

# Clinical Usefulness of HCV Core Antigen Assay for the Management of Patients with Chronic Hepatitis C

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## ABSTRACT

**Aim:** The study aimed to evaluate the clinical utility of the chemiluminescent HCV core Ag test compared to viral load assessment in the management of patients with chronic hepatitis C.

**Methods:** A retrospective study was performed at a tertiary-care infectious diseases hospital on samples collected from anti-HCV positive patients. Seventy-six samples were tested with the Architect HCV core Antigen kit and Cobas AmpliPrep/Cobas Taqman HCV kit. The HCV Ag test accuracy was estimated using data from all the HCV RNA tested samples received between January 2011 and December 2012.

**Results:** The HCV Ag test showed a good correlation between the logarithmic values of HCV RNA and HCV Ag ( $R=0.98$ ), with a 100% specificity and PPV, but with reduced sensitivity for viral loads lower than 1,000 UI/mL. In a model using data from 2,478 HCV RNA tested samples and a cut-off of the Ag assay corresponding to 1,000 UI/mL HCV RNA, the Ag test would have a sensitivity of 82.4%, a NPV of 80.9% and a high specificity and PPV (100%) compared to the viral load. The sensitivity would be higher for baseline evaluation compared to on-treatment samples (98.5 vs. 50%). The highest NPV (98%) would be obtained at 48 and 72 weeks after the initiation of treatment, with a sensitivity of 88.2% and 96.1%, respectively.

**Conclusion:** The Architect HCV core Ag assay might be an alternative for the diagnosis of active HCV infection if molecular tests are not available, and a useful method for the evaluation of sustained virological response in treated patients.

**Key words:** hepatitis C – HCV core antigen – HCV viral load.

## INTRODUCTION

Chronic hepatitis C virus (HCV) infection represents an important public health problem, with approximately 180 million people infected worldwide [1]. Several virological tools are used to diagnose and monitor HCV infected patients. Detection of anti-HCV antibodies is a simple, inexpensive and rapid test but has a low sensitivity in the first 6 - 8 weeks of infection or in several clinical conditions, such as chronic immunosuppression or hemodialysis [2]. It is also unable to distinguish between active and past infection.

Detection and quantification of HCV RNA is the essential tool in the management of chronic HCV infected patients. However, PCR based assays are rather expensive and relatively complex so the availability of an accurate, cheaper test would be attractive.

Quantification of the HCV core antigen (Ag) has been suggested as a possible alternative to the HCV viral load. The first versions of the HCV core Ag test did not fulfill these expectations, mainly due to the low sensitivity of these enzyme immunoassays [3]. In recent years, a new HCV Ag test has been developed, the chemiluminescent immunoassay (CLIA), showing improved sensitivity [4].

The aim of the present study was to evaluate the clinical utility of the CLIA HCV core Ag quantification as an alternative to HCV viral load tests in patients with chronic HCV infection. The clinical usefulness was evaluated in distinct groups of samples, defined according to the moment of treatment initiation, by analyzing in each group if the HCV Ag test is sensitive enough to be adequate as a monitoring tool.

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## MATERIAL AND METHODS

This retrospective study was performed at the Prof. Dr. M. Bals National Institute for Infectious Diseases (NIID), a tertiary-care hospital in Bucharest, Romania. Blood samples collected between January 2011 and December 2012 from anti-HCV positive patients were randomly sent to several laboratories for HCV viral load testing, as part of routine evaluations for hepatitis C. The samples received in the Molecular Diagnostic Laboratory of the NIID were centrifuged and the serum samples were stored at -80°C and tested for HCV RNA with the Cobas AmpliPrep/Cobas Taqman system (Roche Molecular, Pleasanton, CA, USA). The lower limit of detection of this assay is 15 IU/ml ( $1.17 \log_{10}$  IU/ml). After HCV RNA testing was complete, the remaining sample was stored at -80°C.

For data analysis each sample was allocated to one of the six groups, defined according to the moment of testing: baseline evaluation and 4, 12, 24, 48 and 72 weeks after the initiation of treatment with pegylated interferon and ribavirin. The proportion of patients with undetectable or detectable HCV RNA levels (in  $\log$  IU/mL) was analyzed in each group.

To estimate the correlation between the HCV Ag and HCV RNA concentrations a number of 76 serum samples were selected for HCV Ag testing: 18 samples with an undetectable HCV RNA and 58 samples with a detectable HCV viral load, ranging from 30 to 14,690,829 IU/mL ( $1.47$  to  $7.16 \log$  IU/mL), with a similar proportion of each  $\log$  HCV RNA (from 1 to 6  $\log$  IU/mL) within this group. Samples were tested on the Architect i2000 analyzer with the Architect HCV core Antigen kit (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's recommendations. This assay detects HCV core Ag with a cut-off of 3.0 fmol/L (1.0 fmol/L of HCV Ag corresponds to 0.02 pg/mL). The upper limit of linearity is 20,000 fmol/L, so dilutions are necessary for samples with a higher concentration.

Linear regression analysis was used to assess the association between the HCV Ag and HCV RNA concentrations in  $\log$  scales. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HCV Ag detection for predicting HCV RNA were calculated for each of the six treatment groups. All data were analyzed with the GraphPad software. A  $p$  value of less than 0.05 was considered statistically significant.

This study was approved by the Institutional Review Board.

## RESULTS

### HCV RNA viral load in different groups of anti-HCV positive patients

A number of 2,478 serum samples from 1,782 anti-HCV positive patients were tested for HCV RNA between January 2011 and December 2012 in the Molecular Diagnostic Laboratory of the NIID. Overall, 42.7% of these samples were undetectable, 42.7% had a viral load higher than 10,000 IU/ml (or  $4 \log_{10}$  IU/ml), 4.4% were between 2 and  $4 \log_{10}$  IU/ml and only 10% of them had a low viral load (i.e. less than 100 IU/ml, or  $2 \log_{10}$  IU/ml) (Table I). As expected, the proportion of samples with low viral load varied considerably according to the moment of testing, ranging from 1.2% at baseline evaluation to 43.4% at 12 weeks of treatment, thus influencing the usefulness of a test with lower sensitivity than PCR.

### Correlation between HCV RNA and HCV core Antigen

The detection and quantification of HCV core antigen was tested in 76 sera; all the HCV RNA negative samples were nonreactive with Architect HCV Ag test. A number of 43 of the 58 HCV RNA positive samples were also reactive ( $>3$  fmol/l) with the Ag test. These samples had a viral load ranging from 148 to 14,690,829 IU/ml, i.e.  $2.17$ - $7.16 \log_{10}$  IU/ml (Table II). The HCV RNA positive samples with nonreactive Ag test had viral loads of 30, 33, 66, 96, 118, 148, 208, 229, 338 and 1,659 IU/ml.

Overall, the HCV Ag test showed a positive result in 3/17 (17.6%) samples with a viral load lower than 1,000 IU/ml, in 8/9 (88.8%) samples with a HCV RNA between 1,000 and 10,000 IU/ml and in all samples with a viral load higher than 10,000 IU/ml. The HCV RNA level corresponding to the cut-off of the Ag test was not calculated with 95% confidence using the probit analysis due to the limited number of samples tested with Architect HCV Ag; however, this level was expected to be between 3 and  $3.5 \log_{10}$  IU/ml.

The correlation coefficient ( $R$ ) of the logarithmic values of HCV RNA and HCV-Ag was 0.98 (slope = 0.8336, intercept = 1.5483) and it was statistically significant ( $P < 0.001$ ) (Fig. 1).

### HCV Ag accuracy in different groups of HCV infected patients

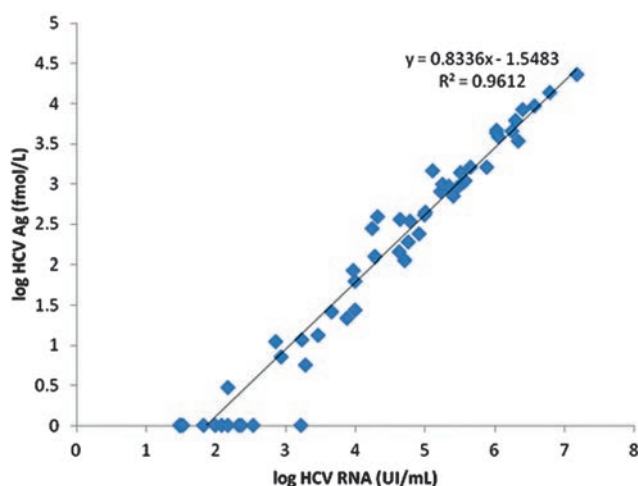
The results of HCV RNA for each group (Table I) were used to calculate the sensitivity, specificity, PPV and NPV of the

**Table I.** Sample distribution according to HCV RNA viral load (in  $\log$  UI/ml) and the moment of testing (baseline and 4, 12, 24, 48, 72 weeks after treatment initiation)

HCV RNA ( $\log$ UI/ml)	Number of samples						Total (n=2478)
	Baseline (n = 825)	4 w (n = 220)	12 w (n = 557)	24 w (n = 176)	48 w (n = 263)	72 w (n = 437)	
Not detectable	158	52	242	97	229	281	1059
< 3	10	37	151	41	4	6	249
3.0-3.99	13	34	54	5	2	3	111
$\geq 4.0$	644	97	110	33	28	147	1059

**Table II.** Comparative results of Architect HCV Ag in HCV RNA positive sera

HCV Ag	No. of HCV RNA positive samples	Median HCV Ag (fmol/ml) (range)	Median HCV RNA (log IU/ml) (range)
Positive (>3 fmol/l)	43	448.7 (3.01-23000)	4.91 (2.17-7.16)
Negative (<3 fmol/l)	15	0 (0-2.55)	1.82 (1.47-3.22)
Total	58	12.6 (0-23000)	4.47 (1.47-7.16)

**Fig. 1.** Correlation between HCV core Ag, measured by Architect (Abbott), and HCV RNA, measured by CAP/CTM (Roche), in logarithmic scales

Architect HCV core Ag test, considering that the cut-off of the Ag assay corresponds to a viral load of 1,000 IU/ml. Overall, the Ag test had a sensitivity of 82.4%, a NPV of 80.9% and high specificity and PPV (100%) when compared to the viral load test (Table III). The sensitivity is higher (98.5%) for baseline evaluation samples compared to on-treatment samples. The highest NPV (98%) was obtained for samples at 48 and 72 weeks after the initiation of treatment.

## DISCUSSION

Nucleic acid tests (NATs), mainly PCR based, with a lower limit of detection of at least 15 IU/ml HCV RNA are the

recommended tool to diagnose active HCV infection and to monitor treatment efficacy, either with pegylated interferon/ribavirin or with new regimens containing direct acting antivirals (DAAs) [5-7]. These assays are fully automated, have a high sensitivity and specificity, and are widely used in the clinical practice.

Detection and quantification of the HCV core antigen, which require less expensive and less complex instruments and facilities, were regarded as an alternative to NATs [8]. The first ELISA assay for HCV Ag detection and quantification was introduced more than a decade ago, but this method showed important variability in HCV RNA/core Ag ratios and failed to demonstrate the sensitivity required to be clinically useful [9]. The enzyme immunoassay version of HCV Ag detected 1.5 pg/ml of HCV core Ag (corresponding to a HCV RNA level of 27,000-30,000 IU/ml) [10]. The use of chemiluminescence technology improved significantly the sensitivity of Ag detection compared to previous assays, a new version (the Architect HCV core Ag) having a lower limit of detection of approximately 0.06 pg/ml (or 3 fmol/L) [10]. A study showed that this cut-off of 3 fmol/L corresponds to a viral load between 428 and 2700 IU/ml, depending on the HCV genotype [11]. This study also showed similar values of prediction in the sustained virologic response when using the criteria of at least 2-log decrease of HCV RNA or HCV core antigen at 12 weeks after the initiation of antiviral therapy [11]. Another study, performed on samples with a low positive HCV RNA (<3.9 log IU/mL) found that the limit of detection of HCV Ag assay corresponded to 1,000 IU/ml HCV RNA, with no influence of the HCV genotype [12].

In the present study, the Architect HCV Ag assay provided positive results in 8/9 samples with a HCV RNA between 1,000 and 10,000 IU/ml, showing good sensitivity for samples with a HCV RNA higher than 1,000 IU/ml. Overall the assay demonstrated 100% specificity and PPV compared to the HCV viral load, and a good correlation between the logarithmic values of HCV RNA and HCV Ag, similar to the results reported in other studies [4, 11].

An important objective of this study was to estimate the usefulness of the Architect HCV Ag assay in different categories of patients with chronic HCV infection. Using an estimated cut-off of the Ag assay of 1,000 IU/ml HCV RNA, the overall sensitivity of the HCV core Ag test was 82.4%, but reached higher levels (>96%) in specific groups of patients, such as

**Table III.** Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HCV Ag detection compared to HCV RNA viral load in different groups of HCV-infected patients. The cut-off of 3 fmol/l HCV core Ag was considered to correspond to a HCV RNA of 1,000 IU/ml.

	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Baseline	98.5 (97.2-99.2)	100 (97.1-100)	100 (99.3-100)	94.0 (89.2-96.8)
4 weeks	77.9 (71.1-83.6)	100 (91.7-100)	100 (96.6-100)	58.4 (48.0-68.1)
12 weeks	52.1 (46.5-57.5)	100 (98.1-100)	100 (97.2-100)	61.5 (56.6-66.2)
24 weeks	48.1 (37.4-58.9)	100 (95.4-100)	100 (89.1-100)	70.3 (62.1-77.3)
48 weeks	88.2 (72.7-95.9)	100 (98-100)	100 (86.5-100)	98.3 (95.5-99.5)
72 weeks	96.1 (91.6-98.4)	100 (98.3-100)	100 (97.0-100)	97.9 (95.4-99.5)
All samples	82.4 (80.4-84.3)	100 (99.5-100)	100 (99.6-100)	80.9 (78.7-83.0)

those evaluated at baseline or at 6 months after the end of treatment.

In newly diagnosed anti-HCV positive patients, the initial HCV RNA testing is indicated for the diagnosis of active or past infection and for a baseline evaluation before the start of the antiviral treatment. According to the data of the present study, the HCV Ag test would provide a positive result in 79.6% of the patients, thus confirming active infection, and a negative one in 20.3% of them, but with 1.2% false negative results. With this approach the confirmation of active infection with the HCV Ag test would save approximately 80% of the HCV RNA tests, with the need to confirm by PCR only HCV Ag negative results, as also suggested by other studies [7]. However, in our opinion this approach is incomplete, because all HCV Ag positive patients with indication for antiviral treatment will also need PCR testing for an accurate baseline measurement of HCV RNA.

For patients under treatment with pegylated interferon or DAAs containing regimens, the evaluation of treatment efficacy must be performed with an assay with a limit of detection lower than 15 IU/ml [7], which is not the case for the actual version of the HCV Ag assay. In this study, the sensitivity of the HCV Ag assay compared to HCV RNA for this group of patients was approximately 50%, with an important number of false negative results, thus limiting the utility of the HCV core Ag assay.

In contrast, in patients at the end of the antiviral treatment or 6 months after the end the small proportion of samples with a low level HCV RNA suggests the clinical utility of the HCV Ag. In the present study HCV Ag had a sensitivity of 88.2% in the first group of patients and 96.1% in the second, with a NPV of approximately 98% in both groups.

This study has several limitations. One limitation is the absence of HCV genotyping, but it is expected that in Romania nearly all the samples belong to genotype 1b, as demonstrated by other studies [13]. Also, due to the limited number of samples tested with the HCV Ag assay, we were not able to calculate the level of HCV RNA which corresponds with 95% confidence to the cut-off (3 fmol/L) of this assay. However, several studies reported HCV RNA levels ranging from 2.5 to 4.5 log IU/ml corresponding to the cut-off of the Architect HCV Ag assay, which is similar to our results (3.0 to 3.5 log IU/ml). In the present study a level of 1,000 IU/ml (3.0 log IU/ml), which is rather low, was used to calculate the values of sensitivity, specificity, PPV and NPV of the HCV Ag assay compared to HCV RNA as reference test. The use of a higher level of HCV RNA corresponding to the cut-off of HCV Ag assay would result in even lower sensitivity and NPV values in the analyzed groups of patients.

## CONCLUSIONS

In our study, the Architect HCV core Ag assay showed a very high specificity and a good correlation with HCV RNA Cobas Ampliprep/ Cobas Taqman results. This assay might

be an alternative for the diagnosis of active infection in anti-HCV positive patients if molecular tests are not available, but a confirmation of HCV Ag negative results with nucleic acid tests is required. HCV core Ag assay is a useful method with reduced costs for the evaluation of sustained virologic response in HCV treated patients.

**Conflicts of interests:** none to declare.

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