SIMULTANEOUS QUANTITATION OF SERUM HBV DNA AND HBeAg CAN DISTINGUISH BETWEEN SLOW AND FAST VIRAL RESPONSES TO ANTIVIRAL THERAPY IN PATIENTS WITH CHRONIC HEPATITIS B

Luiz Caetano DA SILVA, Maria Luiza da NOVA, Suzane Kioko ONO-NITA, João Renatto Rebello PINHO, Roberta SITNIK, Vera Aparecida dos SANTOS & Flair José CARRILHO

SUMMARY

Background: The quantitation of serum HBBeAg is not commonly used to monitor viral response to therapy in chronic hepatitis B. Methods: In this study, 21 patients receiving varying therapies were followed and their viral response monitored by concomitant viral load and HBBeAg quantitation in order to study the meaning and the kinetics of both parameters. Results: It was possible to distinguish between three different patterns of viral response. The first was characterized by a simultaneous decrease in serum HBV DNA and HBeAg. The second pattern was characterized by a decrease in serum HBBeAg but persistent detection of HBV DNA. The third pattern was characterized by undetectable HBV DNA with persistent HBeAg positivity, which points to a non-response (Pattern III-B) except when HBeAg levels showed a slow but steady drop, characterizing a “slow responder” patient (Pattern III-A). Conclusions: The first pattern is compatible with a viral response. A long-term HBeAg seropositivity with a slow and persistent decrease (Pattern III-A) is also compatible with a viral response and calls for a prolongation of anti-viral treatment.

KEYWORDS: Hepatitis B; Serological markers; Treatment response; Treatment follow-up.

INTRODUCTION

The management of chronic hepatitis B has been the subject of many reviews over the last few years. Updates to the recommendations for monitoring patients infected with hepatitis B virus (HBV) were recently published and, in them, as well as recent publications on this subject, serum Hepatitis e antigen (HBeAg) is considered only qualitatively with no evaluation of its expression (quantitation) as a tool for monitoring patients undergoing treatment.

According to KEEFE et al., the quantitation of HBeAg has not been widely used, is expensive, and available data about the predictive value of HBeAg serostatus remains limited. The same authors mention that approximately one third of patients with serum HBV DNA levels lower than 10^4 copies/mL will seroconvert, suggesting a close relationship between HBV DNA suppression and the likelihood of seroconversion. HBeAg-positive chronic hepatitis B, as considered by the classical form, is characterized by a stable high level viremia (10^5-10^6 HBV copies/mL), whereas HBeAg-negative hepatitis B is characterized by a less stable and severe viremia, or less than 10^4 copies/mL.

Several different types of quantitative assays based on signal or target amplification are available for viral DNA, including high sensitivity polymerase chain reaction (PCR)-based methods that measure serum HBV DNA levels with a wide dynamic range. Nowadays, real-time PCR assays are preferably used.

The maximum HBV DNA viral load detected in patients with chronic HBV is not well established and may depend on the quantitative technique used to measure it. Thus, CORDEN et al. measured HBV DNA with the Chiron Amplicor HBV DNA Monitor Assay and found that the viral load was between 4X10^6 and 1.2X10^7 copies/mL. In HBeAg-positive patients, they found HBV DNA levels of up to 10^6 copies/mL. By using in-house end-point dilution PCR based on Kaneko’s method, our group found levels of up to 3X10^6 copies/mL. Also, using real-time PCR, HO et al. found median levels of 1.5X10^4 copies/mL, and JARDI et al. found median a serumb HBV DNA viral load of 9.2X10^4 copies/mL.

For HBeAg-positive chronic hepatitis B patients, the main goals for treatment are HBeAg loss, seroconversion to anti-HBe, normalized alanineaminotransferase (ALT) levels, and the suppression of viremia to undetectable levels. However, it has been consistently observed that lamivudine (LAM) or adefovir dipivoxil (ADV) produces a rapid and dramatic decrease in viremia, but a lower proportion of patients exhibit serum HBeAg loss. This important aspect was also emphasized by
NEUMANN\textsuperscript{26}. It is worth mentioning that the production of HBeAg is not dependent on the formation of HBV DNA, but it reflects the level of HBV core/precore gene expression in the liver\textsuperscript{14}.

The role of HBeAg quantitation has already been addressed by some authors\textsuperscript{13,25}. PERILLO et al.\textsuperscript{28} found that the baseline HBeAg concentration is the best independent predictor of a patient’s response to interferon-alpha (IFN-\(\alpha\)) when compared to baseline HBV DNA levels. Furthermore, it was observed, in this study and another\textsuperscript{12,13} that a steep decline in the HBeAg concentration during the first weeks of therapy was predictive of an antiviral response. A long-term study on the serial quantitation of HBeAg demonstrated that a high serum level (> 100 IU/mL) at week 24 of therapy with peginterferon alpha-2a (Peg-IFN-\(\alpha\)2a) had an excellent negative predictive value; less than 5% of patients achieved HBeAg seroconversion at week 72.\textsuperscript{10}.

During LAM therapy, different types of changing patterns have been observed in relation to levels of HBeAg pretreatment, and they have been categorized into three groups: “decrescendo” (“falling”), “decrescendo-crescendo” (“falling-rising”), and “no changing” or “fluctuating”\textsuperscript{27,31}. These patterns determined by serial monitoring during LAM therapy allowed the prediction of the treatment response as well as early recognition of a viral breakthrough. However, a simultaneous determination of HBV DNA was not performed.

It is worth mentioning that, in HBeAg-positive patients, entecavir was found to be more potent than LAM and resulted in undetectable HBV DNA by PCR in 67% of patients at week 48, compared to 36% of patients treated with LAM\textsuperscript{4}. However, the HBeAg seroconversion rate was similar in the two groups, 21% and 18%. Despite these interesting and valuable observations, serial quantitative determinations of serum HBeAg have not been routinely carried out during the last few years and have not been mentioned in some recent recommendations\textsuperscript{20,25}.

Using serial quantitative PCR for HBV DNA and a simultaneous quantitative HBeAg assay, we previously observed an interesting dissociation between HBV DNA and HBeAg in some patients. Our aim is to show that a simultaneous decrease in HBV DNA and HBeAg levels, in addition to a slow but stepwise decreasing pattern of HBeAg levels in some patients, may predict the negative HBeAg response despite a very prolonged positive response during antiviral therapy. This is particularly important because serum HBV DNA levels may decrease to undetectable levels soon after initiating therapy with LAM or other drugs\textsuperscript{8,26,30,34} and can not be used further for monitoring the treatment response.

**PATIENTS AND METHODS**

**Patients:** Twenty-one HBeAg-positive patients with chronic hepatitis B were enrolled in the study (Table 1). Sixteen patients received LAM and five patients received IFN-\(\alpha\) plus LAM (Table 2).

In this phase of the study, we detected some patterns of the viral response (described below) in 11 of the 16 patients given LAM and in four of the five patients given IFN-\(\alpha\) plus LAM.

Nine patients who did not respond to antiviral therapy, and one who presented with a relapse after LAM withdrawal, were retreated as shown in Table 3. Three patients (5, 6, and 13) had to receive another series of therapy after the second trial.

Patient ages varied from nine to 65 years (mean 38 yr) and they were studied during a period of 34 to 133 months (Table 1). All patients had Hepatitis B surface antigen (HBsAg) in their serum for at least six months and detectable HBV DNA. Patients were excluded if they were also infected with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus (HIV). Patients who withdrew prematurely from the study or had only minimal lesions (inactive HBsAg carrier state) were also excluded. All patients gave informed consent for the study, which was approved by the Institutional Review Board (Hospital das Clínicas - University of São Paulo School of Medicine).

**Treatment schedules and primary endpoints:** Sixteen patients received monotherapy with oral LAM at a dose varying from 150 mg to 300 mg per day for 12 months and 150 mg/day thereafter as previously described.\textsuperscript{4} Three out of four patients with liver cirrhosis received 150 mg/day from the beginning (Table 2). Therapy with IFN-\(\alpha\) (3 to 5 MU three times a week) plus LAM (150 mg/day) was initially given to four patients (Table 2). Another patient was submitted to combined therapy with Peg-IFN-\(\alpha\)2b and LAM because of high serum levels of HBV DNA and HBeAg and a slight increase in ALT. In our experience, patients with such baseline values are prone to develop resistance to monotherapy with LAM\textsuperscript{8,9}. Such patients were included in the IFN-related group (Table 2). Overall, 21 series of antiviral therapy were used during this period (Table 2).

Nine LAM-resistant patients with mutations in the YMDD motif of polymerase gene, six with M204V, two with M204I, and one patient with both, were retreated (Table 3). Adefovir (10 mg/day) was given to three patients as a second series and one patient as a third series (Table 3). Pegylated interferon (Peg-IFN) was given to five patients, four received Peg-IFN-\(\alpha\)2b (1.5 μg/kg of body weight) and one patient received Peg-IFN-\(\alpha\)2a (180 μg), all in association with LAM. Finally, tenofovir was given at a dose of 300 mg/day to four unresponsive patients as a rescue drug.

As for the two patients who experienced a relapse after withdrawal of LAM, one (patient 2) presented with a spontaneous absence of serum...
HBeAg but had a persistence of HBV DNA during the non-treatment period and received a second series of LAM with subsequent clearance of the HBV DNA. The other patient (patient 9) was treated with Peg-IFN-α2b plus LAM and presented with an HBsAg/anti-HBs seroconversion. Finally, another patient non-responder to LAM (patient 16) received LAM associated to lobucavir as a rescue drug (withdrawn from the market).

As shown in Table 3, more than two therapeutic series had to be used in three patients (patients 5, 6, and 13). Overall, 15 series of antiviral therapy were used in the retreatment, resulting in a total number of 36 therapeutic series for the two phases. The primary endpoints were an inability to detect HBV DNA and the loss of HBeAg with seroconversion to anti-HBe.

Table 2
Demographic, baseline data, and patterns of HBV DNA and HBeAg responses to initial therapy with lamivudine (LAM) in 16 patients or interferon-alpha (IFN-α) plus LAM in five other patients with chronic hepatitis B (CH) or liver cirrhosis (LC)

<table>
<thead>
<tr>
<th>Patient/Diagnosis</th>
<th>Age/Sex</th>
<th>HBV DNA (log)</th>
<th>ALT* (XUNL)</th>
<th>First therapy</th>
<th>Viral response (VR) / (Mutation)**</th>
<th>Pattern</th>
<th>Follow-up (months)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/CH</td>
<td>37/M</td>
<td>10.48</td>
<td>4.5</td>
<td>LAM</td>
<td>VR / (NO)</td>
<td>I</td>
<td>102</td>
<td>Seroconversion HBsAg/anti-HBs</td>
</tr>
<tr>
<td>2/CH</td>
<td>37/F</td>
<td>6.48</td>
<td>8.8</td>
<td>LAM</td>
<td>VR / (NO)</td>
<td>I</td>
<td>125</td>
<td>Relapse Re-treatment</td>
</tr>
<tr>
<td>3/CH</td>
<td>45/M</td>
<td>8.63</td>
<td>2.3</td>
<td>LAM</td>
<td>VR / (M204V) (late)</td>
<td>I</td>
<td>125</td>
<td>Breakthrough (late) Re-treatment</td>
</tr>
<tr>
<td>4/LC</td>
<td>65/M</td>
<td>12.48</td>
<td>6.9</td>
<td>LAM</td>
<td>VR / (NO)</td>
<td>I</td>
<td>109</td>
<td>Breakthrough</td>
</tr>
<tr>
<td>5/CH</td>
<td>48/M</td>
<td>8.48</td>
<td>1.7</td>
<td>LAM</td>
<td>BT / (M204V)</td>
<td>II</td>
<td>109</td>
<td>Re-treatment</td>
</tr>
<tr>
<td>6/LC</td>
<td>52/M</td>
<td>8.48</td>
<td>1.5</td>
<td>LAM</td>
<td>BT / (M204V/I)</td>
<td>II</td>
<td>108</td>
<td>Breakthrough Re-treatment</td>
</tr>
<tr>
<td>7/LC</td>
<td>42/M</td>
<td>10.48</td>
<td>3.1</td>
<td>LAM</td>
<td>VR / (NO)</td>
<td>IIIA</td>
<td>90</td>
<td>Seroconversion HBeAg/anti-HBe</td>
</tr>
<tr>
<td>8/LC</td>
<td>59/M</td>
<td>7.96</td>
<td>8.7</td>
<td>LAM</td>
<td>VR / (NO)</td>
<td>IIIA</td>
<td>48</td>
<td>Seroconversion HBeAg/anti-HBe</td>
</tr>
<tr>
<td>9/CH</td>
<td>62/M</td>
<td>12.48</td>
<td>1.7</td>
<td>LAM</td>
<td>VR / (NO)</td>
<td>IIIA</td>
<td>83</td>
<td>VR with relapse Re-treatment</td>
</tr>
<tr>
<td>10/CH</td>
<td>9/F</td>
<td>8.48</td>
<td>3.3</td>
<td>LAM</td>
<td>BT / (M204I)</td>
<td>IIIB</td>
<td>37</td>
<td>Breakthrough. Drop out</td>
</tr>
<tr>
<td>11/CH</td>
<td>34/M</td>
<td>5.41</td>
<td>2.0</td>
<td>LAM</td>
<td>BT / (M204I)</td>
<td>IIIB</td>
<td>95</td>
<td>Breakthrough Re-treatment</td>
</tr>
<tr>
<td>12/CH</td>
<td>11/M</td>
<td>10.48</td>
<td>0.7</td>
<td>LAM</td>
<td>NR / (M204I)</td>
<td>NR</td>
<td>133</td>
<td>Re-treatment</td>
</tr>
<tr>
<td>13/CH</td>
<td>30/M</td>
<td>8.48</td>
<td>2.9</td>
<td>LAM</td>
<td>NR / (M204V)</td>
<td>NR</td>
<td>117</td>
<td>Re-treatment</td>
</tr>
<tr>
<td>14/CH</td>
<td>43/M</td>
<td>10.48</td>
<td>1.7</td>
<td>LAM</td>
<td>NR / (M204V)</td>
<td>NR</td>
<td>86</td>
<td>Re-treatment</td>
</tr>
<tr>
<td>15/CH</td>
<td>44/M</td>
<td>10.48</td>
<td>1.2</td>
<td>LAM</td>
<td>NR / (M204V)</td>
<td>NR</td>
<td>119</td>
<td>Re-treatment</td>
</tr>
<tr>
<td>16/CH</td>
<td>49/M</td>
<td>9.48</td>
<td>8.0</td>
<td>LAM</td>
<td>NR / (M204V)</td>
<td>NR</td>
<td>103</td>
<td>Breakthrough Re-treatment</td>
</tr>
<tr>
<td>17/CH</td>
<td>29/M</td>
<td>12.48</td>
<td>1.5</td>
<td>IFN+LAM</td>
<td>VR / (NO)</td>
<td>I</td>
<td>85</td>
<td>Seroconversion HBeAg/anti-HBe</td>
</tr>
<tr>
<td>18/CH</td>
<td>21/M</td>
<td>6.48</td>
<td>4.2</td>
<td>IFN+LAM</td>
<td>VR / (NO)</td>
<td>I</td>
<td>34</td>
<td>Seroconversion HBeAg/anti-HBe</td>
</tr>
<tr>
<td>19/CH</td>
<td>32/F</td>
<td>7.60</td>
<td>2.0</td>
<td>Peg+LAM ***</td>
<td>VR / (NO)</td>
<td>IIIA</td>
<td>72</td>
<td>Seroconversion HBeAg/anti-HBe</td>
</tr>
<tr>
<td>20/LC</td>
<td>11/F</td>
<td>6.40</td>
<td>0.7</td>
<td>IFN+LAM</td>
<td>VR / (NO)</td>
<td>IIIA</td>
<td>62</td>
<td>Seroconversion HBeAg/anti-HBe</td>
</tr>
<tr>
<td>21/CH</td>
<td>12/M</td>
<td>8.70</td>
<td>0.7</td>
<td>IFN+LAM</td>
<td>NR / (NO)</td>
<td>NR</td>
<td>60</td>
<td>Under treatment. Steady drop of HBV DNA and HBeAg</td>
</tr>
</tbody>
</table>

HBV, Hepatitis B virus; HBeAg, Hepatitis e antigen. * XUNL = upper normal limit; ** VR = viral response (PCR negativation); NR = non-response; *** see text.
Tenofovir

Viral response with relapse
Profound drop of HBeAg

M204V/M204I

HBeAg-non-response
Adefovir
HBeAg/anti-HBe seroconversion
Lamivudine+Lobucavir
M204I
Breakthrough

M204V
HBsAg/anti-HBs seroconversion
Lamivudine
Relapse
I
Clearance of HBVDNA (See table 2)
Under treatment
Under treatment
PegIFN-
Type of previous
I
Relapse
Outcome
IIIB
M204V
HBVDNA persistently positive
NR
IIIA
PegIFN-
PegIFN-
IIIA
PegIFN-
PegIFN-
IIIB
M204V
HBVDNA/anti-HBe seroconversion

** No. according to Table 2; *** NR = non-response; undetectability of HBeAg just before tenofovir; **** See text.

Laboratory methods: Serum samples were available for study at the following time points: initial screening (“baseline”), every three to six months during therapy, and after the completion of therapy. The levels of ALT, HBV DNA, and HBeAg were available at each time point. The serum ALT levels were tested using commercially available assays (Abbott Laboratories). Results are expressed as values times the upper normal limit (x UNL). The semi-quantitation of serum HBeAg was performed prospectively and simultaneously with the determination of serum HBV DNA. Because no commercial assay was available for measuring the HBeAg concentration, a micro-particle enzyme immunoassay (MEIA, AxSYM HBe 2.0, Abbott Laboratories, Abbott Park, IL) was used[13]. The assay is based on two different monoclonal antibodies, and its reference preparation for quantitation of HBeAg makes use of purified recombinant HBeAg as a control. Results are expressed as an index (sample to cutoff = S/CO) luminescent values ratio27.

In 17 patients, the quantitation of HBV DNA was performed by in-house PCR based on Kaneko’s method17,18 and as previously described6,8. The sensitivity of the method was reported to be 3x10^2 copies/mL, and its dynamic range went up to 3x10^6 copies/mL. Quantitative results were estimated based on end-point dilutions. In the last four patients (patients 18 to 21), the serum HBV DNA was determined by the Cobas Amplicor HBV Monitor Test (Roche Diagnostics, Branchburg, NJ) with a linear range from 4x10^2 (lower limit of detection) to 4x10^7 copies/mL7. We have recorded the HBV-DNA levels in copies/mL instead of IU/mL as recently reported19.

A virologic response was defined as a decrease in serum HBV DNA to undetectable levels by PCR and a loss of HBeAg25. Patients who showed neither of these serological features were considered to be non-responders, and those who presented with only one type of response (HBV DNA or HBeAg) were classified according to the patterns as described below. A sustained response was defined as the persistence of a virologic response for six months after the discontinuation of therapy25. The clearance of HBeAg and HBsAg was defined as the absence of the particular antigen in two consecutive samples at least one month apart. Viral polymerase, precore/core, and surface genes were sequenced to determine mutations in the YMDD domain as previously described8,32.

Liver biopsies: Liver biopsy was performed with a Tru-cut (Baxter Health Care, Deerfield, IL, USA) needle in 20 out of 21 patients. The exception was a cirrhotic patient with ascitis and very low platelet counts.

RESULTS

Demographic and baseline characteristics of patients: The demographics, baseline data, and patterns of initial therapeutic response for the patients studied are shown in Tables 1, 2, and 3. Changes in the HBeAg/anti-HBe system were observed in 15/21 patients, and the behavior of serum HBeAg expression during a long-term follow-up will be further detailed.

As shown in Table 2, a relapse after withdrawal, viral breakthrough,

Simultaneous analysis of HBV DNA and HBeAg and characterization of decay patterns: The main goal of our study was to describe the response of HBV to treatment by comparing the patterns of HBV DNA and HBeAg decreases. The results from treated and retreated patients were analyzed together. Overall, 36 series of therapy were obtained.

Three different response patterns could be characterized in our patients: Pattern I was characterized by a simultaneous decrease in serum HBV DNA and HBeAg levels until they were absent within a period of six months (Fig. 1A). If the absence of HBV DNA was followed by the loss of HBeAg in a maximum period of six months, the pattern was still considered as Pattern I.

A concomitant decrease in HBV DNA and HBeAg was observed in 13/36 (36.1%) series as shown in Tables 2 and 3. An example of this pattern is shown in Fig. 1A for patient 15, who had previous resistance to LAM due to the M204V mutation and an excellent response to a combined therapy with Peg-IFN-α2a (180 μg per week) and LAM. Pattern I during LAM or interferon plus LAM was detected in 4/16 patients (15%) and 6/10 patients (60%), respectively ( \( p = 0.10 \), two-tailed Fisher exact test).

Pattern II was characterized by the persistence of viremia (HBV DNA levels above 1,000 copies/mL) simultaneous with undetectable HBeAg (Fig. 1B) for a period greater than six months.

This pattern was observed in 2/36 (5.6%) series. In one patient (patient 5), this pattern was detected during 10 months of therapy with...

Fig. 1 - Viral kinetics in chronic hepatitis B patients. (A) Patient 15, 44 year old male previously resistant to lamivudine (LAM) with a M204V mutation. He exhibited a simultaneous decrease leading to undetectable HBV DNA and HBeAg levels (Pattern I) and sustained HBe-Ag/anti-HBe seroconversion after the administration of combined therapy (Peg-IFN-α2a + LAM). (B) Patient 5, 48 year old male exhibiting a rapid decrease in HBeAg levels but persistent HBV DNA (Pattern II) followed by a breakthrough during therapy. (C) Patient 19, 32 year old female who was treatment-naive and exhibited a rapid decrease in viremia, a slow decrease in HBeAg (still positive at 24 months), and eventual HBeAg/anti-HBe seroconversion at month 26 (Pattern III-A). (D) Patient 11, 34 year old male with persistent levels of HBeAg despite the loss of HBV DNA shown by PCR. He exhibited a breakthrough during LAM administration (Pattern III-B can be observed in both series of therapy). LD (limit of detection) for HBVDNA (*) and HBeAg (**). # “Decrescendo-crescendo” (“Falling-rising”) pattern of HBeAg levels*. ** = see Fig. 1A. Hatched bars represent serum HBV DNA levels above LD and open bars represent HBVDNA levels below LD. Open circles represent HBeAg+, filled circles represent HBeAg-. HBV, Hepatitis B virus; HBeAg, Hepatitis e antigen.
LAM, but HBeAg became positive thereafter (Fig. 1B). In another patient (patient 6), a loss of HBeAg was observed during therapy with LAM and persisted to be negative throughout despite the high levels of HBV DNA over three years. However, a clearance of serum HBV DNA was detected three months after the administration of tenofovir.

Pattern III was characterized by the persistence of detectable HBeAg despite the absence of HBV DNA for a period greater than six months. In some of these patients, a slow but steady decline of HBeAg was observed and was referred to as Pattern III-A (Fig. 1C).

In other patients, HBeAg decreased followed by an increase in serum levels, or it did not change at all, despite the absence of HBV DNA and was referred to as Pattern III-B (Fig. 1D).

The absence of HBV DNA preceding the loss of HBeAg for more than six months was observed in 11/36 therapeutic series (30.6%).

Pattern III-A was observed in seven patients. Prolonged periods of HBeAg positivity with continuously decreasing levels indicate a good, but slow, viral response (Fig. 1C). One patient (No. 19) with chronic hepatitis B, very high viremia (above 4x10^7 copies/mL by the Amplicor Monitor technique), and a high level of HBeAg was submitted to a combined therapy with Peg-IFN-α2b and LAM. As seen in Fig. 1C, HBV DNA became undetectable at the third month. A slow but steady decrease of HBeAg was observed.

Pattern III-B was observed in the other four patients, all of them presenting a viral relapse (Fig. 1D). As seen in Tables 2 and 3, HBeAg/anti-HBe seroconversion was detected in 5/6 patients who exhibited Pattern III-A, whereas no viral response was seen in the four patients exhibiting Pattern III-B.

In non-responder patients with persistent serum HBV DNA and HBeAg, and in patients presenting with Pattern III-B, the levels of HBeAg varied widely either in a “falling-rising” (“decrescendo-crescendo”) pattern or with fluctuating values25,31.

The small number of patients in each group did not allow a statistical comparison among the different patterns.

In one patient, all three patterns of viral response were observed, as seen in Fig. 2.

The ALT levels varied widely independently of the described patterns and were not included in this analysis.

**DISCUSSION**

Based on the simultaneous quantitative determinations of serum HBV DNA and HBeAg, our results suggest that the HBV kinetics can vary widely, depending on the antiviral drugs used for the treatment of HBeAg-positive patients with chronic hepatitis B. The kinetics of HBV have been investigated, but some problems still need clarification22,26.

Our results suggest that patients with positive HBeAg levels are less responsive to LAM or adefovir than combined therapy with interferon and LAM or tenofovir. Thus, Pattern I, which is characterized by the simultaneous decrease of HBV DNA and of HBeAg in a maximum period of six months, is more frequently found during treatment with interferon plus LAM or during tenofovir treatment. This pattern presents a favorable evolution in most cases, though a relapse or breakthrough is occasionally found. Pattern II was detected in very few cases and must be investigated with a larger number of patients. In our patients a breakthrough was found.

Our patients were followed for periods of time varying from 34 months to 133 months. Such long periods allowed us to observe that different patterns of serum HBeAg changes can be present in the same patient, and it allowed us to note how unpredictable the outcomes of patients on and off antiviral therapy are1. Despite the role of HBV DNA quantitation, the rapid and dramatic decrease in viremia after the introduction of nucleoside analogues reinforces the necessity to quantify HBeAg levels during the period of HBeAg positivity as an additional marker for monitoring responses. In some patients, a progressive drop in HBeAg levels may be paralleled with an HBV DNA decrease (Pattern I), but in other patients, the decrease of HBeAg is steady and slow, pointing to a late response (Pattern III-A).

A progressive decrease in serum HBeAg has been referred as a “decrescendo” (“falling”) pattern and a decrease in HBeAg followed by a return to high levels as a “decrescendo-crescendo” (“falling-rising”) pattern27,31. The first pattern is predictive of a viral response, whereas most of the patients who fail to show a continuous decrease in HBeAg levels present with a viral breakthrough or non-response. The characteristics of our study do not allow for a close comparison with the above-mentioned patterns.

Possibly an immune-modulating protein, HBeAg is a nonstructural secreted protein translated from HBV e (precore and core) mRNA. On the other hand, HBeAg, the major protein of the HBV capsid, is translated from HBV pregenomic mRNA31. Since HBeAg and HBeAg share a sequence consisting of 149 amino acids, they are collectively called Hepatitis B core-related antigens. The measurement of these antigens would be particularly useful for monitoring the decline of viral
This study was supported in part by research grants from the Alves de Queirós Family Fund for Research and CNPq.
REFERENCES


Received: 23 March 2009
Accepted: 28 September 2009