Comparison of diagnostic informative value of the methods for the determination of direct (hyaluronic acid) and indirect (alanine aminotransferase, aspartate aminotransferase, de Rithis coefficient) of liver fibrosis markers in patients with chronic viral hepatitis C

L.V. Moroz, I. Yu. Bondaruk

N. I. Pirogov National Medical University Vinnitsa, Ukraine

Abstract

The results of chronic viral hepatitis C patients examinations are described. A comparative characteristic of the diagnostic informative value of direct (hyaluronic acid) and indirect (alanine aminotransferase, aspartate aminotransferase, de Ritis coefficient) of liver fibrosis markers by the use of ROC - analysis is presented. **The objective:** to explore the possibility of non-invasive methods for diagnosing liver fibrosis in patients with chronic viral hepatitis C (CVH C). **Materials and methods.** 66 CVH C with the 1st genotype of the virus were examined. They were either at dispensary observation or ambulatory treatment at Municipal Clinical Hospital №1 (Vinnitsa, Ukraine) and in the clinical department of viral hepatitis at L. V. Gromashevsky Institute of Epidemiology and Infectious Diseases of NAMS (Ukraine). All patients underwent fibroelastography (FEG) on the METAVIR fibrosis score. In all CVH C patients and control group individuals content of hyaluronic acid (HA) in blood serum was determined. ELISA method and "Hyaluronic Acid" kit (Corgenix, Inc., USA) were used. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity was investigated with kinetic method (analyzer and test system Cobas 6000, Roche Diagnostics,

Switzerland). **Results:** AUC for HA content in serum of CVH C patients was in the range 0.720-0.917 (p<0.01). Signs of the cytolytic syndrome were recorded in 86.4% of the patients under examination. AUC for determining the value of ALT in serum in CVH C patients with liver fibrosis F0-F2 and F4 was less than 0.5. Sensitivity and specificity of blood serum ALT determination in the patients under study by the degree of liver fibrosis F3 were respectively 97.71% and 96,42% (AUC = 0.906; p <0.001). The method of determining the value of AST in serum of patients with CVH with fibrosis degree F0-F2 also has a low informativity (AUC<0.5). However, in the group of patients with F3 liver fibrosis, the sensitivity of this method (determination of AST) was 83.51% and specificity was 82.12% (AUC = 0.809, p = 0.0428). The sensitivity and specificity of determining the serum AST value in patients with F4 fibroblasts was 78.23% and 75.25%, respectively (AUC = 0.817 (p = 0.011), were found to be slightly lower). **Conclusions**. High diagnostic informative value of the determination of the content of HA in serum was established for assessing the degree of liver fibrosis in patients with CVH C (AUC = 0.720 – 0.17, p<0,01). High diagnostic informativity for the method of determining the value of ALT in CVH C patients is only established in patients with F3 hepatic fibrosis (AUC = 0.906 (p <0.001)). For the method of determining the value of AST in CVH C patients, statistically reliable diagnostic informative data was established for patients F3 hepatic fibrosis grade (AUC = 0.809, p = 0.042) and F4 (AUC = 0.817, p = 0.011). Diagnostically significant informative of the method under discussion for determining the value of dRk in CVH C patients has been established in F3 hepatic fibrosis: AUC = 0.928 (p = 0,033).

Key words: chronic viral hepatitis C, fibrosis, hyaluronic acid, alanine aminotransferase, aspartate aminotransferase, de Richtis factor.

Urgency of the research. To improve hepatic fibrosis (HF) diagnostic methods by the way of informative markers study is widely spread.

In recent years to determine the concentrations of various molecular compounds in blood serum that directly participate in the mechanisms of HF formation has been suggested. These are so-called direct markers of HF, namely: hyaluronic acid (HA), collagen IV type, matrix metalloproteinases of the 2nd and 9th types, their tissue inhibitor of the 1st type, and also TGF-beta 1 [1, 2, 3].

It is known that the intensity of HA formation, and, consequently, its serum concentration increases with increase of collagen synthesis, which occurs in inflammatory

diseases, including hepatitis. HA elimination occurs by its removing from circulating endothelial cells of hepatic sinusoids, so under expressed fibrosis, which is accompanied by a disturbance of the function of endothelial cells, there is a decrease in the clearance of HA [4].

In connection with this, determination of HA content of blood serum has high prospects as a non-invasive test, which helps to establish the phase of HF, as well as control the processes of fibrosis formation in dynamics.

The high diagnostic value of HF indirect markers (AST, ALT, AST/ALT) is investigated, but only in the context of establishing the activity of the inflammatory process [5].

In the article presented comparative characteristic of the diagnostic informative value of direct (HA) and indirect (ALT, AST, AST / ALT) markers of was performed by the use of ROC analysis.

The objective: to find out the possibility of direct and indirect non-invasive methods for diagnosing HF in CVH C patients.

Materials and methods

To solve the problems mentioned, 66 CVH C patients with virus 1st genotype were examined. There were 30 men (45.5%) and 36 women (54.5%) averagely aged 34.5 ± 1.12 years. The patients were at the dispensary supervision and outpatient treatment at Municipal Hospital N 1 (Vinnitsa) and in the clinical department of viral hepatitis at L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases NAMS of Ukraine.

In order to verify the diagnosis in accordance with modern protocol certain criteria and patients' complaints, anamnestic and clinical examination data were taken into consideration. Diagnosis of CVH C was confirmed by the detection in patients' blood serum HCV-RNA with qualitative and quantitative polymerase chain reaction (PCR).

All the patients underwent complex examinations including general and biochemical blood test with the determination of total bilirubin and its fractions, activity of ALT, AST with the use of kinetic method (analyzer and test system: Cobas 6000, Roche Diagnostics, Switzerland), alkaline phosphatase, gamma-glutamyltransferase, total protein and its fractions, coagulogram, high, low and very low density lipoprotein, cholesterol, triglycerides.

The activity of the necro-inflammatory process and degree of HF using fibroelastography (FibroScan, based in Vinnitsa Central Municipal Clinical Hospital and clinic of efferent therapy, Kyiv) with their further estimation by METAVIR score was made. In 36.4% of cases (24 patients) no signs of HF were found, or the initial fibrosis (F0-F1) was established, and the same number of patients had F2 degree of HF. There were 18.2% (12

patients) with severe (F3) fibrosis. Symptoms of hetapic cirrhosis were detected in 9% of all subjects, suffering CVH C (6 patients).

The control group consisted of healthy subjects (n = 21), representative by gender and age, with F0 degree of HF by METAVIR, with normal liver parameters and absence of liver disease in history.

The determination of HA content in blood serum was done for all CVH C and control group patients. The tests were performed in the research clinical and diagnostic laboratory of Vinnitsa National Medical University by the ELISA method with the use of set "Hyaluronic Acid" (Corgenix, Inc., USA). In the examined CVH C patients this biomarker was determined dynamically in 12 weeks after the beginning of antiviral therapy and in 24 weeks after its completion.

Statistical processing of the results was carried out in the system for statistical analysis of data Statistica 10.0.288.8 Portable.

The estimation of the reliability of the difference between the comparable sample results was carried out according to Student's criterion.

For accuracy prediction by logistic equations and determination of the discriminatory ability of various indicators ROC analysis was performed. It included the construction and analysis of operational characteristics curves, determination of area under the curve (AUC) with standard error of AUC and 95% CI; sensitivity (Se) and specificity (Sp) indexes. ROC analysis was considered adequate for a statistically significant difference in the AUC byvalue 0.5.

Results and discussion

According to our data, the signs of the cytolytic syndrome were recorded in 86.4% or 57 CVH C persons under examination (Fig. 1).
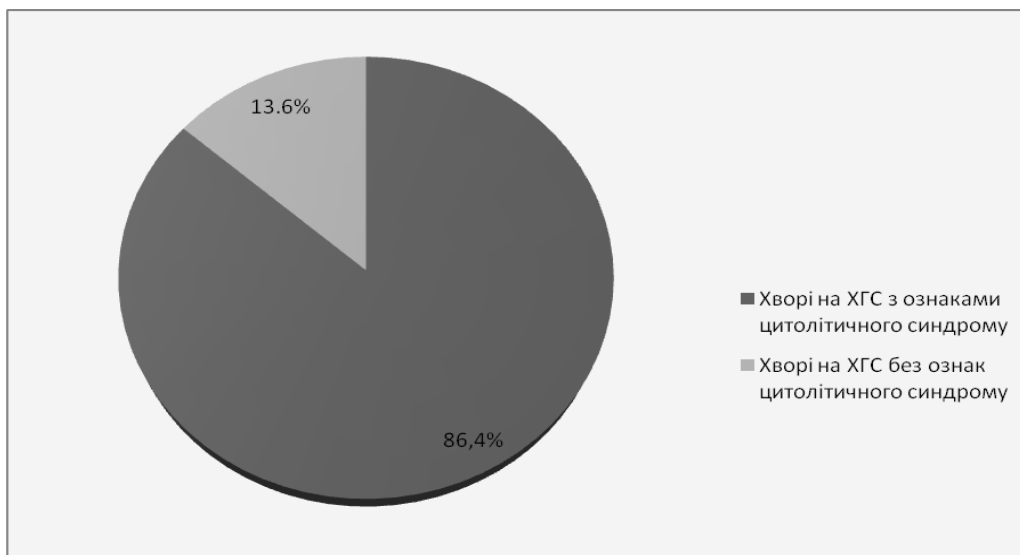
Fig.1. Distribution of CVH C patients with cytolytic syndrome signs

The highest values of ALT and AST in blood serum were found in CVH C patients with F3 HF and HA content 70.43 ± 2.7 ng / ml, and amounted 102.4 ± 1.31 U / L and 78.3 ± 1.12 U / L, respectively ($p < 0.001$; Table 1). Significant differences of these parameters depending on HF degree and blood serum HA content haave not been established.

Table 1

Value of AST, ALT, dRk and HA content in CVH C patients according to the stage of HF

Level of HF	HA content, ng/ml	N	ALT	AST	dRk
Fo-F1	47.75 ± 0.92 *	24	72.7 ± 1.21 *	49.58 ± 1.45 *	0.68 ± 0.35 **
F2	57.62 ± 1.96 *	24	84.4 ± 0.35 *	65.1 ± 0.63 *	0.77 ± 0.29 **
F3	70.43 ± 2.77 *	12	102.4 ± 1.31 *	78.3 ± 1.12 *	0.76 ± 0.43 **
F4	98.47 ± 11.68 *	6	63.3 ± 0.25 *	64.6 ± 0.12 *	1.02 ± 0.19 **
healthy	22.79 ± 2.72 *	21	17.6 ± 2.15	23.4 ± 1.13	1.31 ± 0.38

Note: * - $p < 0.001$ between control group and CVH C patients; ** - $p < 0.05$ between control group and CVH C patients

At the same time, in CVH C group of patients with the presence of severe HF (F3) signs, we had established high sensitivity and specificity in determining the value of ALT in blood serum, which was 97.71% and 96.42% respectively (AUC = 0.906; p < 0.001).
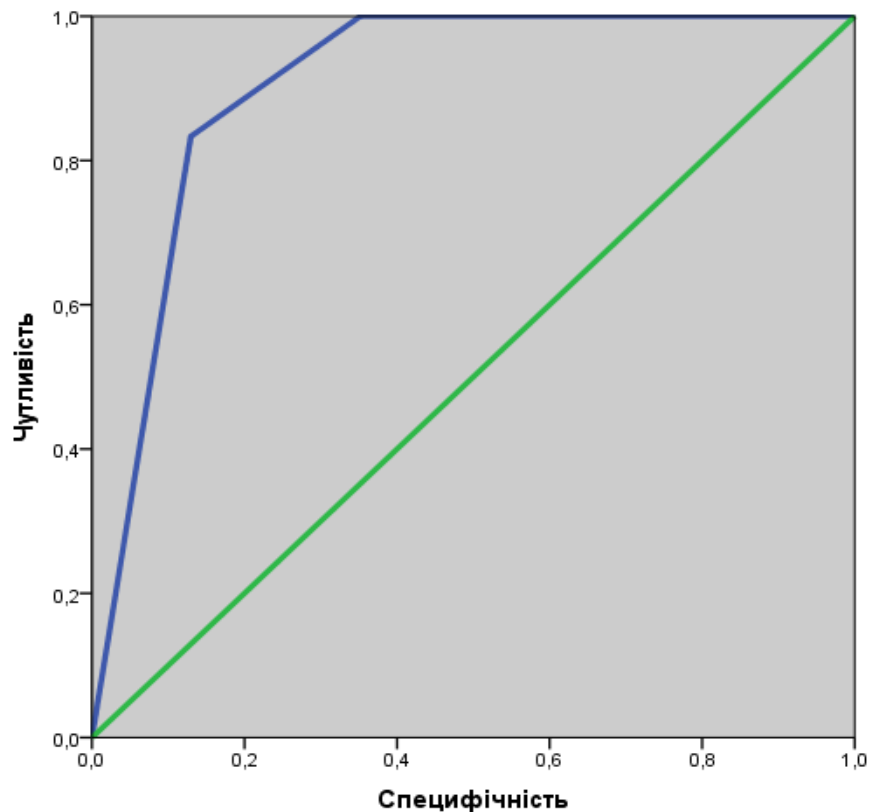
Fig. 2. ROC - curve: determination of ALT in CVH C patients with F3 degree of HF

At the same time, AUC for determining blood serum ALT in CVH C patients with F0-F2 and F4 HF was less than 0.5. This indicates a very low diagnostic informativity of the method mentioned above in this group of patients. .

A similar situation arose with determination of AST value in blood serum in CVH C patients with F0-F2 HF, where it also showed low informativity (AUC<0.5). However, in the group of patients with F3 HF, the sensitivity of this method was 83.51% and specificity was 82.12% (AUC = 0.809, p = 0.0428). A slightly lower was sensitivity and specificity of AST determination in serum in F4 HF patients: 78.23% and 75.25% respectively (AUC = 0.817; p = 0.011) (Fig. 3).
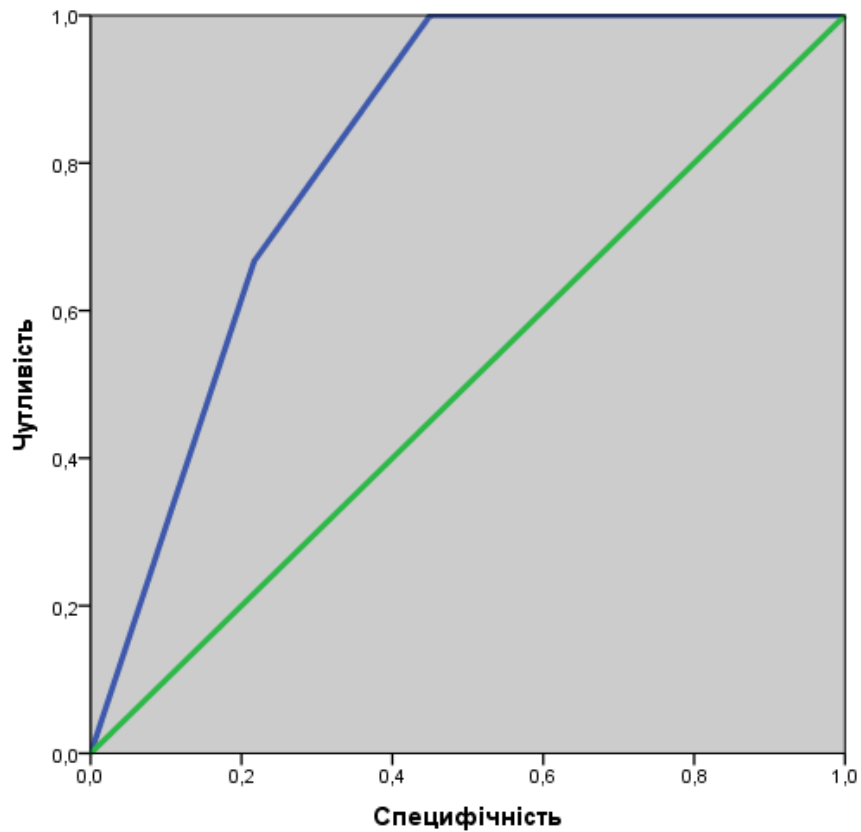
Fig. 3. ROC - curve: determination of AST level in CVH C patients and F4 HF.

The analysis of dRk magnitude ratio has established the gradual growth of this index in CVH C patients with increase of HF degree and, consequently, increase of HA content in blood serum.
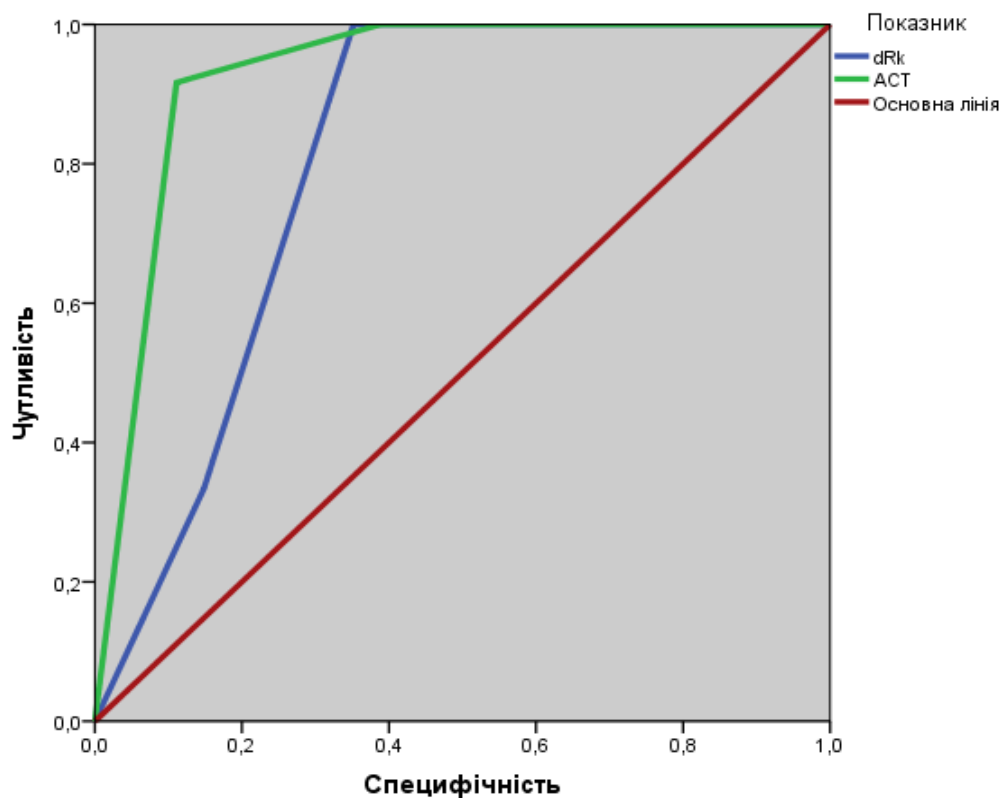
Sensitivity and specificity of dRk determination in CVH C with F2 degree of HF, is although low but reliable (AUC = 0.664; p = 0.043; Se = 58.63% ; Sp = 56.48%) (Fig. 4).
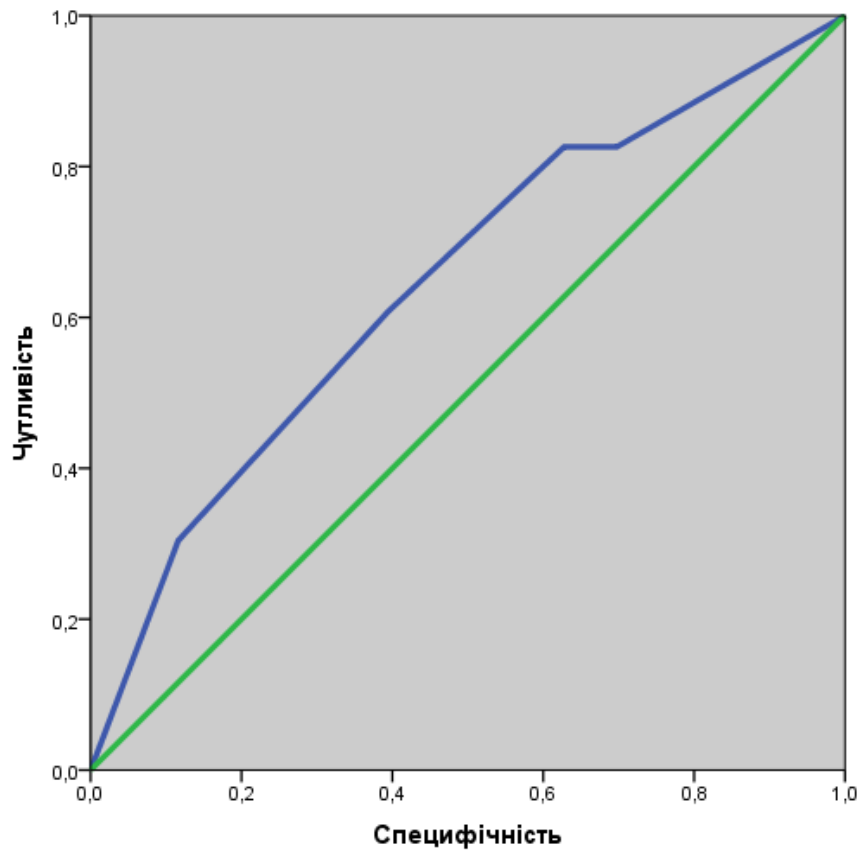
Fig. 4. ROC - curve: determination of dRk level in CVH C patients with HF F2

The sensitivity and specificity of dRk determination (Se = 93.23%; Sp = 91.14%) was significantly higher in CVH C patients F3 HF (AUC 0.928, p = 0.033) (Fig. 5).
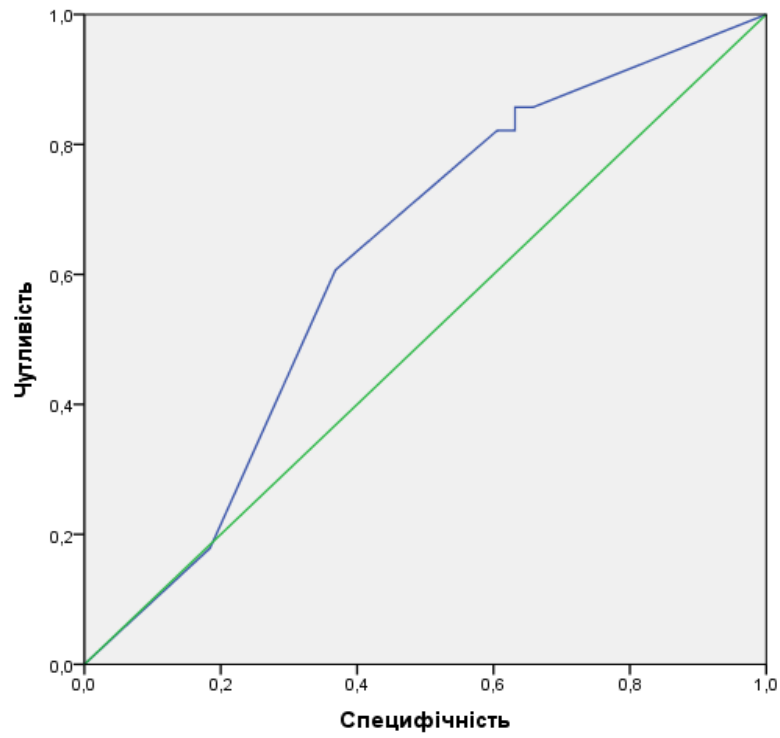
Fig. 5. ROC - curve: determination of AST and dRk level in CVH C patients with F3 HF

According to our data, the sensitivity of HA determination in blood serum of CVH C patients with F0-F2 of HF was 91.23%, and specificity, respectively, 90.46% (Fig. 5). AUC was 0.914 (p <0.001), which corresponded to the high significance of this method of HF diagnostication (Fig. 6).
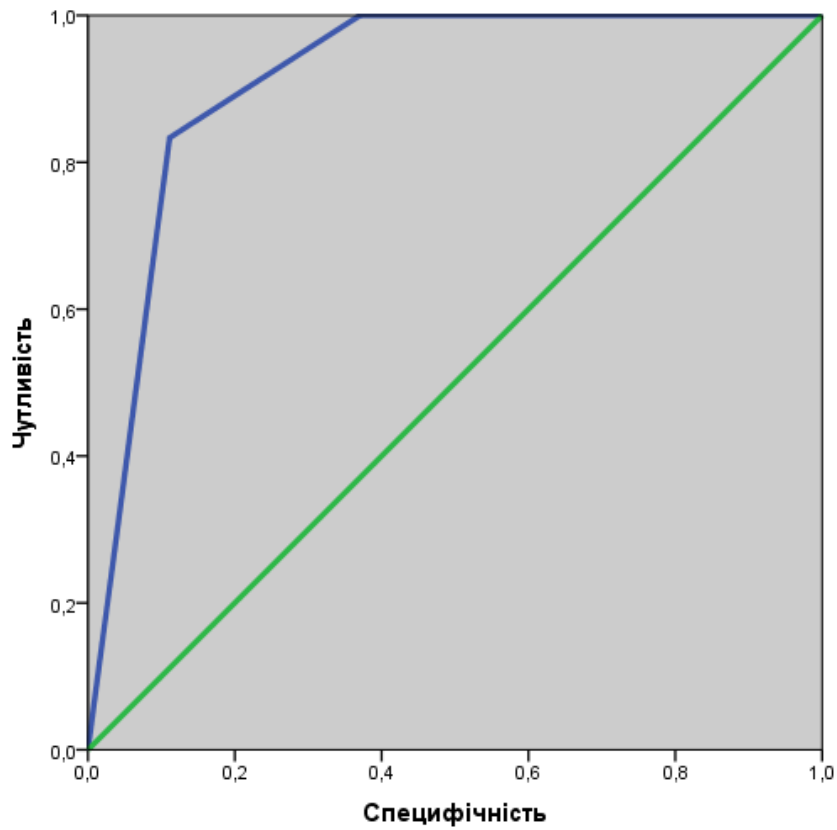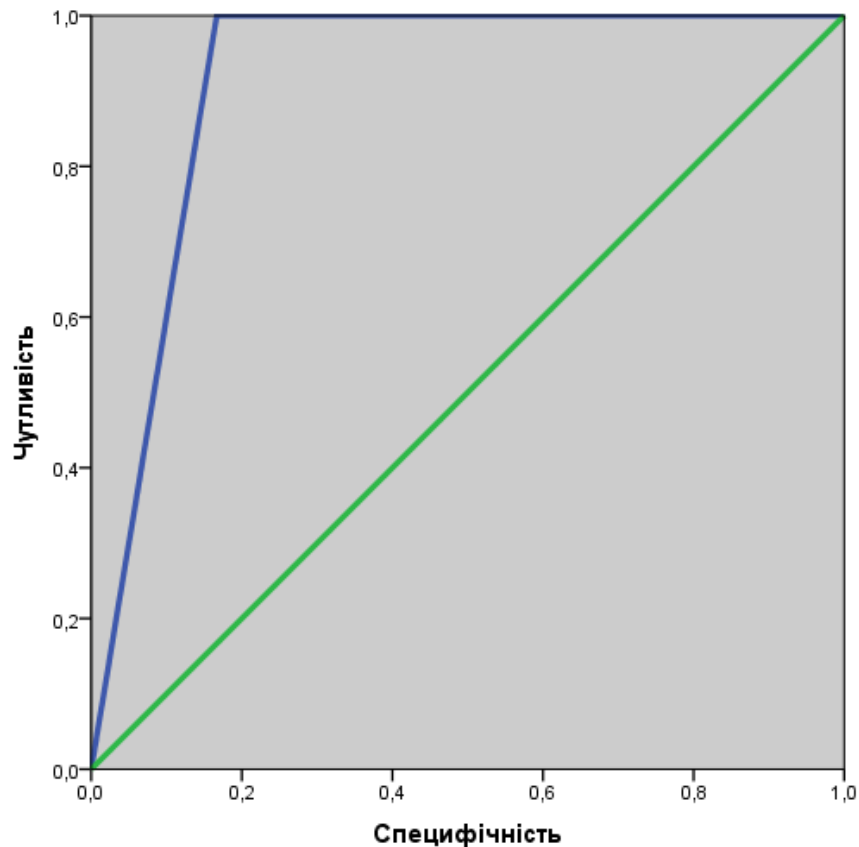
Fig. 6. ROC - curve: determination of HA content in blood serum of CVH C patients with F0-F2 HF

In the group of CVH C patients with a pronounced degree of HF (F3), the significance of HA content determining had a mean value (AUC = 0.720; p = 0.0098). The sensitivity of this method for determining the degree of HF in the patients under study was 72.21%, and specificity was 74.71% (Fig. 7).

Fig. 7. ROC - curve: determination of HA in blood serum of CVH C patients with HF F3

The sensitivity of HA determination in blood serum of CVH C patients F4 degree of HF was 88.23%, and specificity was 92.12%; AUC = 0.917 (p = 0.001) (Fig. 8).

Fig. 8 ROC - curve: determination of HA content in blood serum of CVH C patients with F4 degree of HF.

Conclusions:

1. High diagnostic informative value for the method of ALT determination in CVH C patients has been established only in F3 HF patients (AUC = 0.906; p <0.001).

2. For the method of AST determination in CVH C patients statistically reliable diagnostic informative value was established for F3 HF patients (AUC = 0.809; p = 0.042) and F4 HF (AUC = 0.817, p = 0.011).

3. Diagnostically significant informativity of dRk determination in CVH C patients has been established in those with F3 HF: AUC = 0.928; p = 0.033.

4. High diagnostic informative value of HA determination in blood serum has been established for assessing the degree of HF in CVH C patients (AUC = 0.720 – 0.917; p<0.01).

References:

1. Friedman, S. L. (2003). Liver fibrosis - from bench to bedside. J. Hepatol., 38 (1), 38–53. doi:10.1016/S0168-8278(02)00429-4.

2. Fontana, R .J. & Lok A. S. (2002). Noninvasive monitoring of patients with chronic hepatitis C. Hepatology, 36, 5 (1), 57–64. doi:10.1053/jhep.2002.36800.

3. Castéra, L., Vergniol, J., Foucher, J., Le Bail, B., Chanteloup, E., Haaser, M., de Lédinghen, V. (2005). Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology, 128 (2), 343–350. https://doi.org/10.1053/j.gastro.2004.11.018.

4. Hirata, M. & Akbar, S. M. & Horiike, N. & Onji, M. (2001). Noninvasive diagnosis of the degree of hepatic fibrosis using ultrasonography in patients with chronic liver disease due to hepatitis C virus. Eur. J. Clin. Invest, 31, 528–535. doi:10.1046/j.1365-2362.2001.00840.

5. Rosenberg, W. M., Voelker, M., Thiel, R., Becka, M., Burt, A., Schuppan, D. Arthur, M. J. (2004). Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology, 127 (6), 1704–1713. doi: 10.1371/journal.pone.0051906.