

Hepatitis C Virus Core Antigen for On-treatment Prediction of Sustained Virological Response to Direct Acting Antivirals in Patients with Chronic Hepatitis C

Abstract:

Background and aim: Cirrhotic patients have lower response rates to DAAs with increased frequency of adverse events. This study aims to evaluate the value of HCVcAg as an early predictor of SVR to DAAs in cirrhotic patients to justify the treatment and avoid side effects.

Patients and Methods: This prospective cohort study was conducted on 85 treatment-naive HCV cirrhotic patients who had fulfilled the inclusion and exclusion criteria of the National Treatment Protocol of chronic HCV infection. HCVcAg detection was done on 10th day of treatment. Predictivity of HCVcAg for SVR was assessed in terms of specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and accuracy rates. ROC curve was conducted to assess predictivity of HCVcAg as well.

Results: SVR12 was (91.76%). HCVcAg was negative in 79 patients; 78 of them achieved SVR, while it was positive in 6 patients; all of whom did not achieve SVR. HCVcAg had sensitivity, specificity, PPV, NPV and accuracy rates of 100%, 98.73%, 85.71%, 100% and 98.82% respectively in prediction of SVR. With AUC of 0.929. There was a positive strong significant correlation between HCVcAg and SVR12 ($r = 0.898$, $P = 0.015$).

Conclusion: HCVcAg is a sensitive, specific, accurate, easily available, and affordable on-treatment predictor of SVR in cirrhotic patients with chronic HCV with possible future treatment regimen modification to improve efficacy and tolerability in difficult to treat cirrhotic patients.

Keywords: Hepatitis C Virus Core Antigen; Direct Acting Antivirals; Predictor; SVR; Chronic Hepatitis C.

Introduction

Introduction of directly acting antiviral (DAA) agents has revolutionized the treatment of HCV infection with high efficacy and excellent tolerability (1, 2). Viral eradication is expressed as a sustained virological response (SVR), defined as a negative HCV RNA 12 weeks after stopping DAAs (SVR 12) (3). Despite the fact that DAAs have shown very high efficacy with SVR rates (> 90%) (4), the outcome of DAA-based therapies may be still negatively impacted by certain host and viral factors as presence of comorbidities and/or advanced cirrhosis, presence of baseline or on-treatment resistance-associated substitutions (RASs) (5). Therefore, all latest guidelines still recommend monitoring of treatment efficacy 12-24 weeks after end of therapy. (6, 7).

Patients with cirrhosis have lower response rates than non-cirrhotic patients and increased frequency of serious adverse events with prolonged treatment duration especially in Child' B and C patients (8). Therefore, moving a stepwise earlier during the treatment course of these patients and finding a reasonable predictor of response with possible future on-treatment change of therapeutic regimens is therefore of utmost importance to justify the treatment and avoid the side effects.

HCVcAg is a viral protein released into the blood during HCV assembly. Since 1992, assays for the detection of HCVcAg has been established, gradually improved and proved as a stable, easy to operate, and affordable test (9, 10). The kinetics of HCVcAg are essentially identical to those of HCV RNA (11) as regards the early appearance in blood stream, less pronounced fluctuations of HCVcAg vs the HCV RNA and the later persistence in the circulation in parallel to HCV RNA (12). This would indicate that HCVcAg might be a substitute for HCV RNA testing. (11)

While HCVcAg has demonstrated a lower sensitivity and a high specificity when compared with HCV RNA, this lower sensitivity may provide a clinical advantage over HCV RNA testing in monitoring the response to treatment (13). While DAAs targeting HCV maturation and replication, direct antivirals might already prevent virus formation while HCVcAg is still produced; thereby correlates better with eventual viral clearance (14). Therefore, HCVcAg has been considered an alternative to HCV RNA measurements for monitoring treatment response in the latest EASL guidelines (6).

The aim of this study is to evaluate the value of HCVcAg as an early predictor of SVR to DAAs therapy in chronic HCV patients with cirrhosis paving the way for possible on-treatment modification of therapeutic regimens protecting the patients from adverse events of prolonged ineffective therapy with excess costs and maximizing the chance of cure of delicate group of chronic HCV patients.

Patients and Methods

This prospective cohort study was conducted on chronic hepatitis C patients attending the Viral Hepatitis Treatment Unit at Tanta University Teaching Hospital during the period from May 2017 to May 2018. Local ethical committee approval was taken before starting the study. All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

The study included 85 cirrhotic patients who had fulfilled the inclusion and exclusion criteria of the National Treatment Protocol of chronic HCV infection (December 2016).

All patients were subjected to the following: Full history taking, informed consent, complete clinical examination, Laboratory evaluation included (CBC, liver function tests, serum creatinine and estimated glomerular filtration rate (eGFR) (when required) , alpha-fetoprotein (AFP) and HBsAg.) , and abdominal ultrasound.

Cirrhosis was diagnosed with US, FIB4 value of >3.25 , and/or Fibroscan value of ≥ 12.5 kPascal when available. Evaluation of the severity of liver cirrhosis was assessed with the Child-Turcotte-Pugh (CTP) score.

HCV RNA (PCR) assay (Abbott Real time HCV, Abbott, Chicago, USA with lower limit 12 IU/ml) was done to evaluate viral load at baseline and 12 weeks after end of treatment (SVR12).

HCVcAg detection was done 10 days after starting treatment for every patient (The kit uses a double-antibody sandwich ELISA technique)

Treatment Protocol:

Eighty-five treatment-naive HCV cirrhotic patients were included in the study and given 12 weeks course of triple therapy (sofosbuvir, daclatasvir and ribavirin). Sofosbuvir was administered at the dose of 400 mg (one tablet) once per day, with or without food; daclatasvir was administered at the dose of 60 mg (one tablet) once daily, with or without food and ribavirin tablets (600-1000 mg/day), according to body weight & hemoglobin level.

Study endpoint:

The endpoint of therapy was undetectable HCV RNA in serum or plasma by a sensitive assay (lower limit of detection ≤ 12 IU/ml) 12 weeks (SVR12) after the end of treatment (8).

Statistical analysis

Statistical presentation and analysis of our study was conducted, by IBM SPSS statistics® version.22 and Microsoft excel® 2013 (XLSTAT® version 18.07.39066 and Real-Statistics Analysis Tool®). P value < 0.05 was considered statistically significant. Differences between normally distributed variables were examined by one-way analysis of variance (ANOVA). Tukey's test was used for post hoc analyses. The differences between non-normally distributed variables were examined by Kruskal-Wallis ANOVA using Dunn's test for post hoc analyses. To assess correlation, the Spearman rank correlation test was used. Reservoir observation character (ROC) curve was used to determine the cutoff value. P < 0.05 was considered significant in all tests used.

Results

This study was conducted on 85 cirrhotic patients with chronic HCV who received DAAs in Tanta University Viral Hepatitis Treatment Unit in the period from May 2017 to May 2018. There were 41 males (48.24%) and 44 females (51.76%). The age ranged between 27-75 years with a mean age of 53.882 ± 8.885 as shown in table (1).

Baseline laboratory data including CBC, liver functions, serum creatinine, fasting blood sugar and HbA1c are shown in Table (1). PCR before treatment ranged between 9600-16000000 with mean \pm SD 1506944.718 \pm 2451890.85

SVR12 was achieved in 78 patients (91.76%) versus 7 patients (8.24%) who didn't achieve SVR. HCVcAg detection were done after 10 days from starting treatment for every patient. It was negative in 79 patients; (78 of them achieved SVR and only one patient did not achieve SVR), while it was positive in 6 patients; all of them did not achieve SVR [Tables (2, 3)].

HCVcAg had sensitivity, specificity, positive predictive value, negative predictive value and accuracy rates of 100%, 98.73%, 85.71%, 100% and 98.82% respectively in prediction of SVR [Table (3)]

There was a positive strong significant correlation between HCVcAg and SVR12 as well ($r = 0.898$, $P = 0.015$) [Figure (1)].

Discussion

In the current study, we aimed to evaluate the value of HCVcAg as an affordable, simple early predictor of SVR to DAAs therapy in cirrhotic patients with chronic HCV for possible future on-treatment modification of therapeutic regimens to maximize the chance of cure and to enhance efforts for global elimination of HCV infection.

In this study, SVR was achieved in 78 patients (91.76%) after 12 weeks of end of treatment., which is higher than that reported by *Nelson et al., (2015)* who stated that a 12-week regimen of SOF+ DAC + RBV was associated with an overall SVR rate of 89% (15), and may be comparable with SVR (98% in GT-1, 92% in GT- 2 & 89% in GT-3) reported by *Sulkowski et al., (2014)* (16). However, *Fontaine et al., (2015)* reported SVR 100% in treatment of genotype 4 HCV using the same regimen (17). This difference may be attributed to that all of our patients were cirrhotic and non-easy to treat.

The results of the current study revealed that HCVcAg at the 10th day of treatment was negative in 79 patients (92.94%) which was significantly correlated with the SVR12 results .HCVcAg values on the 10th day of treatment were 100% sensitive, 98.73% specific with positive predictive value of 85.71%, negative predictive value of 100% and AUC of 0.929 in prediction of SVR. All patients who achieved SVR had negative HCVcAg on day 10 of treatment.

These results support the data of *Fujino et al., (2009)* (18) and *Feng et al (2014)* (19) who suggested that monitoring HCV viral load with HCVcAg during antiviral treatment might be an attractive tool for prediction of response in the future. The results also agree with *Nguyen et al., (2016)* (20) who reported that a rapid decline of HCV antigen to negative level at week 2 on telaprevir/boceprevir treatment after commencement of protease inhibitors triple therapy was associated with SVR. They considered HCVcAg testing as a potential alternative for monitoring treatment response during DAA-based regimen (19).They are also in line with the results of *Lamoury et al (2017)* (21) who reported a high sensitivity and specificity of HCVcAg for detection of pretreatment and posttreatment viremia and agree with *Lucejko and Flisiak., (2018)*, who concluded

that early on-treatment testing for HCVcAg can predict SVR12 with SOF+ DCV + RBV in cirrhotic patients (22).

On the other hand, results of the current study do not match with those of *Van Tilborg et al., (2018)* (23), who found that HCVcAg concentration at week 4 on treatment, although decreased in all patients, was not predictive of SVR. These mismatched results might be attributed to the different HCVcAg assay used, different check-points of HCVcAg testing between our study and theirs (10th day vs 4 weeks), higher number of patients in our study (85 vs 33), in addition to the different treatment protocols in their study ledipasvir/sofosbuvir or ombitasvir/paritaprevir/ritonavir ± dasabuvir ± ribavirin.

Given the expected lower response rate in cirrhotic patients, it would be more prudent to identify those patients who would not respond. Our study shows clearly that failure to clear HCVcAg at the studied check-point can adequately predict non-response (non-SVR) of treatment-naïve cirrhotic HCV patients treated with SOF+DCV+RBV for 12 weeks. In our study, all the six patients who were positive for HCVcAg on day 10 of treatment did not achieve SVR (100%). This can be very helpful to guide the decision to change or stop the treatment protocol, which will avoid the side effects and cost of an ineffective regimen with further development of resistant viruses.

Conclusion

Based on the results of the current study, we can conclude that HCVcAg is a sensitive, specific, accurate, easily available, and affordable on-treatment predictor of SVR in patients with chronic HCV with possible future treatment regimen modification and further improvement of DAAs efficacy and tolerability in difficult to treat cirrhotic patients with chronic HCV.

References:

1. Serfaty L, Forns X, Goeser T, Ferenci P, Nevens F, Carosi G, et al. Insulin resistance and response to telaprevir plus peginterferon alpha and ribavirin in treatment-naive patients infected with HCV genotype 1. *Gut*. 2012; 61:1473-80.
2. Sheridan DA, Neely RDG and Bassendine MF. Hepatitis C virus and lipids in the era of direct acting antivirals (DAAs). *Clinics research in hepatologygastroenterology*. 2013;37:10-6.
3. Swain MG, Lai MY, Shiffman ML, Cooksley WG, Zeuzem S, Dieterich DT, et al. A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. *Gastroenterology*. 2010;139:1593-601.
4. Meissner EG, Lee YJ, Osinusi A, Sims Z, Qin J, Sturdevant D, et al. Effect of sofosbuvir and ribavirin treatment on peripheral and hepatic lipid metabolism in chronic hepatitis C virus, genotype 1-infected patients. *Hepatology*. 2015;61:790-801.
5. Paolucci S, Premoli M, Novati S, Gulminetti R, Maserati R, Barbarini G, et al. Baseline and Breakthrough Resistance Mutations in HCV Patients Failing DAAs. *Sci Rep (2017)*. 7, 16017 <https://doi.org/10.1038/s41598-017-15987-1>

6. European Association for the Study of the Liver (EASL). EASL Recommendations on Treatment of Hepatitis C. *J Hepatol*. 2018. Available at <https://doi.org/10.1016/j.jhep.2018.03.026>.
7. AASLD-IDSA HCV Guidance Panel. Hepatitis C Guidance 2018 Update: AASLD-IDSA Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Clinical Infectious Diseases*. 2018;67 (15 November) • 1477- 1492.
8. Charlton M, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, et al. Ledipasvir and sofosbuvir plus ribavirin for treatment of HCV infection in patients with advanced liver disease. *Gastroenterology*. 2015;149(3):649-659
9. Takahashi K, Okamoto H, Kishimoto S, Munekata E, Tachibana K, Akahane Y et al. Demonstration of a hepatitis C virus-specific antigen predicted from the putative core gene in the circulation of infected hosts. *J Gen Virol*. 1992;73:667–72.
10. Chang C, Hung CH, Wang JH and Lu SN. Hepatitis C core antigen highly correlated to HCV RNA. *Kaohsiung J Med Sci*. 2018;34:684–8.
11. Leary, T., Gutierrez, R., Muerhoff, A., Birkenmeyer, L., Desai, S. & Dawson, G.A chemiluminescent, magnetic particle-based immunoassay for the detection of hepatitis C virus core antigen in human serum or plasma. *Journal of medical virology*, 2006;78, 1436-1440.
12. Mederacke, I., Wedemeyer, H., Ciesek, S., Steinmann, E., Raupach, R., Wursthorn, K., et al.. Performance and clinical utility of a novel fully automated quantitative HCV-core antigen assay. *J Clin Virol*, 2009;46, 210-5.
13. Pawlotsky, J. M. Therapy of hepatitis C: from empiricism to eradication. *Hepatology*, 2006, 43, S207-20
14. Tillmann HL. Hepatitis C virus core antigen testing: role in diagnosis, disease monitoring and treatment. *World journal of gastroenterology: WJG*. 2014;20:6701.
15. Nelson, D. R., Cooper, J. N., Lalezari, J. P., Lawitz, E., Pockros, P. J., Gitlin, N., et al. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*, 2015, 61, 1127-35.
16. Sulkowski M, Gardiner D, Rodriguez-Torres M, Reddy K, Hassanein T, Jacobson I et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med*.; 2014, 370(3):211–221.
17. Fontaine H, Hezode C, Zoulim F, Samuel D, Bourliere M, Haour G, et al. Efficacy of the oral sofosbuvir based combinations in HCV genotype 4 mono-infected patients from the French observational cohort ANRS CO22 Hepather. Abstract LP28 presented at: 50th Annual Meeting of European Association for the Study of the Liver; 2015, April 22–26, Vienna, Austria.
18. Fujino T, Nakamuta M, Aoyagi Y, Fukuizumi K, Takemoto R, Yoshimoto T, et al. Early decline of the HCV core antigen can predict SVR in patients with HCV treated by pegylated interferon plus ribavirin combination therapy. *Journal of digestive diseases*. 2009;10: 21-5.

19. Feng, B., Yang, R.-F., Xie, Q., Shang, J., Kong, F.-Y., Zhang, H.-Y., et al. Hepatitis C virus core antigen, an earlier and stronger predictor on sustained virological response in patients with genotype 1 HCV infection. *BMC gastroenterology*, 2014, 14, 47.
20. Nguyen LT, Gray E, O'Leary A, Carr M, De Gascun CF and Irish Hepatitis C Outcomes Research Network. The role of hepatitis C virus core antigen testing in the era of direct acting antiviral therapies: what we can learn from the protease inhibitors. *PLoS One*. 2016;11: e0163900.
21. Lamoury, F. M. J., Soker, A., Martinez, D., Hajarizadeh, B., Cunningham, E. B., Cunningham, P., et al. Hepatitis C virus core antigen: A simplified treatment monitoring tool, including for post-treatment relapse. *J Clin Virol*, 2017;92, 32-38.
22. Lucejko, M. & Flisiak, R. Quantitative measurement of HCV core antigen for management of interferon-free therapy in HCV-infected patients. *Antivir Ther*, 2018, 23, 149-156.
23. Van Tilborg, M., Al Marzooqi, S. H., Wong, W. W. L., Maan, R., Vermehren, J., Maasoumy, B., et al. HCV core antigen as an alternative to HCV RNA testing in the era of direct-acting antivirals: retrospective screening and diagnostic cohort studies. *Lancet Gastroenterol Hepatol*, 2018, 3, 856-864.

Table (1): Baseline demographic and laboratory data of the study population:

Patients characteristics		Range or number (Total number= 85)	Mean \pm SD or % (Total number= 85)
Age (years)		27-75	53.88 \pm 8.89
Sex	Male	41	48.24
	Female	44	51.76
Child score	A	85	100
Laboratory Investigations			
Hb (mg/dl)		10.2 - 16	12.77 \pm 1.34
WBCs (X103)		2.4 – 16	5.95 \pm 2.28
Platelets (X103)		101 – 290	135.14 \pm 51.99
Total bilirubin (mg/dl)		0.3 – 2.5	1.04 \pm 0.46
Albumin (g/dl)		2.7 – 4.6	3.79 \pm 0.53
ALT		31 – 205	60.63 \pm 34.59
AST		42 – 216	70.27 \pm 40.74
AFP		0.3–208	18.76 \pm 32.22
INR		1 – 1.4	1.09 \pm 0.12
Serum Creatinine (mg/dl)		0.4 – 1.7	0.75 \pm 0.20
Fasting blood glucose (mg/dl)		65 – 215	107.55 \pm 58.48

Table (2): HCVcAg at 10th day on-treatment:

	N	%
Negative	79	92.94
Positive	6	7.06
Total	85	100.00
	Mean ± SD	Range
Negative	0.23 ± 0.04	0.12 – 0.35
Positive	1.06 ± 0.54	0.49 – 1.65
P value	<0.001*	

Table (3): HCVcAg at 10th day on treatment and PCR at 12 weeks after end of treatment (SVR12)

SVR12	HCV core Ag			Chi-Square	
	Negative	Positive	Total	X ²	P-value
	N (%)	N (%)	N (%)		
Responders	78 (98.73%)	0	78 (91.76%)	71.935	<0.001*
Non-responders	1 (1.27%)	6 (100%)	7 (8.24%)		
Total	79 (100%)	6 (100%)	85 (100%)		
ROC					
Sensitivity	Specificity	PPV	NPV	Accuracy	
100	98.73	85.71	100	98.82	

*statistically significant at $p \leq 0.05$, PPV: positive predictive value, NPV: negative predictive value

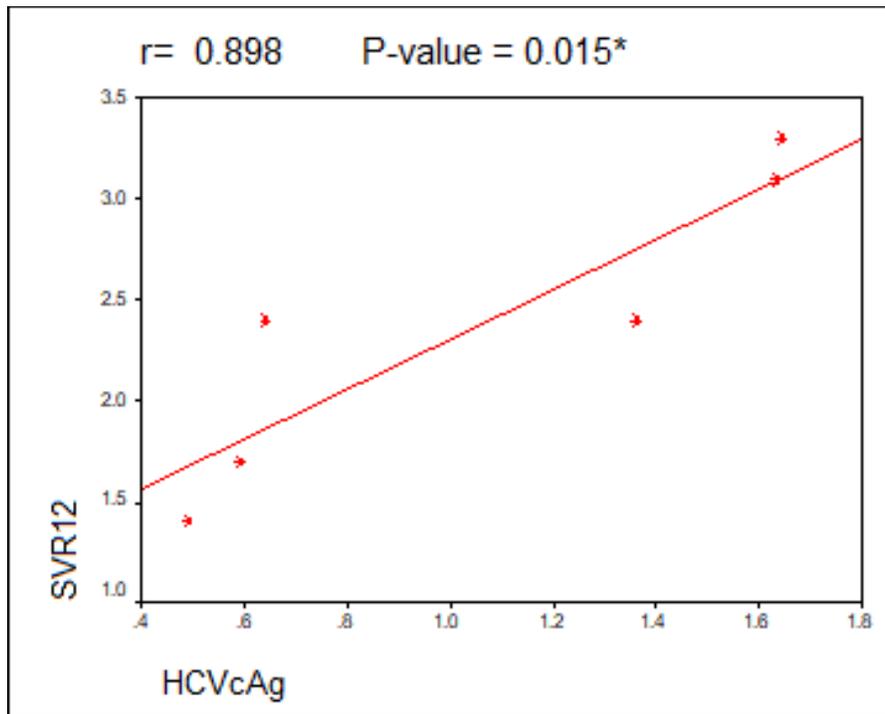


Figure (1): Correlation between HCVcAg and SVR12