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MAJOR ARTICLE

Levels of Antibodies to Hepatitis B Core Antigen Are Associated With Liver Inflammation and Response to Peginterferon in Patients With Chronic Hepatitis B

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Background. Emerging evidence suggests a pivotal role for B-cell responses in the natural history of chronic hepatitis B. Serum levels of antibodies to hepatitis B core antigen (anti-HBc) vary across infection stages, but their role in predicting response to antiviral therapy is uncertain.

Methods. Anti-HBc levels were assessed before peginterferon (PEG-IFN) therapy in patients with chronic hepatitis B who either started *de novo* PEG-IFN (n = 299; 195 hepatitis B e antigen [HBeAg] positive) or started PEG-IFN as add-on to an existing nucleo(s)tide analogue backbone (n = 91; all HBeAg-positive). Associations were explored between anti-HBc and (1) serum biomarkers, (2) liver histological findings, and (3) treatment response.

Results. We studied 390 patients. The hepatitis B virus (HBV) genotype were A, B, C, and D in 24%, 9%, 16%, and 49%, respectively; 72% of patients were Caucasian. Among currently untreated HBeAg-positive patients, anti-HBc was correlated with HBV DNA, hepatitis B core-related antigen (HBcrAg), hepatitis B surface antigen (HBsAg), and HBV RNA, but not with alanine aminotransferase (ALT). Higher anti-HBc was associated with more severe histological inflammatory activity ($P < .001$), irrespective of HBeAg status. After *de novo* PEG-IFN, higher anti-HBc levels were associated with HBeAg loss, sustained response, HBsAg decline, and HBsAg clearance ($P < .050$). Among patients treated with add-on PEG-IFN, higher anti-HBc was associated with HBeAg loss ($P = .01$).

Conclusions. Serum anti-HBc levels correlate with histological inflammatory activity. Higher anti-HBc levels were associated with favorable treatment outcomes. These findings suggest that anti-HBc could be used to select patients most likely to respond to immunomodulatory therapy.

Clinical Trials Registration. NCT00114361, NCT00146705, NCT00877760, and NCT01532843.

Keywords. hepatitis B; serum biomarkers; anti-HBc; B cell; liver inflammation.

The natural history of chronic hepatitis B (CHB) infection is marked by distinct clinical phases, which are characterized by different patterns of serum hepatitis B e antigen (HBeAg) status, viral load, and transaminase levels reflecting the highly complex host-virus interplay [1].

The immune system appears to act as a double-edged sword in patients with CHB; in an attempt to clear infected cells, it causes liver inflammation and injury that may result in development of liver fibrosis and, ultimately, cirrhosis [2]. Emerging evidence suggests that, besides the innate immune system and virus-specific T cells, B cells play a role in the defense against hepatitis B virus (HBV) [2–4]. A recent study [5] showed that that the humoral immune response among patients with CHB is mainly mediated by hepatitis B core antigen (HBcAg)–specific memory B cells and not hepatitis B surface antigen (HBsAg)–specific B cells. Furthermore, serum levels of antibodies to HBcAg (anti-HBc) varied across the different phases in the natural history of chronic hepatitis B (CHB), with higher levels observed during phases with more pronounced liver inflammation [5].

The relationship between serum anti-HBc levels and hepatic inflammation is compelling, as currently used biomarkers (such as alanine aminotransferase [ALT]) are correlated rather poorly with histological activity [6]. This is especially relevant in the light of studies suggesting that circulating immune markers may predict response to immunomodulatory therapy [7, 8].

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We therefore aimed to study the association between serum levels of anti-HBc and (1) other serum biomarkers, (2) histological inflammatory activity, and (3) response to immunomodulatory therapy in patients with CHB.

METHODS

Study Population

This study included patients with CHB who participated in four global randomized controlled trials (the 99-01, PARC, ARES, and PEGON studies). Trial design and inclusion criteria have been described in detail elsewhere [9–12]. In short, the 99-01 study included HBeAg-positive patients (n = 266) who were randomized to *de novo* peginterferon (PEG-IFN) treatment with either PEG-IFN alpha-2b (100 µg/wk) alone or in combination with lamivudine for 52 weeks [9]. In the PARC study, HBeAg-negative patients (n = 133) were randomized to *de novo* PEG-IFN treatment with either PEG-IFN alpha-2a (180 µg/wk) monotherapy or PEG-IFN plus ribavirin (1000–2000 mg) combination therapy for 48 weeks [10]. The ARES study enrolled HBeAg-positive patients (n = 175), who started with entecavir (0.5 mg/d) monotherapy, and were subsequently randomized to receive either PEG-IFN alpha-2a add-on therapy from week 24 to week 48 (n = 85) or to continue entecavir monotherapy (n = 90) [11]. In the PEGON study (n = 77), HBeAg-positive patients who have been treated for at least one year with nucleo(s)tide analogue (NA) therapy were enrolled and randomized to receive 48 weeks of add-on PEG-IFN therapy (n = 39) or to continue NA monotherapy (n = 38) [12].

All patients had CHB, defined as HBsAg-positivity for at least six months. For the 99-01, PARC, and ARES studies, additional inclusion criteria comprised serum HBV DNA levels of >10 000 copies/mL (±2000 IU/mL) and ALT ≥1.3× (ARES study) or ≥1.5–2× (99-01 and PARC studies) the upper limit of normal (ULN) at baseline [9–11]. Additional inclusion criteria of the PEGON study included serum HBV DNA levels <2000 IU/mL and ALT levels <5 ULN during NA therapy [12]. The original study protocols have been approved by the medical ethical committees and are in line with the Declaration of Helsinki of 1975. All patients provided written consent. For the current study, we selected patients who received *de novo* PEG-IFN (patients from 99-01 and PARC) or add-on PEG-IFN (patients enrolled in the add-on PEG-IFN arms from ARES and PEGON) as shown in Supplementary Figure 1.

Biochemistry and Virology

Anti-HBc (immunoglobulin [Ig] G) was measured at baseline (ie, before initiation of PEG-IFN; pretreatment levels) and at end of PEG-IFN treatment (EOT levels), using the Lumipulse G ChemiLuminescent Enzyme Immunoassay anti-HBc assay (Fujirebio Europe; lower limit of detection [LLOD] 15 IU/mL). HBsAg was quantified using the Abbott Architect assay, with a LLOD of 0.05 IU/mL. For HBV DNA the LLOD was 400 copies/mL (approximately 80 IU/mL; in-house TaqMan PCR assay, Rotterdam, the Netherlands) for 99-01 [9], 35 copies/mL (approximately 10 IU/mL; Taqman, Roche Diagnostics, Basel, Switzerland) for PARC [10], and 20 IU/mL (Cobas TaqMan 48, Roche Diagnostics, Basel, Switzerland) for ARES [11] and PEGON [12] participants. HBV RNA (University Hospital Leipzig, Germany) was measured using rapid amplification of complementary DNA (cDNA)-ends (RACE)-based real-time polymerase chain reaction (LLOD 800 copies/mL) [13, 14]. Hepatitis B core-related antigen (HBcrAg) was quantified using the Lumipulse G HBcrAg assay (Fujirebio Europe) according to the manufacturer's instructions, with a lower limit of quantification (LLOQ) of 1000 U/mL (3 log) and an LLOD of 2 log [15]. Serum interferon-γ inducible protein 10 (IP-10) was quantified using an enzyme-linked immunosorbent assay (Alta Analytical Laboratory, San Diego, USA). ALT was quantified using automated techniques at the participating centers [9–12].

Liver Histology

Pretreatment liver histology was assessed in patients treated with *de novo* PEG-IFN (ie, those enrolled in the 99-01 or PARC studies). Liver inflammation was scored using to the histological activity index (HAI; range 0–18) [6, 16]. HAI scores were categorized as no inflammation (HAI 0–3), mild inflammation (HAI 4–8), or moderate-severe inflammation (HAI 9–18) [17, 18]. Liver fibrosis classification was based on the Ishak fibrosis stage [16].

Definitions of Treatment Response

Treatment response was assessed at the end of PEG-IFN treatment (EOT) and at six months after PEG-IFN withdrawal (end of follow-up [EOF]; in patients receiving *de novo* PEG-IFN only). On-treatment ALT flares were defined as an increase in serum ALT to ≥5× ULN during PEG-IFN treatment [18, 19]. Outcomes assessed at EOT included HBeAg loss and decline in HBsAg (≥1 log from baseline). Outcomes assessed at the EOF included sustained response (HBV DNA <2000 IU/mL) and HBsAg loss.

Statistical Analysis

Analyses were performed in the overall population and stratified by treatment strategy (*de novo* or add-on PEG-IFN) or baseline HBeAg status. Descriptives are presented as numbers (with percentages), medians (with interquartile range [IQR]) and means (with standard deviation [SD]). Correlations between pretreatment anti-HBc levels and age, HAI score, and pretreatment serum ALT, IP-10, HBV DNA, HBsAg, HBcrAg, and HBV RNA levels were assessed using Pearson correlation coefficient in the subset of patients treated with *de novo* PEG-IFN (stratified by HBeAg status). Associations between anti-HBc levels and histology or treatment

Table 1. Characteristics of the Patients with Pretreatment Antibodies Against Hepatitis B Core Antigen

Characteristic	Patients, No. (%)[a]		
	De Novo PEG-IFN, HBeAg-Positive (n = 195)	*De Novo* PEG-IFN, HBeAg-Negative (n = 104)	Add-on PEG-IFN, HBeAg-Positive (n = 91)
Age at inclusion, median (IQR), y	33 (25–44)	41 (33–49)	30 (24–38)
Male sex	153 (78.5)	75 (72.1)	65 (71.4)
Race			
Caucasian	149 (76.4)	98 (94.2)	33 (36.3)
Asian	31 (15.9)	4 (3.8)	56 (61.5)
Other	15 (7.7)	2 (1.9)	2 (2.2)
HBV genotype			
A	74 (37.9)	14 (13.5)	4 (4.4)
B	15 (7.7)	0 (0.0)	21 (23.1)
C	23 (11.8)	3 (2.9)	35 (38.5)
D	76 (39.0)	82 (78.8)	31 (34.1)
Other	7 (3.6)	5 (4.8)	-
Pretreatment liver inflammation			
None (HAI 0–3)	37/155 (23.9)	18/98 (18.4)	-
Mild (HAI 4–8)	106/155 (68.4)	72/98 (73.5)	-
Moderate-severe (HAI 9–18)	12/155 (7.7)	8/98 (8.2)	-
Study treatment			
PEG-IFN monotherapy	104 (53.3)	51 (49.0)	-
PEG-IFN + LAM	91 (46.7)	-	-
PEG-IFN + RBV	-	53 (51.0)	-
NA + add-on PEG-IFN	-	-	91 (100)
Baseline laboratory results			
ALT level, median (IQR), U/L.	130 (89–186)	94 (65–183)	102 (63–169)
Mean (SD) values			
Anti-HBc, log IU/mL	3.80 (0.46)	4.16 (0.39)	2.88 (0.73)
HBsAg, log IU/mL	4.41 (0.60)	3.86 (0.50)	3.72 (0.66)
HBV DNA, log IU/mL	8.37 (0.83)	6.08 (1.21)	2.74 (1.49)
HBV RNA, log copies/mL	6.79 (1.11)	4.38 (0.98)	4.85 (1.50)
HBcrAg, log U/mL	8.35 (0.70)	5.00 (1.42)	8.11 (0.76)
Treatment response			
On-treatment ALT flares[b]	102/194 (52.6)	48/103 (46.6)	6/90 (6.7)
HBeAg loss at EOT[c]	78 (40.0)	-	16/90 (17.8)
HBsAg decline at EOT (≥1 log)	53/174 (30.5)	19/102 (18.6)	8/89 (9.0)
Sustained response[d]	37/170 (21.8)	25/95 (26.3)	-
HBsAg loss[e]	16/173 (9.2)	1/100 (1.0)	3 (3.3)

Abbreviations: ALT, alanine aminotransferase; anti-HBc, antibodies against hepatitis B core antigen; EOT, end of PEG-IFN treatment; HAI, histological activity index; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, quantitative hepatitis B surface antigen HBV, hepatitis B virus; IQR, interquartile range; LAM, lamivudine; NA, nucleos(t)ide analogue; PEG-IFN, peginterferon; RBV, ribavirin; SD, standard deviation.

[a]Data represent no. (%) unless otherwise specified. Denominators are provided where they differ from those in the column headings.

[b]On-treatment ALT flare was defined as ALT ≥5× the upper limit of normal during PEG-IFN therapy.

[c]HBeAg loss at the EOT in pretreatment HBeAg-positive patients.

[d]Sustained response was defined as HBV DNA level <2000 IU/mL 6 months after the EOT.

[e]HBsAg loss was defined as HBsAg clearance at the end of follow-up (ie, 6 months after the EOT).

outcomes were assessed using continuous data (with associations assessed using Student *t* test, analysis of variance, logistic regression and area under the receiver operating characteristic curve [AUROC]), and, because no cutoffs are defined in current literature, after categorization into three groups of equal size (low, intermediate, and high).

In addition, baseline anti-HBc levels were also combined in the previously reported baseline scoring system by Lampertico et al [20]. This point-based model is calculated based on age ≥ 45 (0 points) or <45 (1 point) years; male (0 points) or female (1 point) sex; HBsAg level >25 000 (0 points), >7500 to ≤25 000 (1 point), >1250 to ≤7500 (2 points), or ≤1250 IU/mL (4 points); HBV DNA >5 log (0 points) or ≤5 log IU/mL (2 points); and ALT ratio >1–7× (0 points) or either ≤1× or >7× ULN (1 point). Scores were categorized as low (0–1 points), moderate (2–3 points) and high (≥4 points).

Multivariable analyses were performed by entering anti-HBc levels (as units of 0.1 log IU/mL) and other potential predictors (including age, sex, HBV genotype A, HBeAg status at baseline, and serum ALT, HBsAg, and HBV DNA levels at baseline) into a

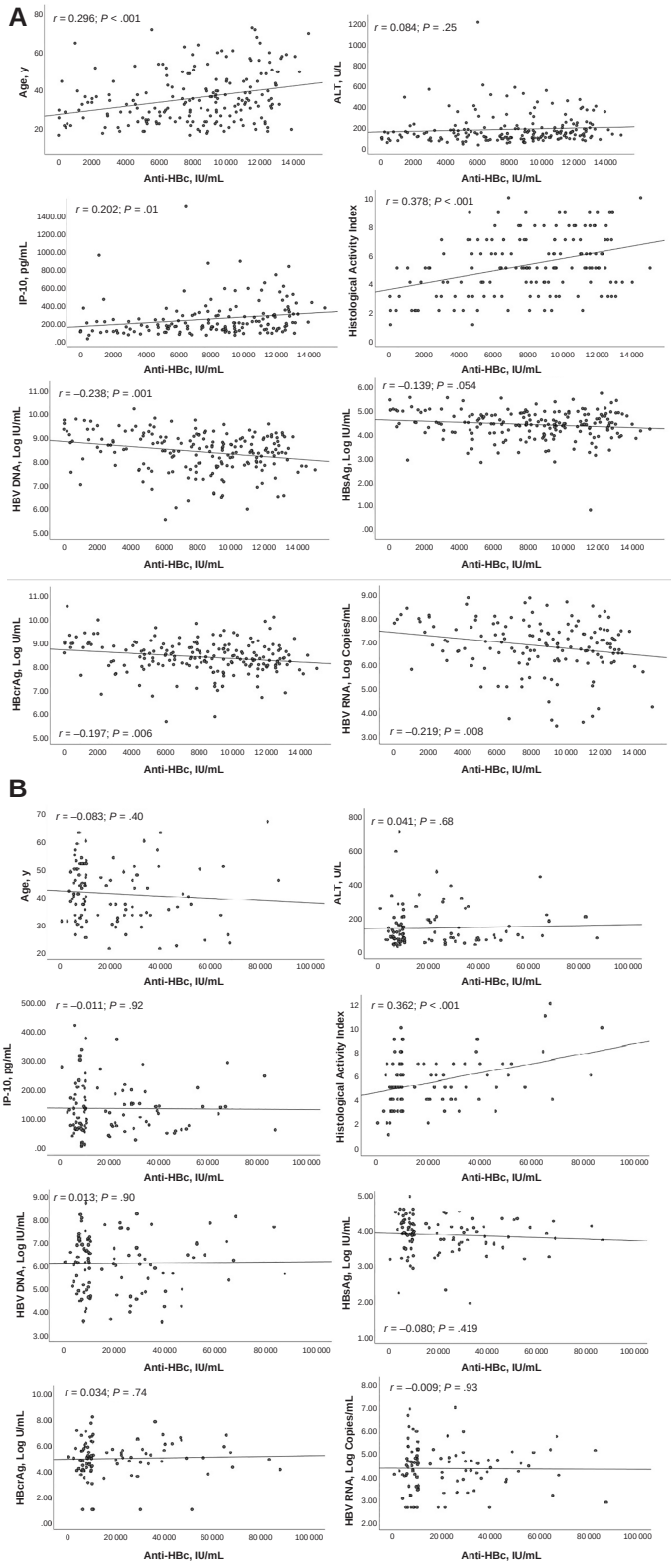

Figure 1. Correlation of levels of antibodies against hepatitis B core antigen (anti-HBc) with age, alanine aminotransferase (ALT), histological activity index, and markers of viral replication among hepatitis B e antigen (HBeAg)–positive (*A*) and HBeAg-negative (*B*) patients. Abbreviations: HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; HBV hepatitis B virus; IP-10, interferon γ inducible protein 10.

backward selection–based logistic regression model. Differences were considered statistically significant at $P < .05$. IBM SPSS software for Windows (version 25.0; SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis, and Graph Pad Prism software (version 5 for Windows; GraphPad Software, San Diego, California, USA) was used for graphic representation of the results.

RESULTS

Patient Characteristics

In total, we enrolled 390 patients, including 299 treated with *de novo* PEG-IFN (195 HBeAg-positive) and 91 treated with add-on PEG-IFN. Patient characteristics are displayed in Table 1. The HBeAg-positive *de novo* PEG-IFN cohort included predominantly Caucasianpatients (76.4%), with genotype A or D (37.9% and 39.0% respectively). The HBeAg-negative *de novo* PEG-IFN cohort included predominantly Caucasianpatients (94.2%), with genotype D (78.8%). The add-on PEG-IFN cohort included predominantly Asian patients (61.5%), with genotypes A, B, C, and D in 4.4%, 23.1%, 38.5%, and 34.1%.

Anti-HBc Levels Correlated With Age, Serum IP-10, and Markers of Viral Replication but Not With ALT

Among untreated HBeAg-positive patients, positive correlations were observed for anti-HBc levels with age and pretreatment serum IP-10 levels, but not with ALT. Negative correlations were observed with markers of viral replication, including HBV DNA, HBcrAg, HBsAg, and HBV RNA levels (Figure 1A). Serum anti-HBc levels were not correlated with any of the serum biomarkers in untreated HBeAg-negative patients (Figure 1B). Mean anti-HBc levels varied significantly across HBV genotype. Anti-HBc levels were highest among patients with HBV genotype A and lowest among those patients with HBV genotype D: 3.98 log vs 3.61 log IU/mL ($P < .001$) among HBeAg-positive and 4.44 log vs 4.16 log IU/mL ($P = .03$) among HBeAg-negative patients (Supplementary Figure 2).

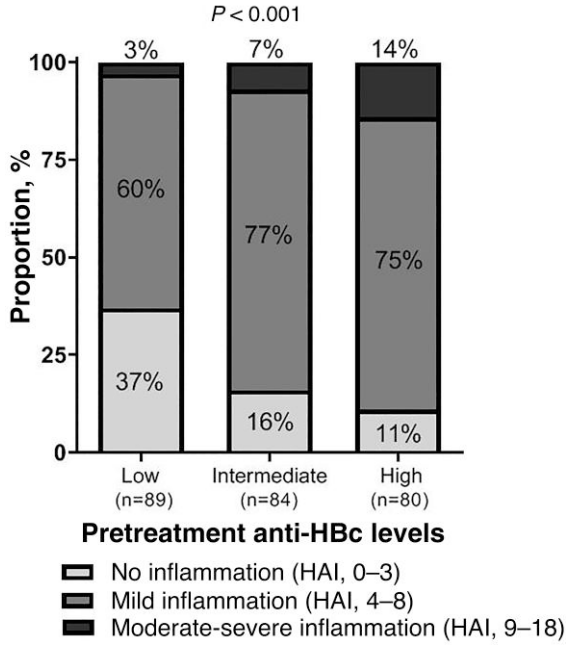

Figure 2. Relationship between levels of antibodies against hepatitis B core antigen (anti-HBc) and intrahepatic inflammatory activity. Liver inflammation was defined as no inflammation (histological activity index [HAI] 0–3), mild inflammation (HAI 4–8), or moderate-severe inflammation (HAI 9–18). Anti-HBc levels were categorized as low, intermediate, or high (<3.82, 3.82–4.0, or ≥4.0 log IU/mL, respectively, for hepatitis B e antigen (HBeAg)–positive and <3.95, 3.95–4.40, or ≥4.40 log IU/mL for HBeAg-negative patients) to create 3 groups of equal size.

Serum Anti-HBc Levels Correlated With Intrahepatic Inflammatory Activity

Among the 253 patients with pretreatment liver histology data available, anti-HBc levels were correlated with the severity of inflammatory activity ($r = 0.38$ for HBeAg-positive and $r = 0.36$ for HBeAg-negative patients; $P < .001$; Figure 1). Among the 89 patients with the lowest pretreatment anti-HBc levels, only 3 patients (3.4%) had moderate to severe inflammation (HAI 9–18) compared with 11 of 80 (13.8%) with the highest anti-HBc levels ($P < .001$; Figure 2; AUROC 0.666 [95% confidence interval 0.550–0.781]; $P = .014$). Similar results were obtained in multivariable logistic regression (adjusted odds ratio for moderate-severe inflammation: 1.24 [95% confidence interval 1.04–1.48]; $P = .015$).

Decreased Serum Anti-HBc Levels During PEG-IFN–Based Antiviral Therapy

Baseline anti-HBc levels were higher in untreated patients (ie, the patients receiving *de novo* PEG-IFN; mean [SD], 3.93 [0.47] log IU/mL) than in patients receiving NA therapy (ie, patients receiving add-on PEG-IFN; 2.88 [0.73] log IU/mL; $P < .001$). Furthermore, PEG-IFN therapy significantly reduced serum anti-HBc levels: mean (SD) declines from baseline to EOT were 0.25 (0.36) log among HBeAg-positive patients treated with *de novo* PEG-IFN, 0.47 (0.41) log in HBeAg-negative patients treated with *de novo* PEG-IFN, and 0.29 (0.28) log among patients who received add-on PEG-IFN ($P < .001$).

Association of Higher Pretreatment Anti-HBc Levels With Favorable Treatment Outcomes

De Novo PEG-IFN

Pretreatment anti-HBc levels were higher in patients with favorable outcomes after PEG-IFN therapy (Figures 3 and 4). Patients with the highest anti-HBc levels achieved sustained response in 35% and HBsAg loss in 13%, compared with 13% and 2% among patients with the lowest anti-HBc levels ($P \leq .004$; Figure 3). Interestingly, HBeAg-positive patients with on-treatment ALT flares had higher pretreatment anti-HBc levels (Figure 4).

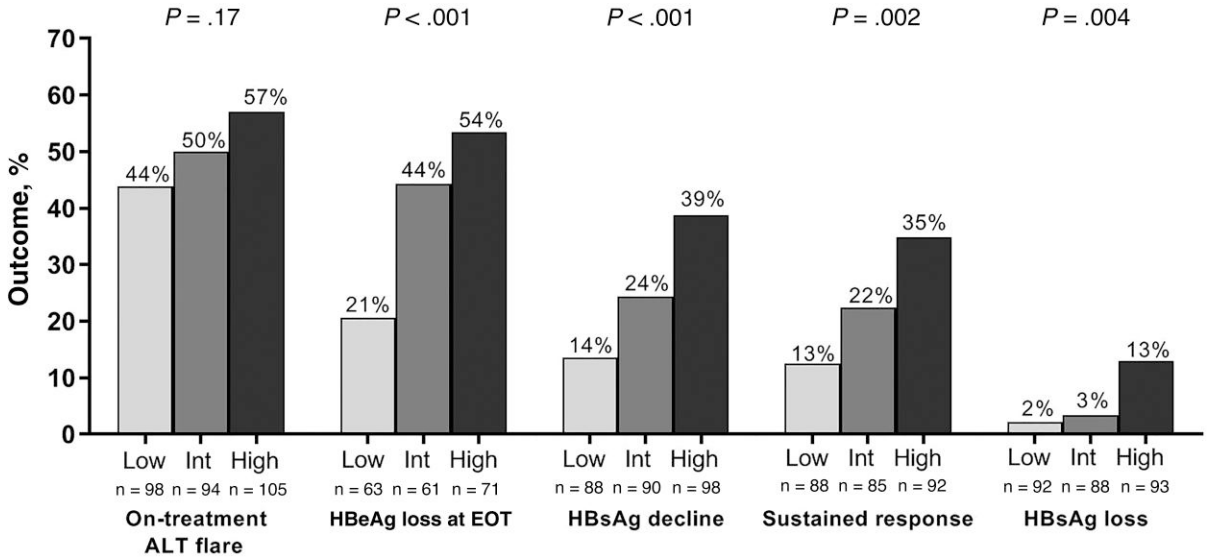

Figure 3. Treatment outcome according to pretreatment level of antibodies against hepatitis B core antigen (anti-HBc level). Anti-HBc levels were categorized as low, intermediate (Int), or high (<3.82, 3.82–4.0, or ≥4.0 log IU/mL, respectively, for hepatitis B e antigen [HBeAg]–positive and <3.95, 3.95–4.40, or ≥4.40 log IU/mL for HBeAg-negative patients) to create 3 groups of equal size. An on-treatment alanine aminotransferase (ALT) flare was defined as an increase in serum ALT to ≥5× the upper limit of normal during peginterferon (PEG-IFN) treatment. Hepatitis B surface antigen (HBsAg) decline was defined as a decline of ≥1 log at the end of PEG-IFN treatment (EOT). Sustained response was defined as HBV DNA levels <2,000 IU/mL six months after the EOT. HBsAg loss was defined as HBsAg clearance at the end of follow-up (six months after the EOT).

The association between higher anti-HBc levels and favorable treatment outcomes was generally consistent after stratification by HBeAg status, although associations were less pronounced in the smaller HBeAg-negative subset (Supplementary Figure 3). Consistent results were obtained in multivariable analysis (Table 2).

In addition, findings were consistent when anti-HBc levels were included in the baseline scoring system of Lampertico *et al* [20]. Among patients with a predicted low (score 0–1) or moderate (score 2–3) probability to response, but high levels of anti-HBc (≥4.0 log among HBeAg-positive and ≥4.40 log among HBeAg-negative patients) were associated with a higher probability of sustained response and HBsAg loss (Figure 5).

Add-on PEG-IFN

Among patients treated with add-on PEG-IFN, anti-HBc levels were significantly higher in patients with than in those without subsequent HBeAg loss (3.12 log vs 2.84 log IU/mL; $P = .01$). Anti-HBc levels did not predict on-treatment HBsAg decline. HBsAg loss was not achieved in any patients in the PEG-IFN add-on cohort.

DISCUSSION

There is emerging evidence suggesting that B cells play a pivotal role in the natural history of CHB [2, 5, 21]. In the current study, higher serum anti-HBc levels were correlated with other immune markers, such as IP-10, and were associated with more severe liver inflammation on liver biopsy. Furthermore, higher pretreatment anti-HBc levels were associated with favorable responses to PEG-IFN therapy. These findings suggest that serum anti-HBc levels could be a valuable new serum biomarker to monitor immune activity in patients with CHB.

During an acute HBV infection, the innate immune response is triggered first, followed by activation of the adaptive immune system. This generally leads to functional cure (ie, HBsAg loss) among adults [2, 22]. However, among patients with CHB in whom functional cure is not achieved, alterations in both innate and adaptive immune responses are observed [2]. The important role for B cells in the immune control over HBV has been demonstrated in clinical practice through the risk for HBV reactivation among patients treated with B-cell–depleting agents, such as rituximab, and by detailed analysis of their phenotype and function ex vivo [2, 4, 5, 23, 24]. B-cells secrete antibodies targeted against various antigens including antibodies against HBsAg (anti-HBs), HBeAg (anti-HBe) and HBcAg (anti-HBc).

A previous study showed that serum levels of anti-HBc vary across the natural history of CHB, with higher levels observed in disease states with more active inflammation. In our cohort, serum anti-HBc levels were correlated with other immune markers, such as serum levels of IP-10, and higher serum levels of anti-HBc were also associated with more severe hepatic inflammation at liver biopsy. Higher anti-HBc levels were also associated with lower levels of markers of viral replication and covalently closed circular DNA (cccDNA) transcriptional

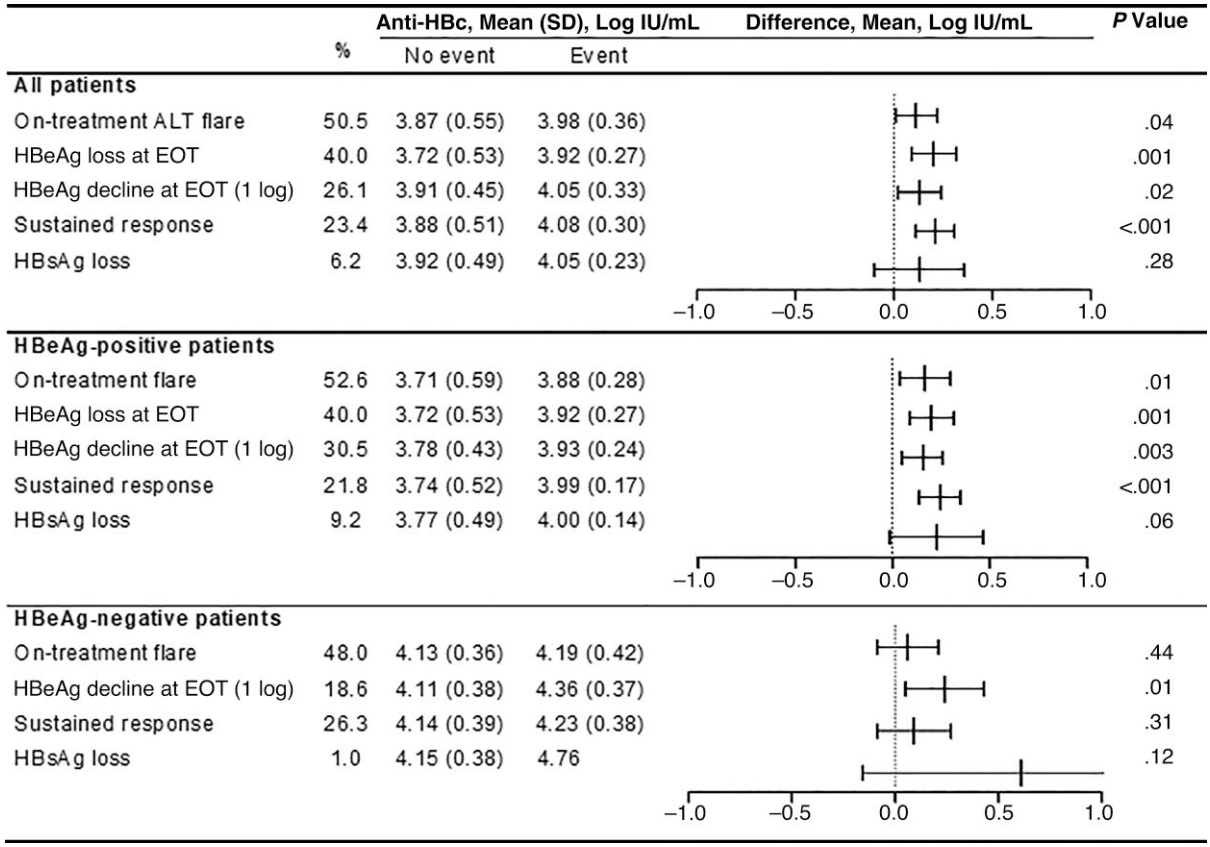

Figure 4. Pretreatment levels of antibodies against hepatitis B core antigen (anti-HBc) according to treatment response. An alanine aminotransferase (ALT) flare was defined as an increase of serum ALT to ≥5× the upper limit of normal during peginterferon (PEG-IFN) treatment. Hepatitis B surface antigen (HBsAg) decline was defined as a decline of ≥1 log at the end of PEG-IFN treatment (EOT); sustained response, as HBV DNA levels of <2000 IU/mL six months after the EOT. Abbreviations: HBeAg, hepatitis B e antigen; SD, standard deviation.

activity, such as HBV DNA, HBV RNA, HBcrAg, and HBsAg [25–27]. Taken together, these findings highlight an association between B-cell activation and control over HBV replication. The observed associations with intrahepatic inflammation suggest that there may also be an important clinical diagnostic application for anti-HBc assessment, as currently used biomarkers (such as ALT) are poorly correlated with findings at liver histology [6]. High serum anti-HBc levels may reflect increased liver inflammatory activity, which could potentially influence decisions regarding initiation of antiviral therapy or performance of liver biopsy [28, 29].

Another interesting observation in our study was that antiviral therapy reduced serum anti-HBc levels. One year of PEG-IFN therapy was associated with a significant decline in serum anti-HBc levels, and patients currently receiving NA therapy had the lowest anti-HBc levels in the cohort. These findings are in line with previous studies that showed a more profound on-treatment decline in anti-HBc levels among HBeAg-positive patients treated with NAs than with PEG-IFN [5, 8]. Thus, antiviral agents seem to affect anti-HBc levels, although the exact mechanism is unclear and may differ between PEG-IFN and NAs. Previous studies hint that PEG-IFN therapy might influence the number of B cells or B-cell function directly or via bone marrow suppression [30, 31]. Whether NA have a direct effect on B-cell production or function is uncertain, but the observed effects on anti-HBc levels may also be due to the rapid decline in viral load [32].

In our cohort, higher levels of anti-HBc were associated with a higher probability of favorable outcomes after treatment with PEG-IFN. Among patients treated with *de novo* PEG-IFN, findings were consistent for multiple end points, including HBeAg clearance, sustained HBV DNA suppression, HBsAg decline and HBsAg loss. In the subset of patients treated with add-on PEG-IFN, higher anti-HBc levels also predicted on-treatment HBeAg clearance. These findings are in line with those of a previous Asian study, comprising HBeAg-positive patients treated with PEG-IFN or NA therapy, which demonstrated that anti-HBc levels of 4.4 log IU/mL were associated with an increased chance of HBeAg seroconversion at EOT [8]. Interestingly, in our study, higher pretreatment anti-HBc levels were also associated with a higher chance of on-treatment ALT flares, which previous studies have shown to be pivotal in

Table 2. Association Between Antibodies Against Hepatitis B Core Antigen and Treatment Outcomes in Multivariable Analysis in Patients Receiving *De Novo* Peginterferon

	All			HBeAg-Positive			HBeAg-Negative		
Outcome[a]	aOR	95% CI	*P* Value	aOR	95% CI	*P* Value	aOR	95% CI	*P* Value
On-treatment ALT flare	1.09	1.02–1.17	.014	1.12	1.02–1.23	.016	1.05	.92–1.20	.50
HBsAg decline at EOT	1.18	1.07–1.31	.001	1.14	1.00–1.31	.058	1.19	1.03–1.37	.02
HBeAg loss at EOT	1.13	1.00–1.28	.049	1.13	1.00–1.28	.049	…	…	…
Sustained response	1.13	1.04–1.23	.006	1.30	1.01–1.66	.04	1.09	.96–1.24	.18
HBsAg loss	1.37	0.95–1.98	.091	1.27	0.86–1.88	.227	-[b]	-	-

Abbreviations: ALT; alanine aminotransferase; aOR, adjusted odds ratio; CI, confidence interval; EOT, end of PEG-IFN treatment; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peginterferon

[a]On-treatment ALT flare was defined as an ALT level ≥5× the upper limit of normal during PEG-IFN treatment; HBsAg decline, as a decline of ≥1 log six months after the EOT; sustained response, as HBV DNA levels of <2000 IU/mL six months after EOT; and HBsAg loss, as loss of HBsAg at any time during treatment or off-treatment follow-up.

[b]Insufficient number of events for multivariable analysis.

achieving sustained response and HBsAg loss with immunomodulators [33]. When seen in the light of the associations between anti-HBc levels and intrahepatic inflammatory activity, our findings provide further support for the hypothesis that the pretreatment immune status is an important determinant of response to immunomodulatory therapy. This hypothesis warrants further exploration, especially in studies involving novel immunomodulatory agents.

Our findings were consistent in multivariable analysis and when anti-HBc levels were combined in the baseline scoring system [20], including age, sex, and HBsAg, HBV DNA, and ALT levels, supporting the robustness of our results. However, our study has several potential limitations. Although our cohort is relatively large and enrolled patients from four randomized controlled trials, stratification by HBeAg status resulted in limited numbers of subjects and

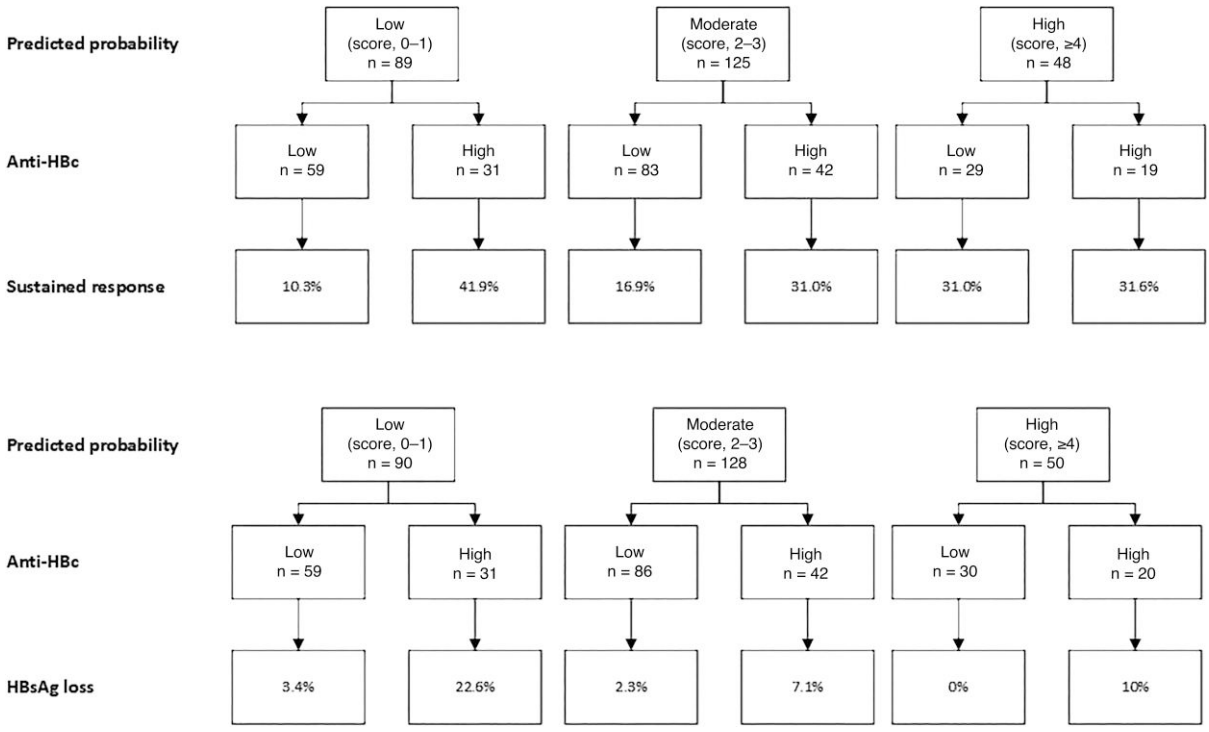

Figure 5. Treatment outcome according to pretreatment levels of antibodies against hepatitis B core antigen (anti-HBc) and predicted probability. The predicted probability was based on the baseline prediction model including age, sex, hepatitis B surface antigen (HBsAg), hepatitis B virus (HBV) DNA, and alanine aminotransferase levels [20]. Anti-HBc levels were categorized as low versus high; <4.0 versus ≥4.0 log for hepatitis B e antigen (HBeAg)–positive patients and <4.40 versus ≥4.40 log for HBeAg-negative patients. Sustained response was defined as an HBV DNA level <2000 IU/mL 6 months after the end of peginterferon treatment; HBsAg loss, as loss of HBsAg 6 months after the end of treatment.

events per subgroup, increasing the risk of type 2 statistical error. However, the association between higher anti-HBc levels and favorable outcomes after antiviral therapy was consistent across subcohorts, supporting the robustness of our findings (Figure 4 and Supplementary Figure 3). Furthermore, the anti-HBc assay we applied assessed only IgG anti-HBc, and it is unclear whether there is a difference in diagnostic performance with assays that also measure IgM anti-HBc. It is also important to note that our *de novo* PEG-IFN studies enrolled predominantly Caucasians, whereas the add-on studies enrolled predominantly Asian patients. External validation of our findings in cohorts with other ethnicities and genotypes is therefore warranted.

In conclusion, our study shows that serum anti-HBc levels are correlated with intrahepatic inflammatory activity. Higher serum anti-HBc levels are associated with favorable outcomes after PEG-IFN therapy. These findings provide further support for the importance of B cells in control of HBV infection and suggest that assessment of anti-HBc levels may have important clinical applications.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. S. M. B., R. J. d. M., and A. B., and M. J. S. conceived the study. J. O. and A. B. performed laboratory analysis. S. M. B. and M. J. S. performed statistical analysis. S. M. B. and M. J. S. made the graphic images. S. M. B. and M. J. S. wrote the manuscript, which was revised by all authors. All authors had access to the study data and have reviewed and approved the final manuscript.

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References

1. Song LW, Liu PG, Liu CJ, et al. Quantitative hepatitis B core antibody levels in the natural history of hepatitis B virus infection. Clin Microbiol Infect **2015**; 21:197–203.
2. Khanam A, Chua JV, Kottilil S. Immunopathology of chronic hepatitis B infection: role of innate and adaptive immune response in disease progression. Int J Mol Sci **2021**; 22:5497.
3. Vanwolleghem T, Hou J, van Oord G, et al. Re-evaluation of hepatitis B virus clinical phases by systems biology identifies unappreciated roles for the innate immune response and B cells. Hepatology **2015**; 62:87–100.
4. Wang Q, Sachse P, Semmo M, et al. T- and B-cell responses and previous exposure to hepatitis B virus in 'anti-HBc alone' patients. J Viral Hepat **2015**; 22:1068–78.
5. Vanwolleghem T, Groothuismink ZMA, Kreefft K, Hung M, Novikov N, Boonstra A. Hepatitis B core-specific memory B cell responses associate with clinical parameters in patients with chronic HBV. J Hepatol **2020**; 73:52–61.
6. Sonneveld MJ, Brouwer WP, Hansen BE, et al. Very low probability of significant liver inflammation in chronic hepatitis B patients with low ALT levels in the absence of liver fibrosis. Aliment Pharmacol Ther **2020**; 52:1399–406.
7. Sonneveld MJ, Arends P, Boonstra A, Hansen BE, Janssen HL. Serum levels of interferon-gamma-inducible protein 10 and response to peginterferon therapy in HBeAg-positive chronic hepatitis B. J Hepatol **2013**; 58:898–903.
8. Fan R, Sun J, Yuan Q, et al. Baseline quantitative hepatitis B core antibody titre alone strongly predicts HBeAg seroconversion across chronic hepatitis B patients treated with peginterferon or nucleos(t)ide analogues. Gut **2016**; 65:313–20.
9. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet **2005**; 365:123–9.
10. Rijckborst V, ter Borg MJ, Cakaloglu Y, et al. A randomized trial of peginterferon alpha-2a with or without ribavirin for

HBeAg-negative chronic hepatitis B. Am J Gastroenterol **2010**; 105:1762–9.
11. Brouwer WP, Xie Q, Sonneveld MJ, et al. Adding pegylated interferon to entecavir for hepatitis B e antigen-positive chronic hepatitis B: a multicenter randomized trial (ARES study). Hepatology **2015**; 61:1512–22.
12. Chi H, Hansen BE, Guo S, et al. Pegylated interferon alfa-2b add-on treatment in hepatitis B virus envelope antigen-positive chronic hepatitis B patients treated with nucleos(t)ide analogue: a randomized, controlled trial (PEGON). J Infect Dis **2017**; 215:1085–93.
13. van Bömmel F, Bartens A, Mysickova A, et al. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. Hepatology **2015**; 61:66–76.
14. van Campenhout MJH, van Bömmel F, Pfefferkorn M, et al. Host and viral factors associated with serum hepatitis B virus RNA levels among patients in need for treatment. Hepatology **2018**; 68:839–47.
15. van Campenhout MJH, Rijckborst V, Brouwer WP, et al. Hepatitis B core-related antigen monitoring during peginterferon alfa treatment for HBeAg-negative chronic hepatitis B. J Viral Hepat **2019**; 26:1156–63.
16. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol **1995**; 22:696–9.
17. Rozario R, Ramakrishna B. Histopathological study of chronic hepatitis B and C: a comparison of two scoring systems. J Hepatol **2003**; 38:223–9.
18. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology **2018**; 67:1560–99.
19. Ghany MG, Feld JJ, Chang KM, et al. Serum alanine aminotransferase flares in chronic hepatitis B infection: the good and the bad. Lancet Gastroenterol Hepatol **2020**; 5:406–17.
20. Lampertico P, Messinger D, Oladipupo H, Bakalos G, Castillo M, Asselah T. An easy-to-use baseline scoring system to predict response to peginterferon alfa-2a in patients with chronic hepatitis B in resource-limited settings. Antivir Ther **2018**; 23:655–63.
21. Zhang ZQ, Shi BS, Lu W, Huang D, Wang YB, Feng YL. Quantitative serum HBV markers in predicting phases of natural history of chronic HBV infection. J Virol Methods **2021**; 296:114226.
22. Thimme R, Wieland S, Steiger C, et al. $CD8^+$ T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol **2003**; 77:68–76.
23. Kusumoto S, Arcaini L, Hong X, et al. Risk of HBV reactivation in patients with B-cell lymphomas receiving obinutuzumab or rituximab immunochemotherapy. Blood **2019**; 133:137–46.
24. Salimzadeh L, Le Bert N, Dutertre CA, et al. PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection. J Clin Invest **2018**; 128: 4573–87.
25. Mak LY, Wong DKH, Cheung KS, Seto WK, Lai CL, Yuen MF. Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection. Aliment Pharmacol Ther **2018**; 47:43–54.
26. Giersch K, Allweiss L, Volz T, Dandri M, Lutgehetmann M. Serum HBV pgRNA as a clinical marker for cccDNA activity. J Hepatol **2017**; 66:460–2.
27. Chuaypen N, Sriprapun M, Praianantathavorn K, et al. Kinetics of serum HBsAg and intrahepatic cccDNA during pegylated interferon therapy in patients with HBeAg-positive and HBeAg-negative chronic hepatitis B. J Med Virol **2017**; 89:130–8.
28. Hou FQ, Song LW, Yuan Q, et al. Quantitative hepatitis B core antibody level is a new predictor for treatment response in HBeAg-positive chronic hepatitis B patients receiving peginterferon. Theranostics **2015**; 5:218–26.
29. Zhou J, Song L, Zhao H, et al. Serum hepatitis B core antibody as a biomarker of hepatic inflammation in chronic hepatitis B patients with normal alanine aminotransferase. Sci Rep **2017**; 7:2747.
30. Aspord C, Bruder Costa J, Jacob MC, et al. Remodeling of B-cell subsets in blood during pegylated IFNα-2a therapy in patients with chronic hepatitis B infection. PLoS One **2016**; 11:e0156200.
31. de Groen RA, Groothuismink ZM, Liu BS, Boonstra A. IFN-λ is able to augment TLR-mediated activation and subsequent function of primary human B cells. J Leukoc Biol **2015**; 98:623–30.
32. Boeijen LL, Spaan M, Boonstra A. The effects of nucleoside/nucleotide analogues on host immune cells: the baseline for future immune therapy for HBV? Antivir Ther **2020**; 25: 181–91.
33. Sonneveld MJ, Zoutendijk R, Flink HJ, Zwang L, Hansen BE, Janssen HL. Close monitoring of hepatitis B surface antigen levels helps classify flares during peginterferon therapy and predicts treatment response. Clin Infect Dis **2013**; 56:100–5.