„CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY

BUCHAREST

DOCTORAL SCHOOL

MEDICINE

The evaluation of the dynamics of liver fibrosis biomarkers and metabolic profile in patients with HCV cirrhosis who registered SVR using Interferon-free regimens

THE SUMMARY OF THE DOCTORAL THESIS

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THE SUMMARY OF THE DOCTORAL THESIS

The evaluation of the dynamics of liver fibrosis biomarkers and metabolic profile in patients with HCV cirrhosis who registered SVR using Interferon-free regimens

The doctoral thesis called **"*The evaluation of the dynamics of liver fibrosis biomarkers and metabolic profile in patients with HCV cirrhosis who registered SVR using Interferon-free regimens"*** is divided into two parts: a general part, with two chapters that address data from the specialized literature regarding the subject of this doctoral research, and a part of personal contributions, structured into six subsections detailing the objectives of the doctoral research, the specific methodology of the study, the results, conclusions, and practical implications of this doctoral research.

THE GENERAL PART

CHAPTER 1: General information about HCV infection and the dynamics of liver fibrosis associated with HCV infection

Chapter 1 of the doctoral research contains general data from the specialized literature regarding the epidemiology of hepatitis C virus (HCV) infection [1], clinical aspects and manifestations associated with HCV infection [2], as well as data on the management of chronic HCV infection [3]. The second part of this chapter presents a synthesis of data concerning the mechanisms and clinical consequences of fibrogenesis and fibrosis regression in the context of HCV infection [4,5].

Chapter 2: Methods of evaluating the liver fibrosis

The evaluation of liver fibrosis represents an important measure in the care of a patient with chronic liver damage and is necessary to evaluate the prognosis. In advanced stages of liver pathology, complications such as ascites, portal hypertension and progression to hepatocellular carcinoma may occur. In current clinical practice, there are various methods for evaluating the

degree of liver fibrosis, which are described in detail in chapter 2 of the doctoral research. This chapter contains data from the specialized literature regarding liver biopsy puncture (PBH) (still considered a reference method for evaluating the degree of liver fibrosis, but which is no longer currently used) [6], but also a synthesis regarding non- - invasive for the evaluation of liver fibrosis widely used in current clinical practice. A series of direct serum biomarkers, fibrosis scores used in staging the degree of fibrosis, as well as imaging methods for evaluating the degree of liver fibrosis are described [7,8].

PERSONAL CONTRIBUTIONS

CHAPTER 3: Working hypothesis and objectives of the study

The assessment of liver fibrosis is an important element in the monitoring algorithm of a patient with chronic HCV liver disease after obtaining SVR under DAA. This behavior is absolutely necessary in the case of patients with advanced degrees of fibrosis (F3 or F4), because following antiviral therapy with DAA, the patient is considered cured, virologically, but the histological and functional liver changes persist, and complications such as ascites, portal hypertension and evolution towards hepatocellular carcinoma can occur in these advanced phases of the disease, including after obtaining RVS.

The vast majority of studies in the specialized literature use fibrosis scores such as APRI [9] or FIB-4 [10] to evaluate the dynamics of the degree of liver damage, in a smaller percentage of studies use FibroTest. Another method for assessing the dynamics of the fibrosis is imaging, most commonly transient elastography (TE).

This study aims to evaluate the dynamics of the degree of liver fibrosis over a period of up to 7 years after the end of DAA antiviral therapy in a group of patients with HCV liver cirrhosis compared to a group of patients with chronic HCV hepatitis.

The objectives of this doctoral research will be included in 3 distinct studies:

1. Evaluation of the dynamics of APRI and FIB-4 fibrosis scores in patients with HCV liver cirrhosis, Child A class, who recorded SVR under interferon-free therapeutic regimens - comparative study with a group of patients with chronic HCV hepatitis who recorded SVR under DAA

2. Evaluation of the dynamics of liver fibrosis by FibroMax in patients with HCV liver cirrhosis, Child A class, who recorded SVR under interferon-free therapeutic regimens - compared to a group of patients with chronic HCV hepatitis, who recorded SVR under DAA

3. Dynamic evaluation of the post-SVR metabolic profile in patients with liver cirrhosis with HCV Child A class, who recorded SVR under interferon-free therapeutic regimens - compared to a group of patients with chronic HCV hepatitis.

CHAPTER 4: General methodology of the research

This chapter includes data about the study plan: the description of the two groups of patients enrolled in the doctoral research (cirrhosis patients and patients with chronic HCV hepatitis), the inclusion and exclusion criteria and the dynamic evaluation and monitoring plan of these patients.

This study is a prospective, comparative one, between 2 groups of patients, who were enrolled between November 2015 and January 2020 and were subsequently monitored until June 2024. The two groups of patients were included as follows:

- Group 1: 170 patients with liver cirrhosis with HCV (FibroMax F3-F4 and F4) Child A class under monitoring at the National Institute for Infectious Diseases "Professor Doctor Matei Balș" (INBIMB) Bucharest

- Group 2: 99 patients with chronic HCV hepatitis (F1-F3) under monitoring at INBIMB

The evaluation of patients included several stages (monitoring visits): at the initiation of antiviral therapy, 6 months after the end of treatment (EOT – end of treatment), 1 year after EOT, 2 years after EOT, 3 years from EOT and 7 years from EOT.

The data used in the study were collected and centralized in the Excel program (Microsoft) and the statistical processing of the obtained data was carried out in the IBM-SPSS Statistics® version 22 program.

CHAPTER 5: Study 1: The evaluation of liver regression using APRI and FIB-4 in patients with liver cirrhosis vs patients with chronic hepatitis C, after SVR using DAA therapies

In this first study, the regression of liver fibrosis was evaluated in the two groups of patients using APRI and FIB-4 fibrosis scores. Afterwards, a comparison was made with the data obtained in the specialized literature.

The results obtained are as follows:

The patients in the two groups were compared at the time of initiation of DAA antiviral therapy using APRI and FIB-4 scores, and a statistically significant difference was recorded between the median scores of patients with cirrhosis and the median scores of patients with chronic hepatitis, $p<0.001$ for both scores. Thus, in the case of the APRI score, at the time of initiation of antiviral therapy, the median for patients with liver cirrhosis was 1.15 compared to the group of patients with chronic hepatitis - 0.75. In the case of the FIB-4 score, a median of 3.41 is observed for patients with cirrhosis, compared to 2.28 for patients with chronic hepatitis.

In the case of the APRI score, the studies published so far describe that a value of ≥1 has a sensitivity of 76% and a specificity of 72%; and when a value of ≥2 is considered, this score has a sensitivity of 46% and a specificity of 91% [9]. In this doctoral research, an APRI score of ≥1 recorded a sensitivity of 59% and a specificity of 65% compared to FibroTest, this being the reference method proposed by the National Protocol in force for staging the degree of fibrosis and classifying the patients for initiation of antiviral therapy with DAA. When considering the APRI threshold value ≥2, the sensitivity is 31%, with a specificity of 87%. The area under the curve (AUROC) was calculated, and in this study it had a value of 0.666 (95% CI 0.6-0.732), an optimal threshold value of 0.867 and for this score, the sensitivity obtained was of 68%, with a specificity of 57%. This AUROC shows that APRI provides satisfactory results compared to FibroTest.

Analyzing previous studies that used the FIB-4 score, the results showed that when it has a value of <1.45, the negative predictive value in excluding liver cirrhosis is 94.7%, with a sensitivity of 74.3% and a specificity of 80.1% . If FIB-4 has a value of ≥3.25, the positive predictive value is 82.1% in confirming liver cirrhosis, with a sensitivity of 37.56% and a specificity of 98.2% [11]. In the present study, the ROC curve was performed for the FIB-4 score in comparison with the FibroTest score, recording an AUROC value of 0.678, which means a satisfactory result in differentiating liver cirrhosis from lower degrees of liver fibrosis. For the preset threshold value above 3.25, FIB-4 recorded a sensitivity of 52% and a specificity of 73% in detecting liver cirrhosis. Taking into account the ROC curve obtained in this study compared to FibroTest, an optimal threshold value of 2.32 was observed, which has a sensitivity of 71% and a specificity of 53% in discriminating liver cirrhosis from other degrees of fibrosis.

For each group of patients, the dynamics of APRI scores and the dynamics of FIB-4 scores during the 7 years of monitoring were subsequently analyzed. APRI scores show a marked decrease at the 6-month post-EOT visit, the difference remaining statistically significant until the end of the 7-year post-EOT follow-up period for both groups of patients ($p<0.001$).

A statistic similar to the APRI score was also performed in the case of the FIB-4 score, both for the group of patients with liver cirrhosis and for those with chronic HCV hepatitis. In the case of group 1, more than 50% of the patients had FIB-4 scores above 3.25 at the start of antiviral therapy, a proportion that decreased at the following visits, only a quarter of patients still presenting FIB-4 values compatible with liver cirrhosis . The same dynamic of decrease was observed in the case of group 2 of patients with chronic HCV hepatitis. There is a small number of patients who were evaluated at the last 2 visits in the case of group 2, and so the data cannot be interpreted as statistically relevant.

The APRI score shows statistically significant correlations with the FIB-4 score at all follow-up visits ($p<0.001$ for each visit). The correlations of the APRI score with the FibroTest score were studied and it was observed that there were statistically significant correlations except for the 1-year post-EOT visit ($p=0.002$, $p=0.032$, $p=0.169$, $p=0.030$, $p=0.031$, $p =0.003$).

The FIB-4 score shows statistically significant correlations with the FibroTest score except for the last follow-up visit, the one at 7 years post-EOT ($p<0.001$, $p<0.001$, $p=0.015$, $p=0.012$, $p=0.005$, $p=0.103$).

In the group of non-cirrhotic patients, neither the alpha-fetoprotein, nor the APRI score, nor the FIB-4 score are statistically correlated with the FibroTest score, which suggests that these scores can correctly discriminate liver cirrhosis from the other degrees of fibrosis, but cannot tell the difference between F1-F3.

CHAPTER 6: Study 2: The evaluation of fibrosis regression using FibroTest in patients with liver cirrhosis vs HCV chronic hepatitis, after SVR using DAA therapies

The main objective of this study is to monitor the dynamics of liver fibrosis using FibroMax in patients with HCV liver cirrhosis, Child A class, who recorded SVR under therapeutic regimens with DAAs - compared to a group of patients with chronic HCV hepatitis who achieved SVR under DAA.

Results:

A separate analysis was performed on patients with cirrhosis and patients with chronic hepatitis at the time of initiation of antiviral therapy and subsequently 6 months after EOT, to determine if there were statistically significant differences between the two follow-up visits. The results showed that there is a statistically significant difference between the two evaluation moments ($p<0.001$ for group 1 and $p=0.03$ for group 2).

A statistically significant difference was recorded between the age groups <65 years and ≥65 years ($p<0.001$). Thus, younger patients have a greater decrease in the FibroTest score than those over 65 years of age. The elderly can also have liver damage in the context of other pathologies, such as hepatic steatosis associated with metabolic dysfunction (MAFLD). In the group of patients with chronic hepatitis, there are no statistically significant differences even between patients over 65 years old, compared to those under 65 years old.

In order to fulfill the objective of this study, the results obtained for each monitoring visit were analyzed, both for FibroTest and for ActiTest, for both groups. Statistical analysis was performed to identify the differences between the results obtained at the monitoring visits, but especially between the results from the moment of initiation of antiviral therapy and those from the end of the monitoring period.

Regarding the FibroTest, in the case of patients with liver cirrhosis, it is noticed that there is a statistically significant difference between the results obtained at the time of initiation of antiviral therapy and those 6 months post-EOT ($p<0.001$); Between 1 year and 2 years post-EOT ($p=0.04$), between 2 and 3 years post-EOT ($p=0.018$). Later, from 3 to 7 years post-EOT, statistically significant differences between visits are no longer recorded ($p=0.733$). Analyzing the entire monitoring period, there is a statistically significant difference ($p<0.001$) between the baseline visit and the one 7 years post-EOT.

Analyzing the ActiTest, there is a clear difference between the time of initiation of DAA antiviral therapy and the 6-month post-EOT visit ($p<0.001$), which can be explained by the achievement of SVR and the elimination of the liver aggressive factor, which leads to a marked reduction in the necro-inflammatory activity.

The percentages of the degrees of liver fibrosis in patients from the studied groups was analyzed comparatively for each visit. Thus, it can be observed that at the time of initiation of antiviral therapy, 89% of the patients had fibrosis grade F4, and 11% had F3-F4, being all

categorized as patients with liver cirrhosis. At the 6-month post-EOT visit, a decrease in the percentage of patients categorized as liver cirrhosis (43% - F4 and 3% - F3-F4) is observed, with the detection of intermediate degrees of fibrosis (29% - F3, 17% - F2, 7% - F1-F2 and F0-F1 - 1 patient).

At 1 year post-EOT, the distribution of patients according to degrees of fibrosis remains similar compared to that described at the 6-month post-EOT visit.

At the assessment at 2 years after EOT, the decrease in the proportion of patients with F4 (from 38% to 30%), with an increase in the proportion of patients with F2 (21%), and 3 years after EOT, the distribution of patients is noted: F4 - 25 % of patients, F3-F4 - 7% of patients, F3 - 39% of patients, F2 - 14% of patients, F1-F2 - 11% of patients, F1 - 4% of patients.

At the last follow-up visit, 16 patients had a FibroTest performed showing that only 1 patient still had F4, 10 patients registered F3 (63%), 3 patients had F2 and 2 patients had F1-F2.

In the case of the group of patients with chronic HCV hepatitis, the dynamics of FibroTest and ActiTest were analyzed. In the case of FibroTest, a statistically significant difference was recorded between the visit from the initiation of DAA and the visit from 6 months post-EOT ($p=0.03$), but this difference was not preserved until the end of the monitoring period, the average value of FibroTest at the 1-year post-EOT visit being similar to the one at baseline. A limitation of the study results from the fact that at the last 2 monitoring visits there is a small number of patients (8 patients - for visit 5 and 6 patients for visit 6), so that the statistical analysis could not be performed on this number of patients. For ActiTest, the statistically significant difference recorded between the visits from DAA initiation and the one 6 months post-EOT is maintained during the monitoring period ($p<0.001$).

Detailed analysis by fibrosis grade at each follow-up visit showed that:

- at the visit from the initiation of DAA, the distribution of patients with chronic hepatitis with HCV according to the degree of fibrosis on FibroTest was: F3 - 71% of patients, F2-F3 - 1% of patients, F2 - 15% of patients, F1-F2 - 10% of patients, F1 - 1% of patients and F0 - 2% of patients,

- at the 6-month post-EOT visit - the distribution of patients with chronic HCV hepatitis according to the degree of fibrosis at FibroTest was: F4 - 5% of patients, F3-F4 - 2% of patients, F3 - 29% of patients, F2 - 36% of patients, F1-F2 - 24% of patients, F1 - 5% of patients.

Later, it is observed that the proportion of patients with advanced degrees of liver fibrosis is maintained. At the last 2 follow-up visits, a very small number of patients were evaluated (8 and 6 patients, respectively).

In 2014, Poynard et al proposed a staging of the degrees of liver fibrosis according to FibroTest and TE results, and the stage of liver cirrhosis was divided into 3 sub-stages: F4.1: >0.74 - ≤0.85 ; F4.2: >0.85 - ≤0.95 and F4.3: >0.95 – 1 [12].

At the initiation of antiviral therapy, a small number of patients were registered in the F4.3 substage (16 patients), none of whom had this stage at subsequent evaluations, obtaining a regression of fibrosis within the liver cirrhosis stage, and 2 patients they even showed regression to lower stages of liver fibrosis. The patients in the F4.2 sub-stage presented in equal proportions, either the maintenance of the same degree of fibrosis, or regression to F4.1 or regression from the liver cirrhosis stage. The most obvious regression was recorded in patients in the F4.1 sub-stage: 78% of patients recorded regression from the stage of liver cirrhosis.

A percentage of 20.3% of patients maintained the same degree of liver fibrosis, 78.1% of patients registered regression from the stage of cirrhosis, and 1 patient showed an increase in the degree of liver fibrosis to F4.2.

Comparing the group of patients without liver cirrhosis, it can be observed even from the assessment at 6 months post-EOT that there are patients who show increases in the degree of liver fibrosis (7 patients out of the 45 evaluated 6 months post-EOT, 8 patients out of the 32 patients at 1 year post-EOT and 5 patients of the 16 patients at 2 years post-EOT). At the evaluations from 3 years and 7 years post-EOT, a small number of patients were analyzed by FibroTest, but even under these conditions, an increase in the degree of fibrosis by 1 degree can be noted in 2 of the 8 patients at 3 years post - EOT and in 1 patient out of the 6 patients at the evaluation 7 years post-EOT.

CHAPTER 7: Study 3: The evaluation of the dynamics of metabolic profile in patients with HCV liver cirrhosis vs patients with HCV chronic hepatitis, after SVR using DAA therapies

Studies have shown that among patients with chronic HCV hepatitis, the prevalence of hepatic steatosis is 40-86%, compared to 20-50% found in other chronic liver diseases [13].

An important aspect in the case of patients who obtained SVR after DAA antiviral therapies is the study of the dynamics of hepatic steatosis and metabolic changes, which have as important an impact on the progression of the disease as hepatic fibrosis.

The objective of this study is to evaluate the dynamics of the post-SVR metabolic profile in patients with liver cirrhosis with HCV, compared to those with chronic hepatitis with HCV, by determining the dynamics of the SteatoTest, the level of cholesterol, triglycerides and blood sugar.

This study focused on evaluating the dynamics of SteatoTest and NashTest scores.

Results:

It is known that chronic HCV infection is associated with hypocholesterolemia, and obtaining an SVR leads to the appearance of hypercholesterolemia, compared to untreated or non-responder patients [14]. The same dynamics can be observed in the case of the patients evaluated in this study, both in the case of patients with liver cirrhosis with HCV, but also those with chronic hepatitis with HCV.

In the case of patients with liver cirrhosis, the average value at the initiation of antiviral therapy was 150.7 mg/dL, and at the end of the monitoring period, the average value reached 186.8 mg/dL, ($p=0.012$). In the case of patients with chronic hepatitis with HCV, there are differences between the monitoring visits, but they are not as great as in the case of patients with liver cirrhosis, these having no statistical significance.

Regarding the dynamics of triglyceride values, there are no statistically significant differences between the values obtained at the initiation of antiviral therapy and the end of the monitoring period at 7 years post-EOT in any of the studied groups.

When evaluating blood glucose values, in the case of patients with cirrhosis with HCV, although between initiation and 6 months post-EOT there is a statistically significant difference ($p=0.018$) between blood glucose values registering a decrease from a median value of 104 mg/dL at a value of 103 mg/dL, this trend is no longer found until the end of the monitoring period (median of 105 mg/dL). In the case of patients with chronic HCV hepatitis, there are no statistically significant differences, even if there were variations in the median values between follow-up visits.

During the monitoring visits, along with the assessment of the degree of liver fibrosis and the necro-inflammatory activity determined by the viral infection, FibroMax also evaluated the

degree of hepatic steatosis by calculating the SteatoTest as well as the necro-inflammatory activity determined by metabolic diseases calculated by the NashTest.

From the evaluation of the results at each monitoring visit, it is observed that there is a difference between the initiation of antiviral therapy and visit 2, visit 3, visit 4 and visit 5, but subsequently a significant increase is observed between visit 5 and visit 6 of monitoring, thus that between the moment of initiation of DAA and the end of monitoring there is no longer a statistically significant difference regarding the average value of SteatoTest ($p=0.138$). This result could also be influenced by the smaller number of patients evaluated 7 years post-EOT.

After the 2-year post-EOT visit, no patient registered grades of S2-S3 or S3 steatosis, and at the 7-year post-EOT visit, no patient registered grades of hepatic steatosis S2 or more. Of the 35 patients (23%) with S3 steatosis at baseline, at 6 months only 3 patients still had this degree of steatosis, and 1 year after EOT, 3 patients had S3 steatosis.

In the group of patients with chronic HCV hepatitis, the mean values of SteatoTest recorded statistically significant differences between the visit from the initiation of antiviral therapy and the visit from 6 months post-EOT ($p<0.001$), a difference that is maintained until the end of the monitoring ($p=0.038$). The degrees of hepatic steatosis in the group of patients with chronic HCV hepatitis varied at the initiation of DAA therapy from S0 (15% of patients) to S3 (11% of patients). Starting with the 1-year post-EOT follow-up visit, no patient had S2-S3 or S3 steatosis grades, and at the last follow-up visit, steatosis grades were S0 in 67% of patients, and S1 and S2 in equal proportions of 17% of patients.

The necro-inflammatory activity caused by metabolic diseases (NashTest) registers a statistically significant decrease only in the first 6 months post-EOT in the case of the group of patients with cirrhosis with HCV, a difference that is not maintained until the end of the monitoring period ($p=0.089$) .

In the case of patients with chronic HCV hepatitis, NashTest did not register any statistically significant difference, neither during the monitoring visits, nor between the first and last visit ($p=0.175$). There is a statistically significant difference between the visit from the initiation of the antiviral treatment and the visit from 2 years post-EOT ($p=0.041$).

Chapter 8: Conclusions and personal contributions

Chapter 5: Study 1: The evaluation of liver regression using APRI and FIB-4 in patients with liver cirrhosis vs patients with chronic hepatitis C, after SVR using DAA therapies

The conclusions of this study were:

1. A statistically significant decrease in the APRI score was observed in both groups of patients with cirrhosis and those with chronic HCV hepatitis, between the time of initiation of DAA antiviral therapy and 6 months post-EOT. In the group of cirrhotic patients, the APRI score continues to show statistically significant differences up to 3 years after EOT, but subsequently remains at stationary values until the 7-year evaluation. In the group of patients with chronic HCV hepatitis, the differences recorded between follow-up visits 6 months after EOT are no longer statistically significant.

2. The FIB-4 score registered persistent and statistically significant decreases throughout the monitoring period both in the group of patients with cirrhosis and in the group of patients with chronic HCV hepatitis.

3. Both APRI and FIB-4 prove to be useful, easy, quick and inexpensive tools for the diagnosis of HCV cirrhosis, but they cannot specifically differentiate other degrees of fibrosis, considering this a limitation of these scores.

4. FIB-4 may also be useful for monitoring patients with liver cirrhosis after EOT, especially since it has been shown that there is a statistical correlation between this score and FibroTest throughout the monitoring period within the cohort patients with liver cirrhosis.

Chapter 6 – Study 2: The evaluation of fibrosis regression using FibroTest in patients with liver cirrhosis vs HCV chronic hepatitis, after SVR using DAA therapies

The conclusions of this study were:

1. In the group of patients with liver cirrhosis, during the 7 years of monitoring after SVR, a regression of liver fibrosis is observed. This process of regression of liver fibrosis seems to be more important in the first 6 months post-EOT, being directly correlated with the significant reduction of necro-inflammatory activity (assessed by ActiTest).

2. The annual dynamic evaluation showed that the reduction in FibroTest and ActiTest scores continues up to 3 years post-EOT, after which it remains at stationary values until the 7-year post-EOT evaluation.

3. In non-cirrhotic patients, no statistically significant decrease in the FibroTest score is detected. A limitation of the study was the small number of non-cirrhotic patients who remained in follow-up up to 7 years post-EOT.

4. The FibroTest score has a greater sensitivity in advanced liver disease and in cirrhotic patients compared to patients with lower degrees of fibrosis.

5. The results of this study emphasize the importance of long-term monitoring of patients with chronic HCV infection who have achieved SVR. In the group of patients with liver cirrhosis at baseline, there are both patients who show regression of liver fibrosis to lower degrees (even up to F1), but there are also patients who maintain the same advanced degree of liver fibrosis (liver cirrhosis). In the group of patients with chronic hepatitis, there were patients who maintained the degree of fibrosis from the baseline, patients who showed a decrease in the degree of fibrosis by 1 or 2 degrees, but most importantly, there were patients in whom it was registered increasing the degree of liver fibrosis by one degree.

Chapter 7 – Study 3: The evaluation of the dynamics of metabolic profile in patients with HCV liver cirrhosis vs patients with HCV chronic hepatitis, after SVR using DAA therapies

The conclusions of this study were:

1. In the group of patients with liver cirrhosis with HCV, from a metabolic perspective, when evaluating the SteatoTest and NashTest scores, a statistically significant decrease is observed in the first 6 months post-EOT. Although there are differences regarding the SteatoTest and NashTest grades obtained during the follow-up visits, the average values obtained do not show statistical significance between the time of initiation and the end of the 7-year follow-up period.

2. In the group of patients with chronic HCV hepatitis, only the SteatoTest evaluation shows a statistically significant difference between the initiation of DAA antiviral therapy and the first 6 months post-EOT. There is a statistically significant difference in the SteatoTest score between the assessment at DAA initiation and that at 7 years post-EOT.

3. The dynamic evaluation of cholesterol and triglyceride values provides results similar to those in the specialized literature, the strength of this study being the longer period of dynamic monitoring of these patients.

4. The mean values of total cholesterol showed statistically significant increases, with a rapid increase in the first 6 months post-EOT, a slower increase up to 1 year and maintaining the plateau until the end of the monitoring period. Both in the group of cirrhotic patients, but especially in that of non-cirrhotic patients, the proportion of the female sex was higher, a fact that contributes to an increased average value of cholesterol in the dynamics.

5. The dynamics of triglyceride values do not show statistically significant differences neither in the case of patients with liver cirrhosis, nor in the case of patients with chronic HCV hepatitis.

6. The increase in cholesterol values has a major impact on cardiovascular diseases, because atherosclerotic plaques can be influenced by the LDL fraction, which will increase the risk of important cardiovascular complications.

7. Long-term metabolic evaluation of patients is necessary and the decision to initiate lipid-lowering therapy should be taken at the right time to prevent cardiovascular complications. An inter- and multidisciplinary approach is necessary for patients with liver cirrhosis and chronic HCV hepatitis, after obtaining SVR under antiviral therapies with DAAs, requiring a good collaboration between the gastroenterologist/infectious disease doctor who monitored the antiviral therapy and the doctor specializing in diabetes and diseases nutrition, cardiologist, as well as with the family doctor.

The limits of the present doctoral research resulted mainly from the interruption of monitoring due to the COVID-19 pandemic, when patients could no longer be evaluated in our clinic, there being a break in the monitoring of patients between 3 years post-EOT and 7 years post-EOT, later many of the patients were lost from the records.

An unsolved problem in the monitoring of patients with chronic HCV infection post-SVR is the collection of data on the number of patients who presented with liver decompensation, who developed hepatocellular carcinoma or who died due to liver disease. This evaluation of complications at a distance, together with the continuation of dynamic monitoring of patients

up to a period of at least 10 years post-EOT, will represent future directions of post-doctoral research.

Practical implications

The results obtained in this doctoral research allow developing a monitoring algorithm for patients with chronic HCV infection who obtained RVS12 (Table 8.1).

Patients with chronic HCV infection (both those with liver cirrhosis and those with chronic HCV hepatitis) will be monitored both from the point of view of liver fibrosis, tracking its regression or progression, and from the point of view of necro-inflammatory activity , of the steatosis score and of the lipid and carbohydrate metabolic profile. Depending on the results of these evaluations, patients will be placed in several categories, each category to be monitored according to a specific monitoring algorithm.

In the event that increases in cholesterol or triglyceride values are observed, the patient should be referred to a specialist in Nutrition and Metabolic Diseases to initiate lipid-lowering therapy in order to prevent cardiovascular complications. In addition, these patients require a cardiological evaluation taking into account the contribution of chronic HCV infection to the development of cardiovascular diseases. Patients with known pre-existing cardiac pathology may benefit from more frequent evaluations (every 6 months). If increases in blood glucose or glycosylated hemoglobin values are observed during the routine evaluation, the patient should be referred to a Diabetes specialist in order to initiate specific treatment, since the complications associated with diabetes are silent and with slow progression. Results regarding the dynamics of the lipid profile were detailed in Chapter 7.

Regarding the AFP value, when an increase in its value is observed compared to the previously determined values, an abdominal ultrasound should be performed as a first step, then depending on the AFP value and the result of the abdominal ultrasound, CT or MRI with liver visa and contrast substance capable of detecting hepatocarcinoma lesions.

Patients with Child A class liver cirrhosis under monitoring by Infectious Diseases doctors, in case they present progression to Child B or C class liver cirrhosis, or in case of liver decompensation or CHC, will be referred to Gastroenterology for specific therapeutic conduct and continuous liver monitoring.

Table 8.1: Monitoring algorithm of patients with chronic HCV infection who obtained SVR12

	Patients with cirrhosis or F3 fibrosis	**Patients with chronic hepatitis (fibrosis <F3)**
The time interval between 2 assessments	Every 6 months	Once a year
Non-specific blood tests	• Complete blood count • Peripheral blood smear • Prothrombin time • Transaminases • Cholestasis enzymes • Complete lipid profile • Protein electrophoresis • Glucose and glycosylated hemoglobin • LDH	• Complete blood count • Peripheral blood smear • Prothrombin time • Transaminases • Cholestasis enzymes • Complete lipid profile • Protein electrophoresis • Glucose and glycosylated hemoglobin • LDH
Specific blood tests	• AFP • cryoglobulines • HCV-RNA (if the patient has risk factors for re-infection) • Screening for HBV (HBsAg/AcHBs, IgM and IgG HBc) or HIV (AcHIV) co-infections • FibroTest, APRI, FIB-4	• AFP • cryoglobulines • HCV-RNA (if the patient has risk factors for re-infection) • Screening for HBV (HBsAg/AcHBs, IgM and IgG HBc) or HIV (AcHIV) co-infections • FibroTest, APRI, FIB-4
Imaging methods	• Abdominal ultrasound • Depending on the AFP value: superior imaging methods (CT/MRI) • Elastography • Upper digestive endoscopy - Depending on the presence/absence of pre-treatment esophageal varices, the number of platelets and the degree of rigidity determined at TE	• Abdominal ultrasound • Depending on the AFP value: superior imaging methods (CT/MRI) • Elastography

Results from this doctoral research were the subject of 3 original articles, published in ISI and BDI magazines, with a cumulative impact factor of 4.

Articles published in ISI journals:

- **Leuştean, A**., Popescu, C., Nichita, L., Tilişcan, C. and Aramă, V., 2021. Dynamics of APRI and FIB-4 in HCV cirrhotic patients who achieved SVR after DAA therapy. *Experimental and Therapeutic Medicine*, *21*(1), pp.1-1. https://doi.org/10.3892/etm.2020.9531 (IF 2,4) original article, first author – Chapter 5; 8 citations. https://www.spandidos-publications.com/10.3892/etm.2020.9531
- **Leuştean, A.**, Olariu, MC., Mihai, N., Tiliscan, C., Molagic, V., Duport-Dodot, I., Stratan, LM., Arama SS., Arama, V., 2024. Regression of liver fibrosis in HCV cirrhotic patients treated with Interferon-free therapies. *Farmacia*, 72(4). https://doi.org/10.31925/farmacia.2024.4.10 (IF 2022: 1,6) original article, first author - Chapter 6. https://farmaciajournal.com/wp-content/uploads/art-10-Leustean_Olariu_Arama_826-831.pdf

Article published in CNCSIS B+ journal:

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