THE PROGNOSTIC VALUE OF BLOOD MARKERS IN PREDICTION OF THE PROBABILITY OF THE DEVELOPMENT OF FIBROTIC PROCESS IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS

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Summary

Introduction. Recently, a negative trend of increasing the levels of prevalence, disability and mortality caused by chronic viral hepatitis C (HCV) infection has been determined. Around the world is defined 0.6-10.0 % suffer from chronic HCV (about 71 million with an annual increase of 1.75 million cases). Even higher are the levels of HCV seropositivity, which according to the WHO are about 100 million people (1.6 % of the world population). The WHO identified the need to improve the diagnosis of chronic HCV and to identify its asymptomatic forms and irreversible consequences (liver fibrosis and liver cirrhosis). Increasing the effectiveness of existing and developing new diagnostic approaches to improve early detection of chronic HCV and its consequences (liver fibrosis) is an urgent issue.

The aim. To determine the prognostic possibilities of blood markers for the diagnosis of the development of the fibrotic process in chronic viral hepatitis C.

Materials and methods. 78 people were examined: 47 (main group) – with chronic HCV and 31 – without chronic HCV (comparison group).

Results. Probable associations with increased risks of development of LF in chronic HCV were: increased Mean Corpuscular Volume (MCV) (OR=4.305; 95.0 % CI 1.187-15.619; p=0.026) and Platelets (OR=0.955; 95.0 % CI 0.922-0.989; p=0.011), which indicated increased chances of developing LF in chronic HCV when exceeding the standard MCH indicators (by 4.305 times) and reduced chances – when increasing Platelets (on 4.5 %).

Conclusions. Based on the research, it was determined that blood markers can be used as a significant predictor of the development of LF of patients with chronic HCV. Increased levels of MCH and Platelets in blood serum characterize a significant relationship with the development of LF in patients with chronic HCV, which indicates a significant influence of blood markers on the pathogenesis of LF in patients with chronic HCV.

Key words: chronic viral hepatitis C, clinical blood analysis, liver fibrosis, prognostic value, probabilities of development of the fibrotic process

INTRODUCTION

Recently, a negative trend of increasing the levels of prevalence, disability and mortality caused by chronic viral hepatitis C (HCV) infection has been determined [1, 2]. Around the world, depending on geographic characteristics, is defined 0.6-10.0 % suffer from chronic HCV (about 71 million [3, 4] with an annual increase of 1.75 million cases [5]). Even higher are the levels of HCV seropositivity, which according to the WHO are about 100 million people [6] (1.6 % [95.0 % confidence intervals (CI) 1.3-2.1 %] of the world population).

High seropositivity is explained by the frequent chronicity of HCV, which according to the WHO is 55.0-85.0 % [7]. Chronic HCV provokes the development of an inflammatory reaction in the liver, which in 10.0-20.0 % of cases transforms into liver fibrosis (LF) and liver cirrhosis (LC) [8] and can cause hepatocellular carcinoma (HCC) and liver failure [9] and death [6] from...
the effects of LC and HCC [10]. Annual mortality from chronic HCV is 580-745 thousand cases [11].

High mortality in chronic HCV is determined not only by the frequent development of LF and KC, but also by a high level of rather long absence of obvious clinical manifestations of chronic HCV within 20-30 years after infection [12]. Therefore, the WHO identified the need to improve the diagnosis of chronic HCV and to identify its asymptomatic forms and irreversible consequences (LF, LC, HCC) [13].

Taking this into account, increasing the effectiveness of existing and developing new diagnostic approaches to improve early detection of chronic HCV and its consequences (primarily LF, which provokes the development of LC and HCC) is an urgent issue. Therefore, determining the possibility of diagnosing the asymptomatic course of chronic HCV by establishing the probability of the development of a fibrotic process is an urgent problem of modern medicine, which can be solved by establishing the prognostic value of blood markers in patients with HCV.

The aim. To determine the prognostic possibilities of blood markers for the diagnosis of the development of the fibrotic process in chronic viral hepatitis C (HCV).

MATERIALS AND METHODS

We conducted a randomized controlled single-center prospective study case-control, which is based on the analysis of the results of study of the analysis of the probabilities of the development of the fibrotic process against the background of chronic HCV by determining the prognostic value of blood markers. The study was conducted on the basis of the regional clinical infectious disease hospital of the city of Kharkiv. It was examined for this 78 people (36 (46.2 %) women and 42 (53.8 %) men) were examined, who were divided into two research groups: the main group (19 (40.4 %) women and 28 (59.6 %) men with chronic HCV and LF) and control group (respectively 17 (54.8 %) and 14 (45.2 %) women and men without chronic HCV and LF).

All respondents before the start of the study were fully informed about the voluntariness of their participation in this study and the complete confidentiality of the information received from them. The respondents surveyed by us took part in the study entirely of their own free will, which was confirmed by their personal signature in the relevant informed consent. Inclusion criteria were: reaching 18 years of age, presence (main group) or absence (control group) of chronic HCV and LF, consent to participate in the study. Exclusion criteria were: not reaching 18 years of age, absence (main group) or presence (control group) of chronic HCV and LF, presence: diffuse and focal diseases, diabetes and other endocrine pathology, allergic reactions, systemic connective tissue diseases, acute and chronic inflammatory diseases of internal organs, severe decomposed somatic pathology, psychiatric and oncological diseases, acute cardiovascular disorder, thyrotoxic crisis, acute and significant decapsulation of carbohydrate metabolism; availability: unsatisfactory physical condition, pregnancy and breastfeeding, chronic alcoholism, refusal to participate in the study and refusal to comply with all prescriptions. The average age of the main group was 45.0 [39.0; 51.0], and the control group – 48.0 [43.0; 51.0] years old. The duration of chronic HCV from the moment of detection in the main group was at the level of 3.0 [1.0; 7.0] years.

Determination of indicators of clinical blood analysis was carried out using generally accepted methods. The presence of chronic HCV was confirmed by the detection of antibodies (IgG, IgM) to HCV. Acoustic impulse-wave (ARFI) ultrasound study (Siemens, Erlangen, Germany) of focal liver lesions was used to diagnose LF.

Medical-statistical calculation of the obtained results was performed using the IBM SPSS 25.0 application program package for Windows.

Qualitative indicators were described in the form of absolute and relative (%) values. When characterizing the central tendency and variability of quantitative traits, the median (Me) and 25.0 % (LQ) and 75.0 % (UQ) quartiles were determined. The obtained results were presented in the form of Me [Lq; Uq].

Comparison of two independent groups of quantitative indicators was performed using the Mann-Whitney U-test (Mann-Whitney U-test).

Associations of indicators with the binomial dependent variable were determined using multiple logistic regression analysis with the calculation of β coefficients, standardized β coefficients (odds ratio (OR) and their 95.0 % CI). The quality check of the obtained models was carried out by calculating the Nagelkerke R2 criterion (Nagelkerke R2).

In the regression analysis, univariate and multivariate analysis were used (methods of simultaneous inclusion and stepwise exclusion of Wald variables in the mathematical model to obtain the most likely independent predictors).

Coding of groups in regression models was as follows: group without chronic HCV – reference group; group with chronic HCV – comparison group.

Significance level (p) in the study was taken as lower than 0.05.

The work is a fragment of research work The Department of Infectious Diseases Kharkiv National Medical University «Development of a system of early diagnosis and optimization of treatment of patients with acute respiratory diseases with pneumonia» (№ state registration 0121U110931), deadline: 2021-2023, project leader – Head of the Department of Infectious Diseases Kharkiv National Medical University, Doctor of Medical Sciences, professor Kateryna V. Yurko.
RESULTS

At the beginning of the study, we determined the characteristics of the clinical blood analysis of patients with chronic HCV compared to controls – Table 1. Were determined the levels of Erythrocytes, Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets, Thrombocrit, Color Index (CI), Erythrocyte Sedimentation Rate (ESR), Leukocytes, Rod-Nuclear (R/N) and Segment-Nuclear (S/N) Neutrophils, Eosinophils, Lymphocytes and Monocytes – Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control group (n=31)</th>
<th>Main group (n=47)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes, 10¹²/l</td>
<td>4.85 [4.43; 5.39]</td>
<td>4.78 [4.46; 5.16]</td>
<td>0.400</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td>142.0 [133.0; 151.0]</td>
<td>144.0 [133.0; 155.0]</td>
<td>0.382</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.43 [0.41; 0.45]</td>
<td>0.44 [0.41; 0.5]</td>
<td>0.400</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>90.7 [88.2; 92.5]</td>
<td>90.0 [86.0; 93.0]</td>
<td>0.471</td>
</tr>
<tr>
<td>MCH, pg/l</td>
<td>28.3 [27.4; 29.4]</td>
<td>30.7 [29.5; 32.2]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCHC, g/l</td>
<td>338.0 [328.0; 345.0]</td>
<td>338.0 [330.0; 347.0]</td>
<td>0.530</td>
</tr>
<tr>
<td>Platelets, 10⁹/l</td>
<td>275.0 [245.0; 293.0]</td>
<td>208.0 [166.0; 243.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thrombocrit</td>
<td>0.21 [0.18; 0.27]</td>
<td>0.18 [0.16; 0.21]</td>
<td>0.009</td>
</tr>
<tr>
<td>CI</td>
<td>0.91 [0.89; 0.94]</td>
<td>0.90 [0.87; 0.94]</td>
<td>0.323</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>3.0 [1.0; 4.0]</td>
<td>9.0 [5.0; 15.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leukocytes, 10⁹/l</td>
<td>5.91 [4.66; 6.71]</td>
<td>6.70 [5.20; 7.80]</td>
<td>0.057</td>
</tr>
<tr>
<td>R/N Neutrophils, %</td>
<td>7.0 [6.0; 8.0]</td>
<td>2.0 [1.0; 3.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S/N Neutrophils, %</td>
<td>79.0 [76.0; 81.0]</td>
<td>55.0 [44.0; 65.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>1.0 [1.0; 1.0]</td>
<td>1.0 [1.0; 3.0]</td>
<td>0.033</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>8.0 [6.0; 10.0]</td>
<td>36.0 [25.0; 40.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monocytes, %</td>
<td>5.0 [3.0; 6.0]</td>
<td>6.4 [4.0; 8.0]</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Defined probably lower values were determined in the control group compared to main group: MCH (respectively 28.3 [27.4; 29.4] and 30.7 [29.5; 32.2] pg/l; p<0.001); ESR (respectively 3.0 [1.0; 4.0] and 9.0 [5.0; 15.0] mm/hr; p<0.001); Leukocytes (respectively 5.91 [4.66; 6.71] and 6.70 [5.20; 7.80] 10⁹/l; p=0.057); Lymphocytes (respectively 8.0 [6.0; 10.0] and 36.0 [25.0; 40.0] %; p<0.001) and Monocytes (respectively 5.0 [3.0; 6.0] and 6.4 [4.0; 8.0] %; p=0.012). Other values of clinical blood analysis, on the contrary, in the control group were higher compared to main group: Platelets – 275.0 [245.0; 293.0] and 208.0 [166.0; 243.0] 10⁹/l; p<0.001; Thrombocrit – 0.21 [0.18; 0.27] and 0.18 [0.16; 0.21]; p=0.009; R/N Neutrophils – 7.0 [6.0; 8.0] and 2.0 [1.0; 3.0] %; p<0.001 and S/N Neutrophils – 79.0 [76.0; 81.0] and 55.0 [44.0; 65.0] %; p<0.001. Eosinophil levels were similar in both groups: 1.0 [1.0; 1.0] and 1.0 [1.0; 3.0] %; p=0.033 respectively control group and main group – Table 1.

Determination of associations of blood parameters of patients with chronic HCV with existing LF by univariate logistic regression analysis established reliable predictive influences on the development of LF of increased levels MCH (OR=1.598; 95.0 % CI 1.116-2.289; p=0.011) and Platelets (OR=0.982; 95.0 % CI 1.007-1.069; p=0.021), which indicated increased chances of developing LF in chronic HCV when exceeding the standard MCH indicators (by 1.598 times) and reduced chances – when increasing Platelets (on 1,8 %) – Table 2.

### Table 2

#### Associations indicators of clinical blood analysis of the examined with existing LF

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95.0 % CI)</td>
<td>p</td>
</tr>
<tr>
<td>Erythrocytes, 10¹²/l</td>
<td>0.413 (0.109-1.560)</td>
<td>0.192</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td>0.986 (0.944-1.030)</td>
<td>0.521</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.992 (0.862-1.142)</td>
<td>0.915</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>1.598 (1.116-2.289)</td>
<td>0.011</td>
</tr>
<tr>
<td>MCH, pg/l</td>
<td>0.994 (0.950-1.041)</td>
<td>0.807</td>
</tr>
<tr>
<td>Platelets, 10⁹/l</td>
<td>0.982 (0.967-0.997)</td>
<td>0.021</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>1.070 (0.979-1.170)</td>
<td>0.137</td>
</tr>
<tr>
<td>Leukocytes, 10⁹/l</td>
<td>0.994 (0.743-1.330)</td>
<td>0.968</td>
</tr>
<tr>
<td>R/N Neutrophils, %</td>
<td>1.109 (0.794-1.548)</td>
<td>0.544</td>
</tr>
<tr>
<td>S/N Neutrophils, %</td>
<td>1.030 (0.980-1.083)</td>
<td>0.247</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.871 (0.590-1.286)</td>
<td>0.487</td>
</tr>
<tr>
<td>Basophils, %</td>
<td>0.916 (0.311-2.704)</td>
<td>0.874</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>0.960 (0.903-1.020)</td>
<td>0.183</td>
</tr>
</tbody>
</table>
Multivariate logistic regression analysis confirmed predictive influences on the development of LF of increased levels MCH (OR=4.305; 95.0 % CI 1.187–15.619; p=0.026) and Platelets (OR=0.955; 95.0 % CI 0.922–0.989; p=0.011), which indicated increased chances of developing LF in chronic HCV when exceeding the standard MCH indicators (by 4.305 times) and reduced chances – when increasing Platelets (on 4.5 %) – Table 2.

**DISCUSSION**

Our results regarding the significant associations blood markers of blood of patients with chronic HCV with existing LF are completely consistent with other conducted studies. Thus, Rasheed H. et al. [14] during the study of 100 people of the control group (without HCV) and 100 patients with chronic HCV, determined, what in cohort of HCV patients, the analysis showed a statistically significant difference in terms of hemoglobin g/dl [p=0.0006], Platelet 10^3/l [p=0.0007], leucocyte count 10^3/l [p=0.0023], hematopoietic cell transplantation % [p=0.0328], neutrophil % [p=0.1574] and neutrophil/lymphocyte ratio [p=0.0324] (p<0.05). Blood samples from 100 controls and 100 HCV cases were collected from various hospitals in Punjab, Pakistan, between August 2021 and January 2022.

Elhiblu M. A. et al. [15] when conducting research on animals with cirrhosis of the liver with clinical signs of liver failure stated, that levels of hemoglobin, lymphocytes, packed cell volume, MCV, MCH and platelet count were significantly lower in liver cirrhosis group than control group while total leukocyte count, neutrophils, and MCH concentration were significantly higher. The mean values of hemoglobin (p<0.05), packed cell volume (p<0.05), lymphocytes (p<0.01), MCV (p<0.01), MCH (p<0.01) and platelets (p<0.01) were significantly lower in liver cirrhosis group than control group, while total leukocyte (p<0.01), neutrophils (p<0.01) and MCHC (p<0.01) were significantly higher than the control group.

Tsai M. H. et al. [16] proved that platelet-related indices significantly differed between the HCV-infected patients and the control group (without HCV). Compared with the control, the HCV-infected patients had significantly lower platelet counts but significantly higher platelet distribution widths, mean platelet volumes, and platelet-large cell ratios. Compared with the control group, the HCV-infected group showed significantly higher red blood cell counts, hemoglobin levels, and hematocrit levels and higher white blood cell counts, lymphocyte counts, and monocyte counts. Correlations with values for key indices and viral load were also determined. Strong predictors of HCV infection were found by using receiver operating characteristics curves to analyze the optimal subsets among red blood cells, monocytes, platelet counts, platelet large cell ratios, and mean corpuscular hemoglobin concentrations.

Rasheed H. et al. [17] during research blood samples from hepatitis B virus (HBV) patients and healthy subjects in Punjab determined what the hemoglobin, hematopoietic cell transplantation, MCHC, MCV, MCH and neutrophil/lymphocyte ratio of patients and controls showed great differences. The white blood cells, red blood cells, platelets, neutrophils, lymphocytes, monocytes, and eosinophils in hepatitis B patients showed no association. The outcome by applying a t-test revealed that there was a statistically significant difference between the cases and controls in hemoglobin g/dl [p<0.0001], hematopoietic cell transplantation % [p<0.0001], MCHC g/dl [p<0.0001], MCV fl [p=0.0116], MCH pg [p=0.0116] and neutrophil/lymphocyte ratio [p=0.5303] (p<0.05). Primary results indicate a promising biomarker to monitor HBV infection by using information from hematological parameters.

**CONCLUSIONS**

Based on the research, it was determined that blood markers can be used as a significant predictor of the development of LF of patients with chronic HCV. Increased levels of MCH and Platelets in blood serum characterize a significant relationship with the development of LF in patients with chronic HCV, which indicates a significant influence of blood markers on the pathogenesis of LF in patients with chronic HCV. The results indicate a direct relationship between the pathogenesis of the comorbidity LF and chronic HCV with MCH and Platelets values, which should be taken into account to ensure therapeutic and preventive measures. Univariate analysis reliably determined trends towards high chances of LF development with increasing MCH values (OR=1.598; 95.0 % CI 1.116–2.289; p=0.011) and platelet levels (OR=0.982; 95.0 % CI 0.967–0.997; p=0.021). According to multivariate analysis, an increase in the chances of developing LF was reliably established with an increase in MCH values – by 4.305 times (OR=4.305; 95.0 % CI 1.187–15.619 0.026) and a decrease in the chances by 4.5 % with a decrease in platelet levels (HR=0.955; 95.0 % CI 0.922–0.989; p=0.011).

**Prospects for Further Research.** Further randomized clinical trials in larger patient cohorts are necessary to provide a more objective assessment of the results.

**COMPLIANCE WITH ETHICAL REQUIREMENTS**

The ethical approval was obtained from Bioethics Committee of the Kharkiv National Medical University. All patients provided written consent to participate in research in accordance with the recommendations of the Ethics Committees for Biomedical Research, Ukrainian Health Legislation and the Declaration of Helsinki of 2000, European Community Directive 86/609 On Human Participation in Biomedical Research.

**FUNDING AND CONFLICT OF INTEREST**

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REFERENCES


REFERENCES (APA)


Резюме

ПРОГНОСТИЧНЕ ЗНАЧЕННЯ МАРКЕРІВ КРОВІ В ПРОГНОЗУВАННІ ЙМОВІРНОСТІ РОЗВИТКУ ФІБРОТИЧНОГО ПРОЦЕСУ У ХВОРИХ НА ХРОНІЧНИЙ ВІРУСНИЙ ГЕПАТИТ С

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Вступ. Останнім часом спостерігається негативна тенденція зростання рівня поширеності, інвалідності та смертності від хронічного вірусного гепатиту С (ВГС). У всьому світі визначено 0,6-10,0 % хворих на хронічний ВГС (близько 71 мільйона з щорічним збільшенням на 1,75 мільйона випадків). Ще вищим є рівні серопозитивності ВГС, які за даними ВООЗ становлять близько 100 млн. осіб (1,6 % населення світу). ВООЗ визначила необхідність удосконалення діагностики хронічного ВГС та виявлення його безсимптомних форм і необоротних наслідків (фіброз печінки та цироз печінки). Тому, підвищення ефективності існуючих та розробка нових діагностичних підходів для покращення раннього виявлення хронічного ВГС та його наслідків (фіброзу печінки) є актуальним питанням.

Мета. Визначити прогностичні можливості маркерів крові для діагностики розвитку фіброзного процесу при хронічному вірусному гепатиті С.

Матеріали та методи. Обстежено 78 осіб: 47 (основна група) – з хронічним ВГС та 31 – без хронічного ВГС (група порівняння).

Результати. Були визначені ймовірні асоціації з підвищеним ризиком розвитку фіброзу печінки при хронічному ВГС: збільшення середнього корпускулярного об’єму (ВШ=4,305; 95,0 % ДІ 1,187-15,619; p=0,026) і тромбоцитів (ВШ=0,955; 95,0 % ДІ 0,922-0,989; p=0,011), що вказувало на підвищення шансів розвитку фіброзу печінки при хронічному ВГС при перевищенні нормативних показників середнього корпускулярного об’єму (у 4,305 рази) та зниження шансів – при підвищенні рівня тромбоцитів (на 4,5 %).

Висновки. На основі проведених досліджень встановлено, що маркери крові можуть бути використані як значущий предиктор розвитку фіброзу печінки у пацієнтів із хронічним ВГС. Підвищення рівня середнього корпускулярного об’єму і тромбоцитів у сироватці крові характеризує значний зв’язок із розвитком фіброзу у хворих на хронічний ВГС, що свідчить про суттєвий вплив маркерів крові на патогенез фіброзу у цих хворих.

Ключові слова: хронічний вірусний гепатит С, клінічний аналіз крові, фіброз печінки, прогностичне значення, ймовірність розвитку фіброзного процесу