ORIGINAL RESEARCH

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Liver enzyme normalization predicts success of Hepatitis C oral direct-acting antiviral treatment

Abstract

Purpose: Monitoring of hepatitis C virus (HCV) treatment response is performed by serial HCV RNA measurements; however, this may not be useful for predicting treatment success or failure with oral direct-acting antiviral agent (DAA) therapies. Liver enzyme levels, which are elevated in chronic HCV and tend to decline on therapy, may serve as a more logistically and economically feasible alternative for monitoring treatment response.

Source: The Ottawa Hospital Viral Hepatitis Clinic patients (n=219), receiving interferon-free oral DAA treatments, were assessed for liver enzymes and HCV RNA levels at baseline, week 4 and ≥ 12 weeks post-treatment. Suppression cut points used for this analysis were ALT ≤ 40 U L⁻¹ and AST ≤ 30 U L⁻¹. The primary outcome was week 12 sustained virologic response (SVR). By our analysis, all indicators had strong PPV (>90%) but limited NPV (<25%).

Principal findings: Along with week 4 HCV RNA, AST ≤ 30 U L⁻¹ and AST:ALT ratio at week 4 were associated with SVR in univariate analysis with similar PPV and NPV to HCV RNA. ALT was not predictive of DAA outcome. In multivariate models, adjusting for cirrhosis and genotype, baseline AST:ALT ratio <0.9 (but none of the week 4 indicators) was significantly associated with SVR.

Conclusion: Our analysis suggests that enzyme levels (particularly AST and AST:ALT ratio) provide a viable alternative to HCV RNA, with robust predictive value in determining treatment success of DAA therapies.

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Chronic hepatitis C (CHC), which affects approximately 200 million people worldwide, poses a significant burden on the medical and economic systems of many countries, including Canada [1–3]. Along with recent treatment improvements, more accurate and less expensive methods for tracking patient progress and predicting treatment success are required.

A key goal of hepatitis C virus (HCV) therapy is to achieve a sustained virologic response (SVR), defined as undetectable serum HCV RNA levels 12 weeks post end of treatment (EOT)[4]. Even with newly approved oral direct-acting antiviral (DAA) regimens replacing interferon (IFN)-based therapy [1,5–8], HCV therapy remains lengthy (~12 weeks) and expensive. It is vital to be able to monitor patient response and predict the likelihood of achieving SVR.

Historically, undetectable serum HCV RNA levels at week 4 (rapid virologic response, RVR) has been used to monitor patient progress and predict the likelihood of SVR [9,10]; however, a recent study on genotype 1- infected patients receiving Sofosbuvir (SOF)-based non-IFN treatments has suggested that undetectable serum HCV RNA levels at week 4 (as well as at EOT) have poor negative predictive value (NPV) and moderate positive predictive value (PPV) [11]. The utility of RVR as a predictive tool in patients receiving IFN-free DAA regimens requires further evaluation and alternative predictors may need to be identified.

Baseline and on-treatment liver enzyme levels (alanine transaminase (ALT) and aspartate transaminase (AST) levels) may be of value as predictive indicators of treatment outcome. HCV and subsequent immune responses to HCV, particularly those of cytotoxic T lymphocytes, cause injury to hepatocytes in the liver, leading to leakage of liver enzymes and other biomarkers into the bloodstream elevating their levels in serum [12,13]. On-treatment normalization of liver enzymes is often observed in CHC patients and is considered to reflect a positive response to therapy.

A study on patients receiving Pegylated Interferon (PEG-IFN) and Ribavirin (RBV) combination treatment showed that, in patients with abnormal baseline ALT levels, normalization of ALT levels to 1.5 times the normal by week 4 of treatment was associated with SVR [14]. Another study showed that patients who achieved SVR had significantly lower ALT levels than non-responders [15]. Similarly, a recent evaluation of patients in our clinic indicated that normal ALT levels at week 4 predicted SVR in patients receiving IFN-based treatments, with moderate PPV but limited NPV [16].

To provide clinically useful data in the DAA era, we retrospectively evaluated the use of liver enzyme levels as

predictors of treatment outcome in CHC patients receiving IFN-free oral DAA treatments.

Methods

Setting and study design

Retrospective analysis of 219 adults with CHC initiating non-experimental oral DAA therapy at The Ottawa Hospital Division of Infectious Diseases Viral Hepatitis Clinic (Ottawa, Canada) between March 1, 2013 and December 31, 2015 was conducted. Patient records housed within the Viral Hepatitis Program Clinical Database were analyzed for baseline and on treatment indicators of virologic response to therapy and changes in liver enzymes. This study was reviewed and approved by the Ottawa Hospital Research Ethics Board [REB #2004-196].

HCV treatment protocol and patient monitoring

Patients were seen in clinical practice and the choice of HCV antiviral regimen was based on the discretion of the treating physician, patient HCV genotype and past treatment history and availability of funding. Patients were assessed for liver enzymes and HCV RNA levels at baseline, treatment week 4, end of therapy and ≥ 12 weeks after completion of therapy, with some patients undergoing additional testing at treatment weeks 2, 6 and 8. HCV RNA assay sensitivity was 15 IU mL⁻¹. Patients were categorized as having quantifiable HCV viremia (≥ 15 IU mL⁻¹) *vs.* Below Lower Limit of Quantification (BLIQ) (< 15 IU mL⁻¹), which included both target detected (TD) and target not detected (TND). Liver enzyme suppression cut points used for this analysis were ALT ≤ 40 U L⁻¹ and AST ≤ 30 U L⁻¹. AST:ALT ratio of 0.9 was considered to be normal based on the sample mean. Liver fibrosis levels by FibroScan or biopsy was categorized using the METAVIR system.

Exclusion criteria

Patients co-infected with hepatitis B virus (HBV), those with incomplete baseline or EOT ALT/AST records, and those with insufficient follow up to determine SVR were excluded from analysis. The majority of patients had undergone liver enzyme testing at treatment week 4. Patients missing week 4 ALT (n=5), AST (n=6) and HCV viral RNA (n=3) were excluded from week 4 analysis.

Data analysis

Categorical variables were described using frequency and percent, and continuous variables using mean $\pm$ standard

deviation. For use in logistic regression, dummy variables were generated for categorical variables, as well as for continuous variables that could be categorized as normal/elevated, such as baseline ALT, AST or AST:ALT ratio or the normalization of ALT, AST or AST:ALT ratio by week 4. Student's *t*-test was used for intergroup comparisons of continuous variables. Chi-square tests were used to compare categorical variables. Statistical significance in a two-tailed test was accepted at $p < 0.05$.

Univariate logistic regression models were run to identify variables associated with SVR at baseline and at week 4. Baseline transient elastography (FibroScan score) and HCV genotype was included as a covariate in adjusted multivariate models to account for fibrosis stage and genotype differences. Sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values for the use of normal liver enzymes and HCV RNA levels at week 4 (and baseline) as predictors of SVR were calculated. PPV was defined as the probability that SVR would occur in patients with normal ALT/AST or HCV RNA (BLQ) values at week 4, and NPV as the probability that SVR would not occur in patients with abnormal ALT/AST or detectable HCV RNA (quantifiable) values at week 4. Receiver Operating Characteristic curves (ROC) were generated and area under the curve (AUC) calculated to test the efficacy of the predictive models. All data were analysed using Stata (version 14).

Results

Population characteristics

Two hundred and nineteen DAA recipients who completed therapy and had SVR data comprised the analysis sample (Table 1). Patients were predominately white (84%), male (63%), with a mean age of 57 (± 9.5) years. Seven percent of patients were HIV-co-infected. The predominant genotypes were 1 (78.5%) and 3 (10%). The mean transient elastography score by FibroScan was 18.3 (± 16.6) kPa. At baseline, 45% of patients were cirrhotic (FibroScan score ≥ 13.0 kPa), 17 % had normal ALT and 19% had normal AST levels.

Combined treatment with SOF and Ledipasvir (LDV) (with or without RBV) was the most common DAA therapy used in genotype 1 patients (72%), followed by SOF/Simeprevir (SMV) (with or without RBV) (26%). Treatment with SOF and RBV was the primary therapy for genotypes 2 (88%) and 3 (73%). The majority of patients (73%) were treatment naïve.

Predictors of Sustained Virologic Response

In total, 201 of 219 patients (92%) achieved SVR. HCV genotype (3/4), race (non-white), and cirrhosis (by biopsy or FibroScan) were associated with reduced odds of achieving SVR in univariate analysis; genotype and cirrhosis remained significant in multivariate models ($p < 0.001$ for genotype, $p < 0.05$ for cirrhosis) (Table 2). Including treatment

FIGURE 1. Mean enzymatic change as a function of HCV antiviral therapy week by sustained virologic response status. W2 to w12 indicates treatment week 2 to treatment week 12; w4 pt and w12 pt indicate 4 weeks post-treatment and 12 weeks post-treatment, respectively; numbers indicate sample sizes at each therapy week.

TABLE 1. Baseline and on treatment patient characteristics of DAA recipients

	Overall		SVR		Not SVR		
Continuous variables	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	p
Age	218	56.9 (9.5)	200	56.9 (9.7)	18	57.5 (6.9)	0.80
HCV RNA (IU mL ⁻¹)	219	3.39x10 ⁶ (5.64x10 ⁶)	201	3.49x10 ⁶ (5.79x10 ⁶)	18	2.35x10 ⁶ (3.36x10 ⁶)	0.41
Biopsy stage	94	2.1 (1.2)	91	2.1 (1.2)	3	3.3 (1.2)	0.07
Baseline ALT (U L ⁻¹)	219	95.1 (71.2)	201	94 (71.1)	18	106.3 (73.1)	0.48
Baseline AST (U L ⁻¹)	219	74.5 (51.2)	201	70.6 (48.7)	18	117.6 (58.9)	<0.001
Fibroscan (kPa)	195	18.3 (16.6)	183	17.5 (16.6)	12	30.4 (12.4)	0.01
Baseline platelet (10 ⁹ L ⁻¹)	202	163.8 (69.5)	185	167.8 (69.4)	17	120.7 (56.3)	0.007
Baseline APRI*	202	0.6 (0.7)	185	0.6 (0.7)	17	1.2 (1.0)	<0.001
	Overall		SVR Achieved				p
Categorical variables	n/N	%	n/N	%			
Sex							0.421
Women	80/219	36.5	75/80	93.8			
Men	139/219	63.5	126/139	90.6			
Race							0.020
White	181/216	83.8	169/181	93.4			
Black	18/216	8.3	14/18	77.0			
Asian	15/216	6.9	14/15	93.3			
Aboriginal	2/216	0.9	1/2	50.0			
HCV genotype							0.000
1	172/219	78.5	167/172	97.1			
2	17/219	7.8	17/17	100.0			
3	22/219	10.0	12/22	54.5			
4	8/219	3.7	5/8	62.5			
HIV co-infection	16/219	7.3	13/16	81.3			0.111
Previous treatment							0.038
IFN experienced	58/219	26.5	51/58	87.9			
DAA experienced	2/219	0.9	1/2	50.0			
DAA regime							0.027
LDV+SOF +/-RBV	133/219	60.7	123/133	92.5			
SOF+SMV +/-RBV	50/219	22.8	49/50	98.0			
SOF+RBV	32/219	14.6	25/32	78.1			
DCV+SOF+RBV	1/219	0.5	1/1	100.0			
OBV/PVT/r +DCV	3/219	1.4	3/3	100.0			

*APRI = [(AST level/Upper Limit of Normal for AST) / Platelet Count] x 100

ALT, alanine transaminase; AST, aspartate transaminase; DCV, Daclatasvir; IFN, Interferon; OBV, Ombitasvir; PVT/r, Paritaprevir with ritonavir; RBV, Ribavirin; SMV, Simeprevir; SOF, Sofosbuvir

TABLE 2. Univariate and multivariate associations between patient characteristics, baseline and week 4 liver enzymes, HCV RNA and Sustained Virologic Response

Variable	Univariate			Multivariate Models*		
	Odds ratio	95% CI	p-Value	Odds ratio	95% CI	p-Value
Patient characteristics						
Female	1.55	(0.53 , 4.51)	0.424	-		
HIV	0.35	(0.09 , 1.35)	0.126	-		
Genotype (ref: others)						
Genotype 3/4	0.04	(0.01 , 0.11)	<0.001	0.03	(0.01 , 0.13)	0.000
Race (ref: Caucasian)						
Black/Asian/Aboriginal	0.34	(0.12 , 0.99)	0.047	0.50	(0.11 , 2.39)	0.386
Cirrhosis (Biopsy Stage 4; kPa $\geq$ 13kPa)	0.06	(0.01 , 0.50)	0.009	0.06	(0.01 , 0.52)	0.011
Baseline Indicators						
Baseline ALT $\leq$ 40 U L ⁻¹	1.02	(0.28 , 3.71)	0.978	-		
Baseline AST, $\leq$ 30 U L ⁻¹	6.62	(1.10 , n/a†)	0.036	0.67	(0.08 , n/a†)	>0.99
Baseline AST:ALT ratio <0.9	19.25	(4.29 , 86.38)	<0.001	8.22	(1.31 , 51.61)	0.025
Baseline RNA <800,000 IU mL ⁻¹	0.93	(0.32 , 2.74)	0.897	-		
Week 4 Indicators						
ALT $\leq$ 40 U L ⁻¹	1.54	(0.51 , 4.61)	0.442	-		
AST $\leq$ 30 U L ⁻¹	9.00	(2.81 , 28.84)	<0.001	1.84	(0.37 , 9.20)	0.458
RNA BLLQ (<15 IU mL ⁻¹)	4.07	(1.17 , 14.13)	0.027	4.37	(0.55 , 35.00)	0.165
AST:ALT ratio <0.9	7.37	(2.05 , 26.49)	0.002	1.17	(0.20 , 6.79)	0.862

*Adjusted for genotype and cirrhosis

†18/18 cases that did not achieve SVR had above normal baseline AST; model fit by exact methods with upper CI not available.

ALT, alanine transaminase; AST, aspartate transaminase; BLLQ, Below Lower Limit of Quantification; CI, confidence interval

experience in models was not statistically significantly associated with SVR (OR = 0.44, 95% CI 0.16-1.16, $p=0.10$) and this did not change when included in multivariate models. At baseline, normal AST (≤ 30 U L⁻¹) and AST:ALT ratio <0.9 were associated with an increased likelihood of SVR in univariate analysis, although both values had wide confidence intervals due to small numbers. Of note, no patient with normal range AST at baseline failed to achieve SVR. Baseline AST:ALT <0.9 remained predictive of SVR after adjustment for cirrhosis and genotype (OR 8.2 [95% CI: 1.3-51.6], $p<0.03$).

At week 4, HCV RNA <15 IU mL⁻¹ (BLLQ) was associated with SVR in univariate analyses (OR 4.1 [95% CI:

1.2-14.1], $p=0.03$), but this effect was not consistent in multivariate models adjusted for genotype and cirrhosis (OR 4.4 [95% CI: 0.6-35.0], $p=0.17$). Similarly, AST ≤ 30 U L⁻¹ and AST:ALT <0.9 at week 4 were predictive of SVR in univariate models (OR 9.0 [95% CI: 2.8-28.8], $p<0.001$ and OR 7.4 [95% CI: 2.1-26.5], $p=0.002$, respectively) but not in multivariate models. By week 4, 78% of patients had normal range ALT, 69% had normal range AST and 92% had undetectable viral load (BLLQ) (Figure 1). HCV RNA levels at week 4 (BLLQ vs quantifiable) exhibited a 93.4% PPV and a 22.2% NPV for SVR with an ROC area of 0.58 (Table 3). Week 4 AST and AST:ALT ratio had greater ROC areas compared with HCV RNA, although statistically significant

TABLE 3. Baseline and Week 4 ALT, AST and HCV RNA, association with SVR, positive and negative predictive values and area under the Receiver Operating Characteristic curve.

Timeline	Variable (cut point for 'normal')	% Above normal (n=219)	SVR-12		Suppression of Variable by Week 4 used to predict SVR		
			Variable Normal at Week 4	Variable Not Normal at Week 4	PPV	NPV	ROC AUC
Baseline	ALT > 40 U L ⁻¹	83.1%					
	AST > 30 U L ⁻¹	80.8%					
	AST:ALT > 0.9	34.3%					
Week 4	ALT > 40 U L ⁻¹	22.0% (47/214)	92.8% (155/167)	89.4% (42/47)	92.8%	10.6%	0.54
	AST > 30 U L ⁻¹	30.5% (65/213)	97.3% (144/148)	80.0% (52/65)*	97.3%	20.0%	0.75
	AST:ALT > 0.9	42.3% (90/213)	97.6% (120/123)	84.4% (76/90)*	97.6%	15.6%	0.72
	HCV RNA > 15 IU mL ⁻¹ ‡	8.3% (18/216)	93.4% (185/198)	77.8% (14/18) †	93.4%	22.2%	0.58
Total (per protocol)			91.8% (201/219)				

*p<0.001; †p=0.018; ‡any detectable virus *versus* BLLQ TD/TND (<15 IU mL⁻¹). ALT, alanine transaminase; AST, aspartate transaminase.

FIGURE 2. Receiver Operating Characteristic curves for sustained virologic response predicted by individual models of week 4 values of AST, ALT, AST:ALT and HCV RNA. Joint test for differences across models ($\chi^2[3 \text{ d.f.}] = 10.8, p = 0.0127$). ALT, alanine transaminase; AST, aspartate transaminase.

differences at conventional levels were not found between AST and HCV RNA ($p=0.09$) or AST:ALT and HCV RNA ($p=0.13$). A joint test of difference in ROC area was statistically significant ($\chi^2[3 \text{ d.f.}] = 10.8$, $p=0.0127$) (Figure 2). AST ≤ 30 at week 4 had 97.3% PPV and NPV of 20.0% for SVR with an ROC area of 0.75. Week 4 AST:ALT ratio had similar PPV (97.6%), NPV (15.6%) and ROC AUC (0.72). ALT $\leq 40 \text{ U L}^{-1}$ at week 4 was less useful as a predictor exhibiting a 92.8% PPV and a 10.6% NPV for SVR and ROC area of 0.54.

Modification of the cut points for normal ALT and AST to 30 U L^{-1} or 20 U L^{-1} , respectively, did not alter the findings substantially and similar associations were found with SVR among the subset of patients with liver enzyme levels above normal at baseline (data not shown). Furthermore, analysis of AST to Platelet Ratio Index (APRI) at baseline and week 4 were associated with SVR in unadjusted models, but this association did not hold when adjusted for genotype and cirrhosis (data not shown).

Discussion

Liver enzymes, often elevated in CHC patients, tend to decline to a normal range during HCV treatment. Based on our analysis in CHC patients receiving IFN-free oral DAA, liver enzymes appear to be a suitable alternative to viral load in monitoring and predicting treatment success. This is consistent with our previous analysis of IFN-treated HCV patients [16]. All four variables (ALT, AST, AST:ALT ratio and HCV RNA) at week 4 had strong PPV ($>90\%$) but low NPV ($<25\%$). AST and HCV RNA at week 4 had higher NPV than the other two variables and models with AST and AST:ALT ratio had higher AUC ROC values (although not statistically significant). All these measures are influenced by the prevalence of the outcome variable indicating a limited ability to identify future treatment failures on highly curative DAA therapies. AST levels and AST:ALT ratio at week 4 appear to be just as strong a predictor of treatment success as week 4 HCV RNA levels. These may thus serve as a readily available and inexpensive marker for treatment success and is a reasonable proxy to end of treatment testing. Our work also indicates that while normal range liver enzymes and undetectable viral loads at week 4 are strong indicators of treatment success, having abnormal liver enzymes or quantifiable viral RNA ($\geq 15 \text{ IU mL}^{-1}$) does not necessarily indicate a failed response to treatment (Table 3).

AST, AST:ALT ratio and HCV RNA at week 4 were associated with SVR by univariate analysis; but when adjusted for baseline FibroScan scores and genotype, these associations

did not hold. Only baseline AST:ALT ratio remained significant in multivariate analysis. A study correlating histological findings in HCV patients not receiving treatment to HCV RNA levels and liver enzymes, found that AST levels were particularly associated with liver fibrosis [17]. Also, AST:ALT ratios can be used to monitor liver disease progression, with values above 1 often indicative of advanced liver fibrosis, particularly in chronic hepatitis C patients [18,19]. Since abnormal AST alone at week 4 does not necessarily indicate a high likelihood of treatment failure, it might be preferable to use a panel of serum markers together to predict SVR. Other markers of chronic liver disease, including GGT, total bilirubin, alkaline phosphatase and albumin, used in tandem with liver enzymes may prove to be a better and more reliable predictor of treatment success [12]. Furthermore, since none of the variables remained significant when models were adjusted for genotype and cirrhosis, the importance of genotype and baseline liver fibrosis as predictors for SVR is highlighted. Analysis of APRI, which is a non-invasive measure of liver fibrosis, similarly displayed association with SVR in unadjusted models, but as expected, a degree of collinearity was introduced when used in models adjusted for cirrhosis, and this association was lost. This suggests the need for earlier detection and treatment of HCV, before liver fibrosis progresses to latter stages.

Overall, our analysis suggests that AST and AST:ALT ratio are suitable substitutes for HCV RNA levels for monitoring response to therapy, particularly exhibiting higher ROC AUC values in predictive models. This will be particularly beneficial for monitoring progress in patients in clinics or medical settings where specialized and expensive viral load testing may not be easily available; for example, in remote areas of Canada and developing regions of the world. A more economically feasible alternative to viral load testing may be well received and prove a successful tool in the treatment and monitoring of HCV treatment around the world, irrespective of access to diagnostic technologies and funding. We acknowledge that monitoring of HCV RNA levels may still have value in identifying rare on-treatment viral breakthroughs that may not be reflected in liver enzyme levels, but in our cohort, liver enzyme monitoring appears to preclude the need for viral load monitoring on-treatment, reducing the on-treatment cost burden of HCV therapy [11].

Limitations of this analysis include biases that may have been introduced through the exclusion of patients with incomplete baseline or EOT values. Other factors that affect liver enzyme levels including concurrent medications and alcohol consumption were not fully explored [14].

Furthermore, our analysis does not take into account genetic polymorphisms that have been shown to affect liver enzyme levels and treatment successes in HCV patients: for example, in *HLA-C* C2-positive individuals, ALT activity decreases more after treatment than in *HLA-C* C2-negative individuals [20]. Due to the high success rates of oral DAA HCV treatments, the proportion of our study cohort failing to achieve SVR was small, which limited the statistical power to identify factors predictive of treatment failure. Nevertheless, this analysis provides valuable insights, which enable the identification of patients likely to achieve treatment success.

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