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Hepatitis C virus core antigen: A simplified treatment monitoring tool, including for post-treatment relapse

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ABSTRACT

Background: Simple, affordable diagnostic tools are essential to facilitate global hepatitis C virus (HCV) elimination efforts.
Objectives: This study evaluated the clinical performance of core antigen (HCVcAg) assay from plasma samples to monitor HCV treatment efficacy and HCV viral recurrence.
Study design: Plasma samples from a study of response-guided pegylated-interferon/ribavirin therapy for people who inject drugs with chronic HCV genotype 2/3 infection were assessed for HCV RNA (AmpliPrep/COBAS Taqman assay, Roche) and HCVcAg (ARCHITECT HCV Ag, Abbott Diagnostics) during and after therapy. The sensitivity and specificity of the HCVcAg assay was compared to the HCV RNA assay (gold standard).
Results: A total of 335 samples from 92 enrolled participants were assessed (mean 4 time-points per participant). At baseline, end of treatment response (ETR) and sustained virological response (SVR) visits, the sensitivity of the HCVcAg assay with quantifiable HCV RNA threshold was 94% (95% CI: 88%, 98%), 56% (21%, 86%) and 100%, respectively. The specificity was between 98 to 100% for all time-points assessed. HCVcAg accurately detected all six participants with viral recurrence, demonstrating 100% sensitivity and specificity. One participant with detectable (non-quantifiable) HCV RNA and non-reactive HCVcAg at SVR12 subsequently cleared HCV RNA at SVR24.
Conclusions: HCVcAg demonstrated high sensitivity and specificity for detection of pre-treatment and post-treatment viraemia. This study indicates that confirmation of active HCV infection, including recurrent viraemia, by HCVcAg is possible. Reduced on-treatment sensitivity of HCVcAg may be a clinical advantage given the moves toward simplification of monitoring schedules.

1. Background

Morbidity and mortality due to HCV-related liver disease continues to increase globally [1]. While direct-acting antivirals (DAAs) provide an unprecedented opportunity to effectively scale up HCV treatment [2], most people with HCV are unaware of their infection [3]. Affordable, simple diagnostic and treatment monitoring algorithms are urgently required to enhance efforts for global elimination of

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HCV infection by 2030 [4,5]. Cost-effective, single-step diagnostic tools for active HCV infection are required to streamline diagnoses and facilitate linkage to care [2,6]. Nucleid-Acid-Tests (NAT) are used currently in clinical practise due to their high specificity, sensitivity and reproducibility, although costs can constrain their utility in resource-limited settings [7]. Likewise, simple tools to confirm cure and detect viral recurrence are critical to facilitate DAA scale-up and HCV elimination, particularly in low and middle income countries.

Assays for the detection of the HCVcAg, a viral protein released into the plasma during viral assembly, have been developed as a more stable, affordable, alternative to HCV nucleic acid tests [8]. Several commercial HCVcAg assays are now available and have demonstrated highly sensitive and specific diagnoses of active HCV infection in a range of populations [9]. Despite extensive evaluation as a diagnostic tool for chronic HCV infection, fewer studies have evaluated the clinical utility of the HCVcAg assay to monitor HCV treatment outcomes [10–13]. The detection of post-treatment viraemia, through either virological failure or reinfection is particularly important in the DAA era.

2. Objectives

This study aimed to evaluate the clinical performance of HCVcAg in plasma to monitor HCV treatment including viral recurrence in people who inject drugs in the ACTIVATE study.

3. Study design

3.1. Study participants

ACTIVATE (NCT01364090) is an international, open-label clinical trial recruited between 2012 and 2014 [14]. Participants were enrolled at 17 sites in 7 countries (Australia, Belgium, Canada, Germany, Norway, Switzerland and the United Kingdom) through drug and alcohol clinics, private practices, hospital clinics and community clinics. Participants had to be more than 18 years of age, have chronic HCV genotype 2 or 3 infection, be HCV treatment-naïve, and have reported recent injecting drug use or receiving opioid substitution therapy (OST). Participants with HIV or HBV coinfection and decompensated liver disease were excluded. Participants received directly observed pegylated interferon alfa-2b (PEG-IFN, 1.5 μg/kg/week) and self-administered ribavirin (RBV, 800–1400 mg daily, weight-based) for 12 or 24 weeks. Participants with rapid virological response (RVR) defined as having undetectable or non-quantifiable HCV RNA (< 15 IU/mL) at week 4 were allocated to 12 weeks of therapy (shortened duration). Participants with quantifiable HCV RNA (≥15 IU/mL) at week 4 received 24 weeks of therapy (standard duration). All study participants provided written informed consent. Ethical (#LNR/15/SVH/286) and governance approval (#15/190) was provided by St. Vincent's Hospital, Sydney Human Research Ethics Committee.

3.2. Study design and definitions

EDTA plasma was obtained from baseline (BSL) or screening if BSL was unavailable, week 4, week 12 (standard treatment arm only), end of treatment (ETR), sustained virological response week 12 (SVR12) or week 24 (SVR24) if SVR12 was unavailable, and follow-up (FU1) visits. Viral recurrence was defined as detectable HCV RNA following ≥1 undetectable result for HCV RNA. Post-treatment relapse was defined by the presence of quantifiable HCV RNA after an ETR, comfirmed as homologous virus on sequencing as described previously [15]. Reinfection was defined by the presence of quantifiable HCV RNA after an ETR and detection of infection with an HCV strain that was distinct from the pre-treatment strain (heterologous virus on sequencing of Core-E2 and/or NS5B regions).

3.3. Study assessments

HCV RNA levels were measured on stored plasma samples tested centrally with the COBAS AmpliPrep/COBAS TaqMan HCV Test (version 2.0, Roche Molecular Systems), which has a lower limit of quantification of 15 IU/mL. HCV genotype and subtype were determined by sequencing of the NS5B region [16]. In brief, 200 μL plasma was extracted using the Roche MagNA Pure LC Total Nucleic Acid Isolation Kit on the Thermo Scientific KingFisher Flex processor as per the manufacturer's instructions. NS5B polymerase chain amplicons (320 bp) were purified and sequenced on the ABI 3730 platform (Ramaciotti Centre for Genomics, Sydney Australia). HCVcAg levels were measured on aliquots of stored plasma using the two-step chemiluminescent microparticle immunoassay ARCHITECT HCV Ag (Ref. 6L47) on the ARCHITECT-i2000R Immunoassay Analyser (Abbott Diagnostics, Illinois, USA). In brief, 200–230 μL was aliquoted into 2 mL screw cap tubes and centrifuged at 10,000*g* for 10 min then transferred into 2 mL sample cups prior to loading. Samples assessed as having HCVcAg between 3 and 10 fmol/mL (ie. the "grey-zone") were retested per manufacturer recommendations where sample was available. For participants with quantifiable HCV RNA, and yet consistently negative HCVcAg results, the HCV Core gene was amplified and sequenced using previously published methods where sample was available [15,17] to identify potential mutations in the antibody binding region of the ARCHITECT HCV Ag assay [18–20].

3.4. Statistical analysis

Log10 transformations of HCVcAg levels ($\log_{10}$ fmol/L) and HCV RNA levels ($\log_{10}$ IU/mL) were used in all analyses. Nonparametric statistical tests were performed given the log10 transformation of both HCVcAg levels and HCV RNA levels were not normally distributed. The distribution of HCVcAg levels at baseline/screening was assessed by HCV genotype using the Wilcoxon–Mann–Whitney test. The correlation of HCVcAg levels and HCV RNA levels at baseline/screening was assessed using Spearman's rho test. The distribution of HCVcAg and HCV RNA levels at each time-point was described using median and interquartile range (Q1–Q3). P values < 0.05 (two-sided) were considered significant in all analyses.

The diagnostic performance of the HCVcAg test was performed by measuring the diagnostic sensitivity, specificity and concordance of HCVcAg in comparison to HCV RNA as the gold standard at all time-points. HCVcAg results were dichotomized as "reactive" (≥3 fmol/L) or "non-reactive" (< 3 fmol/L). HCV RNA results were dichotomized based on two thresholds, including the quantification threshold (15 IU/mL; i.e., ≥15 IU/mL: "quantifiable" < 15 IU/mL: "non-quantifiable"), and a threshold at 3,000 IU/mL. The latter threshold was used given that it has been recently identified in a meta-analysis for high sensitivity, high specificity and good correlation with HCVcAg [9]. All data was analysed using Stata (Stata 14, StataCorp, College Station, USA) and displayed using Prism (Prism 6, GraphPad Software, San Diego, USA).

4. Results

4.1. Participant characteristics

A total of 335 Samples from 92 ACTIVATE study participants were available for inclusion in this study (mean 4 time-points per participant). Participant characteristics are shown in Table 1. The mean age was 41 years (Q1–Q3: 35–49), 83% were male (n = 77), 90% were of Caucasian background (n = 84), 59% reported injecting in the past month (n = 55), and 89% were genotype 3 (n = 83). At baseline, the median HCV RNA level was 6.08 (Q1–Q3: 5.63–6.70) $\log_{10}$ IU/mL and median HCVcAg level was 3.1 (Q1–Q3: 2.5, 3.7) $\log_{10}$ fmol/L (Table 1).

The majority of participants achieved RVR and received shortened

Table 1
Baseline demographic, clinical and virological characteristics of the study population in ACTIVATE (n = 93).

Demographic characteristics	n
Age, median (IQR), yrs	41 (35–49)
Male gender, n (%)	77 (83)
Caucasian ethnicity, n (%)	84 (90)
Injecting in the previous month, n (%)	55 (59)
Treatment duration and virologic response, n (%)	
Treatment arm	
Standard duration treatment (24 weeks)	26 (28)
Shortened duration treatment (12 weeks)	61 (66)
Withdrawal before week4 of treatment	6 (6)
Virologic response	
End of treatment response (ETR)	70 (75)
Sustained virologic response (SVR)	61 (66)
Viral recurrence after ETR (% among those achieving ETR)	5 (7)
Virologic characteristics	
HCV RNA level, median (IQR), Log IU/mL	6.08 (5.63, 6.70)
HCV genotype, n (%)	
Genotype 1	1 (1)
Genotype 2	9 (9)
Genotype 3	83 (89)
HCVcAg level, median (IQR), log fmol/L	3.1 (2.5, 3.7)

duration of treatment (66%, n = 61), 75% of all participants achieved an ETR (n = 70), and 66% achieved a SVR (n = 61). Among participants with an ETR, 7% (n = 5) demonstrated viral recurrence through SVR24. Participant samples were available at baseline (n = 92), week 4 (n = 85), week 12 (n = 16), ETR (n = 75), SVR12 (n = 63), and SVR24 (n = 2).

4.2. HCVcAg distribution and correlation to HCV RNA

Among baseline samples, there was a strong correlation between HCVcAg levels and HCV RNA levels (Fig. 1, Spearman's Rho 0.89, $p < 0.0001$). The distribution for HCVcAg and HCV RNA level was determined for all longitudinal samples (Fig. 2). The median HCVcAg levels in baseline samples was 3.4 (Q1–Q3: 2.7, 3.7) $\log_{10}$ fmol/L and median HCV RNA levels was 6.2 (Q1–Q3: 5.7, 6.7) $\log_{10}$ IU/mL (Fig. 2).

4.3. Diagnostic performance of the HCVcAg assay

The number of samples with reactive HCVcAg (≥3 fmol/L) according to HCV RNA levels can be seen in Fig. 3. Overall, 94% of samples (107/114) with HCV RNA levels greater than 3000 IU/mL were reactive for HCVcAg, and 98% (215/219) with HCV RNA levels less than 3000 IU/mL were non-reactive for HCVcAg. The number of samples with quantifiable RNA with HCVcAg in the "grey zone" (between 3 and 10 fmol/mL) was 8% (9/109) with 4 samples with HCV RNA levels greater than 3000 IU/mL.

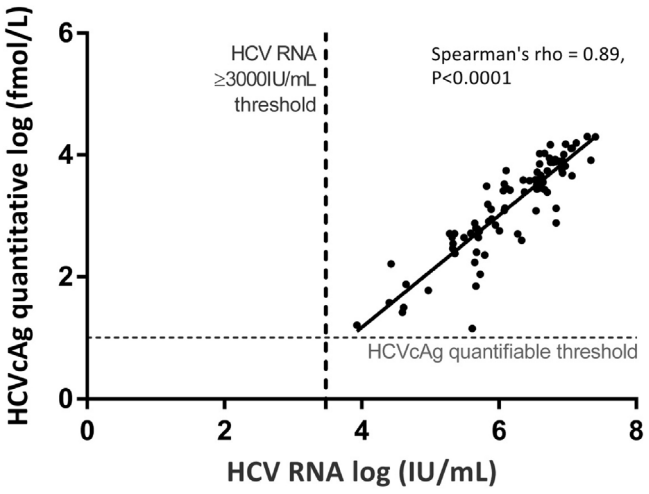

Fig. 1. Correlation of HCVcAg and HCV RNA levels.

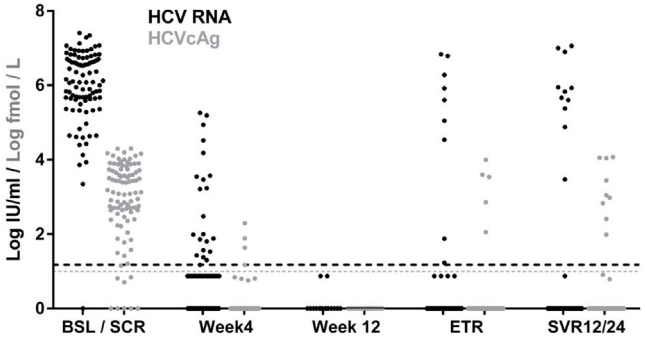

Fig. 2. Distribution of HCV RNA and HCVcAg levels at each study timepoint.

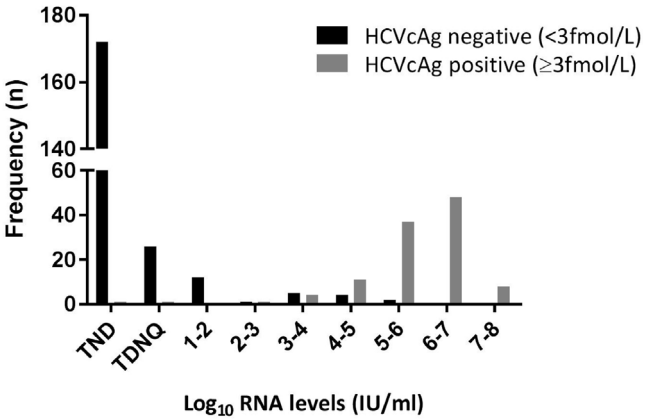

Fig. 3. Frequency of reactive HCVcAg across a range of HCV RNA levels.

4.4. HCVcAg sensitivity and specificity

Using a threshold of quantifiable HCV RNA (≥15 IU/mL), HCVcAg demonstrated consistently high specificity (98–100%) at all time-points and a range of sensitivity (31–100%) (Table 2A). The lowest sensitivity was at week 4 (31% 14–55, 95% CI), followed by ETR (56%, 14–55, 95%CI). At ETR, four participants with quantifiable HCV RNA (range 17–829, 419 IU/mL) were non-reactive for HCVcAg (Tables 3 and 4 and Supplementary Fig. 1). In contrast, at SVR12/24 HCVcAg sensitivity was 100%.

Using a threshold in diagnosing HCV RNA levels ≥3000 IU/mL, HCVcAg demonstrated high specificity (97–100%) and moderate to high sensitivity (71–100%) at all time-points (Table 2B). The lowest sensitivity was at ETR (71% 29–96 CI), at which two participants with HCV RNA above the threshold (34,514 and 829,419 IU/mL) were not reactive for HCVcAg (Tables 3 and 4 and Supplementary Fig. 1). Sensitivity for ETR was higher (86% 42–100 CI) with one participant with quantifiable HCV RNA (154,501 IU/mL) non-reactive for HCVcAg.

HCVcAg was reactive in one participant at baseline (1154 fmol/L) in whom HCV RNA was not detected (ID 1007-47001-03), despite chronic HCV infection being an inclusion criteria for enrolment. The screening sample for this participant had a high HCV RNA level (6.1 million IU/mL) and consistently negative RNA for all subsequent samples. Local RNA results at baseline were unavailable to confirm RNA status and as such, the potential cause of the discrepancy, i.e. A false-positive HCVcAg result, spontaneous clearance prior to treatment or a sample mix-up could not be confirmed.

Table 2
Sensitivity and specificity of HCVcAg in diagnosing quantifiable HCV RNA (A) or HCV RNA ≥ 3000 IU/mL (B) in each time point during treatment and post-treatment follow-up.

(A) Sensitivity and specificity of HCVcAg in diagnosing quantifiable HCV RNA

Treatment	Visit	Samples (n)	Sensitivity% (95% CI)	Specificity% (95% CI)
Pre-treatment	BSL/SCR	92	94 (88, 98)	[a]
On-treatment	Week 4	85	31 (14, 55)	98 (91, 100)
	Week 12	16	100	100
	ETR	75	56 (21, 86)	100
Post-treatment	SVR12/24	65	100	100

(B) Sensitivity and specificity of HCVcAg in diagnosing HCV RNA levels ≥ 3000 IU/mL

Treatment	Visit	Samples (n)	Sensitivity% (95% CI)	Specificity% (95% CI)
Pre-treatment	BSL/SCR	92	96 (89, 99)	[a]
On-treatment	Week 4	85	86 (42, 100)	97 (91, 100)
	Week 12	16	100	100
	ETR	75	71 (29, 96)	100
Post-treatment	SVR12/24	65	100	100

[a] Specificity for baseline/screening could not be calculated due to limited sample size in the true negative group.

Table 3
Numbers of ACTIVATE plasma samples that tested reactive or non-reactive for HCVcAg and HCV RNA (quantifiable or non-quantifiable) at each time-point.

Visit	HCVcAg (> 3 fmol/L)	Quantifiable HCV RNA (≥15 IU/mL)	Non-quantifiable HCV RNA (< 15 IU/mL)
SCR/BSL	Reactive	86	1
	Non-reactive	5	0
Week 4	Reactive	7	1
	Non-reactive	15	62
Week 12	Reactive	1	0
	Non-reactive	0	16
ETR	Reactive	5	0
	Non-reactive	4	66
SVR12/24	Reactive	11	0
	Non-reactive	0	54

4.5. HCVcAg and HCV RNA profiles among those with viral recurrence and non-responders

Five participants experienced post-treatment viral recurrence, defined as participants with end of treatment undetectable HCV RNA followed by quantifiable HCV RNA during post-treatment follow-up (Fig. 4, panels A, B, C, E, F). In all these participants, HCVcAg was reactive at 12 weeks post-treatment. An additional participant (panel D, ID 1007-47001-02) with an ETR had detectable, non-quantifiable HCV RNA at SVR12 and negative HCVcAg, but had a subsequent undetectable HCV RNA result at SVR24 (local laboratory). All six participants (4 shortened treatments, A–D; 2 standard durations, E–F) were non-reactive for HCVcAg and HCV RNA at end of treatment. Thus, HCVcAg demonstrated 100% sensitivity and specificity when compared with HCV RNA for detection of post-treatment viral recurrence.

4.6. HCVcAg sequencing to identify HCV core mutations associated with lower HCVcAg serological result

Eleven participants displayed consistently low HCVcAg in the presence of quantifiable HCV RNA (highlighted in bold in Table 4). Among these 11 participants with consistently low HCVcAg, core sequence data was available for five participants (Table 4, ID 1007-41301-03, 1007-61207-07, 1007-12101-02, 1007-61207-07 and 1007-12501-03). Of these five participants, one participant (ID 1007-41301-03, Supplementary Fig. 1, panel A) was infected by Gt3a HCV with a baseline HCV RNA of 680,987 IU/mL and a HCVcAg measurement of 6.92 fmol/L. The HCV Core sequence for this sample harboured double mutations at amino acids 48 and 49 as A48T and T49A/P. A48T mutation is present in all genotypes (range: 0.35–6.70%). T49A mutation is present in all genotypes (range: 0.1–4.50%), T49P is present in GT2 (as well as Gt1a, GT1b and GT4, range: 0.4–15.7%) [19]. Those mutations were detected in the subsequent time-points (week 4, SVR12 and FU1). Samples from four other participants harboured single mutations, which could impact the performance of the HCVcAg assay detection.

At baseline and screening, five genotype 3a participants (ID 1007-12501-03, ID 1007-61310-03, 1007-12101-02, ID 1007-61207-07, ID

Table 4
Discordant results for HCVcAg (reactive or non-reactive) and HCV RNA (quantifiable or non-quantifiable) at each timepoint, with HCV RNA and HCVcAg levels, core-E2 mutations and clinical outcome in ACTIVATE plasma samples.

Subject-ID	RNA level IU/mL	HCVcAg fmol/L	HCVcAg mutation	ETR	SVR
SCR/BSL					
Quantifiable HCV RNA/Non-reactive HCVcAg					
1007 – 12101-02	13,377	1.35	**mutation (SCR)**	yes	yes
1007 – 12501-03	2225	0.15	**mutation (BSL)**	yes	yes
1007-32801-01	67,431	0	no mutation (BSL)	yes	no
1007-61207-07	42,933	1.73	**mutation (SCR)**	yes	yes
1007-61310-03	7282	2.73	**mutation (SCR)**	yes	yes
Non-quantifiable HCV RNA/Reactive HCVcAg					
1007-47001-03	0	1154.06	not tested	yes	yes
Week 4					
Quantifiable HCV RNA/Non-reactive HCVcAg					
1007 – 12201-02	34	0.94	not tested	yes	yes
1007-41101-02	1631	0	not tested	no	no
1007-41301-03	154,501	0.84	double mutation (BSL/WK4/SVR12/FU1)	no	no
1007-44002-06	27	0.3	not tested	yes	yes
1007-44002-09	73	0	not tested	no	no
1007-44002 – 10	76	0	not tested	yes	yes
1007-47001-08	64	2.76	not tested	yes	yes
1007-47001 – 12	1712	0.8	not tested	no	no
1007-61202-06	24	2.57	not tested	yes	yes
1007-61202 – 10	2909	2.39	not tested	yes	yes
1007-61207-09	20	0.47	not tested	no	no
1007-61212-09	101	0	not tested	no	no
1007-61310-02	15	0	not tested	yes	yes
1007-61310-05	97	0.12	not tested	no	no
1007-61501-06	37	0	not tested	no	no
Non-quantifiable HCV RNA/Reactive HCVcAg					
1007-61202-07	7.5	5.44	not tested	yes	no
ETR					
Quantifiable HCV RNA/Non-reactive HCVcAg					
1007 – 12501-06	829,419	0	not tested	no	yes
1007-41301-03	34,514	0	double mutation (BSL/WK4/SVR12/FU1)	no	no
1007-41301-05	75	0	not tested	no	no
1007-61207-09	17	0	not tested	no	no

Subject ID in bold are participants displaying consistently low HCVcAg in the presence of quantifiable HCV RNA as discussed in the main text.

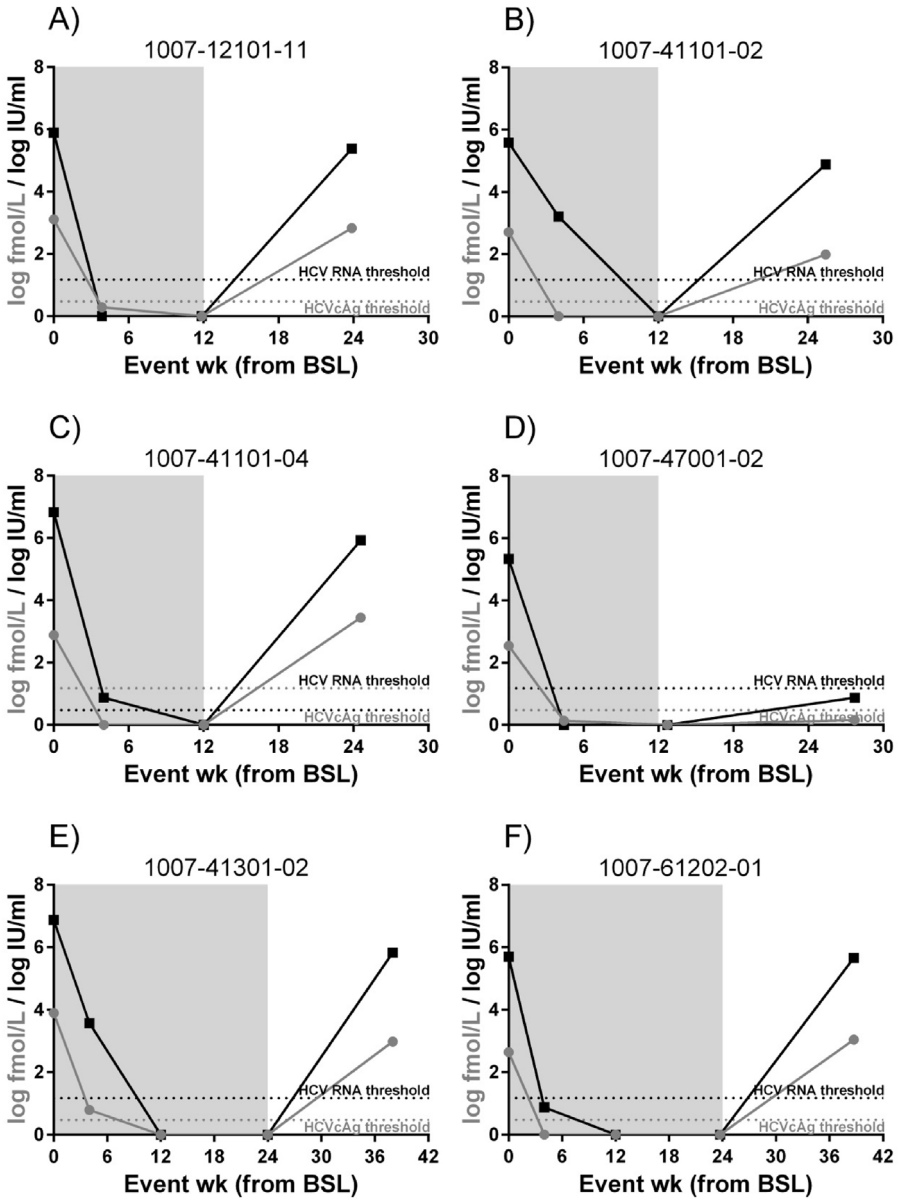

Fig. 4. HCV RNA and HCVcAg levels in a subset of patients with viral recurrence during treatment (grey box) and post-treatment follow-up.

1007-32801-01) had quantifiable HCV RNA at baseline and screening (2225, 7282, 13,377, 42,933, 67,431 IU/mL), but negative HCVcAg (Table 4). These participants continued to be negative for HCVcAg and HCV RNA at subsequent time-points. One sample (ID: 1007-41301-03, Table 4 and Supplementary Fig. 1 panel A) demonstrated quantifiable HCV RNA at week 4 and end of treatment (154,501 and 34,514 IU/mL respectively) but was negative for HCVcAg. This participant had on-treatment virological failure and harboured multiple mutations in the core HCV genome.

4.7. Discordant results between HCV RNA and HCVcAg

Overall, using a threshold of detectable HCV RNA, there were 26 discordant HCVcAg samples (26/333, 7.8%) from 24 participants, including 16 discordant samples at week 4 post-treatments when HCV RNA level is low (Table 4, Supplementary Table 1). Of these discordant samples, 24 had detectable HCV RNA and negative HCVcAg. Using a HCV RNA threshold of 3000 IU/mL, the number of discordant samples was reduced to 7 samples with a range of HCV RNA from 3.9 to 5.9 $\log_{10}$ IU/mL.

5. Discussion

Affordable and effective tools to diagnose HCV infection, confirm cure and detect relapse or reinfection are urgently required. This study is the first to prospectively evaluate the clinical performance of HCVcAg in plasma to monitor HCV treatment and viral recurrence among an international cohort of recent PWID and people receiving OST with chronic HCV infection. While limited to genotype 2/3 HCV infection, this study supports previous studies indicating HCVcAg can be used to identify active HCV infection at baseline/screening among those with chronic HCV infection [9,21–23] and identify those with a sustained virological response at 12 weeks post treatment [10,24]. While these data confirm a previous study by Chevaliez et al. [10] that HCVcAg can accurately detect viral recurrence 12 or 24 weeks post-treatment, this study provides additional comprehensive longitudinal profiles from individuals with recurrent viraemia.

The findings of this study supports previous cross-sectional and analytical studies [9], suggesting that HCVcAg may be an affordable method to screen and diagnose large numbers of people with chronic HCV. Considering greater than 95% of those with chronic HCV

infection have HCV RNA levels above 1,000 IU/mL [25], this study indicates a qualitative HCVcAg assay may be a suitable screening and diagnostic assay for chronic HCV, particularly in a population estimated to have a high prevalence of HCV infection.

This study also supports previous studies indicating a qualitative assay is likely to reliably identify the absence of HCV infection and confirm cure, regardless of the treatment regimen [10,13]. Our study demonstrates a similar HCVcAg analytical sensitivity (94% at baseline) to previous studies (91–98%) [10,26–28] for detection of active HCV infection. With regard to on-treatment monitoring, HCV RNA detectable samples with negative HCVcAg at week 4 had declining HCV RNA levels of < 2,909 IU/mL. Therefore, it could be argued that a reduced sensitivity during treatment may be a clinical advantage, providing a high specificity is maintained. For example, highly sensitive HCV RNA assays that remain positive during early treatment, even among patients who are successfully responding to therapy, may in fact confuse clinical interpretation. Alternatively, less sensitive HCVcAg assays that provide a negative result during early treatment may provide an indication of response to therapy and help guide clinical management. Although the clinical relevance of week 4 data during DAA treatment is debatable [29–33], the potential for HCVcAg to provide an indicator of DAA treatment adherence in the early stages during therapy could be explored further in those where adherence may be of concern. More data would be needed to evaluate potential change of HCVcAg profile in the early phase of the treatment with DAAs disrupting viral production.

This study demonstrated that HCVcAg accurately identified post-treatment relapse 12 or 24 weeks after end of treatment in five participants. A sixth participant who had detectable, but not quantifiable HCV RNA and negative HCVcAg 12 weeks after an end of treatment response, did not have sustained post-treatment viraemia, thus providing further evidence that the lower sensitivity afforded by HCVcAg may be more clinically useful to monitor viral recurrence than highly sensitive RNA assays, as a strategy to avoid ambiguous and potentially irrelevant HCV RNA positive results due to the detection of low, or replication incompetent, HCV RNA. While limited to an interferon-ribavirin regimen, the results are likely transferable to relapse from DAA for which HCV RNA has been shown to rebound rapidly and to high levels [31,34,35].

This study identified a number of samples with discordant HCV RNA and HCVcAg results, including five participants that were “missed” at baseline/screening. In high HCV prevalence populations, where an individual has been at risk of exposure is or has previously resolved or been cured, a two-step diagnostic algorithm could be considered with screening HCVcAg followed by HCV RNA assay in those with negative HCVcAg. This would have the additional advantage of improving detection of acute HCV infection cases prior to anti-HCV antibody seroconversion. In such a setting HCVcAg is a cost effective–effective strategy to replace HCV antibody as a single diagnostic assay for active infection [22,26,36]. For screening HCV in low prevalence setting, a three step algorithm with HCV antibody, followed by HCVcAg (for HCV ab reactive result) and finally HCV RNA test (for HCVcAg non-reactive result) would be cost effective in achieving 100% detection of active viraemia as previously described [37]. This study also identified six genotype 3 participants for whom the HCVcAg assay was consistently negative and HCV RNA levels quantifiable, five of whom could be attributable to amino acids substitutions identified in Core region previously showed to affect HCVcAg serological result [19]. Little data on the prevalence of those mutations are known in the general population and more studies are required to assess the impact of false negative HCV detection due to core antigen mutations on all genotypes. Thus, this provides further evidence indicating a confirmatory testing algorithm for negative HCVcAg results among those at risk may be considered to ensure they are not missed.

This study has a number of limitations. The ACTIVATE study was limited to genotype 2/3 mono infected patients, and the HCVcAg assay would need be assessed in other genotypes and patients coinfected with HIV or HBV, to determine if the results are transferrable. In addition, the potential impact of a higher centrifugation speed (10,000 rpm) during sample preparation on sample quality and error rates is unknown. Despite this, the specificity and sensitivity at baseline/screening matched other studies using Abbott ARCHITECT’s to measure HCVcAg [9]. It is important to acknowledge more data is required to confirm if the core antigen profiles during relapse or reinfection among those treated with DAA are similar and the applicability of this data to DAA era [35]. In addition, all participants are HCV antibody positive, limiting specificity for baseline/screening and determination of positive or negative predictive values. This is important considering specificity may be lower in HCV negative populations. An important limitation was the unavailability of sufficient specimen to repeat samples for which the HCVcAg was between 3 and 10 fmol/L as recommended by the assay protocol, or to sequence the HCV Core region of all samples with discordant results for HCV RNA and HCVcAg.

6. Conclusions

This study provides data indicating the HCVcAg may be a simple and affordable tool to diagnose individuals with chronic HCV infection, confirm cure and detection of post-treatment viraemia and facilitate successful HCV elimination. The reduced sensitivity afforded by the HCVcAg may provide a clinical advantage when assessing treatment response in the presence of low level and intermittent viraemia, as detected by sensitive HCV RNA assays. The potential role for HCVcAg testing, from alternative sample types such as dried blood spots for centralised testing and alternative platforms such as point of care to facilitate testing in remote settings needs to be further explored. Further work is also required in remote, lower- and middle-income settings to determine if specimen handling and testing remains sufficient in real world settings.

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Competing interests

The Kirby Institute is funded by the Australian Government Department of Health and Ageing and the National Health and Medical Research Program Grant (#1053206). GD is a consultant/advisor and has received research grants from Abbvie, Bristol Myers Squibb, Gilead, Merck, Janssen and Roche. JG is a consultant/advisor and has received research grants from Abbvie, Bristol Myers Squibb, Cepheid, Gilead Sciences and Merck/MSD. PB is consultant/advisor and has received research grants from Abbvie, Bristol Myers Squibb, Gilead, Janssen and Merck. GC is an employee and shareholder of Abbott Laboratories, OD is a consultant/advisor and has received research grants from Abbvie, Gilead Sciences and Merck/MSD. GF is a consultant/advisor and has received research grants from Abbvie, Gilead Sciences, Janssen, GSK and Merck/MSD. GR has received research grants from Merck Sharp & Dohme, AbbVie, Janseen Pharmaceuticals,

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Authors have no conflict of interest to declare.

Ethical approval

Ethical (#LNR/15/SVH/286) and governance approval (#15/190) for the ACTIVATE study was provided by St. Vincent's Hospital, Sydney Human Research Ethics Committee.

Contributors

GJD, OD, JG and PM designed the original ACTIVATE study and wrote the protocol. TLA, GJD, JG, designed the current study with input from FL, AS. FL, AS, performed laboratory work with input and help from TLA, DM, PC. The primary analysis was conducted by BH, EC which was reviewed by JG, FL and TLA. FL and TLA wrote the article. All authors reviewed to and approved the final article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2017.05.007.

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