„CAROL DAVILA” University of Medicine and Pharmacy, Bucharest

DOCTORAL SCHOOL

FIELD OF GENERAL MEDICINE

PhD THESIS

-summary-

Coordinator professor:

PROF. UNIV. DR. PETRE I. CALISTRU

PhD Student:

DR. RALUCA M. DÎRȚU

2024

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Evaluation of liver fibrosis, extrahepatic manifestations and quality of life in patients with chronic hepatitis C virus before and after direct-acting antiviral treatment (DAA)

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Epidemiological aspects

Hepatitis C virus is still a public health problem. The distribution is asymmetric and the global prevalence is estimated to be between 0.5% and 6.5%, there are areas with low prevalence and effective screening measures; burden areas difficult to manage, as well as regions with increasing prevalence or difficult to stabilize (1). Seroprevalence studies suggest that between 1990 and 2005 the increase in prevalence was from 122 million infected to 185 million (2). An important aspect of the epidemiological data is also the migration phenomenon. Migration has been a part of society since ancient times and has influenced both the way of life and the epidemiology and control of certain diseases (3). Thus, it is found that, in addition to the measures to increase the diagnosis rate, to increase access to treatment, greater attention to the migration aspect is also necessary (3). A society with a good control of the incidence of hepatitis can be destabilized by the migrant population it receives, thus leading to an increase in the number of infections and putting pressure on the health system. According to studies, this happens due to lack of information, low addressability to the doctor, but also due to the lack of universal screening, diagnosis and treatment measures (3). The hepatitis C virus has the ability to form quasispecies, therefore before starting treatment it is necessary to identify the viral genotype. Depending on the genotypic type, the global distribution is as follows: in Europe and North America genotype 1 predominates (49.1% of cases), with the mention that this genotype is divided into two subclasses: 1a and 1b, the latter being present both in Europe (over 68% of cases) and in Romania (over 99.6% of cases) (4,5). Genotype 3 is the second most prevalent globally, with a percentage of 17.9%, and in Europe, with 25.5% (4). Genotype 4 is common in North Africa and the Middle East, especially in Egypt (as a result of the injection treatment used for schistostomiasis, which infected millions of people with the hepatitis C virus). Genotype 5 is predominant in South Africa (4).

Understanding the mechanisms of viral replication has made possible the development of current interferon-free therapies based on direct-acting antivirals (DAA) (6).

The hepatitis C virus genome is extremely unstable, resulting in permanent errors, correlated with genetic heterogeneity and the appearance of multiple quasispecies in the same infected individual (6,7).

The HVR variable region, located at the N-terminal end of the E2 protein, is responsible for the occurrence of mutations (6,7). Variations in HVR-1 AND HVR-2 are generated very rapidly and may confer adaptive advantages in viral tropism, virulence, or

resistance to therapy (6,7). Mutations in nonstructural proteins, such as NS5A, confer sensitivity to interferon (IFN) treatment. The responsible region is located in the C-terminal portion of the NS5A protein and is called the interferon-responsive region (ISDR) (7).

Chronic C virus infection is generally a slowly progressive disease, characterized by persistence of liver inflammation and correlated with the development of cirrhosis in approximately 20% of patients after 20-30 years of infection (progression rate varying between 2% and 51% after 22 years of persistent infection) (6,8).

The World Health Organization has implemented international programs to diagnose, increase access to treatment and monitor patients with hepatitis C, which aim to eliminate hepatitis C as a public health problem by the year 2030 (9). At the same time, reaching these targets at the global level depends a lot on the local strategies of each individual state. Thus, the "micro-elimination" of hepatitis C virus infection at the level of each region, as well as the establishment of some "target" populations can lead to the establishment of the premises towards a possible global elimination of hepatitis C (9).

In Romania, there are ongoing national programs that provide free treatment to patients diagnosed with hepatitis C in order to establish effective measures of controlling new infections, as well as to increase accessibility to treatments, especially with focus on the population in rural areas, which is not sufficiently informed about the risks of infection, does not have access to medical assistance for social, economic or local infrastructure reasons (9). There are countries that have managed to implement local hepatitis C elimination programs with favorable results, such as Spain, Slovakia, Scotland, Iceland (9). It should be noted that, in order to achieve these targets, other factors are also important, such as demographic, economic, environmental, tourism, etc.

Theme and general objectives

Choice of topic: Chronic hepatitis C virus is still a public health problem worldwide. The effects of chronic infection are not localized only at the liver level, but gradually affect the whole organism, causing the so-called extrahepatic manifestations, such as: skin, neurological, vascular, metabolic manifestations, etc. The treatment of chronic C virus infection, as well as the monitoring of extrahepatic manifestations, but also the evaluation of the quality of life of these patients are important premises in the fight to decrease the incidence of this pathology and regarding the systemic manifestations of the infection.

Objectives:

• I. Assessment of fibrosis before and after starting AAD treatment using One-Dimensional Transient Elastography (Fibroscan) and Fibrotest

• II. Monitoring of extrahepatic manifestations, with emphasis on the measurement of the intima-media index (IMT) by performing Doppler at the level of the common carotid artery (CCA) before the start of AAD treatment and after its completion

• III. Evaluation of the quality of life of patients with hepatitis C virus using the SF-36 and Hospital Anxiety and Depression Scale (HADS) questionnaires before and after treatment with AAD

General research methodology

Statistical analysis

Data collection was performed in Microsoft Office-Excel (v. 2007-2010), and the actual statistical processing was performed in IBM-SPSS Statistics v. 20 and with the online statistical package https://www.medcalc.org/ calc/. The graphic part was made in IBM-SPSS Statistics v. 20 and Microsoft Office-Excell (v. 2007-2010) (70,71,72,73). I used univariate, bivariate and multivariate statistics.

Patients and methods

Study conducted at the Clinical Hospital for Infectious and Tropical Diseases "Dr. Victor Babeș", Bucharest and at the Diagnostic and Treatment Center "Dr. Victor Babeș", Bucharest; the Doppler was performed with the support of the Neurology Clinic of SUU “Elias”, Bucharest;

Prospective longitudinal design: 106 patients included (104 eligible) – study started in January 2019;

Inclusion criteria: stage F1, F2 (Metavir), F3 (Metavir) – naïve patients or previously treated with interferon, F4 (Metavir) – patients with compensated cirrhosis;

Exclusion criteria: decompensated liver cirrhosis (Child-Pugh score > 6 points or presence of complications); liver cancer without therapeutic indication with curative potential; extrahepatic malignancies that do not benefit from potentially curative treatment; HBV-HCV co-infection; HCV-HIV co-infection;

Treatments used: ledispavir/sofosbuvir (HARVONI), ombitasvir/paritaprevir/ritonavir (VIEKIRAX-EXVIERA), grazoprevir/elbasvir (ZEPATIER);

Materials used: Transient elastography (Fibroscan), carotid Doppler, standardized SF-36 quality of life questionnaires, HADS.

Results and discussion

Demographic characteristics of the studied group

Age of patients in the studied group (categories and absolute values)

In the table below (and in the figure) we have the distribution by age groups:

Age	Frequency (no.cases)	Percentage (%)
20-30	2	1.9
31-40	12	11.3
41-50	12	11.3
51-60	32	30.2
61-70	31	29.2
71-80	15	14.2
81+	2	1.9
Total	**106**	**100%**

Tabel 1. Age distribution

As central statistical indicators for age we have:

Average age and 95% CI	**Median (IQR)**	**Minimum**	**Maximum**
57.86 (55.30-60.42)	60.0 (50.75-67.00)	23	82

From the analysis of the distribution of the data (histogram below), we have a non-normal distribution with a shift to the left from the mean (left-skewed).

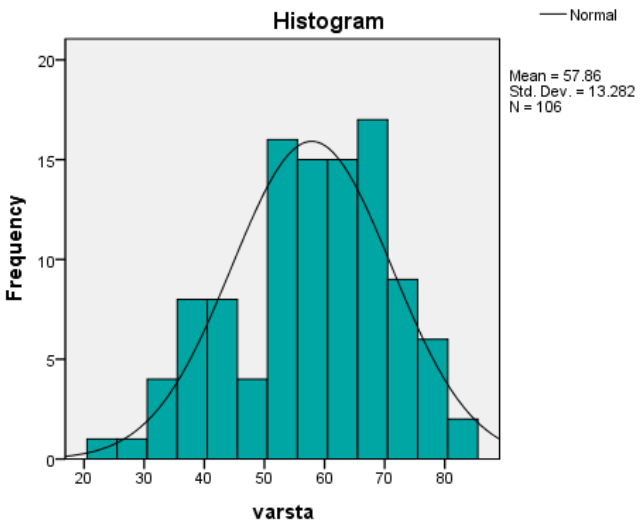

Fig. 1. Shapiro-Wilk Test (confirms the skewed distribution of the data *p=0.029*).

In the analyzed group, women predominated - 67 (63.2%), compared to men - only 39 (36.8%).

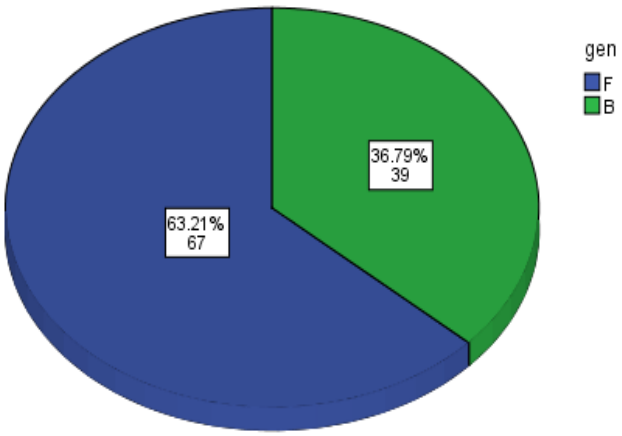

Fig. 2. Gender distribution

Analysis by place of living (univariate analysis)

	Cases	Percentage (%)
Rural area	19	17.9%
Urban area	87	82.1%

Tabel 2. Distribution by means of origin

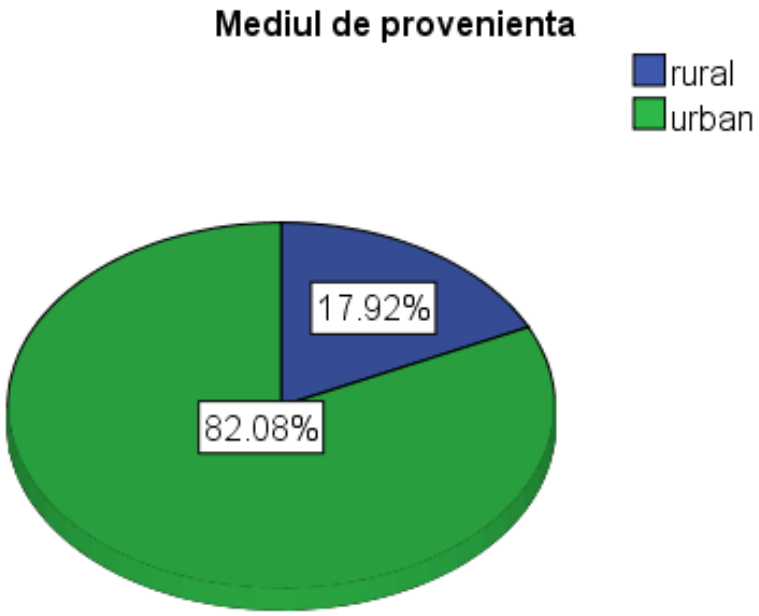

Fig. 3. Distribution by means of origin

Analysis by type of education (primary, secondary, higher)

Education	No. of patients	Percentage (%)
Primary	14	13.2%
Medium	74	69.8%
Superior	18	17.0%

Tabel 3. Type of education

Analysis of comorbidities present in the analysed group

Comorbidities	Cases	Percentage (%)
Cardiovascular	56	52.8
Metabolic	7	6.6
Psychiatric	7	6.6
Others	6	5.7
Without comorbidities	30	28.3
Total	**106**	**100%**

Tabel 4. Distribution of comorbidities in the studied group (univariate analysis)

We observe that cardiovascular ones predominate (52.8%) in a significant percentage even without statistically testing. Patients without comorbidities are also in a significantly high percentage (28.3%), but much less (~1/2) than those with cardiovascular comorbidities. Metabolic, psychiatric and other comorbidities are in very low percentages.

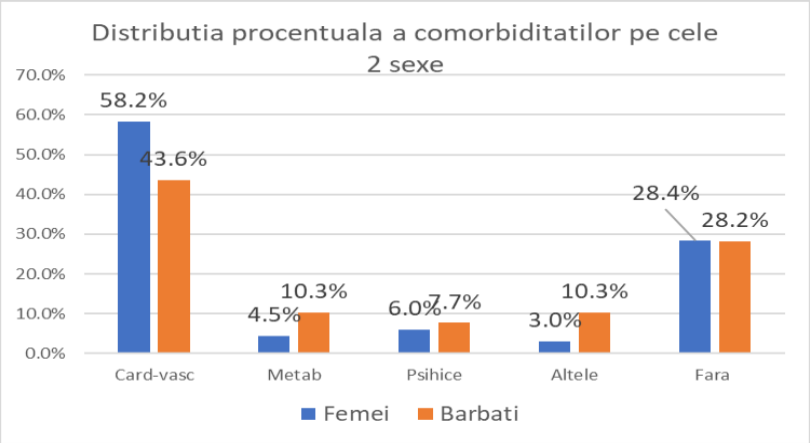

Fig. 4. Percentage distribution of comorbidities by gender

Cardiovascular comorbidities are prevalent in both women and men, with a slightly higher percentage in women (58.2%, compared to 43.6%). The group without comorbidities is also similar in both sexes. There are no statistically significant differences between the disease percentages in the two sexes (*p=0.324*, Pearson chi-square).

BMI by demographic factors

Univariate analysis of BMI

BMI	Cases	Percentage (%)
Underweight	6	5.7
Normal weight	56	52.8
Over weight	44	41.5

Tabel 5. Distribution of patients according to BMI

It can be observed a majority distribution of normal weight cases, but also a fairly large number of overweight cases. Underweight cases being very small in number and bordering on the normal class, for statistical reasons they were merged into the normal weight class.

Bivariate analysis (BMI by other factors)

Bivariate analysis indicates percentage differences with statistical significance (Fisher's exact test, *p=0.024)*.

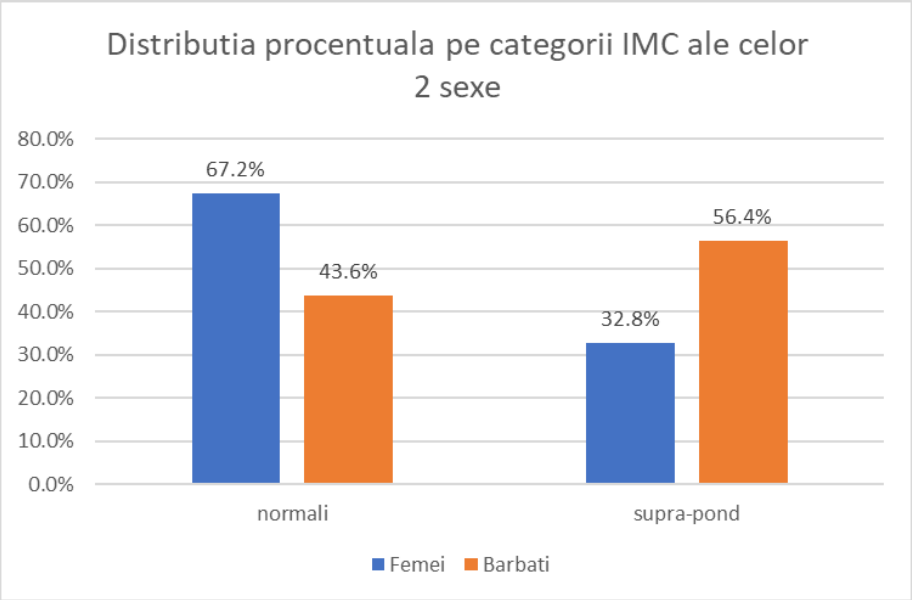

Fig. 5. BMI percentages by gender

The visual analysis of the graph shows us that the percentage of overweight men is higher than those of normal weight, and for women it is an inverse relationship, the percentage of women with normal BMI being higher than the percentage of overweight women.

I. Assessment of liver fibrosis before and after starting AAD treatment using One-Dimensional Transient Elastography (Fibroscan) and Fibrotest

Evaluation of liver fibrosis stages using Fibroscan

Being an important investigation, we analyze it univariately and bivariately (that is, according to various factors that could influence the outcome of the investigation).

Being a repeated analysis over time, we approach it bivariately, i.e. comparing the values before treatment and the values found after treatment, wanting to highlight how the cases are distributed by fibrosis stages, before and after treatment, and how the mean and median values are in kPa before treatment and after treatment within each class.

Comparison of the distribution of cases by fibrosis stage

(104 patients, two of whom could not be investigated because of abdominal adipose tissue)

Fibrosis stage	Before treatment Nr (%)	After treatment Nr (%)
F0	12 (11.3)	58 (54.7)
F1	14 (13.2)	33 (31.1)
F2*	37 (34.9)	9 (8.5)
F3	26 (24.5)	4 (3.8)
F4	15 (14.2)	-

Tabel 6. Distribution of cases by fibrosis stage

Before treatment, in F2 stage, three patients had borderline fibrosis stage (F1/F2). After treatment there were no more borderline classes.

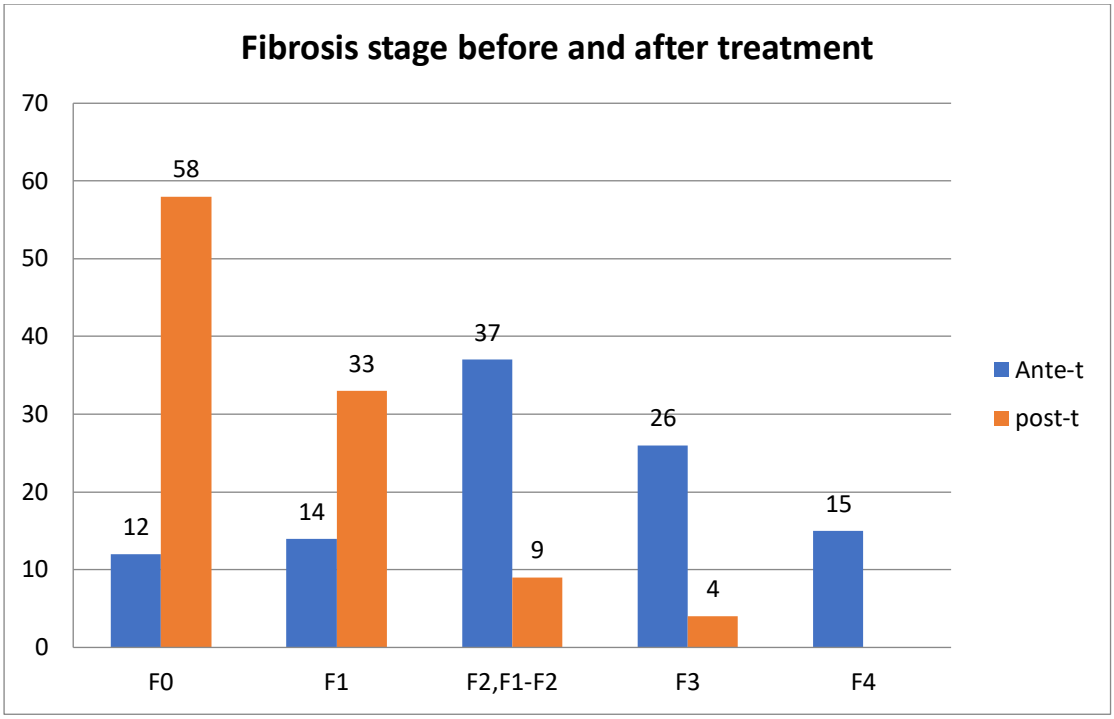

Fig. 6. Fibrosis stages before and after DAA treatment

Comparison of the stage of fibrosis before treatment and after treatment (at 6 months) by analyzing the values in kPa obtained by Fibroscan

By performing the t-test (*paired t-test) an average difference of 5.45 was obtained between the average value before the start of the treatment of 11.100 and the average value after the treatment of 5.641. The difference (5.45, CI95% 3.76-7.15) is statistically significant (t=6.399, df=103, p<0.001).

Percentage distribution on each stage of fibrosis

of age categories after treatment

	Age							
Fibrosis'stage	**20-30 (nr, %)**	**31-40 (nr, %)**	**41-50 (nr, %)**	**51-60 (nr, %)**	**61-70 (nr, %)**	**71-80 (nr, %)**	**81+ (nr, %)**	**Total**
F0	**2 (100)**	**8 (66.7)**	**7 (58.3)**	**16 (53.3)**	**17 (54.8)**	**7 (46.7)**	1 (50.0)	**58**
F1	0 (0.0)	1 (8.3)	**5 (41.7)**	**9 (30.0)**	**10 (32.3)**	**7 (46.7)**	1 (50.0)	**33**
F2	0 (0.0)	1 (8.3)	0 (0.0)	3 (10.0)	4 (12.9)	1 (6.7)	0 (0.0)	**9**
F3	0 (0.0)	2 (16.7)	0 (0.0)	2 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	**4**
F4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**0**
Total (nr, %)	**2 (100)**	**12 (100)**	**12 (100)**	**30* (100)**	**31 (100)**	**15 (100)**	**2 (100)**	**104 (100)**

Tabel 7. Age and stages of fibrosis

From the visual analysis of the tabel above, we notice that percentage-wise, after treatment, most of the patients fell into the lower fibrosis classes (F0-F1) in contrast to the moment before treatment, when there were patients in higher classes as well of fibrosis (F3 and F4). The F4 stage was virtually non-existent after treatment.

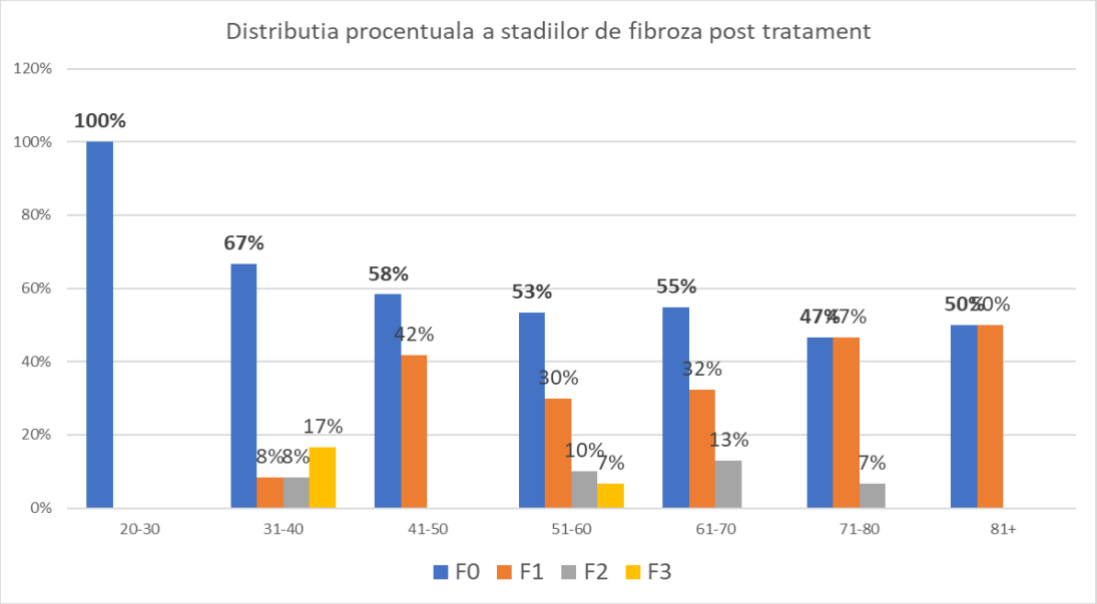

Fig. 7. Percentage distribution of fibrosis stages after treatment

Analiza statistică a stadiilor de fibroză în funcție de tipul de comorbidități

Comorbidities	Stages of fibrosis				
	F0	**F1**	**F2**	**F3**	**F4**
Cardiovascular (nr,%)	4 (33.3)	5 (35.7)	18 (48.6)	21 (80.8)	7 (46.7)
Metabolic (nr,%)	0 (0.0)	0 (0.0)	2 (5.4)	2 (7.7)	2 (13.3)
Psychiatric (nr,%)	0 (0.0)	3 (21.4)	2 (5.4)	0 (0.0)	2 (13.3)
Others (nr,%)	2 (16.7)	0 (0.0)	2 (5.4)	1 (3.8)	1 (3.8)
Without (nr,%)	6 (50.0)	6 (42.9)	13 (35.1)	2 (7.7)	3 (20.0)
Total (n)	**12**	**14**	**37**	**26**	**15**

Tabel 8. Fibrosis stage and type of comorbidities

From the table above, we observe an increased percentage of those with comorbidities present in fibrosis stages F3, F4, especially those with cardiovascular comorbidities. The differences between the proportions are statistically significant (Pearson chi-sq = 28.223, df=16, *p=0.030*).

From the graphic below, we observe for each stage of fibrosis what percentage distribution of comorbidities we have, respectively for each class of comorbidity which stages of fibrosis the

patients present: for example, at the stage of fibrosis F3 we observe that 80% of patients have cardiovascular comorbidities, and at F4 and F2 stages, 46.7% and 48.6%.

II. Evaluation of secondary extrahepatic manifestations of CVD with emphasis on measuring the carotid intima-media index (C-IMT) by using Doppler ultrasound

Doppler ultrasound at the carotid level could only be performed in 47 patients, in the Neurology department of SUU Elias, Bucharest. The monitoring also took place during the COVID-19 Pandemic, that is why many of the patients could no longer have easy access to the hospital (considering that for a long time hospitals entered the circuit exclusively for patients with SARS-CoV infection -2) and could no longer be monitored after treatment or did not accept, therefore they were excluded from the study.

Doppler investigation was performed before and after treatment on the left carotid artery and on the right carotid artery

Doppler test	Before trat Media, CI95%	After trat Media, CI95%	Median diff.	Median Before treatment, IQR	Median after treatment, IQR	Median diff.	T-test	Non-parametric Test
Left	0.943 (0.846-1.040)	0.900 (0.810-0.990)	**0.0431**	0.88 (0.75-1.06)	0.85 (0.72-1.02)	**0.03**	*<0.001*	*<0.001*
Right	0.842 (0.763-0.921)	0.809 (0.732-0.887)	**0.0325**	0.80 (0.65-0.95)	0.75 (0.62-0.91)	**0.05**	*<0.001*	*<0.001*

Tabel 9. Doppler values before and after treatment

Basically, there are statistically significant differences between the pre-treatment mean doppler value versus the post-treatment mean value on both carotids. Mean values are lower after treatment (Paired T-test, left – t=8.197, df 47, $p<0.001$, right – t=8.034, df=46, $p<0.001$).

In non-parametric testing, it is also confirmed by the Wilcoxon signed rank test that we have statistical significance on both the left and the right carotid (left Z= -5.824, *p<0.001*, right Z= -5.309, *p<0.001*)

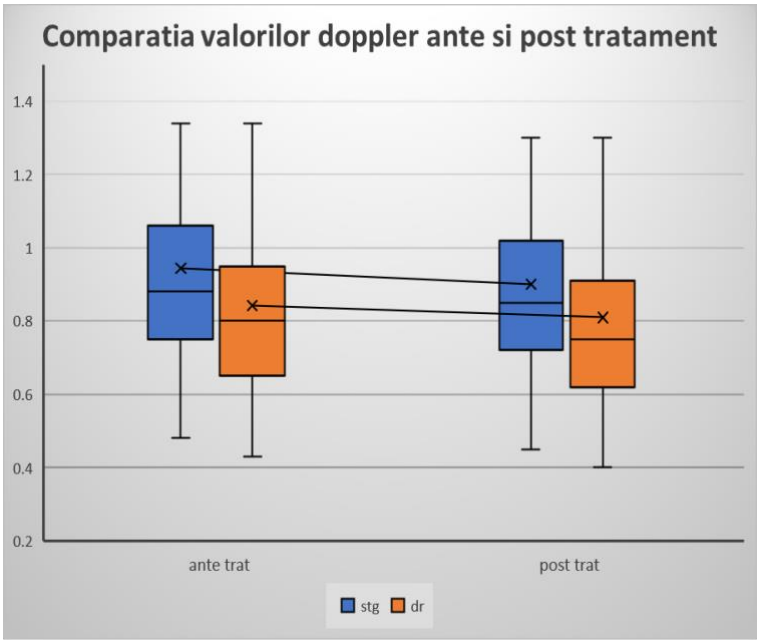

Fig. 8. Comparison of Doppler values before and after treatment

Analyzing the boxplot graphic above, we notice the arrangement of the mean and median values left and right before and after treatment. We also observe the decrease in values after treatment. We also note that on the right the values were lower overall than on the left, both before and after treatment.

Doppler investigation was performed before and after treatment, on the left carotid artery and on the right carotid artery

Doppler test	Before treatment Media, CI95%	After treatment Media, CI95%	Diff.	Median before , IQR	Media after, IQR	Diff.	T-test	Non-parametric test
Left	0.943 (0.846-1.040)	0.900 (0.810-0.990)	**0.0431**	0.88 (0.75-1.06)	0.85 (0.72-1.02)	**0.03**	*<0.001*	*<0.001*
Right	0.842 (0.763-0.921)	0.809 (0.732-0.887)	**0.0325**	0.80 (0.65-0.95)	0.75 (0.62-0.91)	**0.05**	*<0.001*	*<0.001*

Tabel 10. Doppler values before and after treatment

Basically, there are statistically significant differences between the pre-treatment mean doppler value versus the post-treatment mean value on both carotids. Mean values are lower after treatment (Paired T-test, left – t=8.197, df 47, $p<0.001$, right – t=8.034, df=46, $p<0.001$).

In non-parametric testing, it is also confirmed by the Wilcoxon signed rank test that we have statistical significance on both the left and the right carotid (left Z= -5.824, $p<0.001$, right Z= -5.309, $p<0.001$)

III. Evaluarea calității vieții pacienților cu HVC tratați cu AAD prin utilizarea chestionarelor SF-36 și HADS

Statistical analysis of responses to SF-36 questionnaire questions before and after treatment according to age

Answers after treatment	ANOVA test*	Non-parametric test**	Specific differences*** ANOVA
PF post	0.305	0.536	-
BP post	***0.014***	***0.036***	Not affected (53.29) Slightly affected (61.06)
RP post	***0.007***	***0.026***	Not affected (54.31) Slightly affected (62.64)
MH post	***0.018***	***0.011***	Not affected (59.78)*** Slightly affected (51.78)
RE post	***0.029***	***0.031***	Slightly affected (60.50) Moderatly affected (46.14) ***
SF post	0.431	0.800	-
VT post	0.618	0.757	-
GH post	0.313	0.528	-

Tabel 11. Statistical analysis of the answers obtained by applying the SF-36 questionnaire after treatment

** $p<0.05$ is the significance threshold*

*** Median test and Kruska-Wallis test*

****Post hoc analysis (Tukey-test) identifies exactly which response categories differ from each other*

Evaluation of responses to the HADS questionnaire

HADS

- 14 questions: 7 suggestive for anxiety, 7 suggestive for depression

- Simple to apply to patients

- Can provide important data about the patient's emotional state

- Scores: 0-7 points (normal score); 8-10 points (borderline score); 11-21 points (abnormal score)

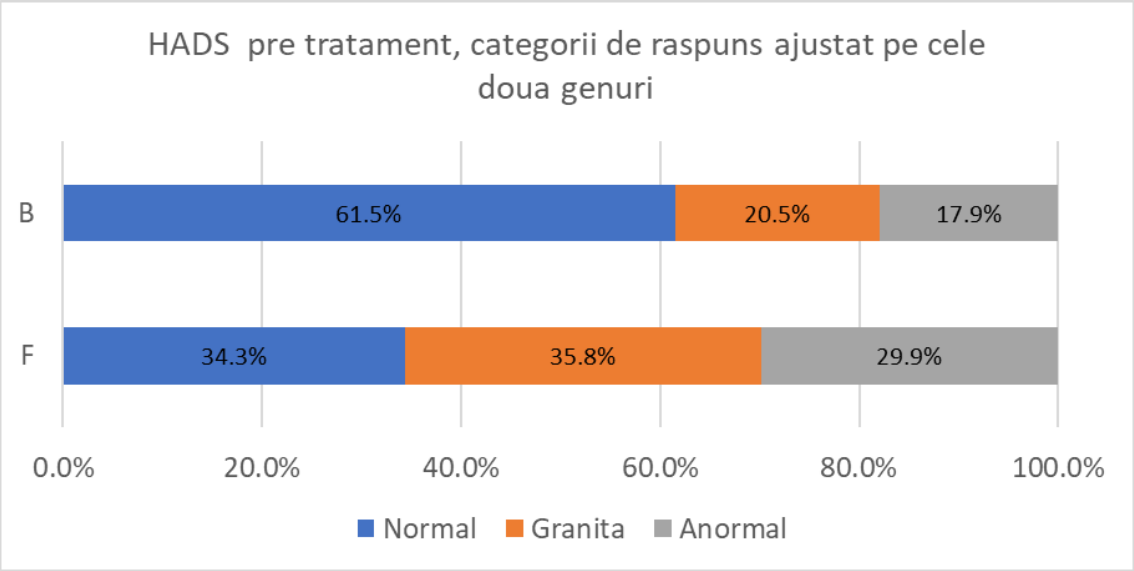

Fig. 9. Pre-treatment HADS scores – gender-adjusted results

			HADS scor 3L post tratament			Total
			Normal	Graniță	Anormal	
Gen	F	Count	38	17	12	67
		% within gen	56.7%	25.4%	17.9%	100.0%
	B	Count	32	2	5	39
		% within gen	82.1%	5.1%	12.8%	100.0%
Total		Count	70	19	17	106
		% within gen	66.0%	17.9%	16.0%	100.0%

Tabel 12. HADS score by gender after treatment

Pearson chi-sq = 8.431, *p=0.015*, statistically significant.

Mantel-Haenszel test of trend =5.101, *p=0.047*, statistically significant.

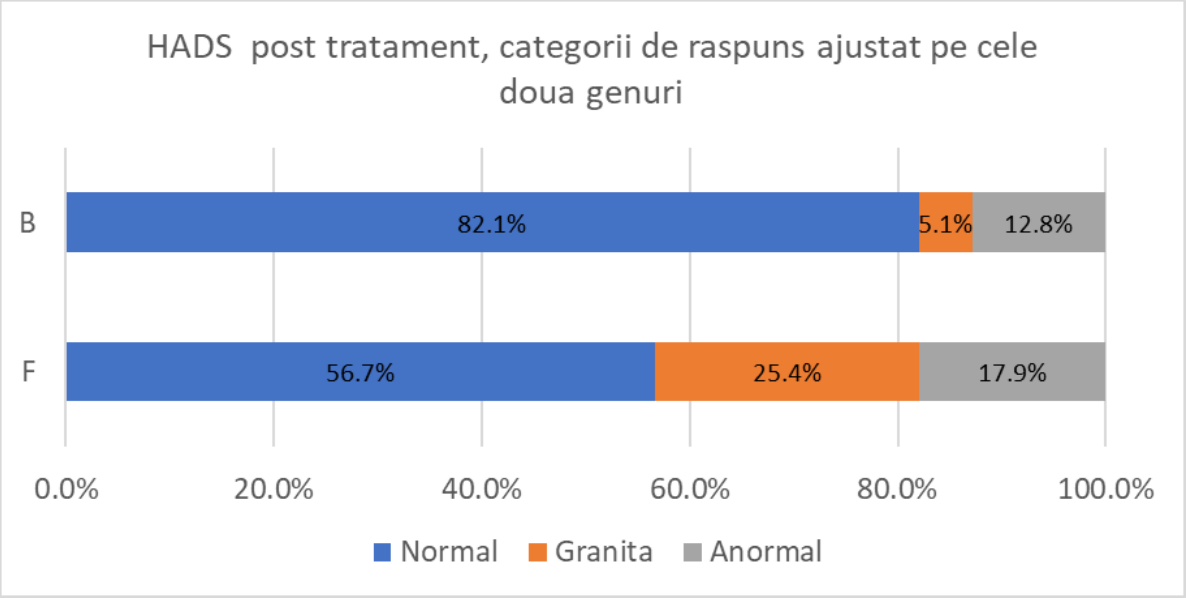

Fig. 10. Post-treatment HADS scores – gender-adjusted results

This analysis of the post-treatment group, although still statistically insignificant, is nevertheless relevant as the pre-treatment one for future studies or expansion of the current group, as it can be seen that patients with a personal psychiatric history show a percentage of responses falling into the "abnormal" categories or "boundary score". At the same time, after the treatment, the percentage of answers with a "normal" score was higher than before the treatment, a result that must be followed in extended groups.

It should be noted that the patients who had an "abnormal" or "borderline" score following the evaluation through these questionnaires were instructed to present themselves for a psychological evaluation at the Clinical Hospital "Dr. Victor Babeș" benefited from psychological counseling, and those with a personal psychiatric history were referred to a psychiatric re-evaluation.

Conclusions

- The era of new direct-acting antivirals has completely changed the evolution of hepatitis C virus, significantly improving the quality of life of patients.

- Hepatitis C patients treated with DAAs should still be monitored and evaluated long-term for extrahepatic manifestations.

- Fibroscan evaluation is a quick, non-invasive, painless method of quantifying the degree of fibrosis.

- Doppler ultrasound can provide important data about cardiovascular risks, as a possible extrahepatic manifestation of CVD.

- Patients with chronic hepatitis C virus may develop anxiety-depressive disorders.

- Quality of life questionnaires can provide data on the physical and emotional state of patients, help improve the doctor-patient relationship and help in the early diagnosis of some psycho-behavioral disorders.

- Cardiovascular comorbidities are prevalent in both women and men, with a slightly higher percentage in women (58.2% compared to 43.6%), so we can state that, in the analyzed group, the presence of comorbidities is higher in percentage, especially cardiovascular on as the age category increases.

- The 15 patients with fibrosis stage F4 before treatment showed a decrease in fibrosis stage as follows:

• 5 patients (33%) progressed to the F1 stage, mean age 66 years (2 men and 2 women)

• 6 patients (40%) passed the F2 stage, average age 60 years (3 men and 3 women)

• 4 patients (27%) progressed to F3 stage, mean age 46 years (3 men and 1 woman)

• no patient after treatment presented stage F4.

- The 26 patients with fibrosis stage F3 before treatment showed a decrease in fibrosis stage as follows:

• 6 patients (23%) progressed to the F0 stage

• 19 patients (73%) progressed to F1 stage

• 1 patient (<1%) progressed to the F2 stage.

- The 37 patients with fibrosis stage F2 before treatment showed a decrease in fibrosis stage as follows:

• 26 patients (70%) progressed to F0 stage

• 9 patients (24%) progressed to the F1 stage

• 2 patients (6%) remained in the F2 stage.

- All 14 patients (100%) with fibrosis stage F1 before treatment showed a decrease in fibrosis stage towards F0.

- The Pearson correlation coefficient is: for the left 0.381, p=0.008 (significant), and for the right it is 0.245, p=0.097. From visual analysis of the graphs we note that there is a

slight trend of positive correlation on both the left and the right, with older age being relatively correlated with higher Doppler values

- Gender does not statistically significantly influence the results obtained during the Doppler assessment, although they have a slightly higher appearance in women than in men on average values, both before and after treatment.

- On multivariate analysis we conclude that it is possible to predict a response to Doppler values after treatment taking into account in particular the Doppler value on the left side and predictive factors such as age, Fibroscan value and duration of treatment.

- Expanding batches and analysis could pave the way for new therapeutic uses of AAD ("old" treatments, "new" actions).

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