

## SIGNIFICANCE OF *UGT1A1\*28* GENOTYPE IN PATIENTS WITH ADVANCED LIVER INJURY CAUSED BY CHRONIC HEPATITIS C

### ZNAČAJ *UGT1A1\*28* GENOTIPA KOD PACIJENATA SA TEŠKOM FIBROZOM I CIROZOM UZROKOVANOM HRONIČNIM HEPATITISOM C

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#### Summary

**Background:** Chronic hepatitis C (CHC) is a significant cause of liver related morbidity and mortality worldwide. The role of genetics in the host response to hepatitis C virus is not elucidated. Genetic variations in *UGT1A1* gene are the most common cause of hereditary unconjugated hyperbilirubinemia-Gilbert syndrome. This is the first study investigating the association of *UGT1A1* TA repeats promoter genotypes with the degree of liver injury, viremia and biochemical markers in CHC patients with advanced liver injury and late virological relapse.

**Methods:** Genetic testing of *UGT1A1* TA repeats promoter genotypes was performed in 42 CHC patients with advanced fibrosis and cirrhosis who achieved sustained virological response and 42 healthy blood donors. CHC patients were evaluated for clinical findings, laboratory tests and imaging.

**Results:** *UGT1A1\*28* genotype (7/7 TA repeats) was observed in 23.8% CHC patients and 16.7% healthy controls with no significant difference in genotype frequencies ( $p=0.49$ ). Pretreatment levels of ferritin and

#### Kratak sadržaj

**Uvod:** Hronični hepatitis C (HHC) je značajan uzročnik morbiditeta i mortaliteta u svetu. Značaj genetskih faktora u patogenezi HHC još uvek nije u potpunosti razjašnjen. Varijacije *UGT1A1* gena su najčešći uzrok nasledne nekonjugovane hiperbilirubinemije – Žilberovog sindroma. Ovo je prva studija koja se bavi ispitivanjem učestalosti TA ponavljanja u promotorskoj regiji *UGT1A1* gena i analizom povezanosti *UGT1A1\*28* genotipa sa stepenom fibroze, viremijom i biohemijskim markerima kod pacijenata sa teškim oštećenjem jetre izazvanim HHC i virusološkim relapsom.

**Metode:** Analizirana su TA ponavljanja u promotorskoj regiji *UGT1A1* gena, kod 42 pacijenta sa teškom fibrozom i cirozom izazvanom HHC, koji postigli stabilan virusološki odgovor i 42 ispitanika u kontrolnoj grupi zdravih dobrovoljnih davalaca krvi. Pacijenti sa HHC su dodatno analizirani kliničkim pregledima, laboratorijskim analizama (hematološki, biohemijski i virusološki) i fibroskenom jetre.

**Rezultati:** *UGT1A1\*28* genotip (7/7 TA ponavljanja) je bio prisutan kod 23.8% pacijenata sa HHC i 16,7% zdravih

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*List of abbreviations:* *UGT1A1*, uridine diphosphate-glucuronosyl transferase 1A1; GS, Gilbert syndrome; TA repeats, thymine-adenine repeats; CHC, chronic hepatitis C; SVR, sustained virologic response; HIV, human immunodeficiency virus; PCR HCV RNA, polymerase chain reaction hepatitis C virus ribonucleic acid; DNA, deoxyribonucleic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; AFP, alpha-fetoprotein; EOT, end of treatment; HCC, hepatocellular carcinoma.

bilirubin were associated with the presence of *UGT1A1\*28* genotype, indicating its potential as a predictive marker. However, in our study, there was no correlation of *UGT1A1\*28* genotype with the degree of fibrosis or viremia. During antiviral treatment, dose reductions and treatment interruptions, as well as treatment success and occurrence of late virological relapse were not related to the presence of *UGT1A1\*28* genotype in CHC patients with severe liver injury.

**Conclusions:** Frequencies of *UGT1A1\*28* genotype are high in both Serbian CHC patients and healthy subjects. The presence of *UGT1A1\*28* genotype was not associated with ribavirin-related adverse effects and had no effect on long term outcome in CHC patients.

**Keywords:** Gilbert syndrome, hepatitis C, hyperbilirubinemia, late relapse, UGT1A1

## Introduction

Enzyme uridine diphosphate-glucuronosyl transferase 1A1 (*UGT1A1*) is responsible for bilirubin conjugation with glucuronic acid, inducing water solubility of bilirubin and allowing urine excretion of the bilirubin-glucuronide conjugates (1). Numerous enzymes from *UGT* family catalyze glucuronidation of xenobiotics, mutagens, and reactive metabolites, acting as indirect antioxidants. Published data have also demonstrated anti-inflammatory and antioxidant functions of its substrate bilirubin (2, 3). The presence of genetic variations in *UGT1A1* gene encoding *UGT1A1* enzyme cause alteration of the enzyme leading to reduced activity. These genetic variations in *UGT1A1* gene are the most common cause of mild hereditary intermittent unconjugated hyperbilirubinemia-Gilbert syndrome (GS) (4). The insertion of thymine-adenine (TA) repeats in the promoter region (wild type consists of 6 TA repeats), reduces gene transcription by 67–82%, with subsequent reduction of enzyme activity by 20–30% (5). *UGT1A1\*28* variant allele contains 7 TA repeats, and individuals with GS possess 7/7 homozygous genotype with two *UGT1A1\*28* alleles. Although GS is considered a benign condition, new pharmacogenetic discoveries have shown that certain *UGT1A1* alleles are responsible for adverse outcomes and higher risks for breast and colorectal cancer due to decreased detoxification of carcinogens. Higher risks for severe drug reactions (irinotecan, nilotinib, pazopanib, belinostat, atazanavir) have been documented in patients with certain homozygous *UGT1A1* alleles and reduced enzyme activity (5–10). Pretreatment pharmacogenomic testing can greatly assist in individualizing therapy and might prompt special caution/different treatment options, due to a higher risk of side effects such as jaundice, diarrhea and neutropenia (11). Other *UGT1A1* genetic variants were also studied as a possible prognostic markers in chronic hepatitis B infection and predictors of onset of hepatocellular carcinoma in chronic hepatitis C (CHC) (12).

ispitanika, ali bez statistički značajne razlike ( $p=0,49$ ). Nivoi feritina i ukupnog bilirubina su bili u korelaciji sa prisustvom *UGT1A1\*28* pre primene antivirusne terapije, što sugeriše prediktivnu ulogu ovog genotipa. U ovoj studiji nije bilo korelacije *UGT1A1\*28* genotipa sa stepenom fibroze i viremijom. Prisustvo *UGT1A1\*28* genotipa nije uticalo na terapijske prekide i redukcije doze antivirusnih lekova, ishod lečenja niti pojavu kasnog virusološkog relapsa kod pacijenata sa HHC i teškim oštećenjem jetre.

**Zaključak:** Učestalost *UGT1A1\*28* genotipa je visoka među srpskim zdravim ispitanicima i pacijentima sa HHC infekcijom. Ispitivani genotip se ne dovodi u vezu sa neželjenim efektima ribavirina niti ima uticaj na ishod lečenja i dugoročnu prognozu pacijenata sa HHC.

**Ključne reči:** Žilberov sindrom, hepatitis C, hiperbilirubinemija, kasni relaps, UGT1A1

Prevalence of GS varies worldwide, estimated in 5–16% European blood donors, but as many cases are unsymptomatic and undiagnosed, true prevalence is probably much higher (13). Although considered a mild condition, there is very little data about GS in chronic liver disease, especially in CHC, including interpretation of bilirubin levels in conjunction with other liver-injury markers and clinical features.

Published data concerning the epidemiology of CHC in Serbian population show that estimated seroprevalence is higher than in most of the developed European countries, approximately 0.19% and that genotypes 1b and 3a are predominate (14, 15). In our country, pegylated interferon and ribavirin have been available since 2003. with high treatment success rates. However we have observed in our clinical practice, supported by published data from foreign authors, that liver-related morbidity and mortality is still high in patients with severe baseline liver damage in spite of achieving sustained virologic response (SVR) (16–18). This particular group of patients has not been evaluated in our surroundings so far. Due to the fact that along with hepatitis C virus, both genetically determined factors and oxidative stress play an important role in liver fibrosis and CHC pathogenesis, there is a question of importance of *UGT1A1* genetic variants as a possible prognostic and therapeutic markers.

Aim of this study was to analyze *UGT1A1* TA repeats promoter genotypes frequency in healthy blood donors and study group of patients with severe liver injury caused by CHC. The second aim was to examine possible association of *UGT1A1\*28* genotype with the degree of liver fibrosis, viremia and biochemical markers in patients who successfully underwent combined antiviral treatment. In order to examine a possible predictive relevance of *UGT1A1\*28* genotype beyond treatment response and SVR, we particularly examined a group of patients with severe fibrosis and cirrhosis who had detectable viraemia in spite of successful treatment.

## Materials and Methods

The study was performed at the referral center – the Hepatology Department of the Clinic for Infectious and Tropical Diseases, Medical Faculty of Belgrade, Clinical Center of Serbia. It included 84 subjects- study group of 42 patients with CHC and 42 healthy blood donors as a control group. The study group was a small part of a long-term follow-up study of a cohort of 325 patients with severe baseline liver damage caused by CHC, who received antiviral treatment and/or retreatment with pegylated interferon and ribavirin between 2003. and November 2015. We contacted patients from this large cohort, who had successfully undergone antiviral treatment and achieved SVR, for a reevaluation during March-July 2017, including clinical examination, fibroscan, virology, liver and genomic biomarkers. Among patients who responded, 42 individuals fulfilled inclusion criteria and gave consent for participation. Two patients from this group had been retreated during mentioned time period and achieved SVR after retreatment, so they were also recruited. The baseline pretreatment data obtained from patient files, included demographic and epidemiological data, clinical findings, laboratory tests and liver histology, before commencement of antiviral treatment, and regular clinical examinations every 6 months including laboratory tests (hematological, biochemical and virology), imaging (ultrasonography yearly in all patients and fibroscan) and upper gastrointestinal endoscopy (in patients with cirrhosis). Among 42 patients who were recruited, 9 had previously been tested during follow-up and were already known to have virologically relapsed, in spite of achieving SVR.

The inclusion criteria for the study group of patients were: patients with a confirmed CHC infection by polymerase chain reaction (PCR) and advanced fibrosis or cirrhosis (METAVIR score >F2) who underwent a complete antiviral treatment and achieved SVR and gave consent for participation. The exclusion criteria for study group were: age (subjects younger than 16 or older than 65 years), mild liver damage on liver histology (METAVIR <2), coinfections (hepatitis B, HIV), other liver diseases (Wilson's disease, haemochromatosis, autoimmune hepatitis, primary biliary cirrhosis, baseline decompensated liver cirrhosis and hepatocellular carcinoma), severe comorbidities (decompensated cardiomyopathies, thyroid dysfunctions, autoimmune diseases, epilepsy, depression, severe neutropenia and thrombocytopenia), active usage of narcotics and alcohol, pregnancy. The study protocol was designed in conformance with the Declaration of Helsinki ethical guidelines and was approved by the Ethical Committee of Belgrade School of Medicine.

The degree of fibrosis was assessed at baseline, using both non-invasive (FibroScan, Echosens, Paris, France) and invasive diagnostic methods (aspiration

liver biopsy, Institute for pathology, Belgrade School of Medicine), and METAVIR score was used to mark necroinflammatory activity of the liver. Viral loads were measured with quantitative PCR HCV RNA (Cobas Amplicor HCV Test version 2.0, Roche Diagnostics, Mannheim, detection: 50 IU/mL) and hepatitis C virus genotype detected (Linear Array HCV genotyping test, Roche Diagnostics) in Virology laboratory, Microbiology Department, Clinical Center of Serbia. Biochemical analysis of blood samples from the study group was performed using Siemens Dimension Xpand® biochemistry analyzer in the Centre for Medical Biochemistry, Department of the Clinic for Infectious and Tropical Diseases, Clinical Centre of Serbia. However biochemical data was not available in healthy control group due to confidentiality.

Control group included healthy 42 blood donors. Informed written consent for the molecular analyses was obtained from each participant. Control group was matched for gender with a study group. A 5ml Na-citrate tube of whole blood was obtained for each subject. All analyzed subjects were of Serbian origin. Molecular genetic analysis was performed at the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade.

DNA was extracted from blood samples using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). The number of TA repeats in *UGT1A1* promoter was genotyped as previously described with slight modifications (19). The amplification reaction was performed in a total volume of 25  $\mu$ L, and the reaction mix contained 20 pmol of each primer, 50–100 ng of genomic DNA, 200  $\mu$ mol/L of each dNTP (Fermentas, ON, Canada), 1 $\times$  PCR reaction buffer (Qiagen), 1 $\times$  Q solution (Qiagen), 2.75 mmol/L MgCl<sub>2</sub>, 1 U HotStar DNA polymerase (Qiagen). The temperature profile of the PCR reactions was for the initial activation of DNA polymerase set at 95 °C for 15 min, followed by 35 cycles of 30 sec denaturation at 95 °C, 30 sec annealing at 63 °C, and 30 sec elongation at 72 °C, ended by a final extension period of 7 min at 72 °C. PCR fragments were visualized on 2% agarose gel and finally analyzed using 15% acrylamide electrophoresis (19:1 acrylamide/bisacrylamide in 1 $\times$ TBE buffer, run at 300 V and 60 mA for 5h) stained with Ag-nitrate (20).

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS®, version 11.5) and included both descriptive and analytical methods. Subjects were grouped and categorized according to the presence of three different genotypes (6/6 wild type, 6/7 heterozygote, 7/7 homozygote), depending on the number of TA repeats in the promoter region of *UGT1A1* gene. Both parametric (Student's test) and nonparametric tests ( $\chi^2$ , Kruskal Wallis, Fisher's exact test) were used, depending on the normality of variables. Linear regression and beta coefficient were also computed for the analysis of association. Values at the p 0.05 level were consid-

ered statistically significant, the confidence interval (CI) was 95% and all performed tests were 2-tailed.

## Results

### Pretreatment and healthy blood donors

A total of 84 subjects were categorized in two groups- study group of 42 patients with CHC and control group of 42 healthy blood donors. In both groups, all subjects were Caucasians of similar ancestry and ethnicity (Serbian), with no significant differences in sex distribution (Table I). In the study group there were 24 (57.1%) male and 18 (42.9%) female, similar to healthy control group- 25 male (59.5%) and 17 (40.5%) female ( $p=0.825$ ). However, there were significant differences regarding age, as the patients' group included older subjects (mean age  $46.2 \pm 9$  years) compared to the control group ( $33.9 \pm 14$  years) with younger healthy subjects ( $p < 0.001$ ).

Frequencies of UGT1A1 TA promoter genotypes are presented in Table I and 7/7 genotype was observed in 10 (23.8%) patients and 7 (16.7%) healthy controls, but there were no significant differences in genotype frequencies between the study group and healthy control group ( $p=0.49$ ).

Further analysis of (pretreatment) characteristics of patients with CHC, including hepatitis C genotypes, viremia and the degree of fibrosis (METAVIR score), did not reveal any statistically significant differences in two patients' groups depending on the finding of UGT1A1\*28 genotype (Table II).

Patients with UGT1A1\*28 genotype had higher average values of total bilirubin ( $p=0.001$ ) and lower values of ferritin ( $p=0.032$ ), but there were no other significant differences in average values of other liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl

**Table I** Demographic data and UGT1A1 genotype frequencies in patients with chronic hepatitis C ( $n=42$ ) and healthy control group ( $n=42$ ).

Variables	CHC <sup>1</sup> patients (n=42)	Healthy control (n=42)	p-value
Sex, n(%)			
Female	18 (42.9%)	17 (40.5%)	0.825
Male	24 (57.1%)	25 (59.5%)	
Age, years (mean $\pm$ SD)	$46.2 \pm 9$	$33.9 \pm 14$	<0.001
Genotype, n(%)			
wild-type 6/6	18 (42.9%)	15 (35.7%)	0.49
heterozygote 6/7	14 (33.3%)	20 (47.6%)	
homozygote 7/7 <sup>2</sup>	10(23.8%)	7 (16.7%)	

<sup>1</sup>CHC-chronic hepatitis C

<sup>2</sup>UGT1A1\*28-Gilbert Syndrome

**Table II** Baseline (pretreatment) characteristics of CHC patients ( $n=42$ ) grouped according to the presence of UGT1A1\*28 genotype and association with hepatitis C virus genotypes, viremia the degree of fibrosis.

Variables	homozygote 7/7 <sup>1</sup> (n=10)	wild-type and heterozygote <sup>2</sup> (n=32)	p-value
HCV <sup>3</sup> genotype, n (%)			
G1	6 (60%)	22 (68.8%)	0.856
G2	1 (10%)	2 (6.3%)	
G3	3 (30%)	8(25%)	
HCV viral load (IU/mL)			
<800.000	3 (30%)	11 (34.4%)	0.798
>800.000	7 (70%)	21(65.6%)	
fibrosis stage <sup>4</sup> , n (%)			
F2	5 (50%)	13(40.6%)	0.501
F3	1(10%)	9(28.1%)	
F4	4(40%)	10(31.3%)	

<sup>1</sup>UGT1A1\*28-Gilbert syndrome

<sup>2</sup>wild type 6/6, heterozygote 6/7

<sup>3</sup>HCV-hepatitis C virus

<sup>4</sup>METAVIR score: F2 – portal fibrosis with few septa; F3 – numerous septa without cirrhosis; F4 – cirrhosis

**Table III** Pretreatment mean values of relevant biochemical markers in patients with CHC grouped according to the presence of UGT1A1\*28 genotype.

Biochemical markers (mean $\pm$ SD)	homozygote 7/7 <sup>1</sup> (n=10)	heterozygote and wild-type <sup>2</sup> (n=32)	p-value
AST (U/L)	$71.5 \pm 57$	$65.4 \pm 38.4$	0.703
ALT (U/L)	$172.7 \pm 102$	$114.5 \pm 93$	0.174
GGT (U/L)	$58.7 \pm 69$	$67.3 \pm 70.9$	0.796
albumin (g/L)	$38 \pm 9.3$	$40 \pm 7.72$	0.339
total proteins (g/L)	$71.7 \pm 7.5$	$72.6 \pm 9.09$	0.598
ferritin ( $\mu$ g/mL)	$66.1 \pm 63.2$	$184.09 \pm 163.3$	0.032
total bilirubin ( $\mu$ mol/L)	$19.5 \pm 7$	$10.98 \pm 5.2$	0.001

<sup>1</sup>UGT1A1\*28-Gilbert syndrome

<sup>2</sup>wild type 6/6, heterozygote 6/7

transferase (GGT), total protein and albumin (Table III). In-depth analysis of total bilirubin levels revealed that 6 (60%) of patients with UGT1A1\*28 genotype had hyperbilirubinemia above  $17 \mu\text{mol/L}$  compared to only 2 (6.3%) of patients who did not have homozygous 7/7 genotype ( $p=0.001$ ). Regression analysis of biochemical markers revealed a statistically significant association of ferritin ( $\beta=0.462$ ,  $p=0.002$ ) and bilirubin levels ( $\beta=0.542$ ,  $p=0.001$ ) with the presence of UGT1A1\*28 genotype.



### Treatment and follow-up of patients

After initial phase and during the antiviral treatment, none of the patients had severe ribavirin-related hemolysis and/or severe indirect hyperbilirubinemia, independently of the presence of *UGT1A1\*28* genotype. However, 19 (45.2%) of patients had temporarily received reduced doses of interferon and 9 (21.4%) of ribavirin. Two patients (4.7%) had a temporary interruption of treatment, due to severe depression and endocrine abnormalities. These dose reductions and treatment interruptions were not related to the presence of *UGT1A1\*28* genotype ( $p > 0.05$ ) and did not affect treatment success, as all patients achieved SVR.

The follow-up period of patients, after achieving SVR ranged from 20 months to 11,3 years (mean was  $66.9 \pm 37$  months). Among the patients who responded to our invitation for reevaluation 14 (33.3%) had detectable viremia, and were considered late relapsers. There was no statistically significant correlation between the occurrence of relapse and presence of *UGT1A1\*28* genotype ( $p = 0.638$ ). Eleven patients (26.2%) had progression of fibrosis verified by fibroscan, and 3 patients in this group had also a virological relapse (27.2%). There was a statistically significant correlation between levels of AST, ALT, GGT and detectable viremia with fibrosis progression ( $p < 0.05$ ).

In patients with late virological relapse mean ALT values were higher (Table IV) compared to patients with stabile SVR ( $p = 0.004$ ) however, there was no statistically significant difference in platelet counts, or other biochemical markers such as AST, GGT, alpha-phetoprotein (AFP) or total bilirubin levels. During follow up period two patients developed decompensation of cirrhosis-with ascites, and one of them a concomitant surgically-resected hepatocellular carcinoma (HCC).

**Table IV** Relevant laboratory markers including biochemical, haematological and tumor markers in patients grouped according to the presence of detectable hepatitis C viremia in spite of achieving SVR.

Laboratory markers (mean $\pm$ SD)	late relapse (n=14)	stabile SVR <sup>1</sup> (n=28)	p-value
AST (U/L)	43.1 $\pm$ 32	27.7 $\pm$ 20.1	0.076
ALT (U/L)	50.3 $\pm$ 39.6	28.3 $\pm$ 21.1	0.004
GGT (U/L)	38.4 $\pm$ 47.7	42.5 $\pm$ 20.2	0.754
total bilirubin ( $\mu$ mol/L)	8.7 $\pm$ 5.4	7.6 $\pm$ 5.1	0.51
AFP <sup>2</sup> (ng/mL)	10.1 $\pm$ 7.1	5.1 $\pm$ 3.3	0.06
Platelets ( $\times 10^9$ /L)	196 $\pm$ 42.1	190 $\pm$ 80	0.796

<sup>1</sup>SVR-sustained virological response

<sup>2</sup>AFP-Alpha-fetoprotein

### Discussion

Gilbert syndrome is the most common genetic cause of hyperbilirubinemia, present worldwide in all ethnicities, with variable frequencies (21, 22). The cause of GS is *UGT1A1\*28* genetic variant, which is also associated with abnormalities of hepatobiliary transport and additional linked variants (in 94% of GS) *UGT1A6\*2* and *UGT1A7\*3*, resulting in reduction in enzyme UGT1A1 activity (23). So far in Serbia there has been no available data concerning the role of this genetic variant in any hepatic disease, especially in patients with severe liver damage. Our study group included patients with CHC with significant liver fibrosis (33% of patients had cirrhosis), as this particular group of patients is of the utmost interest in everyday clinical practice. They present a formidable therapeutic challenge with lower antiviral treatment success rates, higher risk for complications, HCC, loss of work productivity and reduction in health related quality of life (24). Our local success rates of antiviral treatment with pegylated interferon and ribavirin are among the higher- on average 79.7% of patients achieved end of treatment response (EOT) and 70.5% sustained virological response (SVR). This is probably due to younger age of patients, shorter duration of HCV infection, treatment naiveness, good compliance and antiviral drug dose adjustments (25, 26). Published data in Serbian patients show that older age-above 40 years, genotype 1b and presence of cirrhosis are negative predictors of SVR, similar to the results of other European investigators (15, 27, 28).

The frequency of *UGT1A1\*28* genotype in this study was high in both groups- patients with CHC 23.8% and healthy blood donors 16.7%. These frequencies are among the highest of the published data in blood donors ranging from 5% in Iceland, 10% in Portugal, 11.6% in Spain, 15.3% in Croatia and especially compared to Southeast Asia, Melanesia, and the Pacific Islands, ranging from 0 to 5% (5, 13, 29). The recognized prevalence is probably much lower than the real frequencies of the mutations, making GS underdiagnosed, due to incomplete penetrance and other acquired or inherited conditions affecting bilirubin metabolism, which can also alter clinical manifestation (30). One of the specificities of our study population is its genetic homogeneity and similar ethnic background, which might account for differences compared to published data.

So far there have been very few studies analyzing *UGT1A1* promoter genotypes frequencies in patients with CHC. Urbanek P et al. (31) reported frequency of this genetic variant in 15.7% of patients with CHC, Souza et al. (5) 10.4% in a larger-scale patient study. However both of these studies had multiethnic populations with very different genetic background.

Some studies have reported gender differences, especially in serum bilirubin levels, which are proba-

bly the result of hormonally induced discordance in bilirubin clearance (32). They do not appear to be caused by gender specific differences in the *UGT1A1\*28* genotype frequencies (33). There is some data pointing to an association with age, body mass, ethanol consumption, smoking or oral contraceptive use (34, 35). However in our study there were no statistically significant gender differences in *UGT1A1\*28* genotype frequencies in both study and control groups, but we observed that the healthy blood donors were younger than study group. This difference in age has no effect on results, as genetic variants are inherited and not related to age.

Our analysis did not reveal significant relationship between presence of *UGT1A1\*28* genotype and hepatitis C virus related markers- baseline viral load or genotype, as well as associations with baseline liver histology and the degree of fibrosis related, similar to published data (31). This is probably due to the fact that liver fibrosis in chronic hepatitis C is multicausal, including genetically determined factors as well as oxidative stress, immune cell injury and apoptosis. Numerous factors that may explain the lack of association between degree of liver injury and examined *UGT1A1* genetic variants, such as the age of onset of hepatitis C infection and the duration of infection as well as insulin resistance, body mass index or environmental factors (36).

After pretreatment analysis of liver function tests and biochemical markers commonly used for diagnostic orientation in patients with CHC, we found significant associations of the bilirubin and ferritin levels with *UGT1A1\*28* genotype, similar to results by Piekuse L. et al. (37) and de Souza et al. (5). Hyperbilirubinemia caused by reduced *UGT1A1* activity in patients with hepatitis C has been documented in reports of severe ribavirin-induced jaundice, after commencing pegylated interferon and ribavirin therapy, suggesting usefulness of genotyping for *UGT1A1\*28* variant for the decision to continue therapy with ribavirin (38). Elevated bilirubin due to *UGT1A1\*28* variant can be a cause for exclusion of patients from clinical trials, as well as overestimation of liver disease prognostic scores (Child-Pugh and Model for End Stage Liver Disease-MELD) (39, 40). Bilirubin levels have also been observed to correlate with the total number of TA repeats (2, 5). The observed association of *UGT1A1\*28* allele with serum ferritin levels has been previously documented in few studies which included patients with CHC (37). Serum iron markers (ferritin and transferrin) are suggested as surrogate markers for liver fibrosis and necroinflammatory activity. Higher levels of ferritin have been linked to more severe liver inflammation, fibrosis and therefore lower response antiviral treatment rates (41, 42). According to our findings *UGT1A1\*28* genotype was associated with lower ferritin levels, and although we found no association with degree of liver fibrosis, this may point to a group of patients with a better antiviral treatment

response. However no statistically significant differences in mean values of liver function tests- transaminase levels, GGT, total proteins and albumin, or their associations with the presence of *UGT1A1\*28* genotype were observed.

Multiple well studied pharmacogenetic risks associated with *UGT1A1\*28* presence may prompt for different treatment options (6–10). In our study group, during antiviral treatment with pegylated interferon and ribavirin, we observed no episodes of severe hemolysis or high indirect hyperbilirubinemia, independent of the presence of *UGT1A1\*28* genotype. However there have been published reports of individual cases of significant elevation of indirect bilirubin in patients with *UGT1A1\*28* genotype during the initial phase of combined antiviral therapy, prompting for temporary ribavirin dose reduction, but without deterioration in liver function or effect on treatment outcome (43).

Although survival and complications are much improved in patients with successful treatment, there has been overwhelming evidence that in spite of achieving SVR, liver injury can persist and progress. As our patients had a severe baseline liver damage and were involved in a long term follow-up, we were particularly interested in host genetics factors influencing the clinical course and durability of SVR in relation to presence of *UGT1A1\*28* genotype. Published data show that SVR appears durable in the majority of patients at 5 years post-treatment, driven mostly by an increased reinfection risk, with 5-year recurrence rates of 0.95%, 10.67%, and 15.02% in the low-risk, high-risk, and coinfection groups, respectively (44). There are no published data or involvement in any European monitoring systems of HCV infection in Serbia, as well as no long-term follow-up studies, except our clinical observations and patients feedback during a decade of regular check ups. Due to a selection bias and the fact that the patients who already knew of relapse were highly motivated to be reevaluated, the number of patients with late relapse in our study was exceptionally high – we found detectable viremia in 5 new patients and had reconfirmed in 9 that were already known. However we did not observe statistically significant association between the presence of *UGT1A1\*28* genotype and occurrence of relapse. As it is an inherited trait, the analysis would not be affected by selection bias, and although we found no significant correlation, these are the first results on this subject.

As this was a pilot study, main study limitations are the small sample size and the lack of data concerning biochemical tests in healthy control group. However the conclusions are similar to other authors with much larger samples, including the frequency of *UGT1A1\*28* genotype, which is more geographically and ethnically specific for this study population and our region.

In the group of patients with late relapse, we had a response-bias, as most of the patients from this group had previously been evaluated and aware of relapse, so they were especially motivated to respond to our invitation for reevaluation. However, we were not able to exclude reinfection by RNA sequencing, except by retesting virus genotypes. Nevertheless, in this group we had no previous or active intravenous drug users, and the dominant mode of hepatitis C virus infection was through blood transfusion. We used non-invasive methods such as fibroscan and ultrasonography to monitor patients for progress of fibrosis and liver-related complications, as repeated liver biopsies are not ethically justified.

Further investigation is needed on host-related genetic factors and on more patients with hepatitis C, especially in the light of new treatment options that might be available in our country for patients with severe liver damage and hepatitis C relapse.

*Acknowledgements.* This work was supported by Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. III41004).

### Conflict of interest statement

The author stated that she has no conflicts of interest regarding the publication of this article.

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Received: February 1, 2018

Accepted: April 12, 2018