წონით. მენოპაუზის სტატუსის გათვალისწინებით ქალები დაიყო 3 ჯგუფად: I ჯგუფი - 45 ქალი ჰიპერტონიული დაავადებით და ჭარბი წონით პრემენოპაუზის პერიოდში, II ჯგუფი - 50 ქალი მენოპაუზის პერიოდში, რომლის ვადაც არ აღემატება 3 წელს და III (საკონტროლო) ჯგუფი - 20 პრაქტიკულად ჯანმრთელი ქალი პრემენოპაუზის პერიოდში. VEGF გენის ალელური პოლიმორფიზმის - 634 G/C (rs 2010963)-ს კვლევა ჩაატარდა პოლიმერული ჯაჭვური რეაქციის მეთოდით. დადგინდა, რომ VEGF-A - 634 G/C- გენის პოლიმორფიზმის გენოტიპი (rs 2010963) უფრო ხშირია პრემენოპაუზის პერიოდში მყოფ ჭარბი წონის და ჰიპერტონიული დაავადების მქონე ქალებში, ვიდრე მენოპაუზის პერიოდში მყოფ ქალთა ჯგუფში; აგრეთვე,გამოვლინდა,რომ VEGF-ს დონე მნიშვნელოვნად უფრო მაღალია გენი VEGF-A-ს გენოტიპის -634 GG პოლიმორფიზმის (rs 2010963) მტარებელ ქალებში, შედარებით გენოტიპ GC-სთან და CC პაციენტებთან ($p<0,05$). VEGF-A გენის GG- გენოტიპის 634 G/C (rs 2010963) პოლიმორფიზმის მტარებლობა შეიძლება განხილულ იქნას, როგორც ჭარბ წონასთან შერწყმული ჰიპერტონიული დაავადების გულ-სისხლძარღვოვანი რისკის განვითარების ადრეული მარკერი პრემენოპაუზის პერიოდში მყოფ ქალებში.

CLINICAL AND GENETIC PREDICTORS AND PROGNOSTIC MODEL OF RAPIDLY PROGRESSIVE HEPATIC FIBROSIS IN CHRONIC HEPATITIS C

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Chronic hepatitis C (CHC) is one of the most important problems of hepatology due to its extremely high prevalence in the structure of chronic liver diseases worldwide. It is characterized by the continuous progression and for some patients cirrhosis is the end stage of its natural history. CHC development into cirrhosis lasts during several decades, on the average of 20-30 years from the moment of infection, however, the time period can be less than 20 years for some patients, and can increase up to 50 years and more for the others [8,17]. Viral, host and environmental factors have been recognized as the risk factors (RF) associated with a faster rate of fibrosis progression (RFP) in CHC: late age at infection, male gender, chronic alcohol consumption, obesity, insulin resistance and type 2 diabetes, co-infection with HIV and other hepatotropic viruses, iron metabolism disorder, smoking are proved to date [4,8]. Recently, search for genetic determinants, influencing the RFP has become the matter of extensive investigation. The TLR4 and TLR7 gene polymorphisms has been studied, which are of the particular interest with regard to pathogenesis of CHC, i.e., single-stranded RNA, core and nonstructural proteins of the hepatitis C virus (HCV) are their ligands, and, consequently, it is such genes that activate the immune mechanisms in HCV-infection [2,12,13]. The scientific publications elucidate the impact of Asp299Gly polymorphism of the TLR4 gene and Gln-11Leu polymorphism of the TLR7 gene on progression of hepatic fibrosis (HF) in CHC, however, such reports are not numerous and controversial [3,5-6,9-10,14,16,18]. Taking into consideration that the RFP is recognized as one of the major characteristics of a patient, search for and defining of both genetic and other predictors of rapid progression of HF is crucial in prognosis of the clinical course of CHC.

The study was aimed at identification of clinical and genetic predictors and creation of the prognostic model of rapid progression of HF in CHC.

Material and methods. To meet the objectives of our investigation a retrospective cohort study, involving 125 patients with CHC: 48 (38.4%) women and 77 (61.6%) men, age range, 20 to 63 years (mean age, 40.78±0.86 years) have been carried out. CHC have been diagnosed in compliance with the International Classification of Diseases, 10th Edition (ICD-10) and International Classification of Liver Diseases (Los-Angeles, 1994) and verified by the detection of specific HCV serological markers (anti-HCV IgM and IgG, anti-HCV core and anti-NS_3, anti-NS_4, anti-NS_5) using the method of enzyme-linked immunosorbent assay (ELISA) with required detection of HCV-RNA in the serum evaluated by the real-time polymerase chain reaction (PCR) with genetic typing and evaluation of viral load (VL). VL>$4.0x10^5$ IU/ml was considered to be high [8]. Quantitative and qualitative detection of HCV-RNA, as well as genetic typing of HCV has been performed using the TaqMan-48 analyzer (Roche Diagnostics, Switzerland) by the Roche Diagnostics (Switzerland) test system. To exclude co-infection with hepatotropic viruses and HIV the HBsAg, anti-HBcor (total), anti-HDV, anti-HIV were assessed in serum of all patients by the ELISA method. Presumed duration of HCV infection was determined by the results of anamnestic data (past icteric acute hepatitis C,

blood transfusion and its components prior to the introduction of mandatory screening of donors, previous injection drug addiction), and in case of absence of such facts in the patient's history it was determined by the clinical and laboratory findings (the first detection of antibodies to HCV, increased levels of hepatic aminotransferases, specified in the patient's clinical history).

The program of patients' examination included: assessment of complaints, anamnestic data with detailed analysis of medical records, physical examination, common clinical peripheral blood examination, evaluation of biochemical parameters, characterizing the functional state of the liver, and autoimmune and genetic markers. Cytolytic syndrome was assessed by the rates of activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholestasis syndrome – bilirubin, alkaline phosphatase (ALP) and γ-glutamyltranspeptidase (GGTP). Biochemical screens, including evaluation of cholesterol and triglycerides (TG) in addition to the above-mentioned parameters, were performed in GBG STAT FAX-1904 (Japan) automatic biochemical analyzer by the HUMAN's reagents (Germany).

Asp299Gly polymorphic area of the TLR4 gene was genetically typed by the PCR using oligonucleotide primers, and amplification has been performed in "Tertsik" amplifier (JSC "NPO DNA-Technology", Russia), Gln11Leu of the TLR7 gene by the real-time PCR using specific oligonucleotide primers, "DT Lite" amplifier (JSC "NPO DNA-Technology", Russia).

The distribution of the observed frequencies of alleles and genotypes of investigated genes was assessed for compliance with the Hardy-Weinberg equilibrium (HWE), calculation was made on the online calculators available on the links http://gen-exp.ru/calculator_or.php, http://www.oege.org/software/hwe-mr-calc.shtml, using the χ^2 test, balance occurs in $p>0.05$ [19].

Stage of HF on METAVIR score has been evaluated prior to antiviral therapy (AVT) for CHC using the FibroTest methods on Cobas 6000 analyzer (c 501 module), Roche Diagnostics (Switzerland) test system and liver elastometry on "Ultima PA-Expert" (Ukraine) ultrasonic scanner. Rate of hepatic fibrosis progression has been calculated using the T. Poynard formula, dividing the HF stage on METAVIR score by the time over which it was formed, and measured in units per year (units/year) [17]. The median RFP of 125 patients with CHC was 0.200 (0.043-1.000) units/year, on the basis of which the comparison groups have been formed:
- A – CHC patients with rapidly progressive HF (RFP >0.200 units/year), n=62;
- B – CHC patients with slowly progressive HF (RFP ≤0.200 units/year), n=63.

Statistical analyses of the findings were performed using SPSS 17.0 and STATA 11.0 programs. Conventional methods of variation statistics, applied in medicine, were used [1]. The nature of data distribution was checked by the Kolmogorov-Smirnov test. In normal distribution the central tendency were determined using the mean value (M) and its standard error (m), and in abnormal distribution – median (Me) with upper and lower quartiles (interquartile range, Q_1-Q_3). In normal distribution the probability of differences of quantitative results was determined using the Student's *t*-test, and in abnormal distribution by the Mann-Whitney U-test, qualitative results by Fischer's exact test and χ^2 test, depending on the baseline conditions of the analysis. To identify the associations between individual parameters and creation of prognostic models the univariate and multivariate logistic regression analysis was used with calculation of the odds ratio (OR) and its 95% confidence interval [95% CI]. The chances of occurrence of the predicted fact increased if $OR>1$ and decreased when $OR<1$. Binary criterion was selected as the dependent variable: 1 – rapidly and 0 – slowly progressive HF. Each of 46 variables, considered as potential RF has been examined with univariate logistic regression analysis with forced inclusion. In $p<0.05$ the variables were used in multivariate analysis using a stepwise inclusion to identify the reliable predictors and their grading according to the contribution to the model, expressed by the value of χ^2 Wald statistic and regression coefficient [1,11]. Generally, the model of logistic regression [11] specified that probability (P) of assignment to risk group of predicted event can be calculated using the formula (1):

$$P=1/(1+e^{(-y)}),$$

where e – mathematical constant, equal to 2.72;
$y=\alpha+B_1 \cdot X_1+B_2 \cdot X_2+....+B_n \cdot X_n$;
α – regression equation constant;
$B_1 \ldots B_n$ – regression coefficients for independent variables;
$X_1 \ldots X_n$ – independent variables, included to the model.
P=0.5 (null hypothesis) was taken as the point of division: in $P>0.5$ a positive prognosis of the event occurs (high-risk group) [1].

The statistical significance of the prognostic model was determined using the χ^2 test with indication of the number of degrees of freedom (d.f). The ROC-analysis (Receiver Operating Characteristic) has been used to assess its diagnostic power, including estimation of the sensitivity, specificity, the total number of the appropriate assignments, positive and negative predictive value, as well as construction of the ROC-curve with the calculation of the area under it (AUC-area under the curve). According to the conventional expert score, the prognostic capability of the model was considered to be excellent in AUC>0.9; very good – 0.8-0.9; good – 0.7-0.8; satisfactory – 0.6-0.7; unsatisfactory – 0.5-0.6 [15]. Predictive model's compliance with the survey data was assessed using the Hosmer-

Lemeshow goodness of fit test (in p>0.05 the hypothesis about consistency was accepted) [11]. For all the other methods of analysis differences in p<0.05 were considered as statistically significant ones, and when *p was* in the range of 0.05 to≤0.1 the tendency toward reliability was noted.

Results and discussion. The findings of the study have established that most of the examined patients – 108 out of 125 (86.4%) were infected at a young age and 1 genotype of HVC was detected in the overwhelming majority of subjects (64.8%). Almost equal proportion between patients with high and low VL (53.6% and 46.4%, respectively) was observed. The proportion between patients with different periods of infection was as follows: less than 5 years – 50.4%, from 5 to 10 – 16.8%, over 10 – 32.8% (Me=4.0 (1.0-13.5) years). At the time of the examination they have different stages of HF, without prevalence by any (Fig. 1).

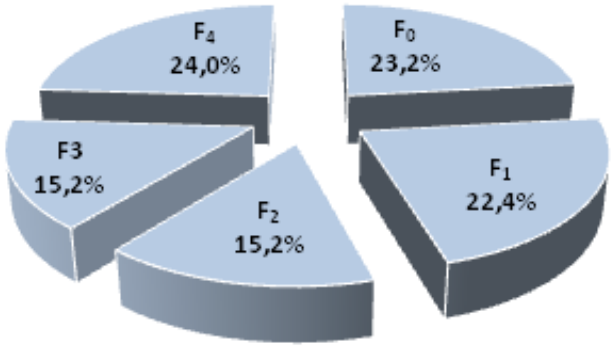

Fig. 1. Distribution of patients with CHC according to the stages of hepatic fibrosis

Fig. 1 shows that absence of HF (F_0) was detected in 29 (23.2%), minor and moderate fibrous lesions (F_1-F_2) were detected in 47 (37.6%) and the advanced HF (F_3-F_4) was detected in 39 (39.2%) patients. On the basis of the calculation of the median RFP of 125 examined subjects (Me=0.200 (0.043-1.000) units/year) the time period from the moment of infection to the progression into F_4 constituted 20 years, which is consistent with the data from scientific publications [8,17].

Findings of the molecular and genetic research have shown that both normal Asp299Asp, Gln11Gln and polymorphic Asp299Gly, Gln11Leu, Leu11Leu genotypes of the TLR4 and TLR7 genes were detected in 125 patients with CHC. Homozygous Gly299Gly genotype of the TLR4 gene has not been detected, showing no contradiction to the scientific data with regard to its low rate in the population [5,7,16]. Herein, among the total number of examined patients Asp299Asp and Asp299Gly genotypes of the TLR4 gene were detected in 106 (84.8%) and 19 (15.2%) patients, respectively, Gln11Gln genotype of the TLR7 gene – in 102 (81.6%), Gln11Leu – in 21 (16.8%), Leu11Leu – in 2 (1.6%). The analysis of distribution of the TLR4 and TLR7 gene genotypes in the comparison groups has established their conformity to HWE equilibrium, indicating about the representativeness of sample and correctness of the variant markers determination (Table 1).

Comparative analysis of demographic and clinical-laboratory characteristics of patients with CHC, supplemented with genetic markers, considering the conventional RF for HF progression, has shown a significant differences between patients with rapidly (group A) and slowly progressive HF (group B) in a number of criteria (Table 2).

Table 1. Distribution of the genotypes of the TLR4 and TLR7 genes in patients with HCH with rapidly and slowly progressive hepatic fibrosis

Group	Gene	Genotype	N.O. (abs.%)	N.E. (abs.%)	HWE, χ^2 d.f.=1
Rapidly progressive HF (A), n=62	TLR4	Asp299Asp	51 (82.3)	51.49 (83.0)	0.59 (p=0.44)
		Asp299Gly	11 (17.7)	10.02 (16.2)	
		Gly299Gly	0 (0.0)	0.49 (0.8)	
Slowly progressive HF (B), n=63		Asp299Asp	55 (87.3)	55.25 (87.7)	0.29 (p=0.59)
		Asp299Gly	8 (12.7)	7.49 (11.9)	
		Gly299Gly	0 (0.0)	0.25 (0.4)	
Rapidly progressive HF (A), n=62	TLR7	Gln11Gln	57 (91.9)	57.1 (92.1)	0.11 (p=0.74)
		Gln11Leu	5 (8.1)	4.8 (7.7)	
		Leu11Leu	0 (0.0)	0 (0.2)	
Slowly progressive HF (B), n=63		Gln11Gln	45 (71.4)	44.59 (70.8)	0.15 (p=0.70)
		Gln11Leu	16 (25.4)	16.83 (26.7)	
		Leu11Leu	2 (3.2)	1.59 (2.5)	

note: N.O. and N.E. – observed and expected number of genotypes; χ^2 test was used to assess the conformity of the observed distribution of the genotypes to the expected one in HWE*; d.f. – number of degrees of freedom*

Table 2. Comparative characteristic of CHC patients with rapidly and slowly progressive hepatic fibrosis

Criteria	Groups		p value
	A (n=62)	B (n=63)	
Age, (M±m)	42.9±1.28	38.6±1.09	0.013
Gender: male/female, (abs.%)	47 (75.8)/15 (24,2)	30 (47.6)/33 (52.4)	0.001
HCV genotype: 1/2 and 3, (abs.%)	44 (71.0)/18 (29.0)	37 (58.7)/26 (41.3)	0.152
ALT, units/l, (Me Q_1-Q_3)	105.5 (61.6-163.7)	64.1 (40.4-110.0)	0.000
AST, units/l, (Me Q_1-Q_3)	74.5 (46.0-112.8)	44.0 (34.0-68.6)	0.000
Type 2 diabetes, (abs.%)	6 (9.7)	1 (1.6)	0.062
Obesity, BMI≥30 kg/m^2. (abs.%)	13 (21.0)	6 (9.5)	0.086
Alcohol intake>40 g/day, (abs.%)	19 (30.6)	7 (11.1)	0.008
Smoke, (абс.%)	11 (17.7)	14 (22.2)	0.531
Stage of HF according to METAVIR: ≤F_2/F_3-F_4, (abs.%)	30 (48.4)/32 (51.6)	46 (73.0)/17 (27.0)	0.005
Genotype of the TLR4 gene: Asp299Asp/Asp299Gly, (abs.%)	51 (82.3)/11 (17.7)	55 (87.3)/8 (12.7)	0.465
Genotype of the TLR7 gene: Gln11Gln/Gln11Leu+ Leu11Leu, (abs.%)	57 (91.9)/5 (8.1)	45 (71.4)/18 (28.6)	0.005

note: p – significance level

Data, presented in the Table 2, shows that in group of patients with rapidly progressive HF the mean age was 4.3 years higher (42.9±1,28) (in group B – 38.6±1.09, p=0.013) with prevalence of men (75.8%) (group B – 47.6%, p=0.001) and patients with alcohol abuse (30.6%) (group B – 11.1%, p=0.008). Patients of group A were diagnosed with type 2 diabetes (9.7%) and obesity (21.0%) more frequently (in group B – 1.6%, and 9.5%, with the tendency to reliability – p=0.062 and p=0.086, respectively). More severe functional changes in liver have been detected in rapidly progressive HF: median value of ALT – 105.5 (61.6-163.7) units/l, AST – 74.5 (46.0-112.8) units/l, in slowly progressive HF – 64.1 (40.4-110.0) and 44.0 (34.0-68.6) units/l, respectively (p=0.000 for both parameters). Group A showed appropriate prevalence of patients with advanced HF (F_3-F_4) – 51.6% (in group B – 27.0%, p=0,005). Moreover, comparison groups differed in the frequency of recording of individual genetic markers. In this way, normal Gln11Gln genotype of the TLR7 gene in group A was detected in 91.9% of patients, polymorphic Gln11Leu+Leu11Leu genotypes were detected in 8.1%, that 3.5 times less frequently than in group B – 71.4% and 28.6%, respectively, p=0.005 (taking into account the low rate of homozygotes relative to polymorphic allele of the TLR7 gene; in comparative analysis the carriers of Gln11Leu and Leu11Leu genotypes conjugated). A normal Asp299Asp genotype of the TLR4 gene in the group of patients with rapidly progressive HF has been registered in 82.3% of patients, polymorphic Asp299Gly – in 17.7% and in the group with slowly progressive HF – 87.3% and 12.7%, respectively, with no statistically significant difference, p=0.465. Thus, comparative analysis showed that along with conventional RF, influencing the RFP in CHC, genetic markers play a major role, too.

Next stage of the study aimed at identification of RF for rapidly progressive HF in CHC and evaluation of their significance has encompassed univariate logistic regression analysis of 46 variables, findings of which are presented in Table 3.

Data from Table 3 shows the following statistically significant associations with rapidly progressive HF in CHC: male gender (OR=3.44 [95% CI 1.60-7.39], p=0.001), increased levels of ALT (OR=4.93 [95% CI 1.54-15.76], p=0.007), particularly, moderate cytolytic activity (OR=2.36 [95% CI 1.08-5.16], p=0.031), AST (OR=3.65 [95% CI 1.41-9.43], p=0.007), GGTP (OR=3.63 [95% CI 1.73-7.61], p=0.001), total bilirubin (OR=3.53 [95% CI 1.47-8.47], p=0.005), ALP (OR=9.18 [95% CI 1.11-75.80], p=0.039), alcohol intake>40 g/day (OR=3.53 [95% CI 1.36-9.17], p=0.009). Considering the fact that among the concomitant pathology of the gastrointestinal tract organs, the associations of chronic cholecystitis and chronic pancreatitis in CHC patients with rapidly progressive HF proved to be the most significant (OR=2.32 [95% CI 1.04-5.13], p=0.038 and OR=1.90 [95% CI 0.92-3.94], p=0,081 respectively), as well as the fact of their recording in the majority of the examined patients (80.0%), the presence of these diseases was incorporated into one RF – chronic cholecystitis and/or pancreatitis, demonstrating the high predictive value (OR=5.30 [95% CI 1.84-15.25], p=0.002). normal Gln11Gln genotype of the TLR7 gene (OR=4.56 [95% CI 1.57-13.22], p=0.005) has been found to be another significant predictor for rapidly progressive HF in CHC, whereas polymorphic Gln11Leu+Leu11Leu were the protective factors, appropriately, (OR=0.21 [95% CI 0.07-0.63], p=0.005). Such variables as obesity and type 2 diabetes were tending to reliability of associations (OR=2.52 [95% CI 0.89-7.13], p=0.081 and OR=6.64 [95% CI 0.77-56.89], p=0.084 respectively). Thus, among 46 potential RF for rapidly progressive HF in CHC, 10 the most significant ones, included into stepwise multivariate logistic regression analysis have been identified. The most qualitative prognostic model (χ^2=49.36, d.f.=6, p=0.000) is presented in Table 4.

Table 3. The analysis of associations of potential risk factors with rapidly progressive hepatic fibrosis in CHC

Risk factors and their grades	Presence in groups (grade 1), abs. %		OR [95% CI]	p
	A (n=62)	B (n=63)		
Viral factors				
1 genotype of HCV (0 – no, 1 – yes)	44(71.0)	37(58.7)	1.71 [0.81-3.61]	0.154
High VL (0 – no, 1 – yes)	31(50.0)	27(42.9)	1.33 [0.65-2.69]	0.424
Host factors				
Male gender (0 – no, 1 – yes)	47(75.8)	30(47.6)	3.44 [1.60-7.39]	0.001
Age 45-59 years (0 – no, 1 – yes)	22(35.5)	15(23.8)	1.76 [0.80-3.83]	0.155
Obesity, BMI≥30 kg/m^2 (0 – no, 1 – yes)	13(21.0)	6(9.5)	2.52 [0.89-7.13]	0.081
Presence of chronic gastrointestinal tract diseases in toto (0 – no, 1 – yes), including:	59(95.2)	55(87.3)	2.86 [0.72-11.33]	0.135
- chronic cholecystitis;	49(79.0)	39(61.9)	2.32 [1.04-5.13]	0.038
- chronic pancreatitis;	42(67.7)	33(52.4)	1.90 [0.92-3.94]	0,081
- chronic gastroduodenitis;	14(22.6)	18(28.6)	0,72 [0.32-1.63]	0.444
- chronic duodenal ulcer;	1(1.6)	4(6.3)	0.24 [0.02-2.22]	0.210
- irritable bowel syndrome;	2(3.2)	2(3.2)	1.01 [0.13-7.45]	0.987
- cholelithiasis;	4(6.5)	3(4.8)	1.37 [0.29-6.43]	0.682
- fatty liver disease;	43(69.4)	36(57.1)	1.69 [0.81-3.54]	0.158
- chronic cholecystitis and/or pancreatitis.	57(91.9)	43(68.3)	5.30 [1.84-15.25]	0.002
Presence of extrahepatic lesions in toto (0 – no, 1 – yes), including:	9(14.5)	8(12.7)	1.16 [0.41-3.25]	0.767
- type 2 diabetes;	6(9.7)	1(1.6)	6.64 [0.77-56.89]	0.084
- autoimmune thyroiditis;	3(4.8)	7(11.1)	0.40 [0.10-1.65]	0.208
- purpura rheumatica.	1(1.6)	1(1.6)	1.01 [0.06-16,61]	0.991
Asthenoneurological syndrome (0 – no, 1 – yes)	58(93.5)	53(84.1)	2.73 [0.80-9.24]	0.105
Vegetative dystonia (0 – no, 1 – yes)	24(38.7)	27(42.9)	0.84 [0.41-1.72]	0.637
Abdominal pain syndrome (0 – no, 1 – yes)	45(72.6)	43(68.3)	1.23 [0.57-2.65]	0.596
Dyspeptic syndrome (0 – no, 1 – yes)	31(50.0)	39(61.9)	0.61 [0.30-1.25]	0.181
Erythropenia (0 – no, 1 – yes)	2(3.2)	3(4.8)	0.66 [0.10-4.13]	0.663
Lower hemoglobin (0 – no, 1 – yes)	7(11.3)	11(17.5)	0.60 [0.21-1.67]	0.329
Leukopenia (0 – no, 1 – yes)	13(21.0)	7(11.1)	2.12 [0.78-5.74]	0.138
Leukocytosis (0 – no, 1 – yes)	1(1.6)	2 (3.2)	0.50 [0.04-5.66]	0.576
Lymphopenia (0 – no, 1 – yes)	1(1.6)	3(4.8)	0.32 [0.03-3.24]	0.340
Lymphocytosis (0 – no, 1 – yes)	16(25.8)	15(23.8)	1.11 [0.49-2.50]	0.796
Monocytosis (0 – no, 1 – yes)	5(8.1)	3(4.8)	1.75 [0.40-7.68]	0.456
Rise in ESR (0 – no, 1 – yes)	1(1.6)	5(7.9)	0.19 [0.22-1.67]	0.135
Thrombocytopenia (0 – no, 1 – yes)	40(64.5)	39(61.9)	1.11 [0.54-2.31]	0.762
ALT level>ULN (0 – no, 1 – yes)	58(93.5)	47(74.6)	4.93 [1.54-15.76]	0.007
Minimal cytolytic activity – ALT up to 3 ULN (0 – no, 1 – yes)	32(51.6)	33(52.4)	0.97 [0.48-1.95]	0.932
Moderate cytolytic activity – ALT 3-10 ULN (0 – no, 1 – yes)	25(40.3)	14(22.2)	2.36 [1.08-5.16]	0.031

High cytolytic activity – ALT>10 ULN (0 – no, 1 – yes)	1(1.6)	-	3.09 [0.12-77.51]	0.491
AST level>ULN (0 – no, 1 – yes)	55(88.7)	43(68.3)	3.65 [1.41-9.43]	0.007
GGTP level>ULN (0 – no, 1 – yes)	41(66.1)	22(34.9)	3.63 [1.73-7.61]	0.001
LDH level>ULN (0 – no, 1 – yes)	7(11.3)	4(6.3)	1.87 [0.52-6.76]	0.336
Total bilirubin level>ULN (0 – no, 1 – yes)	23(37.1)	9(14.3)	3.53 [1.47-8.47]	0.005
ALP level>ULN (0 – no, 1 – yes)	8(12.9)	1(1.6)	9.18 [1.11-75.80]	0.039
Cholesterol level>ULN (0 – no, 1 – yes)	2(3.2)	3(4.8)	0.66 [0.10-4.13]	0.663
TG level>ULN (0 – no, 1 – yes)	5(8.1)	5(7.9)	1.01 [0.27-3.70]	0.979
Environmental factors				
Alcohol intake>40 g/day (0 – no, 1 – yes)	19(30.6)	7(11.1)	3.53 [1.36-9.17]	0.009
Smoke (0 – no, 1 – yes)	11(17.7)	14(22.2)	0.75 [0.31-1.82]	0.532
Genetic factors				
Genotype of the TLR4 gene (0 – Asp299Gly, 1 – Asp299Asp)	51(82.3)	55(87.3)	0,67 [0.25-1.81]	0.434
Genotype of the TLR7 gene (0 – Gln11Leu+Leu11Leu, 1 – Gln11Gln)	57(91.9)	45(71.4)	4.56 [1.57-13.22]	0.005

note: ULN – upper limit of normal, p – significance level

Table 4. Prognostic model of rapidly progressive hepatic fibrosis in CHC

Predictors	B	**m**	χ^2 **Wald**	p	OR	**95% CI**
GGTP level>ULN	1.10	0.45	5.77	0.016	3.01	1.22-7.40
Male gender	0.85	0.47	3.25	0.071	2.34	0.93-5.92
Gln11Gln genotype of the TLR7 gene	1.98	0.64	9.46	0.002	7.27	2.05-25.75
Chronic cholecystitis and/or pancreatitis	1.76	0.64	7.54	0.006	5.82	1.65-20.48
Total bilirubin level>ULN	1.68	0.55	7.16	0.007	4.40	1.48-13.03
AST level>ULN	1.29	0.63	4.13	0.042	3.61	1.04-12.46
α	–5.63	1.18	22.58	0.000	0.004	

note: p – significance level

Data from Table 4 shows that the final prognostic model of rapidly progressive HF in CHC comprises 6 predictors, the major part of which are available to be assessed in the routine clinical practice: GGTP level>ULN, male gender, Gln11Gln genotype of the TLR7 gene, chronic cholecystitis and/or pancreatitis, tolal bilirubin and AST levels>ULN. The findings have become the basis for ROC-analysis, according to which high operational caracteristics of the created model have been defined: sensitivity – 85.5%, specificity – 68.3%, total number of the appropriate assignments – 76.8%, positive and negative predictive value – 72.6% and 82.7%, respectively, The AUC of the ROC-curve constituted 0.840, indicating about "very good" prognostic capability (Fig.2).

Calculation of the Hosmer-Lemeshow goodness of fit test showed the significant result, too: χ^2=5.04, p=0.655.

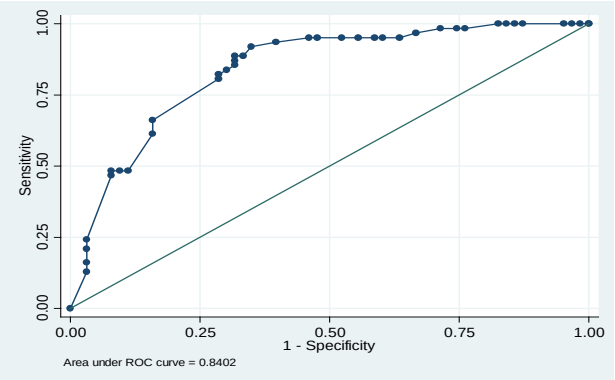

Fig. 2. ROC-curve of the final prognostic model of rapidly progressive hepatic fibrosis in CHC

Therefore, once the numeric values of regression coefficients were entered into the formula (1), the prognostic model of rapidly progressive HF (P) in HCH, depending on the identified RF and their grades, is the following:

$$P = \frac{1}{1+2.72^{-(-5.63+1.10 \cdot X_1+0.85 \cdot X_2+1.98 \cdot X_3+1.76 \cdot X_4+1.48 \cdot X_5+1.29 \cdot X_6)}}$$

, wnere
X_1 – GGTP level>ULN (0 – no, 1 – yes);
X_2 – male gender (0 – no, 1 – yes);
X_3 – genotype of the TLR7 gene (0 – Gln11Leu+Leu11Leu, 1 – Gln11Gln);
X_4 – presence of chronic cholecystitis and/or pancreatitis (0 – no, 1 – yes);
X_5 – tolal bilirubin level>ULN (0 – no, 1 – yes);
X_6 – AST level>ULN (0 – no, 1 – yes).

In this way, when all the abovementioned predictors are defined in a patient, the probability of rapidly progressive HF is 94.5%:

$$\frac{1}{1+2.72^{-(-5.63+1.10 \cdot 1+0.85 \cdot 1+1.98 \cdot 1+1.76 \cdot 1+1.48 \cdot 1+1.29 \cdot 1)}} = 0.945$$

The calculated value P>0.5 allows to assign a patient into the high-risk group of rapidly progressive HF in CHC.

Use of the prognostic model will contribute to high-accuracy prognosis of rapidly progressive HF in CHC and form the risk group, requiring individual approaches to prescribing antiviral therapy for CHC.

Conclusions:
1. The significant clinical and genetic predictors for rapidly progressive HF in CHC have been identified: male gender (OR=3.44 [95% CI 1.60-7,39], p=0.001), increased levels of ALT (OR=4.93 [95% CI 1.54-15.76], p=0.007), particularly, moderate cytolytic activity (OR=2.36 [95% CI 1.08-5.16], p=0.031), AST (OR=3.65 [95% CI 1.41-9.43], p=0,007), GGTP (OR=3.63 [95% CI 1,73-7,61], p=0,001), total bilirubin (OR=3.53 [95% CI 1.47-8.47], p=0.005), ALP (OR=9.18 [95% 1.11-75.80], p=0.039), alcohol intake>40 g/day (OR=3.53 [95% CI 1.36-9.17], p=0,009), Gln11Gln genotype of the TLR7 gene (OR=4.56 [95% CI 1.57-13.22], p=0.005), presence of chronic cholecystitis and/or pancreatitis (OR=5.30 [95% CI 1.84-15.25], p=0.002).
2. The prognostic model of rapidly progressive HF in CHC, comprising 6 predictors (GGTP level>ULN, male gender, Gln11Gln genotype of the TLR7 gene chronic cholecystitis and/or pancreatitis, tolal bilirubin and AST levels>ULN) has been created, demonstrating statistical significance (χ^2=49.36, d.f.=6, p=0.000) and high operational characteristics (sensitivity – 85.5%, specificity – 68.3%, total number of the appropriate assignments – 76.8%, positive and negative predictive value – 72.6% and 82.7%, respectively, the AUC ROC-curve – 0.840).
3. The protective role of the polymorphic Gln11Leu+Leu11Leu genotypes of the TLR7 gene with regard to RFP in CHC (OR=0.21 [95% CI 0.07-0.63], p=0.005) has been established.
4. No associations of Asp299Gly polymorphism of the TLR4 gene with RFP in CHC (OR=0.67 [95% CI 0.25-1.81], p=0.434) have been detected.

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SUMMARY

CLINICAL AND GENETIC PREDICTORS AND PROGNOSTIC MODEL OF RAPIDLY PROGRESSIVE HEPATIC FIBROSIS IN CHRONIC HEPATITIS C

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The search for risk factors for rapid progression of hepatic fibrosis (HF) in chronic hepatitis C (CHC) is a topical scientific and practical task. The purpose of the study is to identify clinical and genetic predictors and create the prognostic model of rapidly progressive HF in CHC.

A retrospective cohort study of 125 patients with CHC has been carried out. The logistic regression and ROC-analysis have been applied for statistical data processing.

The resulting analysis of 46 potential predictors of rapidly progressive HF in CHC identified the following significant ctiteria: male gender – OR=3.44 [95% CI 1.60-7.39], p=0.001; increased levels of alanine aminotransferase (ALT) – OR=4.93 [95% CI 1.54-15.76], p=0.007, particularly, moderate cytolytic activity – OR=2.36 [95% CI 1.08-5.16], p=0.031; aspartate aminotransferase (AST) – OR=3.65 [95% CI 1.41-9.43] p=0.007; γ-glutamiltranspeptidase (GGTP) – OR=3.63 [95% CI 1.73-7.61], p=0.001; total bilirubin – OR=3.53 [95% CI 1.47-8.47], p=0.005; alkaline phosphatase – OR=9.18 [95% 1.11-75.80], p=0.039; alcohol intake>40 g/day (OR=3.53 [95% CI 1.36-9.17], p=0.009), Gln11Gln genotype of the TLR7 gene (OR=4.56 [95% CI 1.57-13.22], p=0.005), presence of chronic cholecystitis and/or pancreatitis (OR=5.30 [95% CI 1.84-15.25], p=0.002). The prognostic model, comprising 6 predictors (level of GGTP>upper limit of normal (ULN), male gender, Gln11Gln genotype of the TLR7 gene chronic cholecystitis and/or pancreatitis, levels of total bilirubin and AST>ULN) have been created, demonstrating the statistical significance (p=0.000) and high operational characteristics (sensitivity – 85.5%, specificity – 68.3%, total number of the appropriate assignments – 76.8%, positive and negative predictive value – 72,6% and 82.7%,, respectively, the AUC ROC-curve – 0.840).

Use of the created model will help to predict the rapid progression of HF in CHC and form the risk-group, requiring individual approaches to prescribing antiviral therapy for CHC.

Keywords: Chronic Hepatitis C, rate of hepatic fibrosis progression, predictors, prognostic model, TLR4 and TLR7 gene polymorphisms.

РЕЗЮМЕ

КЛИНИКО-ГЕНЕТИЧЕСКИЕ ПРЕДИКТОРЫ И ПРОГНОСТИЧЕСКАЯ МОДЕЛЬ БЫСТРО ПРОГРЕССИРУЮЩЕГО ФИБРОЗА ПЕЧЕНИ ПРИ ХРОНИЧЕСКОМ ГЕПАТИТЕ С

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Поиск факторов риска быстрого прогрессирования фиброза печени (ФП) при хроническом гепатите С (ХГС) является актуальной научно-практической задачей. Целью данного исследования была идентификация клинико-генетических предикторов и создание прогностической модели быстро прогрессирующего ФП при ХГС.

Проведено ретроспективное когортное исследование 125 больных ХГС. Для статистической обработки данных применялись логистическая регрессия и ROC-анализ.

В результате анализа 46 потенциальных предикторов быстро прогрессирующего ФП при ХГС идентифицированы значимые: мужской пол – OR=3,44 [95% CI 1,60-7,39], p=0,001; повышенные уровни аланинаминотрасферазы (АЛТ) – OR= OR=4,93 [95% CI 1,54-15,76], p=0,007, в частности, умеренная активность цитолиза – OR=2,36, [95% CI 1,08-5,16]p=0,031; аспартатаминотрансферазы (АСТ) – OR=3,65 [95% CI 1,41-9,43], p=0,007; γ-глутамилтранспептидазы (ГГТП) – OR=3,63 [95% CI 1,73-7,61], p=0,001; общего билирубина – OR=3,53 [95% CI 1,47-8,47], p=0,005); щелочной фосфатазы – OR=9,18 [95% 1,11-75,80], p=0,039; титр; употребление алкоголя >40 г/сутки – OR=3,53 [95% CI 1,36-9,17], p=0,009; генотип Gln11Gln гена TLR7 – OR=4,56 [95% CI 1,57-13,22], p=0,005; наличие хронического холецистита и/или панкреатита – OR=5,30

[95% CI 1,84-15,25], p=0,002. Создана прогностическая модель из 6 предикторов (уровень ГГТП>верхней границы нормы (ВГН), мужской пол, генотип Gln11Gln гена TLR7, хронический холецистит и/или панкреатит, уровни общего билирубина и АСТ>ВГН), которая продемонстрировала статистическую значимость (p=0,000) и высокие операционные характеристики (чувствительность – 85,5%, специфичность – 68,3%, общее количество правильных отнесений – 76,8%, позитивное и негативное предиктивное значение – 72,6%, и 82,7% соответственно, AUC ROC-кривой – 0,840).

Предложенная модель позволит определить быстро прогрессирующий ФП при ХГС и сформировать группы риска для индивидуального подхода при применении противовирусной терапии ХГС.

რეზიუმე

ქრონიკული C ჰეპატიტის პირობებში სწრაფად პროგრესირებადი ღვიძლის ფიბროზის კლინიკო-გენეტიკური პრედიქტორები და პროგნოზირების მოდელი

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ჩატარდა ქრონიკული C ჰეპატიტით 125 ავადმყოფის რეტროსპექტული კოგორტული გამოკვლევა. მონაცემების სტატისტიკური დამუშავებისათვის გამოყენებულია ლოგისტიკური რეგრესია და ROC-ანალიზი

ქრონიკული C ჰეპატიტის დროს სწრაფად პროგრესირებადი ღვიძლის ფიბროზის 46 პოტენციური პრედიქტორის ანალიზის შედეგად იდენტიფიცირებულია მონაცემები: მამრობითი სქესი – OR=3.44 [95% CI 1.60-7.39], p=0.001; ალანინამინოტრანსფერაზას მაღალი დონე (ALT) – OR=4.93 [95% CI 1.54-15.76], p=0.007, კერძოდ, ციტოლიზის ზომიერი აქტივობა – OR=2.36 [95% CI 1.08-5.16], p=0.031; ასპარტატამინოტრანსფერაზას (AST) – OR=3.65 [95% CI 1.41-9.43] p=0.007; γ-გლუტამილტრანსპეპტიდაზას (GGTP) – OR=3.63 [95% CI 1.73-7.61], p=0.001; საერთო ბილირუბინი – OR=3.53 [95% CI 1.47-8.47], p=0.005; ტუტე ფოსფატაზის – OR =9.18 [95% 1.11-75.80], p=0.039; ალკოჰოლის მოხმარება >40 გ/დღე-ღამეში (OR=3.53 [95% CI 1.36-9.17], p=0.009), TLR7 გენის Gln11Gln გენოტიპი (OR=4.56 [95% CI 1.57-13.22], p=0.005), ქრონიკული ქოლეცისტიტი და/ან პანკრეატიტის შემთხვევაში (OR=5.30 [95% CI 1.84-15.25], p=0.002). პროგნოზული მოდელი შექმნილია 6 პრედიქტორისაგან (GGTP დონე >ნორმის ზედა ზღვარზე, მამრობითი სქესი, TLR7 გენის Gln11Gln გენოტიპი, ქრონიკული ქოლეცისტიტი და/ან პანკრეატიტი, საერთო ბილირუბინის და AST დონეები > ნორმის ზედა ზღვარზე). აღნიშნულმა მოდელმა გამოამჟღავნა სტატისტიკური მნიშვნელობა (p=0.000) და მაღალი ოპერაციული მახასიათებლები (მგრძნობელობა – 85.5%, სპეციფიურობა – 68.3%, პოზიტიური და ნეგატიური პრედიქტორული მნიშვნელობა – 72.6% და 82.7%, შესაბამისად, AUC ROC-მრუდი – 0.840).

ავტორების მიერ შემოთავაზებული მოდელი საშუალებას იძლევა ქრონიკული C ჰეპატიტის პირობებში დროულად დადგინდეს ღვიძლის ფიბროზი, განისაზღვროს რისკის ჯგუფები და დაინიშნოს C ჰეპატიტის ვირუსსაწინააღმდეგო ადეკვატური მკურნალობა ავადმყოფის ინდივიდუალური თვისებების გათვალისწინებით.