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Article type : Original Paper

Title: Implications of HCV RNA level at week 4 of direct antiviral treatments for Hepatitis C

Short Running Title: Week 4 viral load and SVR

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Declaration of Funding Sources

The study was funded by a Merit Review grant (I01CX001156), Clinical Science Research and Development, Office of Research and Development, Veterans Affairs (GNI).

Role of Funding Source

The funding source played no role in study design, collection, analysis or interpretation of data.

Declaration of Personal Interests

None

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jvh.12731

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Authors' Contributions and Authorship Statement

All authors approved the final version of the manuscript

George Ioannou is the guarantor of this paper.

George Ioannou: Study concept and design, acquisition of data, statistical analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, obtained funding.

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ABBREVIATIONS:

DBQ = Detectable below quantification

DAQ ≤42 = Detectable above quantification with an HCV RNA level ≤42 IU/mL

DAQ>42 = Detectable above quantification with an HCV RNA level > 42 IU/mL

W4VL = week 4 viral load (the HCV RNA viral load 4 weeks after initiation of antiviral treatment)

ABSTRACT

We aimed to determine whether the HCV viral load after four weeks of treatment (W4VL) with direct-acting antiviral agents (DAAs) predicts sustained virologic response (SVR) in a real-world clinical setting. We identified 21,095 patients who initiated DAA-based antiviral treatment in the national Veterans Affairs (VA) healthcare system from 01/01/2014 to 06/30/2015. Week 4 viral load was categorized as undetectable, detectable below quantification, detectable above quantification with viral load ≤42 IU/mL and detectable above quantification with viral load > 42 IU/mL. Week 4 viral load was undetectable in 36.1%, detectable below quantification in 45.6%, detectable above quantification ≤42 in 9.3%, detectable above quantification >42 in 9.1%. Detectable above quantification was much more common and undetectable week 4 viral load much less common when tested with the Abbott RealTime HCV assay versus the Roche COBAS AmpliPrep/COBAS TaqMan Version 2 assay. Compared to patients with undetectable week 4 viral load (SVR=93.5%), those with detectable below quantification (SVR=91.8%, adjusted odds ratio [AOR] 0.79, p-value=0.001), detectable above quantification ≤42 (SVR=90.0%, AOR 0.63, p-value<0.001) and detectable above quantification >42 (SVR=86.2%, AOR 0.52, p-value<0.001) had progressively lower likelihood of achieving SVR after adjusting for baseline characteristics and treatment duration. Among genotype 1-infected patients who were potentially eligible for 8-week sofosbuvir/ledipasvir monotherapy, we did not find evidence that treatment for 12 weeks instead of 8 weeks was associated with higher SVR, even among those with detectable above quantification. In summary detectable below quantification and detectable above quantification W4VL are very common in real-world practice, contrary to what was reported in clinical trials, and strongly predict reduced SVR across genotypes and clinically-relevant patient subgroups. Whether and how week 4 viral load results should influence treatment decisions requires further study.

Keywords

Response-guided therapy

INTRODUCTION

On-treatment HCV RNA levels were often used in the past to gauge response to interferon-based antiviral treatment and to determine the appropriate duration of treatment, a concept that was known as "response-guided therapy". In the current era of highly effective direct-acting antiviral agents (DAA), it is less clear whether to measure and how to use on-treatment HCV RNA levels. In randomized controlled trials of ledipasvir/sofosbuvir (LDV/SOF) with or without ribavirin, the proportion of patients with quantifiable HCV RNA level (HCV RNA ≥25 IU/ml) at week 4 of treatment, which was defined as a level ≥25 IU/ml, was well below 1%[1-3], suggesting that little information could be gained by this test since it was almost uniformly negative. However, the joint guidelines from American Association for the Study of Liver Diseases/Infectious Disease Society of America recommend measuring HCV RNA viral load 4 weeks after initiating antiviral treatment[4]. In practice, HCV RNA viral loads are frequently measured at 4-week intervals during antiviral treatment, partly as a way to assess compliance.

In a study of real-world clinical practice, week-4 viral load (W4VL) was found to be detectable in approximately 24% of treatment-naïve, genotype 1-infected patients treated with LDV/SOF ± ribavirin[5], in stark contrast to the reports of randomized controlled trials[1, 2]. This study also showed a correlation between W4VL and sustained virologic response (SVR). In two other studies, week-4 virial load was correlated to SVR among patients with genotype 3 HCV infection treated with daclatasvir/sofosbuvir [6] or sofosbuvir/ribavirin [7].

Assuming that the W4VL does predict SVR, it is important to determine the magnitude of this association, and whether it should influence treatment decisions. For example, should patients with a detectable W4VL undergo longer duration of treatment in an effort to improve their SVR? Or, conversely, should patients with a detectable and quantifiable W4VL above a certain level discontinue treatment on the grounds of futility?

Our aim was to determine the extent to which W4VL predicts SVR for different genotypes, treatment regimens, and clinically relevant patient subgroups treated in a real-world clinical setting within the Veterans Affairs (VA) healthcare system. We also aimed to determine whether treating patients with genotype 1 HCV who are now considered eligible for 8 weeks of ledipasvir/sofosbuvir monotherapy with 12 weeks instead of 8 improves SVR in patients with detectable or quantifiable W4VL.

METHODS

Data Source

We extracted data from the VA Corporate Data Warehouse, a national repository of data obtained from the VA electronic medical records[8]. Data extracted included all pharmacy prescriptions, demographics, inpatient and outpatient visits, problem lists, procedures, vital signs, diagnostic tests, and laboratory tests. Data extended back to 10/01/1999 to determine whether patients had received prior HCV treatments and extended forward to 04/15/2016 to allow for completion of treatments and ascertainment of SVR.

Study Population and Antiviral Regimens

Out of 24,089 HCV antiviral regimens initiated in the VA nationally from 01/01/2014 (the month after SOF was approved by the FDA) to 06/30/2015, we excluded 2585 regimens that were no longer used or recommended by the time we analyzed our data (e.g. SOF + pegylated interferon (PEG) /ribavirin (RIBA) and SOF+RIBA for genotype 1-infected patients and all PEG/RIBA regimens). We additionally excluded 409 "duplicate" regimens, in which the same patient appeared to have received one very short "regimen" (e.g. 14-day regimen) followed at a later date by a longer course of the same regimen (these short, "duplicate" regimens were most likely erroneous or postponed prescriptions) leaving 21,095 patients in the current analysis, all of whom were treated with the direct antiviral agents SOF, simeprevir (SMV) + SOF, ledipasvir/sofosbuvir (LDV/SOF) or Paritaprevir/Ritonavir/Ombitasvir/Dasabuvir (PrOD) with or without ribavirin.

Baseline Characteristics

We ascertained age, gender, race/ethnicity, HCV genotype, baseline HCV viral load and important baseline laboratory tests**,** using the value of the test closest to the date treatment was initiated within the preceding 6 months. The FIB-4 score, a marker of hepatic fibrosis, was calculated using

the formula: FIB-4= [age x AST]/[platelets x $ALT^{1/2}$][9]. Cirrhosis, decompensated cirrhosis, hepatocellular carcinoma, alcohol use disorders, substance use disorders, depression, diabetes and HIV infection were identified by appropriate ICD-9 codes (shown in Supplemental Table 1) recorded at least twice in inpatient or outpatient medical records prior to treatment initiation. The ICD-9 codes used to define these comorbidities have been widely used and validated in national VA data [10-20].

Sustained Virologic Response

SVR was defined by an undetectable HCV viral load in all tests performed at least 12 weeks after the end of treatment [21]. If no viral load test was available >12 weeks after the end of treatment, then SVR was defined by a viral load performed 4-12 weeks after the end of treatment, which accounted for an additional 1,126 SVR determinations. This was justified because SVR ascertained based on viral load 4 weeks after the end of treatment was shown to have 98% concordance (positive predictive value 98%; negative predictive value 100%) with SVR ascertained based on viral load >12 weeks after the end of treatment in SOF-treated patients [21]. Duration of therapy and end of treatment were defined by the total duration of DAA prescriptions filled.

Week-4 Viral Load

W4VL was defined by an HCV RNA viral load performed within 21-35 days from the start of treatment and the closest one to 28 days if multiple tests were available. Results were categorized as 1) Undetectable, 2) Detectable below quantification (DBQ), meaning that HCV RNA was present but below the lower limit of quantification of the assay, 3) Detectable above quantification (DAQ), meaning HCV RNA was present and above the limit of quantification (See **Supplemental Figure 1**). The DAQ category was further subdivided into patients with a level ≤42 IU/mL (DAQ≤42)or a level > 42 IU/mL (DAQ>42), 42 IU/mL being the median viral load among those with DAQ.

The vast majority of VA hospitals use either the Roche COBAS AmpliPrep/COBAS TaqMan Version 2 (CAP/CTM Ver. 2) assay, henceforth referred to as the "Roche assay", which has a reported limit of quantification of 15 IU/mL, or the Abbott RealTime HCV (ART) assay, henceforth referred to as the

"Abbott assay", which has a reported limit of quantification of 12 IU/mL. Although the type of assay was not reported, we extrapolated from the reported limit of quantification (LOQ) of the results whether each facility used a Roche assay (when LOQ=15) an Abbot assay (when LOQ=12) or another assay.

We determined for each assay the threshold value of W4VL that corresponded to the top 10% of all W4VLs.

Statistical Analysis

SVR rates and their 95% confidence intervals were determined by W4VL and by subgroups defined by genotype, treatment regimen, prior treatment, cirrhosis and other clinically relevant characteristics. We used multivariable logistic regression to determine whether W4VL was a predictor of SVR after adjusting for the following baseline characteristics: type of HCV RNA assay, age, gender, race/ethnicity, genotype/subgenotype, baseline viral load, regimen, platelet count, serum bilirubin level, serum albumin level, alcohol use disorder, diabetes, cirrhosis, decompensated cirrhosis, HCC, liver transplantation and prior treatment. In addition, we adjusted for duration of treatment since the duration of treatment could have been shortened or extended by the treatment providers based on W4VL and we wanted to determine the association between W4VL and SVR among persons with equal duration of treatment.

Analyses were performed using Stata/MP version 14.1(64-bit) (StataCorp, College Station, TX).

RESULTS

Rates of undetectable, DBQ and DAQ week-4 viral loads

W4VL was undetectable in 26.6% of the patients; DBQ in 33.6%; DAQ in 13.5% (including DAQ ≤42 in 6.8% and DAQ>42 in 6.7%); and missing in 26.2% of the patients (**Table 1**). Among patients with available data on W4VL, undetectable W4VL occurred in 36.1%, DBQ in 45.6% and DAQ≤42 in 9.3% and DAQ>42 in 9.1%.

Rates were very different by HCV RNA assay. Rates of undetectable W4VL were almost half with the Abbott versus the Roche assay (23.5% versus 39.4%) whereas rates of DAQ were almost triple (36.2% versus 13.2%).

The threshold W4VL resulting in the top 10% of W4VLs was >75 IU/mL for Abbot versus >23 IU/mL for Roche.

Characteristics associated with week-4 Viral Load

Baseline characteristics that were associated with high DAQ rate (≥15%) were: high baseline HCV viral load (> 6 million IU/mL), HIV co-infection, liver transplantation, cirrhosis, decompensated cirrhosis, hepatocellular carcinoma, elevated serum bilirubin, elevated INR, elevated FIB-4 score, low serum albumin and low platelet count (**Supplemental Table 2**). Using multivariable analysis, independent predictors of a week-4 viral load that was DAQ included type of assay used (Abbott vs Roche), black race, baseline viral load > 6 million IU/mL, genotype 3 HCV infection, cirrhosis, liver transplantation, low platelet counts, elevated bilirubin and low serum albumin (**Table 2**). Among genotype 1-infected patients, those who received SMV + SOF ± RIBA regimens were less likely to have DAQ or DBQ compared to those treated with other regimens.

Association between week-4 viral load and SVR

Of the 21,095 patients in this study, SVR data were available in 19,286 (91.4%), of whom 89.6% achieved SVR (**Table 3**). The proportion with missing SVR data was similar in those with undetectable (4.8%), DBQ (5.1%), DAQ≤42 (6.4%), or DAQ>42 (6.8%) and overall very low.

Among all patients, SVR was highest in those with W4VL that was undetectable, and progressively lower in those with DBQ , DAQ ≤42 DAQ>42 and missing W4VL, irrespective of HCV RNA assay used. This trend was true for subgroups of patients defined by HCV genotype, presence/absence of cirrhosis, treatment experienced/naïve or HIV co-infection. Since patients with a detectable W4VL may occasionally discontinue treatment, we repeated these analyses limiting to patients who completed 12 weeks of treatment; the SVR rate again progressively declined based on W4VL from undetectable, to DBQ and DAQ , but having a missing W4VL was no longer associated with the lowest SVR (**Table 3**).

We compared SVR rates of the top 10% W4VLs by the Abbot vs. the Roche assay. Patients with W4VL that was DAQ>75 using the Abbot assay had a similar SVR (SVR=83.6%, 95% CI 79.3-87.1) as patients with DAQ>23 using the Roche assay (SVR=85.8%, 95% CI 83.6-87.7).

After adjusting for all baseline characteristics listed in the legend of **Table 4**, including duration of treatment, increasing W4VL was still significantly associated with progressively lower likelihood of achieving SVR compared to undetectable viral load. When limiting to those who completed 12 weeks of treatment, the adjusted odds ratios (AOR) were slightly attenuated, but we found again that increasing W4VL was significantly associated with decreasing likelihood of achieving SVR in a stepwise manner. This pattern of associations persisted among patients with genotype 1 infection and cirrhotic patients but was less evident in patients with genotype 2 or 3 infection or treatment experienced patients.

Sustained virologic response in patients with genotype 1 HCV, comparing 8 versus 12 weeks of Ledipasvir/Sofosbuvir

It has been recommended that genotype 1-infected patients who are treatment-naïve, without cirrhosis and with a baseline HCV viral load ≤6 million IU/mL, can potentially be treated with 8 rather than 12 weeks of LDV/SOF monotherapy[4, 22]. We investigated whether W4VL can further characterize the appropriateness of these shorter regimens. Among patients treated for 8 weeks, those with W4VL DAQ >42 had lower likelihood of SVR than those with undetectable W4VL (AOR 0.37), which was just short of statistical significance (p=0.06) (**Table 5**). Among patients treated for 12 weeks, both patients with DAQ VL>42 and those with DAQ≤42 had lower likelihood of SVR than those with undetectable week-4 viral load, but the difference was not statistically significant. When comparing 12 weeks versus 8 weeks of LDV/SOF monotherapy among these patients who are considered potentially eligible for 8-week regimens, the 12-week regimens were not associated with significantly higher SVR rate, irrespective of the week-4 viral load (**Table 6**).

DISCUSSION

In this large, real-world study of DAA-based antiviral treatments for HCV, on-treatment W4VL was DBQ in 40.4%, DAQ≤42 in 19.4%, and DAQ>42 in 16.8% using Abbott assays and DBQ in 47%, DAQ≤42 in 6.3%, and DAQ>42 in 6.9% using Roche assays, which are much higher rates than what

had been reported in clinical trials. There was a strong "dose-response" association between W4VL and SVR such that SVR was highest in patients with undetectable W4VL (93.5%) followed by DBQ (91.8%), DAQ ≤42 (90%) and DAQ>42 (86.2%). Patients with DBQ, DAQ≤42 and DAQ>42 had progressively lower likelihood of achieving SVR compared to those with undetectable W4VL even after adjustment for baseline characteristics and treatment duration. This was true for all clinically relevant subgroups, such as different HCV genotypes, cirrhotic/non-cirrhotic and treatment naïve/experienced patients. However, the absolute difference in SVR between different categories of W4VL was relatively small, especially among patients who completed 12 weeks of treatment, such that even the worst category of DAQ>42 actually had a substantial SVR of 86.2% among all patients or 89.9% among those who completed 12 weeks of treatment. We did not find any evidence that extending duration of treatment from 8 to 12 weeks of LDV/SOF monotherapy increases SVR in patients with DBQ or DAQ, who were potentially eligible for 8-week regimens.

The proportion of all available W4VLs that were DBQ (40.4% for Abbott and 47.4% for Roche assays) or DAQ (36.2% for Abbott and 10.5% for Roche assays) was much greater than the DBQ rate (18.8-19.1%) and the DAQ rate (0.2-0.7%) reported in the ION1-3 randomized controlled trials[1-3]. Randomized trials may select for lower risk/more compliant patients than real-world patients, so their viral loads could fall more quickly. Perhaps more importantly, these three ION randomized trials used a Roche assay (COBAS TaqMan HCV Test, version 2.0, for use with the High Pure System) that is less sensitive for detecting extremely low levels of virus, with a LOQ of 25 IU/mL, as compared to LOQ of 12 or 15 IU/mL in our study. Subsequent studies reported that W4VLs were undetectable in only 10- 14% using the Abbot assay and 51-55% using the Roche assay among genotype 1-infected patients treated with SMV+SOF±RIBA or daclatasvir+SOF[7], which is closer to our finding of 23.5% (Abbott) and 39.4% (Roche) undetectable W4VL. Taken together with these studies, our findings suggest that DAQ and DBQ at W4VL is a common occurrence that merits further study and that it is much more common with the Abbott than with the Roche assays, as demonstrated by two other recent studies[23, 24]. Whether the higher positivity rate of the Abbott relative to the Roche assay is due to a greater true positive rate or a greater false positive rate or both remains to be determined.

Our results demonstrate that W4VL is a very strong, independent predictor of SVR, irrespective of assay used and among almost all clinically relevant subgroups of patients. Since this was an observational study, it is possible that W4VL might have influenced the duration of treatment and

hence the likelihood of SVR. For example, antiviral treatment might have been discontinued prematurely in the setting of a high W4VL or, conversely, extended for a longer duration in the setting of a detectable or low-quantifiable W4VL. For this reason, we adjusted all our analyses for duration of treatment and additionally limited analyses to patients who completed 12 weeks of treatment, which confirmed that W4VL is strongly associated with SVR independently of duration of treatment.

Many baseline characteristics that are negative predictors of SVR were also found to be predictors of positive W4VL as might be expected, such as genotype 3 HCV, baseline viral load > 6 million IU/mL, black race, cirrhosis, low platelet count and other laboratory markers of liver dysfunction. However, W4VL still predicted SVR even after adjustment for all these baseline characteristics suggesting that W4VL also reflects many other unmeasured patient, viral and provider predictors of SVR both known (such as patient compliance and *IFNL4* ("IL28B") genotype[25, 26]) and potentially unknown. Thus, W4VL incorporates the cumulative impact of many of these known and unknown baseline predictors into a single predictor that is much more efficient and easy to ascertain.

It is necessary to highlight that characteristics such as genotype 3 HCV, cirrhosis and markers of liver dysfunction still predicted SVR even after adjustment for W4VL, demonstrating that their association with SVR was not mediated entirely by W4VL. Furthermore, we found some characteristics that were associated with W4VL but not with SVR or vice versa. For example, liver transplantation was associated with a positive W4VL but not with SVR[27]; genotype 2 HCV and treatment with SMV+SOF±RIBA regimens were associated with undetectable W4VL but were actually associated with lower chance of SVR[28]. A potential explanation for this dissociation between on-treatment response and SVR could be relapse after the end of antiviral treatment, which may occur irrespective of on-treatment response.

For genotype 1-infected patients who are treatment-naïve, without cirrhosis and with a baseline HCV viral load ≤6 million IU/mL, treatment with 8 rather than 12 weeks of LDV/SOF monotherapy can be considered[4, 22], with considerable cost savings. It is controversial whether patients who fulfil the above criteria but have a W4VL that is DBQ or DAQ can still be treated with only 8 weeks or whether they have to extend to 12 weeks. It is tempting to assume that extending the duration of treatment in patients with DBQ or DAQ might increase their likelihood of SVR since DBQ and DAQ

are associated with lower SVR. However, if a positive W4VL is due to the development of viral resistance to an antiviral agent, then prolonging the treatment would not necessarily make a difference. We did not find any evidence that extending duration of treatment from 8 to 12 weeks of LDV/SOF monotherapy increases SVR in patients with DBQ or DAQ, who were potentially eligible for 8-week regimens. Our sample size in these subgroups was small and therefore a type 2 error cannot be excluded reliably. While awaiting further studies, it is reasonable to consider extending treatment to 12 weeks in patients with DAQ, as has been recommended in the most recent (March 2017) update of the VA hepatitis C treatment guidelines[29].

Although DAQ>42 was very strongly associated with lower likelihood of SVR, the absolute SVR rate (86.2%) was still very high in these patients (**Table 3**). Therefore, in agreement with other studies[6, 30], our results suggest that antiviral treatment should not be discontinued early based on a W4VL that is DAQ>42. We do advocate for checking the W4VL, however, in order to document compliance early in these very expensive treatment courses.

One limitation of our study is that W4VL was missing in a significant proportion (26.2%) of patients. Although absence of W4VL data clearly identified patients with higher likelihood of early discontinuation and lower likelihood of SVR, it is unlikely that this systematically biased our comparisons between different categories of W4VL among patients with available data. Also, our study is limited by missing SVR data; however, the rate of missing SVR data was very low among patients with available W4VL and was very similar for undetectable, DBQ, DAQ≤45 and DAQ>45 such that it is unlikely that any systematic bias was introduced in the association between W4VL and SVR rates. We did not have direct documentation of the HCV RNA assay system (Abbott vs. Roche); instead we extrapolated the assay from the reported lower limit of quantification. The striking differences in rates of DAQ that we found between the two assays suggests that we are categorizing them accurately. Another weakness of this study is its observational nature. A randomized trial would have been optimal to answer the question of whether patients with a W4VL that is DAQ benefit from extending treatment from 8 to 12 weeks, but such a trial is probably not feasible due to the very large sample size.

In conclusion, W4VL is detectable and even quantifiable a lot more frequently than what had been reported in clinical trials and a lot more commonly with Abbot than Roche assays. W4VL is a very robust predictor of SVR across almost all clinically subgroups. However, if and how W4VL results should be used to influence treatment decisions requires further study.

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Table 1. Categories of week-4 viral load in patients undergoing DAA-based antiviral treatment, subdivided by type of assay

Week-4 Viral Load		**Type of HCV RNA Assay**			
	All Patients (N=21,095)	**ABBOTT LOQ=12 IU/mL N=4,946**	**ROCHE LOQ=15 IU/mL N=15,730**	**OTHER N=419**	**ALL N=21,095**
Undetectable		856 (17.3%)	4578 (29.1%)	186 (44.4%)	5620 (26.6%)
DBQ		1467 (29.7%)	5513 (35.1%)	109 (26.0%)	7089 (33.6%)
DAQ≤42 IU/mL		704 (14.2%)	736 (4.7%)	2 (0.5%)	1442 (6.8%)
DAQ>42 IU/mL		609 (12.3%)	799 (5.1%)	3 (0.7%)	1411 (6.7%)
Missing		1310 (26.5%)	4104 (26.1%)	119 (28.4%)	5533 (26.2%)
	Patients with available week-4 viral load (n-15,562)	**ABBOTT LOQ=12 N=3,636**	**ROCHE LOQ=15 N=11,626**	**OTHER N=300**	**ALL N=15,562**
Undetectable		856 (23.5%)	4578 (39.4%)	186 (62.0%)	5620 (36.1%)
DBQ		1467 (40.4%)	5513 (47.4%)	109 (36.3%)	7089 (45.6%)
DAQ≤42		704 (19.4%)	736 (6.3%)	2 (0.7%)	1442 (9.3%)
DAQ>42		609 (16.8%)	799 (6.9%)	3 (1.0%)	1411 (9.1%)
DAQ>75 IU/mL for		375 (10.3%)	N/A	N/A	N/A

ABBOTT					
DAQ>23 IU/mL for ROCHE		N/A	1197 (10.3%)	N/A	N/A

DBQ : Detected below quantification

DAQ: Detected above quantification

LOQ: Limit of quantification

Table 2. Predictors of a positive (DAQ or DBQ) week 4 viral load

	DBQ vs Undetectable		**DAQ vs Undetectable**		**(DAQ or DBQ) vs Undetectable**	
	AOR*	**P value**	**AOR***	**P value**	**AOR***	**P value**
HCV RNA ASSAY						
Abbott	**1**		**1**		**1**	
Roche	**0.67**	<0.001	**0.19**	<0.001	**0.45**	<0.001
Other	**0.32**	<0.001	**0.02**	<0.001	**0.18**	<0.001
Male (vs Female)	**1.01**	0.9	**0.84**	0.2	**0.96**	0.7
Age (per year)	**1.0**	0.6	**1.0**	0.9	**1.0**	0.7
Race/Ethnicity						
White, non-Hispanic	**1**		**1**		**1**	
Black, non-Hispanic	**1.18**	<0.001	**1.49**	<0.001	**1.25**	<0.001
Hispanic	**0.92**	0.3	**0.96**	0.7	**0.91**	0.2
Other	**1.0**	1.0	**1.13**	0.5	**1.02**	0.9
Declined or Missing	**0.91**	0.1	**1.21**	0.02	**0.97**	0.6
Treatment Experienced (vs Naïve)	**1.07**	0.1	**1.17**	0.01	**1.09**	0.04

Genotype						
1	**1**		**1**		**1**	
2	**0.91**	0.1	**0.86**	0.01	**0.90**	0.06
3	**1.24**	0.008	**1.12**	0.3	**1.21**	0.01
4	**0.95**	0.8	**1.34**	0.3	**1.02**	0.9
Baseline HCV RNA Viral load >6 million IU/mL (vs ≤6 million IU/mL)	**1.09**	0.03	**1.59**	<0.001	**1.21**	<0.001
Cirrhosis	**1.11**	0.03	**1.25**	>0.001	**1.16**	0.001
Hepatocellular Carcinoma	**1.11**	0.4	**1.19**	0.2	**1.13**	0.3
Liver Transplantation	**1.25**	0.06	**1.45**	0.01	**1.30**	0.02
Diabetes	**0.99**	0.7	**0.87**	0.01	**0.95**	0.2
Alcohol Use Disorder	**0.96**	0.3	**0.95**	0.3	**0.96**	0.3
Platelet Count<100 k/µL	**1.15**	0.02	**1.59**	<0.001	**1.25**	<0.001
Bilirubin > 1.1 g/dL	**0.84**	0.002	**1.34**	<0.001	**0.96**	0.4
Albumin < 3.6 g/dL	**1.18**	0.001	**1.19**	0.009	**1.20**	<0.001
REGIMENS FOR GENOTYPE 1 HCV ONLY:						
LDV/SOF	**1**		**1**		**1**	

LDV/SOF + RIBA	**0.96**	0.5	**1.15**	0.1	**1.00**	0.9
PrOD	**1.11**	0.3	**0.98**	0.9	**1.09**	0.4
PrOD + RIBA	**0.96**	0.6	**1.21**	0.03	**1.02**	0.7
SMV + SOF	**0.71**	<0.001	**0.41**	<0.001	**0.62**	<0.001
SMV + SOF + RIBA	**0.48**	<0.001	**0.33**	<0.001	**0.44**	<0.001

*** Adjusted odds ratio (AOR) simultaneously adjusted for all the characteristics shown in the Table by multivariable logistic regression**

Table 3. SVR rates by week-4 viral load among clinically relevant subgroups.

	All Patients N=19,286	Undetectable	DBQ	DAQ VL≤42 IU/mL	DAQ VL>42 IU/mL	Missing
All Patients	89.6 (89.2-90.1)	93.5 (92.8-94.1)	91.8 (91.1-92.4)	90.0 (88.3-91.5)	86.2 (84.3-88.0)	82.8 (81.6-83.8)
Patients tested with the Abbott Assay	90.4 (89.5-91.2)	94.8 (93.0-96.1)	93.3 (91.8-94.5)	91.7 (89.3-93.6)	86.9 (83.8-89.4)	84.9 (82.8-86.9)
Patients tested with the Roche Assay	89.4 (88.8-89.9)	93.2 (92.4-93.9)	91.5 (90.7-92.2)	88.3 (85.7-90.5)	85.7 (83.0-88.1)	81.9 (80.5-83.2)
All Patients who completed 12 weeks of treatment	92.8 (92.4-93.4)	94.1 (93.3-94.9)	92.8 (92.1-93.5)	91.0 (88.9-92.7)	89.9 (87.5-91.8)	92.5 (91.3-93.5)
Genotype /Regimen						
1	91.1 (90.6-91.5)	94.7 (94.0-95.4)	93.1 (92.4-93.8)	91.9 (90.2-93.3)	87.9 (85.9-89.7)	84.3 (83.1-85.5)
SMV + SOF	84.4 (83.1-85.7)	91.6 (89.3-93.4)	86.4 (84.1-88.3)	85.0 (79.8-89.1)	79.4 (73.7-84.1)	74.0 (70.3-77.4)
SMV + SOF + RIBA	87.0 (84.0-89.6)	91.6 (86.6-94.8)	91.0 (85.5-94.5)	87.9 (70.6-95.6)	80.0 (65.2-89.5)	77.3 (69.1-83.9)
LDV/SOF	92.8 (92.2-93.4)	95.8 (94.9-96.6)	94.9 (94.0-95.7)	93.4 (90.8-95.3)	90.6 (87.6-93.0)	86.9 (85.2-88.3)
LDV/SOF + RIBA	92.0 (90.9-93.0)	95.2 (93.3-96.6)	92.6 (90.7-94.2)	91.9 (87.4-95.0)	90.8 (85.8-94.1)	87.7 (84.6-90.2)

PrOD	94.9 (93.0-96.3)	97.8 (94.2-99.2)	96.2 (93.1-98.0)	N/A*	94.4 (79.2-98.7)	88.5 (82.8-92.5)
PrOD + RIBA	92.5 (91.3-93.5)	94.4 (92.3-96.0)	96.5 (94.8-97.6)	95.4 (91.1-97.7)	89.9 (84.0-93.7)	84.1 (80.6-87.1)
2 SOF + RIBA	86.2 (84.5-87.7)	90.3 (87.7-92.4)	88.1 (85.3-90.4)	87.1 (78.9-92.5)	82.1 (73.4-88.4)	79.0 (75.1-82.5)
3	74.8 (72.2-77.3)	80.4 (75.2-84.7)	79.3 (75.1-83.0)	62.3 (50.1-73.2)	67.6 (55.6-77.7)	67.7 (62.0-73.0)
LDV/SOF + RIBA	77.9 (73.2-82.0)	75.5 (66.1-83.0)	81.6 (73.3-87.7)	76.9 (55.6-89.9)	70.0 (45.0-86.9)	78.1 (67.6-85.9)
SOF + PEG + RIBA	87.0 (80.0-91.8)	95.2 (82.0-98.9)	84.0 (70.6-92.0)	N/A*	N/A*	86.7 (68.0-95.2)
SOF + RIBA	70.6 (66.9-74.1)	79.4 (71.5-85.5)	77.3 (71.7-82.2)	52.6 (36.3-68.4)	66.0 50.9-78.4)	59.3 (51.6-66.5)
4 LDV/SOF or PrOD ± RIBA	89.6 (82.8-93.9)	97.1 (80.8-99.6)	90.5 (76.4-96.5)	N/A*	N/A*	86.7 (68.0-95.2)
No Cirrhosis	92.2 (91.7-92.7)	95.1 (94.4-95.8)	94.5 (93.8-95.2)	93.9 (92.0-95.4)	90.1 (87.7-92.0)	85.4 (84.1-86.6)
Cirrhosis	84.8 (83.9-85.6)	90.0 (88.5-91.3)	86.7 (85.3-88.0)	84.3 (81.0-87.2)	80.9 (77.4-84.1)	77.6 (75.4-79.6)
Decompensated Cirrhosis	79.5 (77.6-81.3)	87.5 (83.8-90.4)	80.6 (77.5-83.4)	79.2 (72.0-85.0)	69.3 (61.8-75.9)	74.7 (70.5-78.5)
Hepatocellular	73.7	80.7	72.2	78.3	63.5	71.3

Carcinoma	(70.0-77.0)	(73.2-86.5)	(65.7-77.9)	(63.5-88.2)	(49.2-75.7)	(63.7-77.9)
HIV	91.1 (88.9-92.9)	96.1 (92.0-98.1)	93.1 (89.5-95.5)	90.6 (78.7-96.1)	89.6 (79.3-95.0)	83.5 (77.2-88.3)
Liver Transplantation	93.8 (91.5-95.5)	96.4 (91.5-98.5)	95.5 (91.6-97.7)	97.4 (82.2-99.7)	98.0 (86.6-99.7)	86.6 (80.0-91.2)
Treatment Naïve	89.6 (89.1-90.1)	93.6 (92.8-94.3)	92.0 (91.2-92.7)	89.9 (87.9-91.6)	86.3 (84.0-88.3)	82.2 (80.9-83.5)
Treatment Experienced	89.7 (88.7-90.5)	93.1 (91.6-94.4)	91.2 (89.8-92.5)	90.3 (86.5-93.1)	86.1 (81.9-89.4)	84.4 (82.2-86.4)

N/A: Not applicable due to fewer than 15 patients in this subgroup

Table 4. Association between week-4 viral load and SVR in multivariable logistic regression models presented overall or by genotype, cirrhosis and treatment experience.

	All Patients				**Patents who completed 12 weeks of treatment**	
Week-4 Viral Load	**Crude Odds Ratio**	**p-value**	**Adjusted† Odds Ratio**	**p-value**	**Adjusted† Odds Ratio**	**p-value**
	All Patients					
Undetectable	**1**		**1**		**1**	
DBQ	**0.78**	<0.001	**0.79**	0.001	**0.83**	0.04
DAQ, VL≤42 IU/mL	**0.62**	<0.001	**0.63**	<0.001	**0.64**	0.002

DAQ, VL>42 IU/mL	**0.43**	<0.001	**0.52**	<0.001	**0.59**	<0.001
Missing	**0.33**	<0.001	**0.73**	<0.001	**0.73**	0.005
	All Patients, Abbott assays					
Undetectable	**1**		**1**		**1**	
DBQ	**0.76**	0.1	**0.75**	0.15	**0.75**	0.2
DAQ, VL≤42 IU/mL	**0.61**	0.02	**0.55**	0.008	**0.45**	0.004
DAQ, VL>42 IU/mL	**0.36**	<0.001	**0.35**	<0.001	**0.36**	<0.001
Missing	**0.31**	<0.001	**0.60**	0.01	**0.68**	0.17
	All Patients, Roche assays					
Undetectable	**1**		**1**		**1**	
DBQ	**0.78**	0.002	**0.81**	0.01	**0.86**	0.15
DAQ, VL≤42 IU/mL	**0.55**	<0.001	**0.62**	0.001	**0.74**	0.1
DAQ, VL>42 IU/mL	**0.44**	<0.001	**0.62**	<0.001	**0.73**	0.1
Missing	**0.33**	<0.001	**0.75**	0.002	**0.70**	0.004
	Genotype 1					
Undetectable	**1**		**1**		**1**	
DBQ	**0.75**	0.001	**0.77**	0.003	**0.81**	0.05
DAQ, VL≤42 IU/mL	**0.63**	<0.001	**0.66**	0.002	**0.60**	0.002
DAQ, VL>42 IU/mL	**0.40**	<0.001	**0.51**	<0.001	**0.54**	<0.001
Missing	**0.30**	<0.001	**0.69**	<0.001	**0.67**	0.002
	Genotype 1, LDV/SOF monotherapy					
Undetectable	**1**		**1**		**1**	
DBQ	**0.81**	0.1	**0.79**	0.1	**0.77**	0.2
DAQ, VL≤42 IU/mL	**0.62**	0.03	**0.57**	0.02	**0.56**	0.06
DAQ, VL>42 IU/mL	**0.42**	<0.001	**0.48**	0.001	**0.47**	0.01
Missing	**0.29**	<0.001	**0.65**	0.006	**0.68**	0.1
	Genotype 1, LDV/SOF + Ribavirin					
Undetectable	**1**		**1**		**1**	

DBQ	**0.63**	0.04	**0.62**	0.04	**0.71**	0.2
DAQ, VL≤42 IU/mL	**0.57**	0.08	**0.53**	0.06	**0.45**	0.02
DAQ, VL>42 IU/mL	**0.49**	0.02	**0.53**	0.07	**0.78**	0.6
Missing	**0.36**	<0.001	**0.70**	0.2	**0.60**	0.08
	Genotype 2					
Undetectable	**1**		**1**		**1**	
DBQ	**0.79**	0.2	**0.75**	0.2	**0.85**	0.5
DAQ, VL≤42 IU/mL	**0.73**	0.3	**0.89**	0.8	**1.0**	1.0
DAQ, VL>42 IU/mL	**0.49**	0.01	**0.60**	0.1	**0.84**	0.7
Missing	**0.40**	<0.001	**0.85**	0.5	**0.71**	0.2
	Genotype 3					
Undetectable	**1**		**1**			
DBQ	**0.94**	0.7	**0.91**	0.6	**N/A**	**N/A**
DAQ, VL≤42 IU/mL	**0.40**	0.002	**0.36**	0.002	**N/A**	**N/A**
DAQ, VL>42 IU/mL	**0.51**	0.02	**0.63**	0.2	**N/A**	**N/A**
Missing	**0.51**	0.001	**0.97**	0.9	**N/A**	**N/A**
	Cirrhosis					
Undetectable	**1**		**1**		**1**	
DBQ	**0.73**	0.002	**0.71**	0.001	**0.73**	0.02
DAQ, VL≤42 IU/mL	**0.60**	<0.001	**0.56**	<0.001	**0.56**	0.005
DAQ, VL>42 IU/mL	**0.47**	<0.001	**0.49**	<0.001	**0.47**	<0.001
Missing	**0.38**	<0.001	**0.67**	0.001	**0.61**	0.002
	Treatment Experienced					
Undetectable	**1**		**1**		**1**	
DBQ	**0.77**	0.06	**0.76**	0.07	**0.76**	0.2
DAQ, VL≤42 IU/mL	**0.69**	0.09	**0.64**	0.07	**0.54**	0.04
DAQ, VL>42 IU/mL	**0.46**	<0.001	**0.62**	0.04	**1.23**	0.7
Missing	**0.40**	<0.001	**0.83**	0.3	**0.68**	0.09

All P values are for comparisons to the reference group (undetectable W4VL) in predicting SVR

*** Genotype 4 infected patients were not modeled separately as there were too few for robust multivariable models**

† AOR: Adjusted odds ratio, by multivariable logistic regression modeling including week 4 viral load category, type of HCV RNA assay, duration of treatment, race/ethnicity, age, genotype/subgenotype, regimen , gender, baseline HCV viral load, platelet count, serum bilirubin level, serum albumin level, alcohol use disorder, diabetes, cirrhosis, decompensated cirrhosis, HCC, liver transplantation and prior treatment.

N/A Not applicable. Most genotype-3 infected patients were treated with 24 weeks of sofosbuvir and ribavirin.

Table 5. Association between week 4 viral load and SVR presented separately among genotype 1 patients treated with 8 or 12 weeks of LDV/SOF.

	Genotype 1 LDV/SOF 8 weeks n=1813			
Week-4 viral load	**Crude Odds Ratio**	**p-value**	**Adjusted† Odds Ratio**	**p-value**
Undetectable	**1**		**1**	
DBQ	**0.94**	0.8	**0.99**	1.0
DAQ **VL≤42 IU/mL**	**1.02**	1,0	**0.83**	0.8
DAQ **VL>42 IU/mL**	**0.39**	0.1	**0.31**	0.03
	Genotype 1 LDV/SOF 12 weeks n=1466			
Undetectable	**1**		**1**	
DBQ	**1.38**	0.4	**1.42**	0.4
DAQ **VL≤42 IU/mL**	**0.47**	0.08	**0.53**	0.2
DAQ **VL>42 IU/mL**	**0.43**	0.08	**0.52**	0.2

† Adjusted odds ratio, by multivariable logistic regression modeling including type of HCV RNA assay, race/ethnicity, age, gender, platelet count, serum bilirubin level, serum albumin level, alcohol use disorder, diabetes.

Table 6. Comparison of 12 versus 8 weeks of treatment with respect to SVR, according to week 4 viral load among genotype 1-infected patients without cirrhosis who are treatment naïve with a baseline viral load<6million.

Regimen	SVR12	Crude Odds Ratio	p-value	Adjusted Odds Ratio †	p-value
Week-4 viral load: Undetectable					
LDV/SOF 8 weeks n=646	**95.8**	1		1	
LDV/SOF 12 weeks n=412	**96.6**	1.24	0.5	1.21	0.6
Week-4 viral load: DBQ					
LDV/SOF 8 weeks n=677	**95.6**	1		1	
LDV/SOF 12 weeks n=524	**97.5**	1.82	0.08	1.89	0.08
Week-4 viral load: DAQ VL≤42 IU/mL					
LDV/SOF 8 weeks n=73	**95.9**	1		1	
LDV/SOF 12 weeks n=128	**93.0**	0.57	0.4	0.60	0.5
Week-4 viral load: DAQ VL>42 IU/mL					
LDV/SOF 8 weeks n=50	**90.0**	1		1	
LDV/SOF 12 weeks n=93	**92.5**	1.37	0.6	1.54	0.6

† Adjusted odds ratio, by multivariable logistic regression modeling including type of HCV RNA assay, race/ethnicity, age, gender, platelet count, serum bilirubin level, serum albumin level, alcohol use disorder, diabetes.