CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY

DOCTORAL SCHOOL

MEDICINE

Evolution of patients with advanced hepatic fibrosis of HCV etiology after treatment with direct acting antivirals

Summary

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1. Working hypotheses and general objectives

This study aims to present the conclusions of a prospective, observational, non-interventional study conducted in the Internal Medicine Department of the Fundeni Clinical Institute, following the evolution of patients with advanced liver fibrosis and functionally compensated cirrhosis (Class Child A), treated with direct antiviral molecules.

The following working hypotheses have been established:

1. Determining the effectiveness of antiviral therapy on the observed group (achieving SVR)
2. Identify the side effects associated with antiviral medication
3. Evolution of biochemical parameters after treatment
4. The evolution of liver fibrosis after treatment
5. Incidence of HCC after treatment
6. Treatment impact over extrahepatic manifestations of chronic infection of HCV etiology after reaching SVR by treatment with direct antiviral molecules (especially on mixed cryoglobulinemia).

The main objective: to demonstrate that successful treatment of chronic hepatitis C virus infection leads to regression of fibrosis, improves the biochemical profile, slows the progression to liver cirrhosis and its complications, decreases the incidence of hepatocellular carcinoma and hepatic death.

2.2. General research methodology. Research plan. Materials and methods

A total of 260 patients were selected. Adult patients, with chronic infection of HCV etiology, viremic (HCV-RNA > 10 UI/ml), with advanced liver fibrosis (F3) and compensated liver cirrhosis, naive / experienced in previous antiviral therapies, who received treatment with direct antiviral molecules

At the start of the study, the nationally approved treatment regimens were: the 3D-Ombitasvir / Ritonavir / Paritaprevir and Dasabuvir regimens, respectively the Sofosbuvir / Ledipasvir fixed dose combinations.

All patients underwent a thorough history (history of infection, adherence to antiviral treatments, adherence to them, old endoscopic investigations, use of oral contraceptives in women of childbearing potential, chronic medication).

The degree of liver fibrosis was assessed by non-invasive methods. The elastographic tests were performed on the same device (FibroScan) within the Fundeni Medical Clinic, using the following cut-offs for the separation in Metavir stages: 5.5 kPa, 7.1 kPa, 9.5 kPa, 14.5 kPa, for F1, respectively F2, F3, F4.

HCV-RNA was determined on COBAS AmpliPrep / COBAS TaqMan HCV (Roche). The result of Fibromax was obtained with the help of Biopredictive. The interpretation of APRI and FIB4a was made according to the recommendations in the articles in which they were presented by their authors, [177] as follows: APRI: 0,5-1,5 F2/F3 , > 1,5 F4 FIB4: 1,45-3,25 F2/F3 > 3,25 F4

The fiber test diagnosed fibrosis at values above 0.69 for advanced fibrosis and above 0.78 for liver cirrhosis.

All patients with a personal history of diabetes benefited from the determination of basal blood glucose and glycosylated hemoglobin (HbA1c%). Immunological tests (serum cryoglobulins, FR, complement, etc.) were determined. Complete biochemical tests were collected: ionogram, renal function tests, coagulation tests (PT, INR), liver function tests, serum albumin. All patients had pre-therapeutic determinations of AFP, the maximum normal value allowed by the laboratory being 10 ng / ml. Patients with an AFP value> 10 ng / ml but <100 ng / ml received standard abdominal ultrasound and contrast agent (SonoVue). Patients with serum AFP values> 100 ng / ml performed abdominal CT / MRI with intravenous contrast agent before initiating direct antiviral therapy.

All patients signed the informed consent. For the development of the study, the approval of the Ethics Council of the Fundeni Clinical Institute was obtained.

The following exclusion criteria were established: uncooperative patients, patients with hepatocellular carcinoma diagnosed by abdominal CT / MRI at the time of DAA initiation, patients with other chronic liver diseases, patients with extrahepatic neoplasms - in the absence of the onco-hematologist, patients with CH etiology HCV class Child B and C.

Patients from risk groups, patients with aberrant sexual practices or patients from the penitentiary environment were not identified in the target group. Patients were evaluated pre-

therapeutic clinically, biologically and imaging at the end of the treatment period (EOT) and 12 weeks after the EOT (SVR). The Audit questionnaire was applied to assess excessive alcohol consumption.

Abdominal ultrasound was performed initially - before establishing the therapeutic decision, at SVR and at 1 year in patients with advanced liver fibrosis (F3). According to international guidelines, ultrasound was repeated at 3-6 month intervals to monitor the risk of hepatocellular carcinoma. All patients benefited from pre-therapeutic HCV-RNA determination at 12 weeks after EOT. Parameters used for monitoring and comparison at 3 months-6 months-1 year were: hepatic stiffness, total bilirubin, transaminases, serum albumin, blood count - platelet count, INR.

Statistical analysis

The results are expressed as an average ± standard deviation (SD) or n (%). The p values < 0,05 were considered to be statistically significant. The Fisher test for nominal variables and Wilcox for continuous variables were used to test the hypotheses in dichotomous samples. The T test was supported by the Hazard Mantel-Handel ratio (MH RH) and its 95% confidence interval as a measure of the impact of the was represented as Kaplan-Meier diagrams. Median and range values were used for the relevant variables. The groups were compared using Mann-Whitney Test for continuous variables and Fisher test for categorical variables. The Wilcoxon rank test was used to compare two samples. Longistic regressive analysis was used to highlight predictive factors of clinical and immunological response Statistical analyzes were performed using GraphPad Prism 9 (GraphPad software, California, USA).

Drug interactions were verified using www.hep-druginteractions.org.

2.3. Treatment protocol

3D regimen: Ombitasvir+Ritonavir+Paritaprevir (12,5 mg/75 mg/50 mg) 2cp/day

Dasabuvir 250 mg 2cp/day, along with food

2D regimen:Ledipasvir/Sofosbuvir 90/400 mg 1cp/day, with / without food

The duration of treatment was 12 weeks for both treatment formulas. None of the patients received DAA-associated Ribavirin.

Treatment-related adverse events, including clinical, biological, or haematological abnormalities that occurred during treatment, were identified, monitored, and reported. The degree of side effects was classified according to the

criteria for side effects of the National Institute of Oncology, version 4.03. The primary safety endpoint was the frequency of adverse effects.

The most common drug interactions (especially in the 3D-treated group) identified were with hypotensive medication (beta-blockers), proton pump inhibitors, benzodiazepines and statins..

2.4. Results obtained

2.4.1. Group characterization

Basal clinical-biological characteristics studied

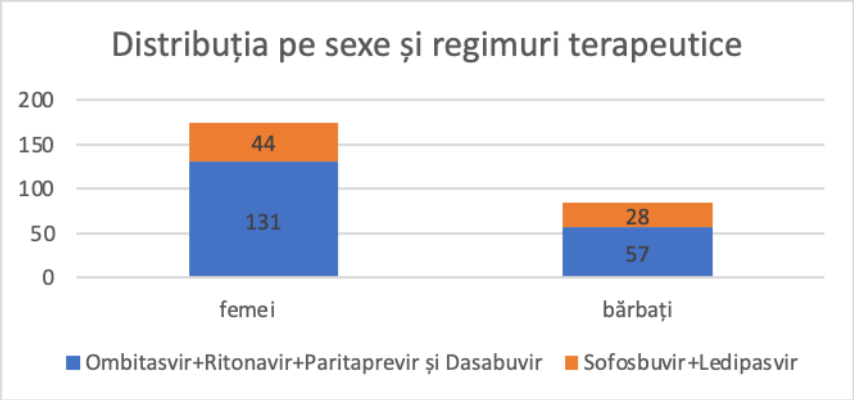
Results

The patients registered belong to the geographical region South-Muntenia, as well as to the region Bucharest-Ilfov.

The basic and demographic characteristics of the studied group are illustrated in the table below.

	Ombitasvir+Ritonavir+Paritaprevir and Dasabuvir	Sofosbuvir+Ledipasvir	P
Nr. patients	188	72	
Age (years)	51,6 ± 9,22	47,2±9,6	0,66
Sex: - women - men	131 (69,6 %) 57 (30,4%)	44 (61,1%) 28 (38,9%)	0,56
Diabetes	32 (17%)	11 (15,2%)	0,85
Hypertension	36 (19,1%)	16 (22,2%)	0,57
Smoking	41 (21,8%)	14 (19,4%)	0,98
Biological data			
Total bilirubin (mg/dl)	1,6±0,31	1,21±0,21	0,01
ALT(UI: 40 U/L)	68,4±40,8	61,2±30,4	0,91
AST(UI: 40 U/L)	57,4±33,7	55,4±21,3	0,75
Albumin (g/dl)	3±0,45	3.1±0,17	0,03
INR	1,6±0,8	1,4±0,5	0,01
Leukocytes ($x10^3/mm^3$)	5,4±1,6	6,2±1,3	0,004
Hemoglobin (g/dl)	12,6±23,6	13,8±21,06	0,01
Platelet ($x10^3/mm^3$)	68,025±18,131	56,703 ±14,370	0,001
PCR-VHC (UI/ML)	1.050.000 (410.387-2.041.000)	945.600 (398.870-2.130.611)	0,76
Degree of fibrosis			
F3-95	83	12	0,001
F4-165	105	60	
Experiment with previous therapies (PegIFN+Ribavirina)	38 (20,2%)	13 (18%)	0,62
SVR	187/188 (99,4%)	68/72 (94,4%)	

The mean age in the group of 3D-treated patients was 51.6 years vs. 47.2 years in the 2D-treated group. Regarding the distribution by sex, the predominance of female, postmenopausal and multiparous sex was observed in the vast majority.



The mean value of pre-therapeutic HCV-RNA (ui / ml) was 1,050,000 (410,387-2,041,000) in the group treated with 3D regimen, respectively 945,600 (398,870-2,130,611) in the group treated with Sof / Led.

95 patients with F3-grade fibrosis were identified, of which 38 patients experienced previous antiviral therapies in group 3D and 13 in group 2 D. Of the 165 patients with liver cirrhosis, 136 patients (82.4%) had esophageal varices. : grade I- 73 patients (61.1%), 41 patients with grade II esophageal varices (31.1%) and 12 patients with grade III esophageal varices (8.8%). grade III benefited from prophylactic banding.

In both groups, the AST and ALT values are increased by 1-1.5xN. Valoarea medie a albuminei serice s-a situat în jurul valorii de 3 g/dl. The average value of total bilirubin (mg/dl) varies between 1,6±0.31 in the 3D treated group and 2.1±0.21 in the 2D treated group. Leukocyte and hemoglobin values showed normal parameters. Platelet counts revealed values between 68.025 ±18.131 in the group proposed to be treated with DAA 3D and 56.703±14.370 in 2D group.

13.46% of obese patients were identified, with a predominance of females in both groups. 18 patients consuming excess alcohol associated with HCV infection, predominantly male.

Discussions

Regarding the geographical distribution, the data from our group are similar to those obtained by Mircea Manuc in a study conducted in 2017 on the epidemiology of HCV infection in Romania (Muntenia, Dobrogea, Moldova, Banat and Crisana showed high prevalences and approx. equals.). His study also confirms the data identified by Liana Gheorghe and her team in 2010 regarding the wide geographical variability of HCV infection in Romania, the South-Muntenia region being an important viral reservoir.

The peak incidence of females in the 5-6 decades of life in our group can be explained by their exposure to unsafe medical procedures (illegal abortions) during the communist period. The results of our study on age and sex distribution differ from those obtained by Manuc et al, where 75% of all patients followed are between 6 and 7 decades of age, 49% of patients being male.

Gender also influences the progression of chronic infection. Men have a higher risk of progression to advanced liver disease, cirrhosis of the liver and hepatocellular carcinoma. Elevated serum testosterone levels have been associated with higher severity of liver fibrosis. For every 1 ng / ml increase in testosterone, there was a 25% increase in the risk of advanced fibrosis.[190]

By contrast, estrogen plays a protective role for women. Comorbid factors associated with menopause, elevated BMI, metabolic syndrome and hepatic steatosis are considered. There is only one study that has shown the acceleration of postmenopausal fibrosis in HCV-infected women. Patients who received hormone stimulation medication had lower stages of fibrosis.[191]

Regarding the age of patients, it has been identified in numerous studies as a major element in the progression of liver disease. In a study led by Prof. Poynard, three factors were associated with an increased rate of progression of fibrosis - age at infection over 40 years, daily consumption of at least 50 g of alcohol and male sex.[192]

It did not identify an association between fibrosis progression and quantitative viral RNA. In our group, the value of the viral load at the time of initiation of treatment was not statistically significant and did not influence the rate of response to treatment..

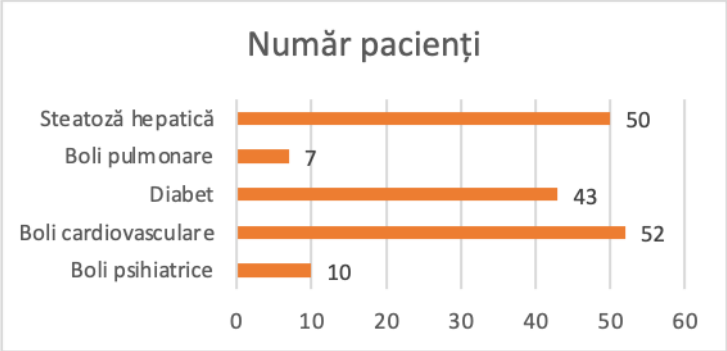
On average, 9% of patients followed reported excessive alcohol consumption. In association with chronic HCV infection exacerbation of liver disease, acceleration of fibrosis progression

and much faster installation of cirrhosis status.In addition, potentiates the installation of side effects in association with DAA. Moreover, it is a proven inducing factor of hepatocellular carcinoma.

2.4.2. Comorbidities monitored patients

Results

The most common comorbidities identified in the investigated group associated with chronic hepatitis C virus infection are illustrated in the graph below.



52 patients with cardiovascular pathology were identified (19.6%): 30 patients with hypertension, 7 patients with coronary heart disease, 11 patients with heart failure, 4 patients with cerebrovascular disease (sequelae stroke). 7 patients had a personal history of pathological lung disease (2.7%). 10 patients with associated psychiatric pathology (2.8%) - the most common being depression. Hepatic steatosis in varying degrees has been described in 50 patients (19.2%). 43 patients presented with type II diabetes at the time of study inclusion: 32 patients in the 3D-treated group and 11 patients in the group treated with Sof / Led.

Discussions

Chronic HCV infection resides in chronic immune and inflammatory stimulation. Studies have shown an association between increased serum levels of these pro-inflammatory cytokines (fibrinogen, IL6, etc.) and increased prevalence of carotid atherosclerosis. In addition, his study demonstrated the connection between serum HCV-RNA levels and elevated serum fibrinogen levels and CRP, suggesting pro-inflammatory status as an independent mechanism for promoting hepatic steatosis. [193] Successful treatment of HCV results in a reduction in pro-inflammatory markers.

There is a direct link between viral load and the risk of cardiovascular disease. The pro-inflammatory status maintained by the virus promotes a profibrogenic environment. Petta

reports the directly proportional relationship between the degree of fibrosis and the extent of atherosclerotic plaques, emphasizing that obtaining SVR is accompanied by amelioration of carotid atherosclerosis. [195] Maruyama proved that the patient with increased viral load and increased histological index of activity has a higher degree of myocardial injury. [196]

[197] In contrast, Volzke's study did not identify a significant association between viremic status and the risk of myocardial infarction, stroke, stenosis or carotid plaque. [198] Younossi and his team showed a significantly higher prevalence of congestive heart failure in the HCV-infected population compared to the control group (3,8% vs 0,9%)[199]

Numerous population-based studies have reported a significantly higher cumulative stroke risk in the HCV-infected patient. A comparative study between a group of HIV-positive HCV patients and a control group (non-HCV patients) revealed a prevalence of 26.2% in the first group vs only 6.6% in the group without C virus. [193] Lee observed that chronic HCV infection is an independent risk factor predictive of cerebrovascular mortality, with a directly proportional relationship between serum RNA level and mortality rate. Younossi did not identify a close association between HCV infection and the risk of stroke. [194]

The latest data on the prevalence of extrahepatic manifestations associated with chronic HCV-infection have defined DM II as one of the most common. Comparing a group of HCV patients with a group of uninfected patients, DM II was present in 15% of cases of HCV patients and in 10% of patients in the control group.[201]

In our study, the prevalence of DM II in HCV-infected individuals was 16.5%, higher than in the meta-analysis published by Yunosuu and his team in 2016..

Another meta-analysis assessed the rate of HCV infection in the diabetic individual. The results showed that diabetic individuals had a 3.5 times higher OR of HCV infection compared to the non-diabetic patient. [202]

The association of diabetes in the chronically infected HCV patient is clinically relevant because it increases the rate of fibrosis, accelerates the progression to liver cirrhosis and the appearance of hepatocellular carcinoma..

Abnormal accumulation of fat in the hepatocyte is often associated with chronic HCV infection. It is important to differentiate between the two main types of hepatic steatosis associated with this category of patients: "metabolic" steatosis vs "viral" steatosis. Metabolic steatosis is generally identified in the individual with chronic HCV genotype 1 infection and is

associated with the elements of the metabolic syndrome. By contrast, viral steatosis is reported in the patient infected with genotype 3, in the absence of steatogenic cofactors. By a similar mechanism, HCV induces insulin resistance.

Another study published in World J Gastroenterology in 2009 indicates HCV is responsible for increased resistance to treatment and promotion of fibrosis by accelerated viral replication associated with hepatocyte lipid accumulation.. [203]

50 patients presented with hepatic steatosis (19.2%) of those followed, 35 patients in the group treated with 3D and 15 patients in the group who received treatment with Led / Sof. Amedeo Lonardo and his team describe a prevalence of hepatic steatosis in the individual with chronic viral hepatitis C up to 55.54%, higher than the general population and higher than the one identified in our study. [204]

Data on the effect of DAA on hepatic steatosis regression are limited.

Regarding the extrahepatic manifestations of psychiatric type, among the patients followed, 10 (3.8%) were identified, lower than in other studies, which reported percentages of up to 50% in the HCV-infected patient. Of these, 7 patients under monitoring and specialized treatment for severe depressive syndrome and 3 patients with anxiety syndrome and repeated episodes of panic attack in the past. None of them had current or previous episodes of hepatic encephalopathy.

Navines et al reported a prevalence of 18.2% of depressive syndrome in HCV-infected individuals - 6.4% of major depressive syndromes, 7% of episodes of generalized anxiety and 5.8% of panic attacks.[206]

The psychological burden of significant chronic suffering in this category should not be neglected. Depression was independently associated with difficulty accessing treatment, thus creating a vicious circle with viral infection.

41 smoking patients (21.8%) among those treated with O / R / P / + D, 14 from the other group (19.4%). Regarding pulmonary comorbidities, 7 patients (2.7%) with COPD were identified. All male patients. 1 Gold II patient, 4 GOLD III patients and 2 Gold IV patients.

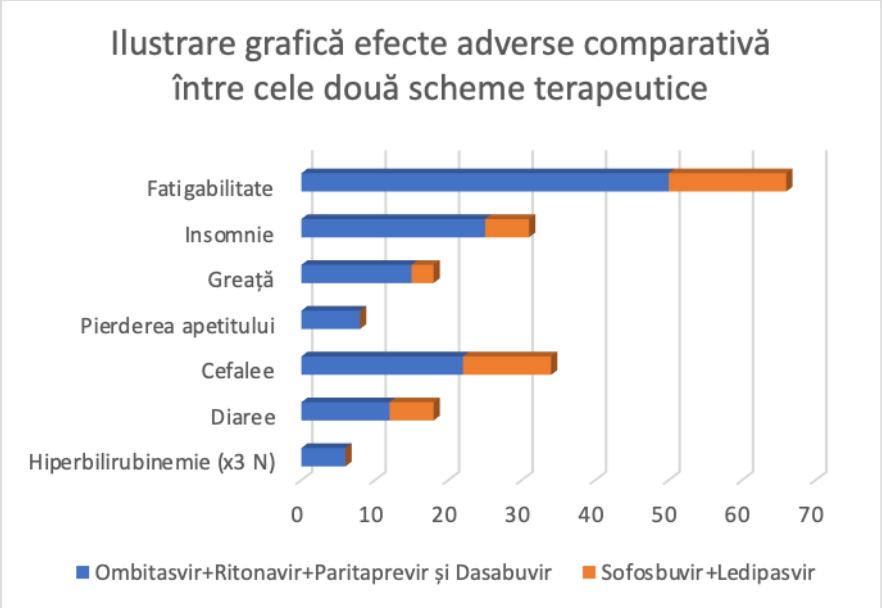
The prevalence identified in our group is similar to that observed in studies worldwide, which is around 3%. It is lower than in Silva's study, which ranks 7.5% among HCV viremic patients.[208] All patients in our group are patients with a history of smoking: 4 active smoking patients and 3 withdrawal patients.

The HCV core protein potentiates IL8 expression in the lung fibroblast. The COPD patient has neutrophilic influx and increased local pulmonary IL8 production, whether or not the patient is HCV viremic.

2.4.3. Adverse effects of antiviral medication directly

Results and discussions

The present study indicated excellent safety and tolerability for both regimens compared to previous Interferon and Ribavirin treatments. Data were obtained by history, clinical examination, telephone interrogation of patients, collection of laboratory samples.



Adverse effects were reported in 138 patients (50.7%). The most common was fatigue - 50 patients (26.6%) in the 3D treated group, 16 patients (22.2%) in the 2D treated group. 25 patients mentioned insomnia (13.2% in the 3D group), 6 patients (8.3% in the 2D group). 34 patients reported headache-22 (11.7% in the 3D group, 12 patients-16.6% in the 2D group).

The following frequency side effects were represented in the 3D group of gastrointestinal complaints: 15 patients complained of nausea, 12 patients- diarrhea, 8 patients showed loss of appetite. In the 3D-treated group, 6 reported diarrhea (7.1%, 3 patients (4%) reported nausea. No patient reported loss of appetite..

The increase in bilirubin was observed only in the group treated with 3D-6 patients (3.19%), being a class effect of protease inhibitors on the organic polypeptide transporter of 2B1 anions

(OATP2B1). The increase in bilirubin was accompanied by pruritus and was remitted by combining ursodeoxycholic acid with treatment. Regarding rebellious insomnia, products based on passionflower and hops were recommended for its control. The side effects disappeared after the end of the antiviral treatment. Our observations are associated with those in the study of Abel-Moneim et al, as well as with those in the study of Sanai et al. [209,210]. All patients complied with treatment, there were no interruptions due to side effects.

2.4.4. Dynamics of fibrosis and the main biochemical parameters after treatment with direct antivirals. Sustained virological response

Results

The table below illustrates the non-invasive markers of liver stiffness used in our study, accompanied by their threshold values..

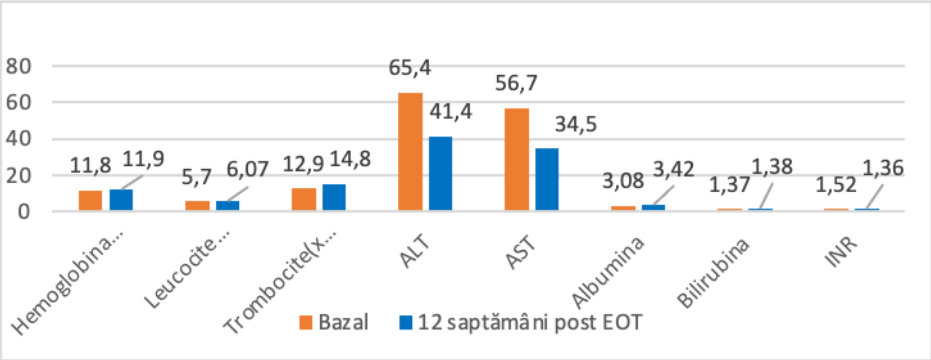
Assessment of the degree of fibrosis

Degree of fibrosis	Nr. patients	Fibroscan	FIB4	APRI	Fibrotest
F3	95	9,5±1,8	2,42±1	1,1±0,4	0,63±0,4
F4	165	15,7±8,9	3,78±0,8	2,68±0,7	0,79±0,8

A total of 260 patients with advanced hepatic fibrosis, 95 with F3 and 165 patients with F4 value of FibroMax were identified.

The response rate is 99.4% in patients treated with Ombitasvir / Ritonavir / Paritaprevir and Dasabuvir - 187/188 patients. Regarding the group treated with Sofosbuvir / Ledipasvir, 68 of the 72 patients followed achieved SVR (94.4%).

Evolution of the main biochemical and immunological characteristics

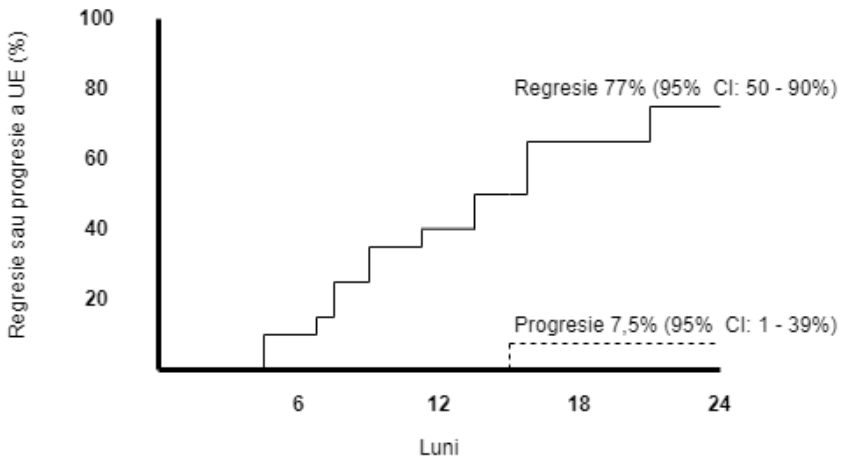


By comparison with the pre-therapeutic level, the following were shown to be: reduction to serum transaminases, improvement of albumin levels, decrease in total bilirubin and significant improvement in platelet counts. The average value of INR decreased from 1.52 to 1.36, a feature with statistical significance. Leukocyte and hemoglobin values remained stationary.

Evolution of Fibrotest, APRI, FIB 4 initially and 12 months after treatment

Variables	Initiation 260 patients	At 12 months post-treatment	P
APRI (%) 1-2 >2	 90 (34,61 %) 170 (65,38%)	 82 (27,30%) 168 (64,61%)	 < 0,001
FIB4 (%) 1,35-3,25 > 3,25	 93 (35,76%) 167 (64,23%)	 75 (28,84%) 156 (60%)	 <0,001
Fibrotest (%) 0,68-0,78 ≥0.78	 95 (36,53%) 165 (63,46%)	 76 (29,23%) 155 (59,61%)	 <0,001

In our study, a decrease in liver stiffness was observed at 12 months post SVR, especially in patients with F3 fibrosis..



Evolution of the degree of esophageal varices according to SVR

Also, regression of the degree of esophageal varices was observed by decreasing to a lower level in patients in whom SVR was achieved, in the interval of 12-18 months post-therapeutic. Cumulated with the correction of platelet levels, elements of regression of portal hypertension can be considered.

Association of clinical-biological parameters with lack of fibrosis regression at> 12 months post SVR

Variables	OR	95% CI PT OR	P
the presence of diabetes	2.11	1.2478-3.5103	0.01
Presence of esophageal varices prior to treatment	2.31	1.4073-3.467	0.001
Serum albumin < 3g/dl	2.97	1.7099-4.5623	0.0001
Platelet < 100.000/mmc	3.88	2.4978-5.9873	0.0001
AST/ALT < 0,8	1.71	1.1324-25798	0.03

Multivariate analysis revealed that the factors associated with non-regression of post-therapeutic fibrosis are the presence of diabetes, the presence of esophageal varices, albuminemia below 3 g / dl, thrombocytopenia < 100.000/mmc and ratio AST/ALT < 0,8.

Discussions

Liver fibrosis regression remains one of the hot topics of study and exploration for liver disease experts in recent decades. Elimination of HCV by treatment with direct antiviral molecules does not completely eradicate the risk of hepatocellular carcinoma, especially in patients with advanced liver fibrosis and liver cirrhosis. Advanced liver fibrosis that precedes the antiviral treatment period is one of the most important predictors of HCC after direct antiviral treatment.

The SVR rates in our group are similar to those in the literature. No significant differences were observed between age groups.

They were identified as unfavorable response factors to treatment: male sex, cirrhotic status, low serum albumin, elevated total serum albumin, thrombocytopenia, personal history of hepatocellular carcinoma.

We identified a number of 5 patients with therapeutic failure, 4 of them male. All cirrhotic patients not experienced in antiviral therapies with previous direct antiviral molecules. Biological: serum albumin ≤ 3 g/dl, BT > 1,5 mg/dl, platelet< 100.000/mmc. Two of the patients had a history of embolized hepatocellular carcinoma.

The serum level of ALT in population studies has been associated with a progressive increase in the risk of death of general and particularly hepatic death. Persistently normal ALT values in the HCV HC patient are associated with lower levels of fibrosis on liver biopsy.

In cross-sectional studies on liver biopsies, serum ALT levels were not correlated with fibrosis severity, but the AST / ALT ratio was identified as a predictive factor. A large study in Taiwan showed that increased ALT was a cumulative risk factor for hepatocellular carcinoma of 1.7% at serum levels. ≤ 15 UI/L. It rose to 4.2% in values between 15-45 %UI and at 13,8% for values ALT ≥45 UI/L. [214].

Results similar to those in our study on the improvement of biological parameters were obtained in previous studies. They theory that the normalization of values of biological parameters after treatment with direct antiviral molecules is associated with the restoration of immune response activity. The reactivated immune response may achieve residual viral clearance in the weeks following treatment. The normalization of the levels of biochemical parameters, mainly of serum albumin, supports the theory according to which intrahepatic

inflammation has a central role in reducing the synthesis capacity of the liver. Therefore, blocking inflammation can lead to recovery of liver function.

Improvement of thrombocytopenia as an indirect marker of portal hypertension in patients with SVR suggests the possibility of regression of liver fibrosis over time in the patient with chronic viral hepatitis C who succeeds in viral eradication.

Our study is consistent with previous real-life studies that investigated OBT / PTV / DSV and SOF / LDV therapy in patients with chronic HCV infection and the SVR rate between 86-100%. SVR in our group of patients was higher compared to the Spanish study conducted by Chamorro-de-Vega, where 93.8% of cirrhotic patients achieved SVR.[216]

We were able to easily follow the evolution of these patients in terms of the evolution of fibrosis 12 months after obtaining SVR.

Low pre-therapeutic levels of ALT and AST indicate a low percentage of necroinflammation and the presence of significant fibrosis. As a percentage of patients remain with fibroscan 18 Kpa, they are still exposed to a high risk of developing complications. FIB4 and APRI showed significant decreases after DAA treatment.

A recent study in non-cirrhotic patients reported an accelerated decrease in APRI and FIB4 from week 2 to week 12 posttreatment..[217]. Another study showed a decrease in elastographic parameters at 18 months post SVR . [218]

Therefore, the observation that FIB4 and APRI decrease post-treatment can be supported by reducing chronic liver inflammation and is reflected biochemically by decreasing transaminases..

2.4.5. The impact of treatment with direct antiviral molecules on HCV-associated mixed cryoglobulinemia

Results

We included 24 adult patients with CM associated with HCV, investigated in the period 2016-2019 in which they have active HCV vasculitis, defined by vasculitic lesions in the skin, joint, kidney, peripheral and / or brain, digestive, pulmonary and / or cerebral nerves. or heart damage (Chapel Hill 2012 criteria). [224,225] Also included were 8 patients with asymptomatic HCV cryoglobulinemia. Exclusion criteria were inactive cryoglobulinemic vasculitis, HIV or HBV infection, decompensated liver cirrhosis.

Clinical evaluation upon inclusion in the study involved age, sex, neurological impairment (peripheral and / or central), skin impairment (Raynaud's phenomenon, purpura, distal ulcers, skin necrosis), arthralgias, myalgia, intestinal transit impairment, renal impairment (proteinuria, hematuria and glomerular filtration rate) and clinical signs of liver damage. All patients were evaluated at 4 weeks interval, up to week 24. Assessment of disease activity and therapeutic response was made using Birmingham vasculitis activity score. [226]

Viral load was determined using Abbot HCV Real Time Assay, with a minimum detection limit of 12 IU / ml. HCV genotyping was performed using NS5b gene sequencing. Laboratory evaluation included complete blood count, serum biochemical profile, rheumatoid factor, quantitative Ig M, total complement and C4 fraction, cryoglobulins (cryocrit over 1% in at least 2 determinations and characterization of cryoprecipitate by immunofixation). Cryoglobulins were classified according to the Brouet classification [227] as type II in the presence of monoclonal Ig M and polyclonal Ig G and type III. Hepatic fibrosis was assessed noninvasively. All patients met the eligibility criteria for DAA treatment: 22 patients with Viekirax / Exviera and 10 patients with Harvoni.

The primary endpoint was complete clinical remission of vasculitis at week 24. The complete clinical response was defined by improving the function of all organs initially affected and the absence of clinical relapse.

Skin and joint improvement has been evaluated clinically (disappearance of purpura and / or ulcers; disappearance of arthralgia or arthritis). Renal impairment was assessed biologically (proteinuria below 0.3 g / 24h, disappearance of haematuria and improvement of GFR > 20 % at week 24, if RFG < de 60 ml/min. at initiation). The improvement of peripheral neurological impairment was appreciated clinically (improvement of pain and paresthesia by analog visual scale, improvement of muscle contraction and initial motor impairment) and / or electrophysiology (improvement of electromyogram abnormalities at week 24, compared to the initial appearance). Total Neuropathy Symtoms Score-6 (NTSS-6) was applied to assess the symptoms of sensory neuropathy.

The partial clinical response was defined at 24 weeks as an improvement in damage to some of the initially affected organs. Those without a clinical response at 24 weeks were defined as therapeutic failure.

Baseline characteristics for inclusion in the study

Basic characteristics	Total lot (n=32)	Patients with cryoglobulinemic vasculitis (n=24)	Asymptomatic patients with circulating cryoglobulins (n=8)	P
Age Years	58 (38-76)	57 (42-72)	64 (56-76)	0,91
Female gender, n (%)	19 (59,3%)	16 (66,6%)	3 (37, 5%)	0,04
Clinical manifestations, n (%)				
Purpura	22 (68,7)			
Arthralgia / arthritis	9 (28,1)			
Muscle weakness	26 (81,2)			
Polyneuropathy	18 (56,2)			
Renal impairment	5 (15,6)			
Sicca syndrome	3 (9,3)			
Abdominal damage	2 (6,2)			
Raynaud's phenomenon	5 (15,5)			
Viral parameters				
HCV genotype, n (%)				
1a	3 (9,3)		-	
1b	29 (90,6)		100	
2				
3				
4				
Basal level HCV-RNA IU/mL	860.200 (14600-10.880.000)	910.000 (26.860-10.880.000)	210.800 (14.600-630.000)	0,02
Biochemical tests				
ALT , IU/mL	76 (41-188)	81 (40-191)	76 (41-153)	0,08
PLT x 10^9 /L	178 (108-246)	127 (74-159)	151 (88-176)	0,05
Transient	14 (9,2-30)	19 (11,9-37)	12,8 (8,2-24)	0,01

elastography, kPa				
Immunological parameters				
Cryocritus (%)	2,5 (1,2-5,1)	3,4 (1,7-5,9)	2,3 (0,9-3)	0,04
C4, g/L	0,07 (0,02-0,15)	0,03 (0,02-0,12)	0,10 (0,07-0,19)	0,03
CH 50, IU/mL	15 (11-29)	13 (11-29)	14 (10-25)	0,06
Rheumatoid factor, IU/mL	31 (10-118)	96 (10-193)	11 (10-26)	0,01
Treatment, n (%)				
Naive	20	12	8	
Null responder	12	12	0	
DAA treatment regimens used, n (%)				
3D	22	12	8	
HARVONI	10	10		
Use of Ribavirin, n (%)				
SVR 12	(30/32) 93,7%	(22/24) 91,6 %	(8/8) 100%	0,01
Immunosuppressive therapy, n (%)				
Corticosteroids		8 (33,3)		
Corticosteroids + cyclophosphamide		4 (16,6)		
Plasmapheresis		3 (12,5)		
Liver fibrosis, n (%)				
F_1	4 (12,6)	2 (8,3)	2 (25%)	
F_2	5 (15,6)	2 (8,3)	3 (37,5%)	
F_3	7 (21,8)	5 (20,8)	2 (25%)	
F_4	16 (50)	15 (62,6)	1 (12,5)	

Annex 1

Comparing the 2 groups, the predominance of the female sex in the total vasculitis is noticeable, but also lower values of C4 in this group (0.03 g / l VS 0.10 g / l with p = 0.05) and higher values of FR (96 u / ml VS 11 u / ml p = 0.01) and cryocrit (3.4% VS 2.3%).

The main clinical manifestations of cryoglobulinemic vasculitis were: purpura (68.7%), arthralgia / arthritis (28.1%), weakness (81.2%), polyneuropathy (56.2%), renal impairment (15, 6%).

Clinical, biological and immunological evolution after antiviral treatment

	Cryoglobulinemic vasculitis n=24			Asymptomatic patients n=8		
	Pretreatment	Post treatment	P	Pretreatment	Post treatment	P
SVR		93,7 %			100 %	
Cryoglobulin (n, %)	24 (100)	11 (45,8)	0,001	8 (100)	3 (37,5)	0,001
Cryocritus (%)	3,4 (1,7-5,9)	0,3 (0-1,5)	0,01	2,3 (0,9-3)	0,1 (0-1,1)	0,01
C_4 reduction (n, %)	20 (83,3%)	5 (20,8 %)	0,001	6 (75 %)	3 (37,5 %)	0,001
Fraction reduction C_4 g/l	0,03 (0,02-0,12)	0,14 (0,09-0,19)	0,01	0,10 (0,07-0,19)	0,14 (0,07-0,20)	0,02
Reduction of activity CH 50 u/ml (n, %)	19 (79,1 %)	8 (33,3 %)	0,001	6 (75 %)	2 (25 %)	0,001
Activity CH 50 u/ml	13 (11-29)	36 (22-51)	0,01	14 (10-25)	33 (15-47)	0,01
Positive rheumatoid factor (n, %)	17 (70,8 %)	11 (45,8 %)	0,001	4 (50 %)	2 (25 %)	0,01
Level UI/ml Rheumatoid factor	96(10-193)	26 (11-89)	0,01	11 (10-26)	10 (10-14)	0,03
ALT	81 (46-191)	25 (18-30)	0,01	76 (41-153)	21 (16-28)	0,01
Platelet x 10^9/l	127 (74-159)	149 (91-210)	0,21	151 (88-186)	152 (90-196)	0,98
GFR ml/min./1,73 m^2	93 (62-93)	94 (65-94)	0,20			
Clinical manifestations (n, %)						
Purpura	22 (68,7)	1 (4,1)	0,01			
Arthralgia	9 (28,1)	1 (4,1)	0,01			
Weakness	26 (81,2)	2 (8,3)	0,01			
polyneuropathy	18 (56,2)	6 (25)	0,01			

Neural impairment	5 (15,6)	1 (4,1)	0,01			
Hematuria	5 (15,6)	1 (4,1)	0,01			
GFR ml/min./1,73 m^2	43 (40-47)	57 (42-61)	0,02			
Proleinuria g/l	1,7 (1,1-2,1)	0,32 (0,1-1,9)	0,62			
BV AS	9 (4-18)	0 (0-5)	0,01			

Of the 5 patients with renal impairment associated with immunosuppressive therapy and plasma exchange (1 case), a complete clinical remission was noted in 3 cases (60%) who also had SVR at 12 weeks, with a significant improvement in the degree of insufficiency. renal (creatinine clearance> 60 mm / min.). In one patient the nephrotic syndrome and creatinine clerance did not change significantly, and in the fifth patient there was an improvement in proteinuria (less than 1 g / 24 h) and creatinine clearance (from 34 ml / min. To 55 ml / min.)

16 out of 18 patients with neurological symptoms were evaluated electromyographically, confirming peripheral polyneuropathy: 6 with multiplex neuropathy, 7 with sensory polyneuropathy and 3 with sensory-motor polyneuropathy.

All 5 patients with renal impairment had renal biopsy, confirming membrane-proliferative glomerulonephritis, 3 of them being on glucocorticoid treatment plus monthly cyclophosphamide pulsterotherapy, and the other 2 only on glucocorticoid treatment. Plasma exchange was performed in 3 patients, one with renal impairment and the other 2 with multiplex neuropathy.

All patients had type 2 cryoglobulins (polyclonal Ig G / monoclonal Ig M). 81.2% (26 of 32) patients had a decrease in C4 and CH 50 activity and all patients with vasculitis had rheumatoid factor present.

In terms of SVR at 12 weeks after the end of antiviral treatment, it was 91.6% (22 of 24) patients in the vasculitis group, compared to 100% in the asymptomatic group ($p = 0.01$).

Purpura, myalgias, arthralgias and muscle weakness were remitted in 91.7% of post-SVR patients, and of the 5 patients with renal impairment 4 obtained remission with nephritic syndrome resolution and improved glomerular filtration rate (43 VS 57 ml / min./1.73 m2). Neurological symptoms improved in 75% of cases. 3 patients with sicca syndrome and 2 with intestinal damage were asymptomatic at the end of the follow-up period. Clinical

improvement was also assessed by decreasing BVASvs3 from an initial mean value of 8 points (3-27) to a value of 3 points (0-11), a decrease with statistical significance ($p < 0.001$).

All immunological parameters improved at 12 weeks after the end of treatment. Circulating cryoglobulins became undetectable in 54.2% of patients with vasculitis and in 62.4% of the group of asymptomatic patients.

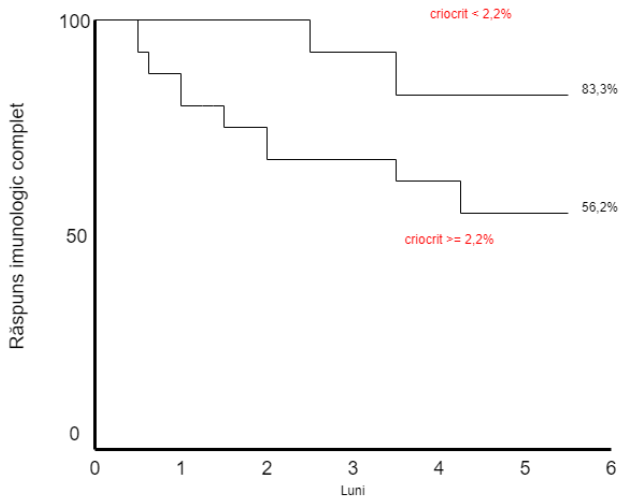
Regarding the post-therapeutic normalization of CH 50, C4 and FR level, it occurred in the group with vasculitis in the proportion of 79.2%, 66.7% and 54.2%, respectively, and in the asymptomatic group in the proportion of 64, 5%, 75% and 75%, respectively.

Most of those with immune response - 19 patients (79.1%) showed an improvement in the clinical picture.

Basic characteristics associated with complete immune response

Variable	Universal analysis OR (95% CI)	P	Multivariable analysis OR (95% CI)	P
F_{1-2}	0.8 (0,21-1,9)	0,05		
Cryocritus < 2,2 %	7,2 (2,8-21)	0,01	9,4 (2,8-38)	0,002
C4 > 0,25 g/l	6,7 (2,3-24)	0,03	8,2 (2,3-31)	0,004
FR < 20 UI/l	1,14 (0,87-1,14)	0,07		
BVAS < 6	3,53 (1,18 -10,59)	0,027	4,68 (2,24-11,43)	0,003

The predictive factors of the clinical and immunological response, relevant by logistic regression analysis were: fibrosis level, cryocritus level, C4 level, FR activity and BVAS v3



Complete immune response depending on the value of the cryocritus

Discussions

Circulating mixed cryoglobulins are detected in 40-60% of patients with chronic HCV infection, while cryoglobulinemic vasculitis is observed in 15% of cases. The prognosis is variable and depends on the degree of kidney damage or the extent of vasculitic lesions.[219, 220]

Patients with mild to moderate forms of HCV-associated glomerulonephritis (stable renal function and / or non-nephrotic proteinuria) should be treated with direct antiviral molecules. Immunosuppressive therapy should be combined in patients with HCV-associated renal disease with resistance to DAA therapy. The use of Sofosbuvir in the treatment of patients with cryoglobulinemic nephropathy has been avoided when GFR ≤30 ml / min, due to the risk of accumulation of the active metabolite GS-331007, which impairs renal function.

SVR is the primary endpoint in these patients because clinical remission of vasculitis is closely associated with viral clearance. Direct antiviral therapy studies have shown a remarkable eradication rate of 90% to 100% depending on the HCV genotype. [222]

In the VASCUVALDIC study that enrolled 24 patients with HCV cryoglobulinemic vasculitis treated with sofosbuvir and ribavirin, the complete clinical response at week 24 was 87.5%.. [230]

In our patients, the SVR rate was 91.6% in patients with cryoglobulinemic vasculitis and 100% in patients with asymptomatic cryoglobulinemia. The favorable virological evolution

was in parallel with the favorable clinical evolution. The five patients with renal impairment were also treated with immunosuppressants (corticosteroids, cyclophosphamide)..

In almost all studies, a complete or partial reduction in clinical symptoms during and after DAA was correlated with SVR. A complete clinical response was defined as an improvement in all affected organs and / or a Birmingham vascular activity score (version 3) of 0. The best response rate was demonstrated in the prospective study by Saadoun et al. [231], in which all patients (no. 41) presented with SVR and a complete clinical response of 90% or partial (10%) after 12 or 24 weeks of Sofosbuvir / daclatasvir. Similar results were reported in the study by Gragnani et al [232] , in which 93% of patients (no. 41/44) had a complete or partial response to vasculitis, based on a SVR of 100%. However, the study by Sollima et al [233] showed that patients with cryoglobulinemic vasculitis treated with DAA and who obtained SVR may have persistent symptoms or may relapse.

DAA has been shown to restore B and T cell homeostasis [234]. However, the clinical symptoms of cryoglobulinemic vasculitis resolve at different rates. In our study, purpura decreased in 62.6% of cases, arthritis / arthralgia in 24% and polyneuropathy in 31.2%. Immunological changes and especially the level of cryoglobulins after anti-HCV treatment dictate the persistence of vasculitic activity. In our study, the cryocritus decreased from a level of 3.4 (1.7-5.9) pretreatment to 0.3 (0-1.5) posttreatment. Bonacci et al [235] found that a basal cryocritus below 2.7% is independently associated with a complete immune response, defined as the absence of circulating cryoglobulins and normalization of complement and / or FR levels. In our study, too, the reduction of cryocritus below 2.2% was correlated with the favorable clinical evolution and the normalization of complement activity. Normalization of complement activity was observed in 66.7% of cases and was accompanied by normalization of the C4 fraction of complement in 79.2% and disappearance of FR in 54.2% of cases..

In our study, five patients had histologically documented glomerular impairment, with 60% of cases showing clinical remission and significant improvement in creatinine clerance (> 60 ml / min.), Combining glucocorticoids and cyclophosphamide with antiviral therapy. maintaining the results at 12 months post-therapeutic. This result indicates that inhibition of viral replication itself is essential in inducing the clinical remission of cryoglobulinemic vasculitis..

Analiza multivariabilă a caracteristicilor de bază asociate cu răspuns imunologic complet a relevat următorii parametri: criocrit < 2,2 %, C_4> 0,25 g/l și PVAS < 6.

Adverse effects were fatigue, insomnia, nausea, pruritus, irritability in 46.8% of patients. These were of low intensity and did not require discontinuation of antiviral therapy. They improved with symptomatic medication.

In conclusion, direct interferon-free and ribavirin-free antiviral therapy generates a virological response in over 95% of cases in patients with HCV cryoglobulinemic vasculitis and is associated with high rates of complete clinical response, moderate immune response and low rate of adverse effects. The study reveals the importance of initiating antiviral treatment in the early stages of HCV infection.

2.4.6. Incidence of HCC in DAA-treated patients

Results

All follow-up patients had standard ultrasound and / or contrast ultrasound as well as normal αFP values (<10 mg / ml) in the study. Of the 260 patients treated and followed for approximately 30 months, a number of 12 patients developed HCC (4.6%, incidence 2.3 / 100 patients / year), higher than that reported by Pons.[241]

Risk factors for the development of hepatocellular carcinoma

	Patients without HCC: 248	Patients with HCC: 12		Univariate analysis HR	Multivariate analysis (95%)
Age	42,7±8,8	54,6±9,3	P	1.11 (0,98-1,12)	1,09 (1,00-1,09)
F/B	171/77 (31%)	4/8 (66,6%)	p: 0,03	0,54 (0,14-2,07)	0,46 (0,10-1,87)
Diabetes	35 (4,1%)	8 (66,6%)	NS	4,21 (0,53-31,78)	
HCV-RNA	997,387±317,876	1389,790±401,370	p: 0,02	0,80 (0,22-2,89)	
AgHBs+/antiHBc+	2/4	2/2	p:0,05	0,96 (0,83-1,07)	
ALT u/l	68±31	72±36	NS	1,8 (0-5,2)	

GGT u/l	79±39	116±47	p: 0,02	3,26 (2,1-6,3)	
Bilirubin mg/dl	0,9 (0,4-4,3)	15 (0,4-5,8)	p: 0,030	2,6 (1,6-4,9)	
Albumin	3,8 (2,4-4,1)	3,5 (2,4-4)	p: 0,035	0,89 (0,80-0,98)	0,92 (0,81-1,03)
Previous interferon treatment	50 (20,1)	1 (83%)	p: 0,001	1,06 (0,6-1,96)	
Esophageal / gastric varices	141 (56,9%)	11 (91,6%)	p: 0,001	1,02 (0,1-1,9)	
Elastography (kPa)	14,5±7,1	22,8±9,3	p: 0,001	1,04 (1,01-1,07)	1,03 (1,01-1,07)
APRI	1.23±0.7	1,8±0,9	p: 0,020	1,2 (1,23-1,84)	1,16 (1,07-1,42)
FIB 4	3,1±1,9	5,6±2,2	p: 0,029	1,7 (0,3-3,84)	2,54 (1,04-6,19)
Post-therapy elastography < 18 kPa ≥ 18 kPa	 174 (70,1) 74 (29,9)	 2 (16,7) 10 (88,3%)	p: 0,001	11 (0,65-4,91)	1,16 (1,02-1,31)
AFP	10,9 (5,9-28)	15,8 (7,9-36)	p: 0,03	1,02 (1,01-1,03)	1,02 (1-1,03)
Child Pugh A	214 (86,2 %)	10 (83,3%)	p: 0,23		
Meld (medie)	6 (6-9)	7 (6-10)	p: 0,12		
Albi score	-2,87	-0,92	p:0,003	1,07	2,35

There is a male predominance in the total HCC and a higher viremia value compared to the group without HCC ($p \leq 0.02$). No significant differences in occult HBV infection. Cytolysis syndrome and bilioexcretory syndrome were similar in both groups. The mean value of post-therapeutic albuminemia in patients without HCC was 3.8 (2.4-4.1 g / dl) vs. 3.5 (2.4-4 g / dl) ($p = 0.001$) in those with HCC.

Elastography at 6 months after the end of treatment had higher values in patients who developed HCC compared to the rest of the group (22.8 ± 9.3kPa vs 14.5 ± 7.1 kPa, p = 0.001). Most patients who developed HCC had values above 18 kPa. Also, the other two non-invasive tests to assess the degree of liver fibrosis showed statistically significant differences.

At the time of diagnosis, most patients were in the early stage of BCLC (66.6%) and the remaining 33.3% in the intermediate stage.

The Meld score was 7 (5-11), and in terms of CHILD class 10 patients were class A and 2 patients class B. The recurrence pattern was heterogeneous.9 patients developed intrahepatic growth with nodular profile (1 single node 6 patients, 2 patients 2 nodules each and one patient multiple nodules). 3 patients developed infiltrative HCC. The mean time to onset of HCC was 16.2 months.

From the point of view of clinical and biological characteristics, the predominant incidence of HCC in males (66.6%), a synthetic function and an affected albuminemia are noted - 3.5 g / dl (2.4-4 g / dl) vs 3.8 g / dl (2.4-4.1), a higher value of αFP (15.8 ng / ml vs 10.9 ng / ml). The Child-Pugh class and the Meld score did not differ between the two groups.

In the univariate analysis, the risk factors for HCC were male, presence of HBsAg / anti HBc, bilirubin level, albuminemia level, αFP level, presence of esophageal / gastric varicose veins, previous treatment with PegIFN + ribavirin, degree of hepatic fibrosis, . Multivariate analysis selected as independent risk factors for male HCC, albuminemia, αFP level, fibrosis level and platelet count. An important predictive factor revealed by our study was the degree of fibrosis, assessed 6 months after the end of treatment. by elastography, APRI test and FIB4 test. Most patients who had elastography values ≥ 18 kPa (83.3%) developed HCC. In patients with a degree of fibrosis <18 kPa we have a reduction in the risk of HCC of 82% (HR: 0.18; 95% CI: 0.07-0.61).

αFP is significantly associated with the development of HCC, with a 20% increase in HR for each 2 ng / ml increase (HR: 2.2, CI: 1.01-1.42, p = 0.036).

Due to the positive predictive value of albuminemia and bilirubinemia, we set out to calculate the ALBI score. □242□ The ALBI score is calculated according to the following formula: (log10 bilirubin [μmol / L] × 0.66) + (albumin [g / L] × −0.0852) and is defined as follows: ≤ -2.6 = grade 1,> - 2.6-≤1.39 = grade 2 and> -1.39 = grade 3.

Discussions

The HCV core protein plays a central role in the overexpression of tumor suppressor genes, activates oncogenesis, disrupts apoptosis. By forming reactive oxygen species and immune modulation, the virus induces epigenetic changes that generate the tumor process, such as hypermethylation of tumor suppressor genes and the release of its own micro-RNA. Genetic and epigenetic changes caused by viral infection may precede SVR and persist indefinitely after SVR. HCC can occur even after long intervals of years since reaching RVS.[256]

Rutlege demonstrated that the annual risk of developing hepatocellular carcinoma in the patient who had RVS was 3.57%, compared with 9.8%. [238] The study conducted by Huang showed a risk of HCC of 3.5% / year in patients with RVS affected and 9.1% in patients with nor-RVS. [239] Waziry demonstrated an occurrence rate of 2.96% / year and a post-clearance recurrence through DAA of 12.6%.[240]

In a prospective study of HCV-infected patients, the annual risk of HCC was 0.11% in patients with stiffness ≤ 10 kPa, 2,9% in patients with stiffness 10-15 kPa, 83% in patients with stiffness 20-25 kPa and 14.4% in patients with hepatic stiffness above 25 kPa. [258]

Among the non-invasive scores, FIB4 ≥ 3,25 is a strong predictor of the risk of liver cancer in both cirrhotic and non-cirrhotic patients. Patients without cirrhosis and with FIB4 ≥ 3,25 have an annual risk of 1.22% of hepatocellular carcinoma. Patients with FIB 4 ≥ 3,25 both before and after SVR have a very high risk of 2% / year of HCC. Those with FIB4 ≤ 3,25 after reaching SVR they have a much lower risk. Patients without cirrhosis and with FIB4 < 3,25 have a 0.2% risk after SVR, suggesting the possibility of looser surveillance at longer intervals.[259]

Multivariable scores for liver cancer risk assessment in patients treated with HCV with direct antivirals have been developed. These combine multiple elements: patient age, obtaining / not RVS, gender, BMI, ethnicity, viral genotype, serum albumin, AST, ALT, PLT, INR and hemoglobin. The model showed that patients with advanced hepatic fibrosis who get SVR may have a risk of more than 1% if they have low serum albumin and platelet levels, a high AST / ALT ratio, are male or older. [115]

The annual risk of HCC in untreated patients with HCV cirrhosis varies between 3 and 4%, and for those with a history of HCC treated by surgical resection or ablation between 15-20%.

Although direct antiviral therapy for HCV has improved liver function in those with SVR and survival in those with cirrhosis and HCC, recent studies have shown an increased rate of de novo and recurrent HCC after DAA therapy. [243, 244,245]

Our study reveals that the degree of liver failure assessed by the ALBI score and the degree of fibrosis are predictive factors of the risk of HCC.

A recent study by Villani [246] demonstrates that DAA therapy induces a rapid reduction in TNFα, IL10 and an increase in VEGF. In particular, IL10 is an immunoregulatory molecule involved in inflammatory processes in chronic HCV infection. Patients with de novo HCC / recurrence after DAA therapy have elevated levels of VEGF and angiopoietin-2 (ANGPT2). Patients with de novo HCV and HCC cirrhosis have increased hepatic expression of AGPT2 prior to DAA treatment. VEGF levels also increase during treatment with DAA. Tumor and nontumor expression of AGPT2 has an inverse relationship with portal velocity and a positive relationship with fibrosis.

DAA therapy activates AGPT2-mediated angiogenesis in patients with predisposition: patients with advanced hepatic fibrosis, extensive splanchnic circulation, and altered intrahepatic and splanchnic blood flow. Not surprisingly, HCC does not occur or recur in patients with small esophageal varices and hepatic stiffness <16 kPa. [247]

Another important factor in the carcinogenesis of these patients is the increase in the volume of visceral fat, which causes insulin resistance, serum increase in leptin and oxidative stress.. [251]

In the study by Guardino et alab, predictors of HCC recurrence were DAA inefficiency, elevated bilirubin, elevated αFP, and BMI.. [252]

The role of elastography in identifying patients at risk for HCC was identified in Conti's Italian study [253] which revealed that a value of over 21.3 kPa and a FIB> 9 is associated with the development of HCC.

The parameters resulting from our study are similar to those in the study of Pons M et al, as well as those in the study of Charette et al. [254] Compared to the results obtained by Charette, where the threshold for hepatic elastography was 10kPa, in our group the statistically significant threshold was 18kPa.

From an imaging point of view, the infiltrative aspect was associated with a much more severe evolution and with the lowest survival. Patients with HCC after DAA therapy require very

strict imaging surveillance (CT in dynamics / MRI) and new therapeutic protocols due to the much more aggressive appearance.

The study by Renzulli M et al was performed on 91 patients with HCC after DAA versus the comparative group of 94 patients with HCC without direct antiviral therapy. Liver status was assessed by FIB4, Child-Pugh classification and Meld score. Patients in the first group were older, the FIB4 score (4.84 ± 3.53) was lower in the HCC group after direct antiviral therapy. The frequency of infiltrative pattern, portal vein thrombosis and regional lymph node metastases was significantly higher in the group with post-DAA HCC. [256]

Also, the αFP value was much higher (5,085 ± 11,883 ng / ml). Patients with post-DAA HCC were significantly higher in advanced stages according to the BCLC classification and with limited treatment options ($p < 0.05$).

In our group, the age of patients with HCC was more advanced, but the degree of fibrosis assessed by FIB4 and APRI was significantly higher than in patients without HCC. The imaging picture was predominantly uni / multinodular, but a characteristic was the microvascular invasion in the case of 8/12 patients (66.6%), in those with nodules with a diameter between 10-20 mm.

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List of published scientific papers

1. Evolution of HCV-associated cryoglobulinemic vasculitis after treatment with direct-acting antivirals **A. Franculescu**, I. Copaci, L. Micu, L. Iliescu; Dept of Internal Medicine, Fundeni Clinical Institute Bucharest. Surg. Gastroenterol. Oncol. 2021;26(4):268-274 DOI: 10.21614/sgo-26-4-373. CiteScore: 0.2

https://www.sgo-iasgo.com/article/evolution-of-hcv-associated-cryoglobulinemic-vasculitis-after-treatment-with-direct-acting-antivirals

2. Clinical relevance of probiotic on long-term maintenance therapy outcomes, I. Copaci, G. Constantinescu, L. Micu, **A. Franculescu-Bertea**, Fundeni Clinical Institute, Department of Internal Medicine; Floreasca Emergency Hospital, Department of Gastroenterology. Bucharest, Romania, FALK Symposium, october 5-6, 2018. Poster

3. Efficacy of Rifaximin vs. dietary fiber on symptoms of uncomplicated diverticular disease of the colon, Authors: Ionel Copaci (Corresponding Author), Gabriel Constantinescu, (Co-author), Mariana Mihaila (Co-author), Laurentiu Micu (Co-author), **Andreea Franculescu-Bertea** (Co-author) Surg Gastroenterol.Oncol.2019;24(5):233-240. DOI: 10.21614/sgo-24-5-233. CiteScore: 0.2

https://www.sgo-iasgo.com/article/efficacy-of-rifaximin-vs-dietary-fiber-on-the-evolution-of-uncomplicated-colonic-diverticular-disease

4. Improvement of liver steatosis achieved by probiotics in patients with nonalcoholic fatty liver disease; Authors: I. Copaci, **A. Franculescu**, L. Micu, G. Chiriac, M. Mihaila, E.L. Iliescu, Journal of Hepatology 68:S585 · April 2018. CiteScore: 25.08

https://www.researchgate.net/publication/324699559_Improvement_of_liver_steatosis_achieved_byprobiotics_in_patients_with_nonalcoholic_fatty_liver_disease

5. A case of esophagogastric cancer: The great masquerader, **Andreea Frânculescu-Bertea**, Ionel Copaci, Elena Laura Iliescu, Elena Simona Ioanițescu, Fundeni Clinical Institute, "Carol Davila" University of Medicine and Pharmacy, Department of Internal Medicine, Bucharest, Romania. E poster