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Diagnostic values of MMP-9 and TGF-1β in assessing the severity of liver fibrosis and the rate of its progression in patients with chronic hepatitis C GT 1 infection

H. V. Venytska[B,C,D], **O. V. Riabokon***[A,F], **Yu. Yu. Riabokon**[C,D,E], **R. O. Shcherbyna**[B,C]

Zaporizhzhia State Medical and Pharmaceutical University, Ukraine

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

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***E-mail:** ryabokonzsmu@ukr.net

Aim. The purpose of our work is to find out diagnostic values of serum MMP-9 and TGF-1β determination for assessing the severity of liver fibrosis and the rate of its progression in patients with chronic hepatitis C genotype 1 (CHC GT1) infection.

Materials and methods. 92 patients with CHC GT1 were examined. The severity of liver fibrosis was assessed by elastometry. The rate of liver fibrosis progression was calculated using the T. Poynard formula. Serum levels of TGF-1β and MMP-9 were measured by ELISA method.

Results. In patients with CHC GT1, the most noticeable changes in the serum parameters of fibrogenesis / fibrinolysis were observed in the presence of F 3–4. The probability of liver fibrosis stages F 3–4 was high at the serum levels of TGF-1β >12.03 pg/ml (p < 0.001), MMP-9 ≤987.20 pg/ml (p = 0.016), TGF-1β/MMP-9 ratio >0.011 (p < 0.001).

Fast liver fibrosis progression was more often registered in F 3–4 than in F 0–2 (62.9 % vs. 16.7 %, p < 0.0001). Increasing rate of liver fibrosis progression in these patients was confirmed by a higher ratio of TGF-1β/MMP-9 compared to that in patients with a slow rate of liver fibrosis progression (p < 0.05). The probability of fast liver fibrosis progression was high at the serum levels of TGF-1β >8.69 pg/ml (p < 0.001), MMP-9 ≤920.65 (p = 0.005), TGF-1β/MMP-9 ratio > 0.011 (p < 0.001).

Conclusions. The diagnostic value of MMP-9 and TGF-1β in assessing the liver fibrosis severity and the rate of its progression in patients with CHC GT1 has been defined. Cut-off levels of MMP-9, TGF-1β and the TGF-1β/MMP-9 ratio for stratification of patients with severe liver fibrosis and the fast rate of its progression have been proposed.

Ключові слова: хронічний гепатит С, вірусна інфекція, фіброз печінки, фактори ризику, цитокіни, діагностика, прогноз.

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Діагностична значущість MMP-9 і TGF-1β під час оцінювання ступеня виразності фіброзу печінки та швидкості його прогресування у хворих на хронічний гепатит С (генотип 1)

Г. В. Веницька, О. В. Рябоконь, Ю. Ю. Рябоконь, Р. О. Щербина

Мета роботи – з'ясувати діагностичну роль визначення MMP-9 і TGF-1β у сироватці крові для оцінювання ступеня виразності фіброзу печінки та швидкості його прогресування у хворих на хронічний гепатит С (генотип 1) (ХГС GT1).

Матеріали та методи. Обстежили 92 хворих на ХГС GT1. Виразність фіброзу печінки оцінювали методом еластометрії. Темп прогресування фіброзу печінки розраховували за формулою T. Poynard. Методом імуноферментного аналізу визначали вміст TGF-1β і MMP-9.

Результати. Найбільш виражені зміни сироваткових параметрів фіброгенезу / фібринолізу у хворих на ХГС GT1 спостерігали за наявності F 3–4. Якщо в сироватці крові вміст TGF-1β становить >12,03 pg/ml (p < 0,001), MMP-9 – ≤987,20 pg/ml (p = 0,016), а коефіцієнт TGF-1β/MMP-9 – >0,011 (p < 0,001), імовірність фіброзу печінки F 3–4 ступеня є значущою. Швидкий темп прогресування фіброзу печінки у хворих на ХГС GT1 частіше визначали при фіброзі F 3–4 ступеня, ніж при F 0–2 (62,9 % проти 16,7 %, p < 0,0001). Збільшення темпу прогресування фіброзу печінки у цих пацієнтів підтверджує вищий коефіцієнт TGF-1β/MMP-9 порівняно з хворими з повільним темпом прогресування фіброзу печінки (p < 0,05). Імовірність швидкого темпу прогресування фіброзу печінки є значущою, якщо вміст TGF-1β у сироватці крові хворих становить >8,69 pg/ml (AUC = 0,864; p < 0,001), MMP-9 – ≤920,65 (AUC = 0,675, p = 0,005), а коефіцієнт TGF-1β/MMP-9 – >0,011 (AUC = 0,861, p < 0,001).

Висновки. Встановили діагностичну значущість MMP-9 і TGF-1β під час оцінювання ступеня виразності фіброзу печінки та швидкості його прогресування у хворих на хронічний гепатит С GT1. Запропоновано межові рівні MMP-9, TGF-1β та коефіцієнта TGF-1β/MMP-9 для стратифікації хворих із тяжким фіброзом печінки та швидким темпом його прогресування.

According to the World Health Organization, viral hepatitis C is one of the main causes of chronic liver disease in the world. The prevalence of chronic hepatitis C (CHC) among the population of the European region is at the level of 1.3 % [1]. The progression of CHC is associated with the cascade activation of profibrogenic mechanisms by the virus resulting in excessive synthesis of collagen fibers which leads to the development of liver fibrosis with subsequent progression to liver cirrhosis [2]. Therefore, in the assessment of CHC course, one of the leading places is the detection of liver fibrosis severity [1,3,4]. In the arsenal of clinicians, there are various methods for detecting the liver fibrosis severity, which are divided into invasive and non-invasive [2,5]. The "gold standard" for diagnosis of

liver fibrosis is a puncture biopsy followed by a histological assessment of liver specimens. However, this method is invasive, expensive, has certain contraindications for its use and limited capacities of dynamic application [2,5]. Therefore, methods for non-invasive diagnosis of liver fibrosis have recently been developed, represented by instrumental (elastometry) and serum (identification of individual fibrogenic factors) methods [2,5,6,7].

Currently, in clinical practice, elastography method is widely used to assess the severity of liver fibrosis, principle of which is based on determining the degree of liver elasticity due to induced mechanical vibrations of medium amplitude and low frequency. It allows to estimate the liver tissue elasticity and draw a conclusion about the degree of fibrotic change expressiveness, correlating the obtained results with the METAVIR scale [5,6,7]. However, during the first years of using this method in clinical practice, a number of factors that could have a significant impact on the accuracy of obtained results, thus to some extent limiting the use of this method. First of all, the presence of abdominal obesity, liver steatosis, and a high level of liver necrotic-inflammatory activity in patients should be considered when choosing this method of examination [8].

Serum markers for assessing severity of liver fibrosis divided into direct and indirect. Their fundamental difference lies in features of formation and release of the corresponding markers [7,9]. Direct markers characterize metabolism in the matrix cells (fibrosis formation and fibrous tissue reversal) and changes that occur in profibrogenic cells (hyaluronic acid, procollagen peptides, tissue inhibitors of metalloproteinases, transforming growth factor-β, etc.). Indirect serum markers of liver fibrosis enter the blood due to inflammation of the liver tissue. Their detection is carried out using routine tests (transaminases, calculation of the De Ritis coefficient, apolipoprotein A1, ferritin, haptoglobin, α2 macroglobulin, etc.). Using combinations of direct and indirect serum markers of liver fibrosis, certain calculated diagnostic indices were also developed, which could also be used to assess the severity of liver fibrosis (aspartate aminotransferase to Platelet Ratio Index – APRI, scale Fibrosis-4 – FIB-4, FibroTest and others) [5,6,7,9].

Currently, there is no standard non-invasive method for determining the severity of liver fibrosis. Therefore, on the one hand, in clinical practice, it is advisable to use a combination of various non-invasive methods of assessing liver fibrosis, and on the other hand, the above-mentioned requires further studies on the search for informative serological markers for assessing the severity of liver fibrosis [4,5,6,9].

However, in clinical practice, it is important to understand not only the severity of liver fibrosis, but also the progression rates of liver fibrotic changes. Poynard T. et al. [10] have presented a developed method of assessing the rate of liver fibrosis progression in patients with CHC, finding the average rate of liver fibrosis progression per year of 0.133 fibrosis units. It is believed that the progression rate of liver fibrosis is influenced by such factors as aging, alcohol consumption and male sex [10,11]. However, the mechanisms underlying the different rates of disease progression are uncertain [10]. To date, in separate studies, an attempt has been made to explain the rate of liver fibrosis progression based on the understanding of immunopathogenetic mechanisms. Thus, researchers [12] associate the slowly progressive CHC disease with an induction of earlier and more pronounced adaptive immune response against hepatitis C virus (HCV) (an earlier peak of cytolysis and seroconversion, transient clearance or reduction of viremia, significant induction of interferon-γ and macrophage inflammatory protein 1β, etc.). In contrast, patients with rapidly progressive CHC showed a reduced or delayed adaptive immune response associated with significantly higher levels of viremia and a persistent increase in proinflammatory and profibrotic chemokines (monocyte chemotactic factor 1, interleukin-8, and interferon-γ-induced protein 10, etc.) [12].

Given the above, the search for new informative serum markers remains relevant not only for assessing the severity of liver fibrosis, but also the rate of its progression in CHC.

Aim

The purpose of our work is to find out diagnostic values of serum MMP-9 and TGF-1β determination for assessing the severity of liver fibrosis and the rate of its progression in patients with chronic hepatitis C genotype 1 infection.

Materials and methods

The study enrolled 92 patients with chronic hepatitis C genotype 1 (CHC GT1) who were examined on the basis of the Municipal Non-Profit Enterprise "Zaporizhzhia Regional Clinical Hospital of Infectious Diseases". The study was open, prospective cohort.

The diagnosis of CHC in all patients was confirmed by the detection of HCV-RNA in blood by the polymerase chain reaction method. GT1 infection was identified in all patients, the median viral load was 229477 (27800; 1143057) IU/ml. There were 52 (56.5 %) women and 40 (43.5 %) men. The age ranged from 27 to 72 years; the median age was 54.5 (44.5; 61.5) years. Exclusion criteria were: co-infection with other hepatotropic viruses (hepatitis A virus, hepatitis B virus) or human immunodeficiency virus, presence of decompensated liver cirrhosis or somatic comorbid conditions in the decompensation stage, no informed consent from a patient to participate in the study.

The study was conducted in compliance with the "Ethical Principles and Guidelines for Scientific Medical Research Involving Human Subjects" provisions approved by the Declaration of Helsinki and the legislation of Ukraine. Patients were included in the study after signing a written informed consent form.

The presumed duration of CHC course was estimated based on clinical and epidemiological anamnestic data, including increased activity of transaminases for a long time in combination with epidemiological history data indicating probable HCV infection. The median duration of CHC was 10.0 (4.0; 17.0) years. Shear wave elastometry was used to assess the liver fibrosis severity in all patients. Liver fibrosis stages F 0–2 were detected in 48 (52.2 %) patients, severe fibrosis with transformation into liver cirrhosis F 3–4 was detected in 44 (47.8 %) patients. According to the results of determining the liver fibrosis stages, the patients were divided into groups: 48 patients with stages F 0–2 liver fibrosis and 44 patients with stages F 3–4.

The formula proposed by T. Poynard et al. was used to calculate the rate of liver fibrosis progression in CHC patients [10]. The rate of liver fibrosis progression was defined as the ratio between the liver fibrosis stage according to the METAVIR scale and estimated probable duration of CHC in years. In this calculation model, it was assumed that patients did not have liver fibrosis at the time of the disease (stage F 0) and the rate of fibrosis progression remained unchanged. A limit value of the calculated coefficient was 0.133 units of fibrosis/year. Accordingly, provided that this coefficient <0.133 units of fibrosis/year, the rate of liver fibrosis was considered slow, and if >0.133 units of fibrosis/year, the rate was fast, respectively. In line with the results of determining the liver fibrosis rate of progression, the patients were allocated into groups: 30 patients with a slow rate and 62 patients with a fast rate of liver fibrosis progression.

Transforming growth factor-1β (TGF-1β) (Elabscience, USA) and matrix metalloproteinase-9 (MMP-9) (Elabscience, USA) were measured by the method of immunoenzymatic analysis in blood serum of patients with CHC and 30 individuals of the control group. Based on the results of these cytokine measurements, the ratio of TGF-1β/MMP-9 was calculated for each CHC patient and the control group. The control group included 30 healthy people aged from 27 to 73 years, the median age was 51.5 (40.0; 60.0), men – 16 (48.0 %), women – 14 (42.0 %). The CHC patient group and the group of healthy individuals did not statistically differ in demographic signs ($p > 0.05$). All individuals of the control group had negative results of laboratory tests for viral hepatitis markers, had a negative result of the test for antibodies to human immunodeficiency virus, did not have concomitant pathology in the stage of decompensation and provided written informed consent to participate in the study. The research was conducted on basis of the Educational and Scientific Medical Laboratory Center with vivarium at Zaporizhzhia State Medical and Pharmaceutical University (scientific consultant – MD, PhD, DSc, Associate Professor R. O. Shcherbyna).

Statistical processing was carried out using the program Statistica 13 for Windows (StatSoft Inc., No. JPZ804I382130ARCN10-J). Normality of distribution was assessed with the Shapiro–Wilk test. The Mann–Whitney test was used to assess the significance of differences between non-normally distributed quantitative variables in independent groups. The results of quantitative data were presented in the form of median and interquartile range – Me (Q_{25}; Q_{75}). The results of qualitative characteristics were presented as an absolute number and a corresponding percentage, abs. (%). The χ^2 method was used to analyze qualitative characteristics. ROC-analysis was performed to cut off the threshold level of an indicator. Spearman's correlation analysis was used to assess relationships between quantitative variables.

Results

According to the study results, the mean serum level of profibrogenic cytokine TGF-1β has been found not to be statistically different between GT1 CHC patients with liver fibrosis stages F 0-2 and healthy individuals ($p > 0.05$), but the mean level of MMP-9, involved in the processes of fibrinolysis, has been found to be lower (1.3 times, $p < 0.05$) than that in healthy persons. At the same time, the mean TGF-1β/MMP-9 ratio in patients of this group was also not significantly different from that of healthy people ($p > 0.05$). In GT1 CHC patients with liver fibrosis stages F 3-4, more significant changes in the examined indicators were documented. So, the serum level of TGF-1β was statistically significantly higher as compared to healthy people (by 2.5 times, $p < 0.05$), and compared to patients with liver fibrosis stages F 0–2 (by 2.2 times, $p < 0.05$). Furthermore, the serum level of MMP-9 was lower not only compared to healthy people ($p<0.05$), but also compared to patients with liver fibrosis stages F 0–2 ($p < 0.05$). The revealed changes in cytokine regulation of fibrogenesis and fibrinolysis processes led to an increase in the ratio between profibrogenic TGF-1β and fibrinolytic MMP-9 parameters in CHC patients. The TGF-1β/MMP-9 ratio in GT1 CHC patients with liver fibrosis stages F 3–4 was higher compared to the corresponding indicator of healthy people (by 4 times, $p < 0.05$) and of CHC patients with liver fibrosis stages F 0–2 (by 3.3 times, $p < 0.05$) (*Table 1*).

In patients with CHC GT1, ROC analysis was performed with the cut-off point estimation to evaluate the diagnostic potential of determining the serum levels of TGF-1β and MMP-9 as well as the TGF-1β/MMP-9 ratio for the detection of liver fibrosis stages F 3–4. Based on the ROC-analysis, a threshold level of TGF-1 β was determined, indicating a high probability of severe liver fibrosis and transformation into liver cirrhosis (AUC = 0.984, $p < 0.001$) in GT1 CHC patients. Namely, if the serum TGF-1β level in patients was >12.03 pg/ml (sensitivity – 94.4 %, specificity – 94.1 %), the probability of liver fibrosis stages F 3–4 was high (*Fig. 1A*). According to the ROC-analysis results, the threshold serum level of MMP-9 was detected ≤987.20 pg/ml (AUC = 0.656, $p = 0.016$), indicating a high probability of liver fibrosis stages F 3–4 presence (sensitivity – 75.0 %, specificity – 54.8 %) (*Fig. 1B*). Next, the threshold level of TGF-1 β/MMP-9 ratio >0.011 (AUC = 0.908, $p < 0.001$) was also found, demonstrating a high probability of liver fibrosis stages F 3–4 (sensitivity – 88.9 %, specificity – 82.4 %) (*Fig. 1C*).

A frequency analysis on different stages of liver fibrosis detection in patients with CHC GT1 depending on the rate of its progression has revealed significantly more frequent severe fibrosis with transformation into liver cirrhosis (F 3–4) and faster liver fibrosis progression as compared to liver fibrosis stages F 0–2 (62.9 % vs. 16.7 %, $\chi^2 = 17.32$, $p < 0.0001$) diagnosed by elastography (*Table 2*).

The analysis results of the serum TGF-1β and MMP-9 levels in patients with CHC GT1 depending on the rate of liver fibrosis progression, has shown the most significant changes in these parameters among patients with the fast rate of liver fibrosis progression. So, in patients with the slow rate of liver fibrosis progression, only the mean level of MMP-9 was lower ($p < 0.05$) than that in healthy individuals with the absence of a statistically significant increase in profibrogenic cytokine TGF-1β ($p > 0.05$) and the TGF-1β/MMP-9 ratio ($p > 0.05$). At the same time, in patients with the fast rate of liver fibrosis progression, the serum level of TGF-1β was higher compared to that in healthy people ($p < 0.05$) and patients with the slow rate of liver fibrosis

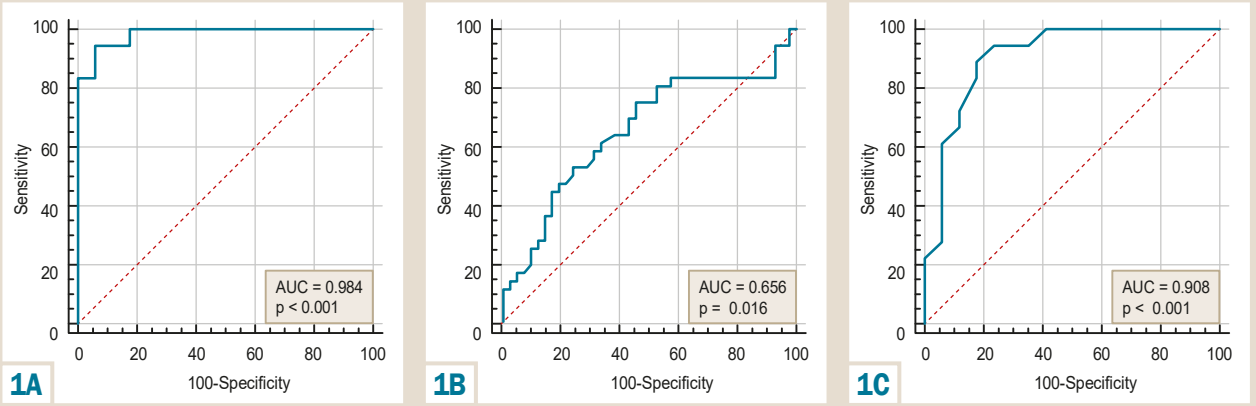

Fig. 1. Diagnostic significance of determining the serum levels of TGF-1β (A) and MMP-9 (B) and the TGF-1β/MMP-9 ratio (C) for assessing liver fibrosis stages F 3–4 in patients with CHC GT1.

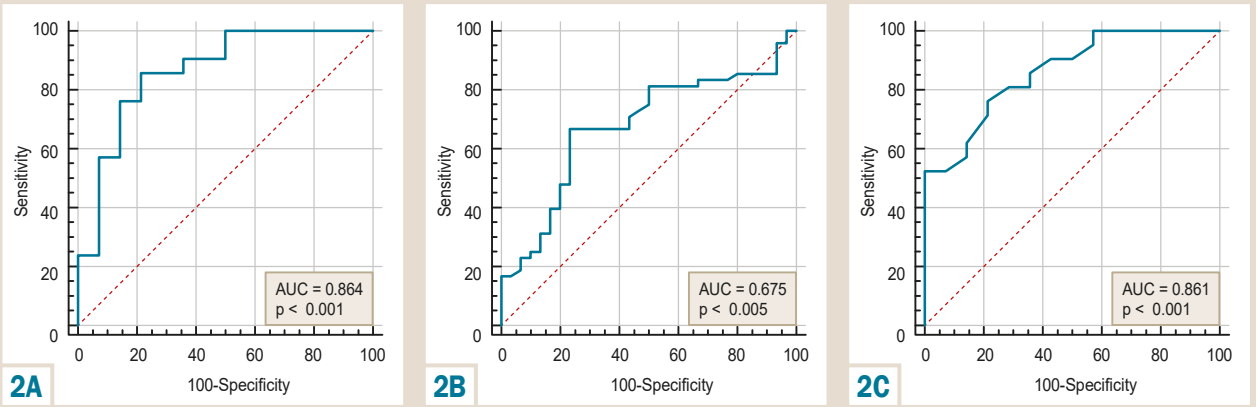

Fig. 2. Diagnostic value of determining the serum levels of TGF-1β (A), MMP-9 (B) and the TGF-1β/MMP-9 ratio (C) to define the rate of liver fibrosis progression in patients with CHC GT1.

Table 1. Serum levels of TGF-1β, MMP-9 and the TGF-1β/MMP-9 ratio in GT1 CHC patients depending on the degree of liver fibrosis, Me (Q_{25}; Q_{75})

Indicator, units of measurement	Healthy people (n = 30)	CHC patients (n = 92)	
		liver fibrosis F 0–2 (n = 48)	liver fibrosis F 3–4 (n = 44)
TGF-1β, pg/ml	6.20 (4.90; 7.00)	7.00 (4.50; 8.50)	15.20 (13.40; 18.40)*,**
MMP-9, pg/ml	1269.43 (1088.70; 1331.50)	994.03 (753.41; 1151.13)*	725.12 (488.74; 994.71)*,**
TGF-1β/MMP-9	0.005 (0.004; 0.006)	0.006 (0.004; 0.010)	0.020 (0.013; 0.035)*,**

*: the difference is significant compared to healthy people (p < 0.05); **: compared to patients with liver fibrosis stages F 0–2 (p < 0.05).

Table 2. The incidence of different stages of liver fibrosis in patients with different rates of its progression, abs (%)

Stage of liver fibrosis	CHC patients (n = 92)	
	with the slow rate of progression (n = 30)	with the fast rate of progression (n = 62)
F 0–2	25 (83.3 %)	23 (37.1 %)*
F 3–4	5 (16.7 %)	39 (62.9 %)*

*: the difference is significant compared to patients with liver fibrosis stages F 0–2 (p < 0.0001).

Table 3. The serum levels of TGF-1β, MMP-9 and the TGF-1β/MMP-9 ratio in GT1 CHC patients depending on the rate of liver fibrosis progression, Me (Q_{25}; Q_{75})

Indicator, units of measurement	Healthy people (n = 30)	CHC patients (n = 92)	
		with the slow rate of progression (n = 30)	with the fast rate of progression (n = 62)
TGF-1β, pg/ml	6.2 0 (4.90; 7.00)	6.1 0 (4.40; 8.70)	14.1 0 (12.30; 16.70)*,**
MMP-9, pg/ml	1269, 43 (1088.70; 1331.50)	1053.77 (926.72; 1209.50)*	814.44 (542.25; 1005.40)*,**
TGF-1β/MMP-9	0.005 (0.004; 0.006)	0.007 (0.004; 0.011)	0.019 (0.012; 0.035)*,**

*: the difference is significant compared to healthy people (p < 0.05); **: compared to patients with the slow rate of liver fibrosis progression (p < 0.05).

progression ($p < 0.05$), as well as the level of MMP-9 was lower compared to that in healthy people ($p < 0.05$) and patients with the slow rate of liver fibrosis progression ($p < 0.05$). Conforming changes in the examined cytokines have led to an increase in the ratio between profibrogenic TGF-1β and fibrinolytic MMP-9 parameters in patients with the fast rate of liver fibrosis progression. The TGF-1β/MMP-9 ratio in these patients was higher compared to the corresponding indicator of healthy people ($p < 0.05$) and patients with the slow rate of liver fibrosis progression ($p < 0.05$) (*Table 3*).

In patients with CHC GT1, the ROC analysis was performed to define the cut-off point for diagnostic value of determining the serum levels of TGF-1β, MMP-9 and the TGF-1β/MMP-9 ratio regarding the fast rate of liver fibrosis progression. Based on the ROC-analysis, a threshold level of TGF-1β was determined indicating a high probability of the fast rate of liver fibrosis progression (AUC = 0.864; $p < 0.001$). Namely, if the serum level of TGF-1β in patients was >8.69 pg/ml, the probability of fast liver fibrosis progression was high (sensitivity – 78.6 %, specificity – 85.7 %) (*Fig. 2A*). The threshold level of serum MMP-9 was detected ≤920.65 pg/ml (AUC = 0.675, $p = 0.005$), indicating a high probability of the fast rate of liver fibrosis progression (sensitivity – 66.7 %, specificity – 76.7 %) (*Fig. 2B*). If the TGF-1β/MMP-9 ratio in GT1 CHC patients was >0.011 (AUC = 0.861, $p < 0.001$), the probability of the fast liver fibrosis progression was high (sensitivity – 76.2 %, specificity – 78.6 %) (*Fig. 2C*).

Correlation analysis has revealed direct correlations between the serum TGF-1β level and γ-glutamyltranspeptidase activity ($r = 0.70$, $p = 0.035$) and thymol test index ($r = 0.40$, $p = 0.022$), as well as between the serum level of MMP-9 and leukocyte ($r = 0.23$, $p = 0.045$) and platelet ($r = 0.27$, $p = 0.02$) counts.

Discussion

The cytokine system, which regulates the intensity of fibrogenesis and fibrinolysis processes, is of primary importance in the pathogenetic mechanisms of CHC progression [2]. It is known that processes of liver fibrogenesis are activated in response to chronic inflammation due to the action of etiological factors [2,13]. As a result of HCV long-term effects, inflammatory processes and liver tissue remodeling are stimulated which is a prerequisite for the fibrosis formation [14]. The processes of fibrogenesis are always accompanied by antagonistically directed processes of fibrinolysis, which promote inactivation or apoptosis of activated stellate cells and lysis of fibrous tissue to preserve physiological functions [13]. It is extremely important to keep the balance between fibrotic and antifibrotic mechanisms to ensure adequate functioning of the organ. When the balance is disturbed, there is an imbalance between these mechanisms with a predominance of fibrogenic processes over antifibrogenic ones. Consequently, excessive synthesis of collagen fibers leads to the development of liver fibrosis with further progression to cirrhotic transformation. This is accompanied by a violation of the exchange between sinusoidal blood and hepatocytes, provoking the development of functional disorders [2,13,14].

Various profibrogenic cytokines (TGF-1β, interleukins 4, 10 and 13, hyaluronic acid, etc.) are important in the pathogenesis of liver fibrosis progression [15]. One of the leading profibrogenic cytokines is TGF-1β, while its activity can be stimulated or inhibited by other cytokines [13,16]. TGF-1β is a member of a large family of pleiotropic cytokines, which induces the activation of hepatic stellate cells followed by their differentiation into fibroblasts, which are directly involved in the fibrogenic processes [16]. In several studies, an increase in serum levels of TGF-1β has been demonstrated with increasing severity of liver fibrosis, and in the presence of liver cirrhosis due to CHC or chronic hepatitis B, the highest levels of this cytokine have been found [17,18,19]. The results obtained in our study regarding the relationship between the serum TGF-1β level and the liver fibrosis severity coincide with the literature data. We have revealed that the highest level of TGF-1β was in GT1 CHC patients with liver fibrosis stages F 3–4. In addition, when applying the ROC-analysis, we have managed to define the threshold value of TGF-1β >12.03 pg/ml, which allowed us to stratify patients with liver fibrosis stages F 3–4. The analysis of literature data focused on the search for informative serum markers to diagnose severe liver fibrosis has shown that it was reasonable to use different serum markers in chronic diffuse liver diseases of different etiology. So, the study [20] has shown that the most informative serum markers for the diagnosis of severe liver fibrosis in patients with chronic alcoholic hepatitis were levels of tumor necrosis factor-α >2.1 pg/ml and protein-bound hydroxyproline >260.5 μmol/l. Another study [21] has shown a number of parameters, in particular, HBeAg status, viral load, the degree of necro-inflammatory activity expressiveness according to alanine aminotransferase indicators, and the degree of tumor necrosis factor-α increase, that should be considered when assessing a risk of severe liver fibrosis in chronic hepatitis B.

The basis of antifibrotic mechanisms is the process of extracellular matrix degradation, which depends on the effect of matrix metalloproteinases (MMPs). Their activity is strictly regulated by corresponding tissue inhibitors of metalloproteinases (TIMPs). MMPs are responsible for tissue remodeling, degradation of extracellular matrix proteins, angiogenesis, cell apoptosis, immune response, promote cell proliferation, migration, and differentiation, etc. MMP-2 (collagenase type IV, gelatinase-A) and MMP-9 (collagenase type IV, gelatinase-B) facilitate the cleavage of collagen type IV, laminin and other components of basement membrane. An imbalance between MMP and TIMP is considered a crucial factor responsible for the production and degradation of extracellular matrix [22]. In our study, the level of MMP-9 has been found to be lower in patients with CHC GT1 than that in healthy individuals and decreased with increasing severity of liver fibrosis. In addition, when applying the ROC-analysis, we have managed to determine the threshold value of MMP-9 ≤987.20 pg/ml, allowing us to stratify patients with liver fibrosis stages F 3–4.

The literature data show that the development of liver fibrosis is characterized by an increase in the level of TIMP-1 and a decrease in the serum activity of type IV collagenase, and the extent of these changes depends on the liver fibrosis stage [22,23,24]. Hence, in the presence of HCV-associated liver cirrhosis, scientists have demonstrated the lowest level of MMP-9 in combination with the highest level of TIMP-1 in blood serum. However, in conditions of the HCV-associated

hepatocellular carcinoma development, the researchers have revealed statistically significantly higher levels of MMP-9 and TIMP-1 compared to those in HCV patients with liver fibrosis stages F 0–3 and F 4 [24].

The literature also presents a study [25] that attempted to compare the diagnostic value of different MMP in assessing the severity of liver fibrosis. According to this research results, it has been shown that the serum level of MMP-7 was more informative in diagnosis of liver fibrosis, and the serum level of MMP-9 did not differ statistically in CHC patients with different stages of liver fibrosis. At the same time, however, high serum concentrations of various inactive MMPs were present in patients with CHC, indicating a limited ability to restrain the liver fibrosis progression [25]. It is assumed that in virus-induced chronic hepatitis, the progression of liver fibrosis is primarily a consequence of insufficient activity of extracellular matrix degradation processes and is not a result of increased synthesis of extracellular matrix components [26].

It is believed that the rate of liver fibrosis progression primarily depends on the degree of expressiveness and prevalence of profibrogenic or antifibrogenic mechanisms [27]. This explains why some patients with chronic hepatitis can develop only mild F 0–2 liver fibrosis within 30 years, while others develop liver cirrhosis within 5 years [27]. In our study, we analyzed the TGF-1β/MMP-9 ratio, which reflects the balance between profibrogenic and antifibrotic mechanisms. Our results have demonstrated a predominance of profibrogenic mechanisms over antifibrogenic ones in patients with CHC GT1, that was the most considerable in patients with severe fibrosis and transformation into liver cirrhosis, as well as in patients with the fast rate of liver fibrosis progression. In addition, when applying the ROC-analysis, we have managed to define the threshold value of the TGF-1β/MMP-9 ratio >0.011, which made it possible to stratify patients with the fast rate of liver fibrosis progression. Other methods of diagnosing the rate of liver fibrosis are proposed in the literature.

So, the study [28] have proposed to consider the episodes of cytolysis syndrome activation in the form of increased alanine aminotransferase, which is associated with the acceleration of the liver fibrosis progression, when calculating the rate of liver fibrosis progression in patients with CHC.

Conclusions

1. In patients with CHC GT1, the most pronounced changes in the serum parameters of fibrogenesis/fibrinolysis are observed in the presence of liver fibrosis stages F 3–4, which confirms the highest level of TGF-1β ($p < 0.05$), the lowest level of MMP-9 ($p < 0.05$) and the highest TGF-1β/MMP-9 ratio compared to those in patients with stages F 0–2 ($p < 0.05$).

2. The probability of liver fibrosis stages F 3–4 is high if the serum TGF-1β level in patients with CHC GT1 >12.03 pg/ml (AUC = 0.984, $p < 0.001$), serum MMP-9 level ≤987.20 pg/ml (AUC = 0.656, $p = 0.016$), and the TGF-1β/MMP-9 ratio >0.011 (AUC = 0.908, $p < 0.001$).

3. The fast rate of liver fibrosis progression in patients with CHC GT1 occurs more often in fibrosis stages F 3–4 than in stages F 0–2 (62.9 % vs. 16.7 %, $p < 0.0001$). Increasing the rate of liver fibrosis progression in these patients is confirmed by the higher TGF-1β/MMP-9 ratio as compared to that in patients with the slow rate of liver fibrosis progression ($p < 0.05$).

4. The probability of fast liver fibrosis progression is high if the serum level of TGF-1β in patients with CHC GT1 >8.69 pg/ml (AUC = 0.864; $p < 0.001$), serum MMP-9 level ≤920.65 (AUC = 0.675, $p = 0.005$), and the TGF-1β/MMP-9 ratio >0.011 (AUC = 0.861, $p < 0.001$).

Prospects for further research. The prospect for further research in this direction, in our opinion, is not only the search for informative serum markers to diagnose the rate of liver fibrosis progression, but also to assess changes in the rate of liver fibrosis progression in patients with CHC who received antiviral treatment.

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Information about the authors:
Venytska H. V., MD, Assistant of the Department of Infectious Diseases, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-3642-0117
Riabokon O. V., MD, PhD, DSc, Professor, Head of the Department of Infectious Diseases, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-7394-4649
Riabokon Yu. Yu., MD, PhD, DSc, Professor of the Department of Children Infectious Diseases, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-2273-8511
Shcherbyna R. O., PhD, DSc, Associate Professor of the Department of Natural Sciences for Foreign Students and Toxicological Chemistry, Head of Educational and Scientific Medical Laboratory Center with vivarium, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-9742-0284

Відомості про авторів:
Веницька Г. В., асистент каф. інфекційних хвороб, Запорізький державний медико-фармацевтичний університет, Україна.
Рябоконь О. В., д-р мед. наук, професор, зав. каф. інфекційних хвороб, Запорізький державний медико-фармацевтичний університет, Україна.
Рябоконь Ю. Ю., д-р мед. наук, професор каф. дитячих інфекційних хвороб, Запорізький державний медико-фармацевтичний університет, Україна.
Щербина Р. О., д-р фарм. наук, доцент каф. природничих дисциплін для іноземних студентів та токсикологічної хімії, керівник Навчально-наукового медико-лабораторного центру з віварієм, Запорізький державний медико-фармацевтичний університет, Україна.

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