Original article

Natural history, outcome, and sustainability of treatment response in chronic viral hepatitis B: Thai multicenter study

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Background: Data regarding the natural history and outcome of treatment of chronic hepatitis B (CHB) patients in Thailand remain inconclusive.
Objective: We aimed to examine the natural history and outcome of therapy in Thai CHB patients treated with nucleoside analogues (NAs) monotherapy or interferon (IFN) monotherapy.
Method: CHB patients without clinical evidence of cirrhosis and alanine aminotransferase (ALT) levels >1.5 times of normal for at least 6 months were enrolled from 6 hospitals (2002 to 2005). Treatment included NAs and IFN. Treatment response was defined as ALT normalization and an HBV DNA level <10,000 copies/ml (or <2,000 IU/ml) and/or HBeAg seroconversion at the end of follow up. The study was approved by the institutional review board of each hospital.
Result: A total of 567 patients with mean age of 46.8 ± 11.9 y were included. The ratio of HBeAg positive to HBeAg negative patients was 1.3:1. Nineteen percent of patients had no HBeAg status. There were 262 HBeAg positive patients (46%) and 197 HBeAg negative patients 35%. Sixty-one percent received NAs while 20% received IFN as a first line treatment and the remaining 19% received no specific medication. For the HBeAg positive patients, HBeAg seroconversion and undetectable HBV-DNA were achieved in 32.8% and 50.5%, respectively in NAs group (on therapy), and HBeAg seroconversion and undetectable HBV-DNA were achieved in 24.3% and 21.4%, respectively in the IFN-treated group (off treatment). For the HBeAg negative patients, undetectable levels of HBV-DNA occurred in 68.8% in the NAs group while undetectable levels of HBV-DNA occurred in 37.5% of patients in the IFN-treated group. HBsAg loss was not found in the NAs group, but 2.8% of patients in IFN group had HBsAg loss. HBV DNA reappeared in the IFN group (off treatment) and NAs groups (on therapy) in 26.6% and 24.3% of patients, respectively. Minor adverse events of therapy were found in 9% of patients. Five percent of patients progressed to Child A cirrhosis and one patient in the NAs group (0.18%) died from causes unrelated to liver disease, during a 3-year follow-up.
Conclusion: The treatment response of Thai CHB patients from multicenter study showed the results similar to previous studies. However, higher durability of treatment with lower rate of reappearance of HBV DNA was observed in Thai CHB patients.

Keywords: Chronic hepatitis B, Thai, multicenter study, natural history, outcome of treatment.

Abbreviations: AFP: alpha-fetoprotein, ALT: alanine transaminase, Anti HBe: antibody to hepatitis B HBeAg, bDNA: branched-chain DNA analysis method, CHB: chronic viral hepatitis B, CT: computed tomography, cIFN: conventional interferon, cp/ml: copies/milliliter, HAI score: histoactivity index by using

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Knodell's score system, HBeAg: hepatitis B-E antigen, HBsAg: hepatitis B surface antigen, HBV DNA: hepatitis B virus DNA, HBV: hepatitis B virus, IFN: interferon, LAM: lamivudine, MR: magnetic resonance, NAs: nucleoside analogues, PCR: polymerase chain reaction, PEG-IFN: Pegylated interferon, pIFN: peg interferon, 3TC: 2′,3′-dideoxy-3′-thiacytidine; lamivudine, US: ultrasonography.

Chronic hepatitis B (CHB) is a worldwide threat and prevalent in Thailand with a carrier rate of 10% during the past 20 years [1, 2]. There is now a clear reduction in the prevalence of CHB infection since the integration of hepatitis B virus (HBV) vaccination into the expanded program of immunization in Thailand [3, 4]. However, the burdened of CHB patients and the increasing risk of morbidity and mortality (25%–40%) are still a major concern [5]. The annual incidence of HCC was 0.1% in asymptomatic hepatitis B surface antigen (HBsAg)-positive individuals or 1% in CHB patients [6]. The HCC incidence increased to 3%–10% in CHB patients with cirrhosis [6]. The probability of developing cirrhosis and complications depended on multiple factors including the duration of HBV replication, baseline viral load, the immune status such as coexisting HIV infection, alcohol intake, and the opportunity to receive antiviral agents for HBV. Benvegnu and colleagues found that there was a significant morbidity and mortality during the first decade after diagnosis of compensated cirrhosis [7]. The natural course of CHB progressing to cirrhosis can be relatively silent and can occur even in younger patients [8]. The spontaneous annual seroconversion rate of HBsAg was 1% and 10% for the HBeAg [9]. Currently, no satisfactory treatment is available for CHB patients. Interferon (IFN) and a number of nucleoside analogues (NAs) including lamivudine, adefovir dipivoxil, entecavir, and telbivudine are already government approved for CHB treatment in Thailand. However, the overall response in Asian patients was only 15%–20% for HBeAg seroconversion [5, 10-12]. Less than 5% of patients showed HBsAg seroconversion [13]. Limitations of the use of antiviral agents such as interferon-alpha (IFN-α) or pegylated interferon-alpha (PEG-IFN-α) are poor tolerability. For NAs limitations are a high rates of emergence of drug-resistant HBV mutants especially for lamivudine resistance, which occurred during the late period of the first year of treatment [10, 11, 14] These HBV mutants, may result in a more severe and progressive clinical course of CHB [11, 15]. Currently, the data on the outcome of treatment of Thai CHB patients remain scant. We aimed to determine the natural history, durability, and outcome of treatment in Thai CHB patients treated with nucleoside analogues (NAs) monotherapy or interferon (IFN) monotherapy with 3-year follow-up.

Methods
Patients

This retrospective multicenter study included consecutive patients with CHB from 6 hospitals including: King Chulalongkorn Memorial University Hospital (KCMH), Siriraj Hospital of Mahidol University (SI), Prince Songklanakarind University Hospital (PS), Ramathibodi Hospital of Mahidol University (RA), King Pramongkrutklao Hospital of the Royal Thai Army (KP), and the Hospital for Tropical diseases of Mahidol University (TM). The study was conducted between January 2002 and December 2004. Patients' ages ranged from 18 to 80 years. CHB was diagnosed by detectable HBsAg and alanine aminotransferase (ALT) levels of at least 1.5 times normal over at least 6 months. Clinical data from follow-up and laboratory tests monitoring at least one time annually were recorded. HBeAg-negative patients must have had a HBV DNA level of at least 2,000 IU/ml.

Patients were excluded if they had hepatitis C virus (HCV) or HIV coinfection, pregnancy, or clinical evidence of compensated or decompensated cirrhosis. Cirrhosis was defined by a serum bilirubin level >2.5 times the upper limit of normal, a prolonged prothrombin time (PT) of ≥3 seconds longer than the normal limit and a serum albumin level <3 g/dl or a history of ascites, variceal bleeding, or hepatic encephalopathy. Post chemotherapy or serious associated diseases including malignancy, poorly controlled diabetes, and end stage renal disease were also excluded. The study was approved by the ethics committees at each participating center.

Liver histology

All patients with available liver biopsy data before starting treatment were evaluated for the degree of necroinflammatory activity and fibrosis using the histological activity index (HAI) or Knodell score index [16]. The Knodell grading system is on a scale from 0 to 22 with the higher scores indicating more severe inflammation. Briefly, the overall Knodell score is the sum of the scores for periportal bridging necrosis (0, 1, 3, 4, 5, 10), intralobular degeneration and focal necrosis (0, 1, 3, 4), portal inflammation (0, 1, 3, 4), and fibrosis (0, 1, 3, 4) [16].

Clinical data and laboratory tests monitoring at follow-up

Data monitoring included review of clinical records, complete blood counts, liver function tests (LFTs), liver histopathological findings, and abdominal ultrasonography at least annually. Measurements of the treatment response included biochemical, virological responses and the development of drug-resistant HBV mutants [17]. A treatment response was defined as ALT normalization and HBV DNA level <10,000 copies/ml (<2,000 IU/ml) at the end of follow-up. HBeAg seroconversion was also recorded in HBeAg-positive patients. Patients treated with NAs were evaluated, while they continued on antiviral agents or other therapy (on-treatment response). Patients treated with IFN were evaluated while they stopped antiviral agents and IFN (off-treatment response).

Primary nonresponse was defined as the inability of NAs to reduce serum HBV DNA by $\geq 1 \log_{10}$ IU/ml after the first 6 months of therapy. Secondary antiviral treatment failure or virological breakthrough, was defined as a $\geq 1 \log_{10}$ IU/ml increase in serum HBV DNA level from nadir in 2 consecutive samples of at least 1 month apart in patients who had responded. All antiviral treatment failures excluded noncompliance with antiviral medications [18].

Primary end-points of this study were death or major cirrhotic complications that occurred during a 3-year follow-up. Major complications of cirrhosis were defined according to the following criteria: (a) HCC was diagnosed by ultrasonography (US) or CT or MRI and AFP above the normal limit with or without histological confirmation; (b) symptomatic ascites was defined by the presence of ascites causing symptoms of abdominal discomfort or spontaneous bacterial peritonitis (SBP), which was identified by abdominal US examination or abdominal tapping; (c) gastrointestinal (GI) bleeding from portal hypertension (PHT) and confirmed by endoscopy; (d) hepatic encephalopathy was diagnosed by clinical findings; (e) decompensated cirrhosis was defined according to transition from Child A to Child B or Child C cirrhosis.

Statistical Analysis

Continuous variables were presented as mean standard deviation (SD) or median (± interquartile range [IQR]) as appropriate. Comparisons between the two groups were performed using an independent *t* test if values were normally distributed or using a Wilcoxon rank sum test if the distribution was not normal. Categorical data were presented as numbers (percentage) and were compared using a Fisher exact test or χ^2 test where appropriate. All tests were two sided, and the chosen level of significance was $P < 0.05$. Statistical analyses were conducted using SPSS version 15.0.

Results
Demographics and clinical features

A total of 567 patients were enrolled and followed up for at least 3 years. Their baseline characteristics are described in **Table 1**. Median follow-up duration was 36 months. Most of the patients lived in Bangkok and the central region of Thailand (73.2%). The patients who received antiviral agents (NAs group and IFN group) were civil-servants (29.3%), laborers (23%), and traders (8%). Only 14.4% of CHB patients had underlying diseases of diabetes mellitus and hypertension. Only 18.3% of patients had a history of HBV infection in their immediate family and 5.5% had a history of receiving blood components. Most CHB patients (82%) were diagnosed while asymptomatic at routine checkups and 10.5% were diagnosed by blood testing as part of blood donation. Only 7.5% of patients were symptomatic complaining of fatigue, tender hepatomegaly or jaundice.

Of the 567 CHB patients, 108 (19%) had no HBeAg status. There were 262 HBeAg-positive patients (46%) and 197 HBeAg-negative patients (35%).

Sixty-one percent received NAs monotherapy (NAs; group1) while 19.9% received IFN-α monotherapy (IFN; group 2) and the remaining 19% received supportive treatment (SUP; group 3). The majority of the NAs group was treated with lamivudine 100 mg per day (69%) while the rest of 25% of patients were treated with lamivudine 150 mg per day and 6% were treated with adefovir 10 mg per day, respectively. Conventional IFN-α accounted for 78% of group 2 while the remainder (22%) was treated with PEG-IFN. Comparing the NAs and IFN groups, there was a statistically significant difference ($P < 0.0001$) in several variables as shown in **Table 2**. Patients treated with NAs (n = 349) had significantly more frequent comorbidities, less liver biopsies, and fewer adverse events from treatment than those receiving IFN.

Baseline laboratory and liver histology findings

Laboratory test results at the first visit are summarized in **Table 1**. The AST/ALT ratio was less than one in most patients. Serum total bilirubin, albumin as well as PT were mostly within the normal range. About half of CHB patients had available results of liver biopsy. The mean (SD) baseline Knodell scores were similar in both HBeAg positive (6.7 ± 3.8) and HBeAg negative patients (7.0 ± 3.7). The Knodell score was less than 8 or mild activity of inflammation was found in 71% of patients. Significant fibrosis (score ≥3) or bridging fibrosis was found in one-third of patients (32.8%).

Biochemical, HBeAg and HBsAg seroconversion and virological responses

For HBeAg-positive patients (n = 262), the proportion of HBeAg seroconversion and undetectable HBV DNA was 32.8% and 50.5% in the NAs group patients respectively, while HBeAg seroconversion and undetectable HBV DNA was found in 24.3% and 21.4% of group 2 patients respectively. Normalization of ALT was found in 69.8% of patients in group 1 and 55.7% of patients in group 2. HBsAg loss was not found in patients from the NAs group, but two patients in the IFN group had HBsAg loss (2.8%) as shown in **Table 3**. For HBeAg negative patients (n = 197), the proportions of undetectable levels of HBV DNA and normalization of ALT were 68.8% and 70.8% in the NAs group, while proportions of patients with undetectable levels of HBV DNA and normalization of ALT were in 37.5% and 90.0% in the IFN group (**Table 4**).

In the SUP group, 77% of patients were labors and most of these (90%) were followed-up regularly without significantly different outcomes compared with patients in the NAs and IFN groups. The main reasons for not receiving antiviral agents were the concern regarding the side effects of this medication, the costs of treatment, and the chance of drug resistance.

Adverse events and major complications

Fewer adverse events were found in the NAs group whereas ten patients (9%) in the IFN group had adverse events including fever, myalgia, flu-like symptoms, weight loss, alopecia, and anorexia. Most subjects could continue therapy with an adjusted dosage of IFN. The reappearance of HBV in patients from the NAs group and IFN group was 24.3% and 26.6%, respectively. Most patients with reappearance of HBV presented with elevated transaminase enzyme. About half of the patients in the NAs group received additional NAs for viral control (**Table 5**). Only 5.6% of patients progressed to Child A cirrhosis during a 3-year follow-up. Five patients in the SUP group developed cirrhosis, compared with 27 patients in the NAs and IFN groups. However, this difference was not statistically significant. One patient in the NAs group died from other cause unrelated to their liver disease (0.18%).

Table 1. Baseline characteristics of the 567 CHB patients enrolled

Major variables (normal values)	Mean ± SD/number of patients (%)
Male:female	2.6:1
HBeAg positive:HBeAg negative	1.3:1
Age (years)	46.9 ± 11.9
ALT (<40 ULN)	75 ± 120
AST (<40 ULN)	52.5 ± 84
AP (<117 ULN)	79.2 ± 35
FBS (<106 mg/dl)	106 ± 38
Knodell score	6.8 ± 3.8
Treatment with	
- Group 1: NAs monotherapy	349
- Lamivudine 100 mg.	69%
- Lamivudine 150 mg.	25%
- Adefovir 10 mg.	6%
- Group 2: IFN monotherapy	110
- PEG-IFN-α	22%
- IFN-α	78%
- Group 3: Supportive care	108

Table 2. Comparison between treatment groups of antiviral agent

Variables	NAs monotherapy (group 1)	IFN monotherapy (group 2)	P
Live in central area	71.2%	75.7%	0.02
Known diagnosis from blood donation	8.4%	18.7%	0.02
Presence of comorbid diseases	15.5%	5.6%	0.03
Presence of liver biopsy	47.8%	74.5%	<0.0001
Adverse events	1.7%	7.0%	<0.009

Pearson, χ^2, $P < 0.05$; statistically significant difference

Table 3. Biochemical response, HBeAg and HBsAg Seroconversion and Virological response at 3-year of follow up in HBeAg positive CHB patients (n = 262)

Variables	NAs (group 1) (n = 192)	Interferon** (group 2) (n = 70)
Normalization of ALT	69.8	55.7
HBV-DNA<2,000 IU/ml	50.5	21.4
HBeAg seroconversion	32.8(on therapy)	24.3(off therapy)
HBeAg loss	14.6	37.0

**S Antigen loss was found 2.8% of interferon monotherapy group (2 patients)

Table 4. Biochemical response, HBsAg seroconversion, and virological response at 3-year of follow up in HBeAg negative CHB patients (n = 197)

Variables	NAs (group 1) (n = 157)	Interferon** (group 2) (n = 40)
Normalization of ALT	70.8	90.0
HBV-DNA<2,000 IU/ml	68.8	37.5

There was no S Antigen loss found both treatment groups

Table 5. The results of 3 years follow-up

Variables	Number of patients (%)
Death	1(0.18%)
Cirrhosis*	32 (5.6%)
Other complications**	9 (1.6%)
Loss follow-up	6.8%
Reappearance of HBV-DNA in NAs group	24.3%
Reappearance of HBV-DNA in IFN group	26.6%

Child A cirrhosis = 30 cases and Child B cirrhosis = 2 cases

Discussion

To our knowledge this is the first multicenter study of Thai CHB patients with a long-term follow-up. The study demonstrated that Thai CHB patients have a potentially progressive disease with 5.6% developing cirrhosis regardless of therapy within 3-years of follow-up. The detection rate of cirrhosis in Thai CHB patients was approximately 1.5% per year. Previous reports showed that the rate of cirrhosis was 2% per year [6, 19]. The dynamic natural history and the high level of HBV DNA at baseline in Asian CHB patients may be important factors for ongoing viral replication and liver damage [20, 21]. In previous studies, the incidence of cirrhosis accounted for approximately twice the rate in HBeAg-negative patients compared with that in HBeAg-positive patients [22]. However, we found no difference in HBeAg status between the groups. The majority (74%) of our patients were diagnosed while asymptomatic and most of them had no risk factors. Thus, an effective early HBV screening system to detect CHB patients should be established for such a highly endemic population.

The outcome of CHB treatment was influenced by several factors for example the presence of bridging fibrosis, which was seen in one-third of our patients [23-25]. Cooksley and colleagues included 8%–10% of patients with Child A cirrhosis while Lau and Janssen enrolled 10%–18% cirrhosis patients. Both studies confirmed the influence of cirrhosis on the outcome of treatment [26, 27]. Other variables including baseline ALT, HBV DNA levels, patient age, sex, and HBV genotype also predicted the outcome of treatment [28]. Recently, quantitative HBeAg was proposed as a new promising predictor for HBeAg seroconversion in HBeAg-positive patients treated with PEG-IFN. This study found that only 4% of the patients who had levels of HBeAg equal or higher than 100 PEIU/ml reached HBeAg seroconversion during 24 weeks of treatment [29]. However, our study did not measure HBeAg level or genotype. The HBV genotype, especially genotypes A and B showed a better outcome from treatment than genotypes C and D [25]. However, HBV genotyping was not tested in our study.

The criteria for treatment response were different in each study. The combined response rate to PEG-IFN and/ or lamivudine therapy was defined by the combined loss of HBeAg, HBV-DNA $<5 \times 10^5$ cp/ml and normalized ALT at 24–52 weeks [23-25, 30]. The difference in duration, dose of medication, and definition of response may change the outcome of treatment and we needed to consider the different definition of treatment response in each study.

One of the important responses is HBsAg loss, which was found mostly in patients from the IFN group (2%–7%) [23-25, 30]. The patients in our IFN-treatment group showed similar numbers of HBsAg loss (2.8%). Patients treated with lamivudine showed the variable response rate at off-therapy at 52 weeks, which varied from 22%–28% [23, 30].

For HBeAg positive patients, extended therapy for 2 and 3 years clearly increased the rate of HBeAg seroconversion from 17% to 40% [10, 12, 31-33]. However, the emergence of YMDD mutants was also increased from 38% at the end of year 2 to 57% at the end of year 3 [10, 12, 31-33]. About 60% of YMDD mutant patients had elevated ALT and one-third of them had an elevated ALT of more than 5 times baseline [33].

For HBeAg-negative patients, HBV DNA suppression and normalization of serum ALT levels have been used as the goal of treatment in most studies [34]. The response rate of the NAs group and the occurrence of genotypic resistance were quite similar to previous studies [15, 35, 36]. Close follow-up for lamivudine resistance, clinical monitoring for cirrhosis, and prompt add-on antiviral therapy or switching therapy when required are needed to assure quality care [15].

For IFN monotherapy, Marcellin and colleagues revealed that PEG-IFN-α2a resulted in better outcomes than lamivudine [37]. A virological response of low serum HBV DNA levels (<20,000 cp/ml) was found in 43% of patients thus treated and sustained suppression of HBV DNA (<400 cp/ml) was also found in 19% of patients [37]. Approximately half of patients treated with PEG-IFN-α2a required dose reduction and 7% of patients discontinued the drug because of adverse events [37]. The frequency of adverse events was found to be 3–4 times higher than that in our study. The better tolerance of Thai patients, lower body weight, and the lower dose of IFN may explain the lower frequency of adverse events found in our study.

The main strengths of our study are that it involves the largest population of Thai CHB patients, a long-term follow-up of 3 years, and a multicenter patient population. Additionally, all patients were well diagnosed with the clinical presentations, radiological findings, and serological testing. Liver histology was

available for more than half of the patients. Nevertheless, our study also has some limitations. First, the duration of 3-year follow-up may be too short to identify important liver complications. Second, the new definition of virological response recommended by an international workshop "Roadmap for management of patients receiving oral therapy for CHB" was not applied to our study [38, 39]. Third, the Thailand consensus recommendation for management of CHB was distributed in 2005. Thus, the treatment choices of CHB have been changed during the study period [17, 28, 40-44].

In conclusion, the treatment response of Thai CHB patients from multicenter study showed similar results to previous studies. However, higher durability of treatment with lower rate of reappearance of HBV DNA was observed in Thai CHB patients.

References

1. Pramoolsinsap C, Pukrittayakamee S, Desakorn V. Hepatitis B problem in Thailand. Southeast Asian J Trop Med Public Health. 1986; 17:219-28.
2. Merican I, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Chien RN, et al. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol. 2000; 15:1356-61.
3. Poovorawan Y, Sanpavat S, Pongpunglert W, Chumdermpadetsuk S, Sentrakul P, Vandepapeliere P, et al. Long term efficacy of hepatitis B vaccine in infants born to hepatitis B e antigen-positive mothers. Pediatr Infect Dis J. 1992; 11:816-21.
4. Poovorawan Y, Chatchatee P, Chongsrisawat V. Epidemiology and prophylaxis of viral hepatitis: a global perspective. J Gastroenterol Hepatol. 2002; 17 (Suppl): S155-66.
5. Lau GK, Carman WF, Locarnini SA, Okuda K, Lu ZM, Williams R, et al. Treatment of chronic hepatitis B virus infection: an Asia-Pacific perspective. J Gastroenterol Hepatol. 1999; 14:3-12.
6. Chu CM. Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol. 2000; 15 (Suppl):E25-30.
7. Benvegnu L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut. 2004; 53:744-9.
8. Yuen MF, Lai CL. Natural history of chronic hepatitis B virus infection. J Gastroenterol Hepatol. 2000; 15 Suppl:E20-4.
9. Zacharakis GH, Koskinas J, Kotsiou S, Papoutselis M, Tzara F, Vafeiadis N, et al. Natural history of chronic HBV infection: a cohort study with up to 12 years follow-up in North Greece (part of the Interreg I-II/EC-project). J Med Virol. 2005; 77:173-9.
10. Leung N. Nucleoside analogues in the treatment of chronic hepatitis B. J Gastroenterol Hepatol. 2000; 15 (Suppl):E53-60.
11. Liaw YF. Therapy of chronic hepatitis B: current challenges and opportunities. J Viral Hepat. 2002; 9: 393-9.
12. Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, et al. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. Gastroenterology. 2000; 119: 172-80.
13. Gish RG, Locarnini SA. Chronic hepatitis B: current testing strategies. Clin Gastroenterol Hepatol. 2006; 4:666-76.

14. Yuen MF, Lai CL. Recommendations and potential future options in the treatment of hepatitis B. Expert Opin Pharmacother. 2006; 7:2225-31.

15. Hadziyannis SJ, Papatheodoridis GV. Hepatitis B e antigen-negative chronic hepatitis B: natural history and treatment. Semin Liver Dis. 2006; 26:130-41.

16. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology. 1981; 1:431-5.

17. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. Hepatology. 2007; 45:1056-75.

18. Lok AS, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology. 2007; 46:254-65.

19. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis. 2005; 25 (Suppl 1):3-8.

20. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004; 350:1118-29.

21. Ribeiro RM, Lo A, Perelson AS. Dynamics of hepatitis B virus infection. Microbes Infect. 2002; 4:829-35.

22. Giovanna F, Bortolotti F, Francesco D. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol. 2008; 48:335-52.

23. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005; 352: 2682-95.

24. Cooksley G. The treatment of hepatitis B e antigen-positive chronic hepatitis B with pegylated interferon. J Hepatol. 2003; 39 (Suppl 1):S143-5.

25. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet. 2005; 365 (9454):123-9.

26. Bedossa P B-SP, Callard P, Chevallier M, Degott C, Deugnier Y, et al. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology. 1994; 20:15-20.

27. Goldin RD, Goldin JG, Burt AD, Dhillon PA, Hubscher S, Wyatt J, et al. Intra-observer and inter-observer variation in the histopathological assessment of chronic viral hepatitis. J Hepatol. 1996; 25:649-54.

28. Bonino F, Marcellin P, Lau GK, Hadziyannis S, Jin R, Piratvisuth T, et al. Predicting response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B. Gut. 2007; 56:699-705.

29. Fried MW, Piratvisuth T, Lau GK, Marcellin P, Chow WC, Cooksley G, et al. HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. Hepatology. 2008; 47:428-34.

30. Chan HL, Leung NW, Hui AY, Wong VW, Liew CT, Chim AM, et al. A randomized, controlled trial of combination therapy for chronic hepatitis B: comparing pegylated interferon-alpha2b and lamivudine with lamivudine alone. Ann Intern Med. 2005; 142:240-50.

31. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology. 2001; 33:1527-32.

32. Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. J Gastroenterol Hepatol. 2004; 19:1276-82.

33. Wong VW, Chan HL, Wong ML, Tam JS, Leung NW. Clinical course after stopping lamivudine in chronic hepatitis B patients with lamivudine-resistant mutants. Aliment Pharmacol Ther. 2004; 19:323-9.

34. Hui CK, Lau GK. Treatment of hepatitis B e antigen-negative patients. Curr Treat Options Gastroenterol. 2007; 10:474-82.

35. Papatheodoridis GV, Dimou E, Dimakopoulos K, Manolakopoulos S, Rapti I, Kitis G, et al. Outcome of hepatitis B e antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with lamivudine. Hepatology. 2005; 42:121-9.

36. Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, et al. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. Hepatology. 2004; 40:883-91.

37. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2004; 351:1206-17.

38. Zhang DZ. [Report of an international workshop: roadmap for management of patients receiving oral therapy for chronic hepatitis B]. Zhonghua Gan Zang

Bing Za Zhi. 2007; 15:876-7.

39. Keeffe EB, Zeuzem S, Koff RS, Dieterich DT, Esteban-Mur R, Gane EJ, et al. Report of an international workshop: roadmap for management of patients receiving oral therapy for chronic hepatitis B. Clin Gastroenterol Hepatol. 2007; 5:890-7.

40. Lacey LF, Gane E. The cost-effectiveness of long-term antiviral therapy in the management of HBeAg-positive and HBeAg-negative chronic hepatitis B in Singapore. J Viral Hepat. 2007; 14:751-66.

41. Gish RG. Improving outcomes for patients with chronic hepatitis B. Hepatol Res. 2007; 37 (s1):S67-78.

42. Lin CL, Kao JH. Hepatitis B viral factors and clinical outcomes of chronic hepatitis B. J Biomed Sci. 2008; 15:137-45.

43. Osborn MK, Han SH, Regev A, Bzowej NH, Ishitani MB, Tran TT, et al. Outcomes of patients with hepatitis B who developed antiviral resistance while on the liver transplant waiting list. Clin Gastroenterol Hepatol. 2007; 5:1454-61.

44. Perrillo RP, Jacobson IM. Halting the natural history of hepatitis B viral infection: a paradigm shift. Semin Liver Dis. 2007; 27 (Suppl 1):3-8.