Prognostic model of rapid hepatic fibrosis progression in men with chronic hepatitis C


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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim of the research was to determine clinical and genetic predictors and to create a prognostic model for the rapid hepatic fibrosis progression in men with chronic hepatitis C.

Materials and methods. A cross-sectional study which included 111 male patients with chronic hepatitis C was conducted. The patient examination program included: assessment of complaints and anamnestic data, physical examination, complete blood count, biochemical test, the stages of hepatic fibrosis according to METAVIR and genetic studies (detecting carriers alleles 11Gln or 11Leu of TLR7 gene in the genome of the examined men).

Results. It was determined that informative predictors of rapid hepatic fibrosis progression in men with chronic hepatitis C are: ethanol use in a dose of more than 40 g/day (OR = 2.40, P = 0.042), presence of chronic cholecystitis in past history (OR = 2.94, P = 0.013), ALT level above 3 upper limit of normal (OR = 2.49, P = 0.031), the levels of AST, GGT exceeding upper limit of normal (OR = 6.94, P = 0.001 and OR = 4.02, P = 0.001 respectively), hyperbilirubinemia (OR = 3.13, P = 0.010) and carrier state of allele 11Gln of TLR7 gene in the genome (OR = 3.62, P = 0.036). In order to optimize the prognosis of rapid hepatic fibrosis progression in men with chronic hepatitis C a model that demonstrated statistical significance (χ² = 44.73, P < 0.001) and high operational characteristics (sensitivity – 76.8 %, specificity – 74.5 %, the total number of correct predictions – 75.7 %, AUC of the ROC-curve – 0.828), which indicates the feasibility of its practical use, was proposed.

Conclusions. An effective clinical and genetic prognostic model has been created and allows us to predict the probability of rapid hepatic fibrosis progression in men with chronic hepatitis C with high accuracy and to form a group of patients who need high priority antiviral therapy.

Key words: chronic hepatitis C, hepatic fibrosis, men, prognosis, TLR7 gene.

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It is widely known that the development of fibrotic changes in the liver is an integral part of the pathogenesis and natural course of chronic hepatitis C [1]. Currently, hepatic fibrosis is considered to be a process when a certain number of external factors interact with a unique combination of host factors, which causes significant differences in the course of chronic hepatitis C. There are virus factors (genotype and HCV quasi species, viral load level), host factors (duration of disease, age over 40 years at the time of infection, male gender, co-infection with hepatitis B virus and/or HIV, metabolic disorders – insulin resistance, hepatic steatosis, type 2 diabetes mellitus, iron metabolism disorders, etc.), as well as external (alcohol abuse, effect of toxins and, in particular, drugs, tobacco smoking and/or cannabinol derivatives) among the factors affecting the rate of hepatic fibrosis progression in chronic hepatitis C [1–4].

Recently, the attention of the researchers has been drawn to the search for genetic determinants that affect the rate of hepatic fibrosis progression in chronic hepatitis C, in particular, the TLR7 gene, which triggers the effector mechanisms of innate immunity and also it effectively regulates the production of IFN Type I which, for its turn, has an antifibrotic effect [5–9]. Conversely, the Gln11Leu polymorphism of the TLR7 gene encodes functionally inferior proteins and is able to reduce the production of IFN-α, thereby disrupting the adaptive immune response, which is realized through the TLR7-dependent signaling pathway [5,7,10,11].

Considering the fact that chronic hepatitis C is detected more often in males, the rate of hepatic fibrosis progression is gender dependent, as well as the availability of data on the influence of the polymorphic 11Leu allele of the TLR7 gene on this process [12–16], scientific and practical interest represents the search for clinical genetic predictors and the creation of a prognostic model for the rapid hepatic fibrosis progression in men.

**The aim of the research**

To determine clinical and genetic predictors and to create a prognostic model for the rapid hepatic fibrosis progression in men with chronic hepatitis C.
use), in the absence of the anamnesis of these facts – on
the basis of clinical and laboratory data (the first detec-
tion of antibodies to HCV and/or hepatic transaminases
elevation the upper limit of normal (ULN), reflected in
outpatient cards).

Biochemical studies were carried out on the automatic
biochemical analyzer GBG STAT FAX-1904 (Japan) with
Human reagents (Germany).

The hepatic fibrosis stage was assessed on the
META VIR scale using the transient elastometry of
shear waves of the liver on the ultrasound scanning
device "Ultima PA-Expert" (Ukraine). The rate of hepatic
fibrosis progression was calculated by T. Poniard’s formula
by dividing the stage of hepatic fibrosis by META VIR for
the time, for which it was formed, and measured in units
per year (units/year) [3].

The gene TLR7 was genotyped by real-time al-
lele-specific PCR on the “DT Lite” amplifier (“NPO
DNA-Technology”, LLC, RF) on the basis of the Research
Institute for Genetics and Immunological Grounds of
Pathology and Pharmacogenetics of Ukrainian Medical
Stomatological Academy.

Statistical processing of the findings was carried
out using the Stata software version 11.0 (StataCorp,
College Station, TX, USA, serial number 71606281563).
The verification of the normality of the data distribution
was analyzed by the Kolmogorov-Smirnov criterion. To
determine the central trend, the value of the median with
the upper and lower quartiles was used. The probability
of differences in quality indicators was determined by an-
alyzing contingency tables using Fisher’s exact test and χ²
test, depending on the conditions of the analysis. To create
a prognostic model, 30 indicators ranked in the nominal
scale were considered as potential predictors of the rapid
hepatic fibrosis progression (1 – sign, 0 – none). The
influence of each was estimated by the method of simple
logistic regression with the calculation of the odds ratio
(OR) and its 95 % confidence interval [95 % CI]. Predic-
tors with a significance level of P < 0.05 were included in
a systematic multiple logistic regression analysis, which
resulted in a clinical prognostic model of the rapid hepatic
fibrosis progression in men with chronic hepatitis C. In
general, the model assumes that the dependent variable
(rapid progression of hepatic fibrosis) is associated with
predictors in accordance with the following formula:

\[ P = \frac{1}{1 + e^{-y}} \]

where P is the probability of an error-free prognosis; \( e \) is a mathematical constant, which is equal to 2.72; \( y = \alpha + B_1X_1 + B_2X_2 + \ldots + B_nX_n \); \( \alpha \) is
the constant of the regression equation; \( B_1, B_2, \ldots B_n \) – regression coefficients for independent variables; \( X_1, X_2, \ldots X_n \) – independent variables included in the model.

The statistical significance of the obtained model was
determined by the \( \chi^2 \) criterion, the evaluation of diagnostic
power – using the analysis of the operating characteristics
of diagnostic tests (ROC), which included calculations of
sensitivity, specificity, the total number of correct
predictions and the construction of the ROC-curve with
the definition of the area under it (AUC). The delimitation
point, according to generally accepted criteria, was taken

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with chronic hepatitis C, ( n = 56 )</th>
<th>Slow hepatic fibrosis progression, ( n = 55 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 genotype of HCV</td>
<td>36 (64.3)</td>
<td>28 (50.9)</td>
<td>0.154</td>
</tr>
<tr>
<td>High viral load</td>
<td>32 (57.1)</td>
<td>23 (41.8)</td>
<td>0.912</td>
</tr>
<tr>
<td>Age older 40</td>
<td>29 (51.8)</td>
<td>23 (41.8)</td>
<td>0.239</td>
</tr>
<tr>
<td>Type II diabetes mellitus</td>
<td>5 (8.9)</td>
<td>1 (1.8)</td>
<td>0.206</td>
</tr>
<tr>
<td>Alcohol use in a dose of more than 40 g/day</td>
<td>21 (37.5)</td>
<td>11 (20.0)</td>
<td>0.042</td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td>16 (28.6)</td>
<td>23 (41.8)</td>
<td>0.144</td>
</tr>
<tr>
<td>Smoking cannabino derivatives</td>
<td>11 (20.0)</td>
<td>5 (9.1)</td>
<td>0.175</td>
</tr>
<tr>
<td>Intravenous drug abuse</td>
<td>5 (8.9)</td>
<td>2 (3.6)</td>
<td>0.437</td>
</tr>
<tr>
<td>Overweight, BMI ( \geq 25 ) kg/m²</td>
<td>10 (17.9)</td>
<td>6 (10.9)</td>
<td>0.419</td>
</tr>
<tr>
<td>Carrier state of the ( 11\text{Leu} ) TLR7 allele</td>
<td>4 (7.1)</td>
<td>12 (21.8)</td>
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#### Results

The study found that the examined men with chronic
hepatitis C had various stages of hepatic fibrosis, without
predominance of any of them. Thus, there were 7 patients
(6.3 %) without fibrosis, 19 (17.1 %) with \( F_1 \) stages,
\( F_2 \) – 33 (29.7 %), \( F_3 \) – 23 (20.7 %) and \( F_4 \) – 29 (26.1 %). By the duration of HCV infection, the patients were di-
vided as follows: less than 5 years – 39 (35.1 %), from
5 to 10 – 24 (21.6 %) and more than 10 – 48 (43.3 %).

Based on the obtained data, the median rate of hepatic
fibrosis progression was determined and amounted to
0.222 (0.125–1.000) units/year. Depending on the rate of
hepatic fibrosis progression, patients with rapid (fibrosis
progression rate \( \geq 0.222 \) units/year) – 56 (50.5 %) and
slow (fibrosis progression rate \( < 0.222 \) units/year) hepatic
fibrosis progression – 55 (49.5 %) were identified.

Further, the main characteristics of the examined men
with rapid and slow progression of hepatic fibrosis were
analyzed, taking into account the well-known risk factors
affecting this process and genetic markers (Table 1).

According to the data presented in table 1, alcohol
abuse – 37.5 % (with slow hepatic fibrosis progression –
20.0 %, \( P = 0.041 \)) and carrier state of the \( 11\text{Leu} \) TLR7
allele – 7.1 % (with slow hepatic fibrosis progression –
21.8 %, \( P = 0.033 \)) were significantly more frequent
identified among patients with a rapid hepatic fibrosis
progression. No statistically significant differences were
found for the remaining characteristics.

Further, a simple logistic regression analysis was

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Here is an example of calculation for a patient with the presence of all the specified predictors:

$$P = \frac{1}{1 + e^{(-4.67 + 1.97 \cdot 1 + 2.22 \cdot 1 + 1.1 \cdot 1 + 1.58 \cdot 1 + 1.18 \cdot 1)}} = 0.970$$

Thus, in this case, the probability of rapid hepatic fibrosis progression is 97.0%.

**Discussion**

Nowadays, hepatic fibrosis is considered as a process when a number of extraneous factors interact with a unique combination of the host’s ones and causes significant differences in a natural course of chronic hepatitis C. Progression of the disease into cirrhosis occurs over several decades, on average – 20–30 years from the time of infection [1]. The prognosis of chronic hepatitis C is based on the idea of the rate of hepatic fibrosis progression, which is proposed to be calculated by dividing the stage of hepatic fibrosis (in units) by the duration of the disease (in years) from the moment of infection to the study. Such calculations that indicate the stability of the rate of hepatic fibrosis progression are the basis for predicting the period of cirrhosis formation. Summarizing the data of a large-scale study, T. Poynard (1997) identified three options for the progression of fibrosis, each of which is observed in about a third of patients with chronic hepatitis C: rapid (cirrhosis develops within 20 years after HCV-infection), average (cirrhosis develops in 30 years after HCV-infection) and slow rate (cirrhosis develops in more than 50 years) [3]. But a number of other researchers divide this process exclusively into rapid and slow [17–20]. The rate of hepatic fibrosis progression is the main characteristic of the patient since the patients with the rapid progression of fibrosis to cirrhosis are the first candidates for antiviral therapy of chronic hepatitis C. Along with universally recognized risk factors that have an influence on the rate of hepatic fibrosis progression, significant role belongs to genetic markers. Comparison of genetic studies with clinical materials demonstrated the existence of a significant effect of the genetic polymorphism on this process in patients with chronic hepatitis C, however, the analysis of the complex impact of clinical data and genetic polymorphism was carried out in only a few works [2,13,17,20–23].

As a result of our study we have created the prognostic model of the rapid progression of hepatic fibrosis in men with chronic hepatitis C. The predictors included in the model are consistent with data from the scientific literature. Thus, the study confirmed the well-known fact of influence on the rate of hepatic fibrosis progression of such a factor as alcohol abuse [1,3]. There are no doubts about the data on the influence of increased levels of such functional indicators as AST, GGT and total bilirubin, because they are nondiagnostic biochemical markers of fibrogenesis – they indicate activity of inflammation in liver tissues and disruption of its synthetic function and allow indirectly estimate a hepatic fibrosis progression is 97.0%.

**Table 2.** Resulting prognostic model of rapid hepatic fibrosis progression in men with chronic hepatitis C

<table>
<thead>
<tr>
<th>Predictors</th>
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<th>χ² Wald</th>
<th>P</th>
<th>OR</th>
<th>95 % CI</th>
</tr>
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<tr>
<td>Carrier state of allele 11Gln TLR7</td>
<td>1.97</td>
<td>7.02</td>
<td>0.008</td>
<td>7.23</td>
<td>1.67–31.28</td>
</tr>
<tr>
<td>AST level above ULN</td>
<td>2.22</td>
<td>12.41</td>
<td>&lt;0.001</td>
<td>9.25</td>
<td>2.68–31.87</td>
</tr>
<tr>
<td>GGT level above ULN</td>
<td>1.10</td>
<td>5.17</td>
<td>0.023</td>
<td>3.02</td>
<td>1.16–7.83</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>1.58</td>
<td>7.69</td>
<td>0.006</td>
<td>4.88</td>
<td>1.59–14.96</td>
</tr>
<tr>
<td>Ethanol use in a dose of more than 40 g/day</td>
<td>1.18</td>
<td>4.87</td>
<td>0.027</td>
<td>3.28</td>
<td>1.14–9.42</td>
</tr>
<tr>
<td>Constant (α)</td>
<td>−4.67</td>
<td>19.81</td>
<td>&lt;0.001</td>
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Here is an example of calculation for a patient with the presence of all the specified predictors:

$$P = \frac{1}{1 + e^{(-4.67 + 1.97 \cdot 1 + 2.22 \cdot 1 + 1.1 \cdot 1 + 1.58 \cdot 1 + 1.18 \cdot 1)}} = 0.970$$
the 11Leu allele as a profibrogenic factor, and E. Askar (2010), who denies the influence of this polymorphism on fibrogenesis in chronic hepatitis C [10,14].

The use of the proposed clinical and genetic prognostic model allows predicting the probability of rapid hepatic fibrosis progression in men with chronic hepatitis C with high accuracy and forming a group of patients who need to receive antiviral therapy in the first place on the basis of simple characteristics, most of which are used in a routine clinical practice.

Conclusions

1. Informative predictors of rapid hepatic fibrosis progression in men with chronic hepatitis C are: ethanol use in a dose of more than 40 g/day (OR = 2.40 [95 % CI 1.02–5.63], P = 0.042), presence of chronic cholecytitis in past history (OR = 2.94 [95 % CI 1.25–6.87], P = 0.013), ALT level above 3 ULN (OR = 2.49 [95 % CI 1.08–5.74], P = 0.031), the levels of AST and GGT exceeding ULN (OR = 6.94 [95 % CI 2.55–18.86], P < 0.001 and OR = 4.02 [95 % CI 1.82–8.87], P = 0.001 respectively), hyperbilirubinemia (OR = 3.13 [95 % CI 1.31–7.46], P = 0.010) and carrier state of allele 11Gln of TLR7 gene in the genome (OR = 3.62 [95 % CI 1.09–12.06], P = 0.036).

2. In order to optimize the prognosis of rapid hepatic fibrosis progression in men with chronic hepatitis C a model that demonstrated statistical significance ($\chi^2 = 44.73$, P < 0.001) and high operational characteristics (sensitivity – 76.8 %, specificity – 74.5 %, the total number of correct predictions – 75.7 %, AUC of the ROC curve – 0.828), which indicates the feasibility of its practical use, was proposed.

Prospects for further research are to study the pathogenetic mechanisms of the influence of the TLR7 gene on the course of chronic hepatitis C.

Conflicts of interest: authors have no conflict of interest to declare.


