Hepatitis C virus core antigen: A potential alternative to HCV RNA testing among persons with substance use disorders

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A R T I C L E   I N F O

Article history:
Received 30 January 2017
Received in revised form 12 April 2017
Accepted 17 April 2017

Keywords:
Drug use disorders
Substance-related disorders
Hepatitis C, chronic

A B S T R A C T

Background: The hepatitis C virus (HCV) core antigen (HCVcAg) may be an alternative diagnostic method to HCV RNA especially in populations such as substance users, the homeless or in resource-limited settings.

Aims: To evaluate performance of HCVcAg test in patients with opioid use disorder (OUD) on methadone in order to document its performance characteristics in the target population and to ensure that its specificity remains consistent across different populations.

Methods: HCVcAg levels from 109 methadone-maintained patients were compared to HCV RNA levels.

Results: Mean age was 53.8 ± 7.8 years, 59.6% were male, 68.8% African American, and 44% HCV-infected. HCVcAg was detectable in 47 of 48 HCV-infected, and undetectable in all HCV RNA negative patients. The HCVcAg assay had sensitivity of 97.9% and specificity of 100%. Correlation with HCV RNA levels was excellent (r = 0.88, 95% CI 0.76; 0.95, p < 0.01).

Conclusion: HCVcAg has excellent performance for the diagnosis of HCV infection in patients with OUD on methadone.

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ARTICLE INFO

1. Introduction

Hepatitis C virus (HCV) infects an estimated 130 million (World Health Organization, 2016) individuals globally and up to five million (Chak, Talal, Sherman, Schiff, & Saab, 2011) individuals in the United States. HCV can lead to cirrhosis, end-stage liver disease, and hepatocellular carcinoma (Micallef, Kaldor, & Dore, 2006). Persons with substance use disorders (PWSUD) have the highest HCV incidence and prevalence as injection drug use is the principal route of HCV transmission (Nelson et al., 2011). Direct acting antivirals (DAAs) for HCV treatment have recently been introduced with antiviral efficacy of at least 90%, minimal side effects, and drastically shortened treatment duration compared to prior interferon containing regimens. These agents offer, for the first time, the potential for global elimination of HCV. To accomplish this auspicious task, new approaches to HCV diagnosis and linkage to care among marginalized populations, such as patients with opioid use disorder (OUD) on methadone are needed (Canary, Klevens, & Holmberg, 2015; Holmberg, Spradling, Moorman, & Denniston, 2013; Ward & Mermin, 2015). Conventionally, an HCV diagnosis has required two steps consisting initially of serologic testing to document HCV exposure followed by nucleic acid testing (NAT) among seropositive individuals to document active infection. Assessment of HCV genotype and fibrosis stage, in addition to serology and NAT, are the minimum additional requirements to determine DAA treatment eligibility. Simplification of the HCV evaluation algorithm to reduce the number of required tests could considerably expand the provision of HCV treatment especially to disenfranchised and marginalized populations such as patients with OUD on methadone.

HCV core antigen (HCVcAg) has been proposed as a potential one-step replacement for serologic and NAT assessments, so that NAT might not be required to identify those with active HCV infection (Freiman et al., 2016). In addition, cost estimates for HCVcAg tests are generally lower (from $10 to $50) than those for HCV RNA assays.
generally obtained on an annual basis, they were available within a 12-month period preceding the collection of blood for HCV RNA and HCVcAg testing.

2.1. HCVcAg assay

The HCVcAg assay (Abbott Diagnostics, Wiesbaden, Germany) was performed as previously described on the ARCHITECT i200SR platform (Abbott Diagnostics, Abbott Park, IL, USA) (Mixson-Hayden et al., 2015). The assay includes a pretreatment step, which disrupts immune complexes and prepares the HCVcAg for capture onto the surface of magnetic microparticles. These microparticles are coated with three distinct monoclonal antibodies that are directed against the HCV core protein. After washing, microparticles containing the captured core protein react with an acridinium conjugate containing two monoclonal antibodies that are directed toward the HCV core protein.

Specimens with concentration values <3.00 fmol/l were considered nonreactive and those with concentration values ≥3.00 fmol/l were considered reactive for HCVcAg. Specimens with concentration values ≥3.00 fmol/l and <10.00 fmol/l were retested in duplicate. If one or both of the duplicates were ≥3.00 fmol/l, the specimen was considered reactive (Mixson-Hayden et al., 2015).

2.2. Statistical analysis

The Bland-Altman technique (Altman & Bland, 1983; Bland & Altman, 1986) was used to assess the agreement of measuring viral activity via the HCV RNA and HCVcAg assays as well as to assess the relationship between HCV RNA results obtained using the Abbott and Cobas real-time instruments. In order to employ the Bland-Altman technique, both the HCV RNA and HCVcAg had to be on the same scale. Therefore, we employed a conversion factor of 1 fmol/l for HCVcAg to equal 500 IU/ml for HCV RNA (Chevaliez et al., 2016). We used log_{10}-transformed data for all analysis. Associations between baseline predictors were investigated using linear regression. Robust regression (Staudte & Sheather, 1990) was utilized to assess the influence of demographic and infection-related variables on the likelihood of a positive HCVcAg result. Robust regression enabled us to solve the problem of non-ignorable outliers and influence points in our sample. This is achieved by assigning weights that reflect the confidence an investigator has in the fact that measurements have the same distribution, a fundamental assumption of the regression method. A weight of 1 indicates complete confidence while weights of <1 indicate various degrees of confidence. The weights are not assigned arbitrarily, but are generated automatically by the robust statistical procedure. Statistical significance level was defined as \( p = 0.05 \) and all statistical comparisons were performed using R (Version 3.2.0 https://cran.r-project.org/).

3. Results

3.1. Demographic and drug use history

Serum samples obtained from 109 injection and non-injection methadone-maintained patients with OUD were tested for the presence of HCVAg. Patients’ mean age was 53.8 ± 7.8 years, 65 (59.6%) were male, 75 (68.8%) were African American, 31 (28.4%) were Hispanic, and 19 (17.4%) were infected with HIV. History of injection drug use was reported by 65 (59.6%) patients, and 11 (10%) injected drugs in the last 6 months. History of illicit non-injection drug use was reported by 103 (94.5%) patients, and 54 (49.5%) used non-injection drugs in the last 6 months. The mean duration of methadone maintenance was 6 ± 5.2 years (Table 1). We found that HCV RNA positive individuals were on OAT for a mean of 8.3 ± 6.3 years compared with 4.5 ± 3.7 years for those who were HCV RNA negative (\( p = 0.001 \)).
### 3.2. HCV infection status

Sixty-five (59.6%) patients were HCV seropositive, and 48 (44%) had a positive HCV RNA test. Eighteen patients had a positive HCV antibody test and undetectable HCV RNA, 17 of whom were spontaneous resolvers while one resolved HCV infection after medical treatment. One patient had a negative HCV antibody test and detectable HCV RNA. Among 48 HCV RNA positive patients, 23 (47.9%) were infected with genotype 1a, 14 (19.2%) with genotype 1b, 5 (10.4%) with genotype 2, and 4 (8.3%) with genotype 3. In one genotype 1-infected patient, the HCV subtype could not be determined. In one patient sample with a low viral load, HCV genotype could not be determined. Fourteen (29.2%) patients were HIV/HCV co-infected.

### 3.3. Correlation results between HCVcAg and HCV RNA

HCVcAg was detected in 47 of 48 HCV RNA positive patients (viral loads ranging from 1920 IU/ml to 16,581,000 IU/ml; mean 3,972,346 ± 4,301,329 IU/ml). HCVcAg was undetectable in one patient with a very low viral load (460 IU/ml). Positive HCVcAg titers ranged from 7 fmol/l to 26,624 fmol/l (mean 7354 ± 7158 fmol/l). The correlation between log_{10}-transformed HCV RNA (Abbott) and log_{10}-transformed HCVcAg was high with r = 0.925 (95% confidence interval [CI] [0.848, 0.967], p < 0.001) (Fig. 1). Similarly, the correlation between the log_{10}-transformed HCV RNA (Cobas) and log_{10}-transformed HCVcAg was high with r = 0.925 (95% CI [0.848, 0.967], p < 0.001) (Fig. 1b). Additionally, correlation between log_{10}-transformed HCV RNA (Cobas) and log_{10}-transformed HCVcAg (Abbott) was also extremely high with r = 0.946 (95% CI [0.891, 0.967], p < 0.001) (Fig. 1c). These correlation results are to be expected because both HCV RNA and HCVcAg measure viral activity. In comparison with the HCV RNA assay, the HCVcAg assay had excellent performance with sensitivity of 97.9%, specificity of 100%, positive and negative predictive values of 100% and 98.4%, respectively.

### 3.4. Agreement between HCVcAg and HCV RNA

To study the agreement between two different methods of measuring viral activity, we use the Bland-Altman plot, which enables us to understand the differences between the two methods of measurement. The plot illustrating the agreement between the converted, log_{10}-transformed HCV RNA (Abbott) and log_{10}-transformed HCVcAg illustrates a bias, on the original scale, of 25%, which is higher than that observed between the converted HCV RNA (Cobas) and HCVcAg (Fig. 2A). The 95% confidence interval for bias, the lower and upper confidence limits are relatively tight (lower limit of agreement is 0.207 and upper limit of agreement is 2.69 on the original scale) indicating a moderate degree of agreement between the methods.

The Bland-Altman plot for comparison between the converted, log_{10}-transformed HCV RNA (Cobas) and log_{10}-transformed HCVcAg levels illustrates a low bias of 0.026 on the log scale, corresponding to approximately 6% on the original scale (Fig. 2B). The upper and lower limits of agreement equal 4 and 0.28 on the original scale, have tight 95% confidence intervals indicating high agreement of measurement of viral activity via HCV RNA (Cobas) and HCVcAg.

The Bland-Altman plot for the comparison between log_{10}-transformed HCV RNA (Cobas) and log_{10}-transformed HCV RNA (Abbott) is illustrated (Fig. 2C). The bias, indicated by the dashed red line, is approximately 0.15 on the log scale, and the blue dashed lines indicate the lower and upper limits of agreement (−0.23 and 0.55, respectively). The associated 95% confidence intervals are fairly tight indicating high agreement of measurement between the Cobas and Abbott platforms.

### 3.5. Multivariable regression

We used robust regression to investigate the potential influence of demographic, virologic and behavioral (such as injection and noninjection drug use) variables on HCVcAg levels as it is important to document that the relationship between HCVcAg and HCV RNA levels does not change among those who likely have higher exposure to potentially cross-reactive antigens. The variables evaluated were chosen to assess the hypothesis whether injection or non-injection drug use as well as active drug use might alter the relationship between HCVcAg and HCV RNA. None of the variables evaluated were found to affect HCVcAg levels except log_{10}(HCV RNA) (Table 2).

### 4. Discussion

In this manuscript, we report excellent performance characteristics of the HCVcAg in the detection of HCV infection in 109 methadone-maintained patients with OUD. The assay had a sensitivity of 97.9%, specificity of 100%, and positive and negative predictive values of 100% and 98.4%, respectively. Correlation coefficients between HCVcAg and quantitative HCV RNA measurements assessed by two assays ranged from 0.88 to 0.93. None of the covariates evaluated in this study were found to significantly affect HCVcAg values in patients with OUD on methadone except log_{10}(HCV RNA). The only HCV RNA positive sample with undetectable HCVcAg had a very low HCV RNA level of 460 IU/ml, which is below the lower limit of detection of the HCVcAg assay. Our results are consistent with previously published data that showed excellent correlation between the HCVcAg assay and HCV RNA test results (Medici et al., 2011; Mibson-Hayden et al., 2015; Ross et al., 2010) especially in situations in which HCV RNA levels are >3000 IU/ml. Since the rate of HCV virion production is estimated to be 10^{12} virions per day (Neumann et al., 1998), most individuals in the acute and chronic phases of HCV infection are highly viremic (RNA levels >3000 IU/ml) and would be expected to have detectable HCVcAg levels (Alanko Blome, Bjorkman, Molnegren, Hoglund, & Widell, 2014; Ticehurst, Hamzeh, & Thomas, 2007).

An estimated 40% of HCV-infected individuals in the United States are unaware of their infection status (Smith et al., 2012). PWSUD increased likelihood of homelessness and of being uninsured with limited access to health care services partially explain their high prevalence of undiagnosed HCV infection and the poor adherence to referrals for engagement into HCV care (Zeremski et al., 2013). The need for a confirmatory HCV RNA test following detection of an HCV seropositive sample further complicates the ability to diagnose HCV among...
PWSUD. In addition, HCV RNA testing is expensive, requires costly equipment and skilled personnel making it difficult to perform in some resource-limited settings. Development of a POC single-step diagnostic test, such as HCVcAg, could greatly facilitate HCV diagnosis. Advantages of the HCVcAg assay, including simplicity, the ability to obtain results rapidly, and low cost, should motivate its development as a POC test that could fill the niche for HCV screening among patients with OUD on methadone. HCVcAg testing could also be leveraged to identify early acute HCV infection. Among active injectors who are more likely to acquire HCV acutely, HCVcAg has been shown to detect HCV infection 1.5 months (range, 38–50 days) earlier than HCV antibody tests (Hosseini-Moghaddam et al., 2012). During the “window period”, these individuals may be unaware that they are HCV infected (Rehermann & Nascimbeni, 2005). HCVcAg is typically detectable in blood one to two days after HCV RNA becomes detectable (Peterson et al., 2000) and, as it is part of the virus, it may circumvent the need for expensive HCV RNA testing. A low cost, rapid, and reliable test could be an important contribution to HCV screening among high-risk populations (Cresswell et al., 2015).

In order to fully translate the antiviral efficacy of DAAs real-world effectiveness, new diagnostic methods are needed to link medically disenfranchised populations, such as PWSUDs, to HCV management. As pan-genotypic HCV therapies are increasingly released and eventually become established as HCV standard of care, as has been the case with the recent release of sofosbuvir/velpatasvir (Feld et al., 2015; Foster et al., 2015), HCV genotyping may eventually not be required as part of DAA procurement. For the foreseeable future, however, it is highly likely that genotype testing will remain as a requirement for DAA-based therapy. Similarly, as increased competition will likely decrease the price of DAAs, fibrosis staging may no longer be required in order to obtain DAA-based therapy. Until this occurs, fibrosis testing is valuable to guide treatment prioritization, especially in areas restricting DAAs to those with advanced fibrosis or cirrhosis. Among inhabitants from LMIC and among medically disenfranchised US populations at high-risk for viral transmission, a “test and treat all with active infection” approach may be pursued. In that situation, a single test to document active HCV infection, such as the HCVcAg, might be the only requirement to determine DAA eligibility. Easing of DAA eligibility requirements would likely substantially increase access to and the number of prescribers of DAAs. As mentioned previously, cost estimates for HCVcAg tests are generally lower than those for HCV RNA assays, which should facilitate HCVcAg uptake in resource-limited settings (Freiman et al., 2016). Indeed, a recent global stakeholder consultative process gave highest-priority target status to development of a POC HCVcAg test (Forum for Collaborative HIV Research, 2015).

At least two other studies in PWSUD have evaluated the relationship between HCVcAg and HCV RNA levels (Mixson-Hayden et al., 2015; Netski et al., 2004). One of these studies assessed HCVcAg levels by enzyme-linked immunosorbent assay (ELISA) (Netski et al., 2004). A recent WHO-commissioned systematic review and meta-analysis, which included a number of entities that manufacture and market diagnostic tests such as HCVcAg, found that HCVcAg platforms that require amplification had improved performance characteristics compared to those that did not (Freiman et al., 2016). This finding is an advantage of our investigation compared to that described by Netski et al., 2004. The review’s authors concluded that HCVcAg has the potential to replace NAT in settings with high HCV prevalence. Recently, Mixson-Hayden et al. (2015) reported an excellent correlation (r = 0.959, p < 0.001) between HCVcAg and HCV RNA levels among a total of 551 anonymous US plasma and serum donors, including 376 samples derived from a cohort of injection drug users ages 18–40 years. Our sample offered an opportunity to assess whether active substance use and the pattern of

Fig. 1. A: Scatter plot of log_{10}(HCV RNA), measured using Abbott® Realtime HCV RNA assay (X-axis) and of log_{10}(HCV Core Ag), measured using Architect Core Antigen assay (Y-axis). B: Scatter plot of log_{10}(HCV Core Ag), measured using Abbott® Realtime HCV RNA assay v 2.0 (X-axis) and of log_{10}(HCV Core Ag), measured using Architect Core Antigen assay (Y-axis).
substance use alters the relationship between HCV RNA and HCVcAg, which was not reported in the work by Mixson-Hayden et al. (2015).

Since the accuracy of the HCVcAg test is directly related to the specificity of core antigen-antibody binding, assessment of its performance characteristics in the target population is important to ensure that other proteins or prior viral exposures do not cause false-positive reactions. The specificity of the immune response is not only important for antigen-antibody reactions, but it is a general phenomenon that applies to the entire repertoire of immune responses. For example, T cells that were thought to be specific for HCV can also be induced by influenza (Wedemeyer, Mizukoshi, Davis, Bennink, & Rehermann, 2001). In other words, both HCV and influenza virus can result in an immune response by T-cells thought previously to be specific only for HCV. Similar observations of variability on the specificity and sensitivity results of HCVcAg testing were noted among patients on hemodialysis. In this situation, obtaining the sample prior to initiation of hemodialysis eliminated filtration through the dialysis membrane and reduced the variability in the experimental results (Hosseini-Moghaddam et al., 2012). These observations support the need to conduct biomarker studies in the population for which they are intended to ensure their accuracy.

Although one of the limitations of this study is the small number of patients with low viral loads (only 2 samples with <100,000 IU/ml), the only patient with undetectable HCVcAg had HCV RNA levels <500 IU/ml. Another patient with HCV RNA levels of 1920 IU/ml had a low value HCVcAg levels of 7 fmol/l. Additionally, the small number of HIV-infected patients and those with HCV genotypes other than 1 limited our ability to evaluate their influence on HCVcAg levels in this population. An additional limitation is the sample of patients with OUD on methadone that may not be generalizable to the entire sample of PWSUD. The fact that only 10% of our sample endorsed injection drug use in the past six months limits our ability to generalize our results to populations with higher rates of active use.

4.1. Conclusions

In conclusion, we propose that the HCVcAg might be a reasonable substitute for HCV RNA testing to diagnose active HCV infection especially in high prevalence populations, such as patients with OUD on methadone. As the vast majority of chronically HCV-infected individuals have viral loads above 3000 IU/ml, the HCVcAg assay may offer one-step screening at the point-of-care that could facilitate HCV diagnosis in the vast majority of patients with OUD on methadone with active HCV infection. With further HCV therapeutic (i.e. pan-genotypic) and policy developments (i.e. elimination of fibrosis stage as a DAA acquisition requirement), HCVcAg might fulfill the need to establish active HCV infection in those pursuing DAA-based HCV therapy, especially in a “treat all with active infection” approach.

Fig. 2. A: Bland-Altman plot assessing the agreement between converted, log10-transformed Abbott realtime PCR assay (HCV RNA) and log10-HCV core antigen using the Architect Core Antigen assay. The Y axis represents the difference between log10(HCV RNA) Abbott and log10(HCVcAg) Abbott. The red dashed line represents the mean difference, and the blue dashed lines represent the lower and upper limits of agreement. The dotted lines on each side of the dashed lines represent the 95% confidence limits for the difference of two measurements and the limits of agreement, respectively. B: Bland-Altman plot assessing the agreement between converted, log10-transformed Cobas® TaqMan® HCV Test v 2.0 (HCV RNA) and log10-HCV core antigen using the Architect Core Antigen assay. The Y axis represents the difference between log10(HCV RNA) Cobas and log10(HCVcAg) Cobas. The red dashed line represents the mean difference, and the blue dashed lines represent the lower and upper limits of agreement. The dotted lines on each side of the dashed lines represent the 95% confidence limits for the difference of two measurements and the limits of agreement, respectively. C: Bland-Altman plot assessing the agreement between HCV RNA log10-transformed Abbott realtime PCR assay (HCV RNA) and log10-transformed Cobas® TaqMan® HCV Test v 2.0 (HCV RNA). The Y axis represents the difference between log10(HCV RNA) Cobas and log10(HCVcAg) Abbott. The X axis represents the average between log10(HCV RNA) Cobas and log10(HCVcAg) Abbott. The red dashed line represents the mean difference, and the blue dashed lines represent the lower and upper limits of agreement. The dotted lines on each side of the dashed lines represent the 95% confidence limits for the difference of two measurements and the limits of agreement, respectively.
Table 2
Results of robust linear regression indicate that the only significant covariate in the linear regression model is the log_{10}(HCV RNA).

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<th>Coefficient</th>
<th>SD</th>
<th>95% confidence interval</th>
<th>p-Value</th>
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<td>0.04</td>
<td>0.83 – 1.01</td>
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Financial support
This investigation was supported in part by Abbott Laboratories, Inc. and the Troup Fund of the Kaleida Health Foundation. The research reported in this manuscript was partially funded through a Patient-Centered Outcomes Research Institute (PCORI) Award (IHS-1507-31640). The statements in this work are solely the responsibility of the authors and do not necessarily represent the views of PCORI, its Board of Governors or Methodology Committee.

Acknowledgements
The authors acknowledge the assistance of Dr. Rositsa Dimova.

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