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TITLE PAGE

Effectiveness of direct-acting agents for hepatitis C and liver stiffness changing after sustained virological response

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List of Abbreviations

CHC, chronic hepatitis C; DAA, direct-acting agents HCV, hepatitis C virus SVR, sustained virologic response; IFN, interferon; TE, transient elastography; LSM, liver stiffness measurement; ITT, intention-to-treat EOT, end-of-treatment; ALT, alanine transaminase; SOF, sofosbuvir; DCV, daclatasvir; RBV, ribavirin; SIM, simeprevir; PEG-IFN, peguilated interferon; IQR, interquartile range; CI, confidence interval; OR, odds-ratio; HR, hazard ratio; ULN, upper limit of normal

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ABSTRACT

Background and Aims: Few studies have evaluated sustained virological response (SVR) rates by direct antivirals (DAAs) and liver stiffness measurement (LSM) changing post-SVR in limited-resource settings. We aimed to describe the effectiveness of DAAs for HCV treatment and to assess the changing of LSM post-SVR

Methods: This retrospective study analyzed data of consecutive HCV-infected patients treated by DAAs from 2015 to 2017 in two tertiary centers in Brazil. SVR rates were reported by intention-to-treat (ITT) and per-protocol analysis. LSM by transient elastography performed before treatment and post-SVR were compared and logistic regression models were performed.

Results: 671 patients [63% female, 62 years (55-68), 89% genotype-1, 8% HIV co-infected and 64% with cirrhosis] were included. Most patients were treated by sofosbuvir/daclatasvir±ribavirin (74%) and sofosbuvir/simeprevir±ribavirin (21%). SVR rates (95%CI) were 94.6% (92.7-96.1) and 97.8% (96.4-98.7) for ITT and per-protocol analysis, respectively. The leading adverse event was anaemia [9.6% (95%CI 7.6-12.1)]. Pre-treatment and post-SVR12 LSM were available in 400 patients. LSM had significantly decreased after SVR [13.6 kPa (IQR, 10.0-21.6) vs 10.2 kPa (7.0-17.6), p<0.001]. A total of 167 patients (42%) decreased at least 30% of LSM post-SVR. Absence of type-2 diabetes [OR=1.52 (95%CI,1.05-2.21), p=0.028] and presence of platelet count ≥ 150 x 10^9/mm^3 [OR=1.75 (1.23-2.50), p=0.002] were independently associated with a significant LSM regression (≥ 30%) post-SVR.

Conclusion: DAAs were highly effective and safe and LSM significantly decrease after-SVR in a real-life cohort in Brazil. Absence of type-2 diabetes and presence of high platelet count were independently associated with LSM decrease post-SVR.

Keywords: hepatitis C, fibrosis, liver stiffness, treatment

INTRODUCTION

Chronic hepatitis C (CHC) remains a major public health issue worldwide.[1] The high efficacy of direct-acting antiviral drugs (DAAs) has revolutionized the management of patients with CHC [2] and the eradication of hepatitis C virus (HCV) has been associated with lower rates of liver-related complications.[3] However, results from clinical trials presumably overestimate benefit and underestimate harm of DAA treatment [4] and few studies have evaluated the sustained virological response (SVR) rates in real-life setting in Latin America. DAA treatment is cost-effective for HCV eradication [5] and interferon-free regimens have been available in the Brazilian Public Health System since June 2015.[6]

The correct determination of liver fibrosis stage has important implications for prognostic, therapeutic and monitoring purposes in patients with CHC.[7] Therefore, assessment of liver fibrosis changes after SVR remains an important landmark to manage patients with CHC after treatment. Few studies have described a clinically significant regression in liver fibrosis post-SVR after interferon (IFN)-based treatment using the liver biopsy as reference.[8,9] However, the performance of liver biopsy after SVR is challenged by the invasiveness of this method and low acceptance by patients. Non-invasive methods can be an alternative to stage liver fibrosis after DAA treatment.[10] Transient elastography (TE) by FibroScan® (EchoSens, Paris, France) is a validated and cost-effective method to liver fibrosis assessment based on liver stiffness measurement (LSM).[11] LSM has been used to predict overall mortality and liver-related events in patients with CHC.[12] In addition, LSM before treatment has been useful for predicting occurrence of hepatocellular carcinoma [13] and LSM at the end-of-treatment can predict incidence of pruritus in patients who achieved SVR.[14] More recently, few studies have been correlating SVR by DAA with regression in liver stiffness.[15-23] However, the proportion of LSM reduction after SVR and factors associated with this condition evaluated in a large sample real-world cohort remains controversy. We aimed to report effectiveness and safety of

DAA treatment and to assess the impact of SVR in LSM, as well as factors associated with changing of LSM post-SVR, in patients with CHC.

MATERIAL AND METHODS

Population and study design

This retrospective observational study analyzed data of consecutive HCV-infected patients treated by DAAs in two tertiary centers in Rio de Janeiro (Brazil) from October-2015 to December-2017. Adult patients with CHC, defined by positive HCV-RNA for more than 6 months, were included. Exclusion criteria were hepatitis B co-infection, DAA treatment after liver-transplantation or previous IFN-free treatment. Demographic characteristics, clinical features, co-morbidities and co-medications, DAA regimens, adverse events during treatment, laboratorial results and LSM pre-treatment and post-SVR12 were collected by trained investigators. All patients were included in the intention-to-treat (ITT) analysis for effectiveness and safety of DAA regimens. Patients with loss of follow-up or death and those with less than 12 weeks after end-of-treatment (EOT) in the moment of the analysis or with missing data for SVR were excluded from the per-protocol analysis. SVR rates were reported as overall and stratified by genotype, previous treatment, DAA regimen and presence of cirrhosis or HIV co-infection. For the analysis of changing LSM after SVR, patients with DAA treatment failure, unreliable or missing LSM and those with TE performed with different probes before treatment and post-SVR were excluded. The study was approved with waiver of informed consent by the Ethical Committee from the National Institute of Infectious Diseases Evandro Chagas [IRB number 51736815.3.0000.5262].

Effectiveness and safety of DAA treatment

Patients were treated by the following regimens according to the Brazilian guidelines for HCV treatment[6]: Sofosbuvir / Daclatasvir ± Ribavirina (SOF/DCV± RBV), Sofosbuvir / Simeprevir ± Ribavirina (SOF/SIM±RBV); Ombitasvir / Paritaprevir / Ritonavir / Dasabuvir; Sofosbuvir/Ribavirina (SOF/RBV) or Sofosbuvir/PEG-interferon/Ribavirina (PEG-IFN/SOF/RBV). Sustained virological response was defined by undetectable HCV-RNA 12 weeks after EOT (SVR12).

Central obesity, dyslipidemia, blood hypertension, diabetes and metabolic syndrome were defined according to the International Diabetes Federation criteria.[24] Cirrhosis was defined by clinical findings, imaging, liver biopsy (METAVIR F4) or LSM ≥ 12.5 kPa. The following adverse events were assessed during DAA treatment: (i) acute liver injury [alanine aminotransferase (ALT) ≥ 5 times the upper limit of normal (ULN)]; (ii) anemia [hemoglobin < 10g/dL], (iii) cardiac arrythmia, (iv) kidney injury [increase of basal creatinine of at least 50%][25], (v) liver-related complications [hepatic encephalopathy, variceal bleeding, hepatocellular carcinoma] and (vi) overall mortality.

LSM changing after SVR

Data of LSM by TE performed by experienced operators were collected before treatment and post-SVR. TE was considered reliable when the following criteria had been met: (i) 10 successful measurements; (ii) an interquartile range (IQR) lower than 30% of the median value of LSM and (iii) a success rate of more than 60%. Spots for each measurement were chosen by the respective operator during examination and they were not recorded. All TE exams were performed in patients with at least a 3h-fasting status. Liver stiffness was considered as the median of all valid measurements. Results from the most recent TE exam obtained prior to DAA treatment and after SVR were used in the analysis if multiple exams were available in the medical records. LSM pre-treatment and post-SVR were converted to the

METAVIR score as proposed by Castera et al[26]: LSM <7.1 kPa for F0F1; LSM=7.1–9.4 kPa for F2; LSM=9.5–12.4 kPa for F3 and LSM ≥12.5 kPa for F4. Significant regression of LSM was defined of at least 30% of decrease of post-SVR LSM compared to pre-treatment values.

Statistical analysis

Continuous variables were reported as median (IQR) and discrete variables were reported as absolute (n) and relative frequency (%). Comparisons between groups were assessed by Mann-Whitney for quantitative comparisons and Chi-square test for qualitative comparisons. SVR rates [95% confidence interval, CI] were expressed for the ITT and per-protocol analysis. Repetitive measures were compared and assessed by Wilcoxon signed-rank for paired continuous and McNemar for paired discrete variables. Logistic regression models adjusted for confounding factors were performed to identify factors associated with treatment failure and adverse events [odds-ratio, OR]. Time-dependent Cox proportional hazard models were performed to identify factors associated with significant LSM regression after SVR12 [hazard-ratio, HR]. Significance level was determined when p value ≤ 0.05 assuming two-tailed tests. Statistical analyses were performed using STATA (2017; StataCorp LP, College Station, TX, USA).

RESULTS

A total of 671 patients [63% female, median age of 62 years (IQR, 55-68) 89% genotype-1, median ALT of 79 UI/L (IQR, 52-124), 8% co-infected by HIV and 64% with cirrhosis] were included in the intention-to-treat (ITT) analysis (Table 1). The following regimes were used: SOF/DCV±RBV (74%), SOF/SIM±RBV (21%), Ombitasvir, Paritaprevir/Ritonavir/Dasabuvir (3%), SOF/RBV (1%) and SOF/PEG-IFN/RBV (1%). RBV was associated with DAA drugs in 353 patients (53%). A total of 22 patients were excluded

for the per-protocol analysis due to loss of follow-up (n=8), follow-up lower than 12 weeks after end-of-treatment (n=7), missing data of SVR12 (n=6) and death during treatment (n=1). From patients included in the per-protocol analysis of DAA effectiveness (n=635), reliable LSM with the same probe pre-treatment and post-SVR12 were available for 400 (63%) subjects. Figure 1 summarizes the flow-chart of the study.

Sustained virological response rates

Overall SVR12 rates (95%CI) were 94.6% (92.7-96.1) and 97.8% (96.4-98.7) for ITT and per-protocol analysis, respectively. Table 2 summarizes the SVR12 rates according to presence of cirrhosis, HIV co-infection, genotype, previous treatment and DAA regimens for ITT and per-protocol analysis. Considering the per-protocol analysis (n=649), there were no significant statistical differences of SVR12 rates for cirrhotic patients compared to non-cirrhotic [99.2% (96.7-99.8) vs 97.1% (94.9-98.3), p=0.079]; patients with HIV co-infection compared to HCV-mono-infected [97.9% (96.3-98.8) vs 97.8% (85.5-99.7), p=0.975] or naïve compared to experimented patients [98.2% (95.3-99.3) vs 97.6% (95.7-98.7), p=0.639]. SVR12 rates for patients with genotypes 1, 2 and 3 were 98.5% (97.0-99.2), 90.9% (53.6-98.9) and 96.0% (85.2-99.0), respectively. The overall SVR12 rates were 98.3% (96.7-99.2) for SOF/DCV ± RBV and 96.4% (91.6-98.5) for SOF/SIM ± RBV. Supplementary table 1 summarizes the SVR12 rates for patients treated with SOF/DCV and SOF/SIM with and without RBV. HCV infection by non-1 genotype was significantly associated with treatment failure (n=14) [vs genotype 1, OR=4.90 (95%CI 1.40-17.18, p=0.013] in a multivariate analysis adjusted for confounding factors (Supplementary Table 2).

Safety

One-hundred and seven patients [16.0% (95%CI 13.4-18.9)] experimented at least one adverse event during DAA treatment. Anaemia [9.6% (7.6-12.1)] and kidney injury [6.00% (4.4-8.0)] were the leading causes of adverse events. Liver-related complications [hepatic encephalopathy, variceal bleeding and hepatocellular carcinoma] and death were registered in 2.2% (n=15) and 0.2% (n=1) of treated patients, respectively. The prevalence (95%CI) of at least one complication was significantly higher in patients with cirrhosis compared to non-cirrhotic patients [19.4% (15.9-23.5) vs 9.8% (6.7-14.3), p =0.001] (Table 3). The use of RBV [vs no RBV use, OR=4.00 (95%CI 1.99-8.04, p<0.001] and treatment for 24 weeks [vs 12 weeks, OR=2.21 (1.23-3.97), p=0.008] were significantly associated with occurrence of at least one complication in a multivariate analysis (Table 4).

Changing of LSM after HCV eradication

Pre-treatment and post-SVR12 LSM were evaluated in 400 patients [64% female, median age of 62 years (IQR, 56-68), median ALT of 78 UI/L (IQR, 54-122), 59% with cirrhosis and 71% treated by SOF/DCV ± RBV]. The median time between end-of-treatment and LSM post-SVR12 was 10 (4-13) months. Patients with cirrhosis, as determinate by TE before treatment (LSM ≥ 12.5 kPa) had a higher proportion of low platelet count (< 150 x 109/mm3) compared to those without cirrhosis [54% vs 19%; p < 0.001]. Overall LSM had significantly decreased after SVR12 compared to before treatment [13.6 kPa (IQR, 10.0-21.6) vs 10.2 kPa (7.0-17.6), p< 0.001]. This significant decreasing of LSM after SVR12 was observed in patients classified as METAVIR F3 [11.0 kPa (10.3-11.8) vs 8.0 kPa (6.8-10.2), p<0.001] and F4 [21.3 kPa (15.6-28.4) vs 17.1 kPa (8.8-22.1), p< 0.001] but not in F0F1 [5.9 kPa (4.9-6.5) vs 5.8 kPa (4.8-6.8), p=0.955] or F2 [8.2 kPa (7.8-8.8) vs 6.9 kPa (6.1-9.5), p=0.197] according to the pre-treatment LSM. A total of 38% (n=152) of patients had at least

one fibrosis stage regression and 8% (n=32) had at least one fibrosis stage progression after SVR12 compared to LSM performed before treatment. In addition, 31% (n=67) of cirrhotic patients according to LSM before treatment were reclassified as non-cirrhotic by LSM post-SVR ($p<0.001$).

The median changing of LSM post-SVR12 was -22% (-40% to +3%) compared to LSM before treatment. A total of 42% (n=167) of patients had a significant regression (≥ 30%) of LSM post-SVR12 compared to pre-treatment LSM. Patients with cirrhosis defined by pre-treatment LSM (≥ 12.5 kPa) had high proportion of type-2 diabetes (64% vs 49%, $p<0.001$) and lower platelet count (77% vs 40%, $p<0.001$) compared to those without cirrhosis (LSM < 12.5 kPa). In the time-dependent analysis [Cox model], the absence of type 2 diabetes [HR=1.52 (95%CI 1.05-2.21), p=0.028] and presence of high platelet count (≥ 150 x 10^9/mm^3) [HR= 1.75 (1.23-2.50), p=0.002] were significantly associated with a significant regression of LSM post-SVR12 adjusted for confounding factors (Table 5). In this model, baseline fibrosis stage defined by LSM was not associated with significant LSM regression. In a sensitivity analysis using the combination of LSM and platelet count to predict LSM regression, the presence of a negative-Baveno VI criteria (LSM < 20 kPa and platelet count ≥ 150 x 10^9/mm^3) at baseline [HR=1.46 (1.02-2.10), p=0.038] at baseline and absence of type 2 diabetes [HR=1.54 (1.06-2.24), p=0.023] were independently associated with a significant regression of LSM post-SVR12.

DISCUSSION

The present study highlights the high effectiveness of DAAs and the decrease of LSM post-SVR in a resource-limited setting, such as Brazil, the only country where DAA are available on the Public Health System in Latin America. In addition, to the best of our

knowledge, this is the largest sample size study that evaluated the changing of LSM after HCV treatment.

International clinical trials [2] and real-life observational studies [27] have reported high SVR rates for HCV eradication by SOF-based regimens. Recent studies reported up to 96% overall SVR rates using DAA regimens in São Paulo [28] and Southern of Brazil.[29,30] The present study confirmed these high overall SVR rates of SOF/SIM and SOF/DCV in a larger sample of Brazilian patients followed in two tertiary centers in Rio de Janeiro. The lower SVR rates in genotype-2 patients compared to other genotypes might be explained by the recommendation of SOF/RBV in these patients according to the Brazilian guidelines.[6] We observed discordant results regarding the impact of cirrhosis in SVR compared to the study from Miotto et al [28], despite a high prevalence of cirrhotic patients and a similar cirrhosis definition in both analyses. The incidence of at least one adverse event was significantly higher in patients with cirrhosis compared to non-cirrhotic patients and mainly driven by use of RBV. We confirmed low rates of severe adverse events or liver-related complications during DAA treatment.

Overall, observational studies or randomized controlled trials have described a significant decrease in LSM after HCV eradication. The heterogeneity among the studies might explain the discrepancies on the impact of necro-inflammatory activity and pre-treatment fibrosis stage in LSM regression. Most studies that evaluated the kinetics of LSM post-SVR included a limited sample size of patients with different ethnical characteristics[18,20,21,31] and variable spectrum of liver fibrosis stages.[19,32] In addition, few studies evaluated patients with specific conditions, such as presence of portal hypertension [33], 1b-genotype[18], post liver transplantation[32] or HIV co-infection.[23,34] In the present study, at least 30% of LSM reduction was used as a threshold indicative of clinically relevant fibrosis regression after SVR. In paired measures, we observed a significantly LSM regression post-SVR in patients with advanced fibrosis but not in those with minimal fibrosis defined by pre-treatment LSM levels. However,

the baseline fibrosis stage defined by LSM was not associated with LSM regression after SVR (Table 5). Few studies described that baseline LSM [23] and histological assessment [35] are predictor factors of liver stiffness regression post-SVR. On the other hand, few studies observed a decrease in liver stiffness independently of the baseline fibrosis stage.[16,21]

LSM regression might be related to suppression of hepatic inflammation post-SVR rather than regression of fibrosis.[36] However, the correlation of LSM reduction with elimination of necro-inflammatory activity post-SVR remains controversial. In the present study, baseline aminotransferase levels were not associated with significant LSM regression (Table 5), reinforcing previous publications.[17,23] On the other hand, few studies have reported a significant relationship between LSM reduction and normalization of ALT levels post-SVR[16,35] that might be associated with an early evaluation of fibrosis by LSM after SVR coinciding with the necro-inflammatory resolution after HCV clearance.

In the present study, absence of type-2 diabetes and presence of high platelet count were independently associated with significant LSM regression in the multivariate time-dependent analysis [Cox model] (Table 5). Type-2 diabetes has been associated with more advanced liver disease, and a risk factor for hepatocellular carcinoma in non-cirrhotic patients with SVR.[7] A recent study described that SVR in patients with type 2 diabetes significantly reduced incidence of extra-hepatic complications regardless of cirrhosis supporting antiviral therapy in these patients.[37] We acknowledge that the prevalence of type-2 diabetes in our study was higher than those described in previous publications of DAA effectiveness [16,20,21,34] or in Brazilian general population.[38] Low platelets count has been considered as a surrogate marker of advanced liver disease and portal hypertension, especially when associated with high levels of LSM.[39] In our study low platelets count was associated with lower rates of LSM reduction after SVR. On the other hand, the presence of a negative Baveno VI criteria [39] based on LSM (< 20 kPa) and platelet count ($\geq 150 \times 10^9/mm^3$) was associated with a higher risk of LSM

regression. These findings were similar to few studies that demonstrated scarce improvements in portal hypertension regardless HCV eradication.[40] Patients with portal hypertension remained at high risk for liver decompensation in the first 5 years after SVR and low platelet count was associated with sever outcomes.[41] Previous studies suggest that LSM have a high correlation with portal hypertension in pre-treatment scenario, but LSM thresholds after SVR must be described and further validated.[33,40] Up to 30% of patients with cirrhosis based on LSM on pre-treatment exam, would be classified as non-cirrhotic patients after SVR. This finding might be associated with the artificial determination of LSM thresholds in studies with HCV-infected patients. The classical 12.5 kPa LSM cut-off had 38% of false-positive for diagnosis of cirrhosis in a study in a limited sample size of CHC patients after SVR using liver biopsy as the reference.[42] LSM thresholds for detection of cirrhosis in people living with HCV infection might be inadequate after SVR.

The major limitations of this study were the retrospective study design and the lack of liver biopsy to assess fibrosis pre-treatment and post-SVR. Liver biopsy has been challenged by limited feasibility, potential complications and sampling error. In addition, the risk of performing a liver biopsy in SVR patients may not be justified. The relative short follow-up time of this cohort hinders the impact of the dynamics of LSM on liver-related complications. Another criticism might be the lack of effectiveness analysis of newer single-pill or pan-genotypic regimens (SOF/ledispavir, SOF/velpastavir), because these regimens were not available in Brazil between 2015 and 2018. We acknowledge a risk of inter-observer variability between pre-treatment and post-SVR LSM and a potential selection bias to evaluate LSM changing after SVR since the prevalence of cirrhosis was significantly lower in patients included compared to excluded for this analysis (Supplementary Table 3). This might be explained by the fact that LSM was not mandatory to access DAA treatment in patients with

cirrhosis detected by liver biopsy or presence of portal hypertension according to the Brazilian guidelines.

The main strength of this study relies on the fact that we included the largest sample size to evaluate effectiveness of DAA treatment (n=671) and changing of LSM after SVR (n=400) in Latin America. Medical records were reviewed by trained investigators and TE examinations were performed in a fasting status by experienced operators. For the analysis of LSM changing post-SVR, we only included patients who had TE exams by the same probe to avoid the potential bias of difference of LSM related to technical aspects.

In conclusion, DAAs were highly effective and safe for HCV treatment and LSM significantly decrease after-SVR in a real-life cohort in Latin America. DAA regimens, such as SOF/DCV or SOF/SIM, can be used in a resource-limited setting to eliminate HCV leading to the decrease of the burden of HCV in low-to-middle income countries. Absence of type-2 diabetes and high platelet count were independently associated with significant LSM regression post-SVR in this large sample size study. However, further long-term longitudinal studies are necessary to assess the impact of this LSM reduction in overall mortality and incidence of liver-related outcomes.

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Table 1. Characteristic of patients treated by DAAs

	All (n=671)
Female gender †	420 (63)
Age, years ¶	62 (55-68)
Type-2 diabetes †	236 (35)
Hypertension †	366 (55)
Dyslipidemia †	54 (8)
HCV genotype †	
Genotype 1	600 (89)
Genotype 2	11 (2)
Genotype 3	51 (8)
Genotype 4 or unknown	9 (1)
HCV viral load, log ¶	5.93 (5.38-6.95)
HIV co-infection †	53 (8)
Previous treatment †	
Treatment naive	441 (66)
Peg-interferon / Ribavirina	195 (29)
Peg-interferon / Ribavirina / Boceprevir or Telaprevir	33 (5)
Liver stiffness measument (LSM), kPa ¶	13.4 (9.9-21.8)
F0F1 (LSM < 7.1 kPa) †	59 (12)
F2 (LSM = 7.1 - 9.4) †	49 (10)
F3 (LSM = 9.5 - 12..4) †	126 (25)
F4 (LSM ≥ 12.5) †	269 (53)
Cirrhosis †	427 (64)
Child-Pugh score ¶	5 (4-6)
Child-Pugh B/C †	54 (15)
MELD score ¶	9 (8-11)
MELD score > 10 †	144 (41)
HCV treatment regimen [a]	
Sofosbuvir/Daclastavir ± Ribavirin	499 (74)
Sofosbuvir/Simeprevir ± Ribavirin	138 (21)
Ombitasvir/Veruprevir/ritonavir/Dasabuvir	22 (3)
Sofosbuvir/Ribavirin	7 (1)
Sofosbuvir/Peg-interferon/Ribavirin	5 (1)
Biochemistry	
ALT, UI/L ¶	79 (52-124)
AST, UI/L ¶	68 (44-107)
Total bilirubin, mg/dL ¶	0.7 (0.5-1.1)
Albumin, mg/dL ¶	3.7 (3.4-4.0)
INR ¶	1.14 (1.06-1.25)
Glucose, mg/dL ¶	98 (88-115)
Creatinine, mg/dL ¶	0.8 (0.7-1.0)
Sodium, mEq/L ¶	140 (138-142)
Platelet count, $x10^3/mm^3$ ¶	156 (106-204)

Data expressed as † n (%) and ¶ median (IQR, interquartile range); HCV, hepatitis C virus; LSM, liver stiffness measurement; MELD, model for end-stage liver disease; INR, international ratio. Child-Pugh and MELD score were calculated only in patients with cirrhosis (n=427). LSM: not available (n=136), unreliable (n=32) and reliable (n=503). Missing data (n): type-2 diabetes (9), hypertension (7), dyslipidemia (29), HCV viral load (101), Child-Pugh score (75), MELD score (73), ALT (41), AST (34), total bilirubin (63), albumin (87), INR (113), glucose (60), creatinine (53), sodium (97), platelet count (14).

Table 2. Sustained virological response (SVR) rates by intention-to-treat (n=671) and per-protocol (n=649) analyses

	Intention-to-treat (ITT) SVR		Per-protocol SVR	
	n/N	**% [95%CI]**	**n/N**	**% [95%CI]**
Overall	635/671	94.6% [92.7-96.1]	635/649	97.8% [96.4-98.7]
Cirrhosis				
No	236/244	96.7% [93.6-98.4]	236/238	99.2% [96.7-99.8]
Yes	399/427	93.4% [90.7-95.4]	399/411	97.1% [94.9-98.3]
p value		0.070		0.079
Decompensated cirrhosis				
No	288/298	96.6% [93.9-98.2]	288/295	97.6% [95.1-98.9]
Yes	46/54	85.2% [72.9-92.5]	46/49	93.9% [82.4-98.0]
p value		< 0.001		0.148
Genotype				
Genotype 1	571/600	95.2% [93.1-96.6]	571/580	98.5% [97.0-99.2]
Genotype 2	10/11	90.9% [53.6-98.9]	10/11	90.9% [53.6-98.9]
Genotype 3	48/51	94.1% [83.1-98.1]	48/50	96.0% [85.2-99.0]
p value		0.776		0.096
G1 sub-type				
Genotype 1a	213/222	95.9% [92.4-97.9]	213/214	99.5% [96.7-99.9]
Genotype 1b	212/227	93.4% [89.3-96.0]	212/217	97.7% [94.6-99.0]
Unknown sub-type	210/222	96.7% [92.3-98.6]	210/218	98.0% [93.9-99.4]
p value		0.486		0.071
HIV co-infection				
No	591/618	95.6% [93.7-97.0]	591/604	97.9% [96.3-98.8]
Yes	44/53	83.0% [70.3-91.0]	44/45	97.8% [85.5-99.7]
p value		< 0.001		0.975
Previous treatment				
Treatment naive	414/441	96.0% [92.6-97.9]	414/424	98.2% [95.3-99.3]
Treatment experimented	219/230	93.9% [91.2-95.8]	219/225	97.6% [95.7-98.7]
p value		0.237		0.639
DAA regimen				
SOF/DCV± RBV	469/499	94.0% [91.5-95.8]	469/477	98.3% [96.7-99.2]
SOF/SIM± RBV	133/138	96.4% [91.6-98.5]	133/138	96.4% [91.6-98.5]
Ombitasvir/Veruprevir ritonavir/Dasabuvir	22/22	100% [-]	22/22	100% [-]
SOF/RBV	6/7	85.7% [37.8-98.3]	6/7	85.7% [37.8-98.3]
PEG-IFN/SOF/RBV	5/5	100% [-]	5/5	100% [-]
p value		0.425		0.116

SVR rates were compared between groups by Chi-square test. CI, confidence interval; SOF/DCV± RBV, Sofosbuvir/Daclastavir ± Ribavirina; SOF/SIM± RBV, Sofosbuvir/Simeprevir ± Ribavirina; 3D, Ombitasvir / Veruprevir / ritonavir / Dasabuvir; SOF/RBV, Sofosbuvir/Ribavirina; PEG-IFN/SOF/RBV, Sofosbuvir/Peg-interferon/Ribavirina. Cirrhosis was defined by clinical, imaging tests, presence of oesophageal varices or liver stiffness measurement > 12.5 kPa. Decompensated cirrhosis was defined as Child-Pugh classification B or C. SVR rates stratified by decompensated cirrhosis were calculated only in patients with cirrhosis (n=427) and had missing data for 75 patients.

Table 3. Prevalence of adverse events during treatment by direct-acting agents in HCV-infected patients (n=671)

	All (n=671)		No cirrhosis (n=244)		Cirrhosis (n=427)		p value
Adverse events (AE)	**n**	**% [95%CI]**	**n**	**% [95%CI]**	**n**	**% [95%CI]**	
Acute liver injury [ALT>5xULN]	3	0.5% [0.1-1.4]	1	0.4% [0.1-2.9]	2	0.5% [0.1-1.9]	0.913
Anemia	64	9.6% [7.6-12.1]	13	5.3% [3.1-9.0]	51	12.0% [9.2-15.5]	0.005
Arrytmia	3	0.5% [0.1-1.4]	1	0.4% [0.1-2.9]	2	0.5% [0.1-1.9]	0.912
Acute kidney injury	40	6.0% [4.4-8.0]	11	4.5% [2.5-8.0]	29	6.8% [4.8-9.6]	0.227
Hepatic Encephalopathy	9	1.3% [0.7-2.6]	0	0% [-]	9	2.1% [1.1-4.0]	0.023
Variceal bleeding	5	0.8% [0.3-1.8]	0	0% [-]	5	1.2% [0.5-2.8]	0.091
Hepatocellular carcinoma	1	0.2% [0.1-1.1]	0	0% [-]	1	0.2% [0.1-1.7]	0.450
Death	1	0.2% [0.1-1.1]	0	0% [-]	1	0.2% [0.1-1.7]	0.450
At least one AE during treatment	**107**	**16.0% [13.4-18.9]**	**24**	**9.8% [6.7-14.3]**	**83**	**19.4% [15.9-23.5]**	**0.001**

Definition of AE:

Missing (n): anemia (9), arritmya (8), acute kidney injury (9), hepatic encephalopathy (9), variceal bleeding (9), hepatocellular carcinoma (10)

Table 4. Factors associated with at least one adverse event (AE) during treatment by direct-acting agents in HCV-infected patients (n=671)

	Univariate analysis				Multivariate analysis	
Variables	**No AE (n=564)**	**At least one AE (n=107)**	**OR [95%CI]**	**p value**	**OR [95%CI]**	**p value**
Male gender (vs female)	207 (38)	34 (32)	0.75 [0.48-1.16]	0.190		
Age, years (per 10-years)	61 [54-67]	65 [57-69]	1.01 [0.97-1.05]	0.736		
Type 2 diabetes (vs no)	196 (35)	40 (37)	1.09 [0.71-1.68]	0.683		
Hypertension (vs no)	296 (53)	70 (66)	1.72 [1.11-2.66]	0.014	1.02 [0.99-1.05]	0.247
Dyslipidemia (vs no)	39 (7)	15 (14)	2.16 [1.14-4.08]	0.018	0.99 [0.98-1.01]	0.608
HIV co-infection (vs no)	49 (9)	4 (4)	0.41 [0.14-1.16]	0.092	0.50 [0.06-3.98]	0.510
Genotype 1 (vs others)	503 (89)	97 (91)	1.18 [0.58-2.38]	0.651		
Treatment experimented (vs naive)	193 (34)	35 (33)	0.93 [0.60-1.44]	0.744		
Ribavirin use (vs no)	285 (51)	86 (80)	4.01 [2.42-6.64]	< 0.001	4.00 [1.99-8.04]	< 0.001
Cirrhosis (vs no)	344 (61)	83 (78)	2.21 [1.36-3.59]	0.001		
Decompensated cirrhosis (vs compensated)	37 (9)	18 (21)	2.68 [1.45-4.98]	0.002	1.38 [0.66-2.89]	0.385
24 weeks treatment (vs 12 weeks)	79 (14)	41 (39)	3.87 [2.45-6.11]	< 0.001	2.21 [1.23-3.97]	0.008

Table 5. Cox-proportional analysis to identify factors associated with a decrease in at least 30% of liver stiffness measurement post-sustained virological response at 12 weeks (SVR12) after direct-acting agents (DAA) treatment in patients with pre- and post-SVR12 transient elastography (n=400)

	Univariate analysis		Multivariate analysis	
	HR [95%CI]	**p value**	**HR [95%CI]**	**p value**
Demographic characterisitcs and metabolic features				
Male gender	reference			
Female gender	1.05 [0.76-1.46]	0.753		
Age ≥ 45 years	reference			
Age < 45 years	1.26 [0.77-2.04]	0.356		
Type-2 diabetes	reference		reference	
Absence of type 2 diabetes	1.59 [1.11-2.29]	0.012	1.52 [1.05-2.21]	0.028
Hypertension	reference			
Absence of hypertension	0.78 [0.57-1.07]	0.118		
Dyslipidemia	reference			
Absence of dyslipidemia	0.99 [0.57-1.73]	0.991		
HCV and DAA treatment features				
Cirrhosis	reference			
No cirrhosis	1.30 [0.94-1.80]	0.113		
HCV mono-infection	reference			
HIV-HCV co-infection	1.51 [0.90-2.54]	0.121		
Genotype 2, 3 or 4	reference			
Genotype 1	1.70 [0.87-3.33]	0.122		
Treatment experimented	reference		reference	
Treatment naive	1.37 [0.98-1.90]	0.063	1.35 [0.97-1.90]	0.080
No ribavirina	reference		reference	
Ribavirin use	0.75 [0.55-1.03]	0.073	1.01 [0.72-1.40]	0.989
Treatment for 24 weeks	reference			
Treatment for 12 weeks	1.54 [0.90-2.62]	0.114		
Pre-treatment liver stiffness measurement (LSM)				
F0F1 (LSM < 7.1 kPa)	reference			
F2 (LSM = 7.1 - 9.4)	1.19 [0.46-3.10]	0.715		
F3 (LSM = 9.5 - 12..4)	1.69 [0.80-3.59]	0.170		
F4 (LSM ≥ 12.5)	1.22 [0.59-2.53]	0.590		
Pre-treatment laboratory parameters				
ALT < 40 UI/L	reference			
ALT = 40-119 UI/L	1.22 [0.71-2.09]	0.480		
ALT = 120-199 UI/L	0.73 [0.38-1.37]	0.324		
ALT ≥ 200 UI/L	0.96 [0.37-2.47]	0.926		
Platelet count < 150 x10^3/mm^3	reference		reference	
Platelet count ≥ 150 x10^3/mm^3	1.78 [1.27-2.49]	0.001	1.75 [1.23-2.50]	0.002

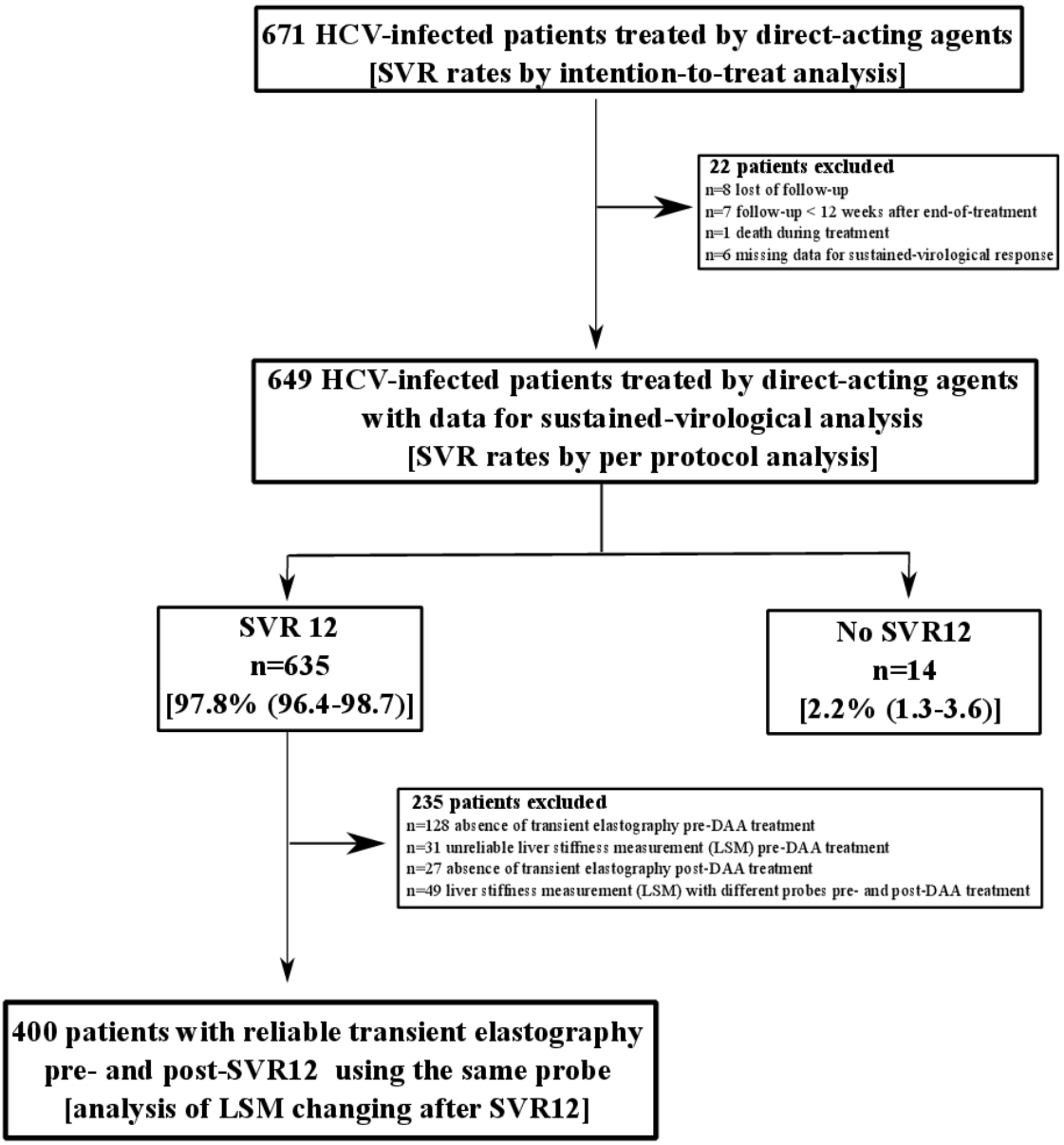

Figure 1. Study flow chart of patient's inclusion of analysis on DAA effectiveness and LSM changing after SVR