Carcinoma. *Gastroenterol Hepatol (NY)* 2015; **11**: 38-46
- 5 **Singal AG**, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. *PLoS Med* 2014; **11**: e1001624 [PMID: [24691105](#) DOI: [10.1371/journal.pmed.1001624](#)]
- 6 **Wallace MC**, Preen DB, Short MW, Adams LA, Jeffrey GP. Hepatocellular carcinoma in Australia 1982-2014: Increasing incidence and improving survival. *Liver Int* 2019; **39**: 522-530 [PMID: [30230194](#) DOI: [10.1111/liv.13966](#)]
- 7 **Llovet JM**, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 2005; **25**: 181-200 [PMID: [15918147](#) DOI: [10.1055/s-2005-871198](#)]
- 8 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: [8594428](#) DOI: [10.1056/NEJM199603143341104](#)]
- 9 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [DOI: [10.1016/S0016-5085\(01\)80268-7](#)]
- 10 **Pompili M**, Saviano A, de Matthaeis N, Cucchetti A, Ardito F, Federico B, Brunello F, Pinna AD, Giorgio A, Giulini SM, De Sio I, Torzilli G, Fornari F, Capussotti L, Guglielmi A, Piscaglia F, Aldrighetti L, Caturelli E, Calise F, Nuzzo G, Rapaccini GL, Giulianti F. Long-term effectiveness of resection and radiofrequency ablation for single hepatocellular carcinoma  $\leq 3$  cm. Results of a multicenter Italian survey. *J Hepatol* 2013; **59**: 89-97 [PMID: [23523578](#) DOI: [10.1016/j.jhep.2013.03.009](#)]
- 11 **Cho YK**, Rhim H, Noh S. Radiofrequency ablation vs surgical resection as primary treatment of hepatocellular carcinoma meeting the Milan criteria: a systematic review. *J Gastroenterol Hepatol* 2011; **26**: 1354-1360 [PMID: [21679247](#) DOI: [10.1111/j.1440-1746.2011.06812.x](#)]
- 12 **Li L**, Zhang J, Liu X, Li X, Jiao B, Kang T. Clinical outcomes of radiofrequency ablation and surgical resection for small hepatocellular carcinoma: a meta-analysis. *J Gastroenterol Hepatol* 2012; **27**: 51-58 [PMID: [22004366](#) DOI: [10.1111/j.1440-1746.2011.06947.x](#)]
- 13 **Xu XL**, Liu XD, Liang M, Luo BM. Radiofrequency Ablation vs Hepatic Resection for Small Hepatocellular Carcinoma: Systematic Review of Randomized Controlled Trials with Meta-Analysis and Trial Sequential Analysis. *Radiology* 2018; **287**: 461-472 [PMID: [29135366](#) DOI: [10.1148/radiol.2017162756](#)]
- 14 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rod s J; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: [11592607](#) DOI: [10.1016/s0168-8278\(01\)00130-1](#)]
- 15 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: [11172350](#) DOI: [10.1053/jhep.2001.22172](#)]
- 16 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: [20175033](#) DOI: [10.1055/s-0030-1247132](#)]
- 17 **Tsilimigras DI**, Bagante F, Sahara K, Moris D, Hyer JM, Wu L, Ratti F, Marques HP, Soubrane O, Paredes AZ, Lam V, Poultsides GA, Popescu I, Alexandrescu S, Martel G, Workneh A, Guglielmi A, Hugh T, Aldrighetti L, Endo I, Pawlik TM. Prognosis After Resection of Barcelona Clinic Liver Cancer (BCLC) Stage 0, A, and B Hepatocellular Carcinoma: A Comprehensive Assessment of the Current BCLC Classification. *Ann Surg Oncol* 2019; **26**: 3693-3700 [PMID: [31267302](#) DOI: [10.1245/s10434-019-07580-9](#)]
- 18 **Ho CM**, Hu RH, Lee PH, Wu YM, Ho MC. Long-term survival in patients with T2 hepatocellular carcinoma after primary curative resection can be further stratified by tumor size. *Medicine (Baltimore)* 2014; **93**: e203 [PMID: [25501076](#) DOI: [10.1097/MD.0000000000000203](#)]
- 19 **Schoenberg MB**, Bucher JN, Vater A, Bazhin AV, Hao J, Guba MO, Angele MK, Werner J, Rentsch M. Resection or Transplant in Early Hepatocellular Carcinoma. *Dtsch Arztebl Int* 2017; **114**: 519-526

- [PMID: [28835324](#) DOI: [10.3238/arztebl.2017.0519](#)]
- 20 **Marrero JA**, Fontana RJ, Barrat A, Askari F, Conjeevaram HS, Su GL, Lok AS. Prognosis of hepatocellular carcinoma: comparison of 7 staging systems in an American cohort. *Hepatology* 2005; **41**: 707-716 [PMID: [15795889](#) DOI: [10.1002/hep.20636](#)]
  - 21 **Park JW**, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS, Sherman M. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015; **35**: 2155-2166 [PMID: [25752327](#) DOI: [10.1111/liv.12818](#)]
  - 22 **Cillo U**, Vitale A, Bassanello M, Boccagni P, Brolese A, Zanusi G, Burra P, Fagioli S, Farinati F, Rugge M, D'Amico DF. Liver transplantation for the treatment of moderately or well-differentiated hepatocellular carcinoma. *Ann Surg* 2004; **239**: 150-159 [PMID: [14745321](#) DOI: [10.1097/01.sla.0000109146.72827.76](#)]
  - 23 **DuBay D**, Sandroussi C, Sandhu L, Cleary S, Guba M, Cattral MS, McGilvray I, Ghanekar A, Selzner M, Greig PD, Grant DR. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg* 2011; **253**: 166-172 [PMID: [21294289](#) DOI: [10.1097/sla.0b013e31820508f1](#)]





## Focal nodular hyperplasia associated with a giant hepatocellular adenoma: A case report and review of literature

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**Author contributions:** Gaspar-Figueiredo S and Kefleyesus A performed the literature review, collected all the data related to the case report, and recorded/edited the video-vignette related to the case report; Sempoux C did the anatomopathological examination/appraisal; Halkic N and Uldry E did the surgical appraisal; all authors have read and approved the final manuscript.

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### Abstract

#### BACKGROUND

Focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) are well-known benign liver lesions. Surgical treatment is usually chosen for symptomatic patients, lesions more than 5 cm, and uncertainty of diagnosis.

#### CASE SUMMARY

We described the case of a large liver composite tumor in an asymptomatic 34-year-old female under oral contraceptive for 17-years. The imaging work-out described two components in this liver tumor; measuring 6 cm × 6 cm and 14 cm × 12 cm × 6 cm. The multidisciplinary team suggested surgery for this young woman with an unclear HCA diagnosis. She underwent a laparoscopic left liver lobectomy, with an uneventful postoperative course. Final pathological examination confirmed FNH associated with a large HCA. This manuscript aimed to make a literature review of the current management in this particular situation of large simultaneous benign liver tumors.

#### CONCLUSION

The simultaneous presence of benign composite liver tumors is rare. This case highlights the management in a multidisciplinary team setting.

**Key Words:** Liver; Focal nodular hyperplasia; Hepatocellular adenoma; Composite tumor; Video vignette; Case report

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**Core Tip:** Focal nodular hyperplasia and hepatocellular adenoma (HCA) are frequent but non-malignant tumors. There is rarely indication for surgery. Combination of these two masses is a very unusual situation. Their diagnosis is mainly based on radiology. Oral contraception is a risk factor for HCA. Malignant transformation of HCA is the predominant argument for surgery. All these cases, especially composite tumors, must be discussed in a multidisciplinary team.

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## INTRODUCTION

Focal nodular hyperplasia (FNH) has become a pretty well-known disease in the past two decades. It is defined by a benign hyperplastic nodule with a central scar, appearing in the normal liver parenchyma, and is composed of normal hepatocytes in a multinodular structure[1]. Its incidence is between 0.6%-3%, predominantly affecting females patients (80%-90%) in their third or fourth decade. The pathophysiology is thought to be due to an increased arterial flow that leads to secondary hepatocellular hyperplasia[2,3]. The correlation with oral contraceptives (OCs) is unproven but very likely, given that OCs are taken almost exclusively by women (sex ratio 9:1) and the proven correlation between OCs and change in lesion size[4,5].

Hepatocellular adenoma (HCA) is a benign lesion with a malignant potential between 4% and 8%, according to recent works of Farges *et al*[6] and Sempoux *et al*[7]. It classically arises in a noncirrhotic liver, in young females with an OC background. However, the understanding of HCA has evolved dramatically and we now know that it can also develop in patients with non-alcoholic steatohepatitis, certain vascular malformations, or alcoholic cirrhosis. Moreover, there are a wide variety of subtypes of this complex disease, making it very difficult to establish treatment guidelines[8-10].

In this present article, we aimed to describe the detailed management of a rare simultaneous case of FNH and HCA and a brief review of the literature.

## CASE PRESENTATION

### Chief complaints

A 34-year-old woman in general good health, with a medical history of oral contraceptives (desogestrel, ethinylestradiol) for 17 years consulted her general practitioner (GP) for a check-up.

### History of present illness

She was completely asymptomatic.

### History of past illness

She had no past illness.

### Personal and family history

The patient had no past medical history except a knee orthopedic surgery 1 year before, had a stable weight with normal body mass index (21.1 kg/m<sup>2</sup>) and no familial medical history.

### Physical examination

During the examination, her GP found a mobile and palpable abdominal mass of more than 10 cm in diameter, with no skin bulging at the Valsalva's maneuver (Figure 1).



Figure 1 Pre-operative patient's supine and stand-up picture – no external signs of tumor.

### Laboratory examinations

The blood exams were normal, except for an elevation in alkaline phosphate level of 519 U/L (normal range = 36-108). Tumoral markers were normal.

### Imaging examinations

Abdominal ultrasound revealed an aspecific giant mass next to the left hepatic lobe. A computed tomography (CT scan) revealed a double mass attached to the left lobe of the liver. The first one had the typical characteristics of FNH and the second one of uncertain dignity. Further magnetic resonance imaging (MRI) confirmed a 6 cm x 6 cm mass suggestive of FNH in the inferior part of segment III. This 6 cm lesion was right next to a second one measuring 14 cm x 12 cm x 6 cm which dignity was unclear. The differential diagnosis was between an HCA, a hepatocellular carcinoma (fibrolamellar variant), or an atypical FNH (Figures 2-5).

## FINAL DIAGNOSIS

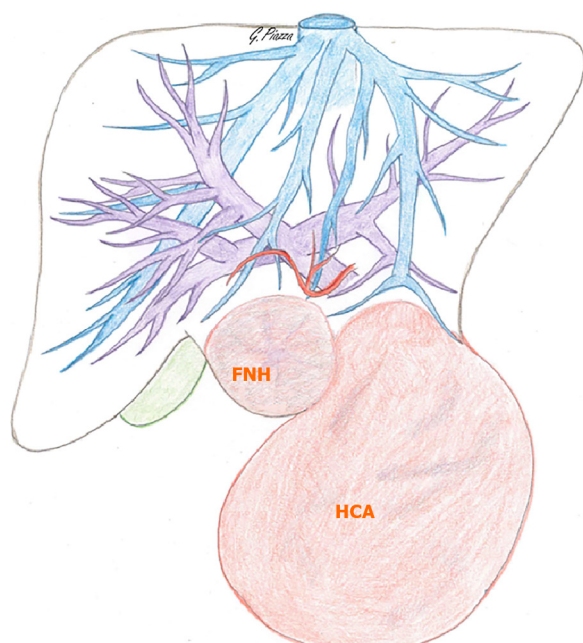
The pathologist's report confirmed the diagnosis of 6 cm FNH resected with good margin and showed a non-beta-catenin-mutated HCA (inflammatory subtype with more risk of malignant transformation) (Figure 6).

## TREATMENT

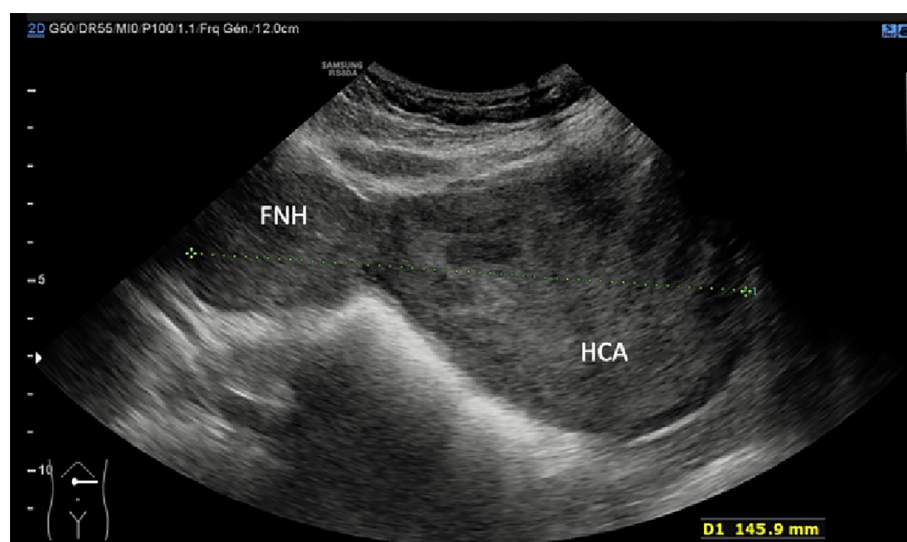
Indication for surgery was retained during a multidisciplinary team (MDT) meeting as the first option for definitive diagnosis and treatment.

The surgery was completed without complication. We summarize hereafter the key points of the minimally invasive procedure. After inserting 4 trocars for the laparoscopy (para-umbilical, right and left flank, subxiphoid) and staying away from the large dual mass which limited the range movements, we performed an ultrasound confirming a pedunculated mass (FNH) highly vascularized attached to segment III and a second component pedunculated between segment II and III. The mass showed no adhesion with the segment IV and the gallbladder allowing a left lobectomy. Dissection was performed with ultrasonic shears (Ultracision Harmonic, Ethicon Inc., NJ, United States) and transection was completed with a 60mm stapler (tri-staple vascular cartridge, Endo-GIA, Medtronic, Minneapolis, MN, United States). We extracted the specimen with both lesions through a suprapubic (Pfannenstiel) incision. The operative time was 122 min. Blood loss was minimal (50 mL) (Video 1).

The postoperative course was uneventful and the patient was discharged on postoperative day 3.



**Figure 2** Preoperative drawing – tumor and liver major vessels' relationship (credits: Dr. Giulia Piazza). FNH: Focal nodular hyperplasia; HCA: Hepatocellular adenoma.



**Figure 3** Ultrasonography with a sagittal view of focal nodular hyperplasia and hepatocellular adenoma. D1: Greater axis length. FNH: Focal nodular hyperplasia; HCA: Hepatocellular adenoma.

## OUTCOME AND FOLLOW-UP

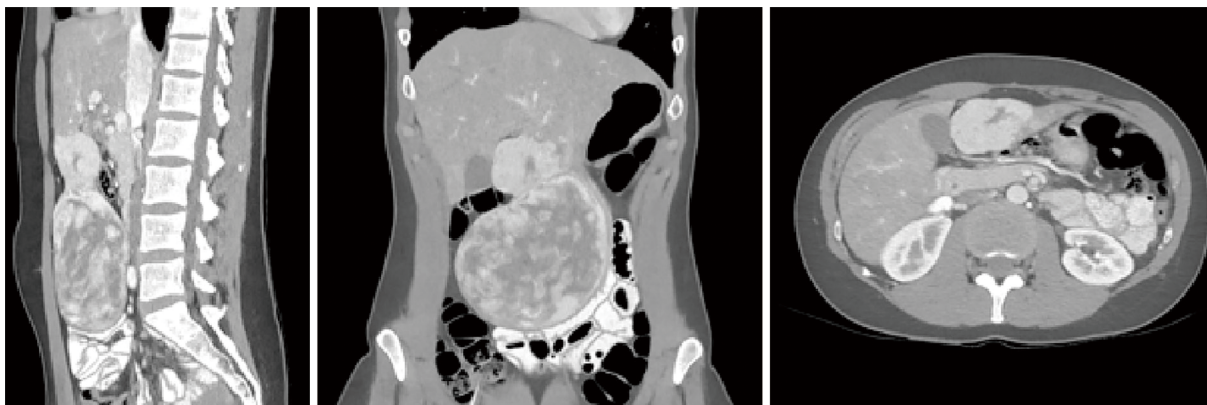
The MDT meeting proposed a 1-year MRI follow-up with oral contraceptive discontinuation.

One month after surgery, the patient was good without any complaint, her scar evolution was satisfactory and there was no sign of an early incisional hernia.

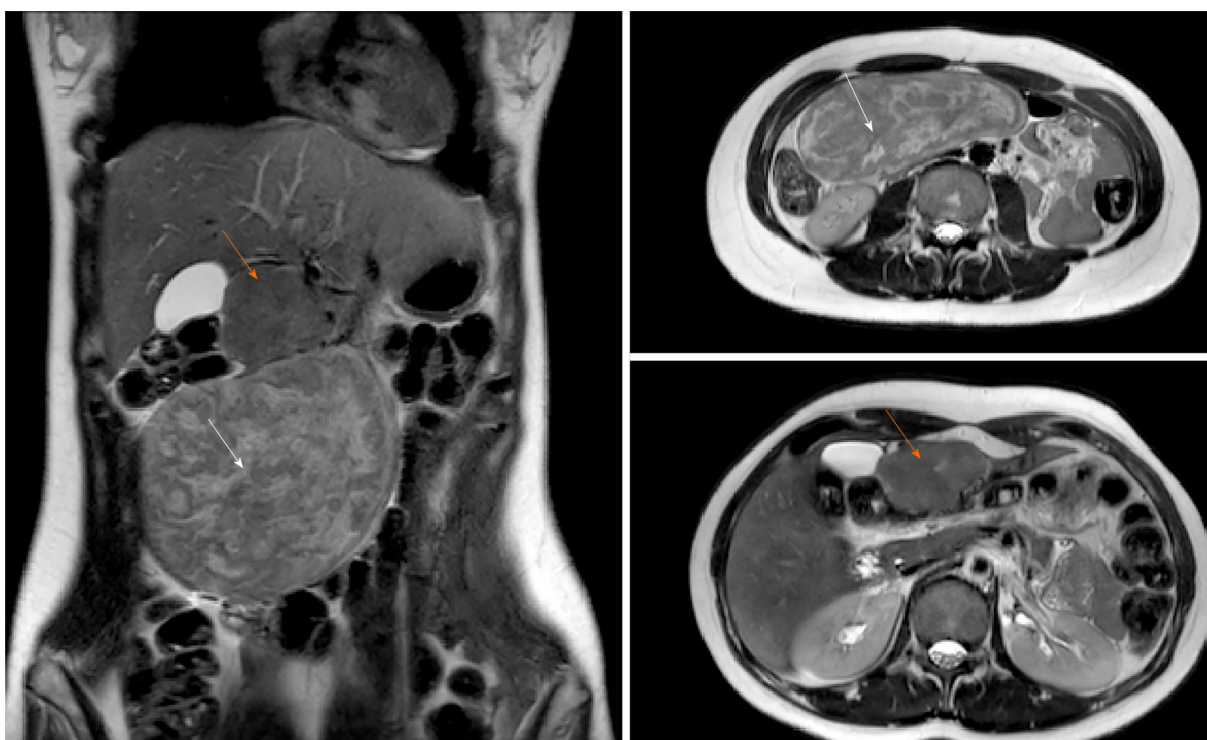
## DISCUSSION

The interest of this case lies in the simultaneous discovery of 2 adjacent but pathologically different benign liver lesions: the first one (FNH) without a strong indication for surgery and the second one requiring surgery because of its uncertain diagnosis.





**Figure 4** Computed tomography late portal phase, with a multiplanar reconstruction of focal nodular hyperplasia and hepatocellular adenoma.

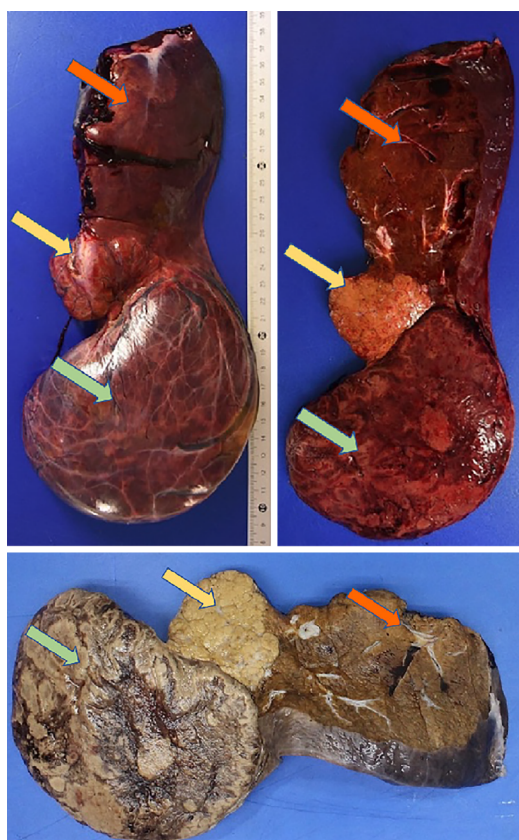


**Figure 5** Magnetic resonance imaging – T2 sequence. Orange arrow: Focal nodular hyperplasia; White arrow: Hepatocellular adenoma.

FNH has no recognized risk of malignant transformation or bleeding and usually has an uneventful course. Therapeutic abstention is usually recommended for asymptomatic patients with a definitive diagnosis[11]. Surgical management is reserved for symptomatic patients or with diagnosis uncertainty despite a complete workup[12,13]. Twelve cases of spontaneous rupture of FNH are described and considering these extremely rare events, conservative treatment is the actual well-established standard of care [English-language literature until 2019; NCBI.gov with terms “spontaneous; rupture; FNH]. Close follow-up is however recommended for FNH more than 5 cm. Some authors advocate for upfront surgery with FNH larger than 5 cm[14-16]. However, we do not recommend a surgical resection in our daily practice but advocate for a close follow-up strategy. In the present case report, the diagnosis of FNH of the segment III lesion was radiologically typical and in the absence of the HCA component, a 1-year MRI follow-up would have been recommended.

On the contrary, the risk of malignant transformation of HCA is 4%-5%. As reported by Sempoux *et al*[7], risk factors for complications of HCA (bleeding or malignant transformation) are the size (> 5 cm), male gender, activating mutation in  $\beta$ -catenin,





**Figure 6** Anatomopathological pictures (top: fresh sample; bottom: formalin-fixed sample), sagittal section plane. Yellow arrow: Focal nodular hyperplasia; Green arrow: Hepatocellular adenoma; Orange arrow: Left liver (segment II).

and specific clinical background (glycogen storage disease, androgens, vascular diseases). The resulting recommendations for surgery are based on initial size (> 5 cm), imaging or histological signs of malignancy, size progression after OC discontinuation, and male patients. Selected patients and those who are not fit for surgery can benefit from embolization[17-19]. When the diagnosis cannot be achieved with imaging, a percutaneous biopsy or resection may be required[20].

Moreover, Bröker *et al*[21] 2012 advocated the surgery for adenoma greater than 5 cm with patients who had planned a pregnancy. Our patient didn't have a pregnancy plan but size and uncertainty of diagnosis were our principal arguments for surgery.

We made a literature review of the simultaneous cases of FNH and HA. Although there is some case reports in the eighties, the article was not available for consulting [22-25]. Table 1 summarizes the other cases with enough data.

Case 1 was operated on because of the lack of obvious radiological evidence[26]. The authors of case 2 don't clearly explain the indication for the operative procedure but they interestingly explain the possible same pathophysiological etiology for 4 different simultaneous hepatic masses[27].

Shih *et al*[28] made a left hepatectomy for a case with common features between FNH and HA and operate for the uncertainty of diagnosis.

The French group of Laurent *et al*[29] found in their records 5 over 30 patients operated for "benign hepatocytic nodules" with simultaneous HNF and adenoma. All of them went under surgery when the radiology reports an HA or unidentified mass. The diagnosis of FNH was already known at the time of the surgical procedure except for one case where the FNH was too small[29].

Concerning the surgical technique, the laparoscopic approach is relatively recent. Unfortunately, Shih *et al*[28] didn't report this in their paper although they did the same procedure for a similar patient. Despite the lack of high-level evidence data (randomized control trials, meta-analysis), current literature about laparoscopic *vs* open liver surgery for benign tumors suggests an advantage for the minimal-invasive technique[30,31]. On the other hand, evidence for laparoscopic malign liver resection is much more consistent. Furthermore, safety, feasibility, and long-term results confirmed the advantages of laparoscopy for malign liver tumors[32-34].

Table 1 Summary of current literature review

No.	Ref.	Sex, age	OC	Pathology - Size (cm)	Location (segment)	Symptoms	Treatment
#1	Dimitroulis <i>et al</i> [26], 2012	F, 18 yr	No	FNH - 2.5 HA - 6	S3 S5-6	RUQ pain	Wedge resection Lt S5-6
#2	Di Carlo <i>et al</i> [27], 2003	F, 25 yr	No	FNH - < 5 HA - NA HH - > 4 HCy - NA	S4 S4 S2 S5	RUQ pain	<i>En bloc</i> (+ gallbladder) Enucleation Enucleation <i>En bloc</i> (+ gallbladder)
#3	Shih <i>et al</i> [28], 2015	F, 40 yr	Yes	FNH - 6 HA - 9.5 & small ones (max 1.5 cm)	III III for the largest, small ones on both lobes	Abdominal pain	LH
#4	Laurent <i>et al</i> [29], 2003	F, 45 yr	Yes	FNH - 1 HA - NA	S3 S7	Fatigue	Lt S3 segmentectomy + wedge Lt RH
		F, 40 yr	Yes	FNH - 5 FNH - 4 NA - 3 HA - 3	S6 S7 Left lobe Left lobe	None	Biopsy Biopsy Lt LH Lt LH
		F, 38 yr	Yes	HA surrounded by FNH - 13	Right lobe	None	Lt RH
		F, 29 yr	Yes	HA - 5 × 1 FNH - 1	S1 (bleeding), S2, 3, 7, 8 S6	Abdominal pain + shock	Lt LH + S1
		F, 41 yr	Yes	HA - 1 FNH - 1	RL RL	Abdominal pain	Lt RH
#5	Our case-report	F, 38 yr	Yes	6 × 614 × 12 × 6	S3	None	Ls LL

FNH: Focal nodular hyperplasia; HA: Hepatic adenoma; HCy: Hepatic hydatid cyst; HH: Hepatic hemangioma; RL: Right lobectomy; LH: Left hepatectomy; LH: Left hepatectomy; LL Left lobectomy; RUQ: Right upper quadrant; Lt: Laparotomy; Ls: Laparoscopic; F: Female; OC: Oral contraception.

## CONCLUSION

We hereby report a laparoscopic resection of a macro-adenoma associated with focal nodular hyperplasia. The review of the literature shows that the simultaneous presence of these two masses is rare and that every case must be discussed in a multidisciplinary board. Factors like age, pregnancy wish, size, and uncertainty of diagnosis must be considered for shared decision in the setting of a multidisciplinary team. The laparoscopic approach should be preferred as much as possible.

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## REFERENCES

- 1 Rebouissou S, Bioulac-Sage P, Zucman-Rossi J. Molecular pathogenesis of focal nodular hyperplasia and hepatocellular adenoma. *J Hepatol* 2008; **48**: 163-170 [PMID: 17997499 DOI: 10.1016/j.jhep.2007.10.003]
- 2 Fukukura Y, Nakashima O, Kusaba A, Kage M, Kojiro M. Angioarchitecture and blood circulation in focal nodular hyperplasia of the liver. *J Hepatol* 1998; **29**: 470-475 [PMID: 9764996 DOI: 10.1016/S0168-8275(98)00100-0]

- 10.1016/s0168-8278(98)80067-6]
- 3 **Gaiani S**, Piscaglia F, Serra C, Bolondi L. Hemodynamics in focal nodular hyperplasia. *J Hepatol* 1999; **31**: 576 [PMID: 10488724 DOI: 10.1016/s0168-8278(99)80057-9]
  - 4 **Giannitrapani L**, Soresi M, La Spada E, Cervello M, D'Alessandro N, Montalto G. Sex hormones and risk of liver tumor. *Ann N Y Acad Sci* 2006; **1089**: 228-236 [PMID: 17261770 DOI: 10.1196/annals.1386.044]
  - 5 **Fukahori S**, Kawano T, Obase Y, Umeyama Y, Sugasaki N, Kinoshita A, Fukushima C, Yamakawa M, Omagari K, Mukae H. Fluctuation of Hepatic Focal Nodular Hyperplasia Size with Oral Contraceptives Use. *Am J Case Rep* 2019; **20**: 1124-1127 [PMID: 31358723 DOI: 10.12659/AJCR.916398]
  - 6 **Farges O**, Ferreira N, Dokmak S, Belghiti J, Bedossa P, Paradis V. Changing trends in malignant transformation of hepatocellular adenoma. *Gut* 2011; **60**: 85-89 [PMID: 21148580 DOI: 10.1136/gut.2010.222109]
  - 7 **Sempoux C**, Balabaud C, Bioulac-Sage P. Pictures of focal nodular hyperplasia and hepatocellular adenomas. *World J Hepatol* 2014; **6**: 580-595 [PMID: 25232451 DOI: 10.4254/wjh.v6.i8.580]
  - 8 **Calderaro J**, Nault JC, Balabaud C, Couchy G, Saint-Paul MC, Azoulay D, Mehdaoui D, Luciani A, Zafrani ES, Bioulac-Sage P, Zucman-Rossi J. Inflammatory hepatocellular adenomas developed in the setting of chronic liver disease and cirrhosis. *Mod Pathol* 2016; **29**: 43-50 [PMID: 26516697 DOI: 10.1038/modpathol.2015.119]
  - 9 **Bioulac-Sage P**, Sempoux C, Balabaud C. Hepatocellular adenoma: Classification, variants and clinical relevance. *Semin Diagn Pathol* 2017; **34**: 112-125 [PMID: 28131467 DOI: 10.1053/j.semdp.2016.12.007]
  - 10 **Blanc JF**, Frulio N, Chiche L, Sempoux C, Annet L, Hubert C, Gouw AS, de Jong KP, Bioulac-Sage P, Balabaud C. Hepatocellular adenoma management: call for shared guidelines and multidisciplinary approach. *Clin Res Hepatol Gastroenterol* 2015; **39**: 180-187 [PMID: 25434466 DOI: 10.1016/j.clinre.2014.10.003]
  - 11 **Ashby PF**, Alfafara C, Amini A, Amini R. Spontaneous Rupture of a Hepatic Adenoma: Diagnostic Nuances and the Necessity of Followup. *Cureus* 2017; **9**: e1975 [PMID: 29492364 DOI: 10.7759/cureus.1975]
  - 12 **Navarro AP**, Gomez D, Lamb CM, Brooks A, Cameron IC. Focal nodular hyperplasia: a review of current indications for and outcomes of hepatic resection. *HPB (Oxford)* 2014; **16**: 503-511 [PMID: 24127684 DOI: 10.1111/hpb.12169]
  - 13 **Jung JM**, Hwang S, Kim KH, Ahn CS, Moon DB, Ha TY, Song GW, Jung DH. Surgical indications for focal nodular hyperplasia of the liver: Single-center experience of 48 adult cases. *Ann Hepatobiliary Pancreat Surg* 2019; **23**: 8-12 [PMID: 30863802 DOI: 10.14701/ahbps.2019.23.1.8]
  - 14 **Kinoshita M**, Takemura S, Tanaka S, Hamano G, Ito T, Aota T, Koda M, Ohsawa M, Kubo S. Ruptured focal nodular hyperplasia observed during follow-up: a case report. *Surg Case Rep* 2017; **3**: 44 [PMID: 28315131 DOI: 10.1186/s40792-017-0320-4]
  - 15 **Velísková M**, Vlk R, Halaška MJ, Minajev G, Krejčí T, Rob L. [Rupture of focal nodular hyperplasia in the 37th week of pregnancy - case report]. *Ceska Gynekol* 2018; **81**: 218-221 [PMID: 27882766]
  - 16 **Li T**, Qin LX, Ji Y, Sun HC, Ye QH, Wang L, Pan Q, Fan J, Tang ZY. Atypical hepatic focal nodular hyperplasia presenting as acute abdomen and misdiagnosed as hepatocellular carcinoma. *Hepatol Res* 2007; **37**: 1100-1105 [PMID: 17608671 DOI: 10.1111/j.1872-034X.2007.00164.x]
  - 17 **Dokmak S**, Paradis V, Vilgrain V, Sauvanet A, Farges O, Valla D, Bedossa P, Belghiti J. A single-center surgical experience of 122 patients with single and multiple hepatocellular adenomas. *Gastroenterology* 2009; **137**: 1698-1705 [PMID: 19664629 DOI: 10.1053/j.gastro.2009.07.061]
  - 18 **van Vledder MG**, van Aalten SM, Terkivatan T, de Man RA, Leertouwer T, Ijzermans JN. Safety and efficacy of radiofrequency ablation for hepatocellular adenoma. *J Vasc Interv Radiol* 2011; **22**: 787-793 [PMID: 21616431 DOI: 10.1016/j.jvir.2011.02.024]
  - 19 **Karkar AM**, Tang LH, Kashikar ND, Gonen M, Solomon SB, Dematteo RP, D'Angelica MI, Correa-Gallego C, Jarnagin WR, Fong Y, Getrajdman GI, Allen P, Kingham TP. Management of hepatocellular adenoma: comparison of resection, embolization and observation. *HPB (Oxford)* 2013; **15**: 235-243 [PMID: 23374365 DOI: 10.1111/j.1477-2574.2012.00584.x]
  - 20 **European Association for the Study of the Liver (EASL)**. EASL Clinical Practice Guidelines on the management of benign liver tumours. *J Hepatol* 2016; **65**: 386-398 [PMID: 27085809 DOI: 10.1016/j.jhep.2016.04.001]
  - 21 **Bröker ME**, Ijzermans JN, van Aalten SM, de Man RA, Terkivatan T. The management of pregnancy in women with hepatocellular adenoma: a plea for an individualized approach. *Int J Hepatol* 2012; **2012**: 725735 [PMID: 23320183 DOI: 10.1155/2012/725735]
  - 22 **Reichlin B**, Stalder GA, Rüedi T, Bianchi L. [Co-occurring liver cell adenoma and focal nodular hyperplasia due to contraceptives. Case report]. *Schweiz Med Wochenschr* 1980; **110**: 873-874 [PMID: 6250211]
  - 23 **Bartók I**, Decastello A, Csikós F, Nagy I. Focal nodular hyperplasia of the liver and hepatic cell adenoma in women on oral contraceptives. *Hepatogastroenterology* 1980; **27**: 435-440 [PMID: 6259038]
  - 24 **Defrance R**, Zafrani ES, Hannoun S, Saada M, Fagniez PL, Métreau JM. [Association of hepatocellular adenoma and focal nodular hyperplasia of the liver in a woman on oral contraceptives]. *Gastroenterol Clin Biol* 1982; **6**: 949-950 [PMID: 6295869]
  - 25 **Tajada M**, Nerín J, Ruiz MM, Sánchez-Dehesa M, Fabre E. Liver adenoma and focal nodular

- hyperplasia associated with oral contraceptives. *Eur J Contracept Reprod Health Care* 2001; **6**: 227-230 [PMID: [11848652](#)]
- 26 **Dimitroulis D**, Charalampoudis P, Lainas P, Papanikolaou IG, Kykalos S, Kouraklis G. Focal nodular hyperplasia and hepatocellular adenoma: current views. *Acta Chir Belg* 2013; **113**: 162-169 [PMID: [24941710](#) DOI: [10.1080/00015458.2013.11680905](#)]
- 27 **Di Carlo I**, Urrico GS, Ursino V, Russello D, Puleo S, Latteri F. Simultaneous occurrence of adenoma, focal nodular hyperplasia, and hemangioma of the liver: are they derived from a common origin? *J Gastroenterol Hepatol* 2003; **18**: 227-230 [PMID: [12542613](#) DOI: [10.1046/j.1440-1746.2003.02840.x](#)]
- 28 **Shih A**, Lauwers GY, Balabaud C, Bioulac-Sage P, Misdraji J. Simultaneous occurrence of focal nodular hyperplasia and HNF1A-inactivated hepatocellular adenoma: a collision tumor simulating a composite FNH-HCA. *Am J Surg Pathol* 2015; **39**: 1296-1300 [PMID: [26274031](#) DOI: [10.1097/PAS.0000000000000477](#)]
- 29 **Laurent C**, Trillaud H, Lepreux S, Balabaud C, Bioulac-Sage P. Association of adenoma and focal nodular hyperplasia: experience of a single French academic center. *Comp Hepatol* 2003; **2**: 6 [PMID: [12812524](#) DOI: [10.1186/1476-5926-2-6](#)]
- 30 **Wabitsch S**, Kästner A, Haber PK, Benzing C, Krenzien F, Andreou A, Kamali C, Lenz K, Pratschke J, Schmelzle M. Laparoscopic Versus Open Liver Resection for Benign Tumors and Lesions: A Case Matched Study with Propensity Score Matching. *J Laparoendosc Adv Surg Tech A* 2019; **29**: 1518-1525 [PMID: [31670608](#) DOI: [10.1089/lap.2019.0427](#)]
- 31 **Schmelzle M**, Krenzien F, Schöning W, Pratschke J. Laparoscopic liver resection: indications, limitations, and economic aspects. *Langenbecks Arch Surg* 2020; **405**: 725-735 [PMID: [32607841](#) DOI: [10.1007/s00423-020-01918-8](#)]
- 32 **Jiang S**, Wang Z, Ou M, Pang Q, Fan D, Cui P. Laparoscopic Versus Open Hepatectomy in Short- and Long-Term Outcomes of the Hepatocellular Carcinoma Patients with Cirrhosis: A Systematic Review and Meta-Analysis. *J Laparoendosc Adv Surg Tech A* 2019; **29**: 643-654 [PMID: [30702362](#) DOI: [10.1089/lap.2018.0588](#)]
- 33 **Yin Z**, Fan X, Ye H, Yin D, Wang J. Short- and long-term outcomes after laparoscopic and open hepatectomy for hepatocellular carcinoma: a global systematic review and meta-analysis. *Ann Surg Oncol* 2013; **20**: 1203-1215 [PMID: [23099728](#) DOI: [10.1245/s10434-012-2705-8](#)]
- 34 **Cipriani F**, Rawashdeh M, Stanton L, Armstrong T, Takhar A, Pearce NW, Primrose J, Abu Hilal M. Propensity score-based analysis of outcomes of laparoscopic versus open liver resection for colorectal metastases. *Br J Surg* 2016; **103**: 1504-1512 [PMID: [27484847](#) DOI: [10.1002/bjs.10211](#)]



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