*Piotr Ząbek[1], Jolanta Opoka-Kegler[2], Magdalena Baka[2], Tomasz Dyda[1], Grzegorz P. Stańczak[1], Janusz J. Stańczak[1]*

# PREVALENCE OF HEPATITIS C VIRUS MUTANTS RESISTANT TO PROTEASE INHIBITORS AMONG POLISH HCV GENOTYPE 1-INFECTED PATIENTS*

[1]Molecular Diagnostics Laboratory, Hospital for Infectious Diseases
[2]Outpatient Clinic of Infectious Diseases, Warsaw, Poland

## ABSTRACT

**AIM.** The aim of this study was to evaluate prevalence of hepatitis C virus (HCV) harbouring mutations associated with decreased susceptibility to protease inhibitors (Boceprevir/Telaprevir) among Polish untreated patients infected with HCV genotype 1.

**MATERIAL AND METHOD.** Population sequencing was used, sequencing data were interpreted by web based geno2pheno algorithm. A total of 91 serum samples were obtained from patients infected with HCV genotype 1, admitting Outpatient Clinics of Hospital of Infectious Diseases, Warsaw.

**RESULTS.** Sequencing analysis of the NS3 protease catalytic domain was successful in 85 out of 91 subjects. In seventy three (85.9%) out of 85 samples wild-type HCV was detected; in 12 (14.1%) samples mutations associated with clinically observed Boceprevir/Telaprevir-decreased susceptibility were detected.

**SUMMARY AND CONCLUSIONS.** Obtained results document the presence of HCV strains harbouring protease inhibitors (PIs) resistance-associated mutations among Polish therapy-naïve patients. The determined prevalence of drug resistant HCV variants is 14.1%. Further and continuous surveillance is necessary to estimate how pre-existing and emerging drug resistance mutations influence clinical outcome in triple-therapy experienced patients.

**Key words***: hepatitis C Virus, Boceprevir, Telaprevir, drug resistant mutants*

## INTRODUCTION

Chronic hepatitis C (CHC) is one of the major problems of public health services worldwide. More than 170 million people are infected with hepatitis C virus (HCV) according to WHO estimations; each year 3 million persons are newly infected (1). Spontaneous recovery from acute HCV infection is rare, and depends on host and viral factors, around 70% of HCV infected individuals will develop CHC (2). Untreated HCV infection may lead to chronic liver disease, cirrhosis or hepatocellular carcinoma, and is a leading cause of liver transplantation (3). Until the year 2011 standard therapy scheme was based on combination of pegylated-IFN-α (Peg-IFN- α) and ribavirin (RBV); and the length of treatment between 16-72 weeks, depending on virus genotype, baseline viraemia, rapid and early viral response to therapy (4). However, only about 50% of patients infected with HCV genotype 1 or 4 achieve sustained viral response (SVR), or 85% in the case of genotype 2 and 3. Additionally, treatment with Peg-IFN-α and RBV causes numerous adverse side effects, like fever, anemia and depression (5). Clinical observations combined with experience obtained during HIV infection treatment allowed to design new classes of anti-HCV drugs.

In 2011, new drugs of NS3/4A protease inhibitors class (PIs), boceprevir (BOC) and telaprevir (TPV), for therapy of HCV genotype 1-infected (G1) patients, were approved by Food and Drug Administration, and soon after that by European Medicines Agency. Clinical trials documented that addition of BOC or TPV to standard therapy scheme increased SVR ratio to about 70% in previously untreated G1-infected patients (6, 7). Combination of different antiviral drugs is necessary because of rapid selection of drug resistance variants

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during PIs monotherapy (8). Genomic analysis of RNA HCV revealed that strains containing mutations related to reduced susceptibility to PIs are pre-existing minority variants, present before initiation of therapy, rather than as a result of spontaneous *de novo* replication during treatment (9, 10). High genetic diversity of HCV may lead towards appearance of primary or emerging drug resistance mutations. Additionally intense replication rate of HCV and lack of proof-reading ability of viral RNA-dependent RNA polymerase cause an accumulation of different subpopulations in infected patient - quasispecies (11).

Several mutations in NS3 and NS4 coding regions, affecting HCV susceptibility to TPV and/or to BOC, have been identified until now. The most clinically important are V36A, T54A, R155K and A156V/S which result in high resistance of HCV to approved and tested protease inhibitors (12). The presence of drug resistance-associated substitutions can limit usage of first approved HCV protease inhibitors.

The aim of this study was to evaluate prevalence of primary drug resistance to protease inhibitors among naïve hepatitis C G1-infected patients in Poland.

## MATERIAL AND METHOD

A total of 91 serum samples obtained from chronic hepatitis C patients admitting Outpatient Clinics of Hospital of Infectious Diseases, were analyzed; the clinics treats the patients from different regions of Poland. Mean age of patients was 48 (ranging from 14 to 72) years; 51 (56%) out of them were women. Patients included in the study were G1- infected; 100% of them with subtype 1b. Mean viral load was 5,62 log IU/ml (ranging from 2,62 to 6,99 log IU/ml). None of the patients had ever been treated with standard Peg-IFN+RBV therapy and/or with protease inhibitors. HCV viral load testing was performed using m2000sp/m2000rt system (Abbott Molecular, IL, USA) with RealTime HCV test - the limit of detection (LOD) was 1,08 log IU/ml, upper limit of quantitation was 8,00 log IU/ml (Abbott Molecular, IL, USA). HCV genotyping was done using Versant HCV Genotype LiPa Assay (Siemens, Germany). HCV RNA was isolated from serum by spin column method (High Pure Viral Nucleic Acid Kit, Roche, Switzerland). RT-PCR was performed in 2720 Thermal Cycler (Life Technologies, NY, USA) according to following thermal profile: reverse transcription at 52°C for 30 min; denaturation step at 94°C for 2 min; then 40 cycles at 94°C for 15 sec, 55°C for 30 sec and 68°C for 90 sec; final elongation step at 68°C for 5 min. Amplification and sequencing of HCV NS3 region were carried out with oligonucleotide primers synthesized according to Bartels DJ, et al. (9). Used primers enabled amplification 890 base pairs fragment of NS3A region; quantity of PCR product was analyzed in agarose gel electrophoresis. Sequencing reaction was carried out in following conditions: 25 cycles at 96°C for 10 sec, 50°C for 5 sec and 60°C for 55 sec. The sequencing was performed using 3100-Avant Genetic Analyzer (Life Technologies, NY, USA); the obtained sequences were aligned by SeqScape ver. 2,7 software (Life Technologies, NY, USA). Finally, geno2pheno program (latest version available at the time of analysis) was used for the interpretation of the results, fold change cutoff value was set to 2,0.

## RESULTS

Population-based sequencing of the NS3 protease catalytic domain was successful in 85 of 91 subjects. In case of 6 samples there was no amplification or obtained sequences were of poor quality. Seventy three (85,9%) out of 85 samples were wild-type HCV: no drug resistance mutations were detected according to geno2pheno algorithm. In 12 (14,1%) samples mutations associated with clinically observed PIs-decreased susceptibility were detected. Five (5,88%) out of these mutants harboured the D168E substitution, 2 strains (2,35%) had A87T mutation, both of them responsible for resistance to BOC; 2 samples (2,35%) contained the T54S mutation resulting in possible resistance to TPV. In the case of remaining 3 strains: 2 (2,35%) carried R117H and 1 (1,17%) V55A substitutions, effecting in reduced susceptibility to both PIs. None of these strains carried more than one drug resistance-associated substitution.

## DISCUSSION

Development of new antiviral drugs directly affecting viral pathogens' life cycles was a great success of pharmacological industry. Introduction of new agents allowed efficient replication control of clinically important blood-born viruses - HIV, HBV and, nowadays, HCV. Soon after the introduction of these drugs, similarly to bacterial infections, a new problem was recognised – generation, selection and transmission of viral genetic variants with lowered susceptibility or resistance to used drugs. Implementation of protease inhibitors to chronic hepatitis C standard treatment scheme improves SVR ratio. However, there is a risk of therapy failure caused by transmitted or selected genetic variants of HCV with drug resistance. The aim of this study was to evaluate prevalence of primary drug resistance to protease inhibitors among Hepatitis C genotype 1 infected therapy naïve patients.

Presented results document naturally occurring HCV strains harbouring drug resistance-associated mutations among Polish therapy-naïve patients. The determined prevalence of drug resistant HCV variants is 14,1% in tested samples. The number of tested samples and treatment of the patients from the different regions of Poland ensure fair representativeness of the presented data.

Determined prevalence of genetic variants with lowered susceptibility or resistance to the PIs is similar to those presented in *Vicenti et al.* study (13). However, there are studies documenting lower percentage of drug resistance HCV strains (10). Observed discrepancies could be a result of differences in the groups of patients tested - their number, duration of infection, transmission of drug resistance strains, geographic location.

Existence of fairly high prevalence of HCV strains naturally resistant to protease inhibitors is a risk factor of viral failure during future treatment. Recommended antiviral schemes of chronic hepatitis C therapy with BOC and TPV suggest usage of PIs for short time - such therapy regime prevents mutants' accumulation.

The necessity of routine HCV drug resistance testing is still discussed. The main reasons for this are conflicting data about clinical significance of pre-existing drug resistance mutations and the fact that HCV genetic material is not archived in infected cells, like in the case of HBV or HIV. This situation enables the re-treatment of patients with viral failure with the same therapy regimen when drug resistance strains will be replaced by wild type population. However, such testing could be helpful in some groups of patients i.e. non-responders, relapsers or patients with unfavourable IL-28b polymorphism which was taken into account by HCV DRAG experts (HCV Drug Development Advisory Group) in guidelines specifying different issues of HCV drug resistance monitoring (14).

## SUMMARY AND CONCLUSION

1. The results of presented studies document the existence of HCV strains harbouring drug resistant associated mutations among Polish therapy-naïve patients. BOC and TPV resistant HCV mutants were detected in 14% of tested samples.
2. Currently, because of the lack of sufficient data, further and continuous surveillance is necessary to estimate how pre-existing and emerging drug resistance mutations influence clinical outcome in triple-therapy experienced patients.

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**Address for correspondence**:
Piotr Ząbek
Molecular Diagnostics Laboratory
Hospital for Infectious Diseases
Wolska 37 str
01-201 Warsaw, Poland
tel/fax +48223355278
e-mail - pzabek@zakazny.pl