



## Short communication

# Serum HBsAg changes in HBeAg positive chronic hepatitis B patients with continuous viral load reductions during treatment with adefovir or peg-interferon- $\alpha$ -2a

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## ABSTRACT

Hepatitis B surface antigen (HBsAg) loss under antiviral therapy is rare in chronic hepatitis B patients and the dynamics of serum HBsAg in these patients are not available. The changes in serum HBsAg following treatment with adefovir ( $n = 31$ ) or peg-interferon- $\alpha$ -2a ( $n = 23$ ) were studied in hepatitis B e-antigen (HBeAg) positive chronic hepatitis B patients. Abbott Architect HBsAg assay was used to quantify serum HBsAg. HBsAg levels were significantly decreased during the first 12 weeks of treatment with median change of  $-397.0$  IU/ml and  $-555.4$  IU/ml, respectively for adefovir and peg-interferon- $\alpha$ -2a ( $p = 0.005$  and  $0.001$ , respectively). Beyond 12 weeks, no further significant HBsAg reductions were found even in patients with sustained viral replication inhibition in either group. Three distinct patterns of HBsAg changes were observed in most patients in both treatment groups: biphasic pattern (rapid HBsAg reduction from baseline to week 12); assurgent pattern (higher HBsAg level at week 12 than at baseline); and wavy pattern (HBsAg reduction from baseline to week 12, followed by relapse at week 24 or week 28). These results might offer insights into the possible mechanism(s) underlying the unusual occurrences of HBsAg loss under antiviral therapy.

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Currently, HBV surface antigen (HBsAg) seroconversion is suggested as the marker of complete response to antiviral therapies in chronic hepatitis B (CHB) patients. However, HBsAg seroconversion rate under approved antiviral therapies is very low (Hoofnagle et al., 2007), even though standard interferon- $\alpha$  (Hess et al., 1987; Janssen et al., 1994; Rang et al., 1999), peg-interferon- $\alpha$  (Wursthorst et al., 2006), lamivudine (Kohmoto et al., 2005) and adefovir (ADV) (Werle-Lapostolle et al., 2004) have been associated with decreases in serum HBsAg levels. Currently, there are no detailed kinetic data on serum HBsAg changes in the early stage of antiviral therapies. Therefore, the availability of such data would be valuable for improving our understanding of why the HBsAg seroconversion rate is low under currently available antiviral treatments.

For this purpose, we studied 54 HBeAg positive CHB patients, which included 31 treated with ADV (Zeng et al., 2006) and 23 with peg-interferon- $\alpha$ -2a (PEG-IFN) (Lau et al., 2005) (Table 1). Serum HBV DNA levels were determined with Cobas Amplicor

HBV Monitor Test in both treatment groups (Lau et al., 2005; Zeng et al., 2006). Sera samples were collected at baseline, weeks 12, 28 (ADV)/24 (PEG-IFN), and 48, and were stored at  $-40^{\circ}\text{C}$  prior to HBsAg quantification using the Architect HBsAg assay (Abbott Laboratories, Abbott Park, IL) according to the manufacturer's recommendation. This assay is a two-step immunoassay using chemiluminescent microparticle technology for the quantitative determination of HBsAg in human serum and plasma (Chen et al., 2004; Deguchi et al., 2004; Kohmoto et al., 2005). Continuous variables were expressed as median (range) due to a skewed distribution. Wilcoxon signed rank test and Pearson chi-square test were performed as appropriate using SPSS 10.0 software (SPSS Inc, Chicago, IL). A  $p$ -value less than 0.05 (two-sided) was considered to be significant.

At the end of the 48 weeks observation, no patient in either treatment group had HBsAg loss, whereas 3 and 6 patients achieved HBeAg loss in the ADV and PEG-IFN treatment group, respectively.

In the ADV group, all 31 patients showed viral load reductions within the initial 12 weeks of treatment ( $p < 0.001$ ). HBsAg levels were significantly decreased from baseline to week 12 with a median change of  $-397.0$  IU/ml (the median reduction was 41.2%,  $p = 0.005$ ), although only 64.5% ( $n = 20$ ) of patients had a lower serum HBsAg level at week 12 compared to baseline. In the fol-

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**Table 1**

Clinical and virological profiles of patients at baseline.

Parameters	ADV group	PEG-IFN group
Total number of patients	31	23
Male patients, N (%)	27 (87.1%)	20 (87.0%)
Age* (years)	32 (19–48)	33 (18–52)
ALT* (ULN)	3.05 (1.50–8.88)	4.40 (1.50–8.73)
HBV DNA* (log <sub>10</sub> copies/ml)	8.3 (6.4–13.2)	8.0 (5.7–13.6)
Serum HBsAg* (IU/ml)	2645.4 (4.5–59 613.1)	2426.7 (7.0–43 352.3)

\* All data shown as median (range).

lowing 16 weeks of treatment, all 31 patients showed continuous viral suppression; however, the serum HBsAg changes from week 12 to 28 were not significant (median change was  $-32.4$  IU/ml,  $p=0.087$ ). The changes of serum HBsAg and HBV DNA from treatment weeks 12 to 28 were 13.6% and 0.08% respectively, when compared to those within the first 12 treatment weeks (median,  $p=0.012$  and  $<0.001$ , respectively). No further HBsAg reduction was found ( $p=0.559$ ) during the last 20 weeks (from week 28 to 48) in 22 patients with persistent viral suppression (Fig. 1A). Serum ALT levels in 18 of these 22 patients normalized at week 48 with a median value of 0.63 (ULN) (Fig. 1A).

Similarly, all patients in the PEG-IFN group showed significant viral load reductions ( $p<0.001$ ). HBsAg levels were also significantly decreased from baseline to week 12 with a median change of  $-555.4$  IU/ml (the median reduction was 46.4%,  $p=0.001$ ), although only 69.6% (16/23) of patients had a lower serum HBsAg at week 12 compared to baseline. There was no significant reduction of serum HBsAg levels within the second 12 treatment weeks (median change was  $-56.8$  IU/ml,  $p=0.067$ ) in 23 patients who had persistent decreases of viral loads. The changes of HBsAg and serum HBV DNA within the second 12 weeks were 9.2% and 0.09%, respectively, when compared to those within the first 12 weeks (median,

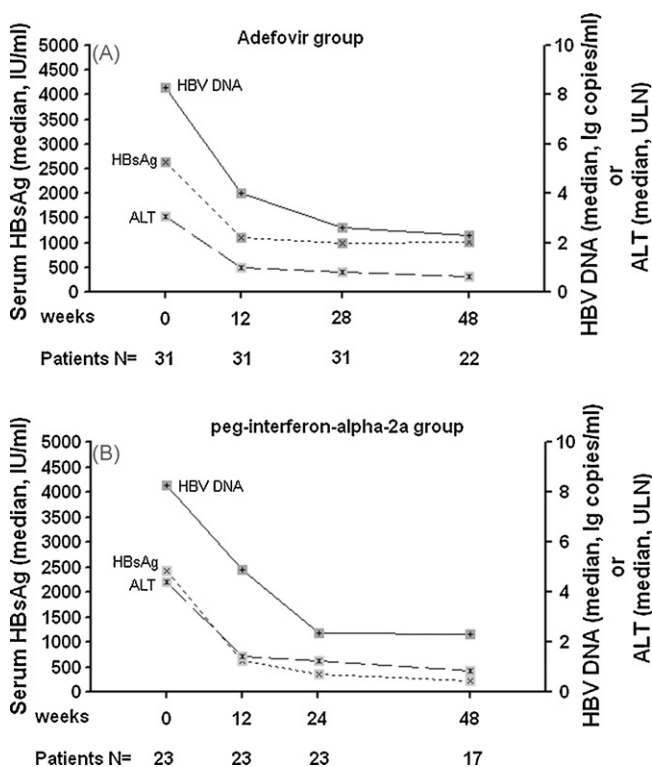
$p=0.001$  and  $<0.001$ , respectively). In 17 patients without even slight viral rebounds, there was no further HBsAg reduction within the last 24 weeks ( $p=0.313$ , Fig. 1B). ALT level at baseline was 4.40 (ULN, median) and decreased to 0.84 (ULN, median) at week 48 with 14/17 patients gaining normalized ALT.

The above results could suggest that, collectively, there are two phases of serum HBsAg reductions: rapid phase (first 12 weeks) and slow phase (following period) under either ADV or PEG-IFN treatment. This may be helpful in understanding why HBsAg seroconversion usually occurs late and infrequently. Considering the much higher HBsAg levels at baseline, the low seroconversion rate of serum HBsAg in CHB patients with short-term antiviral therapy is not unexpected.

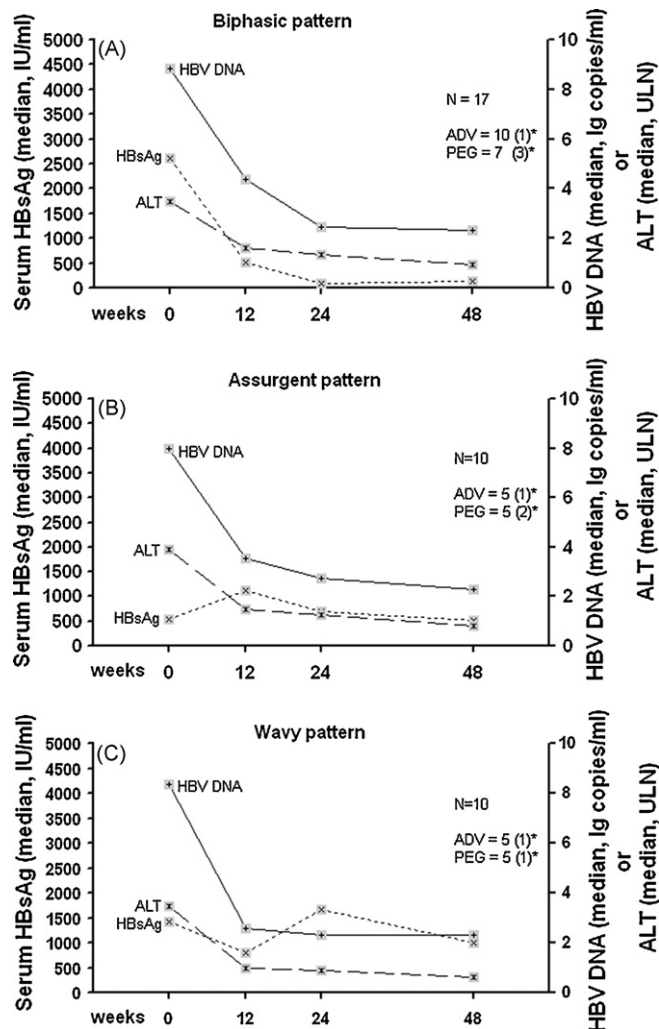
Serum HBsAg reductions have been associated with reductions in intrahepatic HBV DNA or cccDNA (Werle-Lapostolle et al., 2004; Wursthorn et al., 2006). In this study, we found that serum HBV DNA were decreased rapidly and predominantly during the first 12 treatment weeks in both groups, consistent with results from several global trials for ADV (Hadziyannis et al., 2003; Marcellin et al., 2003; Peters et al., 2004) or PEG-IFN (Lau et al., 2005; Marcellin et al., 2004). Patients treated with ADV showed two phases of HBV viral load decline (Mihm et al., 2005; Tsiang et al., 1999), whereas patients treated with PEG-IFN showed multiple phases of decline (Colombatto et al., 2006; ter Borg et al., 2006). Therefore, fast and strong inhibition of viral replication within the first 12 treatment weeks could be the main factor underlying the fast phase of HBsAg reduction. The second phase of serum HBsAg reductions during antiviral treatment could be attributed to the slow decline of HBV cccDNA (Werle-Lapostolle et al., 2004; Wong et al., 2006; Wursthorn et al., 2006), which is the template of transcription of mRNAs of HBV envelope genes.

In patients with continuous viral load reductions, three patterns of serum HBsAg changes under either ADV ( $n=22$ ) or PEG-IFN ( $n=17$ ) were observed (Fig. 2). The biphasic pattern, defined as a rapid reduction of serum HBsAg from baseline to week 12 and insignificant changes afterwards, was found in 43.6% (17/39) patients (Fig. 2A). The assurgent pattern, defined as higher HBsAg level at week 12 than at baseline with minimum increase of 13.98 IU/ml, was found in 10 (25.6%) patients (Fig. 2B). The wavy pattern, defined as a decrease in HBsAg levels from baseline to week 12 followed by a relapse at week 24/28 with minimum increase of 121.43 IU/ml, was found in another 10 patients (Fig. 2C). ADV or PEG-IFN treatment was not associated with those patterns ( $p=0.886$ , Pearson chi-square test). 'Staircase-like' HBsAg reductions were found in two patients and could not be classified under any of these three patterns. ALT evolution patterns appeared to be related with reductions of HBV DNA load but were not correlated with these three distinct patterns of HBsAg changes (Fig. 2).

Despite the tremendous serum HBsAg reduction in the fast phase, the slow phase could be the predominant factor which determines the low and late occurrences of HBsAg loss in patients with the biphasic pattern. For patients with the assurgent or wavy pattern, it is less likely for HBsAg loss to occur under short-term antiviral treatment. In our study, two patients who had HBsAg level below 10 IU/ml at baseline showed the wavy pattern and unfortunately did not achieve HBsAg loss during the 48 weeks of treatment. Similar studies are warranted in larger cohort of patients under long-term nucleos(t)ide analogues (adefovir, lamivudine, entecavir or telbivudine) or prolonged pegylated interferon treatment to uncover all possible patterns of serum HBsAg changes and their associations with HBeAg loss. Our pilot study did not support the association of HBsAg change patterns with HBeAg loss ( $p=0.868$ , Pearson chi-square test, Fig. 2). It is also of interest to study the possible association of HBsAg change patterns with occurrence of HBsAg loss which was found to be increased to 8% at 3-year treat-



**Fig. 1.** Changes of serum HBsAg, HBV DNA, and ALT in HBeAg positive CHB patients undergoing (A) ADV or (B) PEG-IFN treatment. Numbers below X-axis represent the number of patients with continuously decreasing HBV DNA levels.



**Fig. 2.** Three patterns of serum HBsAg changes in HBeAg positive CHB patients with continuously declining HBV DNA load undergoing ADV or PEG-IFN treatment: (A) biphasic pattern, (B) assurgent pattern and (C) wavy pattern. Serum HBV DNA and ALT levels are shown simultaneously. Data at week 28 (ADV group) or week 24 (PEG-IFN group) were combined and shown at week 24. Patient numbers (N) indicating the total number of patients for each pattern together with the number of patients in the ADV or PEG-IFN group are shown on the left of the Y-axis. The number in parentheses represents the number of patients with HBeAg loss at week 48.

ment with PEG-IFN (Marcellin et al., 2007) or to 1% with 4-year ADV treatment (Mao et al., 2008).

Additionally, the existence of three distinct patterns of HBsAg changes in patients with similar viral load changes suggest that viral or host factors other than anti-HBV therapy regimens or ALT evolutions, might exist and determine the discordant dynamics between serum HBsAg and HBV DNA under antiviral treatment.

In conclusion, biphasic reductions of serum HBsAg (collectively) and three distinct patterns of HBsAg changes (individually) were observed in HBeAg positive CHB patients who achieved continuous viral load reductions under ADV or PEG-IFN treatment. These patterns may offer important insights into the low occurrences of HBsAg loss under antiviral treatment.

#### Disclosure statement

None of the authors declares financial relationships with companies that produce drugs or diagnostic reagents used in this study.

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