

IL-28B genetic variation, gender, age, jaundice, hepatitis C virus genotype, and hepatitis B virus and HIV co-infection in spontaneous clearance of hepatitis C virus

Sergii V. Fedorchenko , Anna Klimenko , Tetyana Martynovich , Olga Liashok , Vitaliy Yanchenko 

Department of Viral Hepatitis and AIDS, The L.V. Gromashevskiy Institute of Epidemiology and Infectious Disease, Kiev, Ukraine

Cite this article as: Fedorchenko SV, Klimenko A, Martynovich T, Liashok O, Yanchenko V. IL-28B genetic variation, gender, age, jaundice, hepatitis C virus genotype, and hepatitis B virus and HIV co-infection in spontaneous clearance of hepatitis C virus. *Turk J Gastroenterol* 2019; DOI: 10.5152/tjg.2019.18328

ABSTRACT

Background/Aims: Spontaneous viral clearance observed in some patients is one of the variants of the hepatitis C virus (HCV) infection natural history. We aimed to look at the complexity of factors affecting the spontaneous clearance of HCV (SC HCV).

Materials and Methods: A total of 357 anti-HCV positive patients (309 with chronic hepatitis C and 48 patients with SC HCV) were included into the study. We studied the effects of the interleukin-28B (IL-28B) gene polymorphism, gender, age, the routes of virus transmission, past hepatitis C with jaundice, HCV genotype, and hepatitis B virus (HBV) and HIV co-infection on the outcome of HCV infection.

Results: Based on the study results, the SC HCV was found in 48 individuals (13.4%). The most significant positive factors affecting the SC HCV included IL-28B single nucleotide polymorphism (SNP) rs12979860 (CC) and SNP rs8099917 (TT) (OR 4.03, $p < 0.001$) and (OR 3.14, $p < 0.002$), female gender (OR 2.72, $p < 0.001$), young age (OR 2.30, $p < 0.008$), and past history of jaundice (OR 5.12, $p < 0.001$). The markers of a past HBV infection were found significantly more often in SC.

Conclusion: Positive predictors of the SC HCV include favorable IL-28B genotype, female gender, young age, a history of jaundice, markers of a past HBV infection, the absence of HIV infection, but not the viral genotype.

Keywords: Spontaneous clearance HCV, IL-28B, genotype

INTRODUCTION

Spontaneous viral clearance is one of the variants of the hepatitis C virus (HCV) infection natural history. Its highest rate is observed in acute HCV, ranging from 18% to 63% (1-3), which is significantly higher than in chronic disease, ranging from 3.7% to 17.5% (4). The likelihood of spontaneous clearance (SC) depends on a number of factors, which may be tentatively subdivided into the viral and the host ones. The former may include the virus genotype, the level of viremia, genetic heterogeneity (quasi-species), and the size of the infective dose, and the latter may include gender, age, race, genetic polymorphism (interleukin-28 [IL28B], HLA-A, HLA-C, killer immunoglobulin-like receptors), a history of icteric acute viral hepatitis C, intravenous drug use, HIV and hepatitis B virus (HBC) co-infection, and characteristics of specific cell-mediated and humoral immune response induction. The role of the above-mentioned factors in the development of SC HCV is well known and confirmed by numerous studies conducted in various regions of the world. Positive predictors of SC HCV include female gender, single nucle-

otide polymorphism (SNP) rs 12979860 CC polymorphism of the IL-28B gene, history of jaundice, low-level viremia, and lower viremia and ALT activity by Week 4 from the disease onset (2,5,6). At the same time, a strong specific T-cell-mediated immune response with the production of cytokines and virus neutralizing specific antibodies are observed (7).

So far, there is no single point of view regarding the effect of HCV genotype on the likelihood of spontaneous clearance of acute viral hepatitis C. Thus, several researchers have observed a high likelihood of spontaneous clearance of genotype 1 HCV infection (8,9), the other-with genotype other than 1 (10). The fact that only a small number of those infected develop significant clinical symptoms of the disease makes the study of factors influencing SC of acute hepatitis C even more complicated. In the vast majority of cases, the time of infection remains unknown, with the patients learning about their diagnosis during incidental laboratory evaluation or serological testing. As for the spontaneous

Corresponding Author: **Sergii V. Fedorchenko**; svfedorchenko@rambler.ru

Received: **May 22, 2018** Accepted: **September 8, 2018** Available online date: **February 21, 2019**

© Copyright 2019 by The Turkish Society of Gastroenterology · Available online at www.turkjgastroenterol.org

DOI: 10.5152/tjg.2019.18328

clearance of HCV, we do not know whether it has occurred during acute or chronic disease. Besides the above-mentioned factors, co-infection with other hepatotropic viruses (hepatitis A virus, HBV, and hepatitis D virus), HIV co-infection, and intravenous drug use play important role in patients with chronic disease. The HBV superinfection in patients with chronic viral hepatitis C leads to the manifestation of clinical and laboratory symptoms of liver damage, but it often end with SC HCV (11). HIV co-infection in the setting of significant deficiency of cell-mediated immunity in patients not receiving highly active antiretroviral therapy (HAART) is a negative factor of virus clearance (4). Currently, there is no agreement regarding the impact of intravenous drug use on SC. Several researchers observed high rates of the HCV clearance in active intravenous drug users (IDUs) (12,13), whereas others indicated poor likelihood of viral elimination (14).

The ultimate goal of the current study was to identify the set of factors influencing the SC of HCV: the IL-28B gene polymorphism, gender and age of HCV patients, the route of virus transmission, and HBV and HIV markers.

MATERIALS AND METHODS

The study was conducted between 2008 and 2014, and it included 367 anti-HCV positive patients (309 with chronic hepatitis C [CHC]; 48 with SC HCV; 10 with false positive anti-HCV result). Anti-HCV in serum samples was studied with a third-generation microparticle enzyme immunoassay (HCV version 3.0 Ax sym. Abbott Laboratories, Chicago, IL, USA; MonoLisa HCV Ag-Ab ULTRA, Bio-Rad, ETI-Ab-HCV K-3 ELISA, Sorin Bio Medica, Italy).

Diagnosis of chronic hepatitis C was based on positive results for the presence of anti-HCV and HCV RNA (qualitative test) in serum. The SC HCV was diagnosed when the serum contained anti-HCV and no HCV RNA. The patients were not receiving antiviral therapy. Antibody specificity was confirmed by a verification test (CHIRON RIBA HCV 3.0 SIA, Orto Diagnostic Systems Inc. Raritan NG, USA). To exclude patients with chronic hepatitis C having a low-level viremia from this group (15), all the patients were tested two times within a 5-6 months interval for the presence of the HCV RNA in the serum (qualitative test) with a highly sensitive method of indication, Real-Time HCV TM Abbott (Abbott Laboratories, Abbott Park, Illinois, USA), having the lower threshold of sensitivity 12 IU/mL. A false positive reaction to anti-HCV was determined based on positive test results (ELISA) and

negative verification tests (RIBA). In case of indeterminate RIBA results, the test was repeated in 4-6 months; repeated indeterminate or negative test result was interpreted as false positive. This group included 10 individuals: 4 pregnant women, 2 patients with lymphocytic leukemia, 1 patient with myeloma, and 1 with the history of multiple hemotransfusions.

Specimen collection

Obtained serum samples were stored at -80°C until analysis. For genetic testing, patients were recalled and, if they agreed, the ethylenediaminetetraacetic acid (EDTA) blood was drawn. The EDTA blood was centrifuged at 1500 g for 20 minutes at room temperature within 4 hours of venipuncture, and aliquots were frozen immediately after centrifugation at -80°C until testing was performed.

Virologic testing

The HCV RNA was tested with the qualitative COBAS AmpliCor HCV Test (Roche Molecular Systems, Branchburg, NG; lower limit of detection, 50 IU/mL). The HCV genotype was determined using the VERSANT HCV Genotype 2.0 Assay (LiPA; Siemens Medical Solutions Diagnostics, Tarrytown, NY). All assays were performed according to the manufacturer's instructions.

HCV serotypes and serologic testing for HBV and HIV

Sera from patients with undetectable HCV RNA were tested for type-specific antibodies to HCV genotypes 1, 2, 3, 4, 5, and 6 using the MUREX HCV Serotyping 1-6 Assay (Abbott, Wiesbaden, Germany).

Antibodies directed against the HBV and HIV infection status were carried out the Aksum system (Abbot Laboratories, Chicago, IL, USA) by determining anti-HBs (AUSAB), anti-HBc (Core), and HBsAg (HBsAgV2). Anti-HIV were tested by Abbott PRISM (Abbott Laboratories, Chicago, IL, USA).

Testing for interleukin-28B (IL-28B)

Genomic DNA was isolated from peripheral blood according to the QIAamp DNA Blood Mini Kit from Qiagen (Hilden, Germany). SNPs rs12979860 and rs8099917 in the region of the IL28B gene were analyzed by the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA) with the help of a custom TaqMan SNP Genotyping Assay developed together with Applied Biosystems. Amplicon sequencing was used to validate the genotyping techniques.

Statistical analysis

Database management and statistical analysis were performed using commercially available software systems (Microsoft Office Excel 2010, Microsoft Corp, Redmond, WA; SPSS 2006 for Windows version 16, SPSS Inc, Chicago, IL; and MedCalc 11.4.2.0, Software bvba, Mariakerke, Belgium). Data were analyzed using the Mann-Whitney U test, as well as the χ^2 test, and the degree of association between independent variables of the SC HCV group and CHC was determined calculating the corresponding odds ratio (OR) and its 95% confidence interval (95% CI) by means of simple logistic regression.

To determine whether variables were normally distributed or not, the Kolmogorov-Smirnov test was applied. All P values were two tailed, and those less than 0.05 were considered statistically significant.

Informed consent

Informed consent was obtained from each patient.

RESULTS

Effect of gender, age, history of jaundice, and routes of HCV transmission on spontaneous virus clearance

The SC was diagnosed in 48 individuals (13.4%) evaluating the results of 357 anti-HCV positive patients (13.4%).

The effect of gender, age, and routes of viral transmission was studied in two groups of patients: 309 patients in the CHC and 48 patients in the SC HCV group. Considering the route of transmission, the patients were

subdivided into four main groups: IDUs, recipients of donated blood and its derivatives, patients who underwent surgical and medical procedures, and patients whose route of transmission remained unknown. The IDU group comprised both active users at the time of the study and former users. Hemotransfusions and transfusions of blood components were considered the cause of infection if jaundice or laboratory-confirmed HCV infection (positive result for anti-HCV or anti-HCV seroconversion) had developed within 1 year after the transfusion. Surgical interventions and invasive medical procedures were considered the main route of the HCV transmission in the group of patients with severe comorbidity requiring lengthy stay at the hospital, with other routes of HCV transmission ruled out as much as possible. As follows from the data presented in Table 1, the patient's gender had a significant effect on the outcomes of HCV infection.

In the group of patients with CHC, males prevailed: 198 (64.1 %) males vs. 111 (35.9 %) females. The opposite pattern was observed in the SC group of patients, with females being prevalent: 29 (60.4 %) females vs. 19 (39.6 %) males. Female gender was strongly associated with spontaneous viral eradication (OR, 2.72 [95% CI, 1.46-5.08]; $p < 0.001$).

The analysis of correlation between the SC HCV rate and patient age allowed to state that recovery was more often observed in the group of patients aged 18-35 years: 22 individuals (45.8 %), as compared to patients with CHC (83 [26.9 %]; OR, 2.3 [95% CI, 1.24-4.29]; $p < 0.008$).

Table 1. Effect of the HCV routes of transmission, gender, age, and past history of jaundice on spontaneous HCV clearance

Characteristics	SC HCV n=48	CHC n=309	p	OR	95% CI
Males	19 (39.6 %)	198 (64.1%)	0.0014	0.367	(0.197-0.685)
Females	29 (60.4 %)	111 (35.9%)	0.0013	2.723	(1.460-5.079)
Age					
18-35 years	22 (45.8%)	83 (26.9%)	0.0079	2.304	(1.238-4.287)
36-45 years	9 (18.8%)	108 (34.9%)	0.0276	0.429	(0.201-0.920)
>46 years	17 (35.4%)	118 (38.2%)	0.71	0.888	(0.471-1.674)
Route of transmission					
IDU	16 (33.4%)	129 (41.7%)	0.276	0.698	(0.367-1.325)
Hemotransfusion	2 (4.2%)	41 (13.3%)	0.0727	0.284	(0.066-1.216)
Medical procedures	13 (27%)	78 (25.2%)	0.79	1.1	(0.554-2.185)
Unknown	17 (35.4%)	61 (19.8%)	0.0155	2.23	(1.159-4.290)
History of jaundice	14 (29.2%)	23 (7.5%)	0.001	5.12	(2.410-10.878)

CHC: chronic hepatitis C; IDU: intravenous drug users; OR: odds ratio; CI: confidential interval

We were not able to confirm the impact of IDU on the HCV infection outcomes. At the same time, hemotransfusion-transmitted HCV infection is more often associated with the development of CHC. Thus, hemotransfusion or transfusion of blood products caused infection in 41 (13.3%) patients with CHC, 2 (4.2 %) with spontaneous recovery (OR, 0.28 [95 % CI, 0.06-1.21]; $p < 0.07$). Past acute viral hepatitis C (AHC) with jaundice was diagnosed over 3.5 times more often in the SC HCV (14 [29.2%] patients) than in CHC (23 [7.5 %] patients) (OR, 5.12 [95 % CI, 2.41-10.87]; $p < 0.001$).

IL-28B gene polymorphism in the group of patients with chronic CHC

Two main SNP of IL-28B gene, rs12979860 (CC, CT, TT) and rs8099917 (TT, TG, GG), were studied as genetic predictors of the natural course of the HCV infection.

In evaluating 309 patients (198 men [64.19%] and 11 women [35.9 %]) with chronic hepatitis C for rs12979860 variants of the IL-28B gene polymorphism, we found that the CC genotype was identified in 109 (35.3%), CT genotype in 177 (57.2%), and TT genotype in 23 (7.5%) of patients.

The evaluation of this group for the SNP rs8099917 variant of the IL-28B gene yielded the following results: 179 (57.9%) of patients had the TT genotype, which prevailed, 120 (38.9%) TG genotype, and 10 (3.2 %) GG genotype.

The next stage of our research was to look at the IL-28B gene SNP rs12979860, rs8099917 distribution in the group of patients with CHC in relation to age. By the IL-28B gene rs12979860 polymorphism, we have divided all the male and female patients into groups with the CC, CT, and TT genotype, and by the IL-28B gene rs8099917 into the TT, TG, and GG groups. Based on the data obtained, the group with the genotype CC rs12979860 of the IL-28B gene comprised 71 men (35.8%) and 38 women (34.2%), with the CT genotype 112 (56.6%), and 59 (53.2%), and TT genotype-15 (7.6%) men and 14 (12.6%) women. By the IL-28B gene rs8099917, grouping by the genotype TT, TG, and GG was as follows: 109 (55.0%) men, 68 (61.3 %) women with TT genotype, 83 (41.9%) men and 41 (36.9%) women with TG genotype, and 6 (3.0%) men and 2 (1.8%) women with GG genotype. An analysis of the allele's distribution in the group of patients with chronic HCV in relation to age did not reveal any significant differences.

IL-28B gene SNP rs12979860 and SNP rs8099917 distribution in the group of patients with SC HCV

A total of 48 persons (19 men [39.6%] and 29 women [60.4%]) were included into the group of patients with SC HCV. The evaluation of this group for the IL-28B gene SNP rs12979860 by the CC, CT, and TT alleles produced the following pattern: genotype CC, 33 patients (68.8%); CT, 11 (22.9%) patients; and TT, 4 (8.3%) patients. For the IL-28B gene SNP rs8099917, the results were as follows: genotype TT, 39 (81.3%) patients; TG, 9 (18.7 %) patients; and GG 0 patients.

At the next stage of our research, we studied the IL-28B gene polymorphism distribution in the group of patients with the SC HCV associated with gender. Thus, the SNP rs12979860 distribution of the IL-28B gene in male population was as follows: CC, 14 (73.7%); CT 3 (15.8%); and TT, 2 (10.5%). In the female group, the distribution was as follows: CC, 19 (65.5%); CT, 6 (20.7%); and TT, 4 (13.8%). The distribution among men by the IL-28B gene SNP rs8099917 was as follows: TT, 15 (78.9%); TG, 4 (21.1%); and GG, 0. The same distribution was as follows among women: TT, 24 (82.8%); TG, 5 (17.2%), GG, 0.

A statistical analysis of the rate of the SNP rs12979860 and rs8099917 of the IL-28B gene in the SC HCV patient group in relation to gender has not revealed any significant results.

Influence of the IL-28B gene polymorphism on SC HCV

The influence of the IL-28B gene polymorphism was studied by comparing the gene genotypes SNP rs12979860 (CC, CT, TT) and SNP rs8099917 (TT, TG, GG) occurrence in the groups of patients with SC HCV and with chronic HCV. At the second stage of the study, comparative analysis of the SNP rs12979860 and SNP rs8099917 polymorphism of the IL-28B gene in the SC HCV and chronic HCV groups in relation to gender was conducted (Table 2).

An analysis of the IL-28B gene SNP rs12979860 genotype rate of occurrence revealed a strong association between the CC genotype and spontaneous recovery. Thus, in the group of patients with CHC, it was identified in 109 (35.3%), and with SC HCV in 33 (68.8%) persons (OR, 4.04 [95 % CI, 2.10-7.76]; $p < 0.001$). The SNP rs12979860 CT genotype of IL-28B was found most frequently in CHC (177 [57.2%]) as compared to patients with SC HCV (11 [22.9 %]; OR, 0.22 [95% CI, 0.11-0.45]; $p < 0.001$). The rate of the genotype SNP rs12979860 TT of IL-28B was similar in both groups.

Table 2. Effect of the IL-28B gene SNP rs12979860 and SNP rs8099917 polymorphism on SC HCV

IL-28B gene SNP polymorphism	SC HCV n=48	Chronic HCV n=309	p	OR	95% CI
rs12979860 CC	33 (68.8%)	109 (35.3%)	0.001	4.037	(2.100-7.759)
rs12979860 CT	11 (22.9%)	177 (57.2%)	0.001	0.222	(0.109-0.451)
rs12979860 TT	4 (8.3%)	23 (7.5%)	0.85	1.130	(0.373-3.424)
rs8099917 TT	39 (81.3%)	179 (57.9%)	0.002	3.147	(1.473-6.724)
rs8099917 TG	9 (18.7%)	120 (38.9%)	0.007	0.365	(0.170-0.777)
rs8099917 GG	0	10 (3.2%)			

IL: interleukin; SNP: single nucleotide polymorphism; SC HCV: spontaneous clearance of HCV; OR: odds ratio; CI: confidential interval

An analysis of results of the IL-28B SNP rs8099917 genotype distribution demonstrated the association of SC HCV with genotype TT in 39 (81.3%); of CHC in 179 (57.9%) (OR, 3.15 [95% CI, 1.47-6.72]; $p < 0.002$). The rate of genotype SNP rs8099917 TG occurrence was two times higher in chronic disease: 120 (83.9%) and 9 (18.9%), respectively (OR, 0.7 [95% CI, 0.17-0.78]); $p < 0.007$. The genotype SNP rs8099917 GG was identified only in 10 (3.2%) patients with CHC and was not found in spontaneous recovery.

The study of the influence of the IL-28B gene polymorphism on SC HCV in relation to gender was the next stage of our research. Thus, the SNP rs12979860 CC genotype in CHC and SC HCV in men occurred in 71 (35.8%) and 14 (73.7%) cases, respectively, and in women in 38 (34.2%) and 19 (65.5%) cases, respectively. Significant results were also obtained from the comparison of the SNP rs12979860 CC genotype frequency in SC HCV and CHC groups in relation to gender (men: OR, 1.38 [95% CI, 0.70-2.72]; $p < 0.001$); women (OR, 4.67 [95% CI, 2.39-9.14]; $p < 0.001$).

The genotype SNP rs12979860 CT in CHC was identified in men in 112 (56.6%) cases and in women in 59 (53.2%) cases; for SC HCV, it was identified for men in 3 (15.8%) cases and for women in 6 (20.7%) cases, respectively. In the group of patients with SC HCV, a significant decrease in the SNP rs12979860 CT genotype frequency was observed both in the male and female groups. The TT allele occurred with almost similar frequency both in the male and female groups of patients with SC HCV and CHC.

The study of the IL-28B gene SNP rs8099917 polymorphism in the studied male and female populations demonstrated that the TT allele was identified in 109 (55.0%) men and 68 (61.3%) women with CHC and in 15 (78.9%) men and 24 (82.8%) women in SC HCV (men: OR, 0.84 [95% CI, 0.44-1.60]; $p < 0.001$; and women: OR, 3.54 [95%

CI, 1.89-6.63]; $p < 0.001$). An analysis of the SNP rs8099917 TG in chronic hepatitis C showed the following results: 83 (41.9%) men and 41 (36.9%) women. In SC, it was 4 (21.1%) men and 5 (17.2%) women ($p < 0.001$). The SNP rs8099917 GG genotype was extremely rarely detected in chronic HCV both in men and women, and it was not identified altogether in the SC HCV.

Summarizing this part of the study, we can state that the SNP rs12979860 CC and SNP rs8099917 TT genotypes of IL-28B gene are associated with SC and occur in 1.9 and 1.4 times more often with SC HCV than in CHC. Genotype distribution is not related to the patient gender.

Comparative analysis of genotype distribution and HCV serotypes in CHC and SC HCV

The analysis of the HCV genotype distribution in 309 patients with CHC gave the following results: 1b HCV genotype, 175 (56.6%); 1a, 12 (3.9%); 3a, 113 (36.6%); 3ab, 5 (1.6%); and HCV genotypes 2, 4 (1.3%). For genotype distribution in the group of patients with chronic HCV infection in relation to gender, the following results were obtained: the HCV genotype 1 was found in 122 (61.6%) men; genotype 3 in 73 (36.9%), and genotype 2 in 3 (1.5%) patients. In the CHC group, genotype 1 was found in 65 (58.6%); genotype 3 in 45 (40.5%); and genotype 2 in 1 (0.9%) female patient.

The HCV serotype was identified in 48 SC HCV patients. Serotype 1 was identified in 31 (64.6%) patients, serotype 3 in 17 (35.4%), and serotype 2 was not identified. The serotypes analysis gave the following data: serotype 1 occurred in 12 (63.2%) men and 19 (65.5%) women, and serotype 3 in 7 (36.8%) men and 10 (34.5%) women. Statistical processing of the HCV genotypes frequency results in the group of patients with CHC and serotypes in the group of patients with SC HCV did not produce significant results, nor did their distribution in the groups in relation to gender.

Table 3. Distribution of the HBV and HIV markers in the group of patients with SC HCV and patients with chronic HCV

Markers	SC HCV n=48	CHC n=309	p	OR	95% CI
HBsAg	4 (8.3%)	19 (6.1%)	0.56	1.388	(0.451-4.269)
anti-HBc	25 (52.1%)	73 (23.6%)	0.001	3.514	(0.882-6.560)
anti-HBs	9 (18.8%)	23 (7.4%)	0.01	2.87	(1.239-6.648)
anti-HIV	0	28 (9.1%)			

SC HCV: spontaneous clearance of HCV; OR: odds ratio; CI: confidential interval

An analysis of the obtained results allows us to conclude that the likelihood of SC HCV does not depend on the HCV genotype.

HBV and HIV markers in patients with SC HCV and patients with CHC

In the studied groups, the influence of the past HBV infection was assessed based on the testing of sera for anti-HBc and anti-HBs. The diagnosis of a chronic HBV infection was made based on the HBsAg detection. All positive HBsAg samples were tested for IgM anti-HBc to rule out acute viral hepatitis B. Patients with negative results were included into the study. An HIV infection was diagnosed based on the presence of anti-HIV in the serum.

Table 3 provides data of the HBV and HIV markers identification in the group of patients with SC HCV and patients with CHC.

As follows from the data presented in Table 3, markers of the past HBV infection (anti-HBc and anti-HBs) were identified two times more frequently in the SC HCV group than in patients with CHC (OR, 3.51 [95 % CI, 0.88-6.56]; $p < 0.001$ and (OR, 2.87 [95 % CI, 1.23-6.65]; $p < 0.01$). HBsAg was identified more frequently in SC HCV (4 [8.3%] patients) than in patients with CHC (19 [6.1%] patients) (OR, 1.39 [95% CI, 0.45-4.27]; $p = 0.56$).

Anti-HIV were detected only in the group of patients with a chronic HCV infection (28 [9.1%] patients) and were not identified in SC HCV.

DISCUSSION

This paper presents a comprehensive analysis of viral and host factors influencing the spontaneous recovery in a Slavic population. We do not know whether the SC HCV took place in an acute or chronic HCV infection, since only a few patients have reported the past history of jaundice.

The patient's gender plays an essential role in spontaneous elimination of the virus. Thus, in our study, in case of a favorable outcome, the female-to-male ratio was 1:0.66 and 1:1.78 in chronic disease ($p = 0.0013$). These results are consistent with data obtained in Europe (16), Egypt (17), Australia (9), and China (18). One of the main hypotheses explaining the specifics of immune response in women as compared with that of men considers the effect of sex hormones on the functional activity of lymphocytes, macrophages, dendritic cells, and lymphoid tissue (19). An indirect effect of sex hormones via a specific receptor expressed on the immune competent cell membranes ensures a better control over the outcome of the HCV infection in female population, and a direct effect of sex hormones on the HCV replication is also possible.

The patient age had a significant influence on the outcomes of the HCV infection. Spontaneous recovery occurred more often in younger age (from 18 to 35 years)-22 (45.8%) in HCV and 83 (26.9%) in CHC ($p < 0.008$)-which is consistent with the results of other studies (20).

According to the data obtained, the route of transmission also impacted the HCV infection outcome: a chronic HCV infection developed more often after a hemotransfusion-transmitted infection (41 [13.3%]) than SC HCV (2 [4.2%]) ($p = 0.72$). Hemotransfusion-transmitted infection is associated with a higher infective dose of virus, especially if hemotransfusions were performed before 1990-the time when mandatory HCV-antibody tests started being implemented into the blood banking service. The majority of researchers have not found any difference in the HCV infection outcomes, including children, related to the route of transmission (21,22).

In an overwhelming majority of studies, the history of jaundice was associated with a favorable HCV infection outcome.

Marked necrotic and inflammatory processes in the liver parenchyma with elevated serum enzymes and bilirubin are characteristic for the development of multispecific strong cell-mediated and humoral immune response to viral-specific proteins, which often leads to spontaneous recovery (7,23). A strong specific immune response is determined by a number of genetic factors, such as HLA-A, HLA-B, and IL-28B (24). In AHC, the virus stimulates cell-mediated immune response and INF- α production. IL-28B would be induced by the viral infection itself and further enhanced by INF- α . The IL-28B responder genotypes in this context would allow for a stronger IL-28B induction and thereby increase the ISG induction, with subsequently higher odds for spontaneous recovery. In the study conducted by Tillmann et al. (25), it was found that patients with the SNP rs12979860 CC with AHC jaundice developed more often than in non-CC. In carriers of the CC allele, jaundice was not associated with SC HCV, and at the same time, in patients with the non-CC genotype, jaundice was the criterion of SC HCV.

At present, conclusive data about the effect of IL-28B gene polymorphism on natural course of the HCV infection and response to interferon-containing treatment regimens were obtained. The SNP rs8099917 TT was shown to be closely associated with SC HCV (26,27), as well as with the likelihood of sustainable viral response to Peg INF+ ribavirin therapy in patients with the HCV genotype 1 and 4 infection, but not with the HCV genotypes 2 and 3 (28).

In our study, the rate of SNP rs12979860 genotype CC occurrence in the group of patients with SC HCV was 1.9 times higher than in the group with chronic HCV; and of patients with the SNP rs8099917 genotype TT, it was 1.4 times higher.

The distribution of favorable genotypes in groups did not depend on gender. In studies by van den Berg et al. (16), women with unfavorable IL-28B gene polymorphism rs12979860 CT/TG and SNP rs8099917 TG/GG had similar odds for SC HCV to those of men with favorable or unfavorable genotype. Women with favorable IL-28B polymorphism had the highest odds of SC HCV.

In the presented study, based on evaluation of 357 anti-HCV positive patients, the SC HCV was diagnosed in 48 persons (13.4%). This is significantly lower than reported in other works conducted in European population (26,29). So, far there is no single answer to the question

regarding the role of the HCV genotype in the natural history of the HCV infection. In the AHC development, patients infected with 1b HCV subtype had the highest odds for the development of CHC as compared to those with genotypes 2 and 3 (10,30). At the same time, several researchers have noted high likelihood of spontaneous recovery in patients with genotype 1 HCV from acute viral hepatitis C (9).

The evaluation of large cohorts of anti-HCV positive patients, including those from risk groups, did not allow an unambiguous answer to the question about the role of the HCV genotype in spontaneous viral elimination in chronic disease. Having monitored the natural course of the HCV infection in children for 10 years, Bortolotti et al. (22) noted that infection with the HCV genotype 3 is an independent predictor of SC HCV. In evaluating 496 anti-HCV positive and HBsAg negative IDUs, SC HCV was diagnosed in 38%, with no effect of the HCV genotype on disease outcomes noted (31). In two other extensive studies conducted in Great Britain, serological and molecular-virological evaluation of 749 and 321 anti-HCV patients revealed association between the SC HCV and the HCV genotype 1 (8,20).

An HBV superinfection significantly affects the natural course and outcomes of HCV. Two- to six-years-long monitoring of 24 patients with a chronic HCV infection at the time of acute viral hepatitis B development allowed to state the undetectable HCV RNA level at the end of the first year of the follow up in 7 (29.2 %), after 2 years in 14 (58.3%), and after 3 to 6 years in 18 (75%) patients. In six patients, the SC HCV was stated (18). Majority of works have indicated that markers of an active or past HBV infection are more often found in patients with SC HCV (12). According to our data, an HIV co-infection is a negative factor in spontaneous recovery. Other authors also indicate a low likelihood of HCV clearance in the cohort of patients with an HCV/HIV co-infection not receiving HAART (32). The HAART initiation with the restitution of cell-mediated immunity allows expecting the SC HCV later, as well as an increased likelihood of patients with an HCV/HIV co-infection achieving SVR on antiviral therapy (32).

According our results, the SC HCV was diagnosed in 48 patients (13.4%). The most significant positive factors affecting the SC HCV include the IL-28B gene SNP rs12979860 (CC) and SNP rs8099917 (TT), female gender, young age, and a past history of jaundice, but not the viral genotype.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.V.F., A.K.; Design - O.V.L.; Supervision - S.V.F.; Resources - T.L.M., V.Y.; Materials - T.L.M.; Data Collection and/or Processing - T.L.M., V.Y.; Analysis and/or Interpretation - S.V.F., A.K.; Literature Search - S.V.F.; Writing Manuscript - O.V.L.; Critical Review - S.V.F.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study supported by The National Academy of Medical Sciences of Ukraine.

REFERENCES

- Wang CC, Krantz E, Klarquist J, et al. Acute hepatitis C in a contemporary US cohort: modes of acquisition and factors influencing viral clearance. *J Infect Dis* 2007; 196: 1474-82. [\[CrossRef\]](#)
- Peres-Alvares R, Garcia-Samaniego J, Sola R, et al. Acute hepatitis C in Spain: a retrospective study of 131 cases. *Revista Espanola de Enfermedades Digestivas* 2012; 104: 21-8. [\[CrossRef\]](#)
- Hashem M, Zaghla H, Zakaria Z, et al. High spontaneous clearance of symptomatic iatrogenic acute hepatitis C genotype 4 infection. *J Med Virol Epub* 2018; 90: 1841-7. [\[CrossRef\]](#)
- El-Attar MM, Abadel-Hamid M, Hasam MS. Spontaneous viral clearance of chronic HCV infection in Upper Egypt: A community-based study with a 10 year follow-up. *Arab J Gastroenterol* 2010; 11: 197-201. [\[CrossRef\]](#)
- Deterding K, Gruner N, Buggish P, et al. Delayed versus immediate treatment for patients with acute hepatitis C: a randomised controlled non-inferiority trial. *Lancet Infect Dis* 2013; 13: 497-506. [\[CrossRef\]](#)
- Beinhardt S, Aberle JH, Strasser M, et al. Serum level of IP-10 increases predictive value of IL28B polymorphisms for spontaneous clearance of acute HCV infection. *Gastroenterology* 2012; 142: 78-85. [\[CrossRef\]](#)
- Raghuraman S, Park H, Osburn WO, et al. Spontaneous clearance of chronic hepatitis C virus infection is associated with appearance of neutralizing antibodies and reversal of T-cell exhaustion. *J Infect Dis* 2012; 205: 763-71. [\[CrossRef\]](#)
- Harris HE, Eldridge KP, Harbour S, et al. Does the clinical outcome of hepatitis C infection vary with the infecting hepatitis C virus type? *J Viral Hepat* 2007; 14: 213-20. [\[CrossRef\]](#)
- Grebely J, Page K, Sacks-Davis R, et al. The affects of female sex, viral genotype, and IL28B genotype on spontaneous clearance of acute hepatitis C virus infection. *Hepatology* 2014; 59: 109-20. [\[CrossRef\]](#)
- Cho Yoo-Kyung, Kim Young-Nam, Song Bying-Cheol. Predictors of spontaneous viral clearance and outcomes of acute hepatitis C infection. *Clinical and Molecular Hepatology* 2014; 20: 368-75. [\[CrossRef\]](#)
- Sagnelli E, Coppola N, Pisaturo M, et al. HBV superinfection in HCV chronic carriers: a disease that is frequently severe but associated with the eradication of HCV. *Hepatology* 2009; 49: 1090-7. [\[CrossRef\]](#)
- Grebely J, Conway B, Raffa JD, et al. Hepatitis C virus reinfection in of injection drug users. *Hepatology* 2006; 44: 1139-45. [\[CrossRef\]](#)
- Kaber M, Hammarberg A, Weiland O. Incidence and spontaneous clearance of hepatitis C virus (HCV) in people who inject drug at the Stockholm Needle Exchange-importance for HCV elimination. *J Viral Hepat* 2018; 25: 1452-61. [\[CrossRef\]](#)
- Grebely I, Raffa I, Lai C, et al. Factors associated with spontaneous clearance of hepatitis C virus among illicit drug users. *Canadian J Gastroenterol* 2007; 21: 447-51. [\[CrossRef\]](#)
- Tram NQ, Tomasz M. Occult hepatitis C Virus Infection and its relevance in clinical practice. *J Clin Exp Hepatol* 2011; 1: 185-9. [\[CrossRef\]](#)
- Van den Berg CHV, Grady BPX, Schinkel J, et al. Female sex and IL28B, a synergism for spontaneous viral clearance in Hepatitis C Virus (HCV) seroconverters from a community-based cohort. *PLoS ONE* 2011; 6: e27555. Bakr J, Rekeceuciz C., El Hosseiny M., et al. Higher clearance of hepatitis C virus infection in females compared wit males. *Gut* 2006; 55: 1183-7.
- Kong Fei, Pan Y, Chi X, et al. Factors associated with spontaneous clearance of hepatitis C virus in Chinese population. *BioMed Res Int* 2014; 16: Article ID 527030.
- Klein SL, Jedlicka A, Pecosz A. The Xs and Y of immune responses to viral vaccines. *Lancet Infect Dis* 2010; 10: 338-49. [\[CrossRef\]](#)
- Roffe KJ, Curran MD, Alexander GJM, et al. Spontaneous loss of Hepatitis C virus RNA from serum is associated with genotype 1 and younger age at exposure. *J Med Virol* 2011; 83: 1338-44. [\[CrossRef\]](#)
- Yeung LT, To T, King SM, Roberts EA. Spontaneous clearance of childhood hepatitis C virus infection. *J Viral Hep* 2007; 14: 797-805. [\[CrossRef\]](#)
- Bortolotti F, Verucchi G, Camma C, et al. Long-Term course of chronic hepatitis C in children: from viral clearance to end-stage liver disease. *Gastroenterology* 2008; 134: 1900-7. [\[CrossRef\]](#)
- Yerly D, Heckerman D, Allen TM, et al. Increased cytotoxic T-lymphocyte epitope variant cross-recognition and functional avidity are associated with hepatitis C virus clearance. *J Virol* 2008; 82: 3147-53. [\[CrossRef\]](#)
- Kamal SM, Kassim SK, Ahmed AI, et al. Host and viral determinants of the outcome of exposure to HCV infection genotype 4: a large longitudinal study. *American J Gastroenterol* 2014; 109: 199-211. [\[CrossRef\]](#)
- Tillmann HL, Thompson AJ, Patel K, et al. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. *Gastroenterology* 2010; 139: 1586-92. [\[Cross-Ref\]](#)
- Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; 461: 798-801. [\[CrossRef\]](#)
- Coppola N, Pisaturo M, Sagnelli C, et al. Role of polymorphism in hepatitis C virus chronic infection. *World J Clin Cases* 2015; 3: 807-22. [\[CrossRef\]](#)
- Ahlenstiel G, Booth DR, George J. Clinical significance of IL28B gene variation in hepatitis C virus infection. *Hot Topics in Viral Hepat* 2011; 20: 17-24.

-
28. Chayama K, Hayes CN. Interleukin-28B polymorphisms and hepatitis C virus clearance. *Genome Med* 2013; 5: 2-3. [\[CrossRef\]](#)
29. Hwang SJ, Lee SD, Lu RH, et al. Hepatitis C viral genotype influences the clinical outcome of patients with acute posttransfusion hepatitis C. *J Med Virol* 2001; 65: 505-9. [\[CrossRef\]](#)
30. Keating S, Coughlan S, Connel J, et al. Hepatitis C clearance in an intravenous drug-using cohort in the Dublin area. *Irish J Med Sci* 2005; 174: 37-41. [\[CrossRef\]](#)
31. Stenkovic J, Nystrom J, Falconer K, et al. Occasional spontaneous clearance of chronic hepatitis C virus in HIV-infected individuals. *J Hepatol* 2014; 61: 957-61. [\[CrossRef\]](#)
-