Journal of Clinical Virology 114 (2019) 1–5

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Diagnostic and analytical performance of the hepatitis B core related antigen immunoassay in hepatitis B patients

Gijs J. van Halewijn[a,b], Corine H. Geurtsvankessel[b], Janienne Klaasse[b], Gertine W. van Oord[a], Robert J. de Knegt[a], Margo J. van Campenhout[a], André Boonstra[a], Annemiek A. van der Eijk[b,*]

[a] Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, the Netherlands
[b] Department of Viroscience, Erasmus MC, University Medical Center Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Hepatitis B core related antigen
Diagnostics
Serological marker
Sensitivity and specificity
Hepatitis B virus

ABSTRACT

Background: Novel serological markers for Hepatitis B virus (HBV) infection are needed for prognosis and guidance of therapy.
Objective: We evaluated the diagnostic performance of the Fujirebio Lumipulse G HBcrAg immunoassay on the Fujirebio LUMIPULSE G1200 analyzer.
Study design: Analytical performance was examined using three HBeAg positive HBV samples. Diagnostic specificity was assessed using subpanels of 54 confirmed acute HAV, HCV, HEV, B19, CMV and EBV infections. Diagnostic sensitivity was investigated in well-defined HBV positive patient groups, both treated and untreated, including immunocompromised patients.
Results: The Lumipulse G HBcrAg immunoassay provided a linear measurement at a dilution between 1:100 and1:10,000. Six out of 54 samples showed non-specific reactivity in sera from acute CMV, EBV and HEV infections, of which 2 of them > 3 log U/ml. The highest levels of HBcrAg were measured in HBeAg positive patients, in both treated and untreated as well as in immunocompromised patients. Untreated patients had relatively low serum HBcrAg levels in the inactive carrier phase, which increased upon progression into the HBeAg-negative hepatitis phase. Also, we showed that the applicability of HBcrAg to distinguish between patients with resolved HBV infection and false-positive reactivity to solitary anti-HBc is limited.
Conclusions: Our study demonstrated significant differences in HBcrAg levels depending on HBeAg status, the clinical phase, as well as the treatment status. Specificity of the assay is good; only 2 out of 54 samples showed reactivity above 3 log U/ml. Before implementing the assay in clinical practice, additional research in larger patient cohorts should be carried out.

1. Background and objectives

Chronic hepatitis B virus (HBV) infection is a global health problem affecting more than 350 million people. Prolonged liver inflammation caused by active infection with HBV may result in liver fibrosis, cirrhosis, and hepatocellular carcinoma. Currently, the majority of chronic HBV patients are treated with nucleo(s)tide analogues (NUC). Treatment with NUC is highly effective in suppressing HBV replication, but does not completely eliminate the virus, necessitating life-long therapy for patients [1].

The disease of an individual chronic HBV patient is monitored by various clinical laboratory parameters in serum, including alanine aminotransferase [ALT], hepatitis B e antigen (HBeAg), hepatitis B surface antigen (HBsAg) and HBV-DNA levels, as well as serum antibodies directed against the HBeAg, the HBsAg and the hepatitis B core antigen (HBcAg). These parameters are used to determine the disease phase and consequently the indication for antiviral treatment. Recently, an additional serum marker, hepatitis B core-related antigen (HBcrAg), has been reported to be useful for monitoring HBV treatment response [2–4]. HBcrAg was shown to correlate with serum HBV DNA and to a lesser extent to HBsAg [5]. HBcrAg is a combined measure of three proteins encoded by the precore/core region of the cccDNA: HBcAg, HBeAg and a 22 kDa precore protein (p22cr) [6]. Interestingly, HBcrAg levels have been reported to relate to intrahepatic covalently closed circular DNA (cccDNA) levels, allowing monitoring of residual viral DNA in the liver. In addition, HBcrAg has been suggested to be a predictor of off-treatment responses following cessation of NUC treatment [5,7–11].

* Corresponding author at: Department of Viroscience, Erasmus University Medical Center Rotterdam, PO Box 2040, 3000, CA, Rotterdam, the Netherlands.
E-mail address: a.vandereijk@erasmusmc.nl (A.A. van der Eijk).

https://doi.org/10.1016/j.jcv.2019.03.003
Received 2 November 2018; Received in revised form 24 February 2019; Accepted 3 March 2019

Numerous studies are being conducted to determine the diagnostic value of HBcrAg in clinical practice. However, data regarding analytical performance as well as diagnostic sensitivity and specificity of this test are limited in a European setting [6].

In the current study, we used well-defined sets of clinical samples to evaluate the analytical and diagnostic performance of the Lumipulse G HBcrAg chemiluminescent immunoassay on the LUMIPULSE G1200 for the detection of HBcrAg. Furthermore, we assessed the applicability of the assay by determining serum HBcrAg levels in both acute HBV and chronic HBV patients at various stages of their disease and in immunocompromised patients.

2. Study design

2.1. Measurement of HBcrAg levels

Serum or plasma levels of HBcrAg (expressed as kU/mL or log U/mL) were measured using the Lumipulse G HBcrAg assay (Research Use Only, distributed by Fujirebio Europe, Ghent, Belgium) on a LUMIPULSE G1200 analyser (Fujirebio Inc., Tokyo, Japan). The LUMIPULSE G1200 is a mid-sized fully automated immunoassay instrument, using the chemiluminescent enzyme immunoassay (CLEIA) technique. HBcrAg levels are determined following the manufacturer's instructions. The upper limit of quantitation (ULOQ) of the Lumipulse HBcrAg assay is 7 log U/ml. The lower limit of detection (LLD) is 2 log U/ml, but the assay has been previously validated from 3 log U/ml onwards [5,6]. Samples with HBcrAg levels above 7 log U/mL were diluted with manufacturer's sample diluent and retested.

2.2. Patient samples

Serum and plasma samples were collected retrospectively from patients hospitalized or visiting the outpatient clinic of Erasmus MC, Rotterdam, the Netherlands. Serum samples were archived in a biobank at the Erasmus MC at −20 °C and EDTA-plasma samples were stored at −80 °C. Serological testing of the HBV panels (HBsAg, anti-HBc, HBeAg, anti-HBe and anti-HBs) were performed on the Abbott Architect system from December 2009 until June 2013 and from June 2013 onwards on the DiaSorin, Liaison XL [12]. To determine the analytical performance of the HBcrAg immunoassay, we performed a dilutional linearity experiment. Three random HBsAg positive, HBeAg positive/anti-HBe negative samples were selected.

To assess the diagnostic specificity of the HBcrAg immunoassay, a panel of serum/EDTA plasma samples was selected from patients with acute Epstein–Barr virus (EBV) infections (n = 10), acute cytomegalovirus (CMV) infections (n = 10), acute B19 virus infections (n = 9), acute hepatitis A virus (HAV) infections (n = 5), chronic hepatitis C virus (HCV) infections (n = 10), acute hepatitis E virus (HEV) infection (n = 10), and healthy individuals vaccinated for HBV (n = 10). All samples were negative for anti-HBc antibodies.

To determine the diagnostic sensitivity of the HBcrAg immunoassay, samples from different patient populations were tested. These included samples from acute HBV patients (n = 8), individuals with HBsAg-loss (n = 30) as well as samples from untreated chronic HBV patients at different HBV clinical phases as presented in Table 1 (n = 97 divided over 4 groups). Patients were older in more advanced clinical phases. Immunotolerant patients were mainly female because pregnant women are screened in the Netherlands for HBV. Chronic HBV patients at different clinical phases in the natural history were selected as previously described [13]. In addition, samples from NUC treated patients were tested: NUC treated HBeAg positive patients (n = 20) and NUC treated HBeAg negative patients (n = 19), as well as immunocompromised patients who had undergone transplantations of hematological stem cells or solid organ (heart or kidney) transplantations (2 HBeAg positive and 5 HBeAg negative patients).

To determine if HBcrAg can be detected in serum of patients with resolved HBV infection, anti-HBc-positive, HBsAg negative samples from patients with a confirmed resolved HBV infection were selected. The samples were divided into 3 groups: 1) 10 patients who were anti-HBs positive and anti-HBe positive; 2) 10 patients who were anti-HBs negative and anti-HBe positive; 3) 10 patients who were anti-HBs negative and anti-HBe negative.

2.3. Statistical analysis

IBM SPSS version 24 was used for statistical analyses. HBcrAg was $\log_{10}$ transformed. To compare medians for univariate analyses, we applied the Mann-Whitney U test. P-values were two-sided and considered statistically significant if < 0.05.

3. Results

3.1. Dilutional linearity

To determine the detection range of the Lumipulse G HBcrAg assay, serum samples of 3 HBsAg-positive, HBeAg-positive patients were serially diluted and compared to the calibration curve. As shown in Fig. 1, the linear range is observed when the sample is diluted between 100-fold and 10,000-fold. At higher dilutions, an inflexion of the curve is observed and a leveling effect is seen. The recommendation of the manufacturer for samples over-range is to dilute the sample 100-fold, or in rare cases 400-fold, in sample diluent (SD1) and re-test the sample. This is in concordance with our findings.

3.2. Diagnostic specificity

To determine the diagnostic specificity of the Lumipulse G HBcrAg immunoassay, samples were measured from patients with distinct acute and chronic viral infections, as well as vaccinated healthy controls. As presented in Fig. 2, HBcrAg was undetectable (2 log U/ml) in all samples from healthy individuals vaccinated for HBV. Similarly, all samples from patients with an acute B19 or acute HAV infection or HCV infection (HCV RNA positive) had undetectable HBcrAg levels. In 2 independent experiments, we observed that sera from patients with an acute infection of EBV (n = 1), CMV (n = 3) or HEV (n = 2), with no known medical history of HBV infection, showed HBcrAg levels in the range between 2 log and 3.5 log U/ml. Only 2 samples showed HBcrAg levels > 3 log U/ml. These 6 patients were confirmed to be anti-HBc negative.

3.3. Distribution of serum HBcrAg levels in acute and chronic HBV infection

Next, we determined the concentration range of HBcrAg in various groups of samples obtained from carefully selected HBV patients. Samples from acute and chronic HBV patients were selected. As shown in Fig. 3, all except one patient with acute HBV showed HBcrAg levels higher than 5 log U/ml. The acute HBV patient with a serum HBcrAg level of 2 log U/ml was likely infected with HBV 2 months prior to sample collection. At the time of sampling, the patient was HBsAg positive, HBeAg negative and anti-HBe negative, with an HBV DNA level of 22,500 IU/ml.

Based on the serum levels of HBV DNA, ALT and HBeAg, chronic HBV patients are classified as being in the immunotolerant, immunoactive, inactive carrier and HBeAg negative hepatitis phase [14]. In samples from HBeAg positive patients (immunotolerant and immunoactive phase) relatively high HBcrAg levels were detected with a median value of 7.9 and 7.6 log U/ml, respectively ($p = 0.03$, Table 2). After seroconverting to anti-HBe and progressing to the inactive carrier phase, HBcrAg levels had a much lower median value of 2.3 log U/ml. Surprisingly, serum HBcrAg levels were higher again during the HBeAg negative hepatitis phase with a median value of 4.6 log U/ml ($p < 0.001$).

Table 1
Patient characteristics of chronic HBV patients at different clinical phases.

		Immunotolerant phase	Immunoactive phase	Inactive carrier phase	HBeAg-neg hepatitis phase
N		16	28	28	25
Age (years)		31 (18-39)	31 (19-48)	38 (20-72)	41 (23-66)
Male/Female		2/14	14/14	13/15	18/7
Ethnicity	Caucasian	0	1	2	1
	Asian	0	2	2	3
	African	16	25	17	16
	Other	0	0	7	5
HBV genotype	A-B-C-D-E-unknown	0-7-8-1-0-0	2-5-18-3-0-0	4-6-3-4-3-8	5-2-5-9-1-3
HBV-DNA* Log IU/ml		8.8 (7.9-9.2)	7.5 (5.4-9.1)	2.7 (1.3-4.2)	6.1 (3.9-8.2)
ALT (IU/ml)*		21 (14-36)	65 (34-248)	23 (13-43)	76 (29-243)
qHBsAg* Log U/ml		4.7 (4.0-4.8)	4.2 (3.3-4.9)	3.3 (1.2-4.7)	4.1 (3.0-4.1)
qHBcrAg* Log U/ml		7.9 (7.3-8.2)	7.6 (5.4-8.2)	2.3 (2.0-4.3)	4.6 (2.0-7.4)

*Median (range), ALT; alanine transaminase.

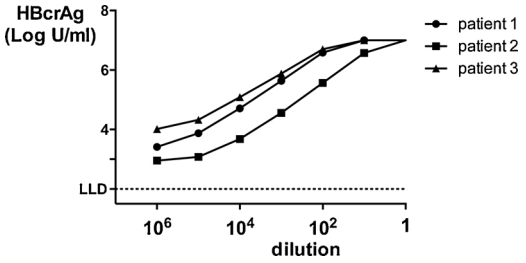

Fig. 1. Linearity of dilution of HBcrAg levels in sera of 3 chronic HBV patients. All patients were HBeAg positive and anti-HBe negative. Both axes are logarithmic. All undiluted samples have a reading of 7 log U/ml, which is the upper limit of detection of the assay.

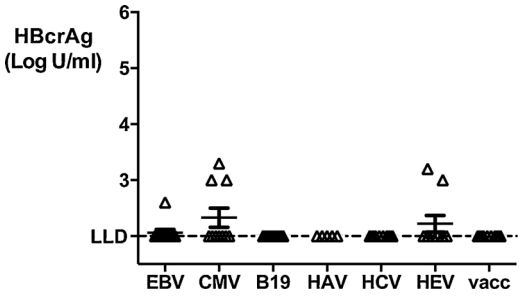

Fig. 2. Specificity of Lumipulse G HBcrAg immunoassay. Dotplot showing the mean, and standard error of the mean of the log HBcrAg concentration in serum of patients with diagnosed acute infections of EBV (n = 10), CMV (n = 10), B19 (n = 9), HAV (n = 5), HEV (n = 10), chronic HCV (n = 10) and healthy controls vaccinated for HBV (n = 10). The lower limit of detection (LLD) of 2 log U/ml is shown as a dashed line.

In the NUC treated group, HBeAg positive patients had significantly higher HBcrAg levels with a median value of 5.3 than HBeAg negative patients with lower levels of 3.7 log U/ml (p = 0.001). Untreated patients who require treatment according to treatment guidelines were compared with treated patients who had the same HBeAg status (Table 2). HBcrAg levels in samples from immunoactive patients were significantly higher than in NUC treated HBeAg positive patients (7.6 versus 5.3 log U/ml; p < 0.001). Similar findings were found for samples from untreated versus treated HBeAg negative hepatitis patients (4.6 versus 3.7 log U/ml; p = 0.004).

Finally, sera from 2 groups of immunocompromised patients, both on NUC therapy, were tested. Relatively high HBcrAg levels were detected in the 2 HBeAg positive NUC immunocompromised patients (8.1 and 8.3 log U/ml), whereas lower, but highly, variable HBcrAg levels were found in the 5 HBeAg negative patients (2.0, 2.6, 2.8, 4.1 and 6.3 log U/ml).

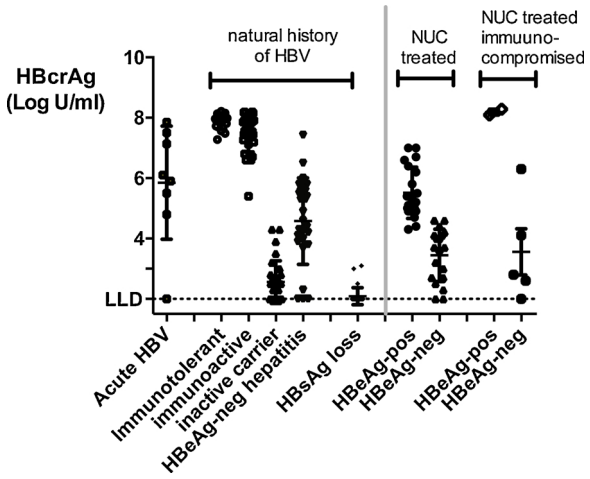

Fig. 3. Fluctuating HBcrAg levels in serum of patients with acute, chronic and resolved HBV infection. HBcrAg was determined in serum of patients with acute HBV (n = 8); patients in the immunotolerant (IT, n = 16), immunoactive (IA, n = 28), inactive carrier (IC, n = 28), and HBeAg-negative hepatitis phase (ENEG, n = 25), with HBsAg-loss (n = 30), and NUC treated patients who were HBeAg-positive (n = 20) or HBeAg-negative (n = 19), and patients who were immunosuppressed and HBeAg positive (n = 2) or HBeAg negative (n = 5). * p < 0.05. The LLD is 2 log U/ml.

3.4. Serum HBcrAg levels in anti-HBc positive, HBsAg-negative individuals

To further explore the applicability of the HBcrAg immunoassay, we determined the serum HBcrAg levels in a group of patients with resolved HBV infections, defined as anti-HBc positive, and HBsAg negative. We compared the HBcrAg levels in patients with 1) detectable serum antibodies of anti-HBe and anti-HBs, 2) anti-HBe antibodies, but not anti-HBs, and 3) anti-HBc antibodies, but no detectable anti-HBe and anti-HBs. As shown in Fig. 4, irrespective of the experimental group, most individuals had undetectable HBcrAg levels in serum (using a cutoff of 2 log U/ml). Only 3 patients had detectable, but very low, HBcrAg levels (2.5, 3.0 and 3.1 log U/ml). Therefore, the applicability of HBcrAg to distinguish between patients with resolved HBV infection and false-positive reactivity to solitary anti-HBc is limited.

4. Discussion

Treatment of HBV is costly, and long-term therapy is a major financial burden on health care systems. To allow clinicians to make individual treatment decisions for their patients there is a need for the development of new diagnostic markers providing insight in the clinical response.

In this study we evaluated the analytical and diagnostic

Table 2
Comparison of serum HBcrAg levels between experimental groups. Mann-Whitney *U* test comparison of median values.

Comparative groups	HBcrAg (log U/ml)	p-value
Untreated immunotolerant (n = 16) vs untreated immunoactive patients (n = 28)	7.9 vs 7.6	p = 0.03
Untreated inactive carrier (n = 28) vs untreated HBeAg-negative hepatitis phase (n = 25)	2.6 vs 4.6	p < 0.001
NUC treated HBeAg positive (n = 20) vs NUC treated HBeAg negative patients (n = 19)	5.3 vs 3.7	p < 0.001
NUC treated HBeAg positive (n = 20) vs untreated HBeAg positive immunoactive patients (n = 28)	5.3 vs 7.6	p < 0.001
NUC treated HBeAg negative patients (n = 19) vs untreated HBeAg negative hepatitis patients (n = 25)	3.7 vs 4.6	p = 0.004

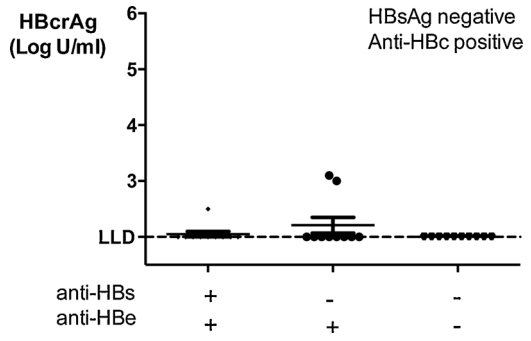

Fig. 4. Low to undetectable serum HBcrAg levels are observed in anti-HBc positive and HBsAg negative individuals. The serum levels are compared between 3 groups of 10 patients each differing in the levels of anti-HBsAg and anti-HBeAg. The LLD is 2 log U/ml.

performance of the Lumipulse G HBcrAg immunoassay. We confirmed the dynamic range of the assay as described by Kimura et al [6]. In addition, cross-reactivity in the HBcrAg immunoassay was observed in a small number of patients with acute viral infections with CMV, EBV and HEV, but without evidence for previous exposure to HBV. These non-specific readings in the lower range of the HBcrAg assay increased the reliable cut-off to 3 log. Our findings are adding to the original findings by Kimura who observed positivity in the HBcrAg immunoassay in samples from anti-HCV positive patients, and using our extensive panel of patients with acute infections we confirmed that the HBcrAg levels in the lower range should be interpreted with caution. However, it should be noted that these acute infections are rare, and that for diagnostic purposes HBcrAg levels will generally be determined in combination with routine HBV serology. The non-specific readings are likely due to cross-reactivity of the reagents with epitopes shared between the various virus components.

We performed a comprehensive analysis of serum HBcrAg levels in well-defined groups of chronic HBV patients, and demonstrated significant differences across HBeAg status, clinical phase, and on-treatment status. Regarding the clinical HBV phases, we demonstrated that the median HBcrAg values were comparable with earlier published studies: in the immunotolerant phase we found HBcrAg levels of 7.9 log U/ml, compared to 8.4 and 8.5 log U/ml in studies published by Maasoumy and Seto [15,16]. This also accounts for the measurements of samples of patients in the immunoactive phase 7.6 log U/ml (our cohort), and 8.1 and 7.9 log U/ml [15,16] as well as the serum HBcrAg levels in our HBeAg-negative patients. The median serum HBcrAg levels of inactive carriers were 2.3 log U/ml in our study, while they were 2.0 and 2.6 log U/ml in the studies by Maasoumy and Seto, respectively. Lastly, in samples of the HBeAg negative hepatitis phase, we detected a level of 4.7 log U/ml HBcrAg, while the earlier studies detected 4.8 and 4.9 log U/ml HBcrAg [15,16]. Thus, our study and earlier studies are consistent in the levels detected in chronic HBV patients at distinct clinical phases, reflecting the reproducibility of the HBcrAg immunoassays across different laboratories using different panels of patient samples. In this respect it is also relevant to mention that in our mixed study population consisting of all HBV genotypes, the levels of serum HBcrAg did not significantly differ among HBV genotype A, B, C and D (data not shown).

Anti-HBc positive individuals who receive chemotherapy or biological therapy, and anti-HBc positive liver allograft recipients are at risk of HBV reactivation. There is a need for a marker that discriminates between patients who are at risk of reactivation and patients who are not. For this reason discrimination between resolved HBV infection and false-positive reactivity is essential [17]. HBcrAg was earlier reported to be a good surrogate marker of intrahepatic cccDNA [5]. However, in our study, in samples of patients who had a documented resolved infection of HBV, no HBcrAg was detected while the livers of these patients probably still harbor cccDNA, as has been reported before. Since we did not test for cccDNA, further research should elucidate the possible correlation between HBcrAg and cccDNA levels in HBsAg negative patients. The three samples with detectable HBcrAg levels were obtained from patients who recently resolved their HBV infection (months prior to sample collection). Interestingly, the HBcrAg levels in the HBeAg-negative hepatitis phase are higher compared to the HBeAg-negative inactive carrier. Again, this is in contrast to earlier findings that intrahepatic cccDNA quantity correlates with serum HBcrAg levels. It has been reported that in the natural history of chronic HBV higher intrahepatic cccDNA levels are observed in the inactive carrier compared to the HBeAg-negative hepatitis phase [18].

In conclusion, our study demonstrated significant differences in HBcrAg levels depending on the HBeAg status, the clinical phase, as well as on the treatment status. Specificity of the assay is good; only 2 out of 54 samples showed reactivity above 3 log U/ml. Before implementing the assay in clinical practice, additional research in larger patient cohorts should be carried out.

Funding

TKI Health Holland (project number LSHM 15032) and Fujirebio Europe provided financial support

Conflicts of interest

The authors have no conflicts of interest to disclose.

Ethical approval

This study was approved by the local medical ethics committee of the Erasmus MC (MEC-2015-306).

Contributions of authors

Gijs J. van Halewijn, Janienne Klaasse, and Gertine W. van Oord performed the experiments and collected data.

Corine H. Geurtsvankessel, Robert J. de Knegt, and Margo J. van Campenhout interpreted the data, contributed to the writing of the manuscript

André Boonstra, and Annemiek A. van der Eijk supervised the project, interpreted the data and wrote the manuscript

Acknowledgements

We thank Chantal Burghoorn-Maas and Sandra Scherbeijn for their

help at various stages of the project, and Fujirebio for providing HBcrAg test kits and providing the LUMIPULSE G1200 on loan for research purposes.

References

[1] L.S.Y. Tang, E. Covert, E. Wilson, S. Kottilil, Chronic hepatitis B infection: a review, JAMA 319 (2018) 1802–1813.
[2] H.L. Janssen, M.J. Sonneveld, M.R. Brunetto, Quantification of serum hepatitis B surface antigen: is it useful for the management of chronic hepatitis B? Gut 61 (2012) 641–645.
[3] M.J. van Campenhout, W.P. Brouwer, G.W. van Oord, Q. Xie, Q. Zhang, N. Zhang, et al., Hepatitis B core-related antigen levels are associated with response to entecavir and peginterferon add-on therapy in hepatitis B e antigen-positive chronic hepatitis B patients, Clin. Microbiol. Infect. 22 (571) (2016) e5–9.
[4] F. Zoulim, B. Testoni, F. Lebosse, Kinetics of intrahepatic covalently closed circular DNA and serum hepatitis B surface antigen during antiviral therapy for chronic hepatitis B: lessons from experimental and clinical studies, Clin. Gastroenterol. Hepatol. 11 (2013) 1011–1013.
[5] L.Y. Mak, D.K. Wong, K.S. Cheung, W.K. Seto, C.L. Lai, M.F. Yuen, Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection, Aliment. Pharmacol. Ther. 47 (2018) 43–54.
[6] T. Kimura, A. Rokuhara, Y. Sakamoto, S. Yagi, E. Tanaka, K. Kiyosawa, et al., Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load, J. Clin. Microbiol. 40 (2002) 439–445.
[7] G.P. Caviglia, A. Smedile, Hepatitis B core-related antigen: a novel biomarker for chronic hepatitis B treatment, Minerva Gastroenterol. Dietol. 63 (2017) 169–171.
[8] D.K. Wong, Y. Tanaka, C.L. Lai, M. Mizokami, J. Fung, M.F. Yuen, Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection, J. Clin. Microbiol. 45 (2007) 3942–3947.
[9] D.K. Wong, W.K. Seto, K.S. Cheung, C.K. Chong, F.Y. Huang, J. Fung, et al., Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA, Liver Int. 37 (2017) 995–1001.
[10] F. Suzuki, H. Miyakoshi, M. Kobayashi, H. Kumada, Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients, J. Med. Virol. 81 (2009) 27–33.
[11] E.Q. Chen, S. Feng, M.L. Wang, L.B. Liang, L.Y. Zhou, L.Y. Du, et al., Serum hepatitis B core-related antigen is a satisfactory surrogate marker of intrahepatic covalently closed circular DNA in chronic hepatitis B, Sci. Rep. 7 (2017) 173.
[12] K.T.D. Thai, H. Gotz, B. Slingerland, J. Klaasse, M. Schutten, C.H. GeurtsvanKessel, An analysis of the predictive value of the HIV Ag/Ab screening assay within the performance characteristics of the DiaSorin LIAISON XL for the detection of blood-borne viruses, J. Clin. Virol. 102 (2018) 95–100.
[13] R.A. de Groen, J. Hou, G.W. van Oord, Z.M.A. Groothuismink, M. van der Heide, R.J. de Knegt, et al., NK cell phenotypic and functional shifts coincide with specific clinical phases in the natural history of chronic HBV infection, Antiviral Res. 140 (2017) 18–24.
[14] Liver EAftSot, EASL, Clinical Practice Guidelines on the management of hepatitis B virus infection, J. Hepatol. (67) (2017) 370–398 2017.
[15] B. Maasoumy, S.B. Wiegand, J. Jaroszewicz, B. Bremer, P. Lehmann, K. Deterding, et al., Hepatitis B core-related antigen (HBcrAg) levels in the natural history of hepatitis B virus infection in a large European cohort predominantly infected with genotypes A and D, Clin. Microbiol. Infect. 21 (606) (2015) e1–10.
[16] W.K. Seto, D.K. Wong, J. Fung, F.Y. Huang, K.S. Liu, C.L. Lai, et al., Linearized hepatitis B surface antigen and hepatitis B core-related antigen in the natural history of chronic hepatitis B, Clin. Microbiol. Infect. 20 (2014) 1173–1180.
[17] Q. Wang, P. Klenerman, N. Semmo, Significance of anti-HBc alone serological status in clinical practice, Lancet Gastroenterol. Hepatol. 2 (2017) 123–134.
[18] B. Werle-Lapostolle, S. Bowden, S. Locarnini, K. Wursthorn, J. Petersen, G. Lau, et al., Persistence of cccDNA duringt he natural history of chornic hepatitis B and decline during adefovir dipivoxil therapy, Gastroenterology 126 (2004) 1750–1758.