Can hepatitis C virus core antigen replace quantitative RNA in the assessment of a sustained virologic response?

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Background
The widespread use of direct-acting antivirals (DAAs) in the treatment of hepatitis C virus (HCV) infection has reduced the need for monitoring HCV-RNA levels because viral kinetics do not predict a sustained virologic response (SVR). However, the performance of cheaper tests, such as the assay to quantify hepatitis C virus core antigen (HCV Ag), has not been determined. Our aim was to assess the accuracy of the HCV core Ag test in predicting the achievement of SVR and its predictive value as a monitoring test in the course of treatment.

Patients and methods
This prospective study was carried out on 90 patients, chronically infected with HCV, who received DAA therapy. Blood samples were collected and the levels of HCV core Ag and HCV-RNA were measured at baseline and at 12 weeks after the end of treatment. We compared the ability of these assays to predict which patients would achieve SVR12.

Results
The baseline level of HCV-RNA was 1 688 529.6±994 697.3 IU/ml (range: 312 700–3 491 100 IU/ml) and HCV core Ag was 179.2±83.5 pg/ml (range: 33.5–315.6 pg/ml). HCV Ag became undetectable in 92.2% 12 weeks after the end of treatment, whereas HCV-RNA became undetectable in 87.8% at the end of treatment ($P<0001$). Seventy-nine out of 90 (87.8%) patients achieved SVR12; the test for HCV Ag identified 63.6% of these patients.

Conclusion
Measurement of HCV core Ag can monitor the efficacy of DAA therapy for HCV infection. Thus, it can be applied in clinical practice.

Keywords:
HCV core antigen, hepatitis C virus, sustained virological response

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Introduction

Hepatitis C virus (HCV) is one of the major causes of death and morbidity worldwide [1]. Recent estimates showed an increase in its seroprevalence over the last decade to 2.8%, corresponding to more than 185 million infections worldwide [2]. Approximately 75% of all cases occur in low-income to middle-income countries [3]. The long-term impact of HCV infection is highly variable, ranging from minimal effects to chronic hepatitis, advanced fibrosis, cirrhosis, decompensated cirrhosis, and hepatocellular carcinoma [4]. The development of direct-acting antiviral (DAA) therapy now allows for safe and effective curative treatment, but treatment is the final step in a long cascade of HCV care that requires virus screening, confirmation, notification of results, and linkage to standard care [5,6]. The presence of HCV antibodies can be found in spontaneous clearance, resolved infection post-treatment, or persistently active disease. Advances in the treatment of HCV infection have led to over 90% cure rates, as defined by the sustained virologic response (SVR), that is, undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after the end of therapy, as assessed by a sensitive molecular method with a lower limit of detection 15 IU/ml. Both SVR12 and SVR24 have been accepted as endpoints of therapy by regulators in the USA and Europe, given that their concordance is 99% [7]. Long-term follow-up studies have shown that an SVR corresponds to a definitive cure of HCV infection in more than 99% of cases. This endpoint, which is associated with significant clinical benefits, was achieved in 90% of patients treated with DAAs, which are designed to target key steps in the HCV replication [8]. In light of the advances in HCV therapy, simplification of diagnosis confirmation, pretreatment diagnostic workup, and treatment

monitoring are required to ensure broad access to these new therapies. Introduction of these highly potent therapies has obviated the need for response-guided therapy and reduced the role of treatment monitoring with highly sensitive quantitative HCV RNA tests. Screening for anti-HCV antibody status often facilitates HCV surveillance in the community [9]. Although simple, such an assay cannot differentiate between past and present infection and requires supplemental HCV RNA testing to confirm active HCV infection and monitor antiviral treatment. Despite its high sensitivity and reliability, an HCV RNA assay involving nucleic acid testing (NAT) and quantitative real-time reverse transcription PCR (RT-PCR) requires skilled laboratory personnel, sophisticated equipment, and expensive reagents [10]. Therefore, routine screening using these tests is limited particularly for many resource-constrained settings. The HCV core protein is antigenic, interacts with numerous cellular proteins, induces specific cellular and humoral responses, and, through various pathways, could play an important role in the pathogenesis of HCV infection. In contrast, testing for hepatitis C virus core antigen (HCV Ag) presents a more attractive alternative owing to the lower cost and short turnaround time. HCV core Ag has been shown to be an indirect marker for HCV replication comparable to the detection of HCV RNA [11]. In addition to serving as a reliable marker to diagnose active HCV infection, HCV core Ag can also be used to evaluate the treatment response to the antiviral therapy [12]. Another advantage of the HCV core Ag assay is that it can often be performed on the same instrument and simultaneously with the anti-HCV assay, an added value when determining the HCV prevalence in the community [13].

The aim of this study was to assess the accuracy of the HCV core Ag test in predicting SVR12 after treatment by DAAs.

Patients and methods

This prospective study was carried out on 90 chronic HCV-infected patients eligible for DAAs. Patients were recruited from the Hepatology and Gastroentrology Unit, Internal Medicine Department, Ain Shams University Hospital, and from the Center For Treatment of Viral Hepatitis at Ain Shams University [one of the National Committee for Control of Viral Hepatitis (NCCVH) centers in Cairo] during the period from August 2017 to April 2018. Eligible patients were men and nonpregnant women, 18 years or older, with chronic HCV infection with positive HCV RNA by PCR. Informed consents were obtained from the patients and the ethical committee of Ain Shams University approved the study.

According to the NCCVH guidelines, patients with Child's C cirrhosis, platelet count less than 50 000/mm^3, hepatocellular carcinoma, except 6 months after intervention aiming at cure with no evidence of activity by dynamic imaging (computed tomography or MRI), extrahepatic malignancy, except after 2 years of disease-free interval, hepatitis B virus coinfected patients, pregnant women or inadequacy of effective contraception, inadequately controlled diabetes mellitus (HbA1c>9%), or patients with evidence of other causes of chronic liver diseases were excluded from the study.

Before treatment and according to the NCCVH guidelines, all the patients included were subjected to a detailed assessment of history and a full clinical examination looking for stigmata of liver cirrhosis.

Laboratory investigations at the baseline included complete blood count, liver profile including aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, albumin, prothrombin time, international normalized ratio, creatinine, fasting blood sugar, and HbA1c in diabetic patients. Hepatitis B surface antigen and HCV-antibody were measured using the third-generation ELISA test.

HCV RT-PCR: before the treatment was started, at 12 weeks after the end of therapy according to the NCCVH Egyptian treatment protocol: (i) Sampling: venous blood samples were drawn into vacutainer tubes (with no additives), centrifuged, and the serum was aliquoted and kept at −70°C till testing. (ii) Procedure: HCV-RNA was detected using a commercially available RT-PCR kit. First, HCV RNA was extracted from serum using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany); then, the extract was added to Qiagen One Step RT-PCR Master Mix (Stratagene, La Jolla, California, USA), and real-time RT-PCR was performed using the Stratagene Mx3000P device (Corbett Research, New South Wales, Australia).

The HCV core Ag concentration was determined (before start of treatment, at 12 weeks after the end of therapy to evaluate SVR) using the Human HCV core Ag ELISA Kit, Sino Gene Clon Biotech Co. Ltd., Hangzhou, China (catalog no: SG-90180).

HCV core Ag results were compared with HCV RNA testing to assess the value of total HCV core Ag as a

marker of viral replication to determine the sensitivity of the core Ag assay relative to molecular biology techniques, and to find the correlation between total HCV core Ag and HCV RNA units.

Abdominal ultrasonography was performed for all the patients included, with a special focus on coarse liver echogenicity, portal vein diameter, presence of any focal lesion, and splenomegaly. Transient elastography: FibroScan (M probe; Echosens, Touch, Paris) was carried out by an experienced examiner in all patients (fasting for≥6 h). During the examination, the patient lay in the dorsal decubitus position with the right arm in maximal abduction to enlarge the intercostal space in which the probe was placed. The median liver stiffness of 10 successful measurements that fulfilled the criteria was noted in kilopascal.

Patients received DAA regimens according to the NCCVH treatment protocols; where patients without liver cirrhosis and those with Child A cirrhosis received sofosbuvir/daclatasvir (SOF/DCV) for 12 weeks, while patients with Child B cirrhosis received SOF/DCV/ribavirin (RBV) for 12 weeks.

Statistical analysis

Statistical analysis was carried out using SPSS 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Statistical presentation and analysis of this study were carried out using the mean, SD, student *t*-test, paired *t*-test, and the linear correlation coefficient. The receiver operating characteristic curve was constructed to obtain the most sensitive and specific cut-off value for HCV core Ag in diagnosing chronic HCV infection.

Results

This prospective study was carried out on 90 chronic HCV infected patients, 51 men and 39 women. Patients were enrolled from different NCCVH treatment centers in Cairo. They received DAA regimens following NCCVH protocols of treatment. Of the total patients studied, 62 (68.89%) received the SOF/DCV regimen, whereas 28 (31.11%) patients received SOF/DCV/RBV.

Baseline quantitative HCV RNA ranged between 312 700 and 3 491 100 IU/ml, with a mean value of 1 688 529.6±994 697.3 IU/ml. 12 weeks after the end of treatment, the quantitative HCV RNA level ranged between 0 IU/ml (undetectable) and 52 300 IU/ml, with a mean concentration of 2041.1±8351.1 IU/ml. There was a significant difference between the baseline HCV RNA level compared with HCV RNA levels at the end of treatment, as indicated by a mean difference of 1 686 488.5±993 230.6 IU/ml ($P<0.001$).

The baseline HCV core Ag level ranged between 33.5 and 315.6 pg/ml, with a mean concentration of 179.2 ±83.5 pg/ml. 12 weeks after the end of treatment, the HCV core Ag level ranged between 0 and 10.5 pg/ml, with a mean concentration of 0.38±1.49 pg/ml. This decline in HCV core Ag concentrations at 12 weeks after treatment compared with the baseline measurement was significant as indicated by a mean difference of 178.8±83.3 pg/ml ($P<0.001$).

A regression analysis yielded the following equation to obtain a relationship between HCV RNA in IU/ml and total HCV Ag in pg/ml:

$$\text{HCV core Ag}\left(\log_{10}\text{pg/ml}\right) = 0.9714 \times \text{HCV RNA}\left(\log_{10}\text{IU/ml}\right) - 3.741.$$

On the basis of this equation, it was found that 1 pg/ml of total HCV core Ag is equivalent to 7888 IU/ml. Thus, total HCV core Ag is a reliable marker of HCV replication, and one core (pg/ml) is equivalent to ~8000 HCV RNA (IU/ml) in clinical samples from HCV-infected patients.

The correlation between HCV RNA (IU/ml) and HCV Ag (pg/ml) varied around this average ratio when individual samples were considered, with the majority of the ratios ranging between 5000 and 12 000 HCV RNA (IU/ml) per core Ag (pg/ml).

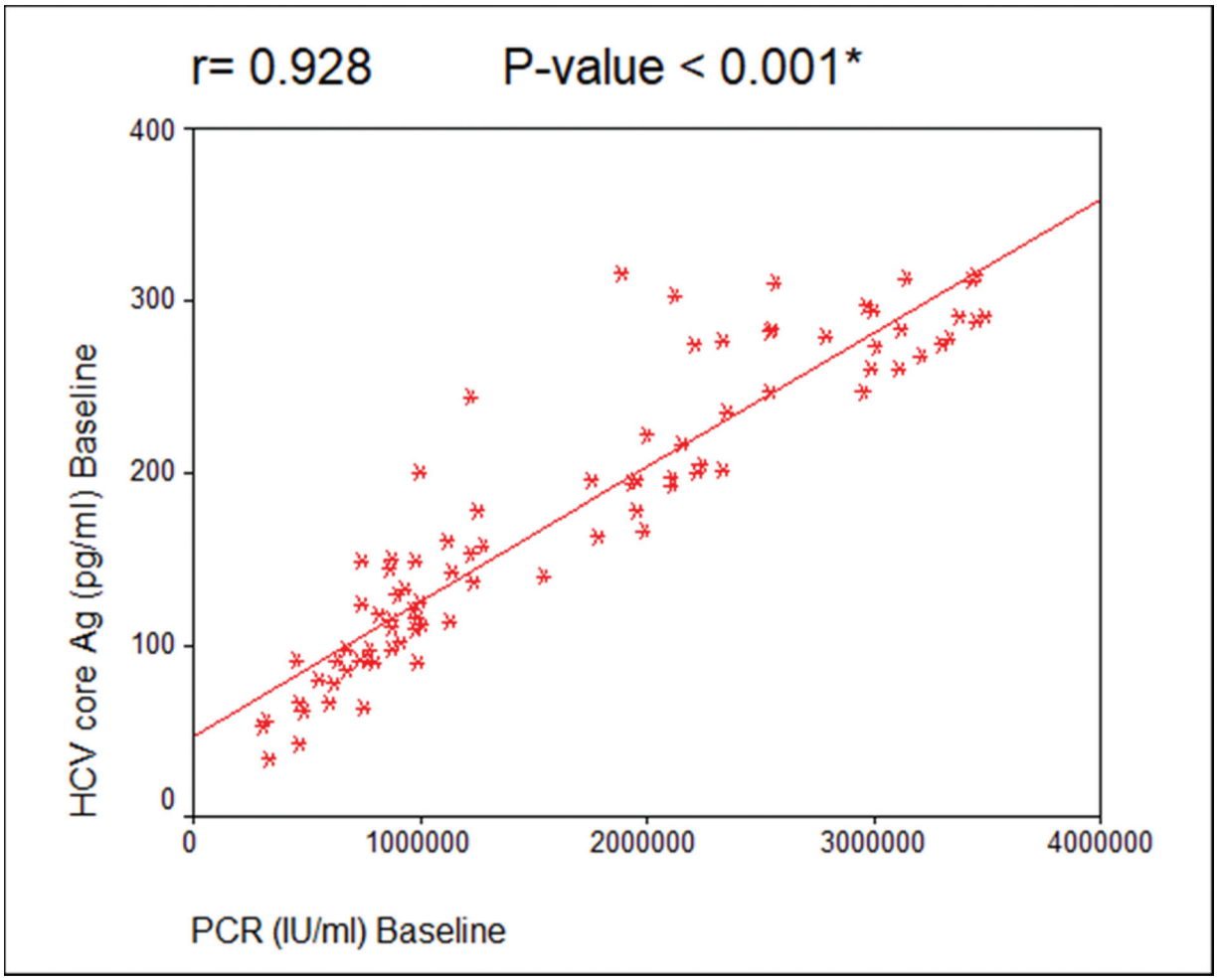
On the basis of the relationship with HCV RNA mentioned above and the calculated cut-off value of this study of 0.71 pg/ml, the total core Ag assay did not detect HCV replication below 5689 IU/ml (~6000 IU/ml).

A strong correlation was observed between baseline HCV RNA and HCV core Ag levels, as indicated by *P* value less than 0.001, where the HCV core Ag concentration was directly proportional to the HCV RNA level as evidenced by $r=0.928$ (Fig. 1).

A strong correlation was observed between HCV RNA and HCV core Ag levels 12 weeks after treatment, as indicated by *P* value less than 0.001, where the HCV core Ag concentration was directly proportional to the HCV RNA level as evidenced by $r=0.987$ (Fig. 2).

At a cut-off value 0.71 pg/ml, the diagnostic performance of the HCV core Ag in differentiating active infection from past infection in HCV sero-

Figure 1



Correlation between baseline hepatitis C virus (HCV) RNA and HCV core antigen levels.

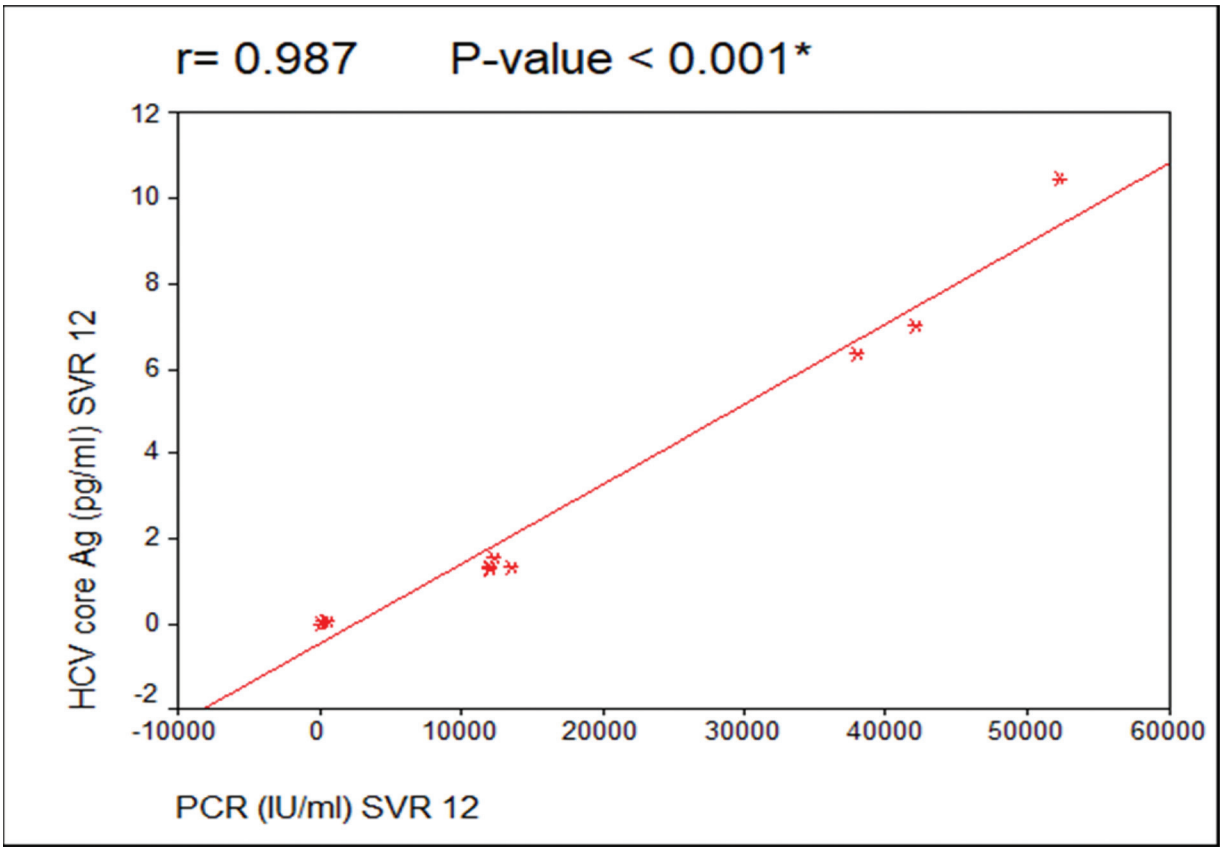
positive patients was shown with a test sensitivity of 63.6%, whereas the specificity was 98.7%. The positive predictive value was 98.6% and the negative predictive value was 89.3%. The accuracy of the test was 90.5% (Fig. 3).

Discussion

Detection and quantification of the HCV core Ag, which require less expensive and less complex instruments and facilities, were considered an alternative to NATs. The first ELISA assay for HCV core Ag detection and quantification was introduced more than a decade ago and had shown a significant relationship with the presence of HCV RNA. At that time, it was suggested that HCV core Ag detection and quantification might be used to follow patients and predict the response to treatment [14].

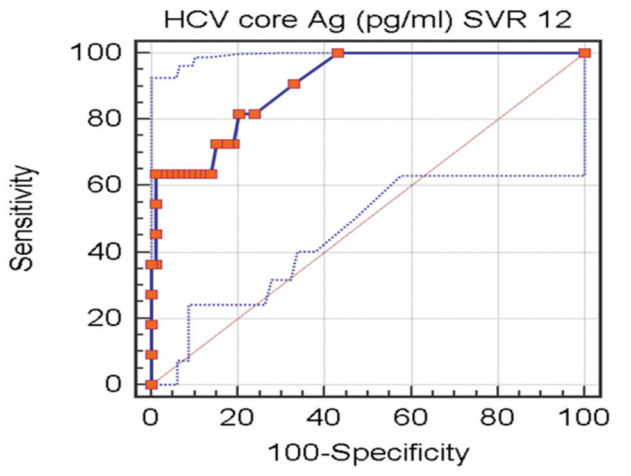
In this study, baseline quantitative HCV RNA among the patients studied ranged from 312 700 to 3 491 100 IU/ml, with a mean concentration of 1 688 529.6±994 697.3 IU/ml. 12 weeks after the end of treatment, SVR was achieved in 79/90 (87.8%) patients; although 11/90 patients had not achieved SVR, their status as nonresponders versus relapsers could not be defined, as end-of-treatment PCR (or core antigen assay) had not been performed in this study. According to the treatment regimen, five out of those 11 patients were on a SOF/DCV/RBV regimen, whereas six patients had been receiving SOF/DCV. At 12 weeks after treatment, the quantitative HCV RNA level in the patients studied ranged between undetectable and 52 300 IU/ml, including patients who had a negative SVR. This decline in PCR compared with the baseline was significant ($P<0.001$), a result that reflects a high efficacy of DAA treatment, provided that an appropriate DAA regimen had been assigned to every patient and patients had been compliant with treatment and follow-up during treatment. However, the proportion of patients who had achieved SVR12 in this study was lower than that reported in other studies, where it was estimated to be over 98% by numerous researchers [15,16], a finding that

Figure 2



Correlation between hepatitis C virus (HCV) RNA and HCV core antigen levels 12 weeks after treatment.

Figure 3



Receiver operating characteristic curve showing the diagnostic performance of the hepatitis C virus (HCV) core antigen for differentiating past HCV infection from active chronic infection.

highlights some limitations in our study. First, data for RASs were not available for all cases and because of the small sample size, the power of the multiple regression analysis was low. Second, because this was a study from one demographic area, the total number of treated case was small. Third, genotype testing was not performed, and yet it was assumed to be genotype 4, as it has been known to be the most prevalent genotype in Egypt.

Interestingly, a significant decline in the HCV core Ag was noted at 12 weeks after treatment compared with its baseline value, as indicated by a mean difference of 178.8±83.3 pg/ml ($P<0.001$).

It is noteworthy that this study showed a strong positive correlation between HCV RNA and HCV core Ag levels at both baseline and 12 weeks post-treatment measurements ($r=0.928$, $P<0.001$ and $r=0.987$, $P<0.001$, respectively).

In a study carried out by Bouvier-Alias *et al.* [17], the enzyme-linked immunosorbent assay version of HCV core Ag kit detected 2.4 pg/ml of HCV core Ag (corresponding to an HCV RNA level of 20 000–22 000 IU/ml). Similar results were found by Aoyagi *et al.* [18] Another study found that, with the use of newer ELISA kits, the limit of detection of HCV core Ag assay corresponded to ~7000 IU/ml HCV RNA.

In this study, the HCV core Ag ELISA kit assay yielded positive results in 7/90 samples collected 12 weeks after the end of treatment, whereas 11/90 were positive for

HCV RNA levels. The four patients in whom HCV core Ag could not be detected had HCV RNA levels of 590, 220, 319, and 119 IU/ml, respectively. Overall, the assay showed a cut-off value 0.71 pg/ml and the diagnostic performance of HCV core Ag in differentiating active infection from past infection in HCV antibody-positive patients was found to have a test sensitivity of 63.6%, whereas the specificity was 98.7%. The positive predictive value was 98.6% and the negative predictive value was 89.3%. The accuracy of the test was 90.5%. A good correlation between the logarithmic values of HCV RNA and HCV core Ag had been observed, similar to the results reported in other studies [19,20]. An important aim of this study was to estimate the usefulness of the ELISA HCV core Ag assay in patients with chronic HCV infection. Using an estimated cut-off of the Ag assay of 6000 IU/ml HCV RNA, the overall sensitivity of the HCV core Ag test was 63.6%, but reached higher levels (>98%) in those evaluated at baseline.In this study, 1 pg/ml of total HCV core Ag was equivalent to ~8000 IU/ml RNA. In previous reports, the 1 pg/ml HCV core Ag equivalent varied between 428 and 8000 IU/ml RNA [20,21]. In one study, the HCV genotype was found to have an effect on the HCV RNA core Ag equivalence value [18] Other possible reasons for the differences in the equivalence values between different studies may include differences between assays and differences between patients; this requires further investigations.

In newly diagnosed anti-HCV-positive patients, the initial HCV RNA testing is indicated for the diagnosis of active or past infection and for a baseline evaluation before the start of the antiviral treatment. According to the data of this study, the HCV core Ag test would yield a positive result in 98.6% of the patients, thus confirming active infection. With this approach, the confirmation of HCV infection with HCV core Ag test will save about 80% of the HCV RNA tests. Only patients with HCV core Ag negative tests need further confirmation by HCV PCR, as also suggested by other studies [22]. However, in our opinion, this approach is incomplete because all HCV core Ag-positive patients with an indication for antiviral treatment will also need PCR testing for an accurate baseline measurement of HCV RNA. For patients under treatment with DAAs, the evaluation of treatment efficacy must be performed with an assay with a limit of detection lower than 15 IU/ml, which is not the case for the actual version of the HCV core Ag assay. In this study, the sensitivity of the HCV core Ag assay compared to HCV RNA for this group of patients was approximately 63.3%, with a significant number of false-negative results, thus, limiting the utility of HCV core Ag assay.

Conclusion

The ELISA HCV core Ag assay showed a very high specificity and a good correlation with HCV RNA results. This assay might be an alternative for the diagnosis of active infection in anti-HCV-positive patients if molecular tests are not available, but a confirmation of HCV core Ag-negative results with NATs is required. The HCV core Ag assay is a useful method with reduced costs for the evaluation of a SVR in HCV-treated patients. HCV core Ag assays with signal amplification have high sensitivity, high specificity, and good correlation with HCV RNA above 3000 IU/ml. HCV core Ag assays have the potential to replace NAT in high HCV prevalence settings. The Architect HCV core Ag assay is highly specific and easy to perform. It represents a valuable screening, diagnostic, and monitoring tool, especially in the era of new all-oral, interferon-free antiviral strategies that do not require high analytical sensitivity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Cooke GS, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, *et al.* Viral hepatitis and the Global Burden of Disease: a need to regroup. J Viral Hepat 2013; 20:600–601.

2 Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age specific antibody to HCV seroprevalence. Hepatology 2013; 57:1333–1342.

3 Gower EEC, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol 2014; 61: S45–S57.

4 World Health Organization. Hepatitis C Fact Sheet. 2015.

5 Ward JW, Mermin JH. Simple, effective, but out of reach? Public Health Implications of HCV Drugs. N Engl J Med 2015; 373:2678–2680.

6 Linas BP, Barter DM, Leff JA, Assoumou SA, Salomon JA, Weinstein MC, *et al.* The hepatitis C cascade of care: identifying priorities to improve clinical outcomes. PLoS One 2014; 9:e97317.

7 Singal AG, Volk ML, Jensen D, Di Bisceglie AM, Schoenfeld PS. A sustained viral response is associated with reduced liver-related morbidity and mortality in patients with hepatitis C virus. Clin Gastroenterol Hepatol 2010; 8:280–288.

8 Pockros PJ. Management of patients who have achieved sustained virologic response for hepatitis C virus infection. Gastroenterol Hepatol (N Y) 2018; 14:305–307.

9 Morisco F, Loperto I, Stroffolini T, Lombardo FL, Cossiga V, Guarino M, *et al.* Prevalence and risk factors of HCV infection in a metropolitan area in southern Italy: tail of a cohort infected in past decades. J Med Virol 2016; 89:291–297.

10 Roth WK, Busch MP, Schuller A, Ismay S, Cheng A, Seed CR, *et al.* International survey on NAT testing of blood donations: expanding implementation and yield from1999 to 2009. Vox Sang 2012; 102:82–90.

11 Florea D, Neaga E, Nicolae I, Maxim D, Popa M, Otelea D. Clinical usefulness of HCV core antigen assay for the management of patients with chronic hepatitis C. J Gastrointestin Liver Dis 2014; 23:393–396.

12 Alonso R, Perez-Garcia F, Ampuero D, Reigadas E, Bouza E. New direct-acting antivirals for patients with chronic HCV infection: can we monitor treatment using an HCV core antigen assay? Diagn Microbiol Infect Dis 2017; 87:243–246.

13 Mixson-Hayden T, Dawson GJ, Teshale E, Le T, Cheng K, Drobeniuc J, *et al.* performance of architect HCV core antigen test with specimens from US plasma donors and injecting drug users. J Clin Virol 2015; 66:15–18.

14 Maasoumy B, Wedemeyer H. Natural history of acute and chronic hepatitis C. Best Pract Res Clin Gastroenterol 2012; 26:401–412.

15 The Global Burden of Hepatitis C. The global burden of hepatitis C. J Clin Pharmacol 2004; 44:20–29.

16 Poiteau L, Soulier A, Rosa I, Roudot-Thoraval F, Hézode C, Pawlotsky JM, *et al.* Performance of rapid diagnostic tests for the detection of antibodies to hepatitis C virus in whole blood collected on dried blood spots. J Viral Hepat. 2016; 23(5):399–401.

17 Bouvier-Alias M, Keyur P, Harel D, Stephanie B, Patrick L, Lawrence B, *et al.* clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepatology 2002; 36:212–215.

18 Aoyagi K, Ohue C, Iida K, Kimura T, Tanaka E, Kiyosawa K, *et al.* A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen. J Clin Microbiol 1999; 37:1802–1808.

19 Morota K, Fujinami R, Kinukawa H, Machida T, Ohno K, Saegusa H, *et al.* A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen. J Virol Methods 2009; 157:8–14.

20 Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C, *et al.* Dynamics of viremia in early hepatitis C virus infection. Transfusion 2005; 45:994–1002.

21 Medici MC, Furlini G, Rodella A, Fuertes A, Monachetti A, Calderaro A, *et al.* Hepatitis C virus core antigen: analytical performances, correlation with viremia and potential applications of a quantitative, automated immunoassay. J Clin Virol 2011; 51:264–269.

22 Kati W, Koev G, Irvin M, Beyer J, Liu Y, Krishnan P, *et al.* In vitro activity and resistance profile of dasabuvir, a nonnucleoside hepatitis C virus polymerase inhibitor. Antimicrob Agents Chemother 2015; 59:1505–1511.