Original Article

MedInform

DOI: 10.18044/MedInform.201524.345

ISSUE 4, 2015

HBsAg-guided extension of Peg-IFN therapy in HBeAg-negative responders

Nina Nikolova, Deian Jelev, Krassimir Antonov, Lyudmila Mateva, Zahariy Krastev.

University Hospital “St. Ivan Rilski” – Sofia, Clinic of Gastroenterology

Abstract

Background: The ideal treatment end point in the management of patients with chronic HBV infection is loss of HBsAg. It could be achieved only in a few cases, especially in HBeAg – negative patients.

The aim of the present study was to evaluate serum HBsAg levels in HBeAg-negative Bulgarian patients responding to the standard therapy with Peg-IFN as an attempt to navigate treatment duration according to the on-treatment HBsAg decline and appearance of anti-HBs.

Methods: Twelve patients with HBeAg-negative chronic hepatitis B were studied. Subjects were treated with Peg-IFN alfa-2a. None of the studied patients fulfilled the “stopping rule” at the 3rd month of Peg-IFN therapy.

Results: In 7/12 patients there was a sharp decline of the HBsAg levels with more than 90% reduction at treatment week-48 (and the levels were <100 IU/ml) and serum HBV DNA was undetectable. In 4 of these patients the therapy was extended for more than 48-weeks. HBsAg-loss was observed in two of them, which occurred at treatment week-48 and week-72, respectively. HBsAg-loss was associated with the development of anti-HBs (>100 IU/L) at treatment week-72 in these patients. In the 3rd patient an anti-HBs titer of 6-10 IU/L was detected at treatment week-72 and after that (96 week). In the 4th patient there was anti-HBs at week-108 (26 IU/L).

Conclusion: Extension of Peg-IFN treatment is reasonable in patients who demonstrated a sharp HBsAg decline during the standard 48-week therapy. Probably, subjects with HBsAg level < 100 IU/ml will mostly gain from prolongation of Peg-IFN. In these cases we propose the term “extended rule”.

Keywords: HBsAg, Interferon, extended therapy

Background

Despite significant advances in antiviral therapies and vaccination programs, hepatitis B virus (HBV) infection is still a major health problem. HBV affects about 2 billion people worldwide causing chronic hepatitis in up to 400 million individuals (1,2,3). In Bulgaria, similarly to other South European countries, about 85% of chronic hepatitis B (CHB) patients are HBeAg-negative and the prevalence of genotype-D is almost 100% (4,5).

Current treatment options for CHB include two different strategies: 1) maintained on-treatment suppression of HBV replication by long-term therapy with nucleoside/nucleotide analogues (NUCs) or 2) attempt for archiving a sustained immune control of the HBV infection by a finite treatment course with pegylated interferon (Peg-IFN) (3).

The ideal treatment end point is loss of HBsAg, but it could be achieved only in occasion in HBeAg-negative patients after long-term therapy with NUCs (6,7). The effect of NUCs is lasting only during therapy and a rapid relapse frequently occurs after NUC discontinuation, so life-long treatment is required (3). The template of HBV transcription is covalently closed circular DNA (cccDNA), which plays a key role in the life cycle of the virus and permits the persistence of infection (8). The persistence of cccDNA in the nucleus of infected hepatocytes, may explain HBV reactivation after discontinuation of anti-viral therapy (8).

The standard 48-week therapy with Peg-IFN leads to a low rate of sustained response (11.8%) in HBeAg-negative patients infected with HBV genotype-D (9). Sustained responders showed greater decline of HBsAg levels than non-responders (10-12). The optimal on-treatment HBsAg cut off level that predicts response in HBeAg-positive patients is probably 1500 IU/ml at treatment week-12 or 24 (13,14). In contrast, an absence of any HBsAg decline together with <2 log reduction in HBV DNA at week-12 was proposed as a stopping rule in HBeAg-negative patients, including those with HBV genotype D infection - response guided therapy (15,16).

Extension of conventional IFN from 1 to 2 years leads to higher rates of sustained virological response (SVR) due to reduced rates of post treatment relapse (17-19). This was recently confirmed also for Peg-IFN therapy (9, 20). However, there are not enough data to define the subset of HBeAg-negative patients who will mostly gain from tailoring of the Peg-IFN therapy. Also the optimal treatment duration and time-point for Peg-IFN discontinuation is unknown.

Aim

The aim of the present study was to evaluate serum HBsAg levels in HBeAg-negative Bulgarian patients responding to the standard therapy with Peg-IFN as an attempt to navigate treatment duration according to the on-treatment HBsAg decline and appearance of anti-HBs.

Methods

Patients and treatment

Twelve Caucasian patients (10 males and 2 females) with HBeAg-negative chronic hepatitis B were studied. The median age was 33 years, ranging between 21 and 55 years.

All subjects were HBsAg-positive for more than 6 months. HDV, HCV and HIV co-infections were excluded. Initially all of them were HBeAg-negative, but viremic and had an elevated ALT level within 6 months prior to therapy. Chronic hepatitis B was histologically proven by liver biopsy in 11 patients. Subjects were treated with Peg-IFN alfa-2a, 180 mcg/weekly. At treatment week-12 all of them were with HBsAg decline (>5%) and had a decrease of HBV DNA level with more than 2 log compared to baseline levels, so none of the studied patients fulfilled the “stopping rule” at 3rd month of Peg-IFN therapy. The baseline patients’ characteristics are presented in table 1.

Table 1. Baseline characteristics

number of pts	12
Age – median, range	33 21-55
Male /female (n)	10/2
ALT U/l –median, range	65 33-268
HBV DNA IU/ml – median, range	428 115 1750-3 340 000
qHBsAg IU/ml – median, range	9 519 268-76 002
Liver histology /METAVIR/: F0 F1 F2 F3	11 patients 0 6 5 0
Liver cirrhosis /F4/	0
Without liver biopsy	1

Methods

HBsAg levels were measured by the fully automated Roche® diagnostic test. The results were expressed as IU/ml. This is an electrochemiluminescence immunoassay.

HBV DNA quantitation was done on patients’ serum (500 µl) by using LightCycler real time PCR assay (Roche Diagnostics). Results were expressed as IU/ml.

Hepatitis B e antigen (HBeAg) and antibody against HBeAg (anti-HBe) status was determined by enzyme immunoassays.

Standard laboratory methods were used for assessment of blood chemistry parameters. The values of ALT <0 U/ml were accepted for normal according to the laboratory report.

Pre-treatment liver biopsy applying the Menghini technique was performed in 11 patients and was evaluated by the METAVIR system. Only one patient did not undergo liver biopsy, but there were no signs of cirrhosis during physical and ultrasound examination.

Written informed consent was obtained from each participating patient prior to enrolment.

Results

Serum HBV DNA and HBsAg levels – initial and during treatment with Peg-IFN alfa-2a are shown in Table 2. The dynamics of ALT is presented in Table 3.

Table 2. HBV DNA and HBsAg levels during the standard treatment with Peg IFN-alfa 2a

Patients	Baseline		3rd month		6th month		9th month		12th month		6 months after the EOT**
	HBV DNA IU/ml	HBsAg IU/ml	HBV DNA IU/ml	HBsAg IU/ml	HBVDNA IU/ml	HBsAg IU/ml	HBV DNA IU/ml	HBsAg IU/ml	HBV DNA IU/ml	HBsAg IU/ml	HBV DNA IU/ml
1	1750	76002	0*	49760	0*	98000	0*	24050	0*	64160	9500
2	519231	11312	577	3251	0*	1046	0*	810	136	806	0
3	30200	10590	221	5458	0*	1714	0*	1158	0*	813	1220
4	3090000	11057	3110	8337	25	376	0*	15	0*	<0,05	***Extended therapy
5	337000	8448	0*	5913	0*	530	0*	373	0*	394	0
6	59800	47744	599	41300	430	61941	ND		464	56309	4056
7	714000	535	0*	187	0*	76	0*	79	0*	52	***Extended therapy
8	21100	859	0*	572	0*	<0,05	0*	<0,05	0*	<0,05	***Extended therapy
9	1862000	312	0*	282	0*	256	0*	106	1995	321	1472
10	674000	1866	0*	1135	0*	14	0*	1,33	0*	<0,05	***Extended therapy
11	67200	268	0*	47	0*	14	0*	<0,05	0*	<0,05	0
12	3340000	11376	1858	10010	0*	10187	0*	10994	0*	9611	53580

* undetectable HBVDNA; ** EOT – end of treatment: ND – not done

***Extended therapy and HBVDNA was undetectable, but the therapy was extended

Table 3. ALT levels during the standard treatment with Peg IFN-alfa 2a

Patients	Baseline	3rd month	6th month	9th month	12th month
	ALT U/L	ALT U/L	ALT U/L	ALT U/L	ALT U/L
1	108	73	70	58	41
2	71	110	88	43	31
3	78	200	114	80	64
4	268	61	110	104	75
5	46	114	65	40	26
6	59	41	35	ND	25
7	55	29	57	41	23
8	55	23	38	40	33
9	118	100	25	23	26
10	33	89	74	65	61
11	58	31	25	20	15
12	143	40	35	30	24

At the end of the standard 48-week therapy with Peg-IFN all patients were with virological response defined by HBV DNA < 2000 IU/ml. In 9/12 of them serum HBV DNA was undetectable (Tabl. 2). HBsAg-loss was observed in one subject (№8, Table 2), who was also with anti-HBs 21 IU/mL at treatment week-48.

During therapy the following patterns of changes in HBsAg levels were observed:

- Fluctuation or slight decrease of HBsAg level with less than 20% compared to baseline value in 4/12 of patients
- Sharp decline of HBsAg level with more than 90% reduction at treatment week-48 in 8/12 of subjects

Virological response in patients without sharp HBsAg decline

Four of the 12 patients (subjects № 1, 6, 9, and 12, table 2, figure 1) were without sharp decline of HBsAg during standard 48-week Peg-IFN-alfa 2a therapy and 3/4 of them relapsed 6 months after treatment discontinuation (defined by HBV DNA level > 2000 IU/ml) – table 2. In all 3 relapsers the baseline HBsAg level was above 10 000 IU/ml and remained high at the end of therapy: 9611 IU/ml in patient №12 (Table 2) and above 55000 IU/ml in subjects № 1 and 6 (Table 2). Only one patient (№ 9) without sharp HBsAg decline achieved sustained virological response 6 months post therapy, but his HBsAg level both at treatment baseline and at the end of the therapy was relatively low (300 IU/mL).

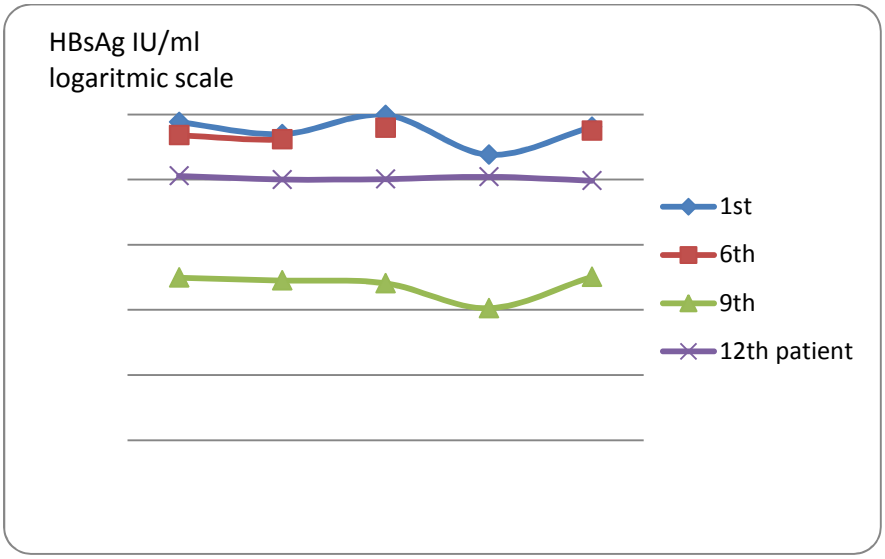

Figure 1. Fluctuations of HBsAg levels in patients without a sharp decline of HBsAg at 3rd month of the standard 48-week Peg-IFN-alfa 2a therapy

Virological response in patients with sharp HBsAg decline

Eight of 12 patients (№ 2, 3, 4, 5, 7, 8, 10, and 11, Table 2) were with sharp decline of HBsAg level and all of them achieved virological response at treatment week-48 (defined as HBV DNA < 2000 IU/mL). In addition serum HBV DNA was undetectable in 7/8 of them (subjects № 3, 4, 5, 7, 8, 10, and 11, Table 2).

In 5/7 of patients with sharp HBsAg decline and undetectable HBV DNA at month-12, HBsAg levels decreased <100 IU/ml (figure 2).

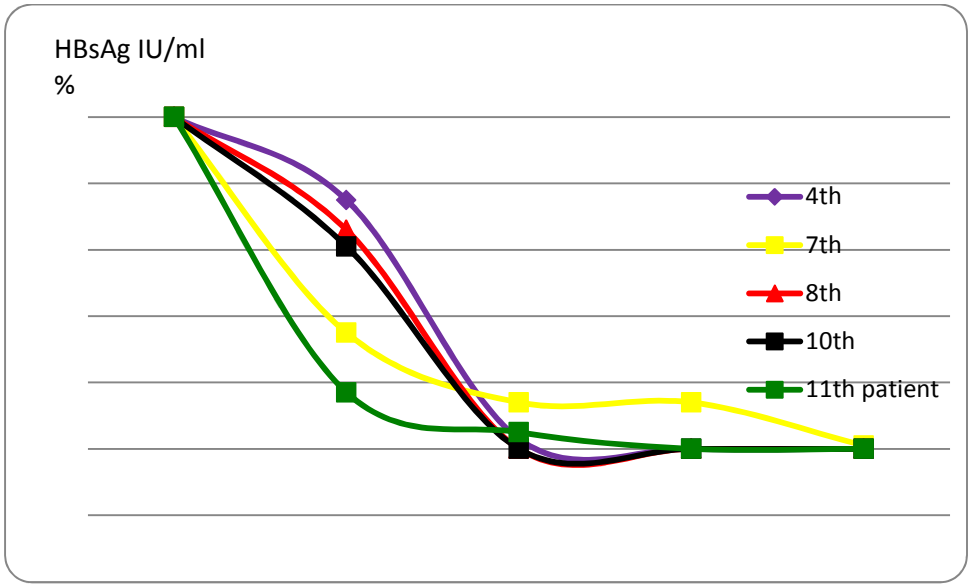

Figure 2. HBeAg-negative patients with chronic hepatitis B, with EOT (Peg-IFN) and HBsAg <100 IU/ml.

Four out of 5 patients with HBsAg level < 100 IU/L agreed to extend Peg-IFN treatment for more then 48-weeks (subjects № 4, 7, 8, and 10, Table 2).

In patient № 4 - HBsAg-loss was observed at treatment week-48. During the extension of the therapy (week 72) anti-HBsAg >100 (118 IU/L) was detected and the therapy was stopped. The patient was lost for further follow-up.

In patient № 8 - HBsAg-loss was observed also at treatment week-48, with anti-HBsAg 21 IU/L. At week 60 – anti-HBsAg was 63 IU/L, and at week 72 – 480 IU/L. Peg-interferon was stopped at week 72. Re-occurrence of HBsAg was found in subject № 8 six months after treatment cessation, which was associated with parallel decline of the anti-HBs titer from 480 to 3 IU/L.

Patient № 7 had anti-HBs - at week-108 (26 IU/L) and therapy is still ongoing. HBsAg is not evaluated. Interferon therapy is still ongoing.

In patient №10 HBsAg is still positive at 96th week of interferon therapy. An anti-HBs titer of 6-10UI/L was detected at treatment week-72 and after that (week-96) and therapy is still ongoing (figure 3).

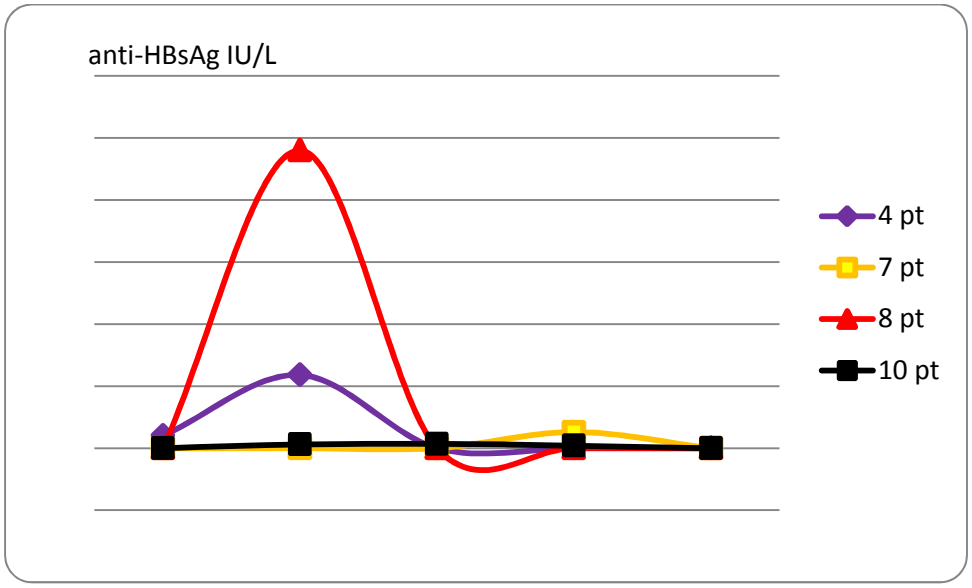

Figure 3. Anti-HBs levels during the extension of Peg-IFN therapy

Discussion

In the present study we evaluated HBsAg levels in the selected group of HBeAg-negative patients who did not fulfill the “stopping rule” at week-12 of Peg-IFN therapy (16). Our results confirmed the beneficial effect that could be achieved by applying the discussed stopping rule in the management of HBeAg-negative patients during standard Peg-IFN therapy. In our serial we achieved a very high rate of end of treatment virological response (100%) and low rate of post-treatment relapse (25%).

We identified two different patterns of HBsAg change during Peg-IFN therapy: 1) fluctuation or slight decrease of HBsAg with less than 20% compared to baseline level and 2) sharp HBsAg decline with >90% than baseline value.

The first pattern was found in all relapsers. We recently reported that HBsAg levels may fluctuate within 20%, spontaneously and separately from the viral load even within a short (3-month) period of the natural course of HBeAg-negative CHB (21). Spontaneous HBsAg fluctuation was quite similar to that observed among relapsers in the present study. One can speculate that the slight decrease of HBsAg levels at treatment week-12 in relapsers might reflect the spontaneous fluctuation of HBsAg rather than the effect of Peg-IFN therapy. In this regard the sharp HBsAg decline during Peg-IFN (second pattern of HBsAg changes identified in the present study) is more informative for the treatment outcome. These data are in line with a previous study which reported that EOT cut-off HBsAg level < 1000 IU/ml is associated with 75% positive predictive value (PPV) for sustained long-term virological response in genotype-D patients treated with Peg-IFN for 48 weeks (22). In our serial all patients with HBsAg level > 1000 IU/mL at treatment week-48 experienced post-treatment relapse. On the other hand all subjects with qHBsAg <

1000 IU/mL at week-48 achieved SVR, irrespective of whether their baseline HBsAg level was above or below 10 000 IU/ml.

In addition, Brunetto et al. (10) showed that an HBsAg level < 10 IU/ml at the EOT was highly predictive of an SVR (80%) at the 24-week post-treatment follow-up and for HBsAg loss (52%) at the 3rd -year post therapy. One third of the patients in our study were with HBsAg < 10 IU/mL at treatment week-48, but HBsAg-loss at the end of the standard 48-week Peg-IFN therapy occurred in only ¼ of them. One possible explanation is that genotype-D is almost 100% in the Bulgarian population (4,5). It was already confirmed that genotype-D patients are more difficult to treat with Peg-IFN (9,23). Recent studies in HBeAg-negatitive subjects predominantly infected with genotype-D demonstrated that the extension of Peg-IFN therapy increases the SVR rate by reduction of relapses and improves HBsAg kinetics and thus enhances HBsAg clearance (9,20).
.
In the light of these data one very important clinical question remains: how to select the right patient for the extended Peg-IFN therapy and when to stop it? It is obvious that there are at least three levels for optimization of Peg-IFN therapy:

1. Careful initial selection of subjects according to the well-known baseline predictors of response. This will help to start Peg-IFN in the right patient at the right time
2. Week-12 stopping rule is helpful to avoid over-treating of non-responders
3. Tailoring of treatment duration according to the individual response by evaluation of HBsAg levels (“extension rule”) might be a useful approach for achieving higher rates of SVR and HBsAg seroconversion.

Extension of Peg-IFN treatment is reasonable in patients who demonstrated a sharp HBsAg decline during the standard 48-week therapy. Probably, subjects with HBsAg level < 100 IU/ml will mostly gain from prolongation of Peg-IFN. In our study HBsAg-seroconversion with anti-HBs > 100 IU/ml was achieved in ½ of patients and additional ¼ was with a borderline titer of anti-HBs. It is still not clear enough when is the optimal time-point to stop Peg-IFN therapy. It is quite logical to navigate treatment duration by using both HBsAg and anti-HBs levels. This approach has been used successfully after add-on Peg-IFN to NUC to achieve HBsAg clearance by extension of Peg-IFN (24). A similar approach was also recently reported in HDV-infected patients who were treated with Peg-IFN (25).

It should be underlined that we observed re-occurrence of HBsAg and disappearance of anti-HBs even after extension of Peg-IFN for 6 months post clearance of HBsAg. This fact clearly suggests that immunomodulation should continue longer in order to achieve durable HBsAg-seroconversion. Hypothetically, some patients may need immunomodulation for additional 12 months or longer after HBsAg-loss and for more than 6 months post development of anti-HBs > 100 IU/ml. Further studies are needed to define and validate the optimal rules for extension of Peg-IFN therapy in HBeAg-negative CHB.

References

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment and current emerging prevention and control measures. J Viral Hepatol 2004; 11: 97–107.

2. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology 2009; 49 (Suppl 5): S45–55.

3. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012; 57: 167–85.

4. Jelev D. Chronic HBV infection: characteristics, natural course and options for treatment. DMSc Thesis. Sofia. 2010.

5. Krastev Z, Tchernev K, Petrova D, et al., Bulgarian Society of Gastroenterology. An open label, multicenter, national non-randomized study to evaluate efficacy and safety of Peginterferon alfa-2a (40 KD) in patients with HBeAg-negative chronic hepatitis B: preliminary results. Bulgarian Hepatogastroenterol. 2006; 2: 90–91.

6. Heathcote EJ, Marcellin P, Buti M et al. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. Gastroenterology 2011; 140:1 32–43.

7. Marcellin P, Gane EJ, Flisiak R, et al., Long term treatment with tenofovir disoproxil fumarate for chronic hepatitis B infection is safe and well tolerated and associated with durable virologic response with no detectable resistance: 8 year results from two phase 3 trials. Oral presentation at AASLD, November, 2014, Bostan, USA.

8. Brechot C, Thiers V, Kremsdorf D, et al. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely ‘‘occult’’? Hepatology 2001; 34: 194–203

9. Lampertico P, Viganò M, Di Costanzo GG, et al., Randomised study comparing 48 and 96 weeks peginterferon α-2a therapy in genotype D HBeAg-negative chronic hepatitis B. Gut. 201; 362: 290–298.

10. Brunetto MR, Moriconi F, Bonino F, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 2009; 49: 1141–1145.

11. Moucari R, Mackiewicz V, Lada O, et al., Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009; 49: 1151-1157.

12. Su TH, Hsu CS, Chen CL, et al. Serum hepatitis B surface antigen concentration correlates with HBV DNA level in patients with chronic hepatitis B. Antivir Ther. 2010; 15: 1133–1139.

13. Liaw YF, Jia JD, Chan HL, et al. Shorter durations and lower doses of peginterferon alfa-2a are associated with inferior hepatitis B e antigen seroconversion rates in hepatitis B virus genotypes B or C. Hepatology. 2011; 54: 1591–1599.

14. Piratvisuth T, Marcellin P, Popescu M, et al., Hepatitis B surface antigen: association with sustained response to peginterferon alfa-2a in hepatitis B e antigen-positive patients. Hepatol Int. 2013; 7 (2): 429–436.

15. Rijckborst V, Hansen BE, Cakaloglu Y, 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: 454–61.

16. Rijckborst V, Hansen BE, Ferenci P, 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.

17. Lampertico P, Del Ninno E, Manzin A, et al., A randomized, controlled trial of a 24-month course of interferon alfa 2b in patients with chronic hepatitis B who had hepatitis B virus DNA without hepatitis B e antigen in serum. Hepatology 1997; 26: 1621–1625.

18. Lampertico P, Del Ninno E, Viganò M, et al., Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003; 37: 756–763.

19. Krastev Z, Jelev D, Antonov K. Long-term supportive cyclical re-treatment in HBeAg-negative IFN responders. J Hepatol. 2005; 42(2): 277–278.

20. Krastev Z, Jelev D, Ivanova A, et al., Maintenance Peginterferon alfa-2a treatment reduces HBsAg level in genotype D HBeAg-negative interferon responders (interim results). J Hepatol; 2011; 54 (Suppl. I): 729.

21. Jelev D, Antonov K, Ivanova A, et al., Natural fluctuations of serum HBsAg level in HBeAg-negative chronic hepatitis B. Falk Symposium Challenges of liver cirrhosis and tumors: prevent it, treat it, and manage consequences. 5-6 October 2012, Mainz, Germany, Abstract book, page 37.

22. Brunetto MR, Marcellin P, Cherubin B, et al. Response to peginterferon alfa-2a(40KD) in HBeAg-negative CHB: on-treatment kinetics of HBsAg serum levels vary by HBV genotype. J Hepatol 2013; 59: 1153–1159.

23. Bonino F, Marcellin P, Lau GK, et al., Predicting response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B. Gut 2007; 56 (5): 699–705.

24. Ouzan D, Pénaranda G, Joly H, et al., Add-on peg-interferon leads to loss of HBsAg in patients with HBeAg-negative chronic hepatitis and HBV DNA fully suppressed by long-term nucleotide analogs. J Clin Virol. 2013; 58: 713–717.

25. Ouzan D, Penaranda G, Joly H, et al., Optimized HBsAg titer monitoring improves interferon therapy in patients with chronic hepatitis delta. J Hepatol 2013; 58:1258–1259.

Corresponding author:

Nina Nikolova
15 Akad. Ivan Geshov Blvd., Sofia 1431, Bulgaria
University Hospital St. Ivan Rilski
Clinic of Gastroenterology
e-mail:nnikolova234@gmail.com