Original Article

Diagnostic Performance and Correlation of Hepatitis C Virus Core Antigen Compared with Hepatitis C Virus RNA

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Background: The hepatitis C virus core antigen (HCVcAg) test is an alternative to measuring hepatitis C virus (HCV) ribonucleic acid (RNA) levels to diagnose active HCV infection. Data are lacking regarding the correlation between HCVcAg and HCV RNA in HCV genotype 6 which are more prevalent in Southeast Asia than in other parts of the world.

Objective: This study aimed to evaluate the diagnostic performance of HCVcAg in detecting chronic hepatitis C virus (HCV) infection, the correlation and association between HCVcAg assay results and HCV RNA levels in Thai patients carrying each HCV genotype.

Materials and Methods: We prospectively enrolled 165 anti-HCV positive patients who visited Srinagarind Hospital from October 2017 to November 2018 for pre-HCV treatment evaluations. All samples were tested using HCVcAg (Architect, Abbott Laboratories). The diagnostic performance of the HCVcAg test was assessed using receiver operating characteristic (ROC) curves to identify the best cutoff value for detection of HCV viremia. A Spearman's correlation and linear regression were used to assess the correlation and association between HCVcAg and HCV RNA.

Results: The most reliable HCVcAg cut-off for diagnosis HCV infection was HCVcAg >6 fmol/L, with a sensitivity of 95.3% (95% confidence interval [CI] 90.5 to 98.1%), specificity of 100% (95% CI 80.5 to 100%), positive predictive value of 100% (95% CI 97.4 to 100%), and negative predictive value of 70.8% (95% CI 48.9 to 87.4%). The AUROC was 0.976 (95% CI 0.955 to 0.997). There was a strong, statistically significant positive correlation between HCVcAg and HCV RNA in all genotypes (r = 0.8915, p<0.001). The regression equation was $\log_{10}$ HCV RNA = 1.3467 $\log_{10}$ HCVcAg + 1.8318 (95% CI for slope and intercept, 1.2359 to 1.4574 and 1.5018 to 2.1619, respectively). The HCV RNA level corresponding to the cutoff of 6 fmol/L of the HCVcAg test was approximately 800 IU/mL.

Conclusion: The HCVcAg assay exhibited excellent diagnostic performance and a good correlation with HCV RNA in all genotypes. This assay can thus be used as a lower-cost alternative tool for diagnosis of active HCV infection in anti-HCV positive patients, allowing these patients greater accessibility to chronic HCV treatment.

Keywords: Chronic hepatitis C, HCV core antigen, Diagnostic performance, Correlation

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Chronic hepatitis C infection is one of the most common blood-borne liver diseases. Approximately 170 million people worldwide are infected with the virus, one-fifth of whom are in Southeast Asia. In Thailand, the highest annual incidence of hepatitis C virus (HCV) infection is in the northeastern region (0.78 per 100,000) followed by the northern, central, and southern regions[1]. We have screened HCV infection by rapid kit for antibody to hepatitis C virus (rapid anti-HCV kit) in the people of northeastern that represent one third population of Thailand. We found that the prevalence rate is 4.2% from 29,278 screened people (unpublished data). One-third of people who are infected with HCV for longer than 20 years will develop cirrhosis, and about 5% of those will eventually develop hepatocellular carcinoma[2]. Therefore, early detection and treatment of HCV infection may effectively reduce the rate of cirrhosis and hepatocellular carcinoma.

Currently, screening for chronic HCV infection in Thailand is conducted using serological tests for positive HCV antibodies (anti-HCV). Although this technique has favorable sensitivity, it is recommended that diagnosis of active infection is confirmed using a nucleic acid test for HCV ribonucleic acid (RNA)[3] to exclude patients without active infection who do not require treatment[4]. However, clinical use of the nucleic test is still limited by its cost, the long turnaround time, and the fact that it requires highly skilled personnel and technology that is only available centralized laboratories.

The HCV core antigen (HCVcAg) assay is a method that has recently been made available for detecting HCV viremia. The HCVcAg forms the internal capsid, which

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is highly conserved and antigenic. During viral assembly, nucleocapsid peptide 22 are released into the plasma and can be detected earlier than antibodies and throughout the course of infection. Previous studies have found that the technique provides good sensitivity and specificity (92.3 to 94.3% and 97.8 to 98.8%, respectively)[5], whereas the procedure is simpler, faster, and more affordable than the HCV RNA test. Several studies have addressed its potential role as an alternative to HCV RNA with correlation values (r) between 0.7 to 0.9[6,7]. The lowest HCV RNA thresholds have varied from 1,000 to 3,000 IU/mL[8,9]. However, data are lacking on the correlation between HCVcAg of HCV genotype 6 (which is more prevalent in Southeast Asia than in other regions) and HCV RNA[10]. The aim of this study is to evaluate the diagnostic performance of the HCVcAg assay in detecting active HCV infection, the correlation and association between its results and those of the HCV RNA test in Thai patients carrying each HCV genotype in order to increase the affordability and accessibility of HCV detection and treatment.

Materials and Methods

This study is a prospective, single-center, diagnostic study. We prospectively enrolled chronic HCV patients who visited Khon Kaen University's Srinagarind Hospital for pre-HCV treatment evaluation between October 2017 and November 2018. Inclusion criteria were age over 18 years, positive anti-HCV (by ELISA), and no history of antiviral therapy for HCV infection. Our protocol was approved by the office of the Khon Kaen University Ethics Committee in Human Research. All participants provided written informed consent. We collected data on the following parameters: patient characteristics, history of alcohol consumption, co-morbidity, complete blood count with platelet count, prothrombin time with INR, liver test results (total bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase), viral hepatitis studies, rapid Anti-HCV kit, HCVcAg, HCV RNA, and HCV genotype.

HCVcAg assay

Blood samples were collected on the same day as the HCV RNA blood test. These samples were centrifuged for plasma then stored at -20°C. They were thawed within 30 days for HCVcAg testing with an Architect i2000 analyzer (ARCHITECT, Abbott Laboratories) according to the manufacturer's recommendation. This assay detects HCVcAg with limit of 3.0 to 20,000 fmol/L.

Rapid anti-HCV kit

Advanced QualityTM rapid Anti-HCV Test (Manufactured by InTec Products, Inc.) is performed in accordance with the manufacturer's instructions.

HCV RNA assays

To determine the concentration of HCV RNA in serum, we used a quantitative reverse transcription (RT-PCR) assay (COBAS AMPLICOR HCV MONITOR version 2.0; Roche Molecular Systems) according to the manufacturer's protocol. This assay has a limit of quantitation of 15 IU/mL (1.17 log10 IU/mL).

Statistical analysis

Baseline demographics and clinical characteristics of patients were summarized using descriptive statistics. For categorical variables, numbers for all categories were presented with percentages. For continuous variables, median and interquartile ranges were presented. The diagnostic performance of the HCVcAg assay was assessed using receiver operating characteristic (ROC) curves. The ROC curves were used to identify the best cutoff values for detection of patients with HCV viremia (by HCV RNA detection). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) with 95% confidence interval (CI), and area under the ROC curve (AUROC) were also calculated to obtain diagnostic accuracy of HCVcAg and rapid Anti-HCV kit. Comparison of HCV genotypes was carried out using a Kruskal-Wallis test. The correlation coefficients and association between HCVcAg and HCV RNA concentrations in log scale were calculated using a Spearman's correlation test and linear regression analysis. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using STATA® 14 (College Station, Texas, USA).

Results

Of the 165 patients who were included in the study, 117 were male (70.9 %) and 48 were female (29.1 %). The median age was 54 (range 47 to 59) years. The median body mass index (BMI) was 23.7 (range 20.9 to 25.8) kg/m^2. Most had no medical comorbidities (97 cases; 58.8%). Three cases each of HIV and HBV coinfection were found (1.8%). The HCV RNA tests of 148 patients were positive. Median HCV RNA and HCVcAg levels were 6.1 (range 5.2 to 6.6) log and 1,359.2 (range 143.5 to 3,730.6) fmol/L, respectively. Genotype 3 was the most prevalent HCV genotype (58 cases; 40.6%), followed by genotype 1 (47 cases; 32.9%) and genotype 6 (38 cases; 24.5%; Table 1).

Patients with HCV genotype 3 had the lowest median HCVcAg level. There was a statistically significant difference in HCVcAg levels between patients with HCV genotype 3 versus those with genotypes 1 and 6 (p = 0.005 and 0.006, respectively). There was no difference between those with genotypes 1 and 6 (p = 0.471; Table 2).

Diagnostic performance

The most reliable HCVcAg cutoff for diagnosis of HCV viremia obtained from the population in this study was HCVcAg >6 fmol/L, with a sensitivity of 95.3%, specificity of 100%, PPV of 100%, NPV of 70.8%, and likelihood ratios for negative test (LR-) of 0.05. The AUROC was 0.976 (Table 3 and Figure 1).

Rapid anti-HCV kit had a sensitivity of 100%, specificity of 17.6%, PPV of 91.4%, NPV of 100%, likelihood

ratios for positive test (LR+) of 1.2, and the AUROC of 0.588 (Table 3).

Correlation and association between HCVcAg and HCV RNA levels

A significant positive correlation between HCVcAg and HCV RNA levels was found (Figure 2) with correlation coefficients of r = 0.8915 (p<0.001), r = 0.9193 (p<0.001), r = 0.7806 (p<0.001), r = 0.9642 (p<0.001), for all genotypes, genotype 1, genotype 3 and genotype 6, respectively. The regression equation was $\log_{10}$ HCV RNA (IU/mL) = 1.3467 $\log_{10}$ HCVcAg + 1.8318 (95% CI for slope and intercept, 1.2359 to 1.4574 and 1.5018 to 2.1619, respectively; Figure 3). On the basis of this equation, the HCV RNA level corresponding to the cutoff of 3, 4, 5, 6 and 7 fmol/L of the HCVcAg test were approximately 300, 450, 600, 800 and 1,000 IU/L, respectively.

Table 1. Baseline characteristics of the 165 patients

Age (years)	54 (47 to 59)
Sex, male	117 (70.9)
BMI (kg/m^2)	23.7 (20.9 to 25.8)
Risk factors for HCV infection	
Injected drug user	33 (20.0)
Tattoo	67 (40.6)
Body piercing	46 (27.9)
Blood transfusion	24 (14.6)
Unsafe sex	43 (26.1)
History of alcohol consumption	115 (70.0)
Underlying disease	
No underlying disease	97 (58.8)
Diabetes mellitus	24 (14.6)
Hypertension	14 (8.5)
Dyslipidemia	4 (2.4)
Hepatocellular carcinoma	9 (5.5)
HIV co-infection	3 (1.8)
HBV co-infection	3 (1.8)
Lab	
HCV RNA (log IU/ml)	6.1 (5.2 to 6.6)
HCVcAg (fmol/L)	1,359.2 (143.5 to 3,730.6)
HCV genotype	
1	47 (32.9)
3	58 (40.6)
6	38 (24.5)
Total bilirubin (mg/dL)	0.7 (0.5 to 1.1)
ALT (U/L)	67 (39 to 103)
AST (U/L)	64 (41 to 99)
ALP (U/L)	96 (76 to 122)
Rapid anti-HCV kit	162 (98.2)

Data presented as median (1st to 3rd quartile range) or number (percentage)
BMI = body mass index, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase

Characteristics of the patients with discordant HCVcAg results

The HCVcAg test resulted in false negatives for seven patients, four of whom had HCV RNA levels lower than 3,000 IU/mL (136, 221, 768 and 2,514 IU/mL). Two of these patients had HCV genotype 3, two had genotype 6, and the HCV genotype was not able to be identified in the remaining three (Table 4).

Discussion

In this study, the HCVcAg tests were performed in a real-world setting. The AUROC of the HCVcAg test compared with that of the HCV RNA test was 0.976, which indicates good diagnostic accuracy. This result was similar to that of a study by Duchesne et al, which found an AUROC of 0.99[12]. We found that a manufacturer's cutoff level of 3 fmol/L resulted in good diagnostic performance with a sensitivity of 95.9% and specificity of 88.2%. We analyzed various HCVcAg cutoff levels and found that a cutoff of 6 fmol/L had a sensitivity of 95.3% and specificity of 100%. Thus, we determined that the optimal cut-off level for our study would be 6 fmol/L when using the HCVcAg test as the gold standard due to its high specificity.

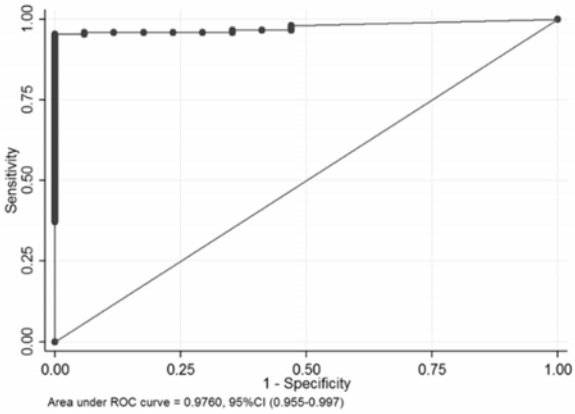

Figure 1. Receiver operating characteristic (ROC) curve of HCVcAg quantification for detecting active hepatitis C virus infection.

Table 2. Serum HCV RNA and HCVcAg levels by HCV genotype

	HCV RNA (log IU/ml)	HCVcAg (fmol/L)
Genotype 1	6.20 (5.69 to 6.56)	2,274.11 (1,002.73 to 6,277.09)
Genotype 3	6.30 (5.59 to 6.71)	1,011.60 (146.46 to 3,326.08)
Genotype 6	6.38 (5.77 to 6.76)	2,328.63 (408.64 to 7,917.66)
p-value	0.486	0.011

Table 3. Diagnostic performance of HCVcAg and rapid Anti-HCV kit compare with HCV RNA with 95% confidence interval (CI)

Cutoff level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
>3 fmol/L	95.9 (91.4 to 98.5)	88.2 (63.6 to 98.5)	98.6 (95.1 to 99.8)	71.4 (47.8 to 88.7)	8.16 (2.22 to 30.00)	0.05 (0.02 to 0.10)
>4 fmol/L	95.9 (91.4 to 98.5)	94.1 (71.3 to 99.9)	99.3 (96.2 to 100)	72.7 (49.8 to 89.3)	16.30 (2.44 to 109)	0.04 (0.20 to 0.10)
>5 fmol/L	95.9 (91.4 to 98.5)	94.1 (71.3 to 99.9)	99.3 (96.2 to 100)	72.7 (49.8 to 89.3)	16.30 (2.44 to 109)	0.04 (0.20 to 0.10)
>6 fmol/L	95.3 (90.5 to 98.1)	100 (80.5 to 100)	100 (97.4 to 100)	70.8 (48.9 to 87.4)	∞	0.05 (0.02-0.10)
>7 fmol/L	94.6 (89.6 to 97.6)	100 (80.5 to 100)	100 (97.4 to 100)	68 (46.5 to 85.1)	∞	0.05 (0.03 to 0.11)
Rapid anti-HCV kit	100 (97.5 to 100)	17.6 (3.8 to 43.4)	91.4 (85.9 to 95.2)	100 (29.2 to 100)	1.2 (1.0 to 1.5)	0

PPV = positive predictive value, NPV = negative predictive value, LR+ = likelihood ratios for positive test, LR- = likelihood ratios for negative test

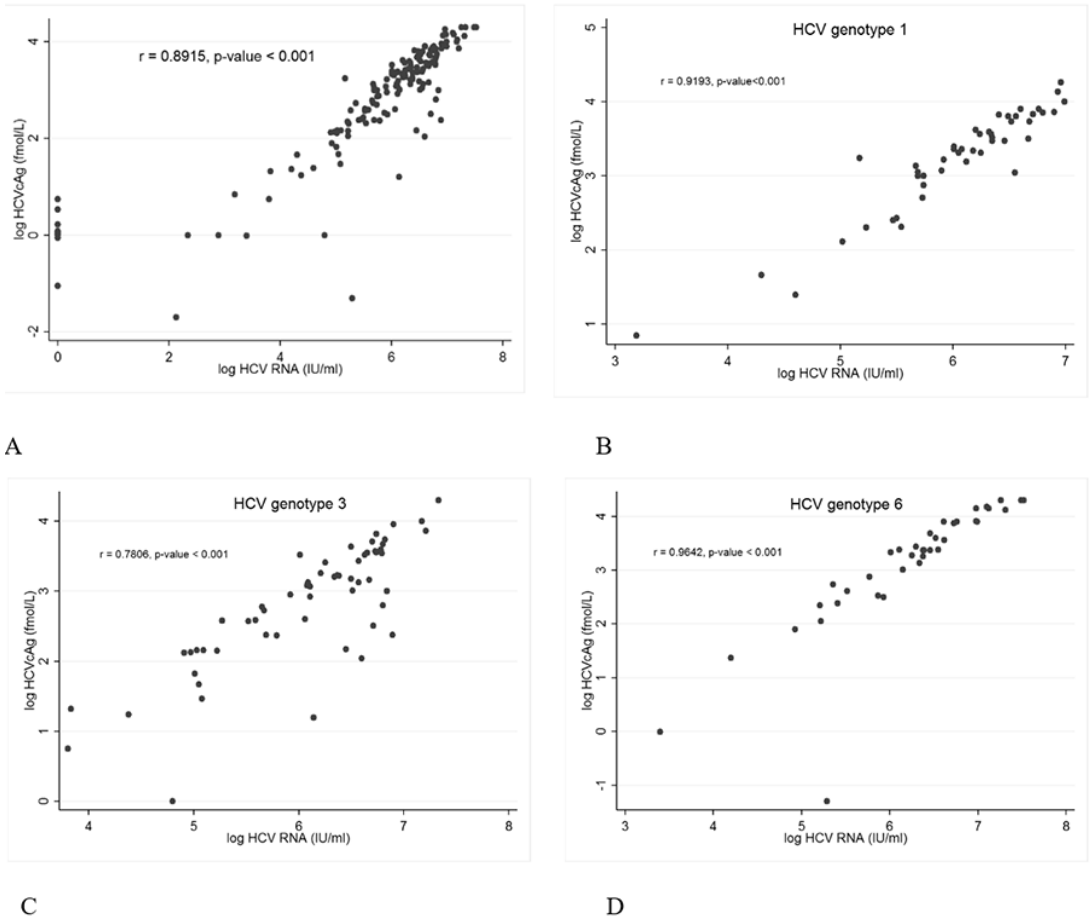

Figure 2. Correlation between HCVcAg and HCV RNA in all genotypes (A), genotype 1 (B), genotype 3 (C), and genotype 6 (D).

A recent meta-analysis found insufficient data regarding HCVcAg test performance in patients with HCV genotype 6[5]. A quarter of patients in this study were genotype 6 and we found that there was a strong correlation between HCVcAg and HCV RNA levels in these patients (r = 0.8915), similar to genotypes 1 and 3.

One previous study found that HCVcAg was well correlated with HCV RNA when HCV RNA levels were higher than 3,000 IU/mL. Most of the false negative results in our study seemed to be due to low viral load. However, most patients with active HCV infections have HCV RNA levels higher than this value. Only 4.7% of the patients in this study had HCV RNA levels below 10,000 IU/mL. Thus, this did not affect effectiveness of this test as a diagnostic tool in a real-world setting.

The HCVcAg test is 3 to 5 times cheaper than the HCV RNA test. Based on the total of 4,193 patients registered in Thailand's National Health Security Office (NHSO) database who underwent HCV treatment over a 4-year period (2012 to 2016)[11], we calculated that switching from the HCV RNA test to the HCVcAg test could save around 10 million baht (Table 5).

Rapid Anti-HCV kit had a high sensitivity of 100% but low specificity of 17.6% which indicates that this test is suitable for screening disease.

Some limitations of this study must be acknowledged. First, we used the HCVcAg test and rapid anti-HCV kit as a two-step diagnostic test in anti-HCV positive patients, meaning that they had high disease prevalence and does not represent a normal population. Second, we had a low number of true negative results. The NPV was 70.8% among patients who had negative HCVcAg test results, which means that about 30% of these patients were misdiagnosed. However, the cost estimate for the HCVcAg test was about ten million baht lower than that of the HCV RNA test, as shown in Table 5. Therefore, implementation of the HCVcAg test should depend on the population, policy, and budget of each individual setting.

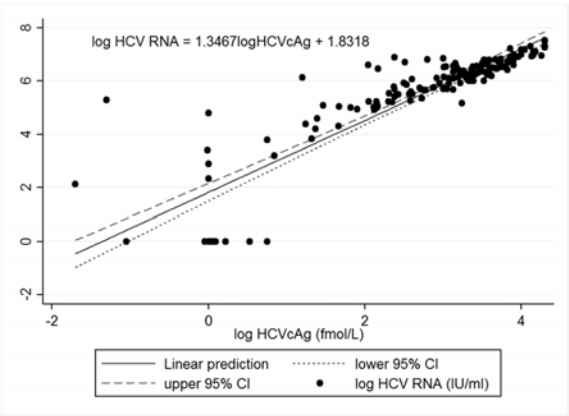

Figure 3. Association between HCVcAg and HCV RNA in all genotypes.

Conclusion

The HCVcAg assay exhibited excellent diagnostic performance and a good correlation with the HCV RNA test in all HCV genotypes. This assay can thus be used as a lower-cost alternative tool for the diagnosis of active HCV infection in anti-HCV-positive patients, allowing these patients greater accessibility to HCV treatment.

What is already known on this topic?

The HCV core antigen test provides good sensitivity, specificity, and a high correlation with the HCV RNA test and can thus be used as an alternative method of diagnosing active HCV infection.

What this study adds?

The most reliable HCVcAg cutoff for diagnosis of HCV infection in the Thai population was HCVcAg >6 fmol/L, which resulted in good diagnostic accuracy and high correlation, as shown in previous studies. The correlation between HCV RNA and HCVcAg levels was not affected by HCV genotype.

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Table 4. Characteristics of the patients with a discordant HCVcAg results (false negative)

Age (year)	Sex	HCV RNA (IU/ml)	HCVcAg (fmol/L)	Genotype
36	Female	136	0.02	Unknown
56	Male	221	0	Unknown
54	Male	768	0	Unknown
74	Female	2,514	0.97	6
64	Female	6,243	5.56	3
48	Male	62,933	0	3
51	Male	195,685	0.05	6

Table 5. Costs of each test and cost difference (in baht) based on the number of hepatitis C patients in Thailand's National Health Office (NHSO) registry (n = 4,193)

HCV RNA*	HCVcAg*	Cost difference
4,193x3,000 = 12,579,000	4,193x500 = 2,096,000	10,482,500

* HCV RNA estimated 3,000 baths/unit, HCVcAg estimated 500 baht/unit

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Potential conflicts of interest

The authors declare no conflicts of interest.

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