

# Chronic HBeAg negative, anti-HBe positive Hepatitis B: an overview EASL 2002

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## Abstract

Virological features of HBeAg negative, anti-HBe positive chronic hepatitis B differ from the HBeAg positive form: mean serum HBV-DNA levels and HBcAg-stained hepatocytes are lower and most of HBV genomes carry mutations that hamper HBeAg secretion at transcriptional or translational levels. HBV genotypes appear to influence the worldwide distribution of the HBeAg defective mutant that prevails in the Mediterranean area. In fact the G to A switch at position 1896 (HBeAg minus HBV) increases the stability of the secondary structure of the encapsidation signal only in genotypes harboring T nucleotides at position 1858. Three major biochemical patterns are observed: acute recurrent hepatitis with intervening periods of biochemical and virological remission, unremitting chronic hepatitis and unremitting chronic hepatitis with acute exacerbations. Therefore single time point observations can cause diagnostic bias; to improve diagnostic accuracy and disease management and reduce the costs of monthly monitoring the combination of molecular biology (HBV-DNA) and immune-assays (IgM anti-HBc) is proposed.

Liver disease caused by HBeAg minus HBV runs usually asymptomatic for 3-4 decades and reaches the stage of histological cirrhosis in patients with median age of 45 years. Cirrhosis progresses to end stage complications in one fourth of the patients in about 10 years: recurrent hepatitis B flares accelerate disease progression. The clinical characterization was performed in patients from the Mediterranean area, therefore the possibility exists that both virological and genetic differences may influence the disease profile and outcome when HBeAg negative/anti-HBe positive chronic hepatitis B is considered from other geographical areas.

## Introduction

In the '80ies, after the hepatitis Delta virus (HDV) epidemics occurred in the Mediterranean area, hepatologists began to look for unknown Non-A, Non-B, Non-D hepatitis agents in anti-HDV negative, HBeAg negative, anti-HBe positive, HBsAg carriers with chronic liver disease (1-2). The intrahepatic detection of hepatitis B core antigen (HBcAg) was the gold standard for etiologic diagnosis of chronic hepatitis B (1-2) and circulating hepatitis B "e" antigen correlated with the nuclear staining of HBcAg in a large number of hepatocytes (1-3). However, intrahepatic HBcAg was detected also (but in a lower number of hepatocytes) and associated with both nuclear and cytoplasmic staining in a few of anti-HD negative, anti-HBe positive patients (2).

In 1980 with the application of molecular hybridization techniques detection of HBV-DNA in the serum became a valid, alternative tool to detect HBV replication as it was shown that serum HBV-DNA was associated with intrahepatic HBcAg in both HBeAg positive and negative patients (2-6). The great majority of HBeAg and anti-HD negative, anti-HBe positive patients with chronic liver disease from the Mediterranean area tested positive for serum HBV-DNA (2-6). In the following years, in a cohort study the clinical and pathological profile of "chronic anti-HBe positive hepatitis B" was characterized as significantly different from that of chronic HBeAg positive hepatitis B (7). Mean HBV-DNA serum levels were lower, the intracytoplasmic staining of HBcAg was more frequent and liver disease was more severe (7).

At the same time molecular biology studies shed new lights on HBV transcription and translation mechanisms giving elements to hypothesize the molecular basis of the atypical serologic profile of these patients (8). In 1989 two studies performed in anti-HBe positive patients of the Mediterranean area showed contemporaneously that the most frequent cause of the discrepancy between serum HBV-DNA and absence of HBeAg was the infection with HBV variants unable to secrete the soluble form of the HBV nucleocapsidic protein (HBeAg minus HBV, 9-10). The mutation is a G to A switch at position 1896 of the precore region of the HBV genome that leads to a translational stop codon in the leader sequence of HBeAg protein resulting in the inhibition of the protein synthesis (8-10). Subsequently many other groups confirmed the observation and identified other mutations able to generate HBeAg defective viruses (11-12). So far 2 major groups of mutations had been described: those occurring in the basic core promoter, which modulate HBeAg secretion at the transcriptional level and those occurring in the pre-core region, which block HBeAg production at the translational level (11-12). Core promoter and stop codon mutants appear to be frequently associated and the interesting question arises as to whether one category of mutants precedes or influences the prevalence of the other.

The prevalence of 1896 stop codon mutants appears to be significantly associated with HBV genotypes harboring a T nucleotide at position 1858 (genotypes B, D, E and a part of the genotype C and F strains) since the generation of the G to A mutation at nucleotide 1896 would stabilize the secondary structure of the encapsidation signal loop (13). On the contrary, in genotypes A and part of genotype C and F strains the presence of C at nucleotide 1858 would significantly reduce the possibility for G to A 1896 variants to be selected, because of lower replication fitness (13). The geographical distribution of HBV genotypes would therefore influence the worldwide distribution of 1896 stop-codon (14). Therefore molecular epidemiology of HBV could be responsible for the different geographical prevalence of HBeAg negative /anti-HBe positive chronic hepatitis B, which appears to be the most common form of chronic hepatitis B in Southern Europe and Asia, where 30% to 80% of patients with chronic hepatitis B are HBeAg negative as compared with Northern Europe and the United States where only 10-40% lack HBeAg (11-14).

Preliminary data using oligo-hybridization technique showed that wild-type and HBeAg minus HBVs may be simultaneously present in a HBV carrier and their relative ratio may undergo dynamic variations over time (15-18). Furthermore, follow-up studies suggested the important association between different circulating ratios of wild-type and HBeAg minus HBVs and pathogenetic events during the course of chronic hepatitis B (17-18). Considering the paramount role of HBeAg in the equilibrium between HBV and the immune-system it will be extremely important to shed new light on the molecular mechanisms that regulate the dynamic changes of the ratio between wild-type and HBeAg defective HBVs.

### Natural history of liver disease

The clinical characterization of anti-HBe positive chronic hepatitis B and the study of its course were performed in patients from the Mediterranean area (7,14,19-21). Therefore the possibility that differences at virological and genetic levels may influence both disease profile and outcome had to be taken into account when considering HBeAg negative/anti-HBe positive chronic hepatitis B from other geographical areas.

In most of anti-HBe positive patients from the Mediterranean area HBV infection occurs in the childhood as suggested by the high rate of intrafamilial HBV infection, the low rates of parenteral exposure and history of acute hepatitis and HBeAg carriership (21). The disease caused by HBeAg minus HBV runs usually asymptomatic for about 3-4 decades and reaches the stage of histological cirrhosis at a median age of about 45 years (21). Thereafter cirrhosis progresses to end stage complications in about one fourth of the patients in about 10 years and recurrent hepatitis B exacerbations accelerate disease progression (21). The virologic and biochemical patterns of chronic anti-HBe positive hepatitis B vary from intermittently to persistently detectable viremia and elevated transaminase levels and three major patterns can be identified (11,21) (Figure n.1, 21):

- ◆ Acute recurrent hepatitis B exacerbations with intervening periods of biochemical and virological remission,
- ◆ Unremitting chronic hepatitis B,

## ◆ Unremitting chronic hepatitis B with acute exacerbations

In spite of an intermitting disease profile associated with frequent and some times long lasting remissions spontaneous recovery of anti-HBe positive chronic hepatitis B is very unusual (11, 14, 19-21). Persistent viral replication is a major cause of chronic liver damage and development of cirrhosis: in a cohort study after a mean follow-up of 10 years about 50% of the patients with chronic hepatitis at baseline developed cirrhosis and persistently detectable HBV-DNA was a factor independently associated with disease progression (21). On the contrary, in patients with cirrhosis development of end point complication was associated with recurrent hepatitis exacerbations (21).

### Diagnosis

Liver disease runs a long lasting asymptomatic course and is diagnosed in over 90% of cases by occasional blood testing. Studies that used uniform and stringent monitoring criteria found major fluctuations of viremia and transaminases in over 50% of the patients (21). HBV-DNA levels fell below the sensitivity limits of the hybridization assay ( $<2.8 \times 10^6$  gen/ml) more than once yearly in about 90% of cases and 6 or more times in 60% of them (21). Similarly, ALT levels showed recurrent flares in 65% of the patients and in 70% of them ALT fell to normal values between flares. These findings point out the need to study disease progression using virologic and biochemical patterns instead of simple baseline values of ALT and HBV-DNA to avoid consistent biases caused by single time-point observations. In addition, the rapid fluctuations of both ALT and HBV-DNA may hamper an accurate monitoring of the pathogenetic events occurring in patients unless a monthly monitoring is performed.

The availability of standardized highly sensitive assays for serum HBV-DNA detection gives a new tool in the setting of diagnosis and monitoring of anti-HBe positive patients (11). However, further studies are mandatory to correctly identify the viremia level correlating with active liver damage and able to discriminate between anti-HBe positive carriers with and without liver disease. Follow-up studies of pedigreed anti-HBe carriers with or without liver disease and different disease patterns will be able to define the cut off (104 or 105 copies/ml.) or, better the gray zone, (103-104 or 104–105 copies/ml.) of clinical relevance. Nevertheless we should not forget older immune-assays. The evidence that IgM anti-HBc, a marker used to reach the etiological diagnosis of acute type B hepatitis, is present also at lower levels during the course of chronic hepatitis B suggests the possibility to use this marker to suspect the presence of HBV disease in anti-HBe positive carriers and to monitor the course of disease activity (22). The availability of standardized, sensitive and quantitative assays allowed the monthly monitoring of patients with recurrent hepatitis exacerbations and showed the high diagnostic accuracy of IgM anti-HBc as a surrogate markers of HBV induced liver damage (23). Furthermore, it has been shown as IgM anti-HBc levels higher than 4 IU PEI are diagnostic for the presence of chronic hepatitis B and may be the diagnostic tool to differentiate the inactive carrier without liver disease from anti-HBe positive patients observed in a low HBV replication phase (24). Therefore the combination of a molecular biology test (HBV-DNA) and an immune-assay (IgM anti-HBc) can be considered to improve the diagnostic accuracy of both etiological diagnosis and monitoring in HBeAg negative/anti-HBe positive HBV carriers.

### Conclusions

HBeAg negative, anti-HBe positive chronic hepatitis B is a clinico-pathological syndrome characterized by the following features:

- ◆ chronic HBV carriership
- ◆ chronic hepatitis at histology or biochemical and ultrasound evidence of chronic liver disease
- ◆ persistent ( from at least one year) anti-HBe positive serology in absence of HBeAg
- ◆ serum HBV-DNA higher than 104-105 (a) genome equivalents or copies per ml (at least intermittently) and / or IgM anti-HBc serum levels higher than 4 Paul Erlich Institute Unit
- ◆ absence of HDV markers (\*)
- ◆ absence of HCV markers (\*)

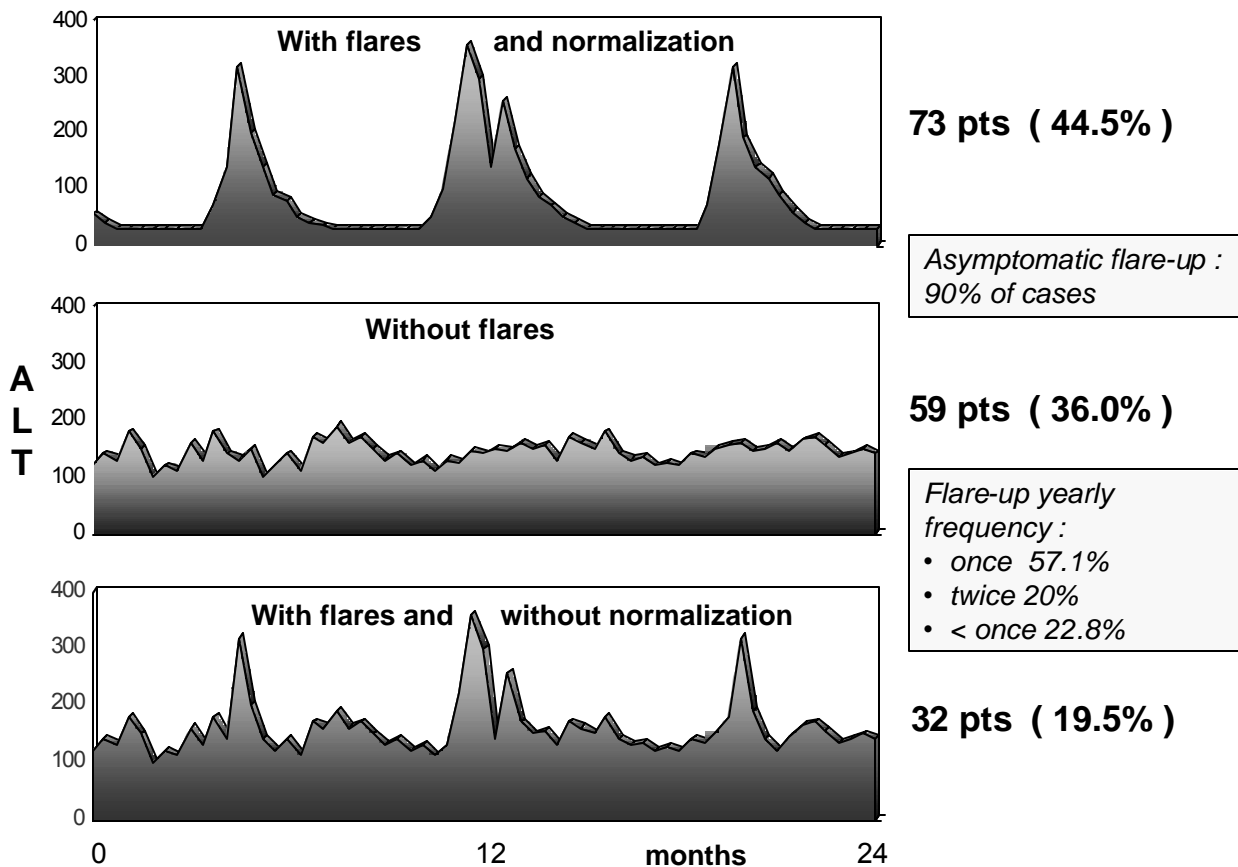
(a) The cut-off has to be defined by prospective studies.

\* In presence of HCV and HDV markers a concurrent chronic hepatitis B etiology of liver disease can be sustained only if HBV-DNA is > than 10<sup>4</sup> genome equivalents or copies per ml and / or IgM anti-HBc serum levels are higher than 4 Paul Erlich Institute Unit .

The most frequent molecular reason that determines the absence of HBeAg is the infection with a HBV variant or mutant unable to secrete "e" antigen, HBeAg minus HBV.

## Legend

**Figure 1. Biochemical patterns in 164 untreated anti-HBe positive patients with chronic hepatitis B: disease profiles were identified during a 23 months (range 12-36) monthly monitoring. (presented at the EASL annual meeting in 1999 and reference n. 21).**



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