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Moving towards core antigen for the management of patients with overt and occult HBV infection

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Abstract

Chronic hepatitis B virus (HBV) infection encompasses a wide virologic and clinical spectrum with heterogeneous outcomes. The natural history of chronic HBV infection ranges from an inactive carrier state (hepatitis B e antigen-negative chronic infection) to progressive chronic hepatitis that may evolve in end-stage liver disease and hepatocellular carcinoma. The issue becomes even more complicated when we consider the unique biology of the virus; the HBV covalently-closed-circular DNA, that acts as virus transcription template, is the key factor responsible of the persistence of the infection even after hepatitis B surface antigen loss. In the last decade, novel serological and immunological biomarkers associated to the core protein of HBV have been approached in different clinical conditions. Remarkable results have been obtained both in the setting of overt and occult HBV infection. Here, we reviewed the meaning and the potential clinical applications of the measurement of core antigen and antibodies.

Key words: Antiviral therapy; Anti-HBc; Biomarkers; HBcrAg; HBV cccDNA.

Hepatitis B virus (HBV) infection is a major health problem worldwide, with an estimated prevalence of 257 million (3.7%) people bearing chronic viral persistence.¹ HBV infection shows a wide spectrum of clinical forms, ranging from mild, poorly symptomatic forms to fulminant hepatitis, end-stage liver disease and hepatocellular carcinoma (HCC), even in pre-cirrhotic liver disease.

HBV is a small, enveloped primarily hepatotropic DNA virus belonging to *Hepadnaviridae* family. After uptake by the hepatocyte, the virus is transported into the nucleus, where the relaxed partially double-strand DNA of HBV forms an episomal supercoiled structure, called covalentlyclosed-circular DNA (cccDNA) (Figure 1). ² The HBV minichromosome acts as template for all viral transcripts: the subgenomic RNAs, the pre-core RNA and the pre-genomic (pg) RNA. ³ Following nuclear export, the subgenomic RNAs are translated into the surface proteins of the virus (hepatitis B surface antigen, HBsAg) and into the regulatory protein HBx. The pre-core RNA gives rise to the pre-core protein precursor of the secretory hepatitis B e antigen (HBeAg), while the pgRNA encodes for the structural protein hepatitis B core antigen (HBcAg) and for HBV polymerase and acts as templated for the transcription of the relaxed HBV DNA. ⁴ According to current guidelines, the expression of definite antigens, the presence of antigen-specific antibodies, in addition to the hepatocyte necroinflammatory activity, ⁵ delineate the course and phases of chronic HBV infection, with consequently different clinical and therapeutic implications. ^{6,7}

HBsAg and HBeAg, together with their specific antibodies and HBV DNA, have traditionally been involved in the assessment of the infection status, defining either a more active viral replication, or a milder course in inactive carriers. More recently, increasing attention has been drawn towards HBcAg and its specific antibody (anti-HBc). As hallmark of viral presence within the liver, both HBcAg and anti-HBc may play a crucial role in specific settings of HBV infection and related liver disease such as monitoring the response to antiviral treatment, investigating the persistence of viral genome after recovery, predicting viral reactivation in patients undergoing druginduced immunosuppression or hepatocellular carcinoma (HCC) development in patients with

cirrhosis under surveillance. Finally, it is particularly intriguing the possible significance of anti-HBc presence in subjects with liver disease of unknown etiology. These topics, where HBcAg and anti-HBc are reported to be primarily involved, will be discussed in this review.

Overt and occult HBV infection

Acute HBV-related hepatitis is mostly self-limiting, leading to a prompt immune response and complete recovery, witnessed by the production of neutralizing antibodies (anti-HBs). Instead, chronic infection is the result of a post-acute, unresolved infection, caused by an impairment in the adaptive T-cell immune activity in response to the immunogenic particles of the virus. ⁸ The complexity of the natural history of HBV infection is the result of the bi-directional, often heterogeneous dialogue between the viral activity and the host immune response, which is highly variable among individuals, and even in the same person at different ages and upon different concomitant clinical conditions. ^{9, 10}

Serum HBV DNA is the hallmark of the replication activity of the virus. The principal endpoint of current therapeutic strategies, which mainly rely on nucleotide analogues (NAs), is the induction of long-term HBV DNA suppression; ^{11, 12} the loss of HBsAg, with or without seroconversion to anti-HBs, is considered the best evidence of deep viral suppression and disease remission, the so-called "functional" cure.¹³ Unfortunately, HBsAg loss is a rare event; even in this occurrence, the virus persists within the liver, as the HBV cccDNA is resistant to current drug treatment and cannot be eradicated. As a matter of fact, novel therapeutic strategies targeting HBV cccDNA are under evaluation at pre-clinical level with the ambitious aim of achieving a "complete" cure. ¹⁴

One crucial point in the biology of HBV is related to the landscape of "occult" virus infection, linked to persistence of intrahepatic HBV cccDNA in subjects testing negative for HBsAg. ¹⁵ Occult HBV infection (OBI) can be accompanied by detectable serum anti-HBc, that is considered a surrogate marker of viral persistence, while HBV DNA is usually undetectable (when

detectable, below a threshold of 200 IU/ml) and transaminases are normal. On the other hand, the evaluation of HBV cccDNA presence in the liver would require a liver biopsy, but several concerns may rise due to the costs and potential risks of the procedure (pain, bleeding, puncturing other organs, death). In addition, no standard assays are available for the measurement of intrahepatic HBV cccDNA so far.

Why should core antigen and anti-core antibodies be highlighted?

In negative HBsAg patients, serum anti-HBc is considered a surrogate of OBI. Among patients showing markers of previous HBV exposure, almost 60% of these patients harbors the HBV in the liver, ¹⁶ whilst only a small proportion of seronegative patients effectively carries occult infection. ¹⁷ The production of anti-HBc depends on efficient T-cell response that elicits protective memory and better immune-mediated virus replication control. ^{18, 19} Patients with OBI and negative anti-HBc may have been exposed to a very low viral concentration not sufficient to stimulate T-cell activity, thus not allowing a proper protective memory. ²⁰ Therefore, the presence of anti-HBc acts either as serological scar in clinically resolved infection, or as indirect marker of latent infection. ²¹

The clinical relevance of OBI derives from the possible reactivation of HBV when the host immune response is compromised, like in patients undergoing immunosuppressive treatment following solid organ transplant or chemotherapy with B-cells depleting agents for hematological neoplasia. ²² Currently, in subjects with markers of previous HBV exposure undergoing immunosuppressive treatment, indication to antiviral prophylaxis is dictated by the sole presence of anti-HBc and intrinsic immunosuppressive potential of therapy (such as monoclonal antibodies anti-CD20 for lymphomas or hematopoietic stem cell transplantation). ²³⁻²⁵

Apart from the implications related to the possible reactivation of HBV in special target populations, the crucial relevance of OBI derives from its major role in the progression of liver damage, as main putative etiologic agent in cirrhosis that are otherwise unexplained (the so-called "cryptogenic" cirrhosis) or in the development of HCC even in low viral replication state. In

addition, viral persistence in silent form may act as cofactor, rather than one innocent bystander, in hepatic diseases of other etiology (namely, hepatitis C virus [HCV] infection or non-alcoholic fatty liver disease [NAFLD]). ²⁶⁻²⁹

HBV infection can cause HCC as consequence of the chronic, low grade intrahepatic inflammation that eventually lead to cirrhosis. In addition, HBV can exert a direct pro-oncogenic action through its propensity of its DNA to integrate into host's genome and thus causing mutations that can escape anti-tumor surveillance. ^{30, 31}Although cirrhosis is recognized as the strongest risk factor for HCC development, ^{32,35} HBV integration into host genome is a parallel mechanism that can explain tumor development in non-cirrhotic subjects. Even in OBI, when the episomal DNA is the only trace of viral presence, integration of HBV DNA fragments is possible, exerting its oncogenic potential related to X or pre-S/S genomic viral regions, one major cause of concern in clinical setting. ^{36, 37} Thus, evaluation of anti-HBc levels appears crucial for the subclinical progression of liver disease and for HCC development; full screening of HBV serology may be recommended. Indeed, some evidence has brought to light that individuals with detectable serum anti-HBc, undergoing resection of HBV-related HCC, have higher risk of intrahepatic recurrence and poorer recurrence-free survival, thus delineating a more aggressive phenotype of HBV-related HCC. ³⁸

More recently, serum anti-HBc has been associated to the development of cirrhosis and HCC in patients with NAFLD, without any other stigmata of HBV infection. In NAFLD, chronic inflammation, depending from both intrahepatic and systemic metabolic derangements, ³⁹ is responsible for progressiveness of liver disease. In this setting, the sole persistence of HBV cccDNA may act as co-factor or superimposed damage that may worsen the outcome, delineating a specific subtype of disease that may require special surveillance. ^{40, 41} Likewise, in chronic HCV infection, that is considered a systemic disease and likely one cause of cryptogenic cirrhosis in its occult form, ⁴² several co-factors have been implied in accelerating the course of liver damage.

Notably, HCV infected patients carrying anti-HBc have increased prevalence of liver cirrhosis at histology or more severe course of liver disease. ⁴³⁻⁴⁵

In this multidimensional background, anti-HBc is the only serum stigmata of OBI and one cornerstone of viral activity both in acute (IgM anti-HBc being the very first immune host response, thus called maker of viral "presence") and chronic (IgG anti-HBc) infection. Thus, its serum quantitation may be considered as a serum non-invasive biomarker of HBV cccDNA and may allow to better define the natural course of the overt infection, as well as to identify and monitor the occult form.

Quantitation of antibodies to hepatitis B core antigen

Serum anti-HBc has proved to have significantly different quantitative levels among the phases of the natural history of HBV chronic infection. ⁴⁶ Viral active replication in the liver and the related immune response, causing hepatocellular necroinflammatory activity and hepatitis, as marked by altered transaminases, is correlated to higher serum levels of anti-HBc. On the contrary, chronic infection without hepatitis is characterized by lower levels of serum anti-HBc. ⁴⁷ This evidence was confirmed by a study conducted by Yuan et al, ⁴⁸ where quantitative anti-HBc levels resulted lower in patients with chronic infection, as compared to those with chronic hepatitis, and were higher in the subgroup of untreated patients, with respect to those who received antiviral treatment. These results are consistent with intrahepatic inflammatory activity. Li and colleagues found that patients with no or mild histological activity had significantly lower levels of quantitative anti-HBc, with respect to those with moderate-to-severe activity. Furthermore, they found that a cut-off value of 4.36 Log IU/mL for HBeAg-positive chronic hepatitis and a cut-off value of 4.62 Log IU/mL for HBeAg-negative chronic hepatitis provided acceptable accuracy for predicting moderate-to-severe histological inflammatory activity.⁴⁹

Remarkably, different levels of anti-HBc are also found between patients with overt infection and those with OBI, being significantly higher in the first and lower in the latter. ⁵⁰

Different serum levels have also been reported between patients carrying OBI, as compared to those with past infection. ⁵¹ Accordingly, quantitation of anti-HBc may be one valuable tool to predict the risk of OBI reactivation in candidates for immunosuppressive therapy, in particular in the setting of liver transplant. ⁵² In a recent study, anti-HBc titer higher than 6.41 IU/mL, together with low levels of anti-HBs, were significantly associated to virus reactivation. ⁵³ A cut off index of 4.4 has been recently proposed to discriminate patients at higher likelihood of HBV cccDNA presence in anti-HBc-positive liver donors. ⁵⁴ Similarly, another study highlighted that in patients undergoing allogenic hematopoietic stem cell transplantation, a cutoff ratio \geq 8 independently predicted HBV reactivation. ⁵⁵

In the therapeutic landscape, quantitation of anti-HBc has brought insightful evidence as well. Total anti-HBc levels significantly declined in patients with chronic hepatitis responder to pegylated interferon (Peg-IFN) and NAs, reaching the lowest levels in long-term responders that achieved HBsAg seroclearance. ⁴⁸ Serum anti-HBc levels resulted the only parameter independently associated to serological, virological and complete response in HBeAg-positive patients undergoing antiviral therapy with Peg-IFN; anti-HBc levels >30.000 IU/mL performed better than HBV DNA suppression in predicting treatment response. ⁵⁶ This evidence has been confirmed in patients undergoing therapy with NAs (namely entecavir [ETV]). Baseline anti-HBc was the strongest predictor of seroconversion, making its quantitative evaluation an intriguing tool to optimize antiviral therapy. ⁵⁷ Conversely, in the study conducted by Tseng et al, anti-HBc titer <100 IU/mL together with HBsAg, evaluated after cessation of NAs therapy, predicted clinical relapse, defined as increased serum transaminases and HBV DNA levels >2000 IU/ml. ⁵⁸

Moreover, significantly different levels of anti-HBc have been reported between remission and reactivation phases, which is typical of the HBeAg-negative hepatitis phase of chronic HBV infection: fluctuating levels of anti-HBc correlated to transaminase levels, which are the hallmark of the transition from infection to hepatitis, making quantitation of anti-HBc a valuable support for monitoring patients' response to therapy. ⁵⁹ In patients with HBeAg-positive hepatitis, baseline anti-

HBc levels were associated to HBeAg loss, with or without seroconversion to anti-HBe, ⁶⁰ while in patients who achieved HBsAg loss following IFN-based therapy, anti-HBc levels measured at treatment withdrawal were predictors of viral recurrence. ⁶¹

Measurement of hepatitis B core-related antigen

An alternative approach for the non-invasive assessment of intrahepatic HBV cccDNA is the investigation of serum HBcAg concentration. HBcAg is normally not secreted and cannot be quantified per se. Nonetheless, a novel serum biomarker, the hepatitis B core-related antigen (HBcrAg), has recently been proposed. HBcrAg combines the antigenic reactivity resulting from HBeAg, HBcAg and a 22 kDa core-related protein (p22cr). ^{62, 63}

Several studies have assessed the correlation between serum HBcrAg and intrahepatic HBV cccDNA. As shown in Table I, this correlation has been evaluated in highly heterogeneous cohorts of patients, showing statistical significance in all cases. ^{55, 64-75} In both naïve and on-treatment patients, a positive correlation between the two parameters has been found, suggesting a potential role for this biomarker in monitoring intrahepatic viral status in course of antiviral treatment. A study conducted by Chuaypen et al showed that the baseline correlation was maintained even after 48 weeks of Peg-IFN therapy. Interestingly, HBcrAg values were correlated to HBV cccDNA in different phases of the disease, both in HBeAg-positive and HBeAg-negative patients. ⁷¹ A Japanese study reported that HBcrAg was significantly correlated to intrahepatic HBV cccDNA even in patients who lost the HBsAg, suggesting a role for this biomarker in OBI. ⁶⁵

A positive correlation between intrahepatic HBV cccDNA and HBcrAg has been found in patients undergoing liver transplantation for HB-related end-stage liver disease. Furthermore, the kinetics of HBcrAg and HBV cccDNA levels were consistent during the post-transplant follow-up period. ⁶⁷ This finding could be remarkable, in particular for the optimization of prophylactic strategy.

In addition, in a Chinese study, quantitation of HBcrAg revealed significantly different concentrations along the natural course of the disease, showing higher performance when compared to HBsAg in distinguishing between the different phases of infection, as resulted from the areas under the curves (AUCs) at specific cut-off levels. ⁷⁶ Different studies conducted on patients with HBeAg-negative chronic infection and HBeAg-negative chronic hepatitis revealed high accuracy of quantitative serum HBcrAg in distinguishing between the two phases of chronic HBV infection (Table II). ⁷⁵⁻⁸¹ Of note, two studies evaluated the accuracy of HBcrAg in discriminating between different histological grading of inflammatory activity in HBeAg-negative patients. Zhang et al found that a HBcrAg value >2.2 Log kU/mL was able to distinguish between patients with grade 1 and those with grade 2-3 (Scheuer score), with high accuracy. ⁷⁷ In addition, Testoni et al found that a HBcrAg >4 Log U/ml was able to discriminate between patients with mild and minimum inflammatory activity. ⁷⁵

HBcrAg is strongly correlated with both HBV cccDNA and HBV DNA levels. This point is of crucial relevance in patients undergoing antiviral treatment with NAs, as HBV DNA during antiviral therapy is mostly undetectable and thus unable to depict the real intrahepatic virologic status. Quantitation of HBsAg has been considered a reliable marker of viral replication decay under antiviral therapy. However, HBsAg can be produced not only from HBV cccDNA but also from HBV DNA sequences integrated into the host genome, while HBcrAg needs the full-length genome of the HBV for its transcription and translation into protein. ⁸² Therefore, HBcrAg is increasingly gathering consensus as the best surrogate for intrahepatic HBV cccDNA. Notably, in longitudinal evaluation, HBcrAg reduction trend was comparable to the magnitude of HBV cccDNA decay along a median period of 6-12 years. ⁷⁰ Another study supported this finding, confirming that in patients treated with NAs the decline of serum HBcrAg was more consistent that quantitative HBsAg in reflecting HBV cccDNA decline. ⁶⁹

Of note, detectable HBcrAg in patients undergoing antiviral therapy has further implications. Treatment with nucleotide analogues suppresses viral replication and should lead to

reduced probability of developing HCC. In one study, presence of HBcrAg during effective therapy was significantly associated with the onset of HCC, being related to a higher intrahepatic viral load. ⁸³ This suggests active HBV replication in HBcrAg positive patients undergoing antiviral therapy and defines one specific subtype of disease which requires special surveillance. ⁸⁴

Conclusions

In conclusion, HBV chronic infection is a polyhedral disease with multiple implications. Persistence of intrahepatic viral genome is the main reason for concern and a peculiar aspect of the biology of the virus. The measurement of HBcrAg and anti-HBc appears to give insightful perspectives for a better management of infection and liver disease.

Quantitative anti-HBc may be a useful tool for the stratification of the risk of HBV reactivation in patients with OBI undergoing pharmacological immunosuppression. In individuals with liver disease of other etiology, or unknown etiology, positive anti-HBc may define a subtype of liver disease that would require special surveillance, especially for the risk of subclinical progression and HCC development. Serum titers of anti-HBc as well as HBcrAg differ significantly among the different phases of HBV natural history. As a matter of fact, HBcrAg allows the correct identification of patients with HBeAg-negative chronic infection (true inactive carriers) that do not require antiviral therapy. In addition, HBcrAg can be implemented to monitor patients under antiviral treatment and may be used to identify patients that could benefit from treatment cessation. In the near future, these novel biomarkers are expected to enrich the armamentarium of clinicians allowing a tailored management of patients with overt and occult HBV infection.

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1 TABLE I. Correlation between serum HBcrAg and intrahepatic HBV cccDNA.

Study	Patients	Treatment	HBcrAg	HBV cccDNA	r	p value
Wong et al. 2007 ⁶⁴	54 (17 HBeAg+ and 37 anti-HBe+)	LAM/ETV	$\begin{array}{ccc} 1180 \ (<1.0 - 9.0 \ x \ 10^5) & 1.3 \ (<0.002 - 23.3) \\ \text{kU/mL} & \text{copies/cell} \end{array}$		0.664	< 0.001
Suzuki et al. 2009 ⁶⁵	57 (16 HBeAg+ and $41 \text{ HBeAg}-)$	LAM/ETV/ADV	4.6 ± 1.6 Log U/mL	4.25 ± 0.91 Log copies/mg	0.692	< 0.001
Hosaka et al. 2010 ⁶⁶	22 HCC-HBV	LAM	/	4.2(3.0-5.0) Log copies/ug	0.479	0.028
Matsuzaki et al. 2013 ⁶⁷	20 HBV-related end-stage liver disease*	LAM	10 with HBcrAg ≥ 3.0 Log U/mL	11 with detectable HBV cccDNA	0.616	<0.001
Chuaypen et al. 2016 ⁶⁸	46 HBeAg+	Naive	8.1 (7.7 – 8.4) Log U/mL	1.6 (1.2 – 1.9) Log copies/cEq	0.546	0.001
Chen et al. 2017 69	139 (111 HBeAg+ and 28 HBeAg-)	ETV	9.2 ± 2.9 Log U/mL	7.33 ± 1.03 Log copies/10 ⁶ cells	0.92	< 0.001
Wong et al. 2017 70	138 (77 HBeAg+ and 61 HBeAg-)	LAM/ETV/ADV	586 (1 – 1.1 x 10 ⁷) kU/mL	1.1 (0.005 - 258) copies/cell	0.70	< 0.001
Chuaypen et al. 2018 71	73 HBeAg- with paired liver biopsies	Baseline	R: 4.1 ± 1.3 NR: 4.4 ± 1.1 Log U/mL	R: 1.6 ± 1.9 NR: 0.8 ± 1.3 Log copies/cEq	0.393	0.001
Chuaypen et al. 2018 71	73 HBeAg- with paired liver biopsies	48 weeks Peg-IFN/ Peg-IFN + ETV		/	0.397	0.001
Wang et al. 2019 72	79 HBeAg+	LAM/ADV	7.9 ± 0.96 Log U/mL	0.67 ± 0.74 Log copies/cell	0.328	0.004
Hasegawa et al. 2019 ⁷³	57 HBeAg+	Naive	3.0(2.0-7.0) Log U/mL	3.0(1.5-5.8) Log copies/ug	0.670	< 0.001
Chen et al. 2019 ⁷⁴	85 HBeAg+	Naive	10.3 (6.0 - 12.3)	7.46 (5.11 - 8.17) Log copies/10 ⁶ cells	0.843	< 0.001
Chen et al. 2019 ⁷⁴	25 HBeAg-	Naive	5.4 (3.3 - 7.2)	6.03 (5.00 - 6.85)	0.865	< 0.001
Testoni et al. 2019 ⁷⁵	93 (32 HBeAg+ and 61 HBeAg-) [†]	Naive	5.3 (4 – 7.6) Log U/mL	0.15 (0.06-1.34) copies/cell	0.52	< 0.001

Caviglia et al. 2020 ⁵⁰	35 chronic	/	3.8 ± 1.8	3.11 ± 1.14	0.733	< 0.001
	HBsAg carriers		Log U/mL	Log copies/10 ⁵ cells		

2 *Correlation analysis was performed on 30 out of 46 patients of the total cohort.

- ³ [†]Correlation analysis was performed on 93 out of 130 patients of the total cohort.
- 4 Abbreviations: ADV: adefovir disoproxil; anti-HBe: antibodies to hepatitis B e antigen; cccDNA: covalently closed circular DNA; ETV: entecavir;
- 5 HBcrAg: hepatitis B core-related antigen; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen; HCC: hepatocellular carcinoma;
- 6 LAM: lamivudine; Peg-IFN: pegylated interferon; r: correlation coefficient.

8 TABLE II. Diagnostic accuracy of quantitative HBcrAg serum levels in discriminating between HBeAg negative chronic infection and HBeAg

9 *negative chronic hepatitis.*

Study	Patients	AUC	Cut-off	Se (%)	Sp (%)	PPV (%)	NPV (%)
Zhang et al. 2016 77	84 HBeAg-	0.96	2.2 [†]	92	96	92	96
	(56 G1 vs 28 G2-G3)*						
Gou et al. 2017 ⁷⁶	158 HBeAg-	0.93	4.1 [‡]	87.9	91.3	/	/
	(100 CI vs. 58 CH)						
Riveiro-Barciela et al. 2017 ⁷⁸	202 HBeAg-	0.67	3‡	97.8	27.3	73	86
70	(135 CI vs. 67 CH)						
Loggi et al. 2018 ⁷⁹	160 HBeAg-	0.87	2.5 [‡]	/	/	87	80
	(75 CI vs. 85 CH)		.*	,	,		
Testoni et al. 2019 ⁷⁵	45 HBeAg- with HBV DNA <2000 IU/mL	0.74	4*	/	/	44	92
	(mild vs. minimal liver disease)		4				
Zhang et al. 2019 ⁸⁰	200 HBeAg-	0.88	1.4*	72.7	95.1	93.5	78
	(101 CI vs. 99 CH)						
Chan et al. 2020 ⁸¹	73 HBeAg-	0.81	4 [‡]	65.7	81.6	/	/
	(38 CI vs. 35 CH)						

10 *histological activity according to Scheuer score.

- 11 [†] Data are expressed as Log kU/mL.
- [‡] Data are expressed as Log U/mL.
- 13 Abbreviations: AUC: area under the curve; CH: chronic hepatitis; CI: chronic infection; HBeAg: hepatitis B e antigen; NPV: negative predictive
- 14 value; PPV: positive predictive value; Se: sensitivity; Sp: specificity.
- 15

16 Figure 1. HBV replication cycle.

18	HBV enter and infects the hepatocytes by binding to the sodium-taurocholate cotransporting peptide
19	(NTCP). Following HBV internalization, the nucleocapsid of the virus is released into the
20	cytoplasm. Through the nuclear pores, the HBV relaxed circular DNA (rcDNA) is released into the
21	nucleus of the hepatocyte where it is converted into HBV covalently-closed-circular DNA
22	(cccDNA). Eventually, HBV rcDNA can integrate into the host genome. The HBV cccDNA acts as
23	a template for the transcription of HBV RNA molecules (all coated at the 5' and polyadenylated at
24	the 3' like cell mRNAs), which include subgenomic RNAs (which can also originate from
25	integrated HBV DNA), the pre-core RNA and the pre-genomic HBV RNA (pgRNA). The formers
26	are translated into HBV surface proteins and HBx regulatory protein; from the pre-core RNA
27	originates the secretory protein HBeAg; nucleocapsid proteins and polymerase originate from
28	pgRNA. The pgRNA is packaged with the viral polymerase in a new nucleocapsid where the
29	reverse transcription from RNA to DNA takes place. Following the assembly of the external
30	lipoprotein coating (envelope), the mature virion can be secreted in the circulation. Alternatively,
31	the HBV rcDNA can move in the hepatocyte nucleus in order to replenish the HBV cccDNA pool.
32	Abbreviations: cccDNA, covalently-closed-circular DNA; HBeAg, hepatitis B e antigen; HBsAg,
33	hepatitis B surface antigen; HBV, hepatitis B virus; NTCP, sodium-taurocholate cotransporting
34	peptide.