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American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

Residual burden of liver disease after HCV clearance in hemophilia: a word of caution in the era of gene therapy.

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Vincenzo La Mura (Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy., Italy) Niccolò Bitto (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Cecilia Capelli (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Camilla Caputo (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Simona Siboni (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Sara Arcudi (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Università degli Studi di Milano, Department of Pathophysiology and Transpl, Italy) Alessandro Ciavarella (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Roberta Gualtierotti (Foundation I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi and Thrombosis center, Milan, Italy, Italy) Anna Ludovica Fracanzani (Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy., Italy) Angelo Sangiovanni (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy) Flora Peyvandi (Foundation I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi and Thrombosis center, Milan, Italy, Italy)

Abstract:
Ruling out advanced fibrosis/cirrhosis is mandatory for persons with hemophilia (PWH) who are candidate to gene therapy. However, clinical evaluation and non-invasive tests may be inaccurate after HCV clearance. We conducted a prospective hepatological screening to detect advanced fibrosis/cirrhosis in PWH after HCV clearance. Any risk factor of chronic liver damage was registered by using biochemical data, liver stiffness measurement (LSM) and ultrasound (US). A pre/post HCV clearance analysis was prospectively conducted in a subgroup of patients with the measurement of LSM, US and non-invasive tests of fibrosis (NITs). We evaluated 119 patients (median age: 53 years; range: 36-87) with a previous HCV infection (108/11 hemophilia A/B). Ninety-six (81%) presented at least one potential risk factor of chronic liver damage. Metabolic risk factors were the most prevalent, 51 cases (44%) having US steatosis. In 21 cases (18%) clinical, biochemical, liver morphology and/or LSM were suggestive of advanced fibrosis/cirrhosis. Furthermore, 10 cases (8%) had esophageal varices, 3(3%) hepatocellular carcinoma. In 57 cases included in the prospective analysis, LSM and NITs were reduced after HCV-clearance ($p<0.05$), but US signs specific of cirrhosis remained unchanged. Overall, 23/80(29%) cases with LSM<10KPa had at least one US sign suggestive of advanced fibrosis/cirrhosis. A similar proportion (18%) was observed for LSM<8KPa. Overall, risk factors of chronic liver damage are frequent after HCV clearance, but LSM and NITs changes after clearance may be inaccurate to rule out advanced fibrosis/cirrhosis. A specific diagnostic work-up is warranted to evaluate liver health in PWH in the era of gene therapy.

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COI notes: VLM: member of advisory board of CSL Behring, Biomarin, speaker of GORE, ALFASIGMA, received grant for research from Gilead and travel grant from Sobi, Sanofi, Takeda. NB: received travel grant from Biomarin. RG: advisory board member/consultant for Pfizer, Roche, Bayer, Novo Nordisk, Takeda, GSK, Biomarin, SOBI. AS: lecturer for Abbvie, MSD. FP: Consultant/Advisory Boards of CSL Behring, Biomarin, Roche, Sanofi, Sobi; Speaker's Bureau or educational programmes/symposia: Takeda/Spark.

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Residual burden of liver disease after HCV clearance in hemophilia: a word of caution in the era of gene therapy

Running title: Burden of liver disease after HCV clearance

Authors: Vincenzo La Mura[1,2], Niccolò Bitto[1], Cecilia Capelli[1], Camilla Caputo[1], Simona Siboni[1], Sara Arcudi[1], Alessandro Ciavarella[1], Roberta Gualtierotti[1,2], Anna Ludovica Fracanzani[2,3], Angelo Sangiovanni[4], Flora Peyvandi[1,2].

Affiliations:
[1]Foundation I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi and Thrombosis Center, Milan, Italy
[2]Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy.
[3]Foundation I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Unit of Internal Medicine and Metabolic Diseases, Milan, Italy
[4]Foundation I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Gastroenterology and Hepatology, Milan, Italy.
Correspondence: Flora Peyvandi, Foundation I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi and Thrombosis Center, flora.peyvandi@unimi.it,
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ABSTRACT

Ruling out advanced fibrosis/cirrhosis is mandatory for persons with hemophilia (PWH) who are candidate to gene therapy. However, clinical evaluation and non-invasive tests may be inaccurate after HCV clearance. We conducted a prospective hepatological screening to detect advanced fibrosis/cirrhosis in PWH after HCV clearance. Any risk factor of chronic liver damage was registered by using biochemical data, liver stiffness measurement (LSM) and ultrasound (US). A pre/post HCV clearance analysis was prospectively conducted in a subgroup of patients with the measurement of LSM, US and non-invasive tests of fibrosis (NITs). We evaluated 119 patients (median age: 53 years; range: 36-87) with a previous HCV infection (108/11 hemophilia A/B). Ninety-six (81%) presented at least one potential risk factor of chronic liver damage. Metabolic risk factors were the most prevalent, 51 cases (44%) having US steatosis. In 21 cases (18%) clinical, biochemical, liver morphology and/or LSM were suggestive of advanced fibrosis/cirrhosis. Furthermore, 10 cases (8%) had esophageal varices, 3(3%) hepatocellular carcinoma. In 57 cases included in the prospective analysis, LSM and NITs were reduced after HCV-clearance ($p<0.05$), but US signs specific of cirrhosis remained unchanged. Overall, 23/80(29%) cases with LSM<10KPa had at least one US sign suggestive of advanced fibrosis/cirrhosis. A similar proportion (18%) was observed for LSM<8KPa. Overall, risk factors of chronic liver damage are frequent after HCV clearance, but LSM and NITs changes after clearance may be inaccurate to rule out advanced fibrosis/cirrhosis. A specific diagnostic work-up is warranted to evaluate liver health in PWH in the era of gene therapy.

KEY POINTS

- Residual risk factors of liver damage after HCV clearance are frequent.
- A specific diagnostic work-up is mandatory for hemophilia gene therapy.

INTRODUCTION

HCV infection is highly prevalent among persons with hemophilia (PWH) treated in the past with plasma-derived products.[1] As a consequence, the early detection and prompt management of HCV infection have been the pillar of liver health.[1,2] The introduction of direct antiviral agents (DAA) has turned the success rate of HCV clearance to 80-100% and PWH are no exception.[3] Unfortunately, although a sustained virological response (SVR) abolishes the risk of liver complications in the early disease stages, advanced fibrosis/cirrhosis at the time of SVR may curb the reduction of such complications as portal hypertension, hepatocellular carcinoma (HCC) and need of transplantation.[4–10] Furthermore, highly prevalent comorbidities and life styles, such as obesity, diabetes and alcohol intake, are per se crucial risk factors for the progression of liver damage in PWH.[11–13]

With this background, although the achievement of SVR is the primary intervention to achieve liver health in HCV-positive patients, selected cases should maintain a close hepatological surveillance.[14] Firstly, patients who at the time of SVR have compensated liver disease in the form of advanced fibrosis/cirrhosis (the so called compensated advanced chronic liver disease, cACLD) must continue the 6-monthly screening for HCC, which remains the most frequent complication despite HCV clearance.[10,15,16] Secondly, patients who had already experienced complications due to portal hypertension (e.g. varices, ascites, variceal hemorrhage, hepatic encephalopathy) have only a partial reduction of portal pressure which, despite HCV clearance, exposes them to a risk of decompensation/further decompensation.[4,6] Lastly, a first event of decompensation should be monitored and prevented in patients with cACLD achieving SVR when another risk factor of liver damage is present (alcohol intake, metabolic comorbidities).[14]

All the forementioned observations are important to implement programs of liver health in the era of gene therapy in hemophilia.[17–19] Indeed, data from clinical trials of this innovative therapy have renewed the traditional alliance between hematologists and hepatologists for the management of PWH in order to better identify the target population and avoid potential liver-related adverse effects. Accordingly, patients with advanced fibrosis/cirrhosis must be excluded from gene therapy but the diagnostic work-up to rule out this condition cannot be limited to the most common non-invasive tests which do not always correspond to a histologically proven downstaging of liver damage after HCV clearance.[20]

We herein report data of a hepatological screening program in a series of HCV-positive persons with hemophilia who had already obtained virus clearance in order to evaluate liver health. The hepatological evaluation was part of the multidisciplinary program of the Joint Ultrasound Evaluation in Hemophilia (the JOINEM study approved by our institution) and was aimed at: 1-detecting the presence of any persistent and/or incidental risk factor of chronic liver damage after HCV clearance, 2-describing the morphological changes of the liver on ultrasound (US) imaging and the trend of liver stiffness measurement (LSM) and of other non-invasive tests (NITs) of fibrosis before and after anti-HCV therapy, 3-optimizing the risk and management of liver-related complications detected at the time of screening by a means of multidisciplinary approach. These objectives are crucial to optimize patient selection in the era of gene therapy with the liver as the target organ of coagulant factor expression.

Patients and Methods

Study cohort and data collection

This study reports data of the first 119 HCV-antibody positive patients addressed to an active hepatological screening program by means of clinical, instrumental and laboratory variables at the Angelo Bianchi Bonomi center for Hemophilia in Milan, Italy, from November 2020 to July 2022. The study was approved by the Milan Area 2 Ethics Committee (199_2021bis). The study was conducted in accordance with the Declaration of Helsinki. At inclusion, all patients were HCV-RNA negative due to eradication by antiviral therapy or spontaneous virus clearance. Comorbidities and risk factors of chronic liver damage (e.g., metabolic, alcohol, other viral etiologies) were systematically recorded. In details, the threshold of risk for alcohol exposure was defined by an alcohol intake above 14 alcoholic units (AU)/week, in agreement with the Italian Institute of Health guidelines on alcohol consumption.[21] A diagnosis of concomitant non-alcoholic fatty liver disease (NAFLD) was made following detection of liver steatosis at US exploration and exclusion of alcoholic liver disease.[22] Autoimmunity and/or cholestatic liver disease were evaluated if suspected after the first assessment. US exploration, LSM by Fibroscan©, FIB-4, APRI, as NITs,[23] were carried

out at the time of screening. The diagnosis of advanced fibrosis/cirrhosis was made according to clinical and radiological criteria (US and LSM) and histological data when necessary. The detection of liver-related complications and clinical complications or decompensation (e.g. endoscopic/radiological signs of portal hypertension, HCC, ascites, bleeding due to portal hypertension, hepatic encephalopathy) were considered suggestive of cirrhosis. Two separate hepatologists (VLM, NB) concurred for a diagnosis of advanced fibrosis/cirrhosis, lack of agreement being solved by a third hepatologist (ALF). The surveillance of esophageal varices, the management of the risk of portal hypertension and related complications was based on the last Baveno VII consensus.[14]

In patients who had obtained HCV clearance after antiviral therapy, US, LSM and NITs were also recorded as the last result available before virus eradication and compared with those obtained at the time of screening for a pre/post-SVR sub-analysis.

Patients with *de novo* HCC were addressed to a tailored approach after a multidisciplinary evaluation by radiologists, oncologists and surgeons.[24] All HCC were classified according to Milan in/out criteria for transplantation based upon the presentation as a single liver nodule less than 5 cm or three nodules less than 3 cm.[25] The control of the bleeding risk associated with hemophilia for any invasive procedure was planned with the hematologists and hepatologists.[26,27] All patients were evaluated for their joint status by means of the Hemophilia Joint Health Score (HJHS) and Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) scoring systems (Supplementary materials).

Statistical analysis

SPSS 28.0 statistical package (IBM) was used for data analysis. All results were presented as medians and minimum-maximum ranges for continuous variables, and as numbers and proportions for categorical variables. Comparisons among groups were made by non-parametric tests. Changes in morphological aspects of the liver, LSM and NITs at two-time points were evaluated before HCV clearance and at the time of hepatological screening by pair-data tests such as Wilcoxon and McNemar tests when appropriate. The statistical significance threshold was $p<0.05$ for all tests used in this analysis.

Data sharing statement

For original data, please contact flora.peyvandi@unimi.it.

RESULTS

Clinical characteristics at inclusion

One hundred and nineteen male subjects (median age: 53, range: 36-87), 108 (91%) with hemophilia A (mild/moderate/severe: 19/13/76), 11 (9%) with hemophilia B (mild/moderate/severe: 1/2/8) underwent hepatological evaluation. Their median HJHS was 15 (range: 0-57), median HEAD-US was 12 (range: 0-59). All patients were screened for the most critical risk factors of chronic liver damage and of biochemical tests needed for the calculation of APRI and FIB-4 to evaluate liver health. One hundred and seventeen patients also underwent abdomen US evaluation, 90 LSM by transient elastography.

The main clinical and biochemical data at baseline presentation are detailed in Table 1. On the whole, 17(14%)/26(22%) patients had transaminases and/or cholestatic enzymes (e.g., gamma-glutamiltransferase, alcaline phospatase) above the physiological range of normality notwithstanding HCV clearance.

At the time of screening, 12 cases (10%) had obtained spontaneous HCV clearance, while the remaining 107 (90%) had obtained clearance after at least one attempt with antivirals. In detail, 40 cases (34%) had experienced treatment failure with interferon-based therapy regimens with/without ribavirin, 64 cases (54%) obtained SVR after DAA. The median age at the time of HCV eradication was 46 years (range: 13-81) and the hepatological screening was conducted 5 years (range: 1-33) after this achievement. HCV genotypes 1a/1b were the most prevalent at the time of successful antiviral therapy (Supplementary Table 1). Thirty-three patients (28%) had a history of HCV as a single viral infection, whereas 53(45%) were HBV/HCV positive, 10(8%) HCV/HIV, 23(19%) HCV/HBV/HIV. All viral infections other than HCV were controlled by antiviral therapy in agreement with the protocols of thearapy.[28,29]

At screening, US analysis revealed steatosis in 51 cases (44%), irregular/nodular surface in 23(20%), caudate lobe hypertrophy in 8(7%), portal vein enlargement in 9(8%) and splenomegaly in 32(27%). The median LSM value was 5.5Kpa (range: 2.3-45), median values of APRI and FIB-4 were 0.35(range: 0.16-1.57) and 1.39 (range: 0.43-7.02).

Risk factors of disease progression after HCV clearance

Table 2 reports data on metabolic comorbidities and alcohol habits at the time of screening. Ninety-two cases (77%) had at least one metabolic condition, arterial hypertension being the most prevalent (46 cases, 39%). The burden of metabolic co-morbidities was proportionally higher with aging (Supplementary Table 2). Eighty-five patients (71%) were maintaining alcohol abstinence at the time of screening. However, the intake, expressed in alcoholic units/week, was 0-6, 7-14 and ≥14 alcoholic units/week in 89 (75%), 16(13%) and 14(12%) patients. On the whole, up to 96 patients (81%) in the present cohort had at least one potential risk factor of chronic liver damage on top of their previous history of HCV infection. Non-alcoholic (NAFLD) or alcoholic liver disease were suspected in 46(39%) and 14(12%) patients.

Liver morphology at US exploration, LSM and NITs before and after SVR

Fifty-seven of 119 cases (48%) had a record of liver US exploration, LSM, NITs (e.g., APRI, FIB-4) both before SVR and at the time of screening (median time difference: 5 (range: 1-16) years) and were thus included in the pre/post-SVR analysis (Table 3) (Figure 1).

The proportion of cases with morphological signs suggestive of cirrhosis did not change before and after SVR for all the most relevant data, with the single exception of portal vein trunk dilation, detectable in 15 cases (26%) before and in 5(9%) after SVR (p=0.006).

Pre-SVR LSM was 8.3(range: 3.6-45.7) KPa, thus significantly higher than post-SVR (5.6 kPa, range: 2.3-45.0)(p<0.001). Accordingly, the number of cases with LSM <8KPa was 28(49%) pre-SVR vs 39(68%) post-SVR (p=0.003). Similarly, the number of patients with LSM <10 KPa was 34(60%) pre-SVR vs 49(86%) post-SVR (p<0.001), confirming that HCV clearance

reduced the LSM independently of the cut-off used as the basis of the most validated threshold of LSM employed to rule out advanced fibrosis/cirrhosis.[23] Similar changes were observed for APRI and FIB-4.

Liver-related complications, interventions and decisions on the hepatological follow-up schedule

In the whole cohort, the number of cases for each liver-related complication detected after the screening were: 10(4%) history of esophageal varices, 4(3%) history of previous decompensation (2 ascites, 3 variceal bleeding), 9(8%) had undefined/non-malignant focal liver lesions, 3(3%) HCCs (2 of them were outside Milan criteria for the transplantation).[16] One of the HCCs occurred in a non-cirrhotic liver and histology revealed parenchymal steatohepatitis around the tumor, likely due to metabolic factors (e.g. diabetes and arterial hypertension). Clinical details for each patient with HCC are provided in supplementary Table 3.

Consistently with the forementioned complications, 5 patients with varices started therapy with carvedilol to prevent decompensation or further decompensation14,30, and 5 patients were addressed to a new endoscopic control before deciding to start bleeding prophylaxis due to portal hypertension. All 9 undefined/non-malignant focal liver lesions were addressed to a 3-monthly imaging follow-up with contrast-enhanced computer tomography or magnetic resonance. All the three patients with HCC were transplanted. Radiofrequency, resection, chemoembolization and/or systemic chemotherapy (e.g.: atezolizumab/bevacizumab) were chosen and/or combined on the basis of a case-by-case decision (Supplementary Table 3). Specifically, downstaging was achieved before transplantation for the 2 Milan-out HCC patients. After transplantation, a patient had extrahepatic HCC recurrence and is now on an oral tyrosine-kinase inhibitor and best-supportive therapy. The others are on close hepatological follow-up without significant post-transplantation complications.

Finally, by considering the combined screening on potential residual risk factors of chronic liver damage, liver morphology, LSM and NITs, 3 patients (2%) could be discharged.

Furthermore, 95(80%), despite not showing advanced fibrosis/cirrhosis, were addressed to annual follow-up due to the persistence of risk factors of chronic hepatitis. The remaining 21 cases (18%) have been considered to have advanced fibrosis/cirrhosis and were thus addressed to a 6-monthly or shorter hepatological follow-up.

Table 4 combines several levels of LSM at the time of screening, and shows the most important variables conditioning the final decision on the presence or not of advanced fibrosis/cirrhosis. Even for the categories at low risk, as defined by LSM<10 KPa or below the more restricted threshold of 8 KPa (Supplementary Table 4), there were US morphological data of the liver and/or complications that suggested the presence of advanced chronic liver disease despite HCV clearance.

DISCUSSION

This study reports data from a hepatological screening program of HCV-infected patients with hemophilia who had obtained eradication of the virus after antiviral therapy or spontaneous clearance. We found that up to 81% of the cases had at least one risk factor of chronic liver damage on top of a previous history of HCV infection and NAFLD or alcoholic liver disease in 39% and 12% of the cohort. In a pre/post-SVR prospective subgroup analysis, despite the consistent reduction of LSM and NITs as marker of fibrosis, up to 39% of cases had at least one morphological sign suggestive of advanced fibrosis/cirrhosis (e.g., irregular/nodular surface of the liver, liver caudate lobe hypertrophy, dilation of the portal trunk, splenomegaly) that, together with residual risk factors of liver damage, demands specialized follow-up despite HCV clearance. Furthermore, because of the hepatological evaluation, 18% of cases were addressed to 6-monthly HCC screening and 14% needed a specialized intervention due to the detection of HCC, undefined/non-malignant focal liver lesions or need to prevent the complications associated with portal hypertension. These data clearly demonstrate that even after SVR a large proportion of PWH should attend a regular hepatological follow-up. The persistence of risk factors of liver damage or the suspicion of a residual advanced fibrosis/cirrhosis at the time of the hepatological screening were the main reasons to address patients to this follow-up.

We found a high prevalence of metabolic comorbidities, which are known risk factors for steatosis/steatohepatitis. Indeed, 77% of cases had at least one metabolic disease, and 39% a diagnosis of NAFLD.[22] Notably, the latter condition was also detectable in cases with no increase of liver enzymes (34% in this series), confirming that NAFLD can be suspected by means of an accurate anamnesis and diagnosed by US even in cases with normal liver enzymes. The high prevalence of a metabolic disease of the liver detected in our cohort is in line with the epidemiological data from the EASL HEPAHEALTH Steering Committee (report 2018), which accounted NAFLD as the leading cause of liver transplantation in western countries.[31,32] In PWH, the prevalence of overweight/obesity is higher than in the past .19,33 because the arthropathy typically associated with the inherited bleeding disorder exposes them to a higher risk of sedentary life and thus of overweight.11,34 Accordingly, 38%/8% of patients in our series were overweight/obese, which increases the risk of diabetes, dyslipidemia, arterial hypertension and, ultimately, of NAFLD, that is the liver expression of the metabolic syndrome.[34,35] We also found that, while the vast majority had a minimal to moderate alcohol intake, 12% of the whole cohort were heavy drinkers (≥14U/week), which is a risk factor for such systemic complications as chronic hepatitis/cirrhosis.[32,36]

We also carried out a prospective pre/post analysis of liver changes as explored by US, LSM and NITs by evaluating two time points at screening and at virus eradication. In this sub-analysis, we found that SVR reduced over time all the non-invasive markers of fibrosis. In particular, the proportion of patients with a LSM below 10 KPa, the threshold commonly used to rule out cACLD/compensated cirrhosis,[14,37] increased from 60% to 86%, with a similar trend for all NITs. However, the proportion of patients with morphological signs of advanced fibrosis/cirrhosis at US exploration did not change between the two time points for all the features, except for portal vein dilatation. Lack of a liver biopsy did not allow us to evaluate which is the most accurate non-invasive strategy in order to rule out an advanced stage of fibrosis/cirrhosis.

In the present cohort 3 HCCs (3%) were found. This is not unexpected, as the risk of HCC persists despite HCV-clearance and international guidelines recommend continuing 6-monthly screening in patients with SVR and advanced fibrosis/cirrhosis.[16] One of the 3 HCCs

diagnosed after the screening was in a non-cirrhotic liver, as demonstrated by the histology around the tumor after liver resection. This patient had diabetes and arterial hypertension, confirming that cases exposed to metabolic risk factors of chronic liver damage should be periodically evaluated for the risk of HCC. Although the therapeutic strategies of HCC have made enormous progress, early detection of this cancer still remains the most efficacious tool to ameliorate survival.[16] We also found that 9 patients (8%) had undefined/non-malignant hepatic liver lesions needing strict imaging follow-up. Real life data after successful therapy with DAA demonstrated that, in line with patients with a previous history of HCC, those with undefined/non-malignant hepatic liver lesions may be at high risk of HCC development.[38] Thus, we recommend a periodical hepatological evaluation in PWH, as also suggested by Isfordink et al. who found that in a cohort of 199 patients with SVR following interferon-based regimens or DAA there was a 21% prevalence of advanced fibrosis and 42% of cirrhosis, plus 4 patients with HCC.[39]

The diagnosis of advanced chronic liver disease is essential to allocate patients to the most appropriate schedule of visits for liver health. Firstly, these patients would benefit from 6-monthly screening for HCC, because early detection warrants curative treatments. Secondly, an adequate stratification of the risk of complications related to portal hypertension is mandatory, because chronic therapy with traditional non-selective beta-blockers/carvedilol and/or repeated sessions of endoscopic band ligation may be needed.14 Liver biopsy is still considered the reference standard to unmask the presence of advanced fibrosis/cirrhosis when a patient falls in the diagnostic grey zone. A few small-sized studies compared LSM and NITs with liver biopsies post HCV eradication,20,40 but the rate of misclassification of advanced fibrosis/cirrhosis was around 60%. Accordingly, guidelines from the European Association for the Study of the Liver (EASL) discourage the routine use of LSM or NITs to detect fibrosis regression after SVR, because these tests are not accurate enough.23 In the present cohort, morphological changes of the liver at US exploration, such as irregular/nodular liver surface and the caudate liver lobe hypertrophy, did not significantly change before and after SVR, Notably, these morphological aspects have 80-100% specificity for cirrhosis.41 However, this high degree of accuracy to rule-in advanced fibrosis/cirrhosis has never been tested after HCV eradication. Therefore, further investigation is needed to

demonstrate whether or not these morphological aspects capture the persistence of advanced fibrosis/cirrhosis better than LSM and/or NITs after successful antiviral therapy. On our opinion, patients classified as having advanced fibrosis/cirrhosis by a comprehensive evaluation of a pre-SVR history of non-invasive assessments (LSM, NITs) together with liver complications and liver morphology should carry on a 6-monthly hepatological evaluation in order to control the risk of HCC and portal hypertension. We also believe that this cautious approach is utmost indicated if a risk factor of chronic liver damage is present. In agreement with international clinical recommendations, liver biopsy should be considered only if histology is needed for the clinical decision process.23 This could be the case for PWH who are candidates to gene therapy, because the detection of advanced fibrosis/cirrhosis is crucial to prevent potential liver-related risks of this approach. However the decision for this invasive test should be taken in highly motivated patients and case by case.17,18,42

Our study has limitations. Although the screening was conducted prospectively, the collection of clinical and instrumental data before SVR was retrospective. This reduced the possibility of extending the pre/post-SVR analysis to the whole cohort. Furthermore, the diagnosis of advanced fibrosis stage/cirrhosis was based on clinical history, US, LSM and NITs, with a significant risk of overdiagnosis. Nevertheless, this kind of misclassification was acceptable in order to reduce the risk of severe liver complications, particularly in cases presenting with clinical and instrumental features suggesting advanced fibrosis/cirrhosis before SVR or in those still exposed to such potential risk factors of chronic liver damage as alcohol and/or metabolic factors. At the same time, the high prevalence of advanced stages of HCC may be, at least in part, influenced by the two years of pandemic, which discouraged patients to attend a regular schedule of visits. Finally, the hepatological screening program is still ongoing, so that we cannot exclude a selection bias justifying the prevalence of advanced chronic liver disease in the cohort herein reported. However, real life data on the incidence of liver-related complications after SVR in HCV patients without hemophilia are in line with the risk observed in the present study.7

In conclusion, liver health is integral to the multidisciplinary care of persons with hemophilia, particularly in the era of gene therapy with two novel adeno-associated viral vector therapies approved for hemophilia A and B which are targeting the liver. This study

demonstrates that even after successful antiviral therapy, PWH still need hepatological evaluation due to the high proportion of cases with residual risk factors of chronic liver damage and the complications associated with advanced stages of the disease. LSM and NITs indeed improve after SVR but may be inaccurate to rule-out advanced fibrosis/cirrhosis. A specific diagnostic work-up led by hepatologists together with hematologists is thus warranted to maintain liver health in PWH. This will also be useful to make the best stratification for cases who might benefit from gene therapy without significant risks for liver health.

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AUTHORSHIP

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Consultant/Advisory Boards of CSL Behring, Biomarin, Roche, Sanofi, Sobi; Speaker's Bureau or educational programmes/symposia: Takeda/Spark.

The remaining authors declare no competing financial interests.

Corresponding author: flora.peyvandi@unimi.it, Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Via Pace 9, 20122, Milan, Italy. Email: flora.peyvandi@unimi.it

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TABLES

Table 1: Main clinical and biochemical data at the time of screening. Continuous variables are presented as medians and minimum-maximum ranges, categorical variables are presented as numbers and proportions.

Age at screening (years)	53 (36-87)
BMI (Kg/m^2)	24.6 (17.3-40.6)
Hemophilia	
A	108 (91%)
mild/moderate/severe	19/13/76 (16%/11%/64%)
B	11 (9%)
mild/moderate/severe	1/2/8 (1%/2%/7%)
Years from eradication	5 (1-33)
Age at eradication or datable clearance	46 (13-81)
Alcohol (≥14 units/week)	14 (12%)
NAFLD	46 (39%)
Patients with alteration of transaminases/cholestasis	17(14%)/26(22%)
Elevated AST (>33 U/L)/ ALT (>41 U/L)	13 (11%)/8 (7%)
Elevated GGT (>36 U/L)/ Elevated ALP (>104 U/L)*	22 (19%)/9 (8%)
AST (U/L)/ ALT (U/L)	25 (15-93)/ 24 (9-80)
GGT (U/L) / ALP (U/L)	20 (6-122)
Cholesterol (mg/dl)	178 (89-253)
HDL (mg/dL) / LDL (mg/dL)	47 (24-85) / 101 (47-180)
Total Bilirubin (mg/dL)	0.6 (0.2-3.6)
Cholinesterase (U/L)	7880 (3563-12232)
Total Proteins (g/dL)/ Albumin (g/dL)	7.3 (6.3-8.10)/ 4.6 (3.5-5.5)
Alphafetoprotein (ng/mL)	2.3 (0.1-61.9)

Hemoglobin (g/dL)	14.1 (10-19.6)
White blood cells (unit/mm^3)	5790 (2330-11650)
Neutrophils(%)/Lymphocytes(%)	57 (22-83)
Platelets (unit/mm^3)	215 (67-442)
Patients with platelets 150.000-100.000/mm^3	7 (6%)
Patients with platelets <100.000/mm^3	5 (4%)
Triglycerides (mg/dL)	96 (40-513)
Glycemia (mg/dL)	89 (67-213)
Creatinine (mg/dL)	0.96 (0.59-1.71)
Na (mmol/L)	141 (123-146)
Liver stiffness (KPa)[§]	5.5 (2.3-45)
APRI score/ FIB-4 score	0.35 (0.16-1.57) / 1.39 (0.43-7.02)
Ultrasound data[#]	
• Liver Steatosis	51 (44%)
• Irregular or nodular liver surface	23 (20%)
• Liver caudate lobe hypertrophy	8 (7%)
• Splenomegaly	32 (27%)
• Portal trunk dilated	9 (8%)
• Focal liver lesions	12 (10%)

BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamil transferase; ALP: alkaline phosphatase; HDL: high density lipoprotein; LDL: low density lipoporotein;

*Data available on 97 patients; [§]Data available on 90 patients; [#]Data available on 117 patients

Table 2: Risk factors of disease progression detected at the time of screening

Alcohol consumption	
Alcoholic unit/week intake	2 (0-35)
Patient distribution per alcohol unit/week intake	
• 0-6	85 (71%)
• 7-14	16 (13%)
• ≥14	14 (12%)
Metabolic	
Type 2 diabetes	8 (6%)
Arterial Hypertension	46 (39%)
Dyslipidemia (triglycerides >150 mg/dl, HDL<40 mg/dl or need of lipid lowering drugs)	51 (43%)

Overweight (BMI 25-30 Kg/m^2)	45 (38%)
Obesity (BMI≥30 Kg/m^2)	10 (8%)
Combined risk factors	
At least 1 potential metabolic risk factor for liver disease	92 (77%)
Three or more metabolic risk factors with alcohol intake<14U/week	25 (21%)
At least 1 potential risk factor for liver disease (metabolic or alcohol)	96 (81%)

BMI: body mass index

Table 3: Liver morphology (US exploration) and liver stiffness measurement (LSM) before and after sustained virological response (SVR)

Parameter	*Pre-SVR*	*Post-SVR*	p
Irregular/nodular surface	13 (23%)	12 (21%)	1.000
Liver caudate lobe hypertrophy	5 (9%)	2 (4%)	0.375
Splenomegaly	20 (35%)	18 (32%)	0.687
Portal vein trunk dilated	15 (26%)	5 (9%)	0.006
At least 1 US sign of cirrhosis	27 (47%)	22 (39%)	0.180
LSM (kPa)	8.3 (3.6-45.7)	5.6 (2.3-45)	<0.001
Patients with LSM<8 kPa (%)	28 (49%)	39 (68%)	0.003
Patients with LSM<10 kPa (%)	34 (60%)	49 (86%)	<0.001

Table 4: Data on post-SVR liver stiffness measurement (LSM) matched with the most important clinical and US features suggestive of advanced fibrosis/cirrhosis

	Post-SVR LSM categories (kPa)			
	<10 *(n=80)*	**10-15** *(n=4)*	**≥15** *(n=6)*	**p** *(linear trend)*
At least one US sign suggestive of cirrhosis	23 (29%)	4 (100%)	5 (83%)	<0.001
Irregular or nodular liver surface	9 (11%)	3 (75%)	5 (83%)	<0.001
Liver Caudate lobe hypertrophy	4 (5%)	1 (25%)	1 (17%)	0.124

Splenomegaly	17 (22%)	3 (75%)	5 (83%)	<0.001
Portal vein dilatation	4 (5%)	1 (25%)	1 (17%)	0.124
History of previous decompensation	1 (1%)	1 (25%)	2 (33%)	<0.001
History of esophageal varices	2 (3%)	3 (75%)	3 (50%)	0.045
NAFLD	29 (36%)	1 (25%)	0	0.068
Alcohol consumption (7-14 U/week)	9 (11%)	0	1 (17%)	0.889
Alcohol consumption (>14U/week)	10 (13%)	1 (25%)	1 (17%)	0.613
At least one metabolic risk factor	58 (73%)	3 (75%)	6 (100%)	<0.001
Platelet count < 150.000/mcL	6 (8%)	1 (25%)	3 (50%)	0.223
Platelet count < 110.000/mcL	1 (1%)	1 (25%)	2 (33%)	<0.001

FIGURE LEGENDS

Figure 1: APRI (A), FIB4 (B) scores and Liver Stiffness (C) variation pre-SVR and post-SVR at screening

Figure 1