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ORIGINAL ARTICLE

# Correlation of Quantitative Assay of HBsAg and HBV DNA Levels During Chronic HBV Treatment

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**Abstract** *Background and aim* Viral load is used for the diagnosis and monitoring the treatment of chronic hepatitis B (CHB). These methods are molecular-based and are expensive. Previous studies suggest that quantitative hepatitis B surface antigen (HBsAg) studied by automated chemiluminescent microparticle immunoassay can be a surrogate marker. In this study, we aimed to investigate whether quantitative HBsAg correlates hepatitis B virus (HBV) DNA levels during CHB treatment. *Methods* The study included 18 patients (13 male, 5 female, mean age: 33 ± 9 years) with CHB. They were given pegylated interferon ± lamivudine for 52 months and serum samples were obtained in weeks 0, 4, 8, 24, 48, 52, and 76. HBV DNA was measured by TaqMan polymerase chain reaction (PCR; Erasmus MC, University Medical Center, Rotterdam, The Netherlands). Quantitative HBsAg was studied by automated chemiluminescent microparticle immunoassay (Architect HBsAg, Abbott, IL). *Results* HBV DNA levels were measured as follows: 9.66, 7.69, 7.06, 5.93, 5.89, 5.88, and 7.27 logarithmic genome equivalent/ml, respectively. The corresponding HBsAg quantitation results were 42,888, 31,176, 37,882, 27,277, 28,279, 29,471, and 31,535 IU/ml, respectively. They showed a significant correlation (canonical correlation = 0.85). *Conclusions* HBsAg studied by automated chemiluminescent microparticle immunoassay correlates with HBV DNA and can be a surrogate marker during the monitoring of the efficacy of HBV treatment.

**Keywords** Chronic hepatitis B · HBsAg quantitation · Automated chemiluminescent microparticle immunoassay

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## Introduction

The development of serological assays to detect hepatitis B surface antigen (HBsAg) has played a major role in the diagnosis of hepatitis B virus (HBV) infection. With other hepatitis B serological assays, a diagnosis of acute or chronic HBV infection, past infection, or successful vaccination can be determined. The quantification of HBV DNA provides a means of monitoring the effectiveness of antiviral therapy and detecting the early development of antiviral drug resistance [1, 2]. Molecular diagnostics are also being applied to HBV-infected liver tissue. The use of molecular techniques to quantify intrahepatic HBV DNA and other key HBV replicative intermediates may provide additional options for monitoring and predicting treatment efficacy. Recent improvements in molecular technology permitted the detection of as few as 10 copies/ml of HBV DNA in serum, leading to redefinitions of chronic HBV infection, as well as the thresholds for antiviral treatment [3]. As the sensitivity of these molecular techniques continues to improve, the challenge will be to standardize these assays, as well as define clinically significant levels of HBV replication. On the other hand, the need to assess viral dynamics several times during the management of HBV treatment makes the cost a challenge. Considering the distribution of HBV especially in the developing world, an

inexpensive and easily performed surrogate marker for the molecular detection of HBV DNA can contribute much to the management of HBV infections in a more practical way.

The treatment of HBV is not successful in most of the cases. Interferon induces hepatitis B e antigen (HBeAg) and HBV DNA clearance in about a third of the patients [4, 5]. On the other hand, inhibitors of DNA polymerase, lamivudine, and adefovir dipivoxil reduce viral load profoundly [6]. However, responses to lamivudine are significantly less durable than those to interferon alfa [7] and drug resistance is almost inevitable in long-term use [8]. Therefore, the study design is based on the rationale that a combination of the immunomodulatory properties of interferon and the strong antiviral potency of lamivudine in a long-term treatment regimen might increase the rate of sustained response in chronic hepatitis B (CHB).

In this study, we aimed to investigate whether quantitative HBsAg correlates with HBV DNA levels during CHB treatment.

## Patients and Methods

HBeAg (+) CHB patients with no evidence of hepatocellular carcinoma or advanced liver diseases were included. These patients were enrolled in a randomized, multi-center trial published elsewhere [9]. Eighteen patients (13 male, five female, mean age: 33 ± 9 years) in our unit were further studied with the permission of the study center. According to the study protocol, HBeAg-positive patients with CHB were assigned combination therapy (pegylated interferon alfa-2b and lamivudine) or monotherapy (pegylated interferon alfa-2b and placebo) for 52 weeks.

The patients were randomly assigned at a one-to-one ratio to receive combination therapy with weekly doses of 100 μg of pegylated interferon alfa-2b (PegIntron, Schering-Plough, Kenilworth, NJ) and a daily dose of 100 mg of lamivudine (Zeffix, GlaxoSmithKline, Greenford, UK) or monotherapy with 100 μg/week of pegylated interferon alfa-2b and placebo, which was similar in appearance to lamivudine and was for daily administration. Patients of body weight 55 kg or less received weight-adjusted dosings of pegylated interferon alfa-2b (1.50 μg/kg weekly for the first 32 weeks and 0.75 μg/kg weekly for the remainder of the treatment period). Randomization was done centrally and stratified by the study center.

During weeks 32–52, the pegylated interferon dose was 50 μg/week in both treatment groups in order to limit the probability of early treatment discontinuation.

According to the study protocol, blood samples were obtained for biochemical studies and viral kinetics at the initial visit and at weeks 4, 8, 24, 48, 52, and 76. Another sample was obtained for HBsAg quantitation and was subsequently stored. HBV DNA was studied in-house using the TaqMan polymerase chain reaction (PCR) assay (Erasmus MC, University Medical Center, Rotterdam, The Netherlands). Biochemical studies were performed by automated techniques.

HBsAg quantitation was studied by automated chemiluminescent microparticle immunoassay (Architect HBsAg, Abbott, IL).

### Statistical Analysis

Categorical variables were compared by using the chi-square test. $P < 0.05$ was accepted as significant. The logarithmic equivalent of every HBV DNA result was calculated. Their correlation with quantitative HBsAg was studied by canonical correlation using the SPSS software package. Canonical correlation is a method to determine how sets of dependent variables are related with sets of independent variables [10]. It shows the strength of the relationship between the clusters.

## Results

When the treatment groups were clear at the end of follow-up, eight of our cases were seen to receive monotherapy (pegylated interferon alfa-2b and placebo) and the remaining ten to receive combination therapy (pegylated interferon alfa-2b and lamivudine).

Six out of 18 patients responded to the therapy. Five of our six responsive cases were seen to be treated with pegylated interferon + lamivudine, while five of 12 non-responders had been given pegylated interferon + lamivudine ($P = 0.12$).

HBV DNA levels were measured as follows: 9.66, 7.69, 7.06, 5.93, 5.89, 5.88, and 7.27 logarithmic genome equivalent/ml, respectively. The corresponding HBsAg quantitation results were 42,888, 31,176, 37,882, 27,277, 28,279, 29,471, and 31,535 IU/ml, respectively. They showed a significant correlation (canonical correlation = 0.85) (Fig. 1). This correlation was also seen in responsive (Fig. 2) and non-responsive (Fig. 3) cases.

Changes in the HBV DNA and HBsAg quantitation results did not differ significantly between the groups who were given combination therapy or monotherapy.

## Discussion

Three types of virus-derived particles can be identified in HBV-infected individuals. First, HBV virions or Dane particles, comprising an outer envelope composed of a

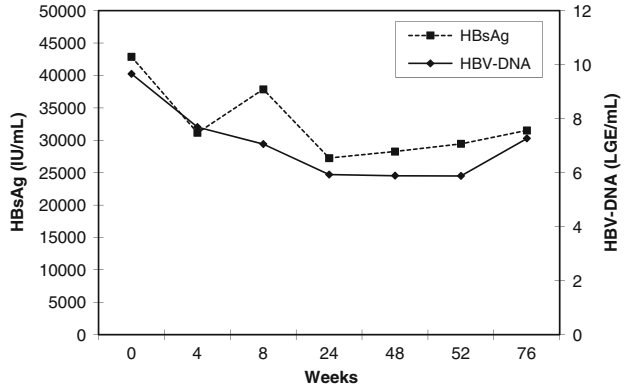


**Fig. 1** Concentration curves of hepatitis B surface antigen (HBsAg) quantitation and hepatitis B virus (HBV) DNA levels in all patients. LGE, logarithmic genome equivalent

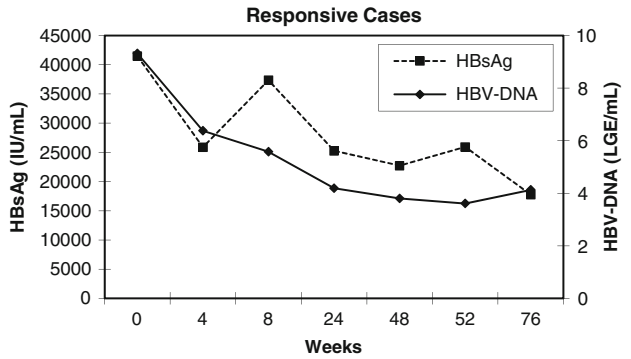


**Fig. 2** Concentration curves of HBsAg quantitation and HBV DNA levels in responsive patients. LGE, logarithmic genome equivalent

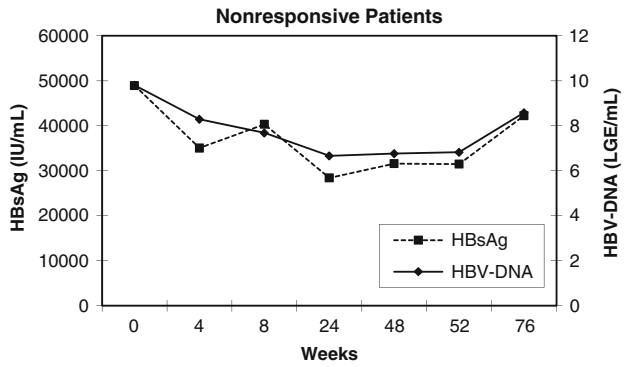


**Fig. 3** Concentration curves of HBsAg quantitation and HBV DNA levels in unresponsive patients. LGE, logarithmic genome equivalent

mixture of glycoproteins, known collectively as HBsAg. This envelope surrounds an inner nucleocapsid made up of the hepatitis B core antigen (HBcAg), which contains the circular partially double-stranded DNA genome. Secondly, spherical particles of nearly 22 nm in diameter and, thirdly, filamentous structures of nearly 20 to 22 nm in diameter and of variable length can also be identified. The spherical and filamentous particles consist of host cell lipid in combination with the virus-derived envelope HBsAg [1]. The particles are found in a $10^4$- to $10^6$-fold excess over the HBV virions, which is why HBsAg has proven to be such a good marker for hepatitis B infection [11]. The purified 22-nm particles are noninfectious but highly immunogenic, and were the active component of the original hepatitis B vaccine [11].

The monitoring of HBeAg and HBV DNA levels in serum is used for assessing the response to antiviral treatment. However, because of the possible presence of HBeAg-negative variants with mutations in the precore or basic core promoter region among some chronic HBV carriers, it is difficult to correlate the absence of HBeAg with the level of HBV replication. Several assays for serum HBV DNA are available. However, these methods involve cumbersome procedures and high costs, and may generate divergent results.

The Architect HBsAg QT is a simple, sensitive, specific, reproducible, and inexpensive method that produces results rapidly and accurately. In their pioneering study, Deguchi et al. [12] performed a quantitative study of HBsAg, HBeAg, HBV DNA, and HBV DNA polymerase in over 733 sera obtained from 43 CHB carriers. The serum HBsAg levels detected by Architect HBsAg QT were found to be higher in HBeAg-positive than in anti-HBe-positive HBV chronic carriers and correlated with the level of serum HBV DNA and HBV DNA polymerase. Chen et al. [13] studied HBsAg by the same method and attempted to reveal the correlation with HBV DNA levels in asymptomatic carriers. HBV DNA was determined by hybridization and PCR. HBsAg levels were shown to correlate with HBV DNA.

Gibb et al. [14] conducted two independent studies to evaluate the performance of two HBsAg immunoassay products performed on the Abbott Architect and Bayer ADVIA Centaur immunoassay analyzers. One was a retrospective study of 484 stored samples and the second was a prospective study of 349 samples from a random population. In their study, a number of discordant samples from HBsAg-positive patients were found, which led to the discovery of a number of HBsAg mutants. Following viral DNA sequencing, these were identified as HBsAg escape mutants. The inability of the Centaur assay to detect such mutants has been shown recently [15]. This data suggest that the Architect polyclonal/monoclonal-based HBsAg immunoassay is superior to the Centaur monoclonal-based immunoassay for the detection of these mutations.

Kohmoto et al. [16] tested the usefulness of a fully automated chemiluminescent microparticle immunoassay (Architect HBsAg QT) for monitoring the serum levels of HBV) during antiviral therapy and measured HBsAg in 20 patients (12 patients had HBeAg and eight did not) with CHB before and during lamivudine treatment. The HBsAg

concentration was significantly higher in HBeAg-positive than in HBeAg-negative patients, and there was a significant correlation between the HBsAg concentration and HBV DNA level. After the start of lamivudine therapy, the HBV DNA levels fell rapidly in all patients and so did the serum HBsAg concentrations, but to a lesser extent. Also, in some patients, the increase in HBsAg preceded the increase in HBV DNA.

Rodella et al. [17] quantitatively determined HBsAg and anti-HBc/IgM by chemiluminescent immunoassays (Abbott Architect) in patients with acute or CHB. In acute hepatitis, the levels of HBsAg were generally greater than 10,000 UI/ml and decreased sharply in the recovery phase. In 35 anti-HBe-positive chronic hepatitis cases, the HBsAg levels were generally lower than 10,000 UI/ml (mean: 2,655), whereas in five HBeAg-positive chronic hepatitis patients, the mean value was 78,756 UI/ml and 90% of specimens exceeded 10,000 UI/ml. They concluded that the quantitative determination of HBsAg and anti-HBc/IgM provides additional information, and may be useful in the differential diagnosis of acute and chronic HBV infections and in the follow-up of chronically infected patients.

In a recent study, Chan et al. [18] studied HBsAg quantitation, intrahepatic cccDNA, and HBV DNA in 26 HBeAg (+) patients treated with pegylated interferon (32-week) and lamivudine (2-year). At baseline, the serum HBsAg levels correlated well with the cccDNA and intrahepatic HBV DNA. Additionally, the reduction of HBsAg had a good correlation with the reduction in cccDNA and the reduction in logarithmic DNA.

Our HBsAg quantitation measurements by automated chemiluminescent microparticle immunoassay revealed a significant correlation with corresponding HBV DNA levels. We conclude that HBsAg quantitation can be a surrogate marker for viral load during the management of chronic HBV infection.

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