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Real-world efficacy and safety of direct-acting antiviral drugs in patients with chronic hepatitis C and inherited blood disorders

Isaac Ruiz^{a,b}, Slim Fourati^{b,c}, Abdelhakim Ahmed-Belkacem^b, Christophe Rodriguez^{b,c}, Giovanna Scoazec^a, Flora Donati^{b,c}, Alexandre Soulier^{b,c}, Vanessa Demontant^{b,c}, Lila Poiteau^{b,c}, Mélissa N'Debi^{b,c}, Murielle François^a, Stéphane Chevaliez^{b,c} and Jean-Michel Pawlotsky^{b,c}

Background Patients with inherited blood disorders (IBLD) have a high risk of hepatitis C virus (HCV) infection. The aim of this work was to assess the efficacy and safety of HCV direct-acting antiviral (DAA)-based treatment in patients with IBLD and chronic HCV infection.

Methods Twenty-seven patients (25 with sickle cell disease, 1 with β -thalassemia and 1 with hemoglobin D-Punjab), including 3 with compensated cirrhosis, were included. They were treated with sofosbuvir in combination with ribavirin, daclatasvir, ledipasvir, or velpatasvir or with grazoprevir/elbasvir for 8 or 12 weeks. In the case of treatment failure, in-vitro assessment of resistance-associated substitutions (RASs) and full-length genome sequence analysis by means of deep sequencing were performed.

Results: Treatment was safe and well-tolerated and there were no drug discontinuations due to DAA-related adverse events. Twenty-five out of the 27 patients (93%) achieved sustained virological response 12 weeks post-treatment. One patient discontinued after 18 days due to adverse events unrelated to the antiviral treatment. One patient infected with 'unusual' genotype 2 subtype 2m relapsed. Subtype 2m naturally carries the NS5A L31M RAS. In a genotype 2a subgenomic replicon model, L31M increased daclatasvir effective concentration 50% (EC_{50}) by 97-fold, but velpatasvir EC_{50} by only 3-fold, without altering the replication capacity. This patient was successfully retreated with sofosbuvir/velpatasvir for 12 weeks.

Conclusion DAA-based regimens are well tolerated and highly efficacious in patients with chronic hepatitis C and IBLD in the real-world setting. Thus, DAA-based antiviral treatment should be prioritized in this thus far neglected population of HCV-infected patients. Eur J Gastroenterol Hepatol XXX: 00–00

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Introduction

Hepatitis C virus (HCV) infection is frequent and associated with substantial morbidity in patients with inherited blood disorders (IBLD), notably those with sickle cell disease or β -thalassemia. A large proportion of patients with IBLD have indeed been infected with HCV, due to

the multiple transfusions required to prevent the complications of the disease [1]. In these patients, transfusion-associated liver iron overload contributes to liver fibrosis progression [2]. Thus, patients with IBLD are exposed to complications of chronic liver disease, including cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC). Their occurrence can be favored by a higher age at infection than in the general population and frequent HIV co-infection and alcohol abuse [3].

The incidence of cirrhosis and its complications has been shown to be substantially reduced after successful antiviral treatment. Pegylated interferon (pegIFN) and ribavirin were contraindicated in patients with IBLD because of the risk of ribavirin-associated anemia [4]. Nowadays, the treatment of chronic hepatitis C is based on combinations of direct-acting antiviral (DAA) drugs without pegIFN and ribavirin. These therapies are not contraindicated in patients with IBLD and the European Association for the Study of the Liver recommends their use in patients with hemoglobinopathies [5]. However, only very few clinical trials with HCV DAAs have been reported in patients with IBLD [6–9].

Here, we report the results of a real-world prospective cohort study aiming to assess the efficacy and safety of DAA-based regimens in patients with both IBLD and chronic HCV infection.

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Keywords: direct-acting antiviral drugs, hepatitis C virus, inherited blood disorders, resistance-associated substitutions, subtype 2m

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Patients and methods

Study design

This study was a retrospective analysis of a prospectively recruited real-world cohort, including all patients with IBLD who were also HCV-infected consecutively treated for their HCV infection at the Henri Mondor University hospital with a DAA-based regimen between 2013 and 2017. Data were collected at baseline, at 4, 8 and 12 weeks of therapy (end-of-treatment), and at 4 and 12 weeks post-treatment. They included hemoglobin level, platelet count, aspartate aminotransferase activity, alanine aminotransferase activity, alkaline phosphatase activity, gamma-glutamyl transferase activity, total, indirect and direct bilirubin levels, serum creatinine and virological parameters, including HCV RNA level and HCV genotype. Clinical information was also recorded, including the presence of diabetes mellitus, kidney disease, heart disease, hypogonadotropic hypogonadism, hypothyroidism, osteoporosis, the presence of cryoglobulins, esophageal varices and/or a history of HCC. Co-administered therapies were reported and their potential interactions with HCV drugs were assessed on the University of Liverpool website (<https://www.hep-druginteractions.org>). Side effects were reported during treatment and until week 12 post-treatment.

The occurrence of vaso-occlusive crises (VOC) during antiviral treatment was recorded. Liver fibrosis was assessed before the start of DAA therapy by means of liver elastography (FibroScan, Echosens, Paris, France). A liver stiffness measurement >12.5 kPa was used as the cut-off for the diagnosis of cirrhosis [10]. Cirrhosis was confirmed by liver biopsy in two-thirds of patients. Resistance-associated substitutions (RASs) were sought at baseline and at treatment failure in patients who failed to achieve a sustained virological response (SVR) 12 weeks after the end of treatment.

Patients

Twenty-seven patients with IBLD and chronic hepatitis C were enrolled. They were infected with HCV genotypes 1, 2, 3, 4 or 6 and their baseline HCV RNA level was $\geq 10,000$ international units (IU)/mL (Table 1). The included patients had chronic hepatitis related to any HCV genotypes with or without compensated cirrhosis, defined by a liver stiffness measurement by FibroScan >12.5 kPa within 12 months of enrollment; they were treatment-naïve or treatment-experienced; and the diagnosis of sickle cell disease was based on electrophoretic or HPLC documentation of hemoglobin S-only phenotype. Patients with decompensated cirrhosis, hepatitis B virus coinfection, active substance abuse or a history of malignancy, those with sickle cell disease and acute pain requiring intravenous analgesics, with a vaso-occlusive pain episode for more than 2 weeks, or with >12 pain episodes during the preceding year were excluded.

Endpoints and outcomes measures

The primary endpoint was the SVR, defined as an undetectable HCV RNA (<12 IU/mL), 12 weeks after the end of treatment (SVR12). HCV RNA levels were measured using the Abbott RealTime HCV assay (Abbott Molecular, Des Plaines, Illinois, USA) with identical lower limits of quantification and detection of 12 IU/mL (1.1 log IU/

Table 1. Demographics and baseline characteristics of the 27 patients with inherited blood disorders and chronic hepatitis C

Demographics	
Male, n (%)	7 (25.9)
Female, n (%)	20 (74.1)
Mean age, years, mean \pm SD	49 \pm 10
BMI, kg/m ² , mean \pm SD	23.1 \pm 3.7
Type of IBLD, n (%)	
Sickle cell disease	25 (92.6)
β -Thalassemia	1 (3.7)
Hemoglobin D-Punjab	1 (3.7)
HCV genotype, n (%)	
1a	1 (3.7)
1b	4 (14.8)
2	11 (40.7)
3a	2 (7.4)
4	8 (29.6)
6	1 (3.7)
HCV RNA level	
Log ₁₀ IU/mL, mean \pm SD	5.7 \pm 0.9
>0.8 $\times 10^6$ IU/mL, n (%)	12 (44.4)
Severity of liver disease	
Fibroscan value, kPa, median (range)	5.6 (3.2–15.7)
Stage of fibrosis (Fibroscan)	
Mild fibrosis: ≤ 7.0 kPa, n (%)	17 (63.0)
Moderate fibrosis: >7.0 to ≤ 9.5 kPa, n (%)	5 (18.5)
Severe fibrosis: >9.5 to ≤ 12.5 kPa, n (%)	2 (7.4)
Compensated cirrhosis: >12.5 kPa, n (%)	3 (11.1)
Child-Pugh score in patients with cirrhosis, mean \pm SD	6.0 \pm 1.0
MELD score in patients with cirrhosis, mean \pm SD	8.0 \pm 3.0
Comorbidities, n (%)	
Kidney disease	1 (3.7)
Hypertension	11 (40.7)
Diabetes mellitus	2 (7.4)
HIV coinfection	1 (3.7)
Prior treatment, n (%)	
PegIFN plus ribavirin	3 (11.0)
PegIFN plus ribavirin plus sofosbuvir	1 (4.0)
Laboratory assessments at baseline, mean \pm SD	
Creatinine, μ mol/L, mean \pm SD	65.3 \pm 23.9
Albumin, g/L, mean \pm SD	39.7 \pm 6.2
Hemoglobin, g/L, mean \pm SD	9.6 \pm 2.5
Platelet count, $\times 10^3/\mu$ L, mean \pm SD	262.7 \pm 93.5
INR, mean \pm SD	1.1 \pm 0.1
Total bilirubin, μ mol/L, mean \pm SD	33.2 \pm 46.1

HCV, hepatitis C virus; MELD, model for end-stage liver disease; pegIFN, pegylated interferon; INR, international normalized ratio.

mL). Virological failure was defined as a lack of SVR12, due to either a virological breakthrough on treatment or a post-treatment relapse (undetectable HCV RNA that becomes detectable again).

Safety was assessed by monitoring adverse events and other safety parameters, including vital signs, physical examinations, 12-lead electrocardiograms and standard laboratory safety tests. The protocol was conducted in accordance with the Declaration of Helsinki and French law for biomedical research and was approved by an Institutional Review Board (Comité de Protection des Personnes Ile-de France V; B12-6).

Genotypic characterization of direct-acting antiviral treatment failures by deep sequencing

In patients who failed to achieve SVR12, blood samples taken at baseline, at treatment failure and before retreatment were tested by means of an original in-house patient-protected shotgun metagenomics method (MetaMIC) based on deep sequencing. Nucleic acid extraction was followed by library preparation using the total RNA and Nextera XT kit (Illumina, San Diego, California, USA) and deep sequencing on a NextSeq500 device (Illumina). Sequences were analyzed by means of in-house software

MetaMIC for quality check, filtering, identification, genome reconstruction and comparisons. The presence of RASs is reported when the corresponding viral population exceeds 15% of the viral quaspecies.

Phenotypic characterization of resistance-associated substitutions selected at treatment failure

Huh7.5 cells were cultured in complete Dulbecco's Modified Eagle Medium (DMEM, ThermoFisher Scientific, Waltham, Massachusetts, USA) supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, 100 µg/mL streptomycin and 0.1 µg/mL amphotericin-β (ThermoFisher Scientific). A genotype 2a subgenomic replicon (SGR) containing the Renilla luciferase reporter gene, APP40-J6/JFH1EMCVIRES-aRlucNeo, was purchased from Apath (New York, USA). The RASs of interest were introduced by means of site-directed mutagenesis using the Quick Change II XL site-directed mutagenesis kit (Agilent Technologies, Santa Clara, California, USA).

For transient HCV SGR expression experiments, wild-type and RAS-containing SGRs were linearized overnight with XbaI (FastDigest, ThermoFisher Scientific) and in-vitro transcribed using the MEGAscript T7 Transcription Kit (ThermoFisher Scientific). Then, 2.0×10^4 Huh7.5 cells were transfected with 250 ng HCV SGR RNA using a trans-IT mRNA transfection kit (Mirus Bio LLC, Madison, Wisconsin, USA). Four hours after transfection, daclatasvir, velpatasvir or sofosbuvir (AGV Discovery, Clapiers, France) were added to the culture medium. Luciferase activity was monitored 48 h post-transfection with the luciferase assay system (Promega, Madison, Wisconsin, USA) to assess RAS-containing HCV SGR susceptibilities to the different DAAs tested, as compared to wild-type HCV SGR. Plots were fitted with a 4-parameter logistic curve using the SigmaPlot v11 software (Systat Software, San Jose, California, USA) and the effective concentrations 50% (EC_{50}) were calculated from the curves. The replication capacity of RAS-containing HCV SGR was assessed by comparing luciferase activity 4 and 48 h post-transfection and expressed as a percentage of wild-type HCV SGR replication capacity.

Statistical analysis

The primary objective of the study was to assess virological efficacy and safety that was measured by the percentage of patients achieving SVR12 and developing clinical or biological adverse events, respectively. Descriptive statistics are shown as means ± SD, median with minimal and maximal range, and percentages. Statistical analyses were performed using the SigmaPlot software.

Results

Patients and treatments

Twenty-seven patients were enrolled, including 25 with sickle cell disease, 1 with β-thalassemia, and 1 with hemoglobin D-Punjab. Their baseline characteristics are shown in Table 1. Four patients had already been treated with pegIFN and ribavirin, plus the first-generation protease inhibitor boceprevir in one case.

The choice of the DAA combination was made at the physician's discretion in the context of his/her current practice. Table 2 shows the treatments received according to the HCV genotype. These treatments included sofosbuvir (400 mg per day) plus ribavirin (400 mg per day), sofosbuvir (400 mg per day) plus daclatasvir (600 mg per day), sofosbuvir/ledipasvir (400 mg/90 mg per day), sofosbuvir/velpatasvir (400 mg/100 mg per day) or elbasvir/grazoprevir (50 mg/100mg per day) for 8 or 12 weeks.

Virological efficacy

Among the 27 patients included, 26 (96.3%) had undetectable HCV RNA at the end of treatment and 25 (92.6%) achieved SVR12 (Fig. 1). One 59 years-old female patient infected with HCV genotype 2 receiving sofosbuvir and daclatasvir stopped treatment at 18 days of administration due to adverse events that were not ascribed to HCV drugs. The HCV RNA levels were 5 002 676, 45 and 793 311 UI/mL, at baseline, at week 1 of treatment and 3 months after stopping treatment, respectively. Thus, of 26 patients who completed the full course of therapy, only one did not achieve SVR.

Another 49 years-old treatment-naïve patient without cirrhosis infected with HCV genotype 2 and treated with sofosbuvir plus daclatasvir for 12 weeks relapsed 4 weeks after the end of treatment. This patient was retreated with sofosbuvir/velpatasvir for 12 weeks and achieved SVR12.

Genotypic and phenotypic resistance analyses

Sequence analysis performed in the patient who relapsed showed that an L31M RAS was present at baseline, at treatment failure and at retreatment baseline in the NS5A region targeted by daclatasvir and velpatasvir.

As shown in Fig. 2, the L31M RAS was introduced into a genotype 2a SGR and the effect of the substitution on drug susceptibility and replication capacity was studied. As expected, the NS5A L31M RAS did not alter sofosbuvir susceptibility. In contrast, it increased daclatasvir EC_{50}

Table 2. Direct-acting antiviral regimens administered at the physician's discretion according to the hepatitis C virus genotype

Genotype	1a	1b	2	3	4	6	Total n (%)
Treatment administered for 12 weeks, n (%)							
Sofosbuvir plus ribavirin			1 (4)				1 (4)
Sofosbuvir plus daclatasvir	1 (4)	1 (4)	8 (30)	1 (4)	1 (4)		12 (44)
Sofosbuvir/ledipasvir		1 (4)			5 (19)	1 (4)	7 (26)
Sofosbuvir/velpatasvir			1 (4)		1 (4)		2 (7)
Grazoprevir/elbasvir		2 (7)			1 (4)		3 (11)
Treatment administered for 8 weeks, n (%)							
Sofosbuvir/ledipasvir			1 (4)				1 (4)
Sofosbuvir plus daclatasvir				1 (4)			1 (4)

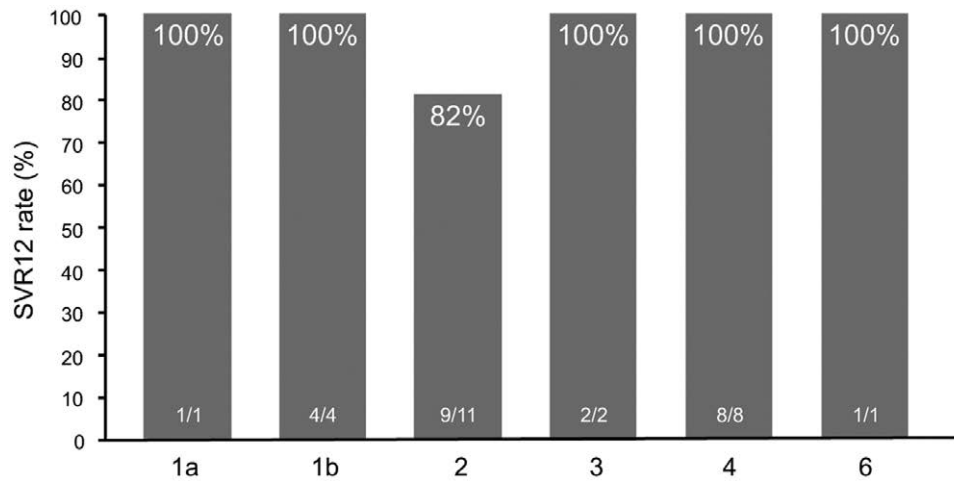
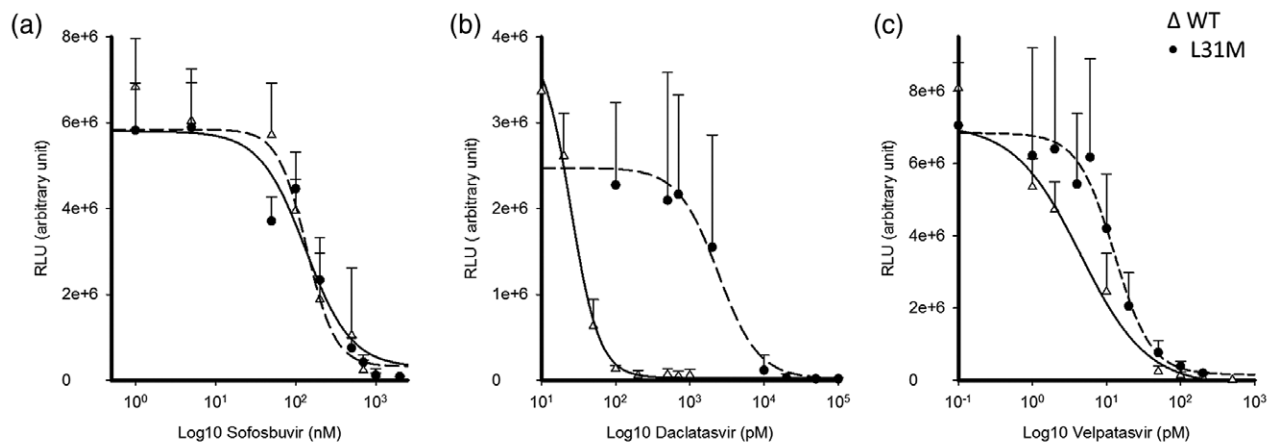


Fig. 1. Virological response according to the hepatitis C virus (HCV) genotype. One patient discontinued therapy at day 18 of treatment due to adverse events. Another patient relapsed after the end of treatment. Both patients were infected with HCV genotype 2 (the latter with subtype 2m).



Genotype 2a replicon	Sofosbuvir		Daclatasvir		Velpatasvir		Replication capacity (%)
	EC ₅₀ (μM)	Fold-change	EC ₅₀ (μM)	Fold-change	EC ₅₀ (pM)	Fold-change	
Wild-type	137.3±35.25	1.0	24.9±1.4	1.0	4.5±1.9	1.0	100
L31M	132.7±39.19	1.0	2415.4±597.6	97.1	12.8±2.0	2.9	27.0

Fig. 2. Effect of the NS5A L31M RAS on hepatitis C virus (HCV) genotype 2a susceptibility to sofosbuvir, daclatasvir and velpatasvir. Dose-dependent curves of antiviral activity of sofosbuvir (a), daclatasvir (b) and velpatasvir (c) against a wild-type (WT) genotype 2a subgenomic replicon harboring an L31 in the NS5A region (open triangle) and a mutated replicon harboring the L31M RAS (black circle). Plots were fitted (dashed line for L31 and solid line for L31M) with a four-parameter logistic curve by means of SigmaPlot v11 software (Systat Software, Inc.) and the EC₅₀s determined from the curves are shown in the Table, together with the fold-increases in EC₅₀ conferred by the different amino acids at position 31 and the corresponding replication capacities.

by approximately 100-fold, whereas velpatasvir susceptibility was reduced by only 3-fold (Fig. 2).

Deep sequencing analysis was performed at baseline, at treatment failure and at retreatment baseline to analyze the full-length genome of this patient. Phylogenetic analysis indicated that the patient was infected with subtype 2m, a rare subtype of genotype 2. Sequence analysis of the NS3A, NS5A and NS5B regions identified three positions with natural polymorphisms at baseline conferring resistance to DAA: an arginine at position 168 in the NS3 protease, a cysteine at position 28 and a methionine at position 31 in the NS5A protein (Table 3). These results explain the failure of sofosbuvir plus daclatasvir treatment and the success of sofosbuvir/velpatasvir retreatment.

Safety and tolerability

Treatment was safe and well-tolerated. Four patients (15%) reported clinical adverse events, including headache in two cases, asthenia in one case and myalgias in two cases. One patient discontinued treatment after 18 days of treatment, due to headache and myalgias without criteria for a VOC. One patient presented with myalgias and arthralgias due to osteoporotic collapse and was hospitalized for orthopedic management while continuing HCV treatment. One patient presented with a VOC 10 days after starting treatment. This patient also presented with a mild veno-occlusive event after starting the HCV treatment. The acute episode was managed in the Emergency Room and did not require further hospitalization. No

Table 3. Amino acid positions commonly associated with reduced susceptibility to direct-acting antivirals in the sequence of the genotype 2, subtype 2m-infected patient at baseline, after sofosbuvir plus daclatasvir failure and prior to retreatment with sofosbuvir/velpatasvir. Gray cells contain amino acids at positions associated with drug resistance, as described for genotype 2 [5]

Gene	Position	Baseline	Post-sofosbuvir and daclatasvir treatment	Before sofosbuvir/velpatasvir retreatment
NS3	168	Arg	Arg	Arg
	170	Ile	Ile	Ile
NS5A	24	Ser	Ser	Ser
	28	Cys	Cys	Cys
	31	Met	Met	Met
	38	Ser	Ser	Ser
	58	Pro	Pro	Pro
	62	Ala	Ala	Ala
	92	Cys	Cys	Cys
	93	Tyr	Tyr	Tyr
	NS5B	159	Leu	Leu
237		Glu	Glu	Glu
273		Thr	Thr	Thr
282		Ser	Ser	Ser
316		Cys	Cys	Cys
320		Leu	Leu	Leu
321		Val	Val	Val
330		Glu	Glu	Glu
392		Ile	Ile	Ile
414		Gln	Gln	Gln
419		Ile	Ile	Ile
421		Val	Val	Val
444		Asn	Asn	Asn
445		Phe	Phe	Phe
446		Glu	Glu	Glu
451		Val	Val	Val
482		Leu	Leu	Leu
486	Ala	Ala	Ala	
494	Ala	Ala	Ala	
495	Pro	Pro	Pro	
499	Ala	Ala	Ala	
556	Gly	Gly	Gly	

relapse was observed. Thus, after a few days, the physician and the patient decided to resume HCV treatment, without a new HCV RNA test. The patient achieved SVR12.

No significant changes in hemoglobin levels were observed during treatment, except in the patient receiving 400 mg of ribavirin who dropped from 11.0 g/dL at baseline to 8.7 g/dL at the end of treatment and 8.2 g/dL at SVR12. No other biological abnormalities were reported.

Discussion

Patients with IBLD are considered as a difficult-to-treat population. Despite the publication of a few clinical trials, conclusive data are lacking as to the optimal HCV DAA regimen in this special group of patients. The present real-world study, including 27 patients treated for 8 or 12 weeks with sofosbuvir-based regimens, shows an SVR12 rate of 93%, with only one virological failure. SVR12 was achieved irrespective of the HCV genotype, the presence of compensated cirrhosis and/or prior treatment failure on pegIFN or DAA-based regimens. The different treatment regimens were safe and well-tolerated, as in previous clinical trials in patients with or without IBLD.

A few clinical trials have included patients with IBLD receiving HCV DAA combinations. In one study, sofosbuvir/ledipasvir was administered for 12 weeks with ribavirin in 14 patients infected with genotype 1, with a 100% SVR12 rate [6]. A pooled analysis of phase II and III clinical trials reported 184 patients with bleeding

disorders and chronic hepatitis C who received either sofosbuvir plus ribavirin or sofosbuvir/ledipasvir with or without ribavirin. The overall SVR12 rate was 85%. Eleven patients (6%) experienced a severe adverse event. Hemarthrosis, muscle hemorrhage and epistaxis were the only hemorrhagic events that occurred in more than one patient. The most frequent laboratory abnormalities were anemia and hyperbilirubinemia associated with ribavirin administration [7]. In a Phase III placebo-controlled study assessing the efficacy and safety of elbasvir/grazoprevir administered for 12 weeks in patients with HCV infection and sickle cell anemia (18%), thalassemia (38%), or hemophilia A/B or von Willebrand disease (44%), 100/107 patients (93.5%) achieved SVR12. Serious adverse events (SAEs) were reported in three patients (2.8%). The hemoglobin levels did not differ between patients receiving grazoprevir/elbasvir and those receiving placebo [8]. Ombitasvir/paritaprevir/tritonavir plus dasabuvir with or without ribavirin was evaluated in 35 treatment-naïve or pegIFN and ribavirin-experienced, cirrhotic or noncirrhotic patients infected with HCV genotype 1. The SVR12 rate was 100% and there were no discontinuations due to adverse events. One patient (2.9%) had a decline in the hemoglobin level <10 g/L [11]. In another study, the SVR rate was 93.5% in 139 patients with IBLD who received DAA therapy [9].

In our study, two patients failed to achieve SVR12. The first patient was a 59-year-old female infected with HCV genotype 2 receiving sofosbuvir and daclatasvir who stopped treatment at 18 days of administration due to adverse events that were not ascribed to the HCV drugs. The second patient who did not achieve SVR12 was a 49-year-old treatment-naïve female without cirrhosis, infected with genotype 2 who was treated with sofosbuvir and daclatasvir for 12 weeks and relapsed 4 weeks after the end of treatment. Resistance testing performed at baseline and at treatment failure showed the preexistence before therapy and persistence after treatment of the L31M RAS. This polymorphism was first described in four patients infected with genotype 2 treated with the NS5A inhibitor IDX719 and shown to confer a 75-fold reduction in drug susceptibility *in vitro* [12,13]. A prevalence of 52, 41 and 86% of the L31M polymorphism was reported in an overall population of 283 patients with genotype 2 infection treated with daclatasvir-based regimens from the Americas, Europe and Asia, respectively. The daclatasvir EC₅₀ in a genotype 2 JFH-1 replicon was 0.02 nM and 11.0 nM in the wild-type and L31M-mutated replicons, respectively. Surprisingly, the presence of baseline NS5A polymorphisms did not appear to affect the SVR rates in patients with HCV genotype 2 infection treated with daclatasvir-based regimens in this study [14]. In a phase 1b 3-day monotherapy, double-blind, randomized, multicenter study of HCV-infected patients treated with velpatasvir in the USA, four of eight genotype 2 patients harbored the L31M polymorphism in their NS5A region. The fold-change in EC₅₀ conferred by L31M was 1.5 ± 0.7, but it was tested in a genotype 1a replicon [15]. In another study in patients with HCV genotype 1–6 infection treated with sofosbuvir/velpatasvir for 12 weeks, pre-existing L31M was found in 130/316 (41%) of patients with genotype 2 infection. All of them achieved SVR12 [16].

We evaluated the effect of the L31M substitution on the susceptibility of an HCV genotype 2a SGR to sofosbuvir,

daclatasvir and velpatasvir, the drugs administered in the patient who failed to achieve SVR12 in our study. L31M has no impact on sofosbuvir susceptibility, but induced a 97-fold change in the susceptibility to daclatasvir, explaining treatment failure with sofosbuvir and daclatasvir in this patient. In contrast, susceptibility to velpatasvir was reduced by only 3-fold by L31M. Thus, because no other RASs were present in this patient, sofosbuvir/velpatasvir for 12 weeks was effective in achieving SVR. This indicates that, given the prevalence of L31M in patients infected with genotype 2, a sofosbuvir/velpatasvir regimen is a more optimal first-line treatment regimen than sofosbuvir plus daclatasvir, if a combination of sofosbuvir and an NS5A inhibitor is to be administered. Further studies are needed in order to confirm this hypothesis.

We decided to use a novel tool based on deep sequencing in order to sequence the full-length HCV genome in this patient, including the NS3, NS5A and NS5B regions. Deep sequencing allowed us to find that the patient was infected with a rare subtype of genotype 2, subtype 2m, and that this subtype carried at least three RASs as natural polymorphisms on its genome. The 168R NS3 RAS has already been reported to confer resistance to last-generation protease inhibitors, including both voxilaprevir and glecaprevir [17]. The 28C NS5A RAS resulted in a 400-fold reduction in genotype 2a sensitivity to NS5A inhibitors [18]. These results explain the failure of sofosbuvir plus daclatasvir treatment and the success of sofosbuvir/velpatasvir retreatment, which therefore appears to be a better first-line therapeutic option than glecaprevir/pibrentasvir in patients infected with the rare subtype 2m.

Our findings must be interpreted in the context of the study's potential limitations. First, this is a monocentric study from a reference center for both HCV infection and IBLD, with a small sample size, but an important proportion of sickle cell disease patients compared to other studies reported in the literature. Second, the choice of the DAA regimen was at the physician's discretion over a long and particular period of time during which HCV treatment paradigms were rapidly changing, explaining the small sample size for each different regimen, including regimens including ribavirin, a drug known to induce anemia.

In conclusion, this real-world study confirms the efficacy and safety of 8 or 12 weeks of highly effective DAA-based regimens in patients with IBLD. HCV treatment should be prioritized in this thus far undertreated population, as universal treatment access is required to achieve the WHO's goal to eliminate HCV infection as a public health threat.

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Conflicts of interest

I.R., A.A-B. and C.R., has served as speakers for AbbVie. S.F. has served as an advisor and/or speaker for AbbVie and MSD. S.C. has served as an advisor for Gilead and MSD. J.-M.P. has served as an advisor and/or speaker

for AbbVie, Gilead, GSK, Merck, Regulus and Siemens Healthcare. For the remaining authors, there are no conflicts of interest.

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