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Significance of the hepatitis C virus core antigen testing as an alternative marker for hepatitis diagnosis in Egyptian patients

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Abstract. – OBJECTIVE: Hepatitis C virus (HCV) core antigen (Ag) quantification by enzyme-immunoassays has been proposed as an economic and simpler alternative to HCV RNA detection. The current study was undertaken to assess the significance of HCV core antigen assay for the diagnosis of chronic HCV infection and monitoring response to antiviral therapy in Egyptian patients.

PATIENTS AND METHODS: Sixty three HCV antibody positive patients and ten interferon-treated patients were included in the current study. The included patients were divided according to their viral load into four groups as follows; group I (n=10): HCV RNA loads ≤ 10000 IU/ml, group II (n=20): HCV RNA loads > 10000 ≤ 100000 IU/ml, group III (n=33): HCV RNA loads >100000 IU/ml and group IV (n=10): interferon-treated HCV patients with a negative HCV RNA. Serum HCV core Ag and RNA loads were assayed and their correlations, including linear regression lines, were calculated.

RESULTS: HCV core Ag exhibited a non-significant ($p > 0.05$) difference between all the studied groups. Concerning, group I patients, HCV core Ag levels and HCV RNA loads were positively correlated, with a correlation coefficient of 0.73 ($p < 0.05$). Group II and III showed stronger correlations; the recorded values were 0.81 ($p < 0.0001$) and 0.94 ($p < 0.0001$) for group II and III, respectively.

CONCLUSIONS: HCV core Ag test can be used as an alternative to HCV RNA tests to evaluate chronic infection when the HCV RNA test is unavailable, but is not reliable enough for treatment monitoring.

Key Words:
HCV core antigen assay, Hepatitis C virus, Viral load.

Introduction

Hepatitis C is a major global health burden and Egypt has the highest prevalence of hepatitis C virus (HCV) worldwide[1]. A high prevalence of chronic HCV infection has been reported in African and Eastern Mediterranean countries, with a prevalence of 14.7% in the general population of Egypt, whereas the HCV prevalence varies from 0.4-0.8% in Sweden, Germany, the Netherlands and France to over 1.9% in the Unites States, and over 5% in some communities in Italy[2-4].

Detection of anti-hepatitis C virus antibodies has limited sensitivity during acute phase: the pre-seroconversion window period. HCV RNA detection techniques are used to overcome this shortfall, but are costly and unavailable widely in developing countries[5]. Although HCV diagnosis through RNA testing has high sensitivity and specificity, and low detection limits such as 15 to 30 IU/ml and can give quantitative results, it has major limitations such as liability of RNA molecules, higher costs, and longer turn-around time as compared with commercially-available HCV core antigen (Ag) testing[6]. In addition, these tests require special technical and laboratory facilities and carry a risk of contamination[7-9].

Considering the complexity of HCV infection diagnosis and the significant cost and turn-around time burden it imposes on clinical laboratories, HCV core Ag testing seems an attractive adjunct to the current battery of laboratory diagnosis that demands more attention[6]. Moreover, Chakravarti et al[5] reported that the estimation of hepatitis C virus core Ag, a protein with highly conserved sequence, by enzyme-immunoassays is an economic and simpler alternative to RNA detection. Therefore, the current study was undertaken to evaluate the significance of HCV core Ag assay for the diagnosis of chronic HCV infection in Egyptian patients. In addition, the study aimed to test the usefulness of HCV core Ag test for monitoring the disease prognosis and response to antiviral therapy.

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Patients and Methods

Patients

The current study was conducted at the Gastroenterology Department of Beni-Suef University Hospital (Egypt) following an approval by the Ethical Committee of Faculty of Medicine, Beni-Suef University which conformed to the ethical guidelines of the 1975 Declaration of Helsinki. In addition, informed consents were signed by all volunteers.

Seventy three HCV patients (mean age ≥ 30 - ≤ 60 years old) were included in the current study. Inclusion criteria included all patients who have proven HCV infection and were negative for Hepatitis B virus (HBV), human immunodeficiency virus (HIV) and Schistosomiasis. Patients with renal failure, congestive heart failure, leukopenia, thrombocytopenia or autoimmune diseases were excluded.

The studied population was divided into 4 groups as follows:

Group I (n=10): HCV patients with HCV RNA loads ≤ 10000 IU/ml.
Group II (n=20): HCV patients with HCV RNA loads > 10000 ≤ 100000 IU/ml.
Group III (n=33): HCV patients with HCV RNA loads > 100000 IU/ml.
Group IV (n=10): Interferon-treated HCV patients with a negative serum HCV RNA.

Laboratory Assays

Liver Function Markers

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed using reagent kit purchased from Spinreact (Girona, Spain) according to the method of Schumann and Klauke[10]. Alkaline phosphatase (ALP) was assayed according to the International Federation of Clinical Chemistry (IFCC)[11] using BioSystem (Barcelona, Spain) commercial kit. Serum albumin and total bilirubin were assayed using kits of Diamond Diagnostic (Egypt) according to the methods of Webster[12] and Kaplan[13], respectively.

HCV core Ag Assay

HCV core antigen was detected with HCV Ag ELISA assay (Monolisa Ultra, Bio-Rad, Marnes la Coquette, France) by strictly following the manufacturer's instructions. Briefly, murine monoclonal antibodies against the hepatitis C capsid were coated on microwells of the solid phase and were conjugated with horseradish peroxidase. Fifty microliters of serum samples were added to the microwells. Antigen was bound by the monoclonal antibodies coated on microplate and by the biotinylated monoclonal antibodies against the capsid hepatitis C antigen. After incubation at 37°C during 90 minutes and a washing step, the peroxidase-labelled antibodies to human IgG and streptavidine-peroxidase were added. Following 30 minutes incubation at 37°C, the unbound enzymatic conjugate was removed by washing step and the antigen-antibody complex was revealed by addition of substrate. The reaction has been stopped after 30 minutes and the absorbance values were read using a spectrophotometer at 450 nm. The absorbance measured for a sample allows to detect the presence or absence of capsid antigen to HCV. The colour intensity is proportional to the quantity of antigen to HCV bound on the solid phase. The results were expressed as the ratio of the absorbance to the cut off; a ratio above 1 is considered a positive result.

Quantitative Assay of HCV RNA Load

HCV RNA levels were determined using the Roche Light Cycler Taqman Master Kit on Roche Light Cycler Real-Time PCR System (Roche Diagnostics GmbH, Mannheim, Germany). Quantitative detection of HCV RNA by real-time polymerase chain reaction (RT-PCR) was performed with a 0.2 ml serum sample according to the manufacturer's instructions. An internal quality control serum was included during RT-PCR.

Statistical Analysis

The data were analyzed using SPSS v.20 (SPSS Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) test was used for comparisons and results were expressed mean ± standard error (SE). A p value of < 0.05 was considered statistically significant. The correlation coefficients for serum levels of HCV RNA load and HCV core Ag were calculated using Pearson's test.

Results

Epidemiological and biochemical data of the studied population were represented in Table I. The recorded data denoted a non-significant dif-

Table I. Characteristics of the HCV patients.

	Group I	Group II	Group III	Group IV	F-Prob.
Gender	Male: 4 Female: 6	Male: 12 Female: 8	Male: 24 Female: 9	Male: 8 Female: 2	–
HCV RNA x10^3 (IU/ml)	4.63 ± 0.85^{b}	51.71 ± 5.90^{b}	659.11 ± 156.88^{a}	–	$p < 0.01$
AST (U/L)	66.70 ± 16.30^{a}	$52.24 \pm 2.30^{a,b}$	$50.82 \pm 3.00^{a,b}$	43.10 ± 3.12^{b}	$p > 0.05$
ALT (U/L)	54.78 ± 5.51^{a}	$51.68 \pm 3.63a$	63.41 ± 4.78^{a}	45.30 ± 2.22^{a}	$p > 0.05$
ALP (U/L)	109.50 ± 12.28^{a}	139.38 ± 15.21^{a}	149.41 ± 13.14^{a}	116.90 ± 18.81^{a}	$p > 0.05$
Albumin (g/dl)	4.01 ± 0.22^{b}	4.47 ± 0.09^{a}	$4.30 \pm 0.07^{a,b}$	$4.24 \pm 0.09^{a,b}$	$p > 0.05$
Total bilirubin(mg/dl)	$0.83 \pm 0.05^{a,b}$	$0.79 \pm 0.02^{a,b}$	0.93 ± 0.05^{a}	0.72 ± 0.07^{b}	$p < 0.05$

Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different.

ference ($p > 0.05$) in serum AST, ALT, ALP and albumin between all studied groups. On the other hand, group IV serum total bilirubin showed a significant difference ($p < 0.05$) when compared to group III patients, however it showed a non-significant difference on comparison with group I and II patients. Regarding serum HCV core Ag, it exhibited a non-significant ($p > 0.05$) difference between all the four groups as represented in Figure 1.

The correlations of HCV RNA loads ($\log_{10}$ IU/ml) with HCV core Ag levels ($\log_{10}$), including linear regression lines, were depicted in Figures 2-4. Concerning group I (HCV RNA load ≤ 10000 IU/ml), HCV core Ag levels and HCV RNA loads were strongly correlated, with a correlation coefficient of 0.73 ($p < 0.05$).

Compared with group I, the correlations between HCV core Ag and HCV RNA loads in group II (HCV RNA load > 10000 ≤ 100000 IU/ml) and group III (HCV RNA load > 100000 IU/ml) were stronger; the recorded correlations were 0.81 ($p < 0.0001$) and 0.94 ($p < 0.0001$) for group II and III, respectively.

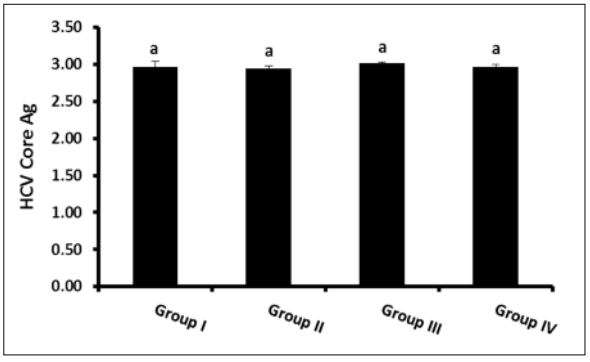

Figure 1. Serum HCV core Ag. Data are expressed as mean ± SE, means which share the same superscript symbol(s) are not significantly different, $p > 0.05$.

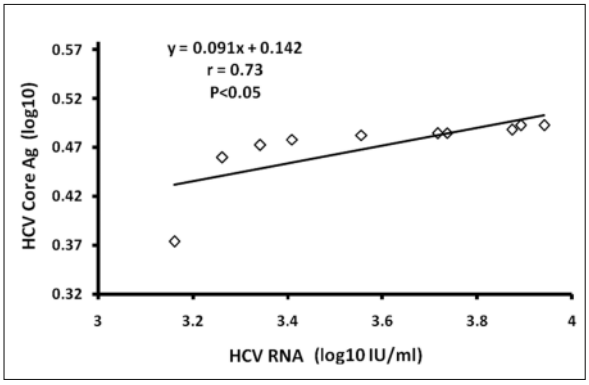

Figure 2. Correlation analysis between HCV RNA and HCV core antigen measurement in group I patients.

Discussion

Hepatitis C virus, a single stranded enveloped RNA virus belonging to the *Flaviviridae* family, is a globally significant pathogen, infecting over 170 million individuals worldwide with about one million new cases being reported annually[14,15]. The Egyptian Demographic Health Survey

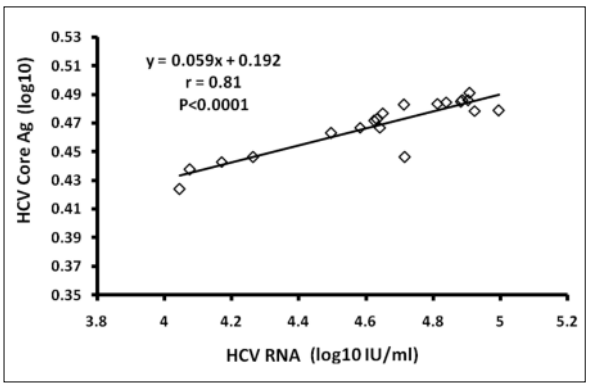

Figure 3. Correlation analysis between HCV RNA and HCV core antigen measurement in group II patients.

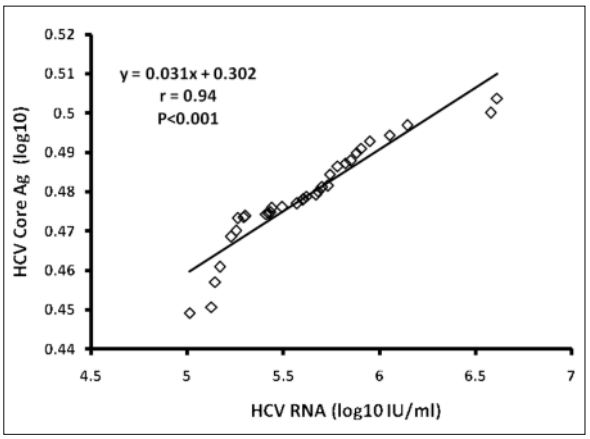

Figure 4. Correlation analysis between HCV RNA and HCV core antigen measurement in group III patients.

(EDHS) estimated HCV prevalence among the 15-59 years age group to be 14.7%[16]. Accordingly, Egypt has the highest HCV prevalence in the world[17-19]. It has been postulated that the epidemic has been caused by extensive iatrogenic transmission during the era of parenteral-antischistosomal-therapy mass-treatment campaigns[20,21]. Today, HCV infection and its complications are among the leading public health challenges in Egypt[22]. More recently, Mohamoud et al[23] concluded that Egypt is confronted with an HCV disease burden of historical proportions that distinguishes this nation from others.

Recently, Kurtulus et al[24] revealed that quantitative tests for the detection of hepatitis C virus RNA levels and HCV core Ag have been mainly used for the management of patients with HCV infection. However, detection of HCV RNA by nucleic acid amplification techniques (NAT) is expensive, time consuming, requires technical expertise and dedicated laboratory area. These reasons restrict the availability or wide scale usage of HCV RNA detection in many laboratories of developing countries[25,26]. Therefore, HCV core Ag quantification has been proposed as a surrogate marker of HCV RNA levels in patients with chronic HCV infection[27-29]. Thus, to reduce the incurred costs and labour associated with HCV RNA testing and also to shorten the turnaround time, the current study aimed to evaluate the clinical diagnostic significance of HCV core Ag as an alternate test for HCV RNA.

When the correlation between HCV RNA and HCV core Ag levels was analyzed, the correlation coefficient (r) between the two tests was quite high in group I, group II and group III; the recorded correlation coefficients were 0.73, 0.81 and 0.94, respectively. The results in the present study are consistent with that recorded by Kesli et al[30] who determined HCV RNA levels using the Qiagen HCV RNA test (Qiagen, Hilden, Germany) and identified a correlation with the Architect HCV core antigen test as 0.864 and Ergünay et al[31] who assayed HCV RNA levels with COBAS Ampliprep/COBAS Taqman HCV Real-time PCR (Roche Diagnostics, Germany) and calculated a correlation coefficient as 0.915. In the same context, a correlation coefficient as 0.75 was reported by Mederacke et al[32] who determined HCV RNA levels using Cobas Taqman or Amplicor HCV Monitor (Roche Diagnostics, Mannheim, Germany). By using different HCV RNA kits, Medici et al[33] determined a correlation coefficient ranging from 0.713 to 0.870 for the HCV core antigen test. Data recorded in our study and the results from other studies reveal that HCV RNA tests and the HCV core antigen test give quite compatible results.

An important finding of the present study is the increased correlation between the two tests with increased HCV RNA levels and the recorded non-significant difference in HCV core Ag between the interferon-treated patients, with negative HCV RNA, and patients with positive HCV RNA. Such findings may be attributed to the fact that HCV virions harbour a single copy of the RNA genome whereas HCV core antigen could be found in both complete virions and RNA free core protein structures as revealed by Schuttler et al[34]. Hence, HCV core antigen test is not of significance for monitoring interferon treatment in HCV patients. Accordingly, a recent study by Kadkhoda and Smart[6] revealed that HCV core Ag test is not deemed reliable enough for treatment monitoring as it could be found singly, in aggregates, associated with disrupted membranes, and complexed with antibodies which may lead to the observed suboptimal coefficient of determination. In contrast, the studies of Bouvier-Alias et al[27] and Maynard et al[35] recommended that HCV core Ag assays could be used to monitor the response to antiviral therapy with comparable results to those of HCV RNA PCR.

Conclusions

HCV RNA testing is more sensitive than HCV core Ag assay, despite the higher stability of the HCV core antigen after freezing or heating than

HCV RNA[36] and its appearance in serum before the appearance of HCV RNA[37]. Therefore, we recommend that the HCV core Ag test can be used as an alternative to HCV RNA tests to evaluate chronic infection when the HCV RNA test is unavailable, but not for clinical management of patients under treatment with antiviral therapy.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Abdel-Moneim A, Morsy BM, Mahmoud AM, Abo-Seif MA, Zanaty MI. Benificial therapeutic effects of Nigella sativa and/or Zingiber officinale in HCV patients in Egypt. EXCLI J 2013; 12: 943-955.
2) Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med 2006; 144: 705-714.
3) Meffre C, Lestrat Y, Delarocque-Astagneau E, Dubois F, Antona1 D, Lemasson J et al. Prevalence of hepatitis B and hepatitis C virus infections in France in 2004: social factors are important predictors after adjusting for known risk factors. J Med Virol 2010; 82: 546-555.
4) Hatzakis A, Wait S, Bruix J, Buti M, Carballo M, Cavaleri M, et al. The state of hepatitis B and C in Europe: report from the hepatitis B and C summit conference. J Viral Hepat 2011; 18(Suppl 1): 1-16.
5) Chakravarti A, Chauhan MS, Dogra G, Banerjee S. Hepatitis C virus core antigen assay: can we think beyond convention in resource limited settings? Braz J Infect Dis 2013; 17: 369-374.
6) Kadkhoda K, Smart G. HCV Antigen testing for the diagnosis of hepatitis C infection: a cost-efficient algorithm. Clin Lab 2014; 60: 677-680.
7) Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. J Clin Microbiol 2002; 40: 4407-4412.
8) Alter MJ, Kuhnert WL, Finelli L. Centers for Disease Control and Prevention. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. MMWR Recomm Rep 2003; 52: 1-13.
9) Chevaliez S. Virological tools to diagnose and monitor hepatitis C virus infection. Clin Microbiol Infect 2011; 17: 116-121.
10) Schumann G, Klauke R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin Chim Acta 2003; 327: 69-79.
11) IFCC. Methods for the measurement of catalytic concentration of enzymes. J Clin Chem Clin Biochem 1980; 18: 521-534.
12) Webster D. A study of the interaction of bromcresol green with isolated serum globulin fractions. Clin Chim Acta 1974; 53: 109-115.
13) Kaplan LA. Glucose. In: Kaplan LA, Pesce AJ. Clinical chemistry: theory, analysis, and correlation. St. Louis, MO: Mosby, 1984; pp. 1032-1036.
14) Strader DB, Wright T, Thomas DL, Seeff LB. American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. Hepatology 2004; 39: 1147-1171.
15) Curry MP, Chopra S. Acute viral hepatitis. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's principles and practice of infectious diseases. Philadelphia: Churchill Livingstone, 2010; pp. 1577-1592.
16) El-Zanaty F, Way A. Egypt Demographic and Health Survey 2008. Egyptian: Ministry of Health. Cairo: El-Zanaty and Associates, and Macro International, 2009.
17) Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5: 558-567.
18) Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol 2007; 13: 2436-2441.
19) Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011; 17: 107-115.
20) Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000; 355: 887-891.
21) Strickland GT. Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors. Hepatolology 2006; 43: 915-922.
22) Miller FD, Abu-Raddad LJ. Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. Proc Natl Acad Sci USA 2010; 107: 14757-14762.
23) Mohamoud YA, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. BMC Infect Dis 2013; 13: 288.
24) Kurtulus B, Gulcin B, Nese K, Zeynep A, Selcuk K, Iftihar K, et al. Correlation between architect hepatitis C virus (HCV) core antigen and HCV Ribonucleic acid levels in Anti- HCV reactive patients in Turkey. Afr J Microbiol Res 2013; 7: 4907-4911.
25) Diment J, Calmann M. Alternatives to nucleic acid testing in the blood transfusion service. Lancet 2002; 360: 1518-1519.
26) Simmonds P, Kurtz J, Tedder RS. The UK blood transfusion service: over a (patent) barrel? Lancet 2002; 359: 1713-1714.
27) Bouvier-Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, Hezode C, Picchio G, Dhumeaux D, Neumann AU, McHutchison JG, Pawlotsky JM. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepatolology 2002; 36: 211-218.

28) Descamps V, Op de Beeck A, Plassart C, Brochot E, François C, Helle F, Adler M, Bourgeois N, Degré D, Duverlie G, Castelain S. Strong correlation between liver and serum levels of hepatitis C virus core antigen and RNA in chronically infected patients. J Clin Microbiol 2012; 50: 465-468.

29) Hosseini-Moghaddam SM, Iran-Pour E, Rotstein C, Husain S, Lilly L, Renner E, Mazzulli T. Hepatitis C core Ag and its clinical applicability: potential advantages and disadvantages for diagnosis and follow-up? Rev Med Virol 2012; 22: 156-165.

30) Kesli R, Polat H, Terzi Y, Kurtoglu MG, Uyar Y. Comparison of a newly developed automated and quantitative hepatitis C virus (HCV) core antigen test with the HCV RNA assay for clinical usefulness in confirming anti-HCV results. J Clin Microbiol 2011; 49: 4089-4093.

31) Ergunay K, Sener B, Alp A, Karakaya J, Hascelik G. Utility of a commercial quantitative hepatitis C virus core antigen assay in a diagnostic laboratory setting. Diagn Microbiol Infect Dis 2011; 70: 486-491.

32) Mederacke I, Wedemeyer H, Ciesek S, Steinmann E, Raupach R, Wursthorn K, Manns MP, Tillmann HL. Performance and clinical utility of a novel fully automated quantitative HCV-coreantigen assay. J Clin Virol 2009; 46: 210-215.

33) Medici MC, Furlini G, Rodella A, Fuertes A, Monachetti A, Calderaro A, Galli S, Terlenghi L, Olivares M, Bagnarelli P, Costantini A, De Conto F, Sainz M, Galli C, Manca N, Landini MP, Dettori G, Chezzi C. Hepatitis C virus core antigen: analytical performances, correlation with viremia and potential applications of a quantitative, automated immunoassay. J Clin Virol 2011; 51: 264-269.

34) Schüttler CG, Thomas C, Discher T, Friese G, Lohmeyer J, Schuster R, Schaefer S, Gerlich WH. Variable ratio of hepatitis C virus RNA to viral core antigen in patient sera. J Clin Microbiol 2004; 42: 1977-1981.

35) Maynard M, Pradat P, Berthillon P, Picchio G, Voirin N, Martinot M, Marcellin P, Trepo C. Clinical relevance of total HCV core antigen testing for hepatitis C monitoring and for predicting patients' response to therapy. J Viral Hepat 2003; 10: 318-323.

36) Tanaka Y, Takagi K, Fujihara T, Kitsugi K, Fujiwara K, Hiramatsu K, Ito Y, Takasaka Y, Sakai M, Mizokami M. High stability of enzyme immunoassay for hepatitis C virus core antigen-evaluation before and after incubation at room temperature. Hepatol Res 2003; 26: 261-267.

37) Leary TP, Gutierrez RA, Muerhoff AS, Birkenmeyer LG, Desai SM, Dawson GJ. A chemiluminescent, magnetic particle based immunoassay for the detection of hepatitis C virus core antigen in human serum or plasma. J Med Virol 2006; 78: 1436-1440.