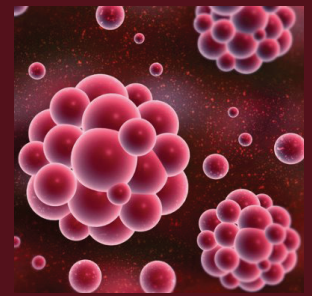


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Review Article: Moving ahead in HCV diagnostics: Need for the introduction of HCV core antigen assay in low and middle-income countries

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Abstract

Hepatitis C virus (HCV) infections are associated with significant morbidity and mortality globally. The diagnosis of HCV is primarily based on indirect serological assays such as enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CIA), and rapid diagnostic tests to detect HCV antibodies. Direct tests detect/quantify components of HCV virions, such as HCV ribonucleic acid (RNA) (nucleic acid test or nucleic acid amplification test [NAT]) and HCV core antigen (HCVcAg). The HCVcAg assay (CIA, Abbott ARCHITECT) is an immune assay used for the quantitative determination of the HCVcAg. This test is simple and fast with the potential to be incorporated into diagnostic guidelines and be used in combination with anti-HCV (CIA) as an effective screening test. HCVcAg can also be used as a potential biomarker for treatment initiation and monitoring patients to assess the treatment response. Apart from this, the scope for implementation of the HCVcAg assay in resource limited setting lies in screening of immune compromised patients where anti-HCV serology is not dependable. However, concerns related to lower sensitivity compared to HCV RNA do exist. Nevertheless, the HCVcAg assay can make a significant difference in the measures taken for the control and eradication of hepatitis C and its complications in India.

Keywords – Hepatitis C virus, immunoassay, HCV core antigen, HCV biomarker, hepatitis screening

Introduction

Hepatitis C virus (HCV) is one of the leading causes of hepatitis, cirrhosis and hepatocellular carcinoma; attributing to significant global of morbidity and mortality. Over 185 million individuals worldwide are infected with HCV and more than two-thirds of these cases are seen in the low-to middle-income countries [1]. In India, HCV presents itself as a significant public health issue with variations in the reported prevalence rates from 0.4%–1.0% [2]. There is also notable genotypic variation (1-7) across that ranges from geographic regions globally. While genotype 1 predominates in Europe, North America, and Australia, genotype 3 is more prevalent in Asian countries namely India, Pakistan and Bangladesh. Despite the high proportion patients in India and availability of highly effective Directly Acting Antiviral (DAA) therapy, only a fraction of the infected patients are formally diagnosed and are put on treatment. This attrition of patients prior to treatment initiation, has been previously attributed to several reasons [3,4]. Firstly, the wide variation in the clinical presentation of disease and no standard diagnostic algorithm. Secondly, lack of routine testing in the community and identification of high-risk regions or individuals. Thirdly, the cost of repeated follow-up testing with qualitative/quantitative nucleic acid amplification tests (NAATs) and finally, the cost of the 12-week course of DAA therapy.

In 2018, the government of India launched the National Viral Hepatitis Control Program (NVHCP) to overcome the above-mentioned challenges. This initiative aims to achieve 90% reduction in incidence and 65% reduction in mortality from existing levels (in line with WHO goals) along with developing comprehensive guidelines on testing strategies and treatment selection [1]. The program identified these lacunae and created national diagnostic guidelines, supporting community/regional surveillance and promoting the use of affordable “generic” DAAs. One

of the key components of formalising the approach to HCV is highlighting who and when to test for active infection. The serological marker available for screening and diagnosis is anti-HCV antibodies. This marker is unable to segregate active infections from spontaneously cleared HCV cases. The current multistep diagnostic algorithm requires physicians to request other NAAT like HCV RNA viral load in adjunct to anti-HCV antibodies, leading to additional delays in management initiation. This step in the cascade of care for HCV has been recognised as a roadblock in the global movement towards elimination of viral hepatitis. The American Association for the Study of Liver Diseases (AASLD) found that only 68% of anti-HCV reactive population are able to confirm infection with a secondary HCV RNA load test [3,4]. Given the substantial proportion of HCV transmission is through percutaneous route, high risk groups like persons who inject drugs (PWIDs), it is imperative that treatment initiation not to be delayed and dependency on patient follow up or high cost tests is reduced. Therefore, the World Health Organization (WHO) diagnostic algorithm for HCV management now includes a new serum antigen marker, HCV core antigen (HCVcAg). Despite being approved for use in India, there remains a paucity of knowledge about this new HCV marker. In this review we aim to discuss the role of HCVcAg assay in India, as well as to formulate strategies to overcome the challenges that might hinder in its implementation.

Diagnosis of Hepatitis C – Current scenario and Need for another biomarker

The diagnosis of HCV is primarily based on two types of laboratory investigations[5]:

- a) Indirect tests – These assays detect the antibodies to HCV
 - i. Enzyme linked immunoassay (ELISA) – first generation (G1) using recombinant HCV C100-3 peptide, second generation (G2) incorporating recombinant antigens from non-structural regions (NS3 and NS4) and HCV core antigen, third generation (G3) was similar to G2 with added NS5 epitope [7]
 - ii. Chemiluminescence immunoassay (CIA)
 - iii. Rapid diagnostic tests for point-of-care testing like lateral flow assay (LFA), flow through assay (FTA)
- b) Direct tests – These tests detect/quantify components of HCV virions, such as
 - i. HCV RNA by nucleic acid amplification (Endpoint or Real Time Polymerase Chain Reaction, RT-PCR)
 - ii. HCV core antigen; can be qualitative or quantitative or included along with antibody detection as fourth generation (G4) ELISA [8]

After initial testing for HCV antibody, confirmation of active ongoing infection by detection of HCV RNA testing is mandatory in all anti-HCV positive patients. Individuals who had a negative result with antibody testing need not undergo further testing and are considered free from HCV infection. However, false negative results must always be suspected and careful consideration of patient immune status (e.g. persons living with HIV/AIDS), window period, ongoing haemodialysis or post-transplant status must be made. False positivity is another situation which is concerning, especially in children aged <18 months born to HCV infected mothers. Variation in testing strategies based on the regional prevalence plays a role in formalising an effective diagnostic algorithm [9]. A number of screening assays have been developed which are able to use finger prick whole blood, dried blood spots or oral fluids for anti-HCV antibody detection. The WHO has even debated a one assay testing strategy versus a two assay testing strategy [1]. One assay testing strategy uses a single assay with high sensitivity and specificity suitable for resource-limited settings. However, a small percentage of test results may be false positive, so appropriate procedures to follow up individuals need to be in place. A two assay testing strategy uses 2 different testing assays to confirm the results of an initial positive sample. It efficiently identifies most uninfected individuals and more definitively rules out exposure to HCV than a one-assay testing strategy. It also improves the positive predictive value when the test results for two different assays are both reactive. However, the costs of implementation and the requirement of follow up testing for HCV viremia remain practical limitation to these strategies. Following a positive serological test, the second step of the diagnostic cascade is determining if the patient is having a current HCV infection by detecting viral RNA in the blood. The commonly used modality for this is real time PCR and is currently the best method to monitor success of DAA therapy. Testing for HCV viremia requires patients to undergo a venepuncture and requires a trained phlebotomist for sample collection. Performance of HCV RNA PCR requires skilled technicians, the test is time intensive and is an expensive test in terms of equipment. Therefore, this is a significant rate limiting step in HCV diagnosis as there still remains a chance that HCV viremia may not be detected due to viral load fluctuations [10-12]. Hence, there is a need to develop a more sensitive and

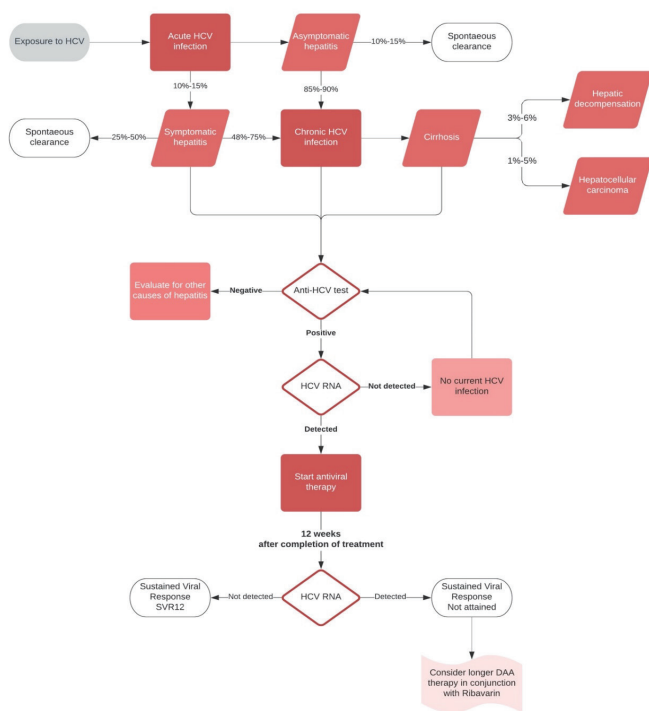


Figure 1: Natural history of hepatitis C and current diagnostic algorithm for HCV [5,6]

Hepatitis C virus causes both acute and chronic infection, as seen in Figure 1. Acute hepatitis C is mostly asymptomatic, with only 10-15% of patients presenting with acute symptomatic hepatitis and jaundice. Spontaneous clearance is seen in 25-50% of those with symptomatic infection and in 10-15% of those with asymptomatic infection. Evolution to chronic hepatitis is observed in 48-75% of those with symptomatic infection and in 85-90% of those with asymptomatic infection. Chronic infection can also lead to nonspecific symptoms, such as nausea, loss of appetite, fever, abdominal pain, and jaundice [5].

specific test for the simultaneous identification of HCV infection and initiation of treatment. HCVcAg is one such marker which has come to the limelight as an affordable, sensitive, specific test in resource-constrained settings.

Hepatitis C Virus (HCV) Core Antigen Assay

HCV core p²² Ag forms a part of the internal capsid of the hepatitis C virus and is released into the blood during viral assembly. This region is highly conserved, antigenic and is associated with the presence of viral capsules for all HCV genotypes [13]. HCVcAg also has somewhat improved stability, given that it measures a protein rather than RNA [3]. HCVcAg has been reported to be detected in patient serum as early as 2-3 weeks following infection, even before anti-HCV antibodies. After the production of anti-HCV antibodies, the HCVcAg forms complexes with the antibodies and becomes undetectable in the serum. This marker has also shown good correlation with a HCV RNA level above 3000IU/mL and its response to antiviral therapy [13-15]. It has been recognised as an alternative marker of indirect HCV viral replication [16]. The Abbott ARCHITECT HCV Ag assay (Abbott Diagnostics, Wiesbaden, Germany) is an automated chemiluminescent microparticle immunoassay (CMIA) and is available in India. The test procedure consists of a pre-treatment step where core antigen is removed from the virion and released into the solution. This solution is then exposed to paramagnetic micro particles coated with anti-HCV antibodies, where the antigen-antibody reaction will occur. This is followed by an introduction of a secondary antibody conjugated with chemiluminescent dye (Acridinium-labelled anti-HCV antibodies). The solution is then exposed to the pre-trigger and trigger solutions to oxidize acridinium and release a chemiluminescent signal measured in relative light units (RLU) (Table 1).

Table 1: Interpretation of the Abbott ARCHITECT HCVcAg assay [17].

Interpretation	HCV antigen(fmol/L)
Reactive	≥ 10.00
Gray zone-reactive	3.00 to 10.00
Nonreactive	< 3.00

The lower detection limit of the HCVcAg assay corresponds to 500 –3,000 IU/mL of RNA in serum or plasma [18]. The accuracy of the test for HCV antigen detection was evaluated in a meta-analysis by Freimanet al. [14] Out of the five index tests studies, the ARCHITECT assay had the highest quality with a sensitivity of 93.4% and specificity of 98.8%. Similar findings were seen in a recent study by Wasitthankasemet al. [16] They evaluated 298 HCC-seropositive individuals for the presence of anti-HCV antibody, HCVcAg and HCV RNA. They showed that irrespective of the genotype orco-infection with hepatitis B, HCVcAg levels correlated well with HCV RNA and has the potential to contribute to the national viral hepatitis elimination strategies. With regard to India, few validation studies on CIA platform have been carried out and unpublished data found specificity ~ 98%–99%, and sensitivity ~ 98.5%–99%, for samples that were with HCV viral load of ≥104 IU/mL. Other studies have looked at HCVcAg ELISA or/and HCVcAg-Ab combination ELISA/immunochromatographic tests in Indian settings with good results in pre-seroconversion phase [19,20].

What do the Guidelines say?

HCVcAg is a recognised marker by most of the international recommending bodies.

APASL

The Asian Pacific Association for the Study of the Liver (APASL) 2016 guidelines specify that the use of HCVcAg testing shortens the window period and can be used for screening of blood and blood products from donors in seroconversion window. The assay can be used depending on the availability of resources as there is good correlation between HCVcAg and HCV RNA levels. However, it is probably not suitable for on-treatment monitoring due to its limited sensitivity [21].

EASL

The European Association for the Study of the Liver (EASL) has recommended HCVcAg be detected during the acute phase of HCV infection and monitoring treatment. HCVcAg can be a surrogate marker for virus replication and can replace HCV RNA when NAAT is unavailable or unaffordable. However, serum levels may vary widely and there may be interludes of up to several weeks of undetectable HCV RNA or HCVcAg. Thus, HCVcAg-negative individuals should be retested for HCV RNA or HCVcAg at 12 and 24 weeks after a negative result to confirm definitive clearance [18].

INASL

The Indian National Association for Study of the Liver (INASL) guidance for antiviral therapy against HCV Infection also clearly states the benefits of HCVcAg. The guidelines say that HCVcAg can be an alternative to HCV RNA testing due to shorter turnaround time, simplicity, cost-effectiveness, utility in blood product screening and utility in treatment monitoring. However, the guidelines specify that further evaluation is needed to ensure that the HCVcAg assay can be used clinically in the absence of HCV RNA testing. Therefore, highlighting it as a surrogate marker that still requires confirmation with HCV RNA [22].

AASLD

The American Association for the Study of Liver Diseases (AASLD) recognises the advantage of HCVcAg due to its lower cost than PCR and improved stability (since RNA is measured instead of protein). However, HCVcAg testing requires instruments in a centralized laboratory and is not available as a point of care card test. Therefore, if point-of-care (POC) core Ag test could be developed at low cost, it could potentially obviate the need for antibody testing and would greatly simplify diagnosis, particularly in resource-limited settings [3].

Clinical Utility of HepatitisC Virus Core Antigen Assay

Quick and cost-efficient

With the availability of the CMIA HCVcAg assay, the diagnosis of hepatitis C can be simpler. With respect to the Indian setting, one of the main benefits of the HCVcAg assay is that it can be performed using the same equipment and sample which are used in the HCV-antibody assay [23]. Moreover, the CMIA has a shorter turnaround time of less than 60 minutes when compared to HCVcAg ELISA. This reduces the risk of losing an infected person to follow-up. Once the anti-HCV antibody is positive, the HCVcAg assay can be immediately carried out, and the results can be obtained immediately so that treatment can be initiated. The anti-HCV assay and HCVcAg assay can also be carried out

concurrently as a screening test in high prevalence communities or regions.

Reduced window period lag in immunocompromised patients

The initial eclipse phase of 1-2 weeks in HCV is the window period where no serological or virologic markers can be identified in the serum. The anti-HCV antibody response is detectable at 6-10 weeks after infection. Third generation ELISAs detecting antibodies targeting NS3, NS5 regions can reduce the window period to 6-8 weeks after exposure. During this serological window, it has been shown that free HCVcAg can be detected in a proportion of individuals. HCV RNA can shorten the window period even further to 7-14 days after infection. HCVcAg assay can be especially beneficial for screening individuals undergoing hemodialysis or those coinfecting with HIV as these groups have longer window periods and are often missed on routine screening [21]. Therefore, screening offered by HCVcAg based assays or HCVcAg-Ab based assays can contribute to increased sensitivity, specificity as well as earlier detection [19].

Assessment of response to treatment

The HCVcAg assay can be used to assess the therapeutic efficacy of antiviral treatment, as shown by Aghemo et al. [24] Concordance between HCVcAg and HCV RNA in identifying patients who would achieve SVR12 after 12 weeks (SVR12) was 40% at week 2, 55% at week 4, and 95% at the end of treatment. Interestingly, HCVcAg was observed to have a positive predictive value for SVR12 of 90% after 2 weeks. Other studies have also showed that the core antigen assay was able to accurately detect 99.6% patients with active viremia and can discriminate between those who achieve SVR12 and those who fail DAA therapy [17,25]. HCVcAg based tests are also included in recent WHO guidelines to assess the SVR in HCV infected patients on treatment [1].

Screening of organ donors

The utility of HCVcAg for screening organ donors – recipients, with the established HCV antibody assay has also been studied [25]. Apart from confirming the presence of active infection in patients with anti-HCV positive test, the HCVcAg assay was able to detect infections missed by the antibody assay and the use of both the tests for organ transplant screening is recommended.

Limitations of HCVcAg assay

There remains certain significant limitations to the HCVcAg assay. Primarily, the sensitivity of HCVcAg, 93.4% and a specificity of 98.7%; this is lower than that of HCV RNA and like any serological marker, will be unable to compete with advanced molecular tests [14]. HCVcAg assay can also not presently replace HCV RNA to identify cases of relapse of HCV infection after DAA therapy and confirmation with molecular tests will remain mandatory. Indian national guidelines for diagnosis and management of viral hepatitis recommends HCV RNA at baseline and the 12 weeks after attaining SVR. Most of the laboratories in the country have the resources to tests for HCV RNA and establishing another serological assay, may not be a justifiable cost. Furthermore, smaller laboratories that do not already have a diagnostic molecular set up, are unlikely to advanced serological technology like CIA. This suggests that initial setting up and running costs HCVcAg assay maybe comparable to HCV RNA. These practical limitations maybe overcome by considering rapid

card tests/immunochromatographic tests (ICT) for HCVcAg in an outpatient setting. However, such ICTs have been unable to detect HCV infection as early as HCV RNA or HCVcAg-Ab ELISA, but have shown improvement over anti-HCV assays [20]. Future research is needed on the utility of the HCVcAg assays in different genotypes and subtypes of HCV, especially during outbreak in high risk groups [27]. Further, the utility of this marker is to be assessed in the setting of co-infection with hepatitis B virus/HIV and detection of HCVcAg using dried blood spots.

Potential Algorithm for the Diagnosis of Hepatitis C using Hepatitis C Virus Core Antigen Assay

The HCVcAg has potential to be the singular screening assay for active and chronic HCV infection. The limitations of HCVcAg assay alone can even be overcome by simultaneous screening for anti HCV and HCVcAg by CIA. The figure 2 below details a possible diagnostic algorithm for detecting hepatitis C antigen using the new assay and possible interpretations (Table 2). This diagnostic algorithm allows for an ideal two assay testing strategy without compromising sensitivity or specificity. This algorithm can be used for screening among immunocompromised individuals and encourages rapid initiation of DAA therapy after HCVcAg positive report.

Figure 2. Possible diagnostic algorithm for detecting hepatitis C antigen using the new assay.

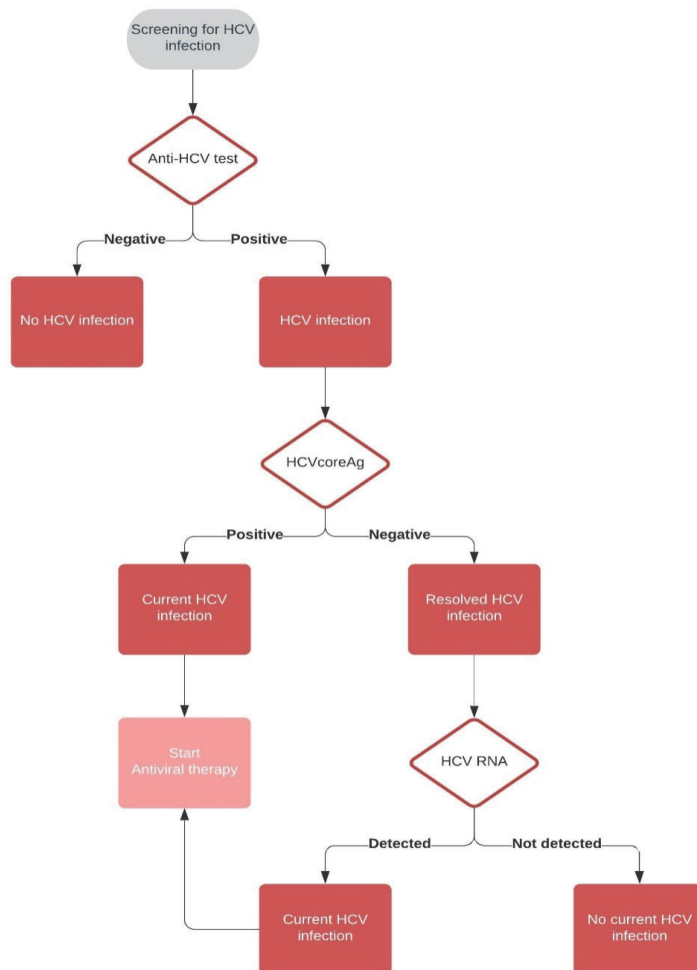


Table 2. Interpretation of HCV Antibody and HCV core antigen assay

Anti-HCV antibody	HCVcAg	Possible Interpretation
Negative	Negative	- No infection
Positive	Positive	- Active (chronic) infection
Negative	Positive	- Early infection - Infection in immunocompromised
Positive	Negative	- Resolved infection - Low level of viremia - False-positive anti-HCV result

Conclusion

The path to eliminating viral hepatitis as a public health problem by 2030 is a difficult and ambitious task. Despite significant advances in diagnosis and availability of affordable effective antiviral therapy, there are still large gaps in the cascade of care. The traditional two step diagnostic process continues to contribute to the attrition of patients seeking HCV treatment and requires modification. Substantial data now establishes the correlation between HCVcAg and HCV RNA. However, for HCVcAg assay to be incorporated into the national and international recommendations, multicentric investigations with a large sample size that is the true representative of the population are needed. The low sensitivity of HCVcAg limits its ability to replace HCV RNA quantitative RT PCR, but the combination of anti-HCV and HCVcAg serological assays using chemiluminescent technology can be a cost-effective way to improve screening. Despite limitations, the future of HCVcAg lies in the design and development of rapid, cheap, sensitive assays which can be done in outpatient and peripheral settings. This kind of POC testing can help avoid repeated visits to the healthcare provider and rapid initiation of DAA therapy to the patient. The HCVcAg assay is now available world over and significant differences can be made by incorporating this assay on the journey to eliminate HCV and its complications.

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