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Efficacy and safety of pegylated-interferon alpha therapy in patients with chronic hepatitis B in recource-limited settings: A Serbian single-center experience

Efikasnost i bezbednost pegilovanog interferona alfa-2a u terapiji hroničnog virusnog hepatitisa B u uslovima ograničenih resursa: iskustvo jednog centra u Srbiji

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Abstract

Background/Aim. In Serbia, pegylated interferon (PEG-IFN) alpha-2a has been registered since 2013 for the treatment of patients with chronic hepatitis B (CHB). Numerous advantages, new experiences during the past five years and lack of any published data in our specific population, have initiated this study, with the aim to examine efficacy and safety of PEG-IFN in patients in a Serbian referral center. **Methods.** This prospective study included 36 patients with CHB who were treated in the Hepatology Department of the Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia in Belgrade, during 2012–2017. Patients had a standard 48-week treatment protocol with PEG-IFN, with measurements of liver enzymes, serology and viraemia before, during, at the end of the treatment and follow-up 6 months afterwards. Treatment outcome was determined using serology (clearance of HBeAg), biochemical [normalization of alanine aminotransferase (ALT)] and virological response [hepatitis B virus (HBV) DNA < 2,000 IU/mL]. **Results.** Virological success in patients with HBeAg positive CHB was achieved in 50% of patients, HBeAg clearance in 62.5%, and normalization of ALT in 37.5% of patients. In patients with HBeAg negative CHB, 38% of the patients achieved virologic success, biochemical success was obtained in 47.6% of the patients and only one (4.7%) patient had HBsAg clearance. **Conclusion.** PEG-IFN is important for treatment of patients with CHB in well-defined situations, and in our population success rates are similar to other published studies. Although safety and tolerability are satisfactory, there is a possibility of more serious side-effects so it is necessary to monitor patients regularly during the treatment.

Key words:
biochemistry; hepatitis b; antigens; hepatitis b, chronic; peginterferon alfa-2; treatment outcome; virology.

Apstrakt

Uvod/Cilj. Pegilovani interferon (PEG-IFN) alfa-2a je registrovan u Srbiji od 2013. godine za lečenje bolesnika sa hroničnim hepatitisom B (HHB). S obzirom na njegove mnogobrojne prednosti u odnosu na dotadašnju terapiju, nova iskustva u periodu od proteklih pet godina i nedostatak publikovanih rezultata u našoj populaciji, cilj rada bio je da se prvi put među bolesnicima sa HHB u Srbiji ispita efikasnost i bezbednost primene PEG-IFN u tercijarnoj zdravstvenoj ustanovi. **Metode.** U prospektivnoj studiji u petogodišnjem periodu od 2012. do 2017. godine analizirano je ukupno 36 bolesnika sa HHB, lečenih standardnim protokolom PEG-IFN tokom 48 nedelja, u Hepatološkom odeljenju Klinike za infektivne i tropske bolesti Kliničkog centra Srbije u Beogradu. Svim bolesnicima su merene bazalne vrednosti transaminaza, serologije i viremije, uključujući praćenje tih parametara tokom terapije, na kraju terapije i u periodu praćenja. Za procenu uspeha terapije analiziran je serološki odgovor (gubitak HBeAg), biohemijski odgovor [normalizacija alanin aminotransferaze (ALT)] i virusološki odgovor na terapiju [supresija DNK hepatitis B virusa (HBV) < 2000 IU/mL. **Rezultati.** Virusološki uspeh terapije kod bolesnika sa HBeAg pozitivnim HHB postignut je kod 50% bolesnika, gubitak HBeAg kod 62,5%, a biohemijski odgovor kod 37,5% bolesnika. Kod HBeAg negativnog HHB, virusološki uspeh terapije postignut je kod 38% bolesnika, biohemijski odgovor kod 47,6%, a samo jedan (4,7%) bolesnik imao je i gubitak HBsAg. **Zaključak.** Primena PEG-IFN u

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lečenju HBV infekcije važna je u dobro selektovanoj grupi bolesnika, a u našoj populaciji lečenih bolesnika procenat uspešnosti terapije sličan je onom od drugih autora. Bezbednost i podnošljivost terapije je dobra, ali se mogu očekivati i ozbiljniji neželjeni događaji zbog čega je neophodno redovno praćenje bolesnika tokom lečenja.

Ključne reči:
biohemija; hepatitis b; antigeni; hepatitis b, hronični; interferon alfa-2a, pegilovani; lečenje, ishod; virologija.

Introduction

Hepatitis B viral infection (HBV) remains a significant challenge in hepatology, in spite of decades of successful immunization worldwide. In Serbia, compulsory immunization against HBV was introduced in 2000 for all newborns, followed by additional campaigns including adolescents and healhcare workers. However, the number of patients with chronic hepatitis B (CHB) infection seeking medical treatment is still significant, although there are only estimates and no published data concerning prevalence in the Serbian population.

Treatment of CHB is primarily oriented towards prevention of disease progression to end-stage liver disease including cirrhosis and hepatocellular carcinoma [1, 2]. Ideally, the ultimate treatment goal is the eradication of viral DNA, which still remains elusive. Current recommendations include two groups of drugs: oral nucleoside/nucleotide analogues (lamivudine, adefovir, entecavir, emtricitabine, tenofovir) and pegylated interferons (PEG-IFN alpha 2a and 2b) [1]. However, both treatment options have certain disadvantages. Although PEG-IFN is a less potent antiviral, it has an additional immunomodulatory effect which may account for durability of sustained virological suppression. The significant advantage of PEG-IFN lies in the absence of drug resistance, clearly defined treatment duration and higher anti HBe seroconversion rates. Other disadvantages, beside less potent antiviral effect, include tolerance issues with more side effects compared to oral analogues [1, 2].

In Serbia, the only treatment options were lamivudine and interferon alpha-2a, until 2012. But as the efficacy and tolerability of interferon are very poor, in everyday practice the single reliable treatment option for patients with CHB was lamivudine. After 2012, two more treatment options became available – second oral analogue tenofovir disoproxyl fumarate (TDF) and, in 2013, PEG-IFN alpha-2a. However, our treatment experience with PEG-IFN during past five years is limited, due to strict selection criteria for its application: elevated alanine aminotransferase (ALT) > 5 times upper limit, basal HBV DNA < 10^7 UI/mL and intermediate necroinflammatory activity in liver histology. Very few patients with CHB fulfilled these criteria, especially due to the fact that in our surroundings virology testing [HBV DNA polymerase chain reaction (PCR)] was often unavailable due to limited resources and funding, not only for treatment of naive patients but also patients who had commenced treatment. Although PEG-IFN was officially registered for the treatment of CHB in 2013, our study included an additional number of 20 patients treated with the donated PEG-IFN in 2012 through a compassionate treatment programme. This treatment group was selected mostly based on clinical judgment, and not always according to the mentioned criteria, especially due to the unavailability of HBV DNA PCR testing.

The aim of study was to present the results of our 5-years treatment experience with PEG-IFN in patients with CHB and to examine its efficacy and possible predictors of sustained virological response concerning tolerability issues and side effects in our study population, as well.

Methods

This study was performed in order to examine the efficacy and safety of PEG-IFN in patients with CHB, who were treated in the Hepatology Department of the Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia in Belgrade during 2012–2017. In total 36 patients consented to participate in this study and fulfilled inclusion criteria: diagnosis of CHB, HBV DNA > 20,000 IU/mL for hepatitis B extractable antigen (HBeAg) positive patients and for HBeAg negative patients, HBV DNA > 2,000 UI/mL, elevated ALT > 5 x upper limit. Exclusion criteria were: hepatitis C and/or HIV coinfections, liver decompensation, active substance abuse, alcohol consumption.

All patients were treated with PEG-IFN alpha-2a 180 μg subcutaneously, once a week for 48 weeks. Pretreatment patient data were collected from patients records, including demographic data (sex, age), previous treatment options, pretreatment levels of ALT and HBV DNA, the presence of HBeAg, and liver histology reports including METAVIR scores. Liver enzymes and full blood counts were analyzed every 4 weeks during treatment, and then during the follow-up period in 12th and 24th weeks after the treatment was finished. Serology, i.e. HBeAg and anti HBe antibodies were analysed every 12 weeks. In 16 patients basal levels of hepatitis B surface antigen (qHBsAg) were available, then retested in 10 patients during duration of the treatment every 12 weeks, at the end of the treatment and during 6-months follow-up period. Level of HBV DNA was determined in all patients in the same time intervals as previously mentioned.

Treatment success was considered as primary and secondary. Primary treatment success was defined in HBeAg positive patients as end-treatment HBeAg seroconversion and viral suppression of HBV DNA < 2,000 IU/mL. In HBeAg negative patients primary treatment success was defined as end-treatment favourable virological response – HBV DNA < 2,000 IU/mL. Secondary treatment success in HBeAg positive patients included HBeAg seroconversion, sustained suppression of HBV DNA < 2,000 IU/mL and clearance of HBsAg 24 weeks after the end of the treatment. In HBeAg negative patients end-treatment success was con-

sidered as sustained viral suppression of HBV DNA < 2,000 IU/mL and HBsAg elimination 24 weeks after treatment. All possible predictors of treatment success were analysed including pretreatment blood tests (liver enzymes, blood count), serology (HBeAg) as well as levels of qHBsAg and HBV DNA before, during and after the treatment.

Biochemical analysis of blood samples was performed using Siemens Dimension Xpand® biochemistry analyzer in the Center for Medical Biochemistry, Hepatology Department of the Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia. Hepatitis B serology (HBsAg, HBeAg, anti-HBeAb) was performed using commercial ELISA tests (Abbot Laboratories, North Chicago, IL, USA) at the Virology Laboratory, Microbiology Department, Clinical Center of Serbia.

HBV DNA was analysed in the same departments using CombasAmpliPrep/CobasTaqMan HBV essay (CAP/CTM version 2.0, Roche Diagnostics Indianapolis, IN, USA with detection levels of 10–107 UI/mL). Quantitative HBsAg was performed in the Biochemistry Laboratory of the Clinical Center "Zvezdara" using Architect HBsAgQT assay (Abbott Diagnostic Germany) with sensitivity levels of 0.05–250 UI/mL. Safety and tolerability of the treatment were assessed during clinical examinations and check-ups, based on the occurrence of side effects and completion of the full treatment protocol. All patients who received at least one dose of PEG IFN were included in safety and tolerability examination.

Statistical analysis was performed using SPSS®, version 11.5 and included both descriptive and analytical methods. Patients were categorised according to the presence of HBeAg into two groups – HBeAg positive and HBeAg negative. Both parametric (Student *t*-test) and nonparametric tests (χ^2, Fisher test) were used, depending on the normality of variables. Linear regression and Spearman's correlation rank were also computed for the analysis of association. Values at the $p \leq 0.05$ level were considered statistically significant, the confidence interval (CI) was 95% and all performed tests were 2-tailed.

All participants provided their informed consent and the study protocol was performed according to the Helsinki declaration, including Ethics Committee permission and institutional approval.

Results

Baseline (pretreatment) patients characteristics

Studied patients (n = 36) were mostly male (86.5%, X2 = 19.7, $p < 0.0001$), with an average age of 37.9 ± 12 years (interval ranging from 18–60 years). Patients were categorised according to the presence of HBeAg into two study groups, with a predominance of HBsAg negative form of CHB (72.2%), but there was no statistically significant difference in age or sex distribution between study groups (Table 1). Most of the patients were treatment naive (83.3%). However, six patients were previously treated with lamivudine, but there was no statistically significant difference in distribution between the two study groups (Table 1). Although most of the patients had a lower degree of fibrosis, four patients had cirrhosis (11.4%), but without differences between study groups ($p = 0.718$). Elevated activity of ALT > 2 x upper limit was registered in most of the patients (72.4%), but there was no significant difference in gradations of enzyme activity between two study groups ($p = 0.308$). Average viraemia was 7.4 log (5.2–8.2) IU/mL, without statistically significant differences between groups (Table 1).

In 44.4% of the patients (16/36) baseline level of qHBsAg was performed, averaging 8,400 UI/mL (345–42,390 UI/mL); however, there was no statistically significant correlation with baseline viraemia ($\rho = -0.082$, $p = 0.589$).

Table 1
Baseline (pretreatment) demographic, clinical and laboratory characteristics of patients with chronic hepatitis B

Variable	Patients			*p*
	total (n = 36)	HBeAg+ (n = 10)	HBeAg- (n = 26)	
Sex, n (%)				
male	31 (86.1)	9 (90)	22 (84.6)	0.676
female	5 (13.9)	1 (10)	4 (15.4)	
Age (years), mean ± SD	38±12	32.1 ± 8.7	40.2 ± 12.6	0.072
Previous treatment, n (%)				
lamivudine	6 (16.7)	3 (30)	3(11.5)	0.317
treatment-naive	30 (83.3)	7 (70)	23 (88.5)	
[1]Liver histology				
F0	4 (11.4)	1 (11.1)	3 (11.5)	0.718
F1	8 (22.9)	1 (11.5)	7 (26.9)	
F2	13 (37.1)	5 (55.6)	8 (30.8)	
F3	6 (17.1)	1 (11.1)	5 (19.2)	
F4	4 (11.4)	1 (11.1)	3 (11.5)	0.972
Elevated ALT, n (%)				
< 2x upper limit	10 (27.8)	1 (10)	9 (34.6)	0.308
2x–5x upper limit	16 (44.4)	6 (60)	10 (38.5)	
> 5x upper limit	10 (27.8)	3 (30)	7 (26.9)	
HBV DNA ($\log_{10}$ IU/mL), mean ± SD	7.4 ± 0.9	7.9 ± 0.4	7 ± 1	0.068

HBeAg – hepatitis B extractable antigen; [1]METAVIR score; HBV – hepatitis B virus; ALT – alanine aminotransferase (upper limit of ALT > 37 IU/L); SD – standard deviation.

Efficacy of PEG- IFN alpha-2a in patients with HBeAg positive CHB

Full treatment protocol of 48 weeks was completed in 8 patients (80%), and in two patients due to the rise in HBV DNA, it was stopped in 12th week. HBeAg clearance after 24 weeks and at the end of the treatment was observed in 50% of patients (4/8), and after follow-up period (6 months after the end of the treatment) in 62.5% of the patients (5/8). However, HBeAg seroconversion with anti-HBeAb was present in only 25% (2/8) of the patients. The only statistically significant baseline factor that influenced HBeAg clearance was the presence of HBcIgM ($p = 0.008$).

Biochemical response, e.g. ALT normalization was achieved at the end of the treatment and after follow-up period in 37.5% (3/8) of the patients (Table 2).

Table 2
Efficacy of pegylated interferon (PEG-IFN) in patients with HBeAg+ chronic hepatitis B (n = 8)

Parameters	End of the treatment (48 weeks)	Follow-up (72 weeks)
Serology response, n (%)		
clearance HBeAg	4 (50)	5 (62.5)
clearance HBsAg	0	1 (12.5)
Virological response, n (%)		
< 2000 IU/mL	1 (12.5)	2 (25)
undetecteble viraemia	3 (37.5)	2 (25)
Biochemical response, n (%)		
ALT normalization	3 (37.5)	3 (37.5)

HBeAg – hepatitis B extractable antigen; HBsAg – hepatitis B surface antigen; ALT – alanine aminotransferase.

In 50% of the patients (4/8) virological suppression was achieved with end-treatment HBV DNA < 2,000 IU/mL, including three patients with undetectable viraemia (Table 2). After the follow-up, virological response of HBV DNA < 2,000 IU/mL was sustained in 50% (4/8) of the patients. The success rates remained unchanged, although one of the patients who had achieved end-treatment success had relapsed (HBV DNK 16,400 UI/mL, ALT > 2x), as another patient who had end-treatment failure had a HBV reduction < 2,000 IU/mL with reduction of ALT > 1.5x upper limit during follow-up period. Undetectable viraemia was sustained in 25% of the patients (2/8), among whom one had HBsAg elimination.

Complete treatment success, eg. secondary success was confirmed in only one (1.25%) patient in this group. This patient had HBeAg clearance after follow-up period of 24 weeks, undetectable viraemia and negative HBsAg. After a year of follow-up period, this patient had achieved seroconversion and anti-HBsAb.

The in-depth statistical analysis did not identify possible predictors of sustained virological response, such as sex, age, previous lamivudine treatment, baseline values of ALT, HBV DNA < 2,000 IU/mL after 12 weeks of the treatment, baseline qHBsAg ($p > 0.05$) (Table 3).

Efficacy of PEG IFN alpha-2a in patients with HBeAg negative CHB

Treatment protocol of 48 weeks was completed in 21 patients with HBeAg negative CHB. In five patients it was stopped before the completion due to early virological failure (in two patients) and serious side-effects (three patients).

Favourable end-treatment biochemical response was achieved in 23.8% (5/21) of the patients, with an additional number of patients who achieved ALT normalization during follow-up period [47.6% (10/21)] (Table 4).

Table 4
Efficacy of pegylated interferon (PEG-IFN) in patients with HBeAg+ chronic hepatitis B (n = 21)

Parameter	End of the treatment (48 weeks)	Follow-up (6 months)
Biochemical response, n (%)		
ALT normalization	5 (23.8)	10 (47.6)
Serology response, n (%)		
clearance of HBsAg	1 (4.7)	1 (4.7)
Virological response, n (%)		
< 2,000 IU/mL	15 (71.42)	7 (33.33)
undetectable viraemia	2 (9.52)	1 (4.76)

HBeAg – hepatitis B extractable antigen; HBsAg – hepatitis B surface antigen; ALT – alanine aminotransferase.

Table 3
Predictors of virological success after follow-up period in patients with HBeAg+ chronic hepatitis B (n = 4)

Parameter	HBeAg+		p^{α}
	HBV DNA < 2,000 IU/mL	HBV DNA > 2,000 IU/mL	
Male, n (%)	4 (100)	3 (75)	0.356
Age (years), mean ± SD	37 ± 11	28 ± 5	0.201
Severe fibrosis$^{\beta}$, n (%)	1 (25)	1 (25)	1.000
Cirrhosis, n (%)	1 (25)	0	0.708
Lamivudine-experienced, n (%)	1 (25)	1	1.000
Baseline HBV DNA (log10 IU/mL), mean ± SD	7.2 ± 1.8	7.6 ± 0.5	0.666
Baseline ALT (U/L), mean ± SD	148 ± 62	177 ± 119	0.624
> 5x upper limit, n (%)	1 (25)	3 (75)	0.437
Baseline HBsAg ($\log_{10}$ IU/mL), mean	4.31	4.62	0.157
HBV DNA ($\log_{10}$ IU/mL , 12th week), mean ± SD	4.08 ± 2.9	5.9 ± 2.1	0.709
HBV DNA decline >2 log 12th week, n (%)	3 (75)	4 (100)	0.748
HBsAg < 150 IU/mL ,12th week, n (%)	0	0	1.000

$^{\alpha}$logistic regression, significance level $p < 0.05$; $^{\beta}$patients with METAVIR > F3; HBV – hepatitis B virus; ALT – alanine aminotransferase (upper limit of ALT > 37 IU/L); SD – standard deviation; HBeAg – hepatitis B extractable antigen; HBsAg – hepatitis B surface antigen.

Table 5

Predictors of virological success after follow-up period in patients with HBeAg- chronic hepatitis B

Parameter	HBeAg-		p^{α}
	HBV DNA < 2,000 U/mL (n = 8)	HBV DNA > 2,000 IU/mL (n = 13)	
Male, n (%)	8 (100)	11 (84.6)	0.266
Age (years), mean ± SD	34.6 ± 15.9	43.7 ± 11	0.136
Severe fibrosis$^{\beta}$, n (%)	3 (37.5)	5 (38.4)	0.764
Cirrhosis, n (%)	2 (25)	1 (7.7)	0.769
Lamivudine-experienced, n (%)	1 (12.5)	2 (15.4)	0.865
Baseline HBV DNA (log_{10} IU/mL), mean ± SD	6.05 ± 1.32	5.85 ± 1.1	0.346
Baseline ALT (U/L), mean ± SD	151 ± 120	148 ± 199	0.947
> 5x upper limit, n (%)	1 (12.5)	5 (38.4)	0.213
Baseline HBsAg (log_{10} IU/mL), mean ± SD	3.5 ± 0.4	3.4 ± 0.09	0.208
HBV DNA (log_{10} IU/mL ,12th week), mean ± SD	2.92 ± 1.45	3.66 ± 1.58	0.639
HBV DNA decline > 2 log 12th week, n (%)	5 (62.5)	9 (69.2)	0.765
HBsAg < 150 IU/mL 12th week, n (%)	0	1 – (7.7)	1.000

$^{\alpha}$logistic regression, significance level $p < 0.05$; $^{\beta}$patients with METAVIR > F3; HBeAg – hepatitis B extractable antigen; HBsAg – hepatitis B surface antigen; HBV hepatitis B virus; ALT – alanine aminotransferase (upper limit ALT > 37 IU/L); SD – standard deviation.

End-treatment HBsAg clearance was achieved in one patient, but with a recurrence of HBsAg during follow-up period. At the end of follow-up, one patient had HBsAg clearance but without seroconversion to anti-HBsAb.

End-treatment virological response, eg. HBV DNA < ,000 IU/mL was achieved in 81% of the patients (17/21), of whom 9.5% (2/21) had undetectable viraemia (Table 4). After a relapse in ten patients during follow-up period, a sustained virological response was maintained in 38% of the patients (8/21), with a patient from this group who had achieved undetectable viraemia and normalization of ALT but without clearance of HBsAg (Table 4). In-depth statistical analysis of possible treatment outcome predictors, did not show a significant correlation between sustained virological response and following variables: age, sex, previous lamivudine treatment, baseline ALT, HBV DNA < 2,000 IU/mL after 12 weeks of the treatment, baseline HBsAg, reduction of HBsAg < 150 IU/mL after 12 weeks of the reatment ($p > 0.05$) (Table 5).

Kinetics of qHBsAg during treatment with PEG IFN

In 16/36 patients we were able to determine baseline qHBsAg in addition to viraemia, and in 10/16 kinetics of qHBsAg was followed during and after the treatment (two patients from this group had HBeAg positive CHB). During treatment, a decline in qHBsAg was observed in 6/10 (60%) of the patients after 12 weeks of the treatment. A further decline of qHBsAg after 24 weeks of the treatment was sustained in 7/10 patients, although we observed levels of HBsAg lower than 20,000 UI/mL in 9/10 patients. However, a continuous decline of qHBsAg even during the follow-up was observed in only one patient, who had achieved a sustained virological response. High baseline and treatment levels of qHBsAg were observed in patients with HBeAg positive CHB (2/10), which remained unchanged for the duration of treatment and follow-up period.

Safety

On-treatment stopping occurred in 7 (19.44%) patients, mostly [in 4 (11.1%) of patients] due to early virological failure after 12 weeks of the treatment. Due to serious side effects, the treatment was stopped in three patients (8.33%): *de novo* diagnosed ovarian cancer, a severe form of depression and debilitating myalgias and arthralgias in a patient who had an early virological response.

In five (17.24%) of the patients there were occasional dosage reductions of PEG-IFN due to the expected side effects – thrombocytopenia in 4 (13.79%) of the patients, neutropenia in one patient (6.89%). During the follow-up period, oral analogues were introduced in five patients (17.24%) due to the rise in liver enzymes over 10x upper limit and risk of hepatic decompensation.

Discussion

The quality of treatment for CHB has been significantly improved in Serbia during past years, especially with the introduction of tenofovir disoproxil fumarate and PEG-INF alfa-2a starting from 2012.

As the treatment with PEG-IFN is covered by state health insurance in Serbia, criteria of the National Health Fund for the administration are low viraemia HBV DNA < 107 copies/mL and elevation of liver enzymes > 2x upper limit [3]. These criteria are mostly fulfilled by younger patients in the immunoeliminatory phase of CHB, e.g. with chronic HBeAg positive hepatitis. However, among Serbian patients, the most predominant are those with HBeAg negative form of CHB, characterized by high viraemia and fluctuating levels of ALT. Unfortunately, due to common shortages and unavailability of HBV DNA PCR testing, as clinicians, we are often faced with a delayed and incorrect diagnosis of CHB in our patients. In this study, we were able to include an additional number of patients who did not fulfil

the National Health Fund criteria, and who were selected based on clinical judgement and treated with donated medication. This enabled us to include patients in the immunoreactive phase of CHB, who were previously not able to receive treatment with PEG-IFN, and reach a total of 36 patients which is a significant number for a single centre experience.

A large portion of patients (80.55%) completed full treatment protocol for the duration of 48 weeks. In seven (19.44%) of the patients treatment was stopped because of lack of early virological response as well as side effects.

Our results showed that in patients with HBeAg positive CHB, treatment with PEG-IFN resulted in HBeAg seroconversion in 62.5% of the patients, stable immunological control in 50% of the patients, with a complete success of the treatment in one patient (12.5%). Previously published results in different European centres have shown PEG-IFN treatment success rates for these patients ranging from 20%–30% [4, 5]. An important aim of treating HBeAg positive patients is the elimination of HBeAg, which was achieved in 62.5% of the patients, among whom a half (4/8) had achieved it during first 24 weeks of the treatment. This particular effect of PEG IFN during the first six months of the treatment has been previously observed by other authors, but the overall treatment success rates are higher after 48 weeks compared to shorter administration [5]. Combined treatment success in this group of patients, eg. HBeAg elimination with HBV DNA < 2,000 IU/mL ,was achieved in 23% of the patients in a study performed by Sonnevald et al. [4], similar to our results of 25%.

However, although we had only one occurrence of HBsAg elimination during follow-up in this group (12.5%), this is still significantly higher than in most published authors who report this in extremely low percentage of treated patients ranging from 3%–7%. One of the limitations for a possible interpretation of this particular result in our population is a small patient sample size. Biochemical response, e.g. ALT normalization in this group was achieved in five (62.5%) of the patients, but after follow-up this percentage was lower, reaching 37.5%. These rates were lower compared to most authors who reported biochemical response rates above 41% in patients with HBeAg positive CHB [4–6]. Possible differences in success rates may be due to different reference limits in numerous studies, as in our study we considered a level of 37 IU/mL normal, and every value measured above this limit at least twice during a three months period was considered elevated.

Virologic success rates of PEG-IFN in patients with HBeAg negative CHB have been reported around 44% [7, 8]. However, in patients with genotype D, these rates are lower, reaching 20% [2]. Although we were unable to perform genotype testing in our study, previously published genotype prevalence studies by Serbian authors report a predominance of genotype D which may explain our success rates of 38% [9, 10].

Elimination of HBsAg is a very rare treatment outcome in this group of patients, published results ranging from 3% after follow-up period of 24 weeks, up to 12% after 5 years [11, 12]. In our study, only one (4%) patient achieved HBsAg elimination, which is similar to results of foreign authors. Biochemical response, e.g. ALT normalization in this group was achieved in five (23.8%) of the patients, and the success rates were even higher after follow-up period (47.6%), similarly to other published results of 51% of patients with HBeAg negative CHB [1].

One of the most important advantages of achieving successful PEG-IFN treatment is its prolonged effect and sustainability of virological suppression even for years after successful completion as well as the rising percentage of HBsAg elimination during follow-up period [12, 13]. Marcellin et al. [12] conducted a 5 year follow-up study of efficacy of PEG-IFN in patients with HBeAg negative CHB and reported a rise in sustained virological response of 28%, as well as HBsAg clearance in 12% of patients with favourable predictors (HBV DNA < 2,000 IU/mL after a one year of follow-up) [12]. In all of our eight (38%) patients with virological response, we were able to confirm that after 1–3 years after the end of the treatment, all of them had sustained virological suppression. These results are in concordance with foreign authors who had much larger patient samples showing that a prolonged stability of achieved HBeAg seroconversion is maintained in more than 80% of patients and virological response sustained in more than 60% of the treated patients [13]. However, in our patients, we did not observe a rise in HBsAg clearance, which has been reported by numerous authors, as none of our patients after 3 years of treatment had achieved HBsAg clearance [11, 13, 14].

Tolerability of PEG-IFN is often a limiting factor, but years of treatment experience in chronic hepatitis C (CHC) patients has improved our possibilities of timely detection and intervention in case of any side-effects. The incidence of side-effects is significantly lower and milder compared to our patients with CHC, as patients with CHB are mostly younger with fewer comorbidities [1, 2]. After the treatment initiation, we observed flu-like symptoms in four patients, as well as neutropenia and thrombocytopenia in five patients, prompting for dosage reduction. We observed a case of drug-induced thyroiditis, which was completely resolved and the patient was treated with PEG-IFN in full, as well as two serious side effects prompting for the secession of the treatment – a patient with an ovarian carcinoma discovered during 4th month of the treatment and a case of severe depression during 32th week of the treatment. There are published data concerning the late occurrence of psychiatric side-effects during PEG IFN treatment [15]. There were no death outcomes or occurrences of hepatocellular carcinoma during the follow-up period.

Current guidelines for treatment of HBeAg positive form of CHB of the European Association of the Study of the Liver (EASL) state certain predictors of successful PEG-IFN treatment including low viraemia, higher activity of liver enzymes, female sex, and genotype A [1, 2]. However, in HBeAg negative patients, there are no clear predictors of successful treatment outcome. In both of our patient groups, we found no such statistically significant predictors, which may be due to the sample size. Yeh et al. [16] have pointed to the importance of previous treatment options as a possible

predictor of successful PEG-IFN treatment. They have shown that patients previously treated with oral analogues have lower PEG-IFN success rates compared to treatment-naive and interferon experienced patients. In our study group, 7 (25%) of the patients have been previously treated with lamivudine, but we found no difference in PEG-IFN success rates compared to the treatment-naive patients.

Current protocols have implemented viraemia kinetic and qHBsAg as major predictors of successful PEG-IFN treatment [2]. Rijckborst et al. [17] have pointed to the importance of HBV kinetics after 12 weeks of the treatment and implementation of the rule of stopping treatment in patients with HBeAg negative form of CHB. The EASL guidelines also underline the rule of stopping in 12th and 24th week of the treatment [2]. These guidelines were followed and we stopped PEG-IFN treatment after 12 weeks of the treatment in 11.11% (4/36) of the patients, of whom three did not fulfill the National Health Fund treatment criteria for PEG-IFN administration (two patients had ALT levels < 2x upper limit and a high viraemia > 108 IU/mL). Current guidelines also state that besides HBV DNA levels, qHBsAg should be measured in order to decide to stop or continue treatment in both forms of CHB. However, as qHBsAg detection was not available at the time, as clinicians we are in doubt if the treatment was stopped prematurely in these patients, especially as it was based solely on HBV DNA levels.

Our treatment experience concerning the role of qHBsAg is very limited and can not be used for a more significant conclusion, as pretreatment qHBsAg was available in only 16 patients, and in only 10 during the treatment and follow-up period. On this small sample, we did not observe any correlation between the decline of viraemia and levels of HBsAg. There are multiple publications pointing out to the importance of qHBsAg at the end of the treatment and during follow-up period, as its continuous decline is correlated with sustainability of virological response, and may predict a possible relapse if there is no decline in levels of HBsAg during follow-up [2, 12, 14].On the other hand, there are published results showing that patients with HBeAg negative CHB and genotype D may benefit from prolonged PEG-IFN treatment (96 weeks instead of 48 weeks) including higher success rates (up to 29%) and HBsAg clearance up to 6% [18]. As this option for prolonged PEG-IFN treatment was not available in our patients who are also mostly HBeAg negative, and considering the local prevalence of genotype D, we suspect that this approach may prove beneficial to patients in Serbia.

This study has some limitations, such as sample size and limited availability of qHBsAg. However, we believe that some of our experiences may prove beneficial to other clinicians who are using PEG-IFN for treatment of CHB patients.

Conclusion

These results are the first published data concerning the efficacy and safety of PEG-IFN in Serbian patients with CHB, as this drug was mostly described and observed in the treatment of patients with CHC. Our modest results showed that although PEG-IFN is important for treatment of patients with CHB in well-defined situations, such as relatively low viraemia, elevated liver enzymes and in younger patients, other treatment predictors are also necessary, especially qHBsAg.

REFERENCES

1. *European Association For The Study Of The Liver*. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; 57(1): 167–85.
2. *European Association for the Study of the Liver*. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017; 67(2): 370–98.
3. *Health Insurance Fund of the Republic of Serbia*. List C. Drugs with a special regime of release. [Cited 2018 May 30] Available from: https://www.rfzo.rs/download/pravilnici/lekovi/Lista%20Cprimena%20od%2001.01.2018..pdf
4. *Sonnevald MJ, Hansen BE, Piratvisuth T, Jia J, Zeuzem S, Gane E*, et al. Response guided peginterferon therapy in hepatitis B e antigen-positive chronic hepatitis B using serum hepatitis B surface antigen level. Hepatology 2013; 58(3): 872–80.
5. *Chon YE, Kim DJ, Kim SG, Kim HG, Bae SH, Hwang SG*, et al. An observational, muticenter, cohort study evaluating the antiviral efficacy and safety in korean patients with chronic hepatitis B receiving pegylated interferon-alfa 2a (Pegasys). Medicine 2016; 95(14): e3026.
6. *Vlachogiannakos J, Papatheodoridis GV*. Optimal therapy of chronic hepatitis B: how do I treat HBeAg-positive patients? Liver Int 2015; 35(Suppl 1): 100–6.
7. *Lampertico P, Rothe V, Caputo A, Papatheodoridis GV*. A baseline predictive tool for selecting HBeAg-negative chronic hepatitis B patients who have a high probability of achieving sustained immune control with peginterferon alfa-2a. Hepatology 2014; 60: 1107A.
8. *Goulis I, Karatapanis S, Akriviadis E, Deutsch M, Dalekos GN, Raptopoulou-Gigi M,* et al. On-treatment prediction of sustained response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B patients. Liver Int 2015; 35(5): 1540–8.
9. *Milosevic I, Delic D, Lazarevic I, Pavlovic IP, Korac M, Bojovic K, Jevtovic D*. The significance of hepatitis B virus (HBV) genotypes for the disease and treatment outcome among patients with chronic hepatitis B in Serbia. J Clin Virol 2013; 58(1): 54–8.
10. *Bojović K, Božić M, Stanojević B, Popović N*. The first resultsof genotyping hepatitis B virus in Serbia and Montenegro, Falk symposium 157 Chronic Hepatitis: metabolic, Cholestatic, Viral and Autoimmune. Freibug; 2006. Abstracts 21.
11. *Lampertico P, Maini M, Papatheodoridis G*. Optimal management of hepatitis B virus infection - EASL Special Conference. J Hepatol 2015; 63(5): 1238–53.
12. *Marcellin P, Bonino F, Yurdaydin C, Hadziyannis S, Moucari R, Kapprell HP,* et al. Hepatitis B surface antigen levels: association with 5-year response to peginterferon alfa-2a in hepatitis B e-antigen-negative patients. Hepatol Int 2013; 7(1): 88–97.
13. *Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F,* et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. Gastroenterology 2008; 135(2): 459–67.
14. *Piratvisuth T, Marcellin P, Brunetto M, Bonino F, Farci P, Yurdaydin C,* et al. Sustained immune control 1 year post-treatment with Peginterferon Alfa -2a [40KD] (PEGASYS) is durable up to 5 years post-teratment and is associated with a hihg rate of

HBsAg clearance in HbeAg-negative chronic hepatitis B. 20th Conference of the Asian Pacific Asspciation for the Study of the Liver (APASL); Bejing, China 2010 March 25–28.

15. *Vigano M, Invernizzi F, Lampertico P.* Optimal therapy of chronic hepatitis B: how do I treat my HBeAg-negative patients? Liver Int 2015; 35(Suppl 1): 107–13.
16. *Yeh ML, Peng CY, Dai CY, Lai HC, Huang CF, Hsieh MY*, et al. Pegylated-interferon alpha therapy for treatment-experienced chronic hepatitis B patients. PLoS One 2015; 10(4): e0122259.
17. *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(5): 1006–11.
18. *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(2): 290–8.

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