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Utility of a commercial quantitative hepatitis C virus core antigen assay in a diagnostic laboratory setting☆

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Abstract

In this study, the utility and impact of hepatitis C virus (HCV) core antigen (Cag) detection via a commercial assay have been evaluated in diagnostic laboratory conditions. In a total of 272 samples from 226 individuals, HCV RNA was detected in 81.3% and anti-HCV antibody prevalence was 86.4%. HCV Cag reactivity was identified in 59.9% of the samples and in 75.8% with detectable RNA. The sensitivity and specificity of HCV Cag assay have been calculated as 75.8% and 95.1%, respectively, and agreement between HCV RNA and HCV Cag was moderate ($\kappa = 0.554$). HCV Cag and RNA levels were highly correlated ($r = 0.915$ and 0.937). A viral load threshold of 10^3 IU/mL has been recognized, above which the correlation with RNA became statistically significant and sensitivity increased to 90.9%. Detection and quantification of HCV core antigen have been observed as a strong alternative to nucleic acid testing for HCV monitorization.

Keywords: Hepatitis C virus; HCV; Core antigen; Quantitative; Assay

1. Introduction

Hepatitis C virus (HCV) is a frequent cause of infectious chronic hepatitis which affects approximately 200 million people worldwide (Shepard et al., 2005). HCV infection is curable by therapy, with the current standard treatment based on the combination of pegylated interferon α and ribavirin (McHutchison et al., 2009). Virologic tools, based on the detection of total anti-HCV immunoglobulins and viral RNA, are essential for the diagnosis and monitorization of HCV infections (Richter, 2002). In the acute phase of infection, anti-HCV antibodies appear on average 2–8 weeks after exposure and persist for life in patients who develop chronic HCV infection. Thus, they provide little data during the long window period and on the extent of viral replication once chronic infection is established (Busch et al., 2005; Pawlotsky, 2002). Detection and quantitation of HCV RNA have been the standard method for the diagnosis and monitorization of chronic HCV infections and for assessing individual patient response to the antiviral drug regimen. In addition, viral RNA is also used as a marker of acute HCV exposure before anti-HCV seroconversion (Busch et al., 2005; Chevaliez, 2011). Although very sensitive and high-throughput systems are available, nucleic acid testing is expensive, labor intensive, and requires technical skill, which limit its use (Scott and Gretch, 2007).

Detection and quantification of HCV core antigen (Cag) by monoclonal antibodies directed against the conserved epitopes of the virus nucleocapsid (core) have been previously described (Aoyagi et al., 1999; Tanaka et al., 1995). Subsequent studies have revealed significant correlations with different HCV-RNA assays, and HCV Cag level has been suggested as a potential marker for viral replication (Bouvier-Alias et al., 2002; Tillmann et al., 2005). Recently, a new and automated version of the Cag assay has been developed and the analytical evaluation of this system revealed increased sensitivity and reduced processing time compared to the previous assays (Mederacke et al., 2009;

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Morota et al., 2009). Since the HCV Cag assay is easy to perform in an immunoassay format, cheap, and less prone to sample carryover contamination compared to nucleic acid tests, it has been introduced as an alternative assay to viral load determination in HCV-infected persons (Park et al., 2010; Ross et al., 2010). The aim of this study was to evaluate the utility of commercial Cag assays for samples that require HCV testing in routine laboratory conditions at a diagnostic laboratory of a major reference hospital.

2. Materials and methods

2.1. Study location and samples

The study was performed in Hacettepe University Hospital, which is one of the largest reference centers in Turkey. Turkey is a nonendemic area for HCV infection and the mean anti-HCV seroprevalence rate in 16 years is reported as 0.38% (Emekdas et al., 2006). The most frequent HCV genotype is 1b, which constitutes 70–94% of all strains in all regions, followed by 1a (3–5%) and non–genotype 1 strains (Altuglu et al., 2008; Selcuk et al., 2006).

Plasma samples sent to the central microbiology laboratory for HCV RNA testing between March 2009 and May 2010 that had been aliquoted and stored in −80°C in serum bank were retrospectively overviewed. All samples with sufficient amounts for further testing were included in the study, which comprised 272 samples of which 226 were from individuals originating from various hospital divisions and units. Eleven samples (11/272, 4.1%) were from pediatric clinics, while 261 (261/272, 95.9%) were from adult patients. The majority of the samples (190/272, 69.9%) were noted to originate from the gastroenterology unit, followed by other internal medicine divisions (33/272, 12.1%; including endocrinology, hematology, neurology, rheumatology, and oncology units), infectious diseases unit (24/272, 8.8%), nephrology/hemodialysis unit (16/272, 5.8%), and surgery divisions (9/272, 3.3%). A total of 75 samples (75/272, 27.8%) have been observed as repeat sera from 29 individuals. The mean number of repeat sera from each person was 3 (range: 2–5). HCV genotype was determined via cycle sequencing in 145 individuals (145/226, 64.2%), in whom the majority of the isolates were observed to belong to genotype 1b (136/145, 93.8%), followed by genotype 1a (5/145, 3.4%), genotype 1b and 1a coinfection (2/145, 1.4%), genotype 3 (1/145, 0.7%), and genotype 4 (1/145, 7.9%). The selected samples were thawed and used for the detection of viral RNA, anti-HCV antibodies, and HCV Cag.

2.2. Detection of HCV RNA and anti-HCV antibodies

Presence and quantitation of HCV RNA in all samples were performed via a commercial automated nucleic acid extraction and real-time polymerase chain reaction (PCR) system (COBAS Ampliprep/COBAS Taqman HCV real-time PCR, Roche Diagnostics, Germany). This assay has been standardized against the first WHO International Standard for HCV RNA (code 96/798), and the determined HCV RNA concentration is reported in international units per milliliter. The assay has a limit of detection of 15 IU/mL with a linear quantitation window of 43–6.9×10^7 IU/mL.

All samples were tested for anti-HCV antibodies by a commercial chemiluminescent microparticle immunoassay (Architect Anti-HCV, Abbott, USA). Samples with a signal-to-cutoff (S/CO) ratio ≥ 1 were regarded as reactive, and samples with a S/CO ratio of 1–10 were further analyzed via a commercial Western blot assay (HCV Blot 3.0, MP Diagnostics, France) for confirmation. All assays were performed according to the manufacturers' recommendations. If an anti-HCV (or confirmation assay) result was available within a 6-month period, the tests were not repeated.

2.3. Detection and quantitation of HCV Cag

HCV Cag levels in plasma were determined by a new-generation commercial quantitative test (Architect HCV Core antigen assay, Abbott, USA), performed as directed by the manufacturer. This test is a chemiluminescent microparticle immunoassay (CMIA) that uses microparticles coated with monoclonal anti-HCV to quantify antigens in serum or plasma samples. The Cag can be detected in the presence of anti-HCV antibodies since samples are initially treated to dissociate the antigen/antibody complexes. The manufacturer's specifications indicate a cut-off of 0.06 pg/mL (3.00 fmol/L) of recombinant c11 antigen (residues 1–160 of the HCV genotype 2a isolate). The upper limit of linearity is 400 pg/mL. The assay employs the same automated detection platform as the other CMIAs of the manufacturer, and the total processing time is around 40 min.

2.4. Statistical analysis

Spearman's correlation coefficient was used to assess the linear association between HCV Ag and HCV RNA concentrations. Pearson's correlation coefficient was used to evaluate correlations between HCV Cag concentrations and viral loads after log transformation. Agreement between HCV RNA and Cag was assessed via the kappa coefficient. The level of statistical significance was considered as $P < 0.05$. All statistical analyses were performed by the SPSS software package version 15.0 (SPSS, Chicago, IL).

3. Results

3.1. HCV RNA, Cag, and anti-HCV antibody reactivity in samples

HCV RNA was detected in 211 (77.6%) of 272 samples. Among RNA reactive samples, 10 (10/211, 4.7%) were observed as positive, despite being equal to or lower than the

reported detection limit of the assay (15 IU/mL). HCV Cag was detected in 163 of all samples (163/272, 59.9%) and in 160 of the samples with detectable RNA (160/211, 75.8%; including reactive samples below the detection limit). Only samples with HCV RNA levels above the commercial assay cutoff were included for sensitivity/specificity and agreement analyses. The overall sensitivity and specificity of HCV Cag assay, when HCV RNA was taken as the gold standard test, have been observed as 75.8% and 95.1%, respectively. The overall agreement between HCV RNA and HCV Cag as evaluated via kappa coefficient was noted as moderate ($\kappa = 0.554$, $P = 0.05$). When samples with viral loads over 10^3 IU/mL were considered separately, the sensitivity increased to 90.9% and the specificity to 100%.

Anti-HCV reactivity was observed in 235 samples (235/272, 86.4%), indicating that the majority of the study group had been previously exposed to HCV. In anti-HCV–negative samples (37/272; 13.6%), HCV RNA and/or Cag reactivity was noted in 19 (19/37; 51.4%). When these samples were further analysed, negative Cag and low HCV loads (mean = 192.8 IU/mL, range = 17.1–775 IU/mL) were observed in 8 samples, suggesting early stages of viral replication. In another patient, Cag positivity and a viral load of 2670 IU/mL were noted. Cag positivity accompanying relatively high viral loads ($\geq 2.5 \times 10^4$ IU/mL) were observed in 8 samples, suggesting acute HCV infections before seroconversion or impaired antibody response, as 5 patients in this group were registered at the medical oncology unit of the hospital. In 2 samples, HCV Cag was reactive, albeit in low levels (0.06 and 0.08 pg/mL), despite all other markers being negative.

The distribution of anti-HCV and HCV Cag reactivity according to various HCV RNA levels is given in Table 1.

3.2. Correlation of HCV RNA and Cag levels

For samples with HCV RNA levels within the linear quantitation range, the correlation of viral load and HCV Cag has been studied. The correlation and statistical significance of HCV Cag levels according to viral loads are given in Table 2. Overall, a high correlation coefficient of 0.915 ($P < 0.001$) has been identified in all samples. It was also observed that the correlation became significant above a viral load threshold of 10^3 IU/mL (Table 2). Only 13 (12.1%) of 107 samples with RNA levels below 10^3 IU/mL were reactive for Cag, whereas 91% (150/165) of the sera were reactive above this RNA level. The association of HCV RNA and Cag levels has also been investigated after logarithmic transformation where the Pearson correlation coefficient revealed a significantly high correlation ($r = 0.937$, $P < 0.001$; Fig. 1).

HCV RNA and Cag kinetics in 2 patients with the highest number of repeat samples are given in Fig. 2.

Table 1
Distribution of anti-HCV and HCV Cag reactivities according to HCV load

Virus load (IU/mL)	Anti-HCV positive	Core antigen		Total
		Positive	Negative	
10^6<=	24 (82.8)	29 (100)	0 (0)	29
10^5–10^6	62 (98.4)	60 (95.2)	3 (4.8)	63
10^4–10^5	52 (96.3)	49 (90.7)	5 (9.3)	54
10^3–10^4	18 (94.7)	12 (63.2)	7 (36.8)	19
10^2–10^3	25 (89.3)	7 (25)	21 (75)	28
16–10^2	13 (72.2)	3 (16.7)	15 (83.3)	18
15>=[a]	9 (90)	0 (0)	10 (100)	10
Negative	32 (62.7)	3 (5.9)	48 (94.1)	51
Total	235 (86.4)	163 (59.9)	109 (40.1)	272

Values are shown as *n* (%).

[a] Reactive but equal or below the detection range of the HCV RNA assay.

Table 2
Correlation and statistical significance of HCV Cag levels according to viral loads

Virus load (IU/mL)	No.	Core antigen (Spearman's rho)	*P* value[a]
10^6<=	29	0.897	<0.001
10^5–10^6	63	0.724	<0.001
10^4–10^5	54	0.404	0.002
10^3–10^4	47	0.595	<0.001
0–10^3	79	0.066	0.561
Total	272	0.915	<0.001

[a] Statistical significance considered as $P < 0.05$.

4. Discussion

Preliminary studies with various HCV Cag assays indicated that the kinetics of Cag were similar to those of HCV RNA in all phases of infection and that the

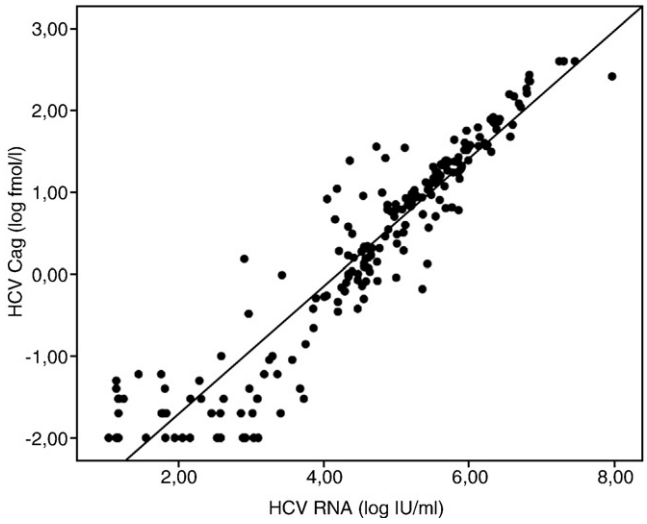

Fig. 1. Scatter plot of HCV Cag ($\log_{10}$ fmol/L) and RNA ($\log_{10}$ IU/mL) in samples with detectable viral load. The correlation of HCV RNA and Cag levels reveals a significantly high correlation ($r = 0.937$, $P < 0.001$).

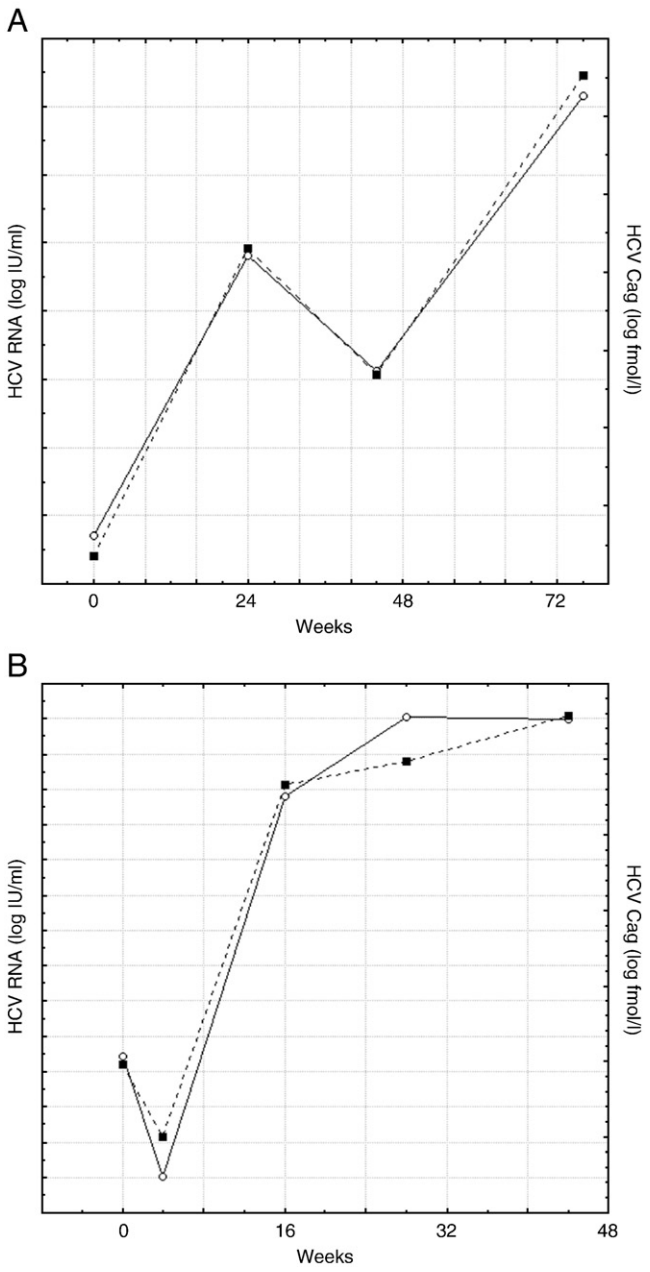

Fig. 2. HCV RNA and Cag kinetics over time in 2 HCV genotype 1b–infected persons with 4 (A) and 5 (B) consecutive samples evaluated in the study (-▪-, HCV Cag; -○-, HCV RNA).

concentrations of viral Cag and RNA were well correlated (Fabrizi et al., 2005; Schuttler et al., 2004; Zanetti et al., 2003). Similar performances to the HCV RNA assays indicated that HCV Cag determination could be considered as an alternative to viral load measurement to reduce the serologic window in acute infections as well as to monitor antiviral therapy (Laperche et al., 2003; Tobler et al., 2005; Veillon et al., 2003). Analytical evaluations of this new-generation of commercial Cag assay in HCV-infected persons and seroconversion panels have revealed the potential of this novel assay in aiding HCV virologic diagnosis and a probable impact in clinical decision making (Morota et al., 2009; Ross et al., 2010). In this study, we aimed to evaluate the utility and effectiveness of the new-generation HCV Cag assay in real-time diagnostic laboratory practice at a reference hospital.

In a total of 272 samples from 226 individuals from various clinics of the hospital, HCV RNA was detected at a rate of 77.6% and anti-HCV antibody prevalence was observed as 86.4%. HCV Cag reactivity was identified in 59.9% of the samples and in 75.8% with detectable viral RNA. An overall sensitivity of 75.8% has been calculated for Cag determination as compared to viral RNA. Previous evaluations of different assays have shown that HCV Cag determination was, indeed, less sensitive than viral RNA detection (Seme et al., 2005; Tobler et al., 2005). This was also valid for the new-generation of commercial assay employed in this study where the analytical sensitivity has been determined as 500–6000 IU/mL of viral RNA (Miedouge et al., 2010; Ross et al., 2010). In accordance with these reports, we have observed a viral load threshold of 10^3 IU/mL, above which 91% (150/165) of the samples were reactive for Cag and only 12.1% (13/107) were reactive below this threshold. Moreover, viral load and Cag correlation have been determined to be statistically significant and the sensitivity of the Cag assay increased from 75.8% to 90.9% above this level. Thus, it can be concluded that HCV Cag assay is much prone to false-negative results below a HCV RNA level of 10^3 IU/mL, despite the presence of viral replication. This observation is further supported by our results from patients with acute infections without anti-HCV antibodies, where HCV Cag was not detected in 8 patients with a mean viral load of 192.8 IU/mL, whereas Cag reactivity could be identified in a patient with a HCV RNA level of 2670 IU/mL. However, HCV Cag assay has been reported not to be less effective than HCV RNA detection in seroconversion panels and reduced the pre-seroconversion period by 4 to 16 days (Ross et al., 2010). In addition, combo assays that simultaneously detect Cag and anti-HCV antibodies have also been developed, which present an alternative to HCV RNA detection for diagnosis of acute HCV infections or for blood screening when nucleic acid-based technologies are not available or feasible (Laperche et al., 2005).

It is known that the presence of Cag and RNA in individual patients may be influenced by different factors, and Cag-to-HCV RNA ratio varies slightly from one person to another (Schuttler et al., 2004). This is partly due to the presence of Cag both in complete virions and in RNA-free core protein structures in peripheral blood (Bouvier-Alias et al., 2002). Moreover, in patients receiving antiviral therapy, HCV Cag/RNA levels may be altered due to RNA-free forms that can be more abundant than complete virions (Miedouge et al., 2010; Pivert et al., 2006). Nevertheless, preliminary studies have suggested that HCV core antigen level is significantly related to HCV RNA within the linear quantitation range

of today's most frequently used commercial nucleic acid tests, including Roche Amplicor HCV Monitor (reverse-transcription PCR) (Morota et al., 2009), Roche Cobas Taqman (real-time PCR) (Park et al., 2010), Versant HCV RNA 3.0 (branched DNA) (Ross et al., 2010). We have observed a high correlation ($r = 0.915$, $P < 0.001$ and $r = 0.937$, $P < 0.001$, after log transformation) in our study using an automated extraction and quantitative real-time PCR system (COBAS Ampliprep/COBAS Taqman HCV Real-time PCR, Roche Diagnostics, Germany). As pointed out earlier, the correlation became significant above a viral load threshold of 10^3 IU/mL.

The influence of HCV genotype on Cag level and viral load is another point of interest. The impact of HCV genotype on HCV Cag assays has been shown in preliminary evaluation studies where the analytical sensitivity of Cag determination has been observed to vary according to the HCV genotype from 3.5 to 13.5 fmol/L (Ross et al., 2010). The correlation is also reported to be independent of the infecting HCV genotype (Mederacke et al., 2009; Miedouge et al., 2010), and a robust correlation was observed between HCV core antigen and HCV RNA values by 2 commercial real-time PCR quantitation assays for all genotypes with the exception of genotype 4, for which a variance of correlation coefficient (0.95 versus 0.80) was identified (Elkady et al., 2010). The majority of HCV genotypes in our study consisted of type 1 strains (types 1b and 1a, 98.6%), while non–genotype 1 strains constituted only 1.4% (2/145, types 3 and 4). Therefore, the effect of HCV genotype variation can be considered as minimal in this study.

In conclusion, detection and quantification of HCV core antigen emerge as a strong alternative to nucleic acid testing, as determined in routine diagnostic laboratory setting in a nonendemic region. Despite having a lower sensitivity compared to HCV RNA below a certain viral load level, it provides the advantage of being a cheaper assay, requiring less turnaround time compared to an automated viral load assay and sample stability (Caliendo et al., 2006; Mederacke et al., 2009). Employment of a frequently used automated CMIA platform can also be considered as an advantage in a diagnostic laboratory. HCV Cag assay can particularly be considered for the follow-up and evaluation of antiviral treatment response in patients infected with HCV. To fully understand the impact of HCV Cag testing in patient management, prospective studies are needed.

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