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EVOLUTION OF FIBROSIS, INFLAMMATION, STEATOSIS AND STEATOHEPATITIS IN HCV INFECTED PATIENTS AFTER TREATMENT WITH DIRECT- ACTING ANTIVIRAL THERAPY (A FIBROMAX® ANALYSIS)

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ABSTRACT

Aim: The aim of this study is to assess the evolution of Fibromax® parameters (fibrosis, inflammation, steatosis, steatohepatitis) in patients with chronic HCV hepatitis at one year after sustained virologic response (SVR) achieved by direct acting antiviral agents (DAA).

Methods: This is a retrospective observational trial including 73 patients with chronic HCV hepatitis, who obtained sustained virologic response under ombitasvir/paritaprevir/ritonavir and dasabuvir, ledipasvir/sofosbuvir or sofosbuvir/velpatasvir. All the patients were evaluated by Fibromax before the initiation of therapy and at 12 months after SVR.

Results and Conclusion: The study included 42 women (57.53%) and 31 men (42.46%), without significant difference in mean age or BMI. We found a higher prevalence of patients with minimal or moderate fibrosis (F1, F2), compared to patients with advanced fibrosis (F3) (63.01% versus 10.95%). At one year follow-up, we found an increased number of patients in the lower levels of fibrosis, inflammation and NASH, with a relatively constant distribution of patients regarding steatosis. Necro-inflammatory activity was significantly diminished, with 58 patients in the no to minimal activity, compared to 37 patients before antiviral therapy. More patients presented N0 and N1 degree of NASH at follow-up than before therapy (63 patients versus 42 patients, p= 0.02). The mean values of Fibrotest (p= 0.03), ActiTest (p<0.01) and NashTest (p=0.02) decreased significantly. The mean value of Steatotest also decreased, but without statistical significance (p=0.12).

Keywords: hepatitis C virus, hemodialysis, anemia, sustained virologic response (SVR)

INTRODUCTION

The global burden of cirrhosis and primary liver cancer, due mainly to chronic hepatitis C (CHC), is increasing and has reached 1.7 million related deaths a year worldwide [1]. The burden of CHC could be greatly reduced, given the emergence of highly effective direct-acting antiviral agents (DAAs) and the availability of effective non-invasive biomarkers for identifying patients with severe liver disease, who should be given priority for the use of these expensive DAAs [2].

Histological assessment of liver disease has been a cornerstone of therapeutic decision making and prognostication in chronic liver disease (CLD) for decades. Liver biopsy is still the established standard for assessment of injury, inflammation, and fibrosis stage, although its role in CLD states, such as chronic hepatitis C (CHC), has been significantly diminished in recent years. The advent of non-invasive approaches for assessment of liver fibrosis, combined with the more recent evolution of simplified direct-acting antiviral (DAA) therapeutic regimens, has now essentially eliminated the need for liver biopsy to differentiate mild from significant (≥F2) disease prior to antiviral therapy for CHC. These non-invasive approaches for assessment of liver fibrosis include various biochemical serum markers, or imaging modalities that provide a physical measure of liver stiffness [3]. There is now increased availability and greater acceptance of non-invasive tests (NITs) as an alternative to biopsy for diagnosis of advanced fibrosis and determination of prognosis in CLD. Current NITs certainly overcome the risks and sampling limitations associated with liver biopsy. However, as these tests become increasingly incorporated into routine clinical practice, there are diagnostic limitations that need to be considered when interpreting results.

Three simple blood tests were developed to provide an estimate of liver fibrosis and its aggravating factors of steatosis and nonalcoholic steatohepatitis, ie, the FibroTest™, SteatoTest™, and NashTest™, respectively. FibroMax™ (Biopredictive, Paris, France) combines five tests on the same result sheet and provides physicians with simultaneous and complete estimation of the liver injury associated with alcoholic or nonalcoholic fatty liver disease [4].

The aim of this study is to assess the evolution of Fibromax® parameters (fibrosis, inflammation, steatosis, steatohepatitis) in patients with chronic HCV hepatitis at one year after sustained virologic response (SVR) achieved by direct acting antiviral agents (DAA).

MATERIALS AND METHODS

This is a retrospective observational trial including 73 patients with chronic HCV hepatitis, (degrees F0 to F3 of fibrosis), who underwent direct acting antiviral therapy from January 2020 to January 2021, obtaining SVR. The therapeutic regimens used were: ombitasvir/paritaprevir/ritonavir and dasabuvir, ledipasvir/sofosbuvir and sofosbuvir/velpatasvir, taking into account that the predominant HCV genotype in the study population is 1b. The exclusion criteria of the study were: patients with compensated or decompensated cirrhosis, patients with HBV or HIV co-infection, patients with auto-immune or inflammatory conditions that may falsely alter test results, patients with obesity or diabetes mellitus, patients with a chronic alcohol use, patients with hematologic or solid malignancies. All the patients were evaluated by Fibromax before the initiation of therapy and at 12 months after SVR. Table 1 presents the cut-off limits used in the trial:

Table 1. Cut-off limits for Fibrotest, SteatoTest, ActiTest, NashTest

FibroTest	SteatoTest	ActiTest	NashTest
F0: 0.0-0.21	S0: 0.00- 0.03	A0: 0.00- 0.17	N0: 0.00-0.25
F0-F1: 0.21-0.27	S0-S1: 0.3- 0.38	A0-A1: 0.17- 0.29	
F1: 0.27-0.31	S1: 0.38-0.48	A1: 0.29- 0.36	N1: 0.25-0.50
F1-F2: 0.31-0.48	S1-S2: 0.48-0.57	A1-A2: 0.36-0.52	
F2: 0.48-0.58	S2: 0.57-0.67	A2: 0.52-0.6	N2: 0.50-0.75
F3: 0.58-0.72	S2-S3: 0.67-0.69	A2-A3: 0.3- 0.62	
F3-F4: 0.72-0.74	S3: 0.69-1.00	A3: 0.62-1.00	N3: 0.75-1
F4: 0.74-1.00			

F0 no fibrosis; F1, F1-F2 minimal fibrosis; F2 moderate fibrosis; F3, F3-F4 advanced fibrosis; F4 severe fibrosis (cirrhosis); S0, S0-S1 no steatosis; S1, S1-S2 mininal steatosis, S2, S2-S3 significant steatosis; S3 severe steatosis; A0, A0-A1 no activity; A1, A1-A2 minimal activity; A2, A2-A3 significant activity; A3 severe activity; N0 no NASH; N1 minimal NASH, N2 moderate NASH; N3 severe NASH.

Statistical analysis was performed using SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp)

RESULTS

The study included 42 women (57.53%) and 31 men (42.46%), without significant difference in mean age or BMI. We found a higher prevalence of patients with minimal or moderate fibrosis (F1, F2), compared to patients with advanced fibrosis (F3) (63.01% versus 10.95%). None of the patients had increased levels of glucose, total bilirubin, or gama-glutammyl transpeptidase (GGT). However, 42.46% of patients had increased values of alanin-aminotranspherase (ALT) or aspartate- aminotranspherase (AST). Mean values are presented in Table 2.

Table 2. Baseline characteristics and mean values of the study group

	Study group (N= 75)
Gender distribution	Male: 31 Female: 42
Mean age	43.56+/- 11.32 years Male: 41.28+/- 8.43 years Female: 44.27+/- 9.12 years
Mean BMI	25.65 +/- 3.18 kg/m^3 Male: 26.37 +/- 3.98 kg/m^3 Female: 25.01 +/- 2.81 kg/m^3
Alpha 1- macroglobulin N: 1.5-3.5 g/L	2.97 +/- 1/03
Haptoglobin N: 0.3-2 g/L	1.62 +/- 0.74
A1 apolipoprotein N: 1.04-2.02 g/L	1.26 +/- 0.52
Total bilirubin N: <17.1 µmol/L	10.4 +/- 4.2
GGT N: 8-61 U/L	32.8 +/- 11.6
ALT N: 5-41 U/L	38.1 +/- 19.4
AST N: 5-38 U/L	27.7 +/- 13.9
Glucose N: 3.33- 5.49 mmol/L	4.06 +/- 0.63
Cholesterol N: 3.11-5.18 mmol/L	4.62 +/- 1.18
Triglycerides N: 0.4-1.7 mmol/L	0.78 +/- 4.65
Degree of fibrosis (FibroTest)	Mean value: 0.23 +/- 0.10 F0: 9 patients F0- F1: 14 patients F1:12 patients F1-F2: 11 patients F2: 19 patients F3: 8 patients
Degree of necro-inflammatory activity (ActiTest)	Mean value: 0.48 +/- 0.21 A0: 4 patients A0-A1: 12 patients A1: 21 patients A1-A2: 29 patients A2: 6 patients A2-A3: 1 patient

Degree of steatosis (SteatoTest)	Mean value: 0.45 +/- 0.22 S0: 2 patients S0-S1: 13 patients S1: 18 patients S1-S2: 31 patients S2: 8 patients S2-S3: 1 patient
Degree of non-alcoholic steatohepatitis (NashTest)	Mean value: 0.64+/- 0.23 N0: 8 patients N1:38 patients N2:27 patients

At one year follow-up, we found an increased number of patients in the lower levels of fibrosis, inflammation and NASH, with a relatively constant distribution of patients regarding steatosis. More patients had minimal or no fibrosis (F0 to F1-F2, 58 patients versus 46 patients), with 5/8 patients remaining in the advanced fibrosis (F3) subgroup. Necro-inflammatory activity was significantly diminished, with 58 patients in the no to minimal activity, compared to 37 patients before antiviral therapy. Regarding steatosis, the patient with S2-S3 degree presented S2 degree at one year follow-up. The distribution of patients did not change significantly. More patients presented N0 and N1 degree of NASH at follow-up than before therapy (63 patients versus 42 patients, p= 0.02)

Furthermore, the mean values of Fibrotest (p= 0.03), ActiTest (p<0.01) and NashTest (p=0.02) decreased significantly. Also, the mean value of Steatotest also decreased, but without statistical significance (p=0.12) (Table 3).

Table 3. Evolution of Fibromax parameters at one year follow-up

	Fibrotest		ActiTest		SteatoTest		NashTest	
	Initial	1 year	Initial	1 year	Initial	1 year	Initial	1 year
Mean value	0.23 +/- 0.10	0.11 +/- 0.07	0.48 +/- 0.21	0.21 +/- 0.14	0.45 +/- 0.22	0.39 +/- 0.24	0.64+/- 0.23	0.44+/- 0.16
Number of patients in each subgroup	F0: 9 F0-F1:14 F1:12 F1-F2: 11 F2: 19 F3: 8	F0: 13 F0-F1:17 F1:16 F1-F2:12 F2: 10 F3: 5	A0: 4 A0-A1:12 A1: 21 A1-A2:29 A2: 6 A2-A3: 1	A0: 10 A0-A1:23 A1: 25 A1-A2:12 A2: 3 A2-A3: 0	S0: 2 S0-S1:13 S1: 18 S1-S2:31 S2: 8 S2-S3:1	S0: 6 S0-S1:16 S1: 21 S1-S2:24 S2: 6 S2-S3:0	N0: 8 N1:38 N2:27	N0: 20 N1:43 N2:10

DISCUSSION

The current opinion is that HCV chronic infection is not only a disease of the liver, but constitute an entire "HCV syndrome" affecting all organs and systems, by a systemic inflammatory state [5]. The mainstay of liver damage in HCV infection is the progression of inflammation to irreversible fibrosis, with microscopic and macroscopic structural damages and ultimately liver failure and hepatocellular carcinoma [6].

Current treatment guidelines recommend DAA therapy for all patients, regardless of the degree of fibrosis, as early as possible, in order to achieve SVR and prevent the progression of liver disease [7]. However, awareness towards the degree of liver fibrosis is highly important in establishing the follow-up and prognosis of patients. While liver biopsy remains the gold standard for assessing liver fibrosis, its disadvantages (small probe, invasiveness) reserves it for clinical trials [8]. New non-invasive tests may accurately assess liver damage, especially in combinations [9]. To this regard, the aim of Fibromax® is to analyze the degree of liver fibrosis and inflammation, as well as steatosis, and liver damage produced by alcoholic and non-alcoholic steatohepatitis [10].

Regression of liver fibrosis is a major desiderate in hepatology, high hopes having been raised after the development of DAA regimens for HCV chronic infection [11]. In previous studies, interferon-based regimens lead to a decrease in FibroTest scores of more than 20% [12, 13], the most important prognostic factor being achievement of SVR. Furthermore, in the DAA era, curing HCV infection has shown to lead to a decrease in liver related mortality and morbidity, even in patients with established cirrhosis [14].

In our trial, we found a significant reduction of FibroTest scores in all groups of patients. This comes to underline the importance of early diagnosis and treatment of HCV, in order to prevent the progression of liver disease. Moreover, a decrease in fibrosis score indicates lower requirements for follow-up, with important reductions in health costs at a national level [15].

HCV promotes liver inflammation by activation of stellate cells and recruitment of immune cells [16]. Chronic destruction of hepatocytes augments hypercytokinaemia and exacerbates the progression of liver disease [17]. Several systemic inflammatory markers have increased values in chronic HCV infection, including tumour necrosis factor- α, interleukins 6, 8, 18 [18]. A recent trial showed that after SVR (achieved by sofosbuvir-based regimens) the serum levels of inflammatory markers decreased significantly [19]. In our study, we found a decrease in overall levels of ActiTest®, as well as a decrease in the number of patients with higher levels and an increase in the number of patients without necro-inflammatory activity.

HCV chronic infection represents a powerful promoter for non-alcoholic steatohepatitis (NASH) [20]. A direct relationship has been established between NASH and HCV genotype 3, while in other cases the risk factors for NASH include insulin resistance and metabolic syndrome [21]. The use of DAAs and cure of HCV infection leads to a decrease in inflammatory processes in the liver, which in turn inhibits liver damage and steato-hepatitis. As such, in our study we found a decrease in NASHTest scores, potentially due to normalization of transaminases. Regarding steatosis, there was no significant difference between values before and after antiviral therapy. This is consistent with the results of a trial based of Fibroscan®, who found higher liver stifness values in patients with HCV associated steatosis than in patients without steatosis, after SVR [22-25]. This may indicate that the metabolic dysregulations induced by HCV may persist indefinitely after the cure of the infection.

In conclusion, our study shows that SVR after DAA in patients with chronic hepatitis improves fibrosis and inflammation scores, and may have a long-term impact on HCV associated liver steatosis.

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Conflict of interest: none

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