Large spontaneous HBV DNA fluctuations and potential usefulness of a single point measurement of combined HBV DNA and quantitative HBsAg for the exclusion of HBeAg negative chronic hepatitis B: A prospective Tunisian cohort study

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Research Article

Keywords: Chronic hepatitis B, HBV DNA, quantitative HBsAg, fluctuation, genotype D

Posted Date: December 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1173592/v1
Abstract

**Background/Aims:** We aimed to describe spontaneous short-term hepatitis B Virus (HBV) DNA fluctuations and to assess the usefulness of quantitative HBsAg (qHBsAg) in Tunisian patients with HBeAg-negative chronic HBV infection.

**Methods:** We included 174 treatment-naïve patients with chronic HBeAg-negative HBV. A one-year prospective follow-up was carried out with serial determinations of HBV DNA, alanine aminotransferase (ALT) levels and qHBsAg. Patients were classified into three groups: inactive carriers (G1), patients with HBeAg negative CHB (G2) and patients with “indeterminant state” (G3). For this latter group, a liver biopsy was indicated.

**Results:** Genotype D was the only detected. During the follow-up, 21.6% and 19.5% of patients with low initial (<2000 IU/mL) and intermediate viral load (2000-20000 IU/mL), experienced a subsequent increase in their HBV DNA levels above 2000 and 20000 IU/mL, respectively. Significant variations of HBV DNA levels ($\geq 0.5 \log_{10} \text{IU/mL}$) were observed in 61.1% of patients at 6 months-interval. Among the 174 patients, 89 (51.1%) belonged to G1, 33 (19%) to G2 and 52 (29.9%) to G3. Fourteen patients have undergone liver biopsy, among whom 7 (50%) showed moderate to severe liver disease. Combination of HBV DNA <2000 IU/mL and qHBsAg <832 IU/mL excluded CHB in 98.4% of cases.

**Conclusions:** This study highlights the large short-term HBV DNA fluctuations in Tunisian patients with HBeAg negative chronic HBV of genotype D. HBV DNA < 2000 IU/mL along with qHBsAg < 832 IU/mL excluded CHB in 98.4% of cases. Significant proportion of patients with “indeterminant state” within genotype D would have HBeAg negative CHB.

Background

Chronic hepatitis B (CHB) is a major cause of liver disease with more than 240 million people worldwide chronically infected with the hepatitis B virus (HBV). Progression of HBV-related liver inflammation to cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) is estimated in 2013 to result in up to 686000 deaths annually [1]. HBV infection is still endemic in Tunisia. However, a gradual shift from high to intermediate-low endemicity is currently observed mainly due to the implementation of the vaccination program since 1995. Indeed, according to a recent national survey on viral hepatitis seroprevalence in the years 2014-2015 [2], hepatitis B surface antigen (HBsAg) carriage rate was 1.8%, confirming the significant decrease in HBV infection seroprevalence, which was 6.5% in the 1990s [3].

During the natural course of hepatitis B e antigen (HBeAg)-negative CHB, fluctuations in both HBV DNA (viral load) and ALT (alanine aminotransferase) levels are often observed, making patient’s classification very difficult. Although significant variations in HBV DNA levels have been well documented during this phase, a little is known about short-term HBV DNA fluctuations. Thus, serial determinations of these two parameters (HBV DNA and ALT levels) for a period of at least one year are mandatory to avoid misclassification of infected patients [4]. Recently, several studies have suggested the potential role of
HBsAg quantification (qHBsAg), in addition to HBV DNA levels for a reliable and accurate determination of the actual HBV infection phase [5–7].

To our knowledge, clinical significance of qHBsAg during chronic HBV infection has not been previously documented in Tunisia and North Africa. The aim of this study was to describe spontaneous HBV DNA fluctuations and to assess the usefulness of qHBsAg in HBV infection diagnosis over a one year prospective follow-up period among Tunisian patients with HBeAg-negative chronic HBV infection.

**Patients And Methods**

**Study population and prospective follow-up**

Patients with chronic HBV infection were consecutively enrolled by the Department of Infectious Diseases, Hedi Chaker University Hospital and the Laboratory of Microbiology, Habib Bourguiba University Hospital, Sfax, Tunisia, from January 2014 to April 2016. Inclusion criteria were: untreated patients above 18 years old, asymptomatic, positive for HBsAg for at least 6 months, negative for anti HBc IgM and HBeAg and positive for anti-HBe. Exclusion criteria were: patients with hepatitis C virus (HCV), hepatitis D virus (HDV), Human immunodeficiency virus (HIV) co-infection or other causes of chronic hepatopathy (alcoholic or autoimmune disease), pregnant women and patient receiving immunosuppressive therapy. Overall, 217 patients were initially recruited and prospectively followed-up every three months (M) over a period of one year (M0, M3, M6, M9 and M12). During the follow-up, 43 patients were secondarily excluded because of unavailable serum sample in 30 cases, anti-viral therapy indication in 6 cases and pregnancy in 7 cases. Thus, 174 patients were finally included in the study. All patients gave written and informed consent to participate in the study, which was approved by the ethical committee of Habib Bourguiba University Hospital.

At inclusion (M0), demographic characteristics (age, gender), liver function test: ALT, aspartate aminotransferase (AST), abdominal ultrasound and virological markers (HBV DNA and qHBsAg) were performed for all patients. During follow up, serial determinations for both HBV DNA and ALT levels were obtained at three times with an interval of 6 months (M0, M6 and M12) at five times with an interval of 3 months (M0, M3, M6, M9 and M12), respectively. Quantification of HBsAg was performed in M0 and M12 for all carriers.

**Laboratory methods**

Liver biochemistries were performed in commercially available multiparametric automated analyzer (DxC 600® (Beckman CoulterInc, Brea, CA, USA). According to this method, the upper limit of normal (ULN) ALT values was 50 IU/L. Abnormal ALT levels were considered if at least two ALT determinations were > ULN throughout the follow-up.

Qualitative HBsAg and HBeAg/anti-HBe determinations were performed by electrochemiluminescent assay (COBAS e411 Analyzer, Roche Diagnostics, Germany). Serum HBV DNA was measured by real-time
PCR using the COBAS TaqMan 48 Analyzer/ COBAS TaqMan HBV test (Roche Diagnostics, Germany) with a detection threshold of 6 IU/mL and a linear range of \(29 - 1.10^8\) IU/mL.

Determination of HBV genotype was performed by multiplex PCR using type-specific primers allowing the identification of genotypes A through F \[8\].

Quantification of HBsAg was performed using the COBAS e411 Analyzer /Elecsys HBsAg II test (Roche Diagnostics, Germany). The 1/100e dilution of the samples allowed the measurement of a value within the range of quantification of \(5 - 13,000\) IU/mL. Samples with HBsAg levels >13,000 IU/mL were diluted to 1/100 to reach the value within the range of quantification and those < 5 IU/mL were retested pure. The detection limit of the test is 0.05 IU/mL.

**Patients classification**

At the end of the one year monitoring, HBV carriers were classified according to their biochemical and virological profiles: patients with persistently low HBV DNA (<2000 IU/mL) and normal ALT (< ULN), were classified in group 1 (G1) as HBsAg inactive carriers (IC) (patients with HBeAg negative Chronic infection). Patients having at least one high viral load (HBV DNA \(\geq\) 20,000 IU/mL) and those with an intermediate viral load (between 2000 and 20,000 IU/mL) with persistently or intermittently abnormal ALAT were classified in Group 2 (G2) as HBeAg negative CHB patients. Group 3 (G3) included patients who were not belonging to G1 nor G2. These patients designated with « indeterminant status » had at least one HBV DNA between 2000 and 20,000 IU/mL with persistently normal ALT or low viral load < 2000 UI/mL with persistently or intermittently abnormal ALAT. For these patients, a liver biopsy was recommended. Patients who had undergone a liver biopsy showing a Metavir histologic score above A2 and/or F2, were secondarily classified in G2.

**Statistical analysis**

The logarithmic transformation was used for quantitative data without normal distribution such as HBV DNA and qHBsAg in order to evaluate its variations over time. Data were analyzed using SPSS version 20. Continuous variables were expressed as medians (ranges) and categorical variables were expressed as percentages. Differences between subgroups were analyzed using Mann–Whitney and Kruskall Wallis non-parametric test when appropriate. A \(p\) value of \(\leq 0.05\) was considered to denote of statistical significance.

For each patient, the largest difference in HBV DNA levels between two consecutive visits (M0-M6, M6-M12) was recorded: a HBV DNA fluctuation \(\geq 0.5 \log_{10}\) IU/mL was considered as significant. Similarly, qHBsAg fluctuation \(\geq 0.5 \log_{10}\) IU/mL between M0 and M12 was considered as significant.

The diagnostic performance of qHBsAg was evaluated by receiver operating characteristic (ROC) curve. The cut-off value to discriminate chronic hepatitis patients from inactive carriers was selected considering the highest sensitivity + specificity sum. Only G1 and G2 patients were included in the analysis, as having a well-defined status at the end of follow-up.
Results

Baseline patient characteristics

Median age of included patients was 35 years (range: 21-66). The sex ratio was 6.56 (151/23). Serial ALT and AST determinations at M0 were 0.52 (0.24-1.88) and 0.62 (0.38-1.98) the ULN, respectively. The median $\log_{10}$ HBsAg titer and HBV DNA levels at baseline were respectively $2.93 (-0.71–4.8)$ and $2.63$ (not detected–6.23) IU/mL. Signs of chronic hepatopathy shown by abdominal ultrasound were noted in four patients (2.3%).

Determination of HBV genotype could be performed for 97 patients. Genotype D was the only found.

HBV DNA fluctuations

HBV DNA monitoring is shown in Table 1. At M0, 125 (71.8%), 41 (23.6%) and 8 (4.6%) of included patients had HBV DNA levels <2000, [2000- 20,000] and $\geq$ 20,000 IU/mL, respectively. Out of 125 patients with low HBV DNA at M0, seven (5.6%) maintained undetectable viral load throughout the follow-up and 27 (21.6%) have experienced at least one fluctuation (at M6 and / or M12), ranging between 2000 IU/mL and 20,000 in 26 cases and reaching more than 20,000 IU/mL in the remaining case. Among the 41 subjects initially having an intermediate viral load, 8 (19.5%) had at least one increase in their HBV DNA above 20,000 IU/mL (in M6 and / or M12). Thus, the proportion of patients with HBV DNA $\geq$2000 IU/mL, increased from 28.2% at M0 (49/174) to 37.3% at M6 (65/174) and to 43.7% at M12 (76/174).

For patients with detectable HBV DNA at inclusion (N=167), the median HBV fluctuation was $0.58 \log_{10}$ IU/mL (range: 0.03 to 3.14 $\log_{10}$ IU/mL). One hundred and two patients (61.1%) have experienced significant fluctuations of their viral load ($\geq 0.5 \log_{10}$ IU/mL) at 6-month interval. Interestingly, 41 out of the 102 patients (40.2%) have shown fluctuation over 1 $\log_{10}$ IU/mL.

Patients classification

At the end of the one year prospective follow–up, 89, 26 and 59 of included patients belonged to G1, G2 and G3, respectively. Liver biopsy was performed for 14 patients of G3, of whom seven patients (50%) showed a metavir histologic score above A2 and/or F2 and therefore were secondarily classified into G2 (Table 2).

Thus, the population study was finally classified into 89 inactive carriers (51.1%), 33 patients (19%) into G2 and 52 patients (29.9%) into G3.

Demographic, biological and virological characteristics of the three groups of patients are shown in Table 3. G1 patients were significantly older, had serum ALT, AST, TB and HBV DNA significantly lower than those belonging to G2 and G3. In addition, G2 patients had serum GGT levels significantly higher than those of G1 and G3. No statistically significant difference was observed between the three groups in terms of PAL, PT, leukocytes, hemoglobin and platelets count.
**HBsAg quantification and ROC curve analysis**

Medians of qHBsAg determined at M0 and M12 were 2.93 [-0.71 to 4.8] and 2.99 [-1.3 to 4.94] log$_{10}$ IU/mL, respectively. Median HBsAg levels (log$_{10}$ IU/mL) were significantly lower in patients belonging to G1 than those belonging to G2 and G3 (table 3).

The diagnostic performance of qHBsAg serum levels was evaluated by ROC curve in order to establish a cut-off value of qHBsAg allowing the identification, at the first visit (M0), of the clinical phase of chronic infection with negative HBeAg (IC or CHB) (Figure 1).

This analysis included two groups of patients G1 and G2. The area under the receiver operating characteristic (AUROC) of qHBsAg to identify CHB in the overall cohort was 0.817 (95% CI: 0.73–0.9, $p<0.0001$). The qHBsAg cut off with the highest sensitivity and specificity was 832 IU/mL (sensitivity: 86.2%, specificity: 68.5, PPV: 47.2%, NPV: 93.8). Thus, a value of qHBsAg <832 IU/mL at M0 allowed us to exclude CHB in 93.8% of cases. Interestingly, the combination of the two parameters qAgHBs <832 IU/mL and HBV DNA levels <2000 IU/mL at M0 allowed us to exclude CHB in 98.4%.

Furthermore, significant qHBsAg fluctuations ($\geq 0.5$ log$_{10}$ IU/mL) were noted in only 15 patients (8.6%) of whom 11 (73.3%) belonged to G1. Of note, three patients achieved HBsAg clearance at M12 with anti-HBs seroconversion. Further follow-up could be performed for 66 among the 89 inactive carriers, during a period ranging from 12 to 48 months. Interestingly, HBsAg clearance was observed in 10 additional patients of whom anti-HBs seroconversion was achieved in 4 cases. Therefore, the annual spontaneous HBsAg loss rate among the inactive carriers was estimated to 4.2%. All patients with HBsAg clearance had baseline qHBsAg levels <100 IU/mL. Annual qHBsAg decline was significantly higher in patients with anti-HBs seroconversion than those without seroconversion (0.91 log$_{10}$ IU/mL vs 0.07 log$_{10}$ IU/mL, respectively, $p=0.001$).

**Discussion**

In the present study, a prospective follow-up during a one-year period of a cohort of 174 treatment-naïve HBeAg negative Tunisian patients was performed. Patients monitoring was based on the determination of serum HBV DNA levels every 6 months and ALT levels every 3 months, which allowed the classification of patients at the end of the monitoring. During the follow-up, 21.6% and 19.5% of patients with low initial (<2000 IU/mL) and intermediate viral load (2000-20000 IU/mL), experienced a subsequent increase in their HBV DNA levels above 2000 IU/mL and 20000 IU/mL, respectively. These results suggest that a chronically infected HBeAg negative patient has a probability of approximately 20% to spontaneously switch from one interval to another during the following year. Overall, the proportion of patients with a viral load >2000 IU/mL passed from 28.2% at the initial visit (M0) to 43.7% at M12. Furthermore, significant variations of HBV DNA levels ($\geq 0.5$ log$_{10}$ IU/mL) were observed in 61.1% of patients at 6 months-interval. Similar results have been reported by Maylin and al, showing that 63% of Senegalese patients experienced a large viral load fluctuation at 4 months-interval [9]. Altogether, our findings
highlight the volatility and the short-term frequency of HBV DNA fluctuations in treatment-naïve HBeAg negative Tunisian patients infected with HBV of genotype D, suggesting that HBV viral load as a single biomarker cannot provide the answer for the actual CHB disease activity, and even a close monitoring of this biomarker may not accurately define the HBV infection phase or help to make decision to treat.

Interestingly, in the present study, nearly 30% of the cohort population fell into “indeterminant state” (G3) after one year follow-up. Significant differences were found between inactive carriers (G1) and patients with HBeAg negative CHB (G2) or with indeterminant state (G3) in relation to demographic and biological characteristics. Similarly, the level of qHBsAg determined at M0 was significantly lower in (G1) than in (G2) or (G3). These findings suggest that patients belonging to G3 group would be closer to G2 than to G1 group; hence, significant proportion of G3 patients would have HBeAg negative CHB.

Indeed, among 14 patients from G3 who have undergone a liver biopsy, 7 (50%) showed moderate to severe liver disease, with Metavir score $\geq$ A2 and/or F2. In other reports, however, indeterminant patients with HBV DNA levels of 2000-10000 IU/mL or 2000 – 20,000 IU/mL and persistent normal ALT concentration were more similar to patients with low HBV DNA in relation to clinical, histological, and laboratory characteristics and therefore, it is concluded that they can be clinically managed as HBV inactive carriers [10, 11]. One reason for the discordant results could be the criteria selected for the definition of patients with “indeterminant state”. Another possible explanation is HBV genotype, given that the above mentioned studies included predominantly Asian patients of genotype A, B and C. Indeed, compared to other genotypes, it was concluded that patients with genotypes D, C, and F1 are more likely to develop complications such as liver cirrhosis and HCC [12]. A study from India has suggested that genotype D was associated with more severe liver diseases and HCC in younger patients than genotype A [13]. Another study from China has also shown that genotype A and B patients have a higher rate of HBsAg sero-clearance than genotype C and D patients [14].

The actual state of patients with viral load between 2000 – 20000 UI/mL and normal ALAT or those having low levels of HBV DNA < 2000 UI/mL with hepatic flares is not well-characterized. These profiles could correspond to inactive carriage, to HBeAg CHB state or even to patients who are likely to subsequently clear HBsAg [15]. Furthermore, it has been recently suggested that for patients with very low level of viremia, an elevation of ALT should prompt investigation into alternate causes such as medications, alcohol and steatosis [11]. In our study, among the 7 patients with abnormal liver biopsy, 4 patients showed fibrosis scores $\geq$ F2. The initial profile of these patients was viral load between 2000 – 20000 UI/mL and normal ALAT in one case and low levels of HBV DNA < 2000 UI/mL with hepatic flares in the remaining 3 cases. Interestingly, all of them showed low qHBsAg levels at M0 (range: 5.85-898.3 IU/ml). Indeed, it has been previously suggested that qHBsAg levels are negatively correlated with advanced stages of liver fibrosis in treatment-naïve African chronic hepatitis B HBeAg-negative patients [16]. Therefore, whether such profiles within genotype D, especially those with low levels of HBV DNA, abnormal ALAT levels and low qHBsAg, are really of good prognosis, should be considered with precaution. These profiles stress the need to be more investigated.
Thus, a single HBV DNA determination at a given time can not reflect the true status of patients so a regular and close monitoring is mandatory to optimize their support. However, the determination of HBV DNA is very expensive and not routinely available in all laboratories in Tunisia. Several studies have shown that qHBsAg could be of a great relevance in phase definition and prediction of HBsAg seroclearance [17–20]. In the present study, we evaluated the performance of qHBsAg serum levels by ROC curve to define, at the first visit (M0), the actual phase of HBeAg negative chronic infection (IC or CHB). A qHBsAg serum level of < 832 UI/mL allowed the exclusion of HBeAg CHB state in 93.8% of cases. Interestingly, the most accurate diagnostic approach was achieved by combining qHBsAg <832 IU/mL and HBV DNA <2000 IU/mL, which excludes CHB in 98.4% of cases. Similar results were reported by Oliveri and al [21] showing that combined single point HBV DNA ≤ 2000 IU/mL/HBsAg ≤ 1000 IU/mL allowed the exclusion of CHB with NPV of 100%. The potential reason for the slight difference in qHBsAg cut-off, compared to several studies which suggested a cut-off of 1000 IU/mL, could be that our group of HBeAg negative CHB patients (G2) was more heterogenous, including patients with both activity and advanced fibrosis scores, hence the presence of low qHBsAg levels in this group of patients, as it was mentioned above.

In our cohort, significant qHBsAg fluctuations ≥ 0.5 log_{10} IU/mL were observed in 8.6% of cases throughout the follow-up. Notably, these fluctuations were primarily found in the inactive carriers group (G1) (73.3%). These results were consistent with those of Brunetto [5] and Tung Hung Su [22] showing that the most wide qHBsAg fluctuations were observed in inactive carriers, especially in the later stage of disease. Indeed, within this group, 13 patients achieved HBsAg seroclearance. As it was previously reported [23, 24], all these patients showed lower baseline qHBsAg < 100 IU/mL, with specifically an annual decline significantly higher for those who achieved anti-HBs seroconversion. These results highlight the potential relevance of qHBsAg for CHB functional cure prediction.

To our knowledge, this study is the first to assess HBV DNA fluctuations and qHBsAg in a cohort of patients chronically infected with HBV of genotype D in North Africa. Strengths of this study are: first, the classification of patients was based on a regular prospective follow up, through the determination of HBV DNA and ALT levels, and not on a single measurement. Second, our group of patients with HBeAg negative CHB (G2) included those with advanced fibrosis based on liver biopsy which provides a more reliable assessment of qHBsAg for phase diagnosis. Nevertheless, several limitations exist: the low numbers of patients within G3 who have undergone a liver biopsy, and the relatively short period of follow up after the first year.

In summary, this study highlighted the large spontaneous fluctuations of HBV DNA during HBeAg negative CHB in HBV infected genotype D Tunisian population, and showed that approximately 20% of patients will spontaneously transit from one HBV DNA interval to another during the following year. A cut-off value of qHBsAg < 832 IU/mL associated with HBV DNA < 2000 IU/mL allowed the exclusion of HBeAg negative CHB in 98.4%, and a cut-off of qHBsAg <100 IU/mL associated with an annual decline of > 0.5 log_{10} IU/mL are good predictors markers of hepatitis B functional cure. Approximately one third of our patients are designated as having an “indeterminant state” during CHB. Low levels of qHBsAg associated
with low HBV DNA and abnormal ALAT may be predictors of advanced fibrosis in this subgroup, which need to be confirmed by further investigations in a large scale population.

Abbreviations

ALT
alanine aminotransferase
AST
aspartate aminotransferase
AUROC
area under the receiver operating characteristic
CHB
chronic hepatitis B
G
group
Anti-HBc
hepatitis B core antibody
Anti-HBs
hepatitis B surface antibody
HBeAg
hepatitis B e antigen
HBsAg
hepatitis B surface antigen
HBV
hepatitis B virus
HBV DNA
hepatitis B virus desoxyribonucleic acid
HCC
hepatocellular carcinoma
HCV
hepatitis C virus
HDV
hepatitis D virus
HIV
human immunodeficiency virus
PCR
polymerase chain reaction
ROC
receiver operating characteristic curve
ULN
Declarations

Ethics approval and consent to participate: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Habib Bourguiba University Hospital (protocol code: 06/15 date of approval: 01 April 2015). Informed consent was obtained from all patients involved in the study.

Consent for publication

All authors give their consent for publication of the present manuscript.

Availability of data and materials: The data presented in this study are available on request from the corresponding author.

Competing interests: The authors declare that they have no competing interests in this section.

Funding: none.

Author Contributions: AC: conceptualization, data curation, formal analysis, investigation, methodology, resources, software and writing (original draft). SG: conceptualization, data curation, formal analysis, methodology, resources, software, validation and writing – review & editing. EE: data curation, methodology and validation. AT: resources. LFK: funding acquisition and project administration. KM: resources. MK: software. AH and MBJ: supervision. HK: funding acquisition, project administration and writing – review & editing. All authors read and approved the final manuscript.

Acknowledgements: We thank Basma Chhaider, Soumaya Aguerbi and Dhouha bougdar for their support during patient recruitment and monitoring and sample collection. We are grateful to Noura Abdelmoula, Najoua Yaich, Houda Moussa, Chefia Chemi, Lamia Charfi and Henda Mguize for instrumental and technical support.

References


Tables

Table 1. HBV DNA levels during the one year-follow-up

<table>
<thead>
<tr>
<th>HBV DNA log_{10} IU/mL</th>
<th>M0</th>
<th>M6</th>
<th>M12</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2000</td>
<td>125 (71.8)</td>
<td>109 (62.7)</td>
<td>98 (56.3)</td>
</tr>
<tr>
<td>[2000- 20 000[</td>
<td>41 (23.6)</td>
<td>54 (31)</td>
<td>59 (33.9)</td>
</tr>
<tr>
<td>≥20 000</td>
<td>8 (4.6)</td>
<td>11 (6.3)</td>
<td>17 (9.8)</td>
</tr>
<tr>
<td>medians/ranges</td>
<td>2.63 [ND – 6.23]</td>
<td>2.73 [ND – 7.92]</td>
<td>2.52 [ND – 7.77]</td>
</tr>
</tbody>
</table>

HBV DNA, hepatitis B virus deoxyribonucleic acid; M, month; N, number ; ND, not detected

Table 2. Patients from G3 group with Metavir histologic score above A2 and/or F2
<table>
<thead>
<tr>
<th>Patient n°</th>
<th>Age (Years)</th>
<th>Abnormal ALT</th>
<th>HBV DNA fluctuation ≥ 2000 IU/mL</th>
<th>qHBsAg at M0 IU/mL</th>
<th>Metavir Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>No</td>
<td>Yes</td>
<td>179.6</td>
<td>A1F2</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>No</td>
<td>Yes</td>
<td>3023</td>
<td>A2F1</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>No</td>
<td>Yes</td>
<td>11270</td>
<td>A2F1</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>No</td>
<td>Yes</td>
<td>24220</td>
<td>A2F2</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>Yes</td>
<td>No</td>
<td>5.85</td>
<td>A1F2</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>Yes</td>
<td>No</td>
<td>50.44</td>
<td>A3F3</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>Yes</td>
<td>No</td>
<td>898.3</td>
<td>A1F2</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid; qHBsAg, quantitative hepatitis B surface antigen.

**Table 3.** Demographic and biological Characteristics of patients groups (G1, G2, G3)
<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1 (N = 89)</th>
<th>G2 (N = 33)</th>
<th>G3 (N = 52)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 (23-63)</td>
<td>34 (25-52)</td>
<td>33 (21-66)</td>
<td>0.014</td>
</tr>
<tr>
<td>ALT (×ULN)</td>
<td>0.48 (0.24-0.92)</td>
<td>0.82 (0.34-1.88)</td>
<td>0.53 (0.24-1.66)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AST (×ULN)</td>
<td>0.61 (0.4-1.58)</td>
<td>0.75 (0.53-1.43)</td>
<td>0.65 (0.38-1.98)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>qHBsAg (IU/mL)</td>
<td>2.4 (-0.71-4.63)</td>
<td>3.5 (0.77-4.76)</td>
<td>3.16 (1.94-4.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HBV DNA (IU/mL)</td>
<td>2.00 (ND-3.24)</td>
<td>3.79 (1.08-6.24)</td>
<td>3.29 (ND-4.12)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HBV DNA/qHBsAg ratio</td>
<td>0.81</td>
<td>1.01</td>
<td>1.0</td>
<td>0.006</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>73 (36-145)</td>
<td>72 (50-124)</td>
<td>76.5 (39-135)</td>
<td>0.69</td>
</tr>
<tr>
<td>Gamma glutamyl transferase (U/L)</td>
<td>18 (6-65)</td>
<td>25 (14-60)</td>
<td>18 (7-77)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>12 (3.7-25)</td>
<td>13 (7-27)</td>
<td>12.7 (7.5-42)</td>
<td>0.001</td>
</tr>
<tr>
<td>Prothrombin time (%)</td>
<td>97 (71-100)</td>
<td>95 (75-100)</td>
<td>97 (61-100)</td>
<td>0.49</td>
</tr>
<tr>
<td>Leukocytes (/mm3)</td>
<td>6420</td>
<td>6420</td>
<td>6470</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>(3990-16400)</td>
<td>(4070-9490)</td>
<td>(3860-10050)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.9 (11-18)</td>
<td>15.3 (11.1-17.3)</td>
<td>14.8 (10.2-18.4)</td>
<td>0.38</td>
</tr>
<tr>
<td>Platelets (/mm3)</td>
<td>213000</td>
<td>219000</td>
<td>226000</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>(117000-43500)</td>
<td>(113000-34500)</td>
<td>(129000-241000)</td>
<td></td>
</tr>
</tbody>
</table>

G, group; G1, inactive carriers; G2, HBeAg negative chronic hepatitis B patients; G3, patients with indeterminant status; ALT, Alanine aminotransferase; ULN, upper limit of normal; AST, Aspartate aminotransferase; qHBsAg, quantitative hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid.

**Figures**
Figure 1

ROC curve analysis of clinical usefulness of qHBsAg. Legend: the diagnostic performance of qHBsAg serum levels was evaluated by receiver operating characteristic (ROC) curve in order to establish a cut-off value of qHBsAg allowing the identification, at the first visit (M0), of the clinical phase of chronic infection with negative HBeAg (chronic hepatitis B or inactive carriage). The qHBsAg cut off to discriminate was selected considering the highest sensitivity + specificity sum. Only G1 and G2 patients were included in the analysis, as having a well-defined status at the end of follow-up. The area under the receiver operating characteristic (AUROC) of qHBsAg to identify CHB in the overall cohort was 0.817.