BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2019, VOL. 76, NO. 4, 190–194
https://doi.org/10.1080/09674845.2019.1654790

Hepatitis C virus core antigen as a possible alternative for evaluation of treatment effectiveness after treatment with direct-acting antivirals

M Łucejko[a], K Tomasiewicz[b], A Olczak[c], M Tudrujek-Zdunek[b], W Halota[c], W Jelski[d], H Donica[e], M Krintus[f], B Mroczko[d] and R Flisiak[a]

[a]Department of Infectious Diseases and Hepatology, Medical University of Bialystok, Bialystok, Poland; [b]Department of Infectious Diseases and Hepatology, Division of Laboratory Diagnostics, Medical University of Lublin, Lublin, Poland; [c]Department of Infectious Diseases and Hepatology, Nicolaus Copernicus University, Torun, Poland; [d]Department of Biochemical Diagnostics, Medical University of Bialystok, Bialystok, Poland; [e]Department of Biochemical Diagnostics, Division of Laboratory Diagnostics, Medical University of Lublin, Lublin, Poland; [f]Department of Laboratory Medicine, Nicolaus Copernicus University, Torun, Poland

ABSTRACT

Background: Chronic hepatitis C is a major public health problem around the world. In monitoring treatment efficacy, although costly and labour-intensive methods of molecular biology are often used, much cheaper and technically easier serological methods evaluating the concentration of HCV core antigen in serum are available. We evaluated HCVcAg quantification as a possible assessment of the treatment efficacy instead of HCV RNA quantification.
Methods: We collected 514 serum samples from treated HCV infected patients. Quantitative evaluation of HCV RNA and HCVcAg was carried out before treatment, at the end of treatment, and at least 12 weeks following treatment termination. HCV RNA was determined by automated assay (Roche COBAS) and HCVcAg quantitation with ARCHITECT ci8200 analyser.
Results: There was a significant correlation between HCVcAg and HCV RNA concentrations at baseline and follow-up visits, but not at the end of treatment. Among samples collected before the treatment, at the end of treatment and follow-up visit, concordance of HCV RNA and HCVcAg reached level of 98.1%, 98.9% and 98.7%, respectively. Diagnostic sensitivity, specificity, positive and negative predictive values of HCVcAg detection were >97%.
Conclusions: HCVcAg measurement could be an alternative for determining HCV treatment efficacy after chemotherapy and could be an option in the diagnosis of HCV infection.

ARTICLE HISTORY
Received 23 April 2019
Accepted 8 August 2019

KEYWORDS
Hepatitis C virus; hepatitis C virus core antigen; HCV RNA; treatment monitoring; polymerase chain reaction; diagnosis of HCV

Introduction

Chronic hepatitis C virus (HCV) infection is a serious global health problem, being one of the main factors leading to liver cirrhosis with subsequent liver failure, and hepatocellular carcinoma, with a global prevalence estimated to be 1%, corresponding to about 71 million viraemic cases [1–3]. Prevalence varies significantly between regions of the world, ranging from 0.5% to 7% of the population, and in 2016 the WHO approved a strategy to eliminate viral hepatitis by 2030 by reducing new infections by 90%, reducing mortality by 65% and increasing the number of treated patients to 80% [4]. An undiagnosed patient population is considered a significant barrier to achieve the goal of this strategy. The introduction of direct-acting antivirals changed principles of chronic hepatitis C treatment, with its high efficacy and excellent tolerability [5]. Despite the emergence of generic drugs, new therapies are not widely available in some low-income countries mainly due to the high costs of therapy [6].

Current diagnosis of hepatitis C infection is based on the detection of serum HCV antibodies using automated serological methods, and although quick diagnostic tests are also approved for screening, confirmation of viral replication is necessary with molecular genetics. According to the EASL recommendations (European Association for the Study of the Liver), HCV core antigen (HCVcAg) assay is recommended when the HCV RNA assay is not available or not affordable [7,8].

HCV RNA monitoring is no longer useful during anti-viral treatment but is still useful in evaluating the efficacy of anti-viral treatment 12 weeks after treatment completion. Although real-time PCR assay is sensitive and reliable, it is time-consuming, requires sophisticated equipped laboratories, qualified laboratory personnel, and relatively expensive reagents. Therefore, measurement of HCVcAg can be an alternative for initial diagnosis. Numerous studies have confirmed that HCVcAg concentration correlate positively with HCV RNA [9–15]. A lower workload, faster turn-around time, and significantly lower costs are advantages of this method [16]. The absence of both HCVcAg and HCV RNA after successful treatment defines HCV infection cure.

Aim of this study was to evaluate the quantitative measurement of HCVcAg in HCV infected patients as a possible assessment of the treatment efficacy for the replacement of sustained virologic response measured with undetectability of HCV RNA.

CONTACT M Łucejko mariusz.lucejko@gmail.com 15-540 Białystok, 14 Żurawia Str Poland

Methods

We obtained 514 serum samples from chronically HCV-infected patients treated with anti-virals. Treatment was selected according to national expert guidelines and National Health Fund reimbursement regulations at the time of patient's management [17]. Quantitative evaluation of HCV RNA and HCVcAg was carried out before HCV treatment (baseline), at the end of treatment, and at least 12 weeks following treatment termination. A sustained virological response (SVR) was defined as undetectable serum HCV RNA at least 12 weeks after treatment termination. Respectively, HCVcAg undetectability at the same time, was referred to definition of sustained serological response (SSR).

Blood samples were collected in EDTA tubes, plasma was separated and stored at −70°C until laboratory evaluation. HCV RNA and HCVcAg concentration measurements were carried out from the same sample. Quantitative detection of plasma HCV RNA used an automated RT-PCR assay (Roche COBAS AmpliPrep HCV platform, Roche Molecular Systems, Pleasanton, CA, USA). The lower level of quantification was 15 IU/ml. HCV genotype was determined by direct sequencing of the PCR product before the start of the treatment. Quantitative detection of HCVcAg was performed on an automated ARCHITECT ci8200 analyser (Abbott Diagnostics, Chicago, IL, USA), using an HCVcAg assay kit (Abbott Laboratories, Abbott Park, IL, USA), in a two-step chemiluminescent microparticle immunoassay. Concentration of HCVcAg is expressed in femtomols (fmol/l) per litre (1.0 fmol/l = 0.02 pg/ml). The detection limit ranged from 0 to 20,000 fmol/L with an upper limit of detection after dilution (1:9) of 180,000 fmol/l. Detection cut-off value is 3.0 fmol/l, a value between 3 and 10 fmol/l is defined as 'grey zone'.

Statistical analysis was performed with Statistica 10 (StatSoft Poland, Cracow, Poland). Data were presented as median and range. The correlation coefficients between HCVAg and HCVRNA were calculated by Spearman's rank test and a comparison between the groups was carried out using the Mann-Whitney U test. Differences were considered significant at $p < 0.05$.

Results

Table 1 shows pre-treatment (baseline), immediate post-treatment, and 12-week post-treatment data. HCVcAg and HCV RNA concentrations correlates were r = 0.75 (p < 0.001) at baseline, r = 0.75 (*P*< 0.001) immediately after treatment, and r = 0.19 (*P*= 0.07) 12 weeks after treatment. Of 265 samples collected before treatment 260 (98.1%) were concordant for HCV RNA and HCVcAg detection. The remaining five samples (1.9%) were positive for HCV RNA but HCVcAg negative. However, HCV RNA concentration in these patients was relatively low and ranged from 375 to 7400 IU/ml. In the same group, one result was obtained in the grey zone of HCVcAg (4.68 fmol/L) and HCV RNA concentration amounted in this case was 968 IU/ml. Of the 92 samples collected at the end of treatment, 90 (98.9%) were concordant. In one of the two discordant samples, a negative HCVcAg sample was found simultaneously with very low HCV RNA concentration of 34 IU/ml. HCV RNA below the quantification limit <15 IU/ml was found in the second case. Of 157 samples collected at 12 weeks after treatment, 155 (98.7%) were concordant. The first discordant sample was HCVcAg negative and HCV RNA detectable but not quantifiable (<15 IU/ml), but in repeated measurement HCV RNA becomes undetectable. The second discordant sample at the FU was HCV RNA undetectable but HCVcAg detectable at the low level of 6.89 fmol/l. This patient, a female, had non-advanced liver fibrosis, undetectable for both HCV RNA and HCVcAg at the end of treatment.

Table 1. Median and range HCV RNA and HCVcAg concentration before and during the treatment.

Time of sample collection	n	HCV RNA (IU/ml)	HCVcAg (fmol/L)
Before treatment	265	1.2×10^6 ($56.3–29.7 \times 10^6$)	2490 (0–40,281)
End of treatment	92	0 ($0–0.142 \times 10^6$)	0 (0–187.4)
12 weeks after treatment	157	0 ($0–13.2 \times 10^6$)	0 (0–12,513)

Abbreviations: HCV RNA, hepatitis C virus ribonucleic acid; HCVcAg, hepatitis C virus core antigen.

Taking HCV RNA measurement as a gold standard, the diagnostic sensitivity, specificity, positive and negative predictive values of HCVcAg detection are shown in Table 2. Altogether, nine discordant samples were found. In eight HCV RNA detectable cases, its concentration ranged from the limit of quantification (<15 IU/ml) to 7,400 IU/ml (Table 3). The relationship between HCV RNA and HCVcAg concentration, based on the linear regression analysis of baseline samples, can be expressed with following equation: HCV RNA (in IU/ml) = 1016 + 300.24 x HCVcAg. Based on this calculation 1 fmol/l of HCVcAg is equivalent to 301 IU/ml, so the detection threshold of HCVcAg in our study is around 904 IU/ml of HCV RNA.

Discussion

The first studies investigating the use of HCVcAg quantification started to be published around 1999 [18]. HCVcAg can be detected 2 days after the appearance of HCV RNA which is detectable within 2 weeks after infection. HCVcAg concentration correlates well with HCV RNA viral load [19,20]. In the Hosseini – Moghaddam et al. study good correlation between HCVcAg and HCV RNA in liver tissue and serum was reported [21]. In HCV/HBV and HCV/HIV co-infected population, some studies showed lack or low correlation between HCVcAg and HCV RNA, however, these studies were limited by small group size [22]. We found a statistically significant correlation between HCVcAg and HCV RNA at baseline and follow-up visits but not at the end of treatment, the latter caused by a small number of samples with detectable

Table 2. Characteristics of HCVcAg testing with respect to HCV RNA detection.

	HCVRNA+ HCVcAg+	HCVRNA- HCVcAg-	HCVRNA+ HCVcAg-	HCVRNA- HCVcAg+	HCVcAg Sensitivity (95% CI)	HCVcAg Specificity (95% CI)	HCVcAg PPV (95% CI)	HCVcAg NPV (95% CI)
Baseline (n = 265)	260 (98.1%)	0	5 (1.9%)	0	98.1% (95.7–99.4)	-	100%	0
End of treatment (n = 92)	1 (1.1%)	89 (96.7%)	2 (2.2%)	0	33.3% (0.84–90.6)	100% (95.4–100)	100%	97.8% (95.2–99)
12 week follow up (n = 157)	8 (5.1%)	147 (93.6%)	1 (0.6%)	1 (0.6%)	88.9% (51.8–99.7)	99.3% (96.3–99.9)	88.9% (52.8–98.3)	99.3% (95.9–99.9)
All samples (n = 514)	269 (52.3%)	236 (45.9%)	8 (1.6%)	1 (0.2%)	97.1% (94.4–98.8)	99.6% (97.7–99.9)	99.6 (97.4–99.9)	96.7 (93.7–98.3)

Abbreviations: HCV RNA, hepatitis C virus ribonucleic acid; HCVcAg, hepatitis C virus core antigen; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Table 3. Characteristics of discordant samples.

Sample number	Time-point of collection	HCV RNA [IU/ml]	HCVcAg [fmol/L]
1	baseline	375	undetectable
2	baseline	968	4.68
3	baseline	5070	undetectable
4	baseline	6190	undetectable
5	baseline	7400	undetectable
6	End of treatment	34	undetectable
7	End of treatment	<15	undetectable
8	12 week follow up	<15	undetectable
9	12 week follow up	undetectable	6.89

HCV RNA and/or HCVcAg, and the presence of very low HCVcAg concentrations in some samples accompanied by undetectable HCV RNA (Figure 1).

One of the disadvantages of HCVcAg assay is relatively low sensitivity compared to nucleic acid testing (~90%, depending on the study) [14,23]. According to the literature the equivalent of the HCVcAg detection level, depending on the HCV genotype, ranges from 500 to 3000 IU/ml of HCV RNA assessed by nucleic acid testing [24]. However, chemiluminescent microparticle immunoassay has better sensitivity than ELISA, both free HCVcAg and HCVcAg plus anti-HCV complex are detected [25]. In our hands, the detection threshold of HCVcAg is ~900 IU/ml of HCV RNA. Moreover, one additional advantage of this method is the stability of HCVcAg in samples compared to HCV RNA when they are stored at room temperature [26]. The agreement between methods was excellent at 98.1%, with nine discordant samples, pointing to the high sensitivity of the assay. Of the nine discordant samples, in five HCV RNA viral load was low, and in two cases it was detectable but not quantifiable (<15 IU/mL). In one case HCVcAg remained in the 'grey-zone' level (between 3 and 10 fmol/l), accompanied by relatively low HCV RNA level of 968 IU/ml. In one case HCVcAg concentration was also in the grey zone, with undetectable HCV RNA at the same time, but in this patient, SVR has finally been confirmed: similar cases have been described [12,13]. Delayed release of antigen traces from hepatocytes resulting in low-level HCVcAg detection may be the most likely explanation. Another issue is detection of low HCVcAg concentrations during anti-viral treatment. Strong inhibition of HCV viral replication, while HCVcAg particles are still produced, could be an explanation [27].

As shown above, with HCV RNA assays as a gold standard, the diagnostic sensitivity of HCVcAg was high and detection in all samples was >97%. Based on the results and taking into account significantly lower costs of HCVcAg testing, this approach should be considered in HCV infections for diagnostic purposes. However, patients with low baseline HCV RNA viral load can cause diagnostic problems because of possible false-negative HCVcAg results. On the other hand, proportion of patients with viremia <400,000 IU/ml is relatively low. For example, among 2472 genotype 1 infected patients,

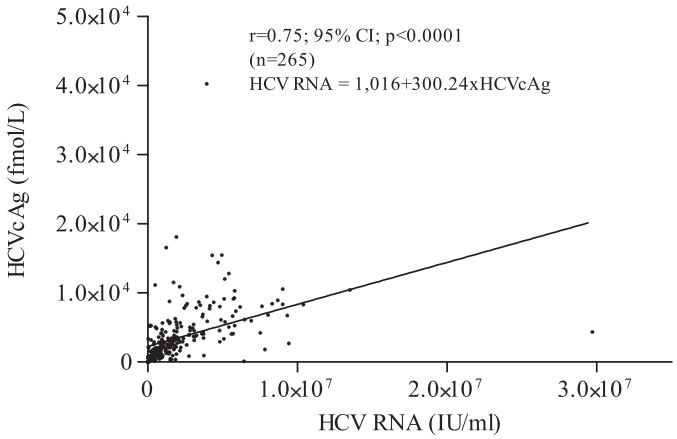

Figure 1. Correlation between HCVcAg and HCV-RNA in samples collected before the treatment.

only 4 had HCV RNA below 1000 IU/ml [28]. Therefore, risk of misdiagnosing an HCV-infected patient seems to be low. However in high-risk infection groups, the HCV RNA test should be prioritized. EASL guidelines, the WHO Global Hepatitis Report 2017 and some national guidelines have included HCVcAg testing for confirmation of acute or chronic the HCV infection, as an alternative to HCV RNA testing [4,7,8].

Several studies have confirmed that anti-viral therapy strongly and quickly suppress HCV viral load to the undetectable level [29,30]. Some have demonstrated the potential utility of HCVcAg and HCV RNA measurements in the early stages of treatment to assess the prediction of response to treatment [13]. On the other hand, some showed that on-treatment levels of HCVcAg and HCV RNA had no predictive value for possible relapse or SVR [12,31,32]. Therefore, most expert groups do not recommend HCVcAg and HCV RNA evaluation during and at the end of anti-viral treatment to predict treatment effectiveness.

We found two cases of complete non-responders, with detectable HCV RNA at the end of treatment. In the first, HCV RNA and HCVcAg were detectable at the end of treatment. This patient did not achieve SVR and had detectable, but 'grey zone', HCVcAg. The second non-responder had HCV RNA of 34 IU/ml at the end of the treatment, while HCVcAg was undetectable. At the follow-up visit, viral load increased to 9.98×10^6 IU/ml. Unfortunately, HCVcAg testing was not performed in this case due to technical reason. Another case had detectable but not quantifiable HCV RNA and with undetectable HCVcAg at the end of treatment and achieved both SVR and SSR. Eleven patients had traces of HCVcAg at the end of treatment (interpreted as negative results), who finally achieved SVR. In summary, from nine patients who did not achieve SVR, only one demonstrated detectable both HCV RNA and HCVcAg at the end of treatment and the other had detectable HCV RNA but not HCVcAg at the end of treatment. This supports the thesis of the inappropriateness of testing patients during and at the end of treatment with antivirals. Furthermore, patients evaluated for the treatment efficacy with HCVcAg levels detectable in a 'grey zone' should be additionally tested for HCV RNA. The most important time-point is the evaluation of the treatment efficacy with either HCV RNA or HCVcAg after the post-treatment follow-up period. In our study concordance between these two tests achieved 88.9% and was slightly lower than in study of van Tilborg et al. [33].

This work represents an advance in biomedical; science because it shows that HCVcAg measurement could be a potential alternative for treatment effectiveness monitoring with HCV RNA after anti-viral regimens and that HCVcAg may also be useful in the diagnosis of HCV infection because it can replace the two-step testing with anti-HCV and HCV RNA.

Summary table

What is known about this subject:

- Despite many disadvantages, HCV RNA measurement, with real-time PCR assay, is still in use to evaluate the efficacy of anti-viral treatment.
- HCVcAg concentration correlate positively with HCV RNA and the method has many advantages such as lower workload, faster results and lower costs.
- Determination of HCVcAg or HCV RNA disappearance after successful treatment corresponds to the definitive cure of HCV infection.

What this paper adds:

- In patients treated with directly acting antivirals regimens HCVcAg measurement could be alternative for treatment effectiveness monitoring with HCV RNA.
- HCVcAg may also be useful in the diagnosis of HCV infection.

Disclosure statement

Mariusz Łucejko, Magdalena Tudrujek-Zdunek, Wojciech Jelski, Helena Donica, Magdalena Krintus: None
Krzysztof Tomasiewicz: advisory/speaker: AbbVie, Alfa Wasserman, BMS, Gilead, Janssen, MSD, Roche
Anita Olczak: advisory/speaker: AbbVie, Gilead, Roche
Waldemar Halota: advisory/speaker/grants recipient: AbbVie, Abbott, Gilead, MSD, Roche
Barbara Mroczko: advisory/speaker/grants recipient: Abbott, Roche, Cormay, Biameditek
Robert Flisiak: advisory/speaker/grants recipient: AbbVie, Abbott, Gilead, MSD, Roche

Funding

This work was supported by the Abbott Diagnostics; Medical University of Bialystok, Poland [N/ST/ZB/18/001/1156].

ORCID

K Tomasiewicz http://orcid.org/0000-0001-7868-2708
M Tudrujek-Zdunek http://orcid.org/0000-0002-5640-5432
W Halota http://orcid.org/0000-0003-2952-2374
W Jelski http://orcid.org/0000-0002-1648-907X
H Donica http://orcid.org/0000-0003-0677-7825
M Krintus http://orcid.org/0000-0003-2627-7473
B Mroczko http://orcid.org/0000-0002-4075-8479
R Flisiak http://orcid.org/0000-0003-3394-1635

References

[1] GBD. Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385:117–171.
[2] Perz JF, Armstrong GL, Farrington LA, et al. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45:529–538.
[3] Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017;2:161–176.

[4] WHO. Global hepatitis report. 2017 [cited Jul 2016]. https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/.
[5] Falade-Nwulia O, Suarez-Cuervo C, Nelson DR, et al. Oral direct-acting agent therapy for hepatitis C virus infection: a systematic review. Ann Intern Med. 2017;166:637–648.
[6] European Union HCV Collaborators. Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: a modelling study. Lancet Gastroenterol Hepatol. 2017;2:325–336.
[7] European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2018. J Hepatol. 2018;69:461–511.
[8] Chevaliez S, Feld J, Cheng K, et al. Clinical utility of HCV core antigen detection and quantification in the diagnosis and management of patients with chronic hepatitis C receiving an all-oral, interferon-free regimen. Antivir Ther. 2018;23:211–217.
[9] Kamal SM, Kassim S, El Gohary E, et al. The accuracy and cost-effectiveness of hepatitis C core antigen assay in the monitoring of anti-viral therapy in patients with chronic hepatitis C genotype 4. Aliment Pharmacol Ther. 2015;42:307–318.
[10] Mederacke I, Wedemeyer H, Ciesek S, et al. Performance and clinical utility of a novel fully automated quantitative HCV core antigen assay. J Clin Virol. 2009;46:210–215.
[11] Garbuglia AR, Lionetti R, Lapa D, et al. The clinical significance of HCV core antigen detection during telaprevir/peg-interferon/ribavirin therapy in patients with HCV 1 genotype infection. J Clin Virol. 2015;69:68–73.
[12] Aghemo A, Degasperi E, De Nicola S, et al. Quantification of core antigen monitors efficacy of direct-acting antiviral agents in patients with chronic hepatitis C virus infection. Clin Gastroenterol Hepatol. 2016;14:1331–1336.
[13] Łucejko M, Flisiak R. Quantitative measurement of HCV core antigen for management of interferon-free therapy in HCV infected patients. Antivir Ther. 2018;23:149–156.
[14] Rockstroh JK, Feld JJ, Chevaliez S, et al. HCV core antigen as an alternate test to HCV RNA for assessment of virologic responses to all-oral, interferonfree treatment in HCV genotype 1 infected patients. J Virol Methods. 2017;245:14–18.
[15] Lamoury FMJ, Soker A, Martinez D, et al. Hepatitis C virus core antigen: a simplified treatment monitoring tool, including for post-treatment relapse. J Clin Virol. 2017;92:32–38.
[16] Cresswell FV, Fisher M, Hughes DJ, et al. Hepatitis C core antigen testing: a reliable, quick, and potentially cost-effective alternative to hepatitis C polymerase chain reaction in diagnosing acute hepatitis C virus infection. Clin Infect Dis. 2015;60:263–266.
[17] Halota W, Flisiak R, Juszczyk J, et al. Polish Group of experts for HCV. Recommendations for the treatment of hepatitis C in 2017. Clin Exp Hepatol. 2017;3:47–55.
[18] Aoyagi K, Ohue C, Lida K, et al. Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol. 1999;37:1802–1808.
[19] Cetinera S, Duranb AC, Kibarb F, et al. Performance comparison of new generation HCV core antigen test versus HCV RNA test in management of hepatitis C virus infection. Transfus Apher Sci. 2017;56:362-6.
[20] Thong VD, Akkarathamrongsin S, Avihingsanon A, et al. The correlation between hepatitis C core antigen and hepatitis C virus RNA levels with respect to human immunodeficiency virus status, hepatitis C virus genotype and interferon-lambda-4 polymorphism. Intervirology. 2015;58:73–79.
[21] Hosseini-Moghaddam SM, Iran-Pour E, Rotstein C, et al. Hepatitis C core Ag and its clinical applicability: potential advantages and disadvantages for diagnosis and follow-up. Rev Med Virol. 2012;22:156-65.
[22] Mederacke I, Potthoff A, Meyer-Olson D, et al. HCV core antigen testing in HIV- and HBV-coinfected patients, and in HCV-infected patients on hemodialysis. J Clin Virol. 2012;53:110–115.
[23] Łucejko M, Grzeszczuk A, Jaroszewicz J, et al. Serum HCV core antigen concentration in HCV monoinfection and HCV/HIV coinfection. Pol Merkur Lekarski. 2013;35:72–76.
[24] Wasitthankasem R, Vichaiwattana P, Auphimai C, et al. HCV core antigen is an alternative marker to HCV RNA for evaluating active HCV infection: implications for improved diagnostic option in an era of affordable DAAs. Peer J. 2017;5:e4008.
[25] Ottiger C, Gygli N, Huber AR. Detection limit of architect hepatitis C core antigen assay in correlation with HCV RNA, and renewed confirmation algorithm for reactive anti-HCV samples. J Clin Virol. 2013;58:535–540.
[26] Ross RS, Viazov S, Salloum S, et al. Analytical performance characteristics and clinical utility of a novel assay for total hepatitis C virus core antigen quantification. J Clin Microbiol. 2010;48:1161-8.
[27] Tanaka Y, Takagi K, Fujihara T, et al. 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.
[28] Tillmann HL. Hepatitis C virus core antigen testing: role in diagnosis, disease monitoring and treatment. World J Gastroenterol. 2014;20:6701-6.
[29] Ticehurst JR, Hamzeh FM, Thomas DL. Factors affecting serum concentrations of hepatitis C virus (HCV) RNA in HCV genotype 1-infected patients with chronic hepatitis. J Clin Microbiol. 2007;45:2426–2433.
[30] Flisiak R, Łucejko M, Mazur W, et al. Effectiveness and safety of ledipasvir/sofosbuvir±ribavirin in the treatment of HCV infection: the real-world HARVEST study. Adv Med Sci. 2017;62:387–392.
[31] Flisiak R, Janczewska E, Wawrzynowicz-Syczewska M, et al. Real-world effectiveness and safety of ombitasvir/paritaprevir/ritonavir ± dasabuvir ± ribavirin in hepatitis C: AMBER study. Aliment Pharmacol Ther. 2016;44:946–956.
[32] Maasoumy B, Vermehren J, Welker MW, et al. Clinical value of on-treatment HCV RNA levels during different sofosbuvir-based antiviral regimens. J Hepatol. 2016;65:473–482.
[33] van Tilborg M, Al Marzooqi SH, Wong WWL, et al. HCV core antigen as an alternative to HCV RNA testing in the era of direct-acting antivirals: retrospective screening and diagnostic cohort studies. Lancet Gastroenterol Hepatol. 2018;3:856–864.