

Research Article

Pegylated Interferon Alfa 2a Treatment in Patients with Chronic Hepatitis B and Genotype D in Jordan

Hamoudi Waseem^{1*}, Mirela Maria H, Al-Azizi Moath, Al Sheikh Mahmoud, Shabaan Hamdi, Laswi Eyad RN and Al Hamed Ahmad RN

Internal Medicine department, Al Bashir Hospital, Jordan

*Corresponding author: Hamoudi Waseem, Internal Medicine department, Gastroenterology & Hepatology unit – Al Bashir Hospital, Amman – Jordan, Email: Waseem6520012001@yahoo.com

Received: August 01, 2014; Accepted: August 20, 2014; Published: August 21, 2014

Abstract

Background & Aims: To evaluate the efficacy of immune-modulation therapy using pegylated interferon Alfa 2a in the treatment of patients infected with HBV.

Patients, Methods: 103 patients diagnosed with chronic hepatitis B at the Gastroenterology & Hepatology unit in Al Bashir Hospital Amman-Jordan between the years 2006 and 2013 were treated with pegylated interferon Alfa 2a 180 mcg once weekly for 48 weeks, 54.3% (56/103) were of male gender and 45.6% (47/103) were female. 45 (43.6%) patients were HBeAg negative, 58 (56.3%) were HBeAg positive.

ALT, HBV DNA levels, HBeAg seroconversion, HBsAg seroconversion were investigated at 12, 24, 36, 48 and 72 weeks. Non responders were defined failure to decrease in the viral load at least 2 logs at 24 weeks of treatment, responders were defined as HBV DNA load less than 2000 IU/ml at week 48, sustained responders were defined as patients who sustained their viral load less than 2000 after 72 weeks of treatment. HBeAg seroconversion was defined as disappearance of HBeAg and seroconversion to HBeAb. HBsAg clearance was defined as disappearance of HBsAg. HBsAg seroconversion was considered as disappearance of HBsAg and seroconversion to HBsAb. Relapsers were those responders that had HBV DNA increase more than 2000 IU/ml and ALT flair after end of treatment.

All patients were of genotype D.

Results: 5.8% of treated patients were sustained responders (6/103), of them 33.3% (2/6) were HBeAg negative patients and 66.6% (4/6) were HBeAg positive patients.

Non-responders counted 68.9% (71/103), of them 53.3% (24/45) were HBeAg negative patients and 46.6% (21/45) were HBeAg positive patients. Relapsers were 25.2% (26/103) (16 HBeAg negative patients and 10 HBeAg positive patients). One patient seroconverted HBsAg to HBsAb (0.9%), and two patients obtained HBsAg clearance (1.9%).

Conclusion: Pegylated interferon therapy for patients with chronic hepatitis B in Jordan showed that the response to this treatment is unsatisfactory, mostly due to high prevalence of genotype D in Jordan.

Longer duration of treatment or sequential therapy may be of help in order to obtain more positive results. More studies regarding treatment with immune modulation therapy are warranted to obtain more representative data.

Keywords: Chronic Hepatitis B; Pegylated Interferon; Genotype D; Sustained responders; Jordan

Introduction

Chronic hepatitis B is prevalent in the world, with estimated chronic carriers of more than 350 millions worldwide [1]. Currently, pegylated interferon alfa 2a or oral nucleos(t)ides are approved for the treatment of chronic hepatitis B with the aim of HBV DNA negativity, HbeAg seroconversion, normalization of ALT and ultimately HbsAg clearance and seroconversion [2,3].

Pegylated interferon-alfa 2a (PEG-IFN) provides potential advantages over nucleos(t)ides analogues in the treatment of Chronic

Hepatitis B (CHB) given its finite course, durability and lack of drug resistance [2,3].

Best predictors of good response for treatment with interferon / Pegylated interferon based treatment are HBeAg positive patients, low viral load, high serum ALT levels, HBV genotype A, B and high activity in liver biopsy [2,3].

The predominant HBV genotype in Middle Eastern countries including Jordan is genotype D [4,5]. Also Jordan has high prevalence of HBsAg negative individuals, which may raise questions regarding

efficacy of treatment with immune modulators for patients with Chronic Hepatitis B.

Jordan has high prevalence of HBV infected people reaching in the med eighties of the last century around 10% (carriers and diseased) [6,7,8], it is estimated now that this prevalence has dropped to 3% due to introduction of vaccination in the med nighties and other measurement to control this disease [9,10]. In order to clarify the issue of efficacy of treating chronic hepatitis B patients with Pegylated Interferon Alfa 2a and its cost effectiveness, we concluded this study over a period of 8 years in order to investigate the efficacy and tolerability of Pegylated Interferon Alfa 2a in chronic hepatitis patients in a clinical setting.

Methods & Material

This study included all eligible chronic hepatitis B patients for treatment who choose treatment with Pegylated interferon therapy and addressed Al Bashir Teaching Hospital – Gastroenterology & Hepatology unit between the years 2006 and 2013; they were offered to choose between nucleotide / nucleoside analogues or treatment with Pegylated Interferon Alfa 2a 180 mcg weekly for 48 weeks after explaining advantages and disadvantages of both regimens.

Al Bashir Teaching Hospital is the biggest tertiary hospital in Jordan that serves more than 40% of Jordanians. This study included all patients who accepted treatment with pegylated interferon 180 mcg. Demographic data, HBV markers including HBeAg, HBV DNA by PCR, ALT levels, synthetic function of the liver and imagistic studies were recorded prior to treatment. HBV genotyping was done for all studied patients.

Follow up tests with HBV DNA by PCR, HBsAg, HBsAb, HBeAg, HBeAb status and ALT levels were recorded at 12, 24, 48 weeks of treatment and 6 months after ending treatment. Complete blood count was investigated weekly for every patient for the first two months then monthly. Side effects were recorded for every patient also. Cirrhotic patients were not included in this study.

Patients who responded favorably to treatment at 6 months or less (decrease in HBV DNA with at least 2 logs and/or seroconverted HBeAg to HBeAb and normalization of ALT) continued treatment for another 24 months (total treatment regimen of 48 weeks) and were considered responders. Patients who did not achieve the treatment endpoints at 6 months were considered non-responders while patients who did respond to treatment regimen and relapsed after stopping the treatment were considered relapses.

The ultimate goal of the treatment was HBsAg clearance and seroconversion to HBsAb.

Other goals of treatment were sustained HBeAg seroconversion to HBeAb in HBeAg positive patients, decrease / undetectably of HBV DNA and normalization of ALT.

Treated patients (responders, non-responders and relapsers) after completion of treatment regimen were tested yearly for liver function tests and HBV status.

Non-responders and relapses were offered alternative therapy using nucleoside/nucleotide analogues.

One hundred and three patients were included in this study; 56

(54.3%) were of male gender and 47 (35.6%) were female.

Fifty eight patients (56.3%) were HBeAg positive and 45 patients (43.6%) were HBeAg negative. Patient's age ranged between 18 and 60 years with median range of 42.1 years (SD=11) (Table 1).

Table 1: Characteristics of 103 Chronic Hepatitis B patients treated with Pegylated interferon 180 mcg.

	No/%
Age(mean/year)	42.1
Sex	
Female	47(45.6)
Male	56(54.3)
HBeAgpositive	58(56.3)
HBeAgnegative	45(43.6)
GenotypeD	103(100)
ALT(mean/IU/L)	96.8
HBVDNA(mean/IU)	278291

Pegylated Interferon Alfa 2a 180mcg Roche® was used subcutaneously on a weekly basis for at least 24 weeks, extended for 48 weeks in responders.

We did not perform liver biopsy prior to treatment for our treated patients because this procedure was not needed for our sitting; all of them had obvious active CHB in HBeAg positive and HBeAg negative patients (ALT 2 time above upper normal values and high serum HBV DNA) [42]. High Serum HBV DNA was considered > 20000 IU/ml for HBeAg positive patients, and > than 2000 IU/ml for HBeAg negative patients [3,42]. Anyhow all patients included in this study had high values of more than 20000IU/ml prior to treatment.

We did not include HBsAg titer in our study as possible predictor of response.

HBV markers were tested using TKA Bellini System (ELISA) with lower detection limit of 1.

HBV DNA quantification were tested using real time amplification PCR type COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 with low detection limit of 20 IU/ml. Roche diagnostics.

HBV genotyping were tested using Line probe genotyping assay (INNO-LiPA HBV Genotyping assay; Innogenetics, Ghent).

Serum Alanine Aminotransferase (ALT) normal limits was between 0-41 IU/ml.

Results

From the 103 treated patients, 68.9% (71/103) were non-responders (did not achieve any goal of treatment at 6 months), of them 53.3% (24/71) were HBeAg negative and 46.6% (21/71) were HBeAg positive. Relapsers counted 25.2% (26/103), of them 61.5% (16/26) were HBeAg negative and 38.4% (10/26) were HBeAg positive (Table 2).

Viralresponse	No/%
SustainedResponders	6(5.8)
Non-Responders	71(68.9)
Relapsers	26(25.2)

Only 6 responders in this study were recorded (5.8%), of them 33.3% (2/6) were HBeAg negative and 66.6% (4/6) were HBeAg positive.

One patient seroconverted HBsAg to HBsAb (0.9%) after 3 years of follow up and was of HBeAg positive patients, and two patients obtained HBsAg clearance (1.9%) after 2 years of follow up and also were of HBeAg positive patients (Table 3). No serious side effects were recorded during treatment and treatment regimen was tolerated well.

Table 3:

	HBeAgpositiveNo/%	HBeAgnegativeNo/%
Sustainedresponders	4(66.6)	2(33.3)
Non-responders	21(46.6)	24(53.3)
Relapsers	10(38.4)	16(61.5)
HBsAgclearance	2(1.9)	
HBsAgseroconversion	1(0.9)	

Ethical Issues

Data collected were treated confidentially, and all participants provided informed consent for this study. This study was approved by the Ministry of Health Ethics Committee (MOH/EC/13700/2014).

Statistical Issues

Quantitative variables are expressed as mean \pm SD. Qualitative variables are expressed as percentage with range. Statistical analyses were performed using SPSS version 15. A value of $P < 0.05$ was considered to be statistically significant.

Discussion

Several international guidelines stated that predictors of good response for treatment with interferon / Pegylated interferon in HBeAg positive patients are low viral load, high serum ALT levels, HBV genotype and high activity in liver biopsy [11,12,13].

Genotype A and B have been showed to be associated with higher rates of anti HBe sero-conversion and HBsAg loss than genotype D and C after treatment with PegIFN [13,14,15,16].

During treatment in those patients a decrease of < 20000 IU/ml at 12 weeks is associated with a 50% chance of anti HBe seroconversion [17].

In HBeAg negative patients there are no pretreatment predictors of virological response. During treatment for those patients, a decrease of < 20000 IU/ml at 12 weeks was associated with a 50 % chance of sustained off treatment response [18,19].

HBV genotypes

Eight genotypes have been identified labeled A through H [20,21]. Genotype A occurs in Africa, Europe and India, genotype B occurs in east and southeast Asia, genotype C occurs in East Asia and the pacific Islands and genotype D occurs in the Mediterranean region, Middle East, central Asia and India and can be used to study anthropological migration patterns in the past [22,5]. Genotype E occurs in West Africa and genotypes F and H in central and South America. The genotype G appears partially defective and invariably occurs together with another genotype [23]. Hybrids of B and C are

found in Asian countries, A and D in Italy and C and D in Tibet and China [27].

Recent data suggest that HBV genotypes may play an important role in the progression of HBV related liver disease as well as response to Interferon therapy [20]. Studies from Asia found that HBV genotype B is associated with HBeAg seroconversion at an earlier age, more sustained remission after HBeAg seroconversion, less active hepatic necroinflammation, a lower rate of progression to cirrhosis, and a lower rate of HCC development compared to genotype C [25,26]. Several studies of standard Interferon alpha and one study of pegylated Interferon alpha therapy reported that genotypes A and B were associated with higher rates of HBeAg seroconversion compared to genotypes C and D [27,12]. Another study of Pegylated Interferon reported that genotype A but not genotype B was associated with a higher rate of HBeAg seroconversion [11]. Studies of nucleos(t) ide analogue therapies have not showed any relation between HBV genotypes and response.

Nevertheless, the guidelines for management of chronic hepatitis B published by the American Association for the Study of Liver Diseases (AASLD) [29], the European Association for the Study of Liver (EASL) [2] and the Asian Pacific Association for the Study of the Liver (APASL) [30] suggest that genotyping the virus is not a recommended part of the management and is best regarded as a research tool. In contrast, individual reviews [31,32] cite the same evidence and recommended that therapy must be based on genotyping. The German guidelines [33] for the management of hepatitis B virus infection strongly support this position. The rationale for this position is because interferon alpha therapy should be evaluated first because it is for finite duration, and the aim is a sustained success of therapy. Because genotype A is likely to achieve this outcome, it is important to identify such patients. The Dutch guidelines [34] mention pegylated interferon should be considered for initial therapy particularly with genotypes A and B, and the Swedish guidelines [35] recommend pegylated interferon as first line treatment in particular for genotypes A and B.

Response to pegylated Interferon

There are few randomized controlled studies that have involved more than 100 patients treated with pegylated interferon in HBeAg positive and negative patients, the majority of those patients were of genotype B and C.

Lau *et al.* [28] included in his study 814 patients, genotypes B and C were predominant, and there were 56 patients with genotype A and 37 patients with genotype D. The HBe antigen seroconversion rate in those receiving pegylated interferon plus placebo was 52% (12/23) in genotype A and 22% (2/9) in genotype D.

Janssen *et al.* [12] was a European study and 34% of patients were genotype A and 39% genotype D, 9% had genotype B and 15% had genotype C and utilized 100 μ g for 52 weeks. Response rates (HBe antigen loss) varied by HBV genotype using univariate analysis ($P = 0.01$): genotype A $n = 42$ patients (response rate 47%); B $n = 10$ (44%); C $n = 11$ (28%); and D $n = 26$ (25%). Using multivariate analysis, patients infected with genotype A were more likely to respond than those with genotype D (odds ratio 2.4, $P = 0.01$) or genotype C (3.6, $P = 0.006$). Patients infected with genotype B were slightly but

not significantly more likely to respond compared with those with genotype C (odds ratio 2.2, $P = 0.18$).

Both of the studies (Lau (28) and Janssen (12)) with genotypes A, B, C and D had shown the best response in genotype A and the lowest response in genotype D.

Marcellin P et al. [36] in HBe antigen-negative patients using pegylated interferon or pegylated interferon with lamivudine or lamivudine monotherapy involving 530 patients in Europe and Asia. Patients received pegylated interferon Alfa 2a 180 μ g for 48 weeks. Genotype was available for 346 patients in the two arms containing PEG interferon, and the largest group was genotype C, then genotypes D and B. Only 6% had genotype A. The combined response (normalization of ALT and DNA <20 000 copies/mL) was 44% for genotype B, 49% for genotype C and 16% for genotype D in patients receiving PEG interferon only. Six months after the end of therapy, approximately 3% of patients in the two PEG interferon groups had lost HBsAg compared to 0% in the lamivudine group. Two hundred and thirty patients from the two groups who received PEG interferon then entered a long-term follow-up study for 5 years. After 5 years, 22% of patients still had ALT normalization, 17% had undetectable DNA (<400 copies/mL) and 12% had lost HBsAg [37].

Analysis of the effects of HBV genotypes on the treatment outcome in the pooled analysis [38] of three large cohorts of hepatitis B patients both HBeAg positive and HBeAg negative treated with interferon alpha produced 1229 patients and included two clinical trials [28,12]. There were 174 patients with genotype A, 245 genotype B, 464 genotype C and 346 genotype D, and treatment was standard interferon or pegylated interferon with or without lamivudine. Overall, sustained virological response was greatest for genotype A ($P < 0.001$) (with 36% and 34% having a sustained virological response for HBeAg positive and negative, respectively). The rates of sustained virological response for HBe antigen-positive patients were 21%, 19% and 15% for genotypes B, C and D, respectively, and in the negative patients 32%, 50% and 21% [38].

What will be the outcome if genotyping is carried out in HBe antigen-positive patients prior to treatment? Patients with genotype A and B will be offered pegylated interferon as first line of therapy as it offers the likelihood of HBeAg seroconversion with a finite course of therapy.

In HBe antigen-negative patients, the same recommendations with respect to genotypes A and B at least will apply. As a result, about a third of patients will be successfully treated, and the disadvantages of nucleoside analogues will be avoided.

In summary, genotyping should be carried out in HBeAg-positive disease to influence choice of treatment and in HBeAg-negative disease where decision to treat depends on genotype.

Another issue is what will happen with patients with failure to Pegylated Interferon treatment? The answer will possibly be by prolongation of treatment with Pegylated Interferon up to 2 years, current data suggest that patients with HBeAg negative chronic hepatitis B should be treated for more than one year [42,43].

Another option of increasing the rate of sustained response is

the current trials of sequential therapy with Entecavir and interferon therapy, which showed a promising good result [40,41].

Conclusion

Our study showed that the sustained response to pegylated Interferon is unsatisfactory, having 5.6% of treated patients with sustained viral suppression, with only one patient who cleared and seroconverted the virus into antibodies and two patients cleared HBsAg without seroconversion. The high number of non-responders 68.9% and relapses 25.2% raised the idea that low response to interferon therapy in our patients is due to genotype D, having in mind that all our treated patients had genotype D indifferent to HBeAg status. So as a recommendation, we recommend our physicians to think of treating their patients with nucleoside /nucleotide analogue or to combine immune modulators with oral drugs sequentially or prolongation of treatment with pegylated Interferon in order to have more satisfactory results. Our study may be criticized being the majority of treated patients are HBeAg positive patients, where experts sustain that HBeAg negative individuals in Jordan are more frequent seen, this may be explained because of undeclared bias of the investigators in selecting patients for treatment during explanation of advantages of this treatment for HBeAg positive patients and this may influenced the choice of the patients for this kind of treatment.

References

1. Vincenzo Puro, Daniel Shouval. Viral Hepatitis. 2005.
2. European Association For The Study Of The Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol.* 2009; 50: 227-242.
3. Lok ASF, Mc Mahon BJ. AASLD practice guidelines. *Chronic hepatitis B. Hepatology.* 2007; 45: 507-539.
4. Suguchi F, Mizokami M, Orito E, Ohno T, Kato H, Suzuki S, et al. A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. *J Gen Virol.* 2001; 82: 883-892.
5. Jazayeri MS, Basuni AA, Cooksley G, Locarnini S, Carman WF. Hepatitis B virus genotypes, core gene variability and ethnicity in the Pacific region. *J Hepatol.* 2004; 41: 139-146.
6. Toukan AU, Sharaiha ZK, Abu-el-Rub OA, Hmoud MK, Dahbour SS, Abu-Hassan H, et al. The epidemiology of hepatitis B virus among family members in the Middle East. *Am J Epidemiol.* 1990; 132: 220-232.
7. Toukan AU. Hepatitis B in the Middle East: aspects of epidemiology and liver disease after infection. *Gut.* 1996; 38: 2-4.
8. Qirbi N, Hall AJ. Epidemiology of hepatitis B virus infection in the Middle East. *East Mediterr Health J.* 2001; 7: 1034-1045.
9. W.Hamoudi. Jordan and hepatitis B virus. Do we have to worry? *World Gastroenterology Organization – Viral hepatitis.* 2007.
10. Belbisi A, Hadadin A, Toukan A, Hamoudi W, Ghazzawi I, Khatib MA, et al. *Jordan National Strategy for Viral Hepatitis.* 2010.
11. 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: 2682-2695.
12. Janssen HL, van ZM, 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; 365:123-129.
13. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-

- positive chronic hepatitis B. A meta-analysis. *Ann Intern Med.* 1993; 119: 312-323.
14. Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. HBV 99-01 Study Group. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol.* 2006; 101: 297-303.
 15. Buster EH, Hansen BE, Lau GK, Piratvisuth T, Zeuzem S, Steyerberg EW, et al. Factors that predict response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa. *Gastroenterology.* 2009; 137: 2002-2009.
 16. 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: 428-434.
 17. 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.
 18. Rijckborst V, Hansen BE, Cakaloglu Y, Ferenci P, Tabak F, Akdogan M, et al. Early on-treatment prediction of response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B using HBsAg and HBV DNA levels. *Hepatology.* 2010; 52: 454-461.
 19. Rijckborst V, Hansen BE, Ferenci P, Brunetto MR, Tabak F, Cakaloglu Y, et al. Validation of a stopping rule at week 12 using HBsAg and HBV DNA for HBeAg-negative patients treated with peginterferon alfa-2a. *J Hepatol.* 2012; 56: 1006-1011.
 20. Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? *Hepatology.* 2004; 40: 790-792.
 21. Norder H, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology.* 2004; 47: 289-309.
 22. Sugauchi F, Mizokami M, Orito E, Ohno T, Kato H, Suzuki S, et al. A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. *J Gen Virol.* 2001; 82: 883-892.
 23. Kato H, Orito E, Gish RG, Sugauchi F, Suzuki S, Ueda R, et al. Characteristics of hepatitis B virus isolates of genotype G and their phylogenetic differences from the other six genotypes (A through F). *J Virol.* 2002; 76: 6131-6137.
 24. Pujol FH, Navas MC, Hainaut P, Chemin I. Worldwide genetic diversity of HBV genotypes and risk of hepatocellular carcinoma. *Cancer Lett.* 2009; 286: 80-88.
 25. Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, et al. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut.* 2004; 53: 1494-1498.
 26. Yu MW, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst.* 2005; 97: 265-272.
 27. Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol.* 2000; 33: 998-1002.
 28. 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: 2682-2695.
 29. Lok ASF, Mc Mahon BJ. AASLD practice guidelines. Chronic hepatitis B. *Hepatology* 2007; 45: 507-538.
 30. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int.* 2008; 2: 263-283.
 31. Perrillo R. Benefits and risks of interferon therapy for hepatitis B. *Hepatology.* 2009; 49: 103-111.
 32. Buster EH, Schalm SW, Janssen HL. Peginterferon for the treatment of chronic hepatitis B in the era of nucleos(t)ide analogues. *Best Pract Res. Clin Gastroenterol.* 2008; 22: 1093-1108.
 33. Cornberg M, Protzer U, Dollinger MM, Petersen J, Wedemeyer H, Berg T, et al. The German guideline for the management of hepatitis B virus infection: short version. *J Viral Hepat.* 2008; 15: 1-21.
 34. Buster EH, van Erpecum KJ, Schalm SW, Zaaier HL, Brouwer JT, Gelderblom HC, et al. Treatment of chronic hepatitis B virus infection - Dutch national guidelines. *Neth J Med.* 2008; 66: 292-306.
 35. Lindh M, Uhnoo I, Bläckberg J, Duberg AS, Friman S, Fischler B, et al. Treatment of chronic hepatitis B infection: an update of Swedish recommendations. *Scand J Infect Dis.* 2008; 40: 436-450.
 36. 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: 1206-1217.
 37. Marcellin P, Piratvisuth T, Brunetto M et al. HBsAg clearance continues to increase after end of treatment with Pegasys +/- lamivudine: 5 year follow up study. *Hepatol Int.* 2009; 3: 108.
 38. Erhardt A, Ludwig AD, Brunetto M et al. HBV genotypes are the strongest predictors of response to interferon alpha treatment: multivariate evaluation in 1229 hepatitis B patients. *Hepatology* 2008; 48: 700-701.
 39. Gish RG, Lau DT, Schmid P, Perrillo R. A pilot study of extended duration peginterferon alfa-2a for patients with hepatitis B e antigen-negative chronic hepatitis B. *Am J Gastroenterol.* 2007; 102: 2718-2723.
 40. Entecavir and pegasys sequential therapy versus pegasys for HBeAg negative chronic hepatitis B. 2012.
 41. Enomoto M, Nishiguchi S, Tamori A, Kobayashi S, Sakaguchi H, Shiomi S, et al. Entecavir and interferon- α sequential therapy in Japanese patients with hepatitis B e antigen-positive chronic hepatitis B. *J Gastroenterol.* 2013; 48: 397-404.
 42. European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol.* 2012; 57: 167-185.
 43. Lampertico P, Viganò M, Di Costanzo GG, Sagnelli E, Fasano M, Di Marco V, et al. Randomised study comparing 48 and 96 weeks peginterferon α -2a therapy in genotype D HBeAg-negative chronic hepatitis B. *Gut.* 2013; 62: 290-298.