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EVALUATION OF LIVER FIBROSIS IN CHRONIC VIRAL HEPATITIS C.

PhD thesis abstract

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THE SYNTHESIS OF THE GENERAL PART

C virus infection is a public health problem worldwide, involving the growing costs and extensive health programs.

C viral infection is the cause of 70% of chronic hepatitis, 40% of decompensated liver cirrhosis, 60% of cases of hepatocellular carcinoma and 40-50% of liver transplant indications.

From WHO epidemiological data it is estimated that C virus infection affects 3% of world population, totaling over 170 million people, 2 million are located in Europe.

The overall prevalence of HCV infection is between 0.1 and 25% with an average of 3%. It is considered that subjects with positive HCV RNA is between 80 and 90% of those identified as HCV-Antibodies positive. In Romania HCV infection is responsible for 64% of chronic hepatitis and 55.8%. of liver cirrhosis. Genotype 1 is the one that prevails.

In most cases is fibrosis with slow progression to cirrhosis in reaching an average of 30 years. Among patients with cirrhosis a rate of 2-3% per year will develop hepatocarcinoma and 1-3% of them decompensated liver disease.

Means to assess liver fibrosis

1. Morphological evaluation- the liver biopsy

In the era of technological progress and development of molecular tests, liver biopsy remains the "gold standard" for assessing liver disease.

Fragment obtained by liver biopsy is subjected to macroscopic examination, optical microscopy, electron microscopy examination, immunohistochemistry techniques, special stain, DNA hybridization studies, microbiological examination.

Liver biopsy is of undoubted value in assessing viral hepatitis C. This confirms the diagnosis of chronic hepatitis C, but may reveal other pathological conditions that steatohepatitis (alcoholic or non-alcoholic), iron-loading syndromes suggestive of hemochromatosis, or can demonstrate the presence of blood suggestive of alpha 1 antitrypsin deficiency.

It can also reveal the presence of dysplasia or hepatocellular carcinoma.

2. Noninvasive markers of liver fibrosis

Since hepatic fibrosis is the most important in assessing the progression of viral hepatitis C and that it is governed by a number of mediators seams useful try to find a noninvasive method for assessing liver fibrosis, which is devoid of risks and repetitive in different stages of disease and treatment.

Study were taken following serological markers:

APRI-SCORE

-Index FORNS

FIB-4-Index

- Fibro Test

- FIBROINDEX

THE SYNTHESIS OF THE SPECIFIC PART

Material and method

In this study were 184 patients diagnosed with chronic hepatitis C who were admitted between January 2002 - March 2009, in the County Emergency Hospital of Sibiu and Military Emergency Hospital Sibiu.

Inclusion criteria

Diagnosis of chronic hepatitis C was made on clinical criteria, ultrasound, biochemical- persistent liver enzyme changes over 6 months (even though they were minimal), the presence of HCV antibodies.

Exclusion criteria

Exclusion criteria consisted of other possible causes of liver disease, HIV co-infection, co-morbidities that could alter hepatic histology and serological markers values study: alcohol consumption exceeds 20 g / day, hemolysis, SD. Gilbert, haematological pathology. Linked to liver biopsy led to the exclusion of patients for lack of agreement and the presence of contraindications for liver biopsy puncture.

Punctures were performed with 1.6 mm needles Menghini type (16 gauge), the transthoracic approach, with local anesthesia prior to post-expiratory apnea.

Histopathological examination of liver fragments obtained was performed in the pathological laboratory of Sibiu County Emergency Hospital.

Liver fragments were fixed in formalin and included in paraffin. There were hematoxylin-eosin stains, tricrom Masson and Perls .

The result of the histopathological examination was measured by Ishak score for the need for consistent analysis of cases and to initiate antiviral therapy.

At a 30 subgroup of the 184 patients, **(group 2),** in addition to serological markers determined in the global group, was evaluated Fibrotest (Timisoara Bioclinica Laboratories). Therefore, to compare data, histopathological examination was necessary to express the result as METAVIR scale, with subsequent comparison of results.

The global group of 184 subjects **(group 1)** were determined values of several serological markers for quantification of liver fibrosis: APRI scores, FORNS, FIB-4, FIBROINDEX and was made comparing the results with those of markers biopsies.

Calculation of scores was made using cut-off values and formulas used in original research published.

Results were obtained according to the protocol validated in laboratories Bioclinica Biopredictive Timisoara.

Data analysis was obtained for both the overall group **(1),** 184 subjects and two groups obtained according to ALT values: with normal ALT **(group 3)** and increased ALT **(group 4),** comparing the results obtained for each group separately.

RESULTS

In this paper we analyzed the data related to global group of 184 patients **(group 1),** which were compared with data obtained from liver biopsies and that obtained from the calculation of APRI, FORNS, FIB-4, FIBROINDEX.

1. Liver fibrosis

It was considered a significant level of fibrosis ≥ 3, obtained Ishak scale. In the group of 184 subjects overall index values on the grid Ishak fibrosis had the following distribution: group consisted of 88 (47.83%) patients with insignificant fibrosis, 96 (52.17%) patients with significant fibrosis of which 3 subjects with cirrhosis (1.6%).

2. Demographics

The group was composed of 121 women (65.8%) and 63 men (34.2%). In the batch average age of female subgroup was significantly increased ($p = 0.040$) than male

subgroup, which does not appear in the detailed analysis on the group index of fibrosis (Ishak) <3 or ≥ 3.

This could be explained by more severe general evolution of viral hepatitis C in males, which could cause symptomatic disease earlier than in females, leading to presentation of a medical service and research.

As expected the mean ages of subjects with fibrosis <3 are lower than in subjects with fibrosis> 3, requiring a certain period of evolution for the development of significant fibrosis.

Patients studied were from rural areas at a rate of 24.5% (45 subjects) and in urban areas at a rate of 75.5% (139 subjects). I have not found significant data in the literature on this, probable due to smaller differences between urban / rural areas in developed countries.

Given the long duration of infection until the time of diagnosis, source of infection could be established in a small number of patients, many remain unknown.

Thus, of the 184 patients 37 (20.1%) underwent blood transfusion, the most likely source of infection is this, 30 subjects (16.3%) of them underwent dental treatment more frequently than others, a total of 19 subjects (10.3%) were health professionals, the most likely cause of infection is occupational exposure, with all the information required in 98 patients in group (53.3%) source of infection could not be established. Note that it could not reveal a possible cause of contagion family occurred in the studied group.

Between the ages of 4 groups there was no significant difference, p = 0.764.

No significant associations were recorded between the different sources of infection and the living environment of the subjects in the group with global or significant fibrosis. In the group with fibrosis <3 the number of urban subjects was significantly higher. This could be due to greater addressability to the doctor in urban areas, so diagnosis in early stage disease.

There is a significant correlation between the type of activity and means of infection in both the global group (p=0,000) and the group with different degrees of fibrosis (p=0,017; respectively p=0,007). In patients who perform physical labor the number of patients with unknown source of infections was significantly higher than those providing intellectual work. Professional concerned cases of infection appeared less

frequently in situations of occupation based on physical labor, they are less exposed to contamination by HCV.

3. Hepatic steatosis

Steatosis was present in 42 of the 184 subjects studied (22.82%), the lower limit of literature data.

Note that there is no significant correlation between age groups and the presence of steatosis, although the literature shows significant correlation with more advanced ages, the presence of fatty liver and, hence, the response to antiviral treatment.

In the group of subjects studied to analyze the influence of gender on the presence of steatosis. Found significant correlation between fatty liver and feminine gender at global group, p-value = 0.040.

No significant correlations were recorded between the presence of steatosis and fibrosis grades.

4. Presence of diabetes

In the group studied diabetes was absent in 151 subjects (82.1%) and present in 33 subjects (17.9%), in a proportion much lower than the literature

The presence of diabetes and liver fibrosis were not significantly associated in our study.

No significant correlations were found between the presence of diabetes and distribution of the gender in the group studied, both in the global group and the groups differentiated by the amount of fibrosis (<3, ≥ 3), p = 0.495; 0.634; 0.192 respectively.

Diabetes- steatosis correlation

In our study we observed the existence of significant correlations between the presence of diabetes mellitus and hepatic steatosis in the overall group (p=0,005) without to keep it the batch analysis differentiated by the absence or presence of liver fibrosis ≥ 3 (p = 0.087 respectively 0.377)

5. Association of obesity

We studied at our group, the presence of obesity and its correlation with the index of fibrosis, necro-inflamatory activity, the presence of steatosis, diabetes mellitus and its relationship with liver function tests. Of the two tests is observed absence of exact match

with the degree of fibrosis obesity led Ishak scale (p=0.407) but there is a close relationship between the absence or presence of a significant degree of fibrosis and the absence or presence of obesity (p=0.036).

Unlike the literature, in our study did not exist correlations between the presence of obesity and fatty liver. It was found, but strong correlation between obesity and steatosis in the group without significant fibrosis, a condition under capacity steatosis without fibrotic damage (p=0.040). Also in the group without the presence of steatosis, obesity significantly influence the development of fibrosis (p=0.006). The presence of obesity was associated with the levels of necro-inflammatory activity (p=0.015).

6. N**ecro-inflammatory activity in global group.** (NIA)

Although necro-inflammatory activity was not the focus of this study is an important element in the characterization of liver damage and in determining whether antiviral usually treatment is necessary, with various studies to validate noninvasive methods of assessing it.

Most of the patients showed moderate necro-inflammatory activity, around 7 per Ishak score .

No significant correlations were recorded between NIA values and selected age groups, between values and distribution of cases by gender.

NIA values and values of liver fibrosis significantly correlated (p=0.007). This result was predictable in the light parallel to the parameters of liver injury. The correlation between the distribution of years and maintain liver fibrosis and fibrosis lots <3 or ≥ 3. (P = 0.001).

There were significant correlations between values and the presence of fatty liver (p=0,025).

7. Relationship between degrees of fibrosis and the laboratory findings

For each of the groups studied was made to determine some biological constants necessary to calculate the value chosen serological markers.

The global group were significantly associated with: changes in platelet count (low, normal) and the index of Ishak fibrosis score appreciated (p=0.031), also the numerical value of platelets with the index of fibrosis (p=0.000)

In the group of patients without diabetes was a significant correlation between the fibrosis and no values. platelets (p=0.002). This correlation was not maintained in the group of subjects with diabetes but was correlated with the absence of hepatic steatosis (p=0,002). Note the importance of determining the number of platelets, in fact an analysis performed in all patients usual, regardless of condition, the initial assessment of the likely degree of liver damage.

AST is well known as importance of its value, its ratio to normal values, in assessing the progression of liver disease.

In assessing the correlation between these parameters and the evolution of liver fibrosis following results were obtained: mean lots AST significantly correlated with hepatic fibrosis in the global group (p=0.005). There were significant correlations in group and patients without and with diabetes (p=0.000; 0.038 respectively), significant result was obtained in the group with absent steatosis (p=0.012).

The global group and the groups without and with diabetes ALT was significantly associated with the presence of liver fibrosis (p=0.047, 0.004, 0.035 respectively). No data has been showing significant correlation between ALT levels in groups differentiated the presence or absence of steatosis.

In our study there wasn't a significant correlation between serum cholesterol levels in either global batch or batches differentiated by absence or presence of diabetes, the absence or presence of hepatic steatosis.

Gammaglutamil transpeptidase values can be, as recent studies, an early marker of oxidative stress, possibly of cardiovascular stress. In our study we found a significantly association between hepatic fibrosis index detected by biopsy and GGT value in global group (p=0,027), group without diabetes (p=0.000) and without steatosis (p = 0.000).

8. Markers of noninvasive liver fibrosis

Markers were calculated values, in the general group **(1)** and normal ALT lots **(3)** and increased ALT lot (group **4),** using the original formulas published. They obtained the following results:

Table no. I. Performance of noninvasive markers to diagnose significant fibrosis (≥ ISHACK 3), the global group 184 patients / 108 Fibroindex

		APRI			FORNS		FIB-4		FIBROINDEX	
Cut-off Value		0.5	1.5	2	4.2	6.9	1.45	3.25	1.25	2.25
Classified cases (%)		58% (n = 107)			59% (n = 108)		70% (n = 128)		69% (n = 51)	
Unclassified cases	Total (%)	42% (n = 77)			41% (n = 76)		30% (n = 56)		31% (n = 57)	
	positive (%)	68% (n = 52)			61% (n = 46)		63% (n = 52)		70% (n = 52)	
Sensitivity		0.80	0.26	0.21	0.89	0.42	0.65	0.28	0.75	0.13
Specificity		0.53	0.82	0.87	0.53	0.88	0.65	0.89	0.55	0.93
PPV		0.65	0.61	0.65	0.67	0.78	0.67	0.73	0.71	0.73
NPV		0.71	0.50	0.50	0.82	0.58	0.63	0.53	0.60	0.42
LR +		1.70	1.44	1.62	1.89	3.5	1.86	2.55	1.67	1.86
LR-		0.38	0.90	0.91	0.21	0.66	0.69	0.81	0.45	0.94
Accuracy		0.67	0.53	0.53	0.72	0.64	0.65	0.57	0.67	0.45
AUROC (95% CI)		0.685 (0.607 ÷ 0.763)			0.765 (0.696 ÷ 0.834)		0.691 (0.614 ÷ 0.768)		0.655 (0.547 ÷ 0.763)	

Calculation of **APRI** score cut-off values of 0.5 and 1.5 led to the classification of 58% of subjects. Since 42% of patients could not be classified and adding the error rates observed with APRI <0.5 and one with APRI> 1.5, it is clear that liver biopsy could be avoided in 30% of subjects, values lower than other studies.

The calculation is **Forns** score considers that it coluld be avoided 37% of liver biopsies.

The analysis of four markers was observed that Forns score was the one who gave the highest value of NPV for the absence of significant fibrosis, the PPV for the presence of significant fibrosis and the highest value of AUROC (0.765), followed by FIB-4 (0.691).

In the literature it is considered that there are differences between the significance values whichever serological markers alanine aminotransferase (ALT), although transaminase levels was considered that the scale does not correlate with histological changes. We did a separate analysis of the lot taken according to this study.

The batch consisted of 142 subjects with increased ALT and 42 subjects with normal ALT. We analyzed the correlation with demographic, biological constants, histological data.

We obtained the following results:

Table no. II: Correlations with demographic data, histology observed with increased and normal ALT

		Increased ALT (142)	Normal ALT (42)	p
Male (%)		50 (35,2)	13 (31,0)	0.607
Urban (%)		108 (76,1)	31 (73,8)	0.767
Source of infection (%)	N	75 (52,8)	23 (54,8)	0.135
	P	11 (7,7)	8 (19,0)	
	S	26 (18,3)	4 (9,5)	
	T	30 (21,1)	7 (16,7)	
Physical Activity (F) (%)		107 (75,4)	32 (76,2)	0.911
Age (mean ± dev.standard)		49.50 ± 10.12	44.90 ± 9.68	**0.009 ****
Low platelets (%)		34 (23,9)	3 (7,1)	**0.009**
Platelets (mean ± dev.standard)		196112.68 ± 60961.46	226690.48 ± 66761.13	**0.010 ***
TRx10 9 / l (mean ± dev.standard)		196.11 ± 60.96	226.69 ± 66.76	**0.010 ***
AST (mean ± dev.standard)		77.11 ± 47.31	26.14 ± 12.73	**0.000 ****
AST / ULN (mean ± dev.standard)		2.1538 ± 1.3778	.7338 ± .3470	**0.000 ****
GAMAGLOB (mean ± dev.standard)		1.50 ± .43	1.21 ± .20	**0.000 ****
Cholesterol (mean ± dev.standard)		176.39 ± 39.76	180.64 ± 37.47	0.527
GGT (mean ± dev.standard)		117.95 ± 81.56	41.00 ± 38.45	**0.001 ***
SPLINE diamonds (mean ± dev.standard)		10.98 ± 1.39	10.63 ± 1.34	0.214
Ishak Fibrosis (%)	0	4 (2.8)	6 (14.3%)	0.000 **
	A	1 (7%)	4 (9.5%)	
	2	47 (33.1%)	26 (61.9%)	
	3	73 (51.4%)	6 (14.3%)	
	4	14 (9.9%)		
	5	3 (2.1%)		
NIA (mean ± dev.standard)		6.97 ± 1.90	6.24 ± 1.71	0.020 *

ALT = alanine aminotransferase, AST = aspartataminotrasferaza, GGT = gamaglutamiltransferaza, NIA= necro-inflammatory activity.

It is noted that there is statistically significant correlation between several characteristics of both groups: mean age of subjects (p = 0.009), with significantly higher number of older subjects in the group with increased ALT (possibly more severe disease

with increasing age), platelet count (p = 0.01), with the proportion of subjects with low platelet count (p = 0.09), with a tendency to decrease with advancing disease or numerical value of AST (p = 0.0), consistent with ALT evolving, with a tendency to overcome the disease progresses to ALT liver cirrhosis. Gamma globulines level was significantly higher in the group with increased ALT (p = 0.00), GGT level was also significantly increased in the group with increased ALT (p = 0.001).

There is also a statistically significant correlation between values and distribution of liver fibrosis according to the ALT. The fibrosis is increased, the number of subjects with increased ALT is greater. (p = 0.0) There was a significant correlation between the value necro-inflammatory activity (NIA) and ALT level (p=0,020).

No significant correlations were found between the two groups regarding distribution by gender, type of activity, type of origin, source of infection.

For each of the plots obtained were analyzed markers determined in the global group: APRI, Forns, FIB-4, Fibroindex. The results are:

Table III: Performance of noninvasive markers for the diagnosis of fibrosis (ISHACK> 3) in the patients with increased ALT

		APRI			FORNS		FIB4		FIBROINDEX	
Cut-off Value		0.5	1.5	2	4.2	6.9	1.45	3.25	1.25	2.25
Classified cases (%)		51.4 (n = 73)			55.6 (n = 79)		65.5 (n = 93)		42.7 (n = 38)	
Unclassified cases	Total (%)	48.6 (n = 69)			44.4 (n = 63)		34.5 (n = 49)		57.3 (n = 51)	
	positive (%)	72.5 (n = 50)			66.7 (n = 42)		67.3 (n = 33)		74.5 (n = 38)	
Sensitivity		0.83	0.28	0.22	0.89	0.42	0.65	0.28	0.74	0.13
Specificity		0.35	0.71	0.80	0.40	0.80	0.50	0.80	0.40	0.89
PPV		0.69	0.62	0.67	0.72	0.79	0.69	0.72	0.74	0.73
NPV		0.55	0.36	0.37	0.68	0.45	0.45	0.40	0.41	0.31
LR +		1.27	0.97	1.16	1.49	2.20	1.31	1.51	1.25	1.16
LR-		0.48	1.01	0.96	0.27	0.72	0.69	0.88	0.63	0.98
Accuracy		0.65	0.44	0.44	0.71	0.56	0.60	0.48	0.64	0.36
AUROC (95% CI)		0.566 (0.465 ÷ 0.667)			0.705 (0.615 ÷ 0.795)		0.596 (0.497 ÷ 0.695)		0.544 (0.410 ÷ 0.678)	

Table no. IV: Performance of noninvasive markers for the diagnosis of fibrosis (ISHACK> 3) in the patients with normal ALT

		APRI			FORNS		FIB4		FIBROINDEX	
Cut-off Value		0.5	1.5	2	4.2	6.9	1.45	3.25	1.25	2.25
Classified cases (%)		80.1 (n = 34)			69.1 (n = 29)		83.3 (n = 35)		68.4 (n = 13)	
Cases classified	Total (%)	19.9 (n = 8)			30.9 (n = 13)		16.7 (n = 7)		31.6 (n = 6)	
	positive (%)	25.0 (n = 2)			30.8 (n = 4)		28.6 (n = 2)		33.3 (n = 2)	
Sensitivity		0.33	0.00	0.00	A	0.33	0.50	-	A	0.0
Specificity		0.81	0.97	0.97	0.72	0.97	0.86	-	0.76	A
PPV		0.22	0.00	0.00	0.38	0.67	0.38	-	0.33	0.0
NPV		0.88	0.83	0.83	A	0.90	0.91	-	A	A
LR +		1.72	0.00	0.00	3.59	11.89	0.58	-	4.25	0.00
LR-		0.83	0.01	0.01	0.00	0.69	3.59	-	0.00	
Accuracy		0.74	0.83	0.83	0.76	0.88	0.81	-	0.79	0.89
AUROC (95% CI)		0.738 (0.549 ÷ 0.928)			0.903 (0.801 ÷ 1004)		0.745 (0.553 ÷ 0.938)		0.853 (0.685 ÷ 1021)	

As can be seen from Tables nr.III and IV, the performance obtained by determining the serological markers of liver fibrosis in the group with increased ALT are less encouraging than for global lot. Values were obtained from our study for all markers, significantly lower than the results of other studies.

Group 2 comparison of the results. (Fibrotest further determination)

In general batch of 184 patients **(group 1)** was selected a sublot 30 subjects **(group 2),** which, in addition to the 4 serological markers analyzed values previously determined and the value of Fibrotest.

The correlation between Fibrotest and fibrosis estimated values is as follows (table no. V):

Table nr.V. Performance of noninvasive markers to diagnose significant fibrosis (METAVIR F2 ≥) in the patients in group 2

		APRI			FORNS		FIB4		Fibro-Index		Fibro-TEST
Cut-off Value		0.5	1.5	2	4.2	6.9	1.45	3.25	1.25	2.25	0.32
Classified cases (%)		40 (n = 12)			70 (n = 21)		70 (n = 21)		39 (n = 11)		100%
Cases classified	Total (%)	60 (n = 18)			30 (n = 9)		30 (n = 9)		61 (n = 17)		0%
	positive (%)	83 (n = 15)			100 (n = 9)		89 (n = 8)		100 (n = 17)		-
Sensitivity		0.69	0.12	0.08	0.65	0.31	0.42	0.12	0.72	0.04	0.77
Specificity		0.25	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00	0.75
PPV		0.86	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00	0.95
NPV		0.11	0.15	0.14	0.31	0.18	0.17	0.15	0.30	0.11	0.33
LR +		0.92	∞	∞	∞	∞	1.68	∞	∞	∞	3.08
LR-		1.24	0.88	0.92	0.35	0.69	0.77	0.88	0.28	0.96	0.31
Accuracy		0.63	0.23	0.20	0.70	0.70	0.47	0.23	0.75	0.14	0.77
AUROC (95% CI)		0.563 (0.25 ° 0.88)			0.962 (0.89 ÷ 1.03)		0.798 (0.51 ÷ 1.09)		0.833 (0.68 ÷ 0.98)		0.659 (0.41 ÷ 0.91)

As can be seen from Table no.V advantage Fibrotest determination is that all patients can be classified.

APRI score leaves not classified 60% of cases, Forns, and FIB-4 scores: 30% of cases and 61% of subjects Fibroindex.

Negative predictive values for the absence of fibrosis were small for all four markers in turn were obtained positive predictive values for the presence of fibrosis very good and 1 for all four markers.

Just as with a lot of the best AUROC value was obtained for Forns index (0.962), followed by Fibroindex (0.833) and Fib -4 (0.798).

APRI and Fibrotest AUROC for value has the meaning encouraging results and the possibility to substitute liver biopsy them. In literature, the original study was obtained WAI for APRI index for detection of significant fibrosis AUROC of 0.88, a much better value to our study (0.563).

Forns original study (2002) obtained for the detection of significant fibrosis AUROC of 0.94, the value of our study was close.

Unexpected results of determination were Fibrotest results are much less encouraging than in many international studies:

Given the results on the global significance for serological markers of liver fibrosis and on the influence of obesity and fatty liver fibrosis, we tried a new marker accuracy assessment result from the combination of the best performing markers, which were added related data to obesity and hepatic steatosis. BMI was calculated at each and ultrasound steatosis was highlighted. They use Forns index and other markers of elements in different proportions. This was applied to the group of 108 subjects who were available gamma globulines value. Cut off values were 34 and 41.5.

The results are listed in Table. VI,VII.

The used formula was:

Forns x √ALT+0.005x AST+0.463x gamaglob+10x obesity+5x steatosis.

Table nr.VI. Performance of noninvasive markers to diagnose significant fibrosis (METAVIR F2 ≥) in the patients in group 5

		APRI			FORNS		FIB4		FIBROINDEX	
Cut-off value		0,5	1,5	2	4,2	6,9	1,45	3,25	1,25	2,25
Classified (%)		54,6 (n=59)			63,0 (n=68)		63,9 (n=69)		47,2 (n=51)	
Unclassified	total (%)	45,4 (n=49)			37,0 (n=40)		36,1 (n=39)		52,8 (n=57)	
	positiv (%)	71,4 (n=35)			70,0 (n=28)		64,1 (n=25)		70,2 (n=40)	
Sensibility (%)		0,78	0,23	0,17	0,86	0,42	0,63	0,23	0,75	0,13
Specificity(%)		0,48	0,79	0,86	0,55	0,82	0,57	0,89	0,55	0,93
PPV (%)		0,68	0,62	0,65	0,73	0,77	0,68	0,75	0,71	0,73
NPV (%)		0,60	0,42	0,42	0,73	0,49	0,51	0,44	0,60	0,42
LR+		1,50	1,10	1,21	1,91	2,33	1,47	2,09	1,67	1,86
LR-		0,46	0,97	0,97	0,17	0,71	0,65	7,00	0,45	0,94
Acuracy		0,66	0,46	0,45	0,73	0,58	0,60	0,50	0,67	0,45
AUROC (95% CI)		0,622 0,508 ÷0,736			0,728 0,629 ÷0,827		0,645 0,535 ÷0,754		0,655 0,547 ÷0,763	

Table nr.VII. Performance of noninvasive markers to diagnose significant fibrosis (METAVIR F2 ≥) in the patients in group 5

Valoare Cut-off		SCOR N	
		34	41.5
Cazuri clasificate (%)		91.67 %(n=99)	
Cazuri neclasificate	total (%)	8.33% (n=9)	
	pozitivi(%)	67% (n=6)	
Sensibilitate		0.938	0.844
Specificitate		0.50	0.568
PPV		0.732	0.74
NPV		0.846	0.714
LR+		1.876	1.954
LR-		0.124	0.275
Acurateţe		0.759	0.731
AUROC (95% CI)		0,744 (0,646÷0,843)	

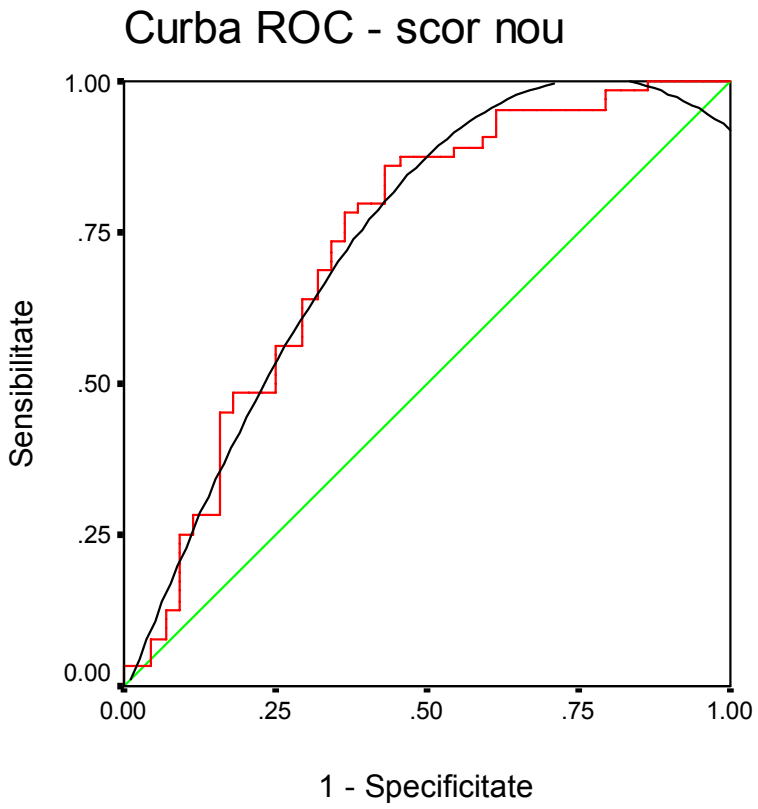

Fig. nr 101

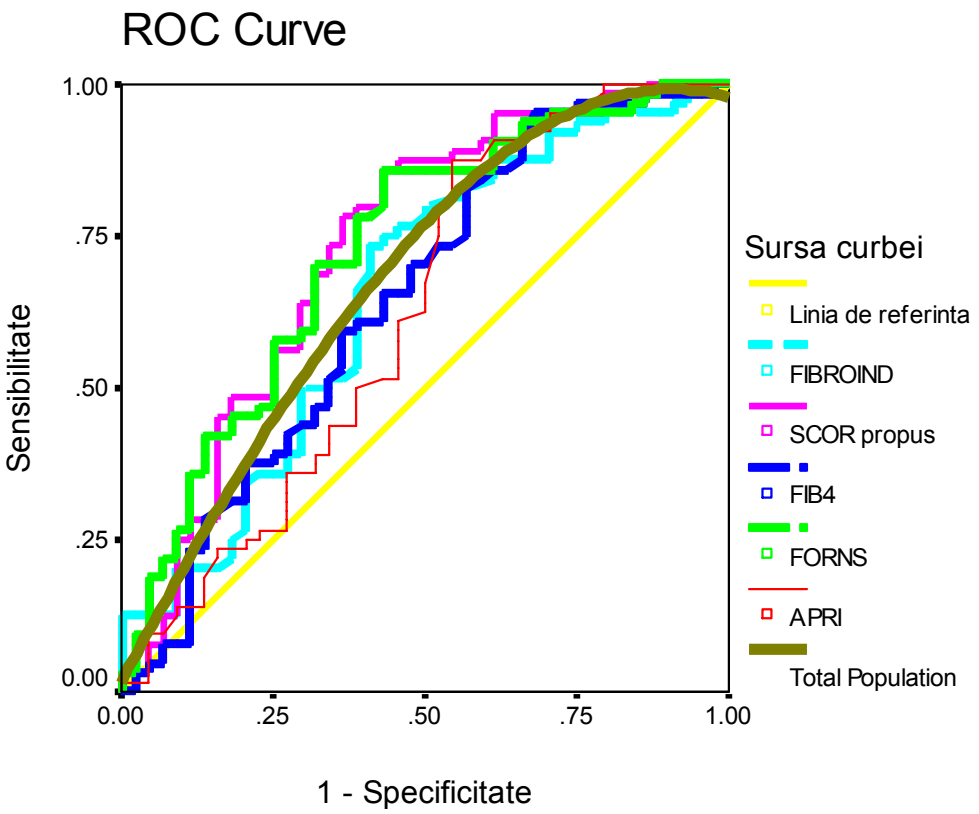

Fig. nr. 102

This was applied to the group of 108 subjects who were available gamma globulines value. Cut off values were 34 and 41.5.

Unlike most of the value achieved when the index FORNS AUROC (0.728), the new marker obtained slightly higher AUROC (0.744).

CONCLUSIONS

1. It was taken to study a group consisted of a representative number of subjects with chronic hepatitis C that was dependent on the given consent to perform a liver biopsy puncture. The group was composed of subjects with significant fibrosis (ISAHK scale values ≥ 3) (52,17%), and subjects without significant fibrosis (47,3%).

2. The distribution by gender was predominantly female gender significantly more likely related to greater addressability to medical services, the possible increased exposure to infection related to obstetric maneuvers, the increased frequency of women in the medical staff.

3. The average age of subjects was consistent with the literature, about the time of infection. The average age of the male of the lot was significantly lower than that of the female lot, possibly more severe due to the evolution of the disease in the masculine gender. Mean age of subjects with fibrosis <3 are lower than those with fibrosis ≥ 3, given the need for a greater period of time to produce significant liver damage.

4. Subjects in the study came mostly from the urban, probably due to greater addressability to their medical practices, including dental disease information possibilities and risks.

5. Viral infection source could be determined approximately in subjects with known history of transfusion, repeated dental treatments, history of traumatic occupational exposure.

6. No significant correlations were recorded between the ages of subjects undergoing different modes of infection.

7. There was a significant correlation between the type of activity and means of infection in both the global group and the fraction of the significant degree of fibrosis (≥ 3. In patients who provide physical labor with unknown source number of infections was significantly higher than those providing intellectual work. Professional concerned cases of infection appeared less frequently in situations of occupation based on physical labor, they are less exposed to contamination by HCV.

8. Steatosis was confirmed by biopsy in 22.8% of subjects. This was not significantly correlated with the degree of fibrosis. Female gender was associated mainly with the presence of steatosis in the overall lot.

9. Diabetes mellitus was diagnosed in 17.9% of subjects studied. Its presence was not associated with hepatic fibrosis grades. A statistically significant correlation was found between the presence of diabetes mellitus and hepatic steatosis in the overall lot without keeping it in batches of fibrosis <3 and ≥ 3.

10. Obesity determinism has important role in liver damage. There is a correlation between the absence or presence of a significant degree of fibrosis and the absence or presence of obesity.

11. Along with the degree of hepatic fibrosis was analyzed necro-inflammatory activity

level. No significant correlations were recorded between necro-inflammatory score (NIA) and age groups of subjects or their distribution by gender .

12. As expected, the degree of fibrosis and degree of necroinflammatory activity recorded score were statistically significantly associated.

13. Were significantly correlated also the degree of necro-inflammatory activity and the presence of steatosis.

14. We pursued the statistical significance of laboratory tests specifying the degree of hepatic fibrosis. The global group platelet value, its variations and index Ishak fibrosis score showed appreciated significant correlation. Hence the importance of a biological marker in assessing the evolution of a trivial disease of considerable severity. In patients without diabetes was a significant correlation between the fibrosis and values of number of platelets, AST, ALT, GGT. (p= 0.002, 0.000, 0,004, 0.000 respectively). Were significantly correlated to liver fibrosis index biopsy detected the GGT value in batches of diabetic absent (p = 0.000) and absent steatosis (p = 0.000). Hence, the conclusion that modification of the usual biochemical tests could be a warning in staging of subjects with chronic hepatitis C.

15. For subjects under study were calculated several noninvasive markers: APRI, Forns, FIB-4, Fibroindex, Fibrotest. To liver biopsy, which cdassifies each subject, non-invasive markers except Fibrotest can not classify all cases. Thus: the global group, APRI classified 80.1% of cases, Forns 69.1%, 83.3% Fib-4, Fibroindex 68.4% of cases. In group 2 (group of 30 subjects who determined Fibrotest), APRI has classified 58% of cases, Forns 59%, 70% Fib-4, Fibroindex 69% of cases. Fibrotest has the quality classification of all subjects.

16. APRI, Forns, FIB-4, Fibroindex scores have relatively low cost price quality using biological association that constant is performed routinely in the evaluation of chronic hepatitis.

17. The results were less optimistic than in the original studies, except Forns index, which was obtained AUROC of 0.765.

18. Unlike other international studies analyzing data from serological markers showed optimistic results highlight liver fibrosis in the group of subjects with increased ALT to normal ALT.

19. Between the assessment made by Fibroindex fibrosis / APRI, FIB-4 / APRI, were found statistically significant correlations.

20. Some results obtained were used to obtain a marker combined with better statistical significance. Thus, by combining with the Forns index and reference ALT in obesity and fatty liver formula was obtained with a marker in determining hepatic fibrosis AUROC 0.744.

21. Serological markers of liver fibrosis, by corroborating data may represent an alternative to liver biopsy, especially in cases where contraindications or refused by the patient.

Personal contributions

- It is the first study of its kind in our area .
- Interesting data were obtained on the correlations between variables fibrosis and demographic factors.
- Demonstrated the importance and influence of obesity on liver disease.
- We highlighted the significance of laboratory values in assessing fibrosis.
- Conflicting results were obtained with other studies on serological markers accuracy between the groups with increased ALT and normal ALT.
- Formula outlined a new serological marker that can improve data interpretation.