Original article

Anti-HBc levels are associated with liver inflammation and response to peginterferon in chronic hepatitis B patients

Running title: anti-HBc levels predict treatment response

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ABSTRACT

Background: Emerging evidence suggests a pivotal role for B-cell responses in the natural history of chronic hepatitis B (CHB). Serum levels of antibodies to HBcAg (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 CHB patients who either initiated *de novo* PEG-IFN (n=299; 195 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 histology and (3)treatment response.

Results: We studied 390 patients. HBV-genotypes were A/B/C/D in 24/9/16/49%, and 72% were Caucasian. Among currently untreated HBeAg-positive patients, anti-HBc correlated with HBV DNA, HBcrAg, HBsAg and HBV RNA, but not with ALT. Higher anti-HBc was associated with more severe histological inflammatory activity ($p<0.001$), irrespective of HBeAg-status. After *de novo* PEG-IFN, higher anti-HBc was associated with HBeAg-loss, sustained response, HBsAg-decline and HBsAg-clearance ($p<0.050$). Among patients treated with add-on PEG-IFN, higher anti-HBc was associated with HBeAg-loss ($p=0.012$).

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

Key words: Hepatitis B, Serum biomarkers, anti-HBc, B cell, liver inflammation

INTRODUCTION

The natural history of chronic hepatitis B (CHB) infection is marked by distinct clinical phases, which are characterised by different patterns of serum 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 defence against HBV.[2-4] A recent study showed that that the humoral immune response among CHB patients is mainly mediated by HBcAg-specific memory B cells and not HBsAg-specific B cells. Furthermore, serum levels of antibodies to hepatitis B core antigen (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]) correlate 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]

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.

PATIENTS AND METHODS

Study population

This study included CHB patients who participated in four global randomised 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 randomised to *de novo* PEG-IFN treatment with either PEG-IFN alpha-2b

100 µg/week alone or in combination with lamivudine for 52 weeks.[9] In the PARC study, HBeAg-negative patients (n = 133) were randomised to *de novo* PEG-IFN treatment with either PEG-IFN alpha-2a 180 µg/week mono-therapy 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 (ETV) 0.5 mg/day monotherapy, and were subsequently randomised to receive either PEG-IFN alpha-2a add-on therapy from week 24 to week 48 (n = 85) or to continue ETV mono-therapy (n = 90).[11] In the PEGON (n = 77), HBeAg-positive patients who have been treated for at least one year with nucleo(s)tide analogue (NA) therapy were enrolled and randomised 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 more than 10,000 copies/ml (± 2,000 IU/ml) and ALT ≥ 1.3 times (ARES study) or ≥ 1.5-2 times (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 < 2,000 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 this study, we selected patients who received *de novo* PEG-IFN (ie. patients from 99-01 and PARC) or add-on PEG-IFN (i.e, the patients enrolled in the add-on PEG-IFN arms from ARES and PEGON) as shown in Supplementary Figure 1.

Biochemistry and virology

Anti-HBc (IgG) was measured at baseline (i.e. before initiation of IFN; pre-treatment levels) and at end of PEG-IFN treatment (EOT levels), using Lumipulse® G CLEIA anti-HBc assay (Fujirebio Europe, lower limit of detection [LLOD] 15 IU/mL). HBsAg was quantified using the Abbott Architect (Abbott Park, IL) with a LLOD of 0.05 IU/mL. For HBV DNA the LLOD was

400 copies/mL (~80 IU/mL; in-house TaqMan PCR assay, Rotterdam, the Netherlands) for 99-01[9], 35 copies/mL (~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] HBcrAg was quantified using Lumipulse® G HBcrAg assay (Fujirebio Europe) according to the manufacturer's instructions, with a lower limit of quantification (LLOQ) of 1,000 U/mL (3 log) and LLOD of 2 log.[15] Serum interferon-y inducible protein 10 (IP-10) was quantified using ELISA (Alta Analytical Laboratory, San Diego, USA). ALT was quantified using automated techniques at the participating centres.[9-12]

Liver histology

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

Definitions of treatment response

Treatment response was assessed at end of PEG-IFN treatment (EOT) and at six months after PEG-IFN withdrawal (end of follow-up [EOF]; in *de novo* PEG-IFN patients only). On-treatment ALT flares were defined as an increase of serum ALT ≥ 5x 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 EOF included sustained response (HBV DNA <2,000 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 (± standard deviation; SD). Correlations between pre-treatment anti-HBc levels and age, HAI score, and pre-treatment 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 outcomes were assessed using continuous data (with associations assessed using student t-test, ANOVA, logistic regression and area under the ROC curve; AUROC), and, since no cut-offs are defined in current literature, after categorisation into three groups of equal size (low/intermediate/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) or <45 years (1); male (0) or female (1); HBsAg >25,000 (0), >7,500-≤25,000 (1), >1,250-≤7,500 (2) or ≤1,250 IU/mL (4); HBV DNA >5 log (0) or ≤5 log IU/mL (2) and ALT reatio >1-7 (0) or either ≤1 or >7 xULN (1). Scores were categorised 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 backward selection based logistic regression model. Differences were considered statistically significant when $p < 0.05$. IBM SPSS for Windows version 25.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Graph Pad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA) was used for graphical representation of the results.

RESULTS

Patient characteristics

In total, we enrolled 390 patients; 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 Caucasian patients (76.4%), with genotypes A or D (respectively 37.9% and 39.0%). The HBeAg-negative *de novo* PEG-IFN cohort included predominantly Caucasian patients (94.2%), with genotype D (78.8%). The add-on PEG-IFN cohort included predominantly Asian patients (61.5%), with genotype A/B/C/D in 4.4/23.1/38.5/34.1%.

Anti-HBc levels correlate 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 pre-treatment serum IP-10 levels, but not with ALT. Negative correlations were observed with markers of viral replication including with HBV DNA, HBcrAg, HBsAg and HBV RNA levels (Figure 1A). Serum anti-HBc levels did not correlate 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 patients with HBV genotype D: 3.98 log vs 3.61 log IU/mL ($p < 0.001$) among HBeAg-positive and 4.44 log vs 4.16 log IU/mL ($p = 0.036$) among HBeAg-negative patients (Supplementary Figure 2).

Serum anti-HBc levels correlate with intrahepatic inflammatory activity

Among the 253 patients with pre-treatment liver histology data available, anti-HBc levels correlated with the severity of inflammatory activity ($r = 0.38$ for HBeAg-positive and $r = 0.36$ for HBeAg-negative patients, $p < 0.001$, Figure 1). Among the 89 patients with the lowest pre-treatment anti-HBc levels, only 3 patients (3.4%) had moderate to severe inflammation (HAI 9-18) compared to 11/80 (13.8%) with the highest anti-HBc levels ($p < 0.001$, Figure 2;

AUROC 0.666, 95% CI 0.550 – 0.781, p = 0.014). Similar results were obtained in multivariable logistic regression (aOR for moderate-severe inflammation: 1.24, 95% CI 1.04 – 1.48, p = 0.015).

Serum anti-HBc levels decrease during PEG-IFN based antiviral therapy

Baseline anti-HBc levels were higher in untreated patients (i.e., the *de novo* PEG-IFN patients, 3.93 log IU/mL (±0.47)) when compared to patients on NA therapy (i.e. add-on PEG-IFN patients, 2.88 log IU/mL (±0.73); p < 0.001). Furthermore, PEG-IFN therapy significantly reduced serum anti-HBc levels: mean declines from baseline to EOT were 0.25 log (±0.36) among HBeAg-positive patients treated with *de novo* PEG-IFN, 0.47 log (±0.41) in HBeAg-negative patients treated with *de novo* PEG-IFN, and 0.29 log (±0.28) among patients who received add-on PEG-IFN (p < 0.001).

Higher pre-treatment anti-HBc levels are associated with favourable treatment outcomes

De novo PEG-IFN

Pre-treatment anti-HBc levels were higher in patients with favourable outcomes after PEG-IFN therapy (Figure 3 and 4). Patients with the highest anti-HBc levels achieved sustained response in 35% and HBsAg loss in 13%, compared to 13% and 2% among patients with the lowest anti-HBc levels (p ≤ 0.004; Figure 3). Interestingly, HBeAg-positive patients with on-treatment ALT flares had higher pre-treatment anti-HBc levels (Figure 4).

The association between higher anti-HBc levels and favourable treatment outcomes were 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*. 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 patients without subsequent HBeAg loss (3.12 log versus 2.84 log IU/mL, p = 0.012). Anti-HBc levels did not predict on-treatment HBsAg decline. None of the patients in the PEG-IFN add-on cohort achieved HBsAg loss.

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 correlated with other immune markers, such as IP-10, and were associated with more severe liver inflammation on liver biopsy. Furthermore, higher pre-treatment anti-HBc levels were associated with favourable 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 (i.e. HBsAg loss) among adults.[2,22] However, among CHB patients 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 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 on liver biopsy. Higher anti-HBc levels were also associated with lower levels of markers of viral replication and cccDNA transcriptional 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) correlate poorly with liver histology.[6] High serum anti-HBc levels may be reflective of having increased degrees of liver inflammatory activity, which could potentially influence decision making regarding initiation of antiviral therapy or performing 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 on NA therapy had the lowest anti-HBc levels in the cohort. These findings are in line with previous studies which 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 impact anti-HBc levels although the exact mechanism is unclear and may differ for PEG-IFN versus 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 favourable outcomes after treatment with PEG-IFN. Among patients treated with *de novo* PEG-IFN, findings were consistent for multiple endpoints, 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 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 pre-treatment anti-HBc levels were also associated with a higher chance of on-treatment ALT flares, which previous studies have shown to be pivotal in 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 pre-treatment 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 multiviate analysis and when anti-HBc levels were combined in the baseline scoring system[20] including age, sex, 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 randomised controlled trials, stratification by HBeAg status resulted in limited numbers of subjects and events per subgroup, increasing the risk of type 2 statistical error. However, the association between higher anti-HBc levels and favourable outcomes after antiviral therapy was consistent across sub-cohorts, supporting the robustness of our findings (Figure 4, Supplementary Figure 3). Furthermore, the anti-HBc assay we applied assessed only IgG anti-HBc, and whether there is a difference in diagnostic performance with assays that also measure IgM anti-HBc is yet unclear. Also, it is 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/genotypes is therefore warranted.

In conclusion, our study shows that serum anti-HBc levels correlate with intrahepatic inflammatory activity. Higher serum anti-HBc levels are associated with favourable 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.

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List of abbreviations

Anti-HBc, antibodies to hepatitis B core antigen; ALT, alanine aminotransferase; CHB, chronic hepatitis B; cccDNA, covalently closed circular DNA; c/mL, copies/millilitre; EOT, end of treatment; EOF, six months after PEG-IFN treatment withdrawal; ETV, entacavir; HBV, hepatitis B virus; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; IU/mL, international units/millilitre; LAM, lamivudine; LLOD, lower limit of detection; NA(s), nucleos(t)ide analogue(s); NK cells, natural killer cells; OR, odds ratio; PCR, polymerase chain reaction; PEG-IFN, peginterferon; RACE, rapid amplification of cDNA ends; RBV, ribavirin; SR, sustained response; ULN, upper limit of normal; U/mL, units/millilitre.

FIGURE LEGENDS

Figure 1. Correlation between anti-HBc levels with age, ALT, histological activity index and markers of viral replication among HBeAg-positive (A) and HBeAg-negative (B) patients

Abbreviations: anti-HBc, antibodies against hepatitis B core antigen; ALT, alanine aminotransferase; c/ml, copies/millilitre; IU/mL, international units/millilitre; HAI, histological activity index; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; HBV hepatitis B virus; IP-10, interferon-y inducible protein 10.

Figure 2. Relationship between anti-HBc levels and intrahepatic inflammatory activity

Liver inflammation was defined as no inflammation (HAI 0-3), mild inflammation (HAI 4-8), and moderate-severe inflammation (HAI 9-18). Anti-HBc levels were categorised as low, intermediate or high (<3.82/3.82-4.0/≥4.0 log IU/mL for HBeAg-positive and <3.95/3.95-4.40/≥4.40 log IU/mL for HBeAg-negative patients) to create 3 groups of equal size.

Abbreviations: anti-HBc, antibodies against hepatitis B core antigen; HAI, histology histological activity index; EOT, end of PEG-IFN treatment; IU/mL, international units/millilitre

Figure 3. Treatment outcome according to pre-treatment anti-HBc level.

Anti-HBc levels were categorised as low, intermediate or high (<3.82/3.82-4.0/≥4.0 log IU/mL for HBeAg-positive and <3.95/3.95-4.40/≥4.40 log IU/mL for HBeAg-negative patients) to create 3 groups of equal size. An on-treatment ALT flare was defined as an increase of serum ALT ≥ 5x ULN during PEG-IFN treatment. HBsAg decline was defined as a decline ≥ 1 log at EOT. Sustained response was defined as HBV DNA levels of < 2,000 IU/mL six months after end of PEG-IFN treatment. HBsAg loss was defined as HBsAg clearance at EOF.

Abbreviations: EOT, end of PEG-IFN treatment; EOF, six months after PEG-IFN treatment withdrawal; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peginterferon; int, intermediate; IU/mL, international units/millilitre

Figure 4. Pre-treatment anti-HBc levels according to treatment response.

An ALT flare was defined as an increase of serum ALT ≥ 5x ULN during PEG-IFN treatment. HBsAg decline was defined as a decline of ≥ 1 log at EOT. Sustained response was defined as HBV DNA levels of < 2,000 IU/mL six months after end of PEG-IFN treatment.

Abbreviations: anti-HBc, antibodies against hepatitis B core antigen; EOT, end of PEG-IFN treatment; EOF, end of follow-up (i.e. six months after PEG-IFN treatment withdrawal); ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peginterferon; IU/mL, international units/millilitre.

Figure 5. Treatment outcome according to pre-treatment anti-HBc levels and predicted probability

The predicted probability was based on the baseline prediction model including age, sex, HBsAg, HBV DNA and ALT levels.[20] Anti-HBc levels were categorised as low versus high; <4.0 versus ≥4.0 log for HBeAg-positive patients and <4.40 versus ≥4.40 log for HBeAg-negative patients. Sustained response was defined as HBV DNA <2,000 IU/mL six months after end of treatment. HBsAg loss was defined as loss of HBsAg six months after end of treatment.

Abbreviations: HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peginterferon; IU/mL, international units/millilitre.

	***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, years (median, IQR)	33 (25-44)	41 (33-49)	30 (24-38)
Male (n, %)	153 (78.5)	75 (72.1)	65 (71.4)
Race (n, %)			
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 (n, %)			
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)	-
Pre-treatment Liver inflammation (HAI score; n, %)			
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 (n, %)			
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)
Laboratory results BL			
ALT$^{\alpha}$ (median, IQR)	130 (89-186)	94 (65-183)	102 (63-169)
Anti-HBc‡ (mean, ±SD)	3.80 (±0.46)	4.16 (±0.39)	2.88 (±0.73)
HBsAg‡ (mean, ±SD)	4.41 (±0.60)	3.86 (±0.50)	3.72 (±0.66)
HBV DNA‡ (mean, ±SD)	8.37 (±0.83)	6.08 (±1.21)	2.74 (±1.49)
HBV RNA$^{\pi}$ (mean, ±SD)	6.79 (±1.11)	4.38 (±0.98)	4.85 (±1.50)
HBcrAg$^{\Sigma}$ (mean, ±SD)	8.35 (±0.70)	5.00 (±1.42)	8.11 (±0.76)
Treatment response (n, %)			
On-treatment ALT flares$^{\infty}$	102/194 (52.6)	48/103 (46.6)	6/90 (6.7)
HBeAg loss EOT$^{\Omega}$	78 (40.0)	-	16/90 (17.8)
HBsAg decline EOT (≥ 1 log)	53/174 (30.5)	19/102 (18.6)	8/89 (9.0)
Sustained response$^{\beta}$	37/170 (21.8)	25/95 (26.3)	-
HBsAg loss$^{¥}$	16/173 (9.2)	1/100 (1.0)	3 (3.3)

Table 1. Patient characteristics of the patients with pre-treatment anti-HBc

$^{\alpha}$ *U/L*

‡ *Logarithmic scale, IU/mL*

[π] Logarithmic scale, copies/mL

[Σ] Logarithmic scale, U/mL

[∞] On treatment ALT flare is defined as ALT >5x the upper limit of normal during PEG-IFN therapy.

[Ω] HBeAg loss at end of treatment in pre-treatment HBeAg-positive patients

[β] Sustained response was defined as HBV DNA < 2,000 IU/mL six months after end of PEG-IFN treatment

[¥] HBsAg loss was defined as HBsAg clearance at EOF.

Abbreviations: HBV, hepatitis B virus; PEG-IFN, peginterferon; LAM, lamivudine; RBV, ribavirin; NA, nucleos(t)ide analogue; anti-HBc, antibodies against hepatitis B core antigen; HBcrAg, Hepatitis B core related Antigen; HBeAg, Hepatitis B e Antigen; HBsAg, quantitative hepatitis B surface antigen; EOF, end of follow-up (i.e. six months after PEG-IFN treatment withdrawal); EOT, end of PEG-IFN treatment; IQR, interquartile range; c/mL, copies/millilitre; IU/mL, international units/millilitre

Table 2. Association between anti-HBc and treatment outcomes in multivariable analysis among *de novo* PEG-IFN patients.

	All			HBeAg-positive			HBeAg-negative		
	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	0.014	1.12	1.02 – 1.23	0.016	1.05	0.92 – 1.20	0.497
HBsAg decline EOT	1.18	1.07 – 1.31	0.001	1.14	1.00 – 1.31	0.058	1.19	1.03 – 1.37	0.017
HBeAg loss EOT	1.13	1.00 – 1.28	0.049	1.13	1.00 – 1.28	0.049	-	-	-
Sustained response	1.13	1.04 – 1.23	0.006	1.30	1.01 – 1.66	0.040	1.09	0.96 – 1.24	0.177
HBsAg loss	1.37	0.95 – 1.98	0.091	1.27	0.86 – 1.88	0.227	-*	-	-

On-treatment ALT flare was defined as an ALT level ≥ 5x ULN during PEG-IFN treatment. HBsAg decline was defined as a decline of ≥ 1 log six months at EOT. Sustained response was defined as HBV DNA levels of < 2,000 IU/mL six months after end of PEG-IFN treatment. HBsAg loss was defined as loss of HBsAg at any time during treatment or off-treatment follow-up. *Insufficient number of events for multivariable analysis.

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

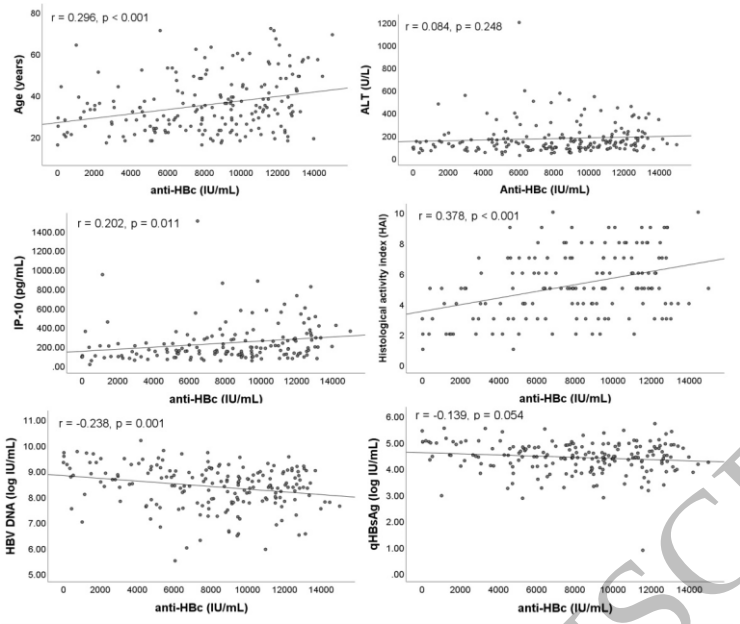

Figure 1A

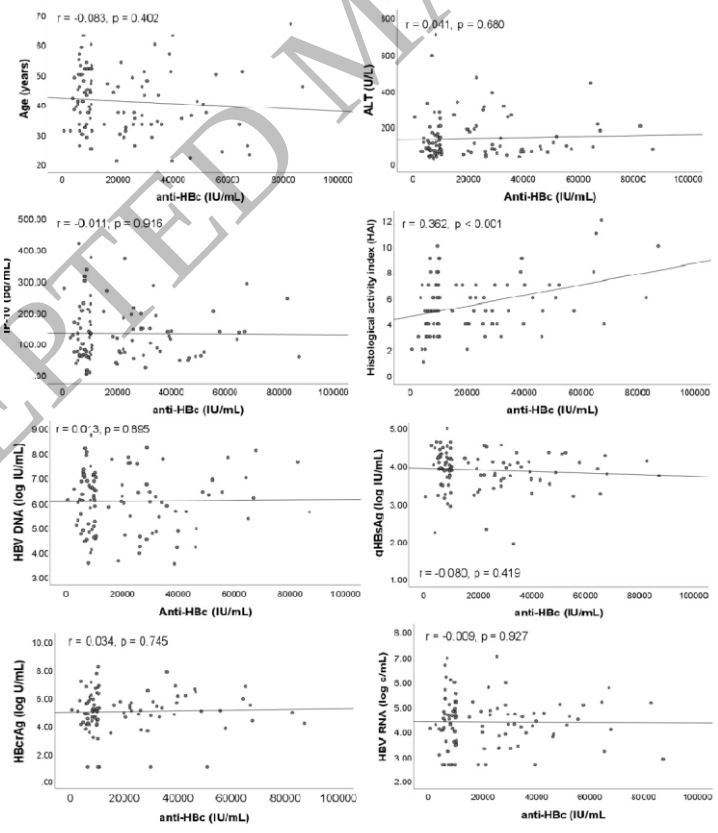

Figure 1B

1

2

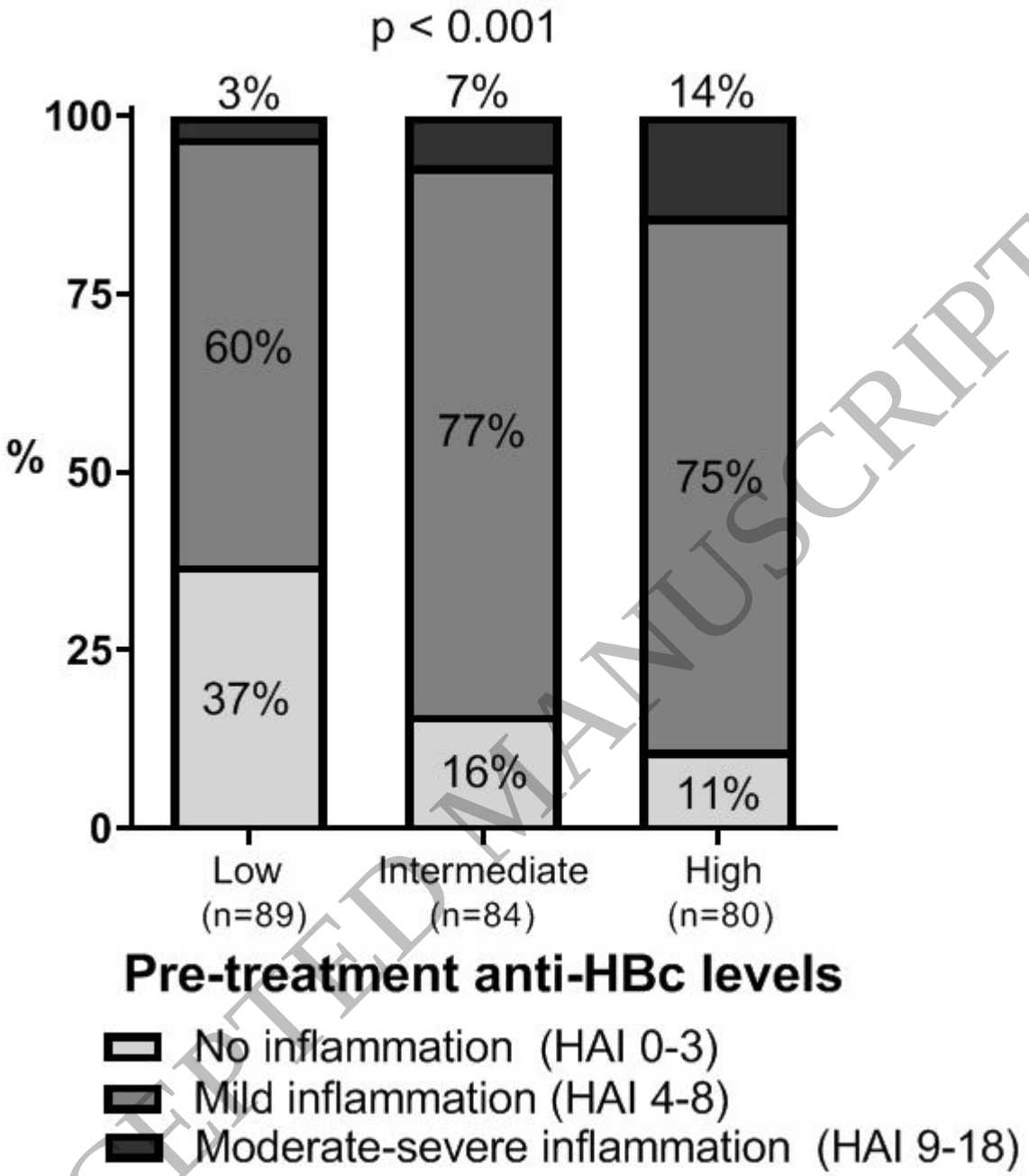

Figure 2

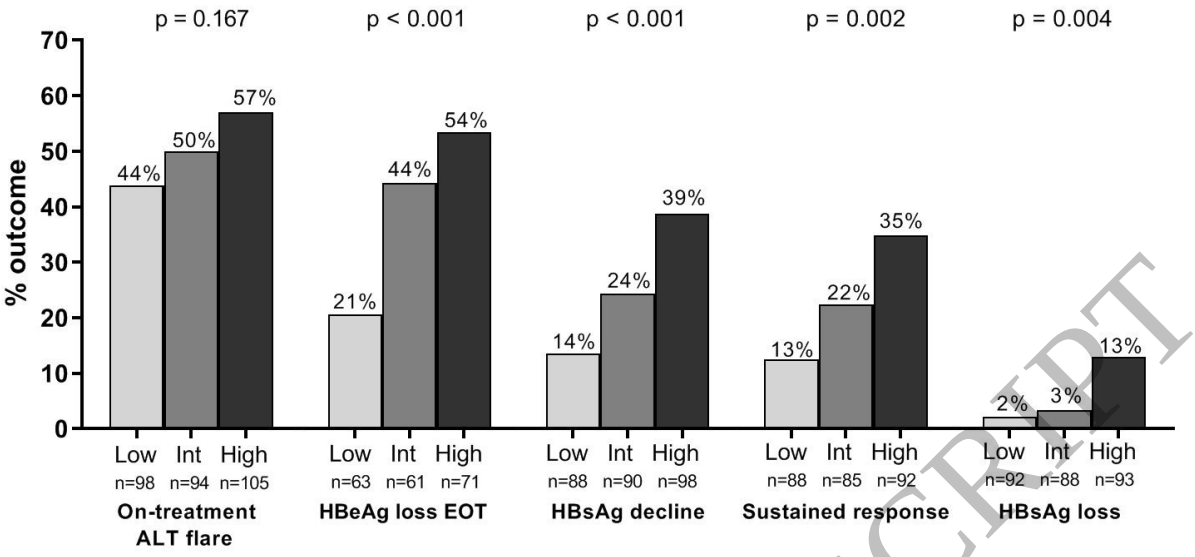

Figure 3

	%	Mean anti-HBc (log IU/mL) No event	Mean anti-HBc (log IU/mL) Event	Mean difference (log IU/mL)	p-value
All patients					
On-treatment ALT flare	50.5	3.87 (±0.55)	3.98 (±0.36)		0.039
HBeAg loss EOT	40.0	3.72 (±0.53)	3.92 (±0.27)		0.001
HBsAg decline EOT (1 log)	26.1	3.91 (±0.45)	4.05 (±0.33)		0.023
Sustained response	23.4	3.88 (±0.51)	4.08 (±0.30)		<0.001
HBsAg loss	6.2	3.92 (±0.49)	4.05 (±0.23)		0.279
HBeAg-positive patients					
On-treatment flare	52.6	3.71 (±0.59)	3.88 (±0.28)		0.012
HBeAg loss EOT	40.0	3.72 (±0.53)	3.92 (±0.27)		0.001
HBsAg decline EOT (1 log)	30.5	3.78 (±0.43)	3.93 (±0.24)		0.003
Sustained response	21.8	3.74 (±0.52)	3.99 (±0.17)		<0.001
HBsAg loss	9.2	3.77 (±0.49)	4.00 (±0.14)		0.060
HBeAg-negative patients					
On-treatment flare	48.0	4.13 (±0.36)	4.19 (±0.42)		0.442
HBsAg decline EOT (1 log)	18.6	4.11 (±0.38)	4.36 (±0.37)		0.013
Sustained response	26.3	4.14 (±0.39)	4.23 (±0.38)		0.312
HBsAg loss	1.0	4.15 (±0.38)	4.76		0.117

Figure 4

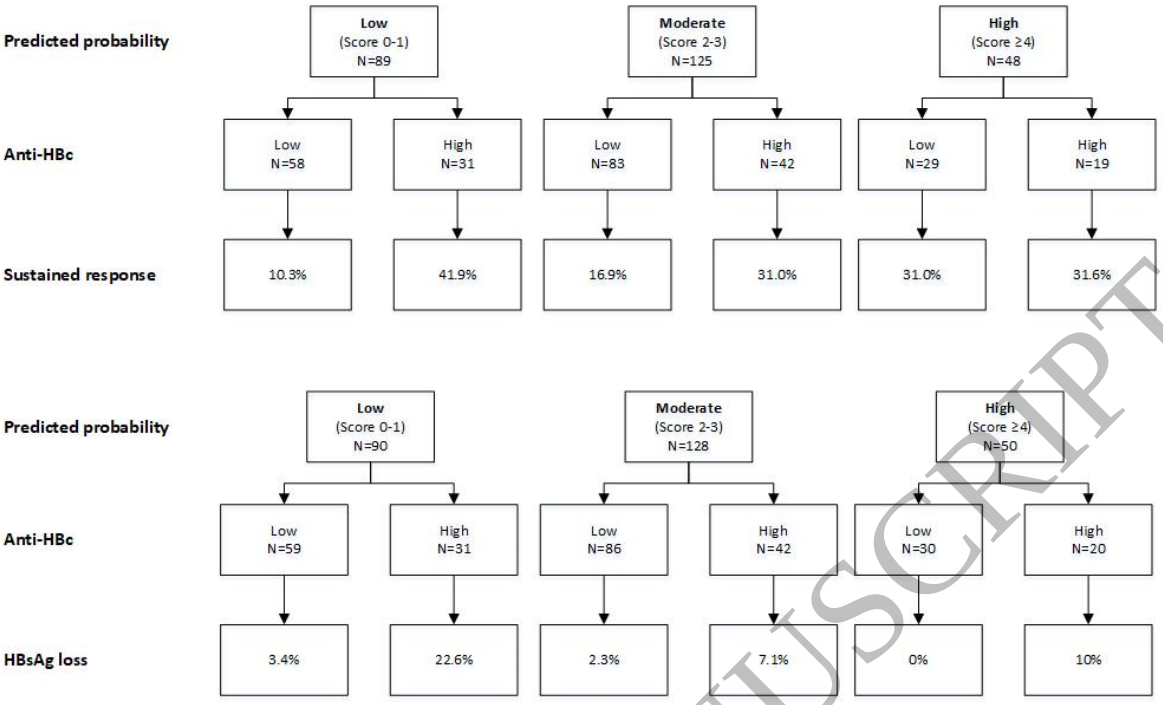

Figure 5