

Received: 2013.09.16
Accepted: 2013.11.07
Published: 2014.02.26

An initial assessment of correlations between host- and virus-related factors affecting analogues antiviral therapy in HBV chronically infected patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDE 1 **Piotr Stalke***
BCDEF 2 **Magda Rybicka***
C 2 **Anna Wróblewska**
B 1 **Marcin Dreczewski**
B 2 **Ewa Stracewska**
G 1 **Tomasz Smiatacz**
ACDEFG 2 **Krzysztof Piotr Bielawski**

1 Department of Infectious Diseases, Medical University of Gdańsk, Gdańsk, Poland
2 Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland

* Equal contribution of both authors

Corresponding Author: Krzysztof Piotr Bielawski, e-mail: bielawski@biotech.ug.edu.pl

Source of support: Ministry of Science and Higher Education, grant no. N N302183639 and Medical University of Gdansk, grant no. ST79

Background: Success in treating hepatitis B virus (HBV) infection with nucleoside analogues drugs is limited by the emergence of drug-resistant viral strains upon prolonged therapy. In addition to mutation patterns in the viral polymerase gene, host factors are assumed to contribute to failure of treatment in chronic HBV infections. The aim of this study was to analyze the correlation between efficacy of antiviral therapy and the prevalence of HBV pretreatment drug-resistant variants. We also analyzed the role of heterogeneity in the promoter region of the IL-10 on the HBV *pol/s* gene polymorphisms and efficacy of analogues-driven therapy.

Material/Methods: HBV DNA was extracted from 54 serum samples from chronic hepatitis B (CHB) patients. Drug-resistance mutations were analyzed using MALDI-TOF mass spectrometry technology (MALDI-TOF MS) and Multi-temperature single-strand conformation polymorphism (MSSCP). IL-10 gene promoter region polymorphisms at positions -1082, -819, and -592 were determined in allele-specific PCR reactions (AS-PCR).

Results: Drug-resistance mutations were detected in 74% of naïve and 93% of experienced patients, but the effect of pre-existence of drug-resistant HBV variants on antiviral therapy was not statistically significant ($p=0.86$). The role of polymorphisms at positions -1082 ($p=0.88$), -819 ($p=0.26$), and -592 ($p=0.26$) of IL-10 promoter region polymorphisms was excluded from the response-predicting factors. The main host factors predicting successful response to antiviral therapy were female sex ($p=0.007$) and young age ($p=0.013$).

Conclusions: The presence of drug-resistant HBV variants in baseline is not a viral predictor of good response to nucleoside/nucleotide analogues therapy. Only low HBV viral load predicted positive response to antiviral therapy. The ideal candidate for antiviral therapy is an immunocompetent, young female with low HBV viral load and elevated ALT activity.

MeSH Keywords: **Polymorphism, Genetic • Drug Resistance • Interleukin-10 • Mass Spectrometry • Hepatitis B, Chronic**

Full-text PDF: <http://www.medscimonit.com/download/index/idArt/889788>

 2778

 4

 2

 37

Background

Hepatitis B virus (HBV) infection, affecting about 2 billion people worldwide, is still a global health problem. Despite the existence of a highly effective vaccine, 240–350 million chronic HBV carriers could develop chronic hepatitis B (CHB) with high risk of liver cirrhosis and hepatocellular carcinoma (HCC) [1–4]. The primary treatment goal in CHB patients is the prevention of liver disease progression. Life-long suppression of HBV replication seems to be the best antiviral strategy [4–6]. However, the existence of covalently closed circular DNA (cccDNA) in infected hepatocytes probably makes the eradication of HBV infection impossible [7,8].

Nucleotide/nucleoside analogues (NAs) and peg-interferon alpha (PegIFN α_{2a}) are 2 major classes of antiviral drugs approved for CHB treatment. NAs agents (e.g., lamivudine, telbivudine, entecavir, adefovir, and tenofovir) can suppress viral load, but long-term therapy can lead to the selection of drug-resistant HBV variants [4,9]. Recent studies showed that there is a possibility of the pre-existence of natural resistance mutations in treatment-naïve patients [10–12]. Viral quasispecies evolve over time under selective pressure (e.g., antiviral therapy). These variants are more viable and spread more rapidly in the liver than does wild-type HBV [1,6,13].

Early detection of drug-resistant HBV variants is crucial for patients treated with low genetic barrier drugs [14,15]. Several methods are currently available to monitor HBV drug resistance (direct sequencing of PCR products, restriction fragment length polymorphism – RFLP, and mutation-specific real-time PCR) but reverse hybridization – Line Probe Assay is the most popular. Among these methods, MSSCP assay (multi-temperature single-strand conformation polymorphism) and MALDI-TOF MS (matrix-assisted laser desorption ionization time of flight mass spectrometry) are the most sensitive, being able to detect HBV mutants, which constitute only 1% of the viral population [16–19].

Development of HBV mutants is also related to the persistence of cccDNA and host factors affecting immune response [13,20]. Polymorphic sites within the IL-10 gene promoter region at positions –1082, –819, and –592 have been described [21–23]. It was published that certain polymorphisms within the IL-10 gene promoter region are associated with the development of chronic HBV infection and effects of IFN α therapy [21,24]. However, the impact of these polymorphisms on the development on drug-resistant HBV strains and response to NAs therapy has not been determined.

The aim of this study was to analyze the correlation between efficacy of antiviral therapy and the prevalence of HBV pretreatment drug-resistant variants. Moreover, the role of

heterogeneity in the promoter region of the IL-10 on the HBV *pol/S* gene polymorphisms and efficacy of analogues-driven therapy was analyzed.

Material and Methods

Study population and sample design

We enrolled into the study 54 consecutive CHB (29 male and 25 female) patients, mean age 48.7 \pm 2 yrs, qualified between January 2011 and June 2012 to antiviral therapy at the Department of Infectious Diseases, Medical University of Gdansk. They were qualified and received treatment accordingly to recommendations of the Polish National Health Service (NFZ). These recommendations require the use of peg-IFN α_{2a} or lamivudine (when cytokines are contradicted) as a primary therapy in HBeAg-negative subjects. A pretreatment HBV drug resistance test is required in analogues therapy, and the detection of lamivudine-resistant strains allows use of entecavir, adefovir, or tenofovir. In HBeAg-reactive patients, entecavir or tenofovir is recommended as a first-line therapy.

The majority of subjects received lamivudine – 30/54 (55%), entecavir 16/54 (30%), tenofovir 7/54 (13%), and combined therapy (lamivudine with adefovir) in 1/54 (2%). The therapy effect was assessed 48 weeks after NAs administration, accordingly to the NFZ recommendation. Patients were classified into 2 groups: those with detectable HBV DNA (non-responders) and those with undetectable HBV DNA (responders). Patients receiving peg-IFN α_{2a} ; HCV or HIV co-infected individuals were excluded from the study.

Whole blood and serum samples for analyzing HBV viral load, HBV *pol/S* region, and IL-10 promoter region polymorphisms were collected before antiviral therapy (baseline). Serum samples taken at week 12, 24, and 48 were stored for HBV DNA quantitative testing. At baseline, biochemical and serological tests and liver biopsy assessments were part of routine analyses.

The study protocol was approved by the local ethics committee and informed consent for participation was obtained from all enrolled subjects.

HBV viral load and *pol/S* region analysis

Whole blood samples were collected into Vacutainer tubes without anticoagulant and then incubated in an upright position for 30–45 min to allow clotting. The clot was then removed by centrifuging at 3500 \times g for 15 min and serum was immediately transferred into a clean Eppendorf tube. All samples were stored at –20°C until further analysis. Viral DNA was extracted from

Table 1. PCR thermal cycling parameters.

Step	Temperature/duration						
	1 st PCR	2 nd PCR	HBV genotyping	rs1082	rs819	rs592	
No. of amplification cycles	35	35	35	33	33	33	
Initial inactivation/denaturation	95°C/10 min	95°C/10 min	95°C/10 min	95°C/3 min	95°C/3 min	95°C/3 min	
Parameters of amplification	Denaturation	94°C/30 sec	94°C/30 sec	94°C/30 sec	95°C/30 sec	95°C/30 sec	
	Annealing	56°C/30sec	57°C/30 sec	57°C/30 sec	57.9°C/30 sec	61.4°C/30 sec	53.7°C/30 sec
	Extension	72°C/30 sec	72°C/30 sec	72°C/90 sec	72°C/60 sec	72°C/60 sec	72°C/60 sec
Final extension	72°C/10 min	72°C/10 min	72°C/10 min	72°C/1 min	72°C/1 min	72°C/1 min	
Hold	4°C	4°C	4°C	4°C	4°C	4°C	

200 µl of serum samples using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany) with a slightly modified manufacturer's protocol: the incubation time with Proteinase K was 1 h instead of 10 min and the final elution volume was 30 µl. Quality and quantity of the DNA was measured using a spectrophotometer (NanoDrop™ ND-1000, Thermo Scientific, USA). The Roche Cobas Amplicor HBV Monitor Assay (Roche Diagnostics, Pleasanton, USA) was used to quantify the level of HBV DNA in serum (sensitivity 34.36 IU/ml) using the manufacturer's protocol.

Multi-temperature single-strand conformation polymorphism (MSSCP)

The MSSCP assay was done by means of the DNA Pointer System (Biovectis, Poland). To increase the sensitivity of HBV YMDD (rt204) variants detection, nested PCR was performed. The reaction mixture for the first step (1st PCR) contained (25 µl): 2 µl of HBV DNA, 1× chelating buffer, 1.2 mM Mg(OAc)₂, 0.2 mM dNTP, 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Germany), 0.1 mg/ml of casein, 0.01% (v/v) formamide, and 0.125 µM of primers: 1F and 1R (1F: 5'-GACTCGTGGTGGACTTCTCTC-3', 1R: 5'-TGATCCTGTGGCAAAGTCC-3'). The reaction mixture for the second step (2nd PCR) consisted of (25 µl): 1× chelating buffer, 1.2 mM MgCl₂, 0.5 mM dNTP, 1 U of AmpliTaq Gold polymerase, 0.25% (v/v) glycerol, 0.4% (v/v) BSA, 0.125 µM of primers: 2F [17] and 2R (5'-TAACAGCGGTATAAAGGCCT-3'), and 2 µl of the first amplification step product. PCR conditions for each step are shown in Table 1. Positive and negative control was used at each step (HBV DNA External Quality Control, PeliSpy™PRO; AcroMetrix, USA). To determine the detection limit, serial dilutions of Quantification Standard QS HBV Real Star® (Altona Diagnostics, Germany) were used: 10⁴ copies/µl (1718 IU/mL), 10³ copies/µl (171.8 IU/mL), 10² copies/µl (17.18 IU/mL), 10 copies/µl (1.718 IU/mL), 5 copies/µl (0.859 IU/mL), 2 copies/µl (0.3436 IU/mL), and 1 copy/µl (0.1718 IU/mL).

One µl of denatured PCR products (159 bp) containing HBV mutant and wild-type (WT) sequences at the codons of interest were loaded onto a 11% polyacrylamide gel (29:1 acrylamide: bisacrylamide) with addition of 5% glycerol. The electrophoresis was run under sequentially changed gel temperature (15°C, 10°C, and 5°C for 900 Vxh each) at a constant voltage of 40V in 0.5×TBE. After silver staining, specific band patterns characteristic for each HBV variant were cut out from the gel, purified using the QIAquick Gel Extraction Kit (Qiagen, Germany), and then sequenced.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

The MALDI-TOF MS-based HBV genotyping assay started with amplification of 754 bp DNA fragment containing 12 codons: 80, 169, 173, 180, 181, 184, 194, 202, 204, 233, 236, and 250 [27–35]. The PCR reaction mixture contained (25 µl): 200 nmol/l of each primer [25], 5 µl of HBV DNA, 1× chelating buffer, 1.2 mM Mg(OAc)₂, 0.2 mM dNTP and 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Germany). Amplification conditions of HBV *pol/S* region analysis are shown in Table 1.

PCR products were then purified from non-incorporated dNTPs by treating with shrimp alkaline phosphatase (SAP) solution (40 min at 37°C and 5 min at 85°C). The iPLEX Gold assay was done in 4 separate primer-extension reactions on the Mass Array genotyping platform (Sequenom Inc., USA) with a standard procedure following the iPLEX kit protocol (Sequenom Inc., USA). To desalt the iPLEX reaction products, a resin kit was used (SpectroCLEAN resin, Sequenom Inc., USA), then cleaned extension products were dispensed onto a 384-element SpectroChip using the Nanodispenser, and mass differences were detected with MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry). All data analyses were carried out with TyperAnalyzer Application, version 4 (Sequenom Inc., USA).

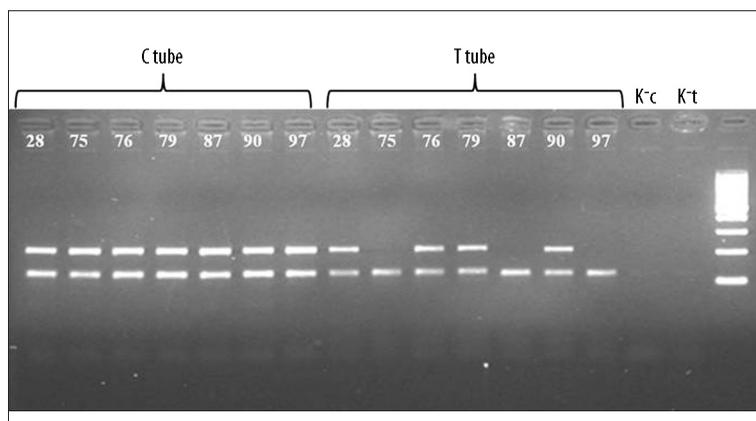


Figure 1. An example of results for 7 patients (28, 75, 76, 87, 90, 97) obtained for rs871. Kc and Kt are negative controls for C and T tubes.

IL-10 promoter region polymorphism

Interleukin 10 promoter region polymorphism DNA was extracted from 500 μ l of whole blood using the QIAamp[®] DNA Blood Mini Kit (Qiagen, Germany). DNA was eluted in 50 μ l of AE buffer. To determine 3 biallelic polymorphisms at positions -1082, -819, and -592 in the IL-10 gene promoter region, allele-specific PCR reactions (AS-PCR) were performed. Each polymorphism (rs) was assessed in 2 separate reactions. While the common primer was the same for both reactions (rs1082: 5'-AAGCTTCTGGCTGGAGTC-3'; rs819: 5'-GGCACATGTTCCACCTCTTC-3'; rs519: 5'-GGGGTCATGGTGAGCACTAC-3'), 2 different primers were designed for each allele (rs1082: **A**: 5'-AACACTACTAAGGCTTCTTTGGGTA-3' and **G**: 5'-AACACTACTAAGGCTTCTTTGGGTG-3'; rs819: **C**: 5'-TACCCTGTACAGGTGATGTACC-3' and **T**: 5'-TACCTTGTACAGGTGATGTACT-3'; rs519: **C**: 5'-TCCAGAGACTGGCTTCTTACAAG-3'). The Human β -globin primers were used as an experimental control (5'-TTGGACCCAGAGGTTCTTTG-3', 5'-GAGCCAGGCCATCACTAAAG-3'). The PCR reaction mixture contained (25 μ l): 1 \times Taq buffer +NH₄SO₄ -MgCl₂ (Thermo Scientific, USA), 2 mM MgCl₂ (Thermo Scientific, USA), 0.2 mM dNTPs (Thermo Scientific, USA), 0.4 μ M of each primer, 5 μ l of BSA (1 mg/ μ l), 1 U DreamTaq (Thermo Scientific, USA), and ~20 ng/ μ l DNA. The PCR conditions are shown in Table 1.

Further analysis involved agarose gel (2%) electrophoresis of PCR products (40 min, 100V). Based on the presence or absence of a PCR product, the patient's genotype was determined. For example, if a product was seen in a "G" tube line without a product in an "A" tube line (rs1082), the patient was a GG homozygote. If PCR products were seen in both lines, the patient was a GA heterozygote (Figure 1).

Statistical analysis

Statistical analysis was done using STATISTICA data analysis software, version 8.0 (StatSoft Inc., USA). All statistical data are presented as a mean \pm standard error of means (\pm SE), or

median value. Standard error was used because distributions of data were skewed. Analysis of differences between variations was done using nonparametric statistics: Mann-Whitney's U test, chi-squared test, and Kruskal-Wallis one-way analysis of variance. Relations between variables were estimated by univariate and multivariate logistic regressions. P-value less than 0.05 was considered statistically significant.

Results

The studied group of 54 consecutive CHB patients consisted of 27 naïve and 27 NAs-experienced individuals. The naïve patients had lower prevalence of HBV drug-resistant strains ($p=0.019$) and were more frequently treated with lamivudine 23/27 (85%) ($\chi^2=20.5$, $p=0.0001$) (Table 2). Entecavir was administered in 4/27 (15%) subjects. Undetectable HBV DNA at week 24 and at week 48 was similar ($p=0.7$ and $p=0.98$, respectively).

Some HBV drug-resistant variants were detected in 20/27 (74%) naïve and 25/27 (93%) NAs-experienced patients in MSSCP assay, confirmed in randomly selected cases by MALDI-TOF MS (Figure 2A, 2B). The HBV drug-resistant variant could affect prescribed drug activity in 13/27 (48%) naïve and 23/27 (85%) NAs-experienced subjects. Commercially available tests (Line Probe Assay – INNOLiPA HBV DR) did not detect drug-resistant strains in naïve patients qualified to NAs therapy [26]. The National Health Service (NFZ) requires pretreatment drug resistance tests in all patients (naïve and experienced) during qualification to NAs therapy.

The efficacy of antiviral therapy in our study did not depend on liver biopsy inflammation activity ($p=0.5$), fibrosis stage ($p=0.99$), polymorphisms promoter IL-10 gene (Table 2), or pre-existence of HBV drug-resistant variants ($p=0.86$). The strongest factor predicting the response to antiviral therapy was baseline viral load, which was significantly lower in the responder than the non-responder group (Table 2). The antiviral therapy

Table 2. Demographic and clinical characteristics in responder v/s nonresponder and naïve v/s NAs-experienced CHB patients.

	Analogues antiviral therapy		Significance p<0.05)	Analogues antiviral therapy		Significance p<0.05)
	responder (n=28)	nonresponder (n=26)		Naïve (n=27)	NAs experienced (n=27)	
Gender (male/female)	10/18	19/7	0.0066	11/16	18/9	0.10
Age (years)	43.4±1.98	54.4±3.51	0.011	47.67±3.3	49.70±2.59	0.62
ALT (IU/l)	136±41	76±11	0.75	120±40	95±20	0.74
Baseline viral load (kIU/ml)	13702±8385	57857±15205	0.0035	33989±12765	35935±12814	0.98
HBe Ag (reactive/nonreactive)	8/20	15/11	0.033	9/18	14/14	0.25
HBe Ab (reactive/nonreactive)	21/7	12/14	0.032	19/8	14/13	0.25
Baseline HBV drug-resistant variants* (Yes/No)	19/9	17/9	0.86	13/14	23/4	0.019
Immunocompromised (Yes/No)	10/17	8/17	0.71	8/19	10/15	0.52
NAs therapy** (naïve/experienced)	13/15	14/12	0.60	NA§	NA##	NA##
Antiviral therapy	Lamivudine	16	0.029	23	7	0.0001
	Entecavir	6		4	12	
	Tenofovir	6		0	7	
	lamivudine + adefovir	0		0	1	
Liver biopsy#	inflammation grade	2	0.50	1.75	2	0.70
	fibrosis stage	1.5	0.99	1.5	1.5	0.42

Data are presented as a mean value ± standard error (SE); # data presented as a median value; * the presence of HBV drug-resistant minor variant before NAs therapy; ** nucleoside/nucleotide analogues therapy; ## not applicable.

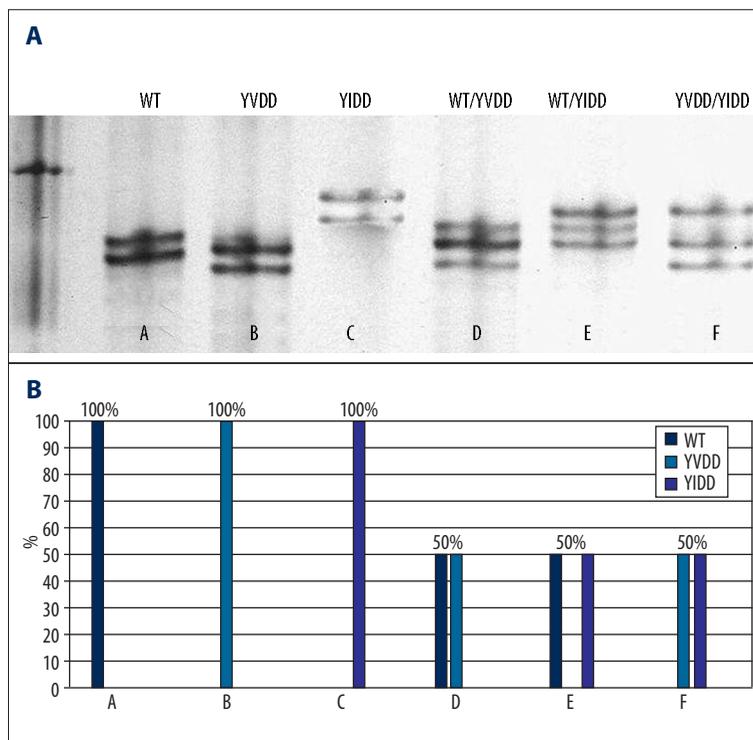


Figure 2. HBV YMDD variants obtained for 7 patients (A–F) during MSCP (A) and MALDI-TOF MS analysis (B).

Table 3. Distribution of alleles promoter region IL-10 in CHB patients.

IL-10 polymorphism	Analogues antiviral therapy		Significance p<0.05)
	Responder (n=28)	Nonresponder (n=26)	
rs1800896 (1082)			
GG	4	4	0.88
AA	3	3	
GA	21	19	
rs1800871 (819)			
CC	20	15	0.26
TT	0	1	
CT	8	10	
rs1800872 (592)			
CC	19	13	0.26
AA	2	1	
CA	7	12	
ATA haplotype IL-10 (present/absent)	8	11	0.3

Table 4. Univariate and multivariate logistic regression analyses of host and viral predicting NAs therapy response factors in CHB patients.

	Analogues antiviral therapy		Univariate analysis		Multivariate analysis	
	Responder (n=28)	Nonresponder (n=26)	OR (95% CI)	p value	OR (95% CI)	p value
Age (year)	43.4±1.98	54.4±3.51	0.95 (0.91–0.99)	0.013	0.90 (0.83–0.98)	0.011
Female gender	18	7	4.88 (1.49–16.04)	0.007	10.3 (1.60–65.81)	0.011
Baseline HBV viral load (kIU/ml)	13.702±8.385	57.857±15.205	0.999987 (0.999997–0.999998)	0.019	0.999961 (0.999993–0.999999)	0.008
Baseline HBeAg (reactive)	8	15	0.29 (0.09–0.90)	0.033		
Baseline ALT (IU/l)	136±41	76±11	1.003 (0.998–1.009)	0.22	1.01 (0.99–1.02)	0.178
Previous NAs* therapy	15	12	1.35 (0.45–4.03)	0.586		
Baseline drug resistance presence **	19	17	1.12 (0.36–3.47)	0.85		
Liver biopsy inflammation activity#	2	2	1.42 (0.60–3.35)	0.41		
Liver biopsy fibrosis stage#	1.5	1.5	0.93 (0.46–1.93)	0.86		
ATA IL-10 haplotype	8	11	0.54 (0.18–1.69)	0.29		
Immunocompetent patient	17	17	0.8 (0.25–2.59)	0.7	0.09 (0.009–0.93)	0.038

Data are presented as a mean value ± standard error (SE); # data presented as a median value; * nucleoside/nucleotide analogues; ** the presence of HBV drug-resistant minor variant before NAs therapy.

was significantly different in responder and non-responder groups (p=0.03), but the frequency of lamivudine administration was similar in both groups (Table 2).

The highest mean viral load (kIU/ml) was detected in patients receiving entecavir (66,138±20,800). Significantly lower concentration of HBV DNA was measured in lamivudine (19,378±9,379)

and tenofovir (11,769±11,539) groups ($p=0.03$). HBeAg reactive was significantly more frequent in the entecavir 11/16 (67%) and tenofovir 4/7 (57%) than in the lamivudine 7/23 (30%) groups ($\chi^2=10.98$, $p=0.0118$).

The univariate and multivariate comparison of variables between patients with good and weak response to antiviral therapy are reported in Table 4. Univariate logistic regression analysis showed the strongest influence of female sex (OR 4.88, 95% CI 1.49-16.04, $p=0.007$) on the response to NAs therapy. Multivariate logistic regression analysis revealed significant correlations ($\chi^2=25.95$, $p=0.00009$) between the following independent variables predicting better response to NAs therapy: younger age (OR 0.90, 95% CI 0.83-0.98, $p=0.011$), female sex (OR 10.3, 95% CI 1.60-65.81, $p=0.011$), lower baseline viral load (OR 0.999961, 95% CI 0.99993-0.99999, $p=0.008$), higher baseline ALT activity (OR 1.01, 95% CI 0.99-1.02, $p=0.178$), and patient's immunocompetence (OR 0.09, 95% CI 0.009-0.93, $p=0.038$).

Discussion

We collected blood samples only from patients randomly qualified to NAs therapy in the Therapeutic Programs of the National Health Service (NFZ). Criteria for qualification to antiviral therapy was different from the EASL CHB statement and probably contributed to the slightly higher rate of therapeutic success of NAs in experienced compared to naïve patients [4,14]. NFZ qualification criteria still promotes lamivudine therapy in subjects without detectable HBV drug-resistant strains, and explains the overrepresentation of these patients in naïve and NAs-experienced individuals. A relatively weak HBV DNA suppression rate in entecavir-treated subjects have an effect on entecavir prescribing in lamivudine-resistant or high viremic HBeAg-reactive patients. These criteria selected patients in the entecavir-treated group who were more difficult to treat.

It was interesting to discover drug-resistant HBV variants in 13/27 (48%) treatment-naïve CHB patients, but without a significant influence on the therapy success rate (Table 1). We found drug-resistant HBV variants significantly more frequently in NAs-experienced than among naïve patients. However, some HBV variants seemed to be non-correlated with previous antiviral therapy and could have appeared spontaneously. Recently a natural prevalence of HBV reverse transcriptase amino acid substitutions in treatment-naïve patients with CHB has been shown [10-12,36,37]. However, these mutations in our subjects were more common. MSSCP and MALDI-TOF MS techniques used in our study had superior sensitivity than did commercially available HBV drug-resistance assays [26].

In our study there were no significant differences in frequencies of genotypes and alleles of IL-10 gene promoter region

at position -1082 G/A, -819 T/C, -592 A/C and the initial response of HBV infection to treatment with NAs. Although IL-10 has been shown to have an important role in chronic inflammation and fibrogenesis, as well as in IFN- α therapy of hepatitis B patients, it cannot become a predictive factor of response to NAs in chronic HBV infection. Moreover, in contrast to IFN therapy, no differential haplotype distribution responsible for IL-10 expression was observed between NAs responders and non-responding patients [21]. This suggests that the association of remaining putative functional single nucleotide polymorphisms (SNPs) (e.g., 2753 A/C and 3575 T/A) in IL-10 and other host genetic factors should be investigated. It is well known that the actions of cytokines may be profoundly conditioned by the presence of other cytokines. Thus, there is a need to identify new predictors for treatment outcome in these patients. New studies of particular combinations of IL-10 SNPs and other host predictors could reveal the relation with susceptibility to nucleos(t)ide analogues and may help in making appropriate treatment decisions [20-23].

In the presented study, female sex and young age were the main host factors predicting successful response to antiviral therapy. The presence of drug-resistant HBV variants at baseline was not a viral predictor of good response to NAs therapy. Only low HBV viral load was useful in identifying the likelihood of response to antiviral therapy. Multivariate analysis connected essential host and viral factors predicting response to NAs drugs, investigated in univariate analysis, with higher ALT activity and absence of immunosuppressive therapy or past bone marrow transplantation. The ideal candidate for antiviral therapy is an immunocompetent, young, female with low HBV viral load and higher ALT activity. Similar observations were previously reported [4,6,12,22].

Conclusions

We did not confirm the influence of presence drug-resistant HBV variants on NAs treatment efficacy in naïve patients. The Polish National Health Service requirement of a pre-treatment drug resistance test during qualification to antiviral therapy has no justification in our data or in commonly accepted knowledge. We did not find any host- or virus-related causes of HBV drug-resistant variants except for previous insufficient NAs therapy. The main HBV-related predicting response factor was low viral load. Host-related factors of good antiviral response predictors were young age, female sex, high ALT activity, and immunocompetence. Our study did not confirm the role of IL-10 promoter region polymorphisms in efficacy of NAs therapy in CHB patients. Natural polymorphism of the host immune system genes seems to be an essential factor affecting elimination of varied pathogens and needs further investigation.

Acknowledgments

Magda Rybicka is the recipient of the European Social Fund in the framework of the project “InnoDoktorant” – Scholarships for PhD students, 5th Edition.

References:

- Seeger C, Mason WS: Hepatitis B virus biology. *Microbiol Mol Biol Rev*, 2000; 64: 51–68
- Bielawski KP, Dybikowska A, Lisowska-Charmuszko U et al: Distribution of HBV genotypes and mutants among hepatitis B infected patients from northern Poland. *Int J Mol Med*, 2004; 14: 301–4
- WHO HBV fact sheet No 204, Revised July 2012, World Health Organisation: <http://www.who.int/mediacentre/factsheets/fs204/en/>
- 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
- Bielawski KP, Stalke P: Molecular epidemiology of chronic hepatitis B virus infection in northern Poland. *J Clin Virol*, 2005; 34(Suppl.1): S63–69
- Chen CJ, Yang HI: Natural history of chronic hepatitis B REVEALed. *J Gastroenterol Hepatol*, 2011; 26: 628–38
- Cai D, Mills C, Yu W et al: Identification of disubstituted sulfonamide compounds as specific inhibitors of hepatitis B virus covalently closed circular DNA formation. *Antimicrob Agents Chemother*, 2012; 56: 4277–88
- Locarnini S, Mason WS: Cellular and virological mechanisms of HBV drug resistance. *J Hepatol*, 2006; 44: 422–31
- Lok AS, Zoulim F, Locarnini S et al: Hepatitis B Virus Drug Resistance Working Group. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology*, 2007; 46: 254–65
- Han Y, Huang LH, Liu CM et al: Characterization of hepatitis B virus reverse transcriptase sequences in Chinese treatment naive patients. *J Gastroenterol Hepatol*, 2009; 24: 1417–23
- Liu BM, Li T, Xu J et al: Characterization of potential antiviral resistance mutations in hepatitis B virus reverse transcriptase sequences in treatment-naive Chinese patients. *Antiviral Res*, 2010; 85: 512–19
- Nguyen MH, Garcia RT, Trinh HN et al: Prevalence of hepatitis B virus DNA polymerase mutations in treatment-naive patients with chronic hepatitis B. *Aliment Pharmacol Ther*, 2009; 30: 1150–58
- Rybicka M, Charmuszko U, Stalke P, Bielawski KP: The influence of hepatitis B virus polymorphism on the progression of chronic liver disease. *Postepy Hig Med Dosw*, 2011; 65: 244–54
- Keffe EB, Dieterich DT, Pawlotsky JM, Benhamou Y: Chronic hepatitis B: preventing, detecting, and managing viral resistance. *Clin Gastroenterol Hepatol*, 2008; 6: 268–74
- Paik YH, Han KH, Hong SP: The clinical impact of early detection of YMDD mutants on the outcomes of long-term lamivudine therapy in patients with chronic hepatitis B. *Antivir Ther*, 2006; 11: 447–55
- Han KH, Hong SP, Choi SH et al: Comparison of multiplex restriction fragment mass polymorphism and sequencing analyses for detecting entecavir resistance in chronic hepatitis B. *Antivir Ther*, 2011; 16: 77–87
- Bielawski KP, Al-Soud WA, Stalke P et al: Determination of lamivudine-resistant variants of hepatitis B virus by denaturing gradient gel electrophoresis: A novel approach to monitoring drug resistance. *Med Sci Monit*, 2008; 14(5): CR281–85
- van Belkum A, Welker M, Erhard M, Chatellier S: Biomedical mass spectrometry in today's and tomorrow's clinical microbiology laboratories. *J Clin Microbiol*, 2012; 50: 1513–17
- Tost J, Gut IG: Genotyping single nucleotide polymorphisms by MALDI mass spectrometry in clinical applications. *Clin Biochem*, 2005; 38: 335–50
- Zoulim F: Hepatitis B virus resistance to antiviral drugs: where are we going? *Liver Int*, 2011; 31(Suppl.1): 111–16
- Wang S, Huang D, Sun S et al: Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis B to interferon alfa. *Virology*, 2011; 20(8): 28
- Gao QJ, Liu DW, Zhang SY et al: Polymorphisms of some cytokines and chronic hepatitis B and C virus infection. *World J Gastroenterol*, 2009; 28(15): 5610–19
- Yan Z, Tan W, Zhao W et al: Regulatory polymorphisms in the IL-10 gene promoter and HBV-related acute liver failure in the Chinese population. *J Viral Hepat*, 2009; 16: 775–83
- Thursz M, Yee L, Khakoo S: Understanding the host genetics of chronic hepatitis B and C. *Semin Liver Dis*, 2011; 31: 115–27
- Luan J, Yuan J, Li X et al: Multiplex detection of 60 hepatitis B virus variants by maldi-tof mass spectrometry. *Clin Chem*, 2009; 55: 1503–9
- Rybicka M, Stalke P, Dręczewski M et al: High-throughput MALDI-TOF Mass Spectrometry as an Alternative Approach to HBV Drug Resistance Monitoring. *J Clin Microbiol*, 2013 [Epub ahead of print]
- Warner N, Locarnini S, Kuiper M et al: The L80I substitution in the reverse transcriptase domain of the hepatitis B virus polymerase is associated with lamivudine resistance and enhanced viral replication *in vitro*. *Antimicrob Agents Chemother*, 2007; 51: 2285–92
- Tenney DJ, Levine SM, Rose RE et al: Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to Lamivudine. *Antimicrob Agents Chemother*, 2004; 48: 3498–507
- Delaney WE, Yang H, Westland CE et al: The hepatitis B virus polymerase mutation rtV173L is selected during lamivudine therapy and enhances viral replication *in vitro*. *J Virol*, 2003; 77: 11833–41
- Ono SK, Kato N, Shiratori Y et al: The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. *J Clin Invest*, 2001; 107: 449–55
- Sheldon J, Camino N, Rodes B et al: Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. *Antivir Ther*, 2005; 10: 727–34
- Allen MI, Deslauriers M, Andrews CW et al: Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. *Hepatology*, 1998; 27: 1670–77
- Bozdayi AM, Uzunalimoglu O, Turkyilmaz AR et al: Y5DD: a novel mutation in HBV DNA polymerase confers clinical resistance to lamivudine. *J Viral Hepat*, 2003; 10: 256–65
- Schildgen O, Sirma H, Funk A et al: Variant of hepatitis B virus with primary resistance to adefovir. *N Engl J Med*, 2006; 354: 1807–12
- Angus P, Vaughan R, Xiong S et al: Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology*, 2003; 125: 292–97
- Singla B, Chakraborti A, Sharma BK et al: Hepatitis B virus reverse transcriptase mutations in treatment Naïve chronic hepatitis B patients. *J Med Virol*, 2013; 85: 1155–62
- Du QW, Ding JG, Sun QF et al: Combination lamivudine and adefovir versus entecavir for the treatment of naïve chronic hepatitis B patients: a pilot study. *Med Sci Monit*, 2014; 20: 751–56

Conflict of interest

None declared.