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

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ABSTRACT

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.

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

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($13 to $100) (Freiman et al., 2016). Excess production of part of the HCV genetic material, specifically nucleocapsid peptide p22 (Dubuisson, 2007), is released into plasma during the assembly of the virus and this protein is detectable starting in the period before development of HCV antibodies (Tedder et al., 2013). As a diagnostic assay for acute HCV infection, HCVcAg can be detected from 1 to 2 days following HCV RNA detection in serum and 33.2 (range: 23–46) days prior to detection of HCV antibodies (Hosseini-Moghaddam et al., 2012). HCVcAg is highly stable across different individuals and has been shown to have high correlation with HCV RNA testing. As such, assessment of HCVcAg levels alone might be sufficient to confirm active HCV infection prior to DAA prescription without requirement for HCV RNA testing. This test could have applicability in resource limited settings such as low and middle income countries (LMIC) and among potentially marginalized populations such as patients with OUD on methadone in developed countries. Development of a point-of-care (POC) HCVcAg test that could be assayed without the need to send samples to an outside laboratory would be particularly helpful toward in the objective of global HCV elimination. Informing the individual of the test results within minutes of the sample being obtained would minimize the loss to follow up and facilitate linkage to care.

Given that development of a POC HCVcAg test has been identified as a high priority for HCV diagnostics by a global stakeholder consultative process (Forum for Collaborative HIV Research, 2015), its performance characteristics in various populations need to be assessed. In fact, a recent World Health Organization commissioned systematic review that evaluated five different platforms used to assess HCVcAg reported substantially improved performance for methods that include amplification compared to standard enzyme-linked immunosorbent assays (Freiman et al., 2016). We sought to evaluate the relationship between HCVcAg compared to HCV RNA measurements as assessed by two separate assays in patients with OUD on methadone. If patients with OUD on methadone in the United States are the likely target population for HCVcAg testing, it is important to document its performance characteristics in this population. Since the specificity of the HCVcAg test depends upon antibody-antigen binding, its performance characteristics need to be documented in different populations to ensure that specificity remains consistent.

2. Materials and methods

This study was approved by the Institutional Review Boards of Weill Cornell Medical College, University at Buffalo, and START Treatment & Recovery Centers. Study subjects were recruited from an opioid agonist therapy (OAT) clinic located in the West Harlem neighborhood of New York City (described in (Zeremski et al., 2014)). All patients were initially enrolled in a larger study with the goal of developing a telemedicine-based model for HCV evaluation and treatment of substance users on OAT (Talal et al., 2016). In preparation for telemedicine-based HCV treatment, all subjects initially participated in an HCV-related educational intervention (Zeremski et al., 2016). Prior to the educational intervention, blood was obtained for HCVcAg and HCV RNA testing. One aliquot was prepared as per performance instructions and was immediately shipped for quantitation of peripheral HCV RNA (Roche COBAS Taqman, Laboratory Corporation of America, Research Triangle Park, NC). The remaining specimen was frozen within 6 h of collection and stored at −80 °C. Samples were assayed in batch for both HCVcAg and a second HCV RNA measurement (M2000-Abbott Molecular, Abbott Park, IL). Patient demographic and life-time drug use data were obtained from a survey completed at entry into the overall study (Zeremski et al., 2014). Information about illicit drug use in the last 6 months was obtained from a questionnaire performed at the end of the educational intervention (Zeremski et al., 2016). HCV and HIV antibody test results were obtained from the electronic medical record. As these tests are 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 indicates 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 HCVcAg. 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 ($p = 0.001$).

Table 1
Demographic and substance use characteristics.

Variable	Level	All patients		HCV RNA positive	
		Count/mean	%/SD	Count/mean	%/SD
Age		53.8	7.80	56.7	8.30
Gender	Male	66	60.55	32	68.09
	Female	43	39.45	15	31.91
Race	White	5	4.59	3	6.38
	Black	75	68.81	30	63.83
	Mixed	2	1.83	13	27.66
	Other	27	24.77	1	2.13
Ethnicity	Hispanic	32	29.36	14	29.79
	Non-Hispanic	77	70.64	33	70.21
Duration on OAT		6	5.20	8.3	6.30
Hx IDU	Yes	65	59.63	39	82.98
	No	44	40.37	8	17.02
Hx non-IDU	Yes	103	94.50	44	93.62
	No	6	5.50	3	6.38
IDU-past 6 months	Yes	11	10.09	6	12.77
	No	98	89.91	41	87.23
Non-IDU-past 6 months	Yes	54	49.54	18	38.30
	No	53	48.62	28	59.57
	Missing	2	1.83	1	2.13

Abbreviations: SD, standard deviation: OAT, opiate agonist therapy; IDU, injection drug use.

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 levels was also high with $r = 0.88$ (95% CI [0.76; 0.95], $p < 0.01$) (Fig. 1b). Additionally, correlation between $\log_{10}$-transformed HCV RNA (Cobas) and $\log_{10}$-transformed HCV RNA (Abbott) was also extremely high with $r = 0.946$ (95% CI [0.891, 0.967], $p < 0.01$) (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

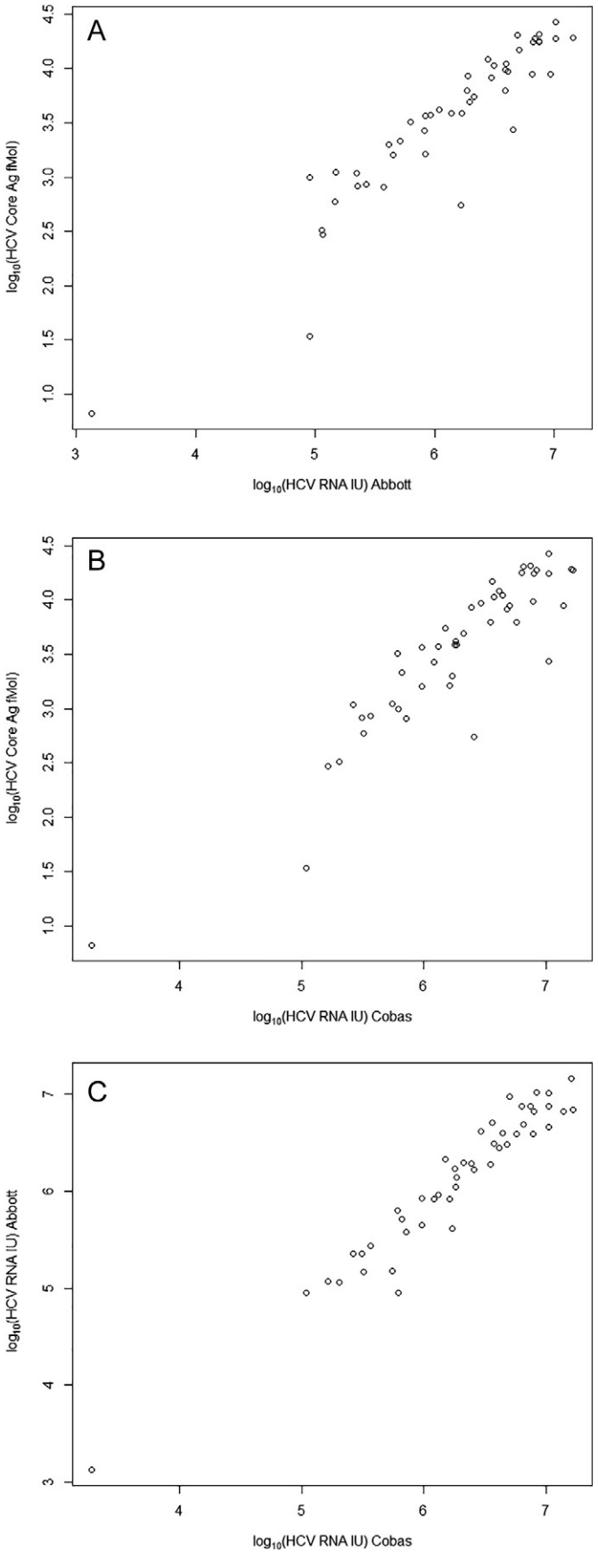
We used robust regression to investigate the potential influence of demographic, virologic and behavioral (such as injection and noninjection drug use) variables on HCVcAg levels as it is important to document that the relationship between HCVcAg and HCV RNA levels does not change among those who likely 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; Mixson-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 RNA), measured using Cobas® TaqMan® HCV Test v 2.0 (X-axis) and $\log_{10}$(HCV Core Ag), measured using Architect Core Antigen assay (Y-axis). C: Scatter plot of $\log_{10}$(HCV RNA), measured using Cobas® TaqMan® HCV Test v 2.0 (X-axis) and of $\log_{10}$(HCV RNA), measured using Abbott® Realtime HCV RNA 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.

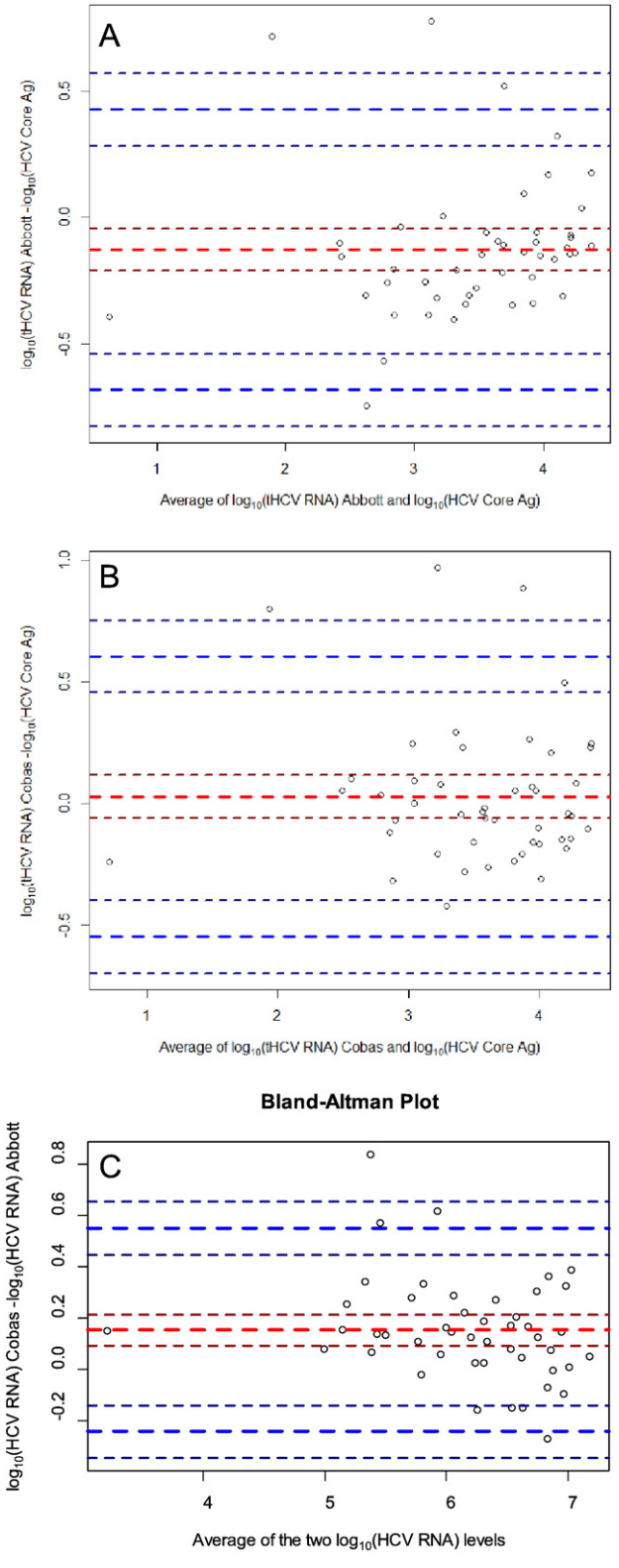


Fig. 2. A: Bland-Altman plot assessing the agreement between converted, $\log_{10}$-transformed Abbott realtime PCR assay (tHCV RNA) and $\log_{10}$-HCV core antigen using the Architect Core Antigen assay. The Y axis represents the difference between $\log_{10}$(tHCV RNA) Abbott and $\log_{10}$(HCVcAg). The X axis represents the average between $\log_{10}$(tHCV RNA) Abbott and $\log_{10}$(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, $\log_{10}$-transformed Cobas® TaqMan® HCV Test v 2.0 (tHCV RNA) and $\log_{10}$-HCV core antigen using the Architect Core Antigen assay. The Y axis represents the difference between $\log_{10}$(tHCV RNA) Cobas and $\log_{10}$(HCVcAg). The X axis represents the average between $\log_{10}$(tHCV RNA) Cobas and $\log_{10}$(HCVcAg). 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 $\log_{10}$-transformed Abbott realtime PCR assay (HCV RNA) and $\log_{10}$-transformed Cobas® TaqMan® HCV Test v 2.0 (HCV RNA). The Y axis represents the difference between $\log_{10}$(HCV RNA) Cobas and $\log_{10}$(HCV RNA) Abbott. The X axis represents the average between $\log_{10}$(HCV RNA) Cobas and $\log_{10}$(HCV RNA) 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).

Variable	Coefficient	SD	95% confidence interval		p-Value
Intercept	−2.18	0.26	−2.70	−1.66	<0.001
HCV RNA (LabCorp) (log10)	0.92	0.04	0.83	1.01	<0.001

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