

PREDICTIVE FACTORS OF METABOLIC CHANGES AFTER INTERFERON-FREE TREATMENT IN HEPATITIS C INFECTED PATIENTS

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ABSTRACT

Chronic hepatitis C virus (HCV) infection is a systemic infection with multiple extrahepatic manifestations, including alterations in lipid and glucose metabolism. Interferon-free treatment reverses metabolic abnormalities through virus eradication, but is associated with significant elevation in serum cholesterol levels with further increased risk for diabetes and cardiovascular diseases. The aim of our study was to assess the correlation between baseline metabolic and hepatic features and changes in lipid and glucose levels after interferon-free treatment in HCV-infected patients.

Keywords: interferon free treatment, hepatic steatosis, NAFLD

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is still associated with an increased morbidity and mortality, with evolution towards hepatic cirrhosis, hepatocellular carcinoma and death (approximately 390,000 deaths reported annually) (1). HCV infection is a systemic infection, with multiple extrahepatic manifestations. Chronic HCV-infected patients often associates type 2 diabetes mellitus, but also an increased risk of atherogenesis and cardiovascular diseases (2). HCV increases insulin resistance by multiple direct (e.g. increase of SOC proteins expression, decrease expression of IRS1 proteins, up-regulation of protein phosphatase 2 A etc.) and extrahepatic (e.g. increase expression of TNF alpha, IL-8, IL-18, disturbance of glucose metabolism in adipose tissues and skeletal muscles etc.) mechanisms (3). HCV up-regulates athero-

genesis through lipid metabolism. HCV and lipid metabolism interaction is now very well described in literature. Endogenous lipids (total cholesterol, LDL-cholesterol, oxysterols – 24-,25-hydroxycholesterol) interfere cu viral lifecycle by a direct antiviral mechanism or by indirect immune-mediated pathways. Chronic HCV infection is frequently associated with hypolipemia and hepatic steatosis. HCV-infected patients have low levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol and lipoproteins, with no significant alterations of HDL-cholesterol and triglycerides (4,5). Genetic factors (e.g. increased expression of PNPLAS gene) or host factors (e.g. alcohol consumption, drugs, metabolic syndrome, insulin resistance) could be responsible for pathogenic mechanism of hepatic steatosis (accumulation of triglycerides within hepatocytes), which is met in over 55%

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chronic HCV infected patients (6). In predisposed patients, such as those with metabolic syndrome (defined by abdominal obesity, dyslipidemia, diabetes mellitus, hypertension), fat accumulation within hepatocytes leads to other hepatic disturbances, such as non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH). More often, NAFLD is an asymptomatic benign condition, but in some cases, it progress to NASH with known evolution to hepatocellular carcinoma and death (7). In addition, NAFLD may enhance the risks of diabetes mellitus, cardiovascular or renal diseases (8). Liver biopsy is the gold standard for NAFLD and hepatic steatosis diagnosis, but as an invasive method and it often associates multiple risks. Currently, other non-invasive methods (e.g. Fibromax, Fibroscan) offer accurate and predictive information, being often used in current diagnosis schemes (9). With an important therapeutic success and a favourable safety profile, even in elderly patients, novel direct acting antiviral agents (DAAs) represent currently a major step in HCV eradication (10). However, recent data revealed that interferon-free treatment produces important metabolic effects (2,8). We evaluated in our study the impact on lipid and glucose profiles of different interferon-free regimens approved for HCV treatment in our country. The association of baseline metabolic and hepatic characteristics with changes in lipid and glucose levels after DAAs treatment was also assessed.

MATERIAL AND METHODS

We included in our study HCV infected patients who had addressed to “Sf. Parascheva” Infectious Diseases Hospital, Iasi, in the period 2018-2019 and were eligible for interferon-free therapy according to national protocol and included in the program reimbursed by National Health Insurance House (11). Treatment naïve or experienced patients with mild, moderate or severe liver fibrosis were eligible for different approved interferon-free treatment regimens. Patients with concomitant diseases which contraindicated previous administration of interferon (depression, immune diseases, uncontrolled type 1 diabetes mellitus etc.) were also included. Patients with decompensated HCV cirrhosis (defined as presence of ascitis, icterus, gastrointestinal bleeding, Child-Pugh 2-3) or dif-

ferent types of cancer were excluded. Concomitant treatments known to interact with antiviral agents (e.g. benzodiazepines, statins, amiodarone, carbamazepine etc.) were also excluded.

Patients included received one of the following DAAs regimens:

- PrOD (25 mg ombitasvir + 150 mg paritaprevir + 100 mg ritonavir in combination with 500 mg dasabuvir, twice daily, for up to 12 weeks in naïve patients with comorbidities or experienced patients);
- SOF/LED (400 mg sofosbuvir and 90 mg ledipasvir, once daily, for up to 12 weeks in naïve patients with comorbidities or experienced patients)
- ELB/GLA (50 mg elbasvir and 100 mg grazoprevir, once daily, for 12 weeks in all categories of patients).

Patients were evaluated at baseline, at the end of therapy (EOT) and 12 weeks after treatment completion to evaluate sustained virologic response (SVR visit), defined as HCV RNA below the limit of detection. Data collected at baseline were demographics, medical and medication history. Blood tests included full blood count, INR, ALT, AST, bilirubin, creatinine, alpha-fetoprotein). In addition, lipid profile (total cholesterol and triglycerides) was assessed at baseline and SVR visit. Hepatitis C RNA was measured at baseline and SVR visit. Metavir score was evaluated based on Fibromax test or liver biopsy. Fibromax is a combination of up to five non-invasive liver tests (namely FibroTest[®], Acti Test[®], SteatoTest[®], NASHTest[®] and AshTest[®]) used to diagnose hepatic fibrosis, viral necro-inflammatory activity, hepatic steatosis, severe alcoholic steatohepatitis (in alcoholic patients) and non-alcoholic steatohepatitis (in overweight, diabetic or insulin resistant patients). Fibromax score is calculated using an algorithm based on the patients' gender, age, weight, height and specific blood biomarkers (ALT, AST, GGT, haptoglobin, blood glucose, lipids, apolipoprotein A1, alpha 1-macroglobulin (9, 12).

STATISTICAL ANALYSIS

Continuous data are expressed as means \pm standard deviation. Differences between lipid profiles, glucose levels, metabolic and hepatic factors at baseline and post-treatment were compared by Stu-

dent t-test. Pearson correlation coefficients were used for correlation between different variables. A p value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using XL-STAT software version 2018.

RESULTS

Patients' demographics

We included in our study 131 HCV infected patients. One patient died from stroke during interferon-free treatment. A total of 105 patients with complete dataset were included in our analysis. From patients analyzed, 62.16% received PrOD, 22.52% SOF/LED and 16.21% received ELB/GRA. Demographic data and baseline characteristics are presented in Table 1.

Hepatic steatosis (based on Steato Test results) correlated with fibrosis and NASH Test results are

presented in Tables 2 and 3. If in 31.43% patients without steatosis, the degree of NASH grade was 0 and in 19.04% cases without steatosis had grade 2 (moderate) fibrosis.

TABLE 2. Contingency of NASH grades and fibrosis

		none	mild	Fibro Test		very severe	Total
				moderate	severe		
NASH TEST	none	8	10	27	6	1	52
	mild	5	5	16	4	6	36
	moderate	1	0	7	3	6	17

TABLE 3. Contingency of NASH grades and steatosis

		none	Steato Test		severe	Total
			mild	moderate		
NASH TEST	none	33	14	5	0	52
	mild	1	17	16	2	36
	moderate	0	3	8	6	17

TABLE 1. Demographic and baseline characteristics by treatment group

Characteristics	PrOD N = 63	SOF/LED N = 17	ELB/GRA N = 25
Gender, male (%)	35.82	23.53	20
Age (years) (mean \pm SD, CV%)	57.91 \pm 12.16 (21)	58.76 \pm 7.66 (13.03)	60.88 \pm 8.46 (13.9)
BMI (kg/m ²) (mean \pm SD, CV%)	26.18 \pm 4.30 (16.42)	28.40 \pm 5.33 (18.76)	27.27 \pm 4.84 (17.75)
Comorbidities (%)			
Diabetes mellitus	56.72	58.82	52.0
Arterial hypertension	17.91	11.76	12
Antiviral treatment history (%)			
Naive	74.62	64.71	68.0
Non-responder interferon therapy	10.45	23.53	12.0
Relapse after interferon therapy	13.43	11.76	20.0
Interferon therapy discontinuation for safety reason	1.49	-	-
Creatinine (mg/dl) (mean \pm SD, CV%)	0.89 \pm 0.14 (15.77)	0.87 \pm 0.13 (15.17)	0.80 \pm 0.10 (12.53)
Urea (mg/dl) (mean \pm SD, CV%)	35.48 \pm 12.24 (39.52)	32.41 \pm 16.23 (50.07)	31.27 \pm 5.91 (18.90)
ALT (IU/l) (mean \pm SD, CV%)	86.0 \pm 59.07 (68.68)	87.82 \pm 79.63 (90.68)	91.78 \pm 77.27 (84.19)
AST (IU/l) (mean \pm SD, CV%)	60.7 \pm 43.41 (71.51)	53.11 \pm 26.16 (49.25)	71.56 \pm 63.39 (88.58)
Gama GT (IU/l) (mean \pm SD, CV%)	60.69 \pm 53.68 (88.46)	57.53 \pm 31.36 (54.51)	50.25 \pm 35.01 (69.67)
Cholesterol (mg/dl) (mean \pm SD, CV%)	162.45 \pm 33.58 (20.67)	171.23 \pm 57.63 (33.66)	161.81 \pm 48.72 (30.11)
Triglycerides (mg/dl) (mean \pm SD, CV%)	97.19 \pm 40.86 (88.46)	105.51 \pm 4.35 (40.14)	89.61 \pm 41.72 (46.56)
Blood glucose (mg/dl) (mean \pm SD, CV%)	127.85 \pm 50.53 (39.52)	126.4 \pm 54.52 (43.14)	104.52 \pm 14.32 (13.70)
Systolic blood pressure (mmHg), median (range)	145 (120-200)	152.5 (110-180)	135 (125-170)
Diastolic blood pressure (mmHg), median (range)	85 (70-110)	90 (60-100)	85 (70-100)

Changes in lipid profile by treatment group

The levels of total cholesterol were increased up to 92% with the highest elevation seen in the PrOD regimen. All three interferon-free treatment regimens produced a statistically rise in serum cholesterol (Table 4).

Triglycerides profile revealed a high variability between patients with decrease as low as 57% or increase up to 150%. The mean increase of serum triglycerides in all three groups was not significant (Table 4).

Changes in fasting blood glucose by treatment group

Fasting blood glucose levels had a high variability between patients with mean increase of 3.37 mg/dl. Changes in glucose levels were not significant regardless of treatment group (Table 4).

Factors affecting lipid and glucose levels

A significant correlation between serum levels of total cholesterol, triglycerides or blood glucose measured at SVR visit and hepatic impairment (steatosis and fibrosis intensities, as revealed by Steato Test, NASH Test and Fibro Test) was found for all three antiviral treatments (Table 5).

Overall, a strong treatment-dependent correlation was noted between cholesterol levels and hepatic steatosis and fibrosis intensity. SOF/LED therapy was significantly correlated with hepatic steatosis ($p = 0.039$) and non-alcoholic steatohepatitis ($p = 0.072$). Weaker, but statistically signifi-

cant correlation was noted for PrOD regimen and intensity of fibrosis ($p = 0.012$), steatosis ($p = 0.003$) and NASH ($p = 0.026$) (Figure 1). Serum triglycerides levels were significantly correlated with body mass index (especially in SOF/LED-treated group, $p = 0.012$, and in a lesser extent for PrOD, $p = 0.04$), with hepatic steatosis (SOF/LED-treated group, $p = 0.024$) and NASH (especially in SOF/LED-treated group, $p = 0.045$, and in a lesser extent for PrOD regimen, $p = 0.039$) (Figure 1). Fasting blood glucose was significantly correlated steatosis (especially in SOF/LED-treated group, $p = 0.045$, and in a lesser extent for PrOD regimen, $p = 0.004$) and NASH (in SOF/LED-treated group, $p = 0.014$, and in a lesser extent for PrOD-treated group, $p = 0.002$). Weaker, but significant association was found also between glycemia and comorbidities (for PrOD group, $p = 0.02$) and baseline ALT (in PrOD group, $p = 0.02$). In ELB/GRA treated-group, we have found a moderate, but significant positive correlation of serum triglyceride and blood glucose level and hepatic fibrosis ($p = 0.014$ and 0.016 , respectively) (Figure 1)

DISCUSSIONS

Hepatic steatosis, which frequently coexists with necro-inflammatory and fibrotic changes, is a relatively common feature of chronic hepatitis C infection, as result of combination between viral factors and different host factors (mainly metabolic syndrome) (8). Hepatic steatosis associated with

TABLE 4. Changes in lipids and metabolic parameters at baseline and SVR (*t*-Student test)

	PrOD		SOF/LED		ELB/GRA	
	p	IC95%	p	IC95%	p	IC95%
Total cholesterol	< 0.0001	[-83.876, -49.237]	0.036	[-94.560, -3.530]	< 0.0001	[-79.834, -33.978]
Triglycerides	0.077	[-34.472, 1.809]	0.216	[-94.143, 22.406]	0.282	[-33.438, 9.906]
Fasting blood glucose	0.815	[-22.482, 17.740]	0.239	[-86.824, 22.874]	0.874	[-12.020, 10.247]

TABLE 5. Associated factors of total cholesterol, triglycerides and blood glucose

	Total cholesterol		Triglycerides		Blood glucose	
	Pearson's r	p	Pearson's r	p	Pearson's r	p
Age	-0.069	0.6118	0.039	0.7776	0.143	0.2922
BMI	-0.180	0.1833	0.409	0.0017	0.195	0.1501
Comorbidities	-0.078	0.5691	0.165	0.2236	0.340	0.0104
Fibro Test	-0.332	0.0125	-0.105	0.4432	0.158	0.2452
Steato Test	-0.301	0.0243	0.339	0.0106	0.497	< 0.0001
NASH Test	-0.206	0.1276	0.374	0.0045	0.489	0.0001
ALT	-0.100	0.4649	-0.129	0.344	0.213	0.1158
AST	-0.119	0.3821	-0.162	0.2329	0.157	0.2482

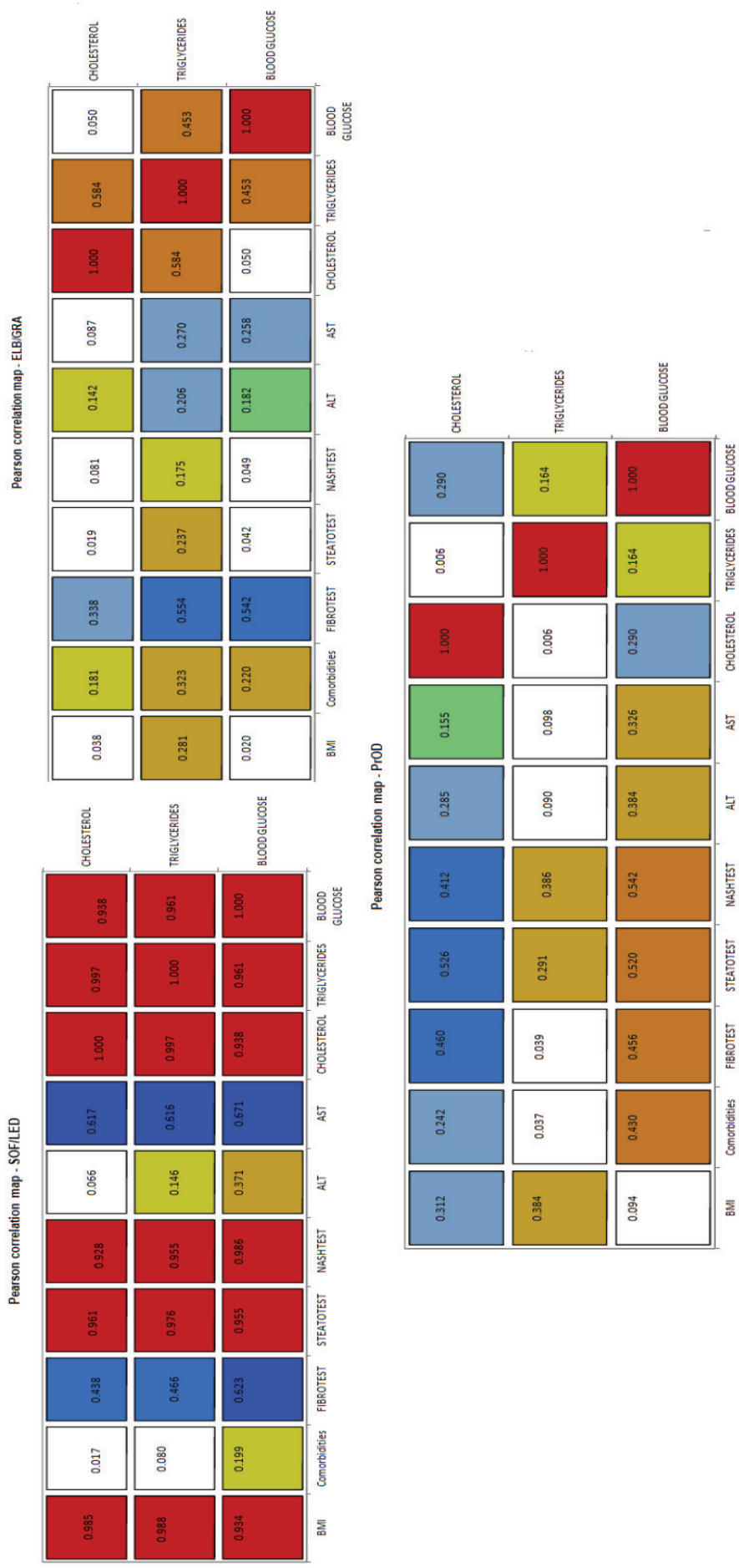


FIGURE 1. Correlation maps between variables according to treatment

viral infections (e.g. HVC, HIV) was named “virus-associated fatty liver disease” (VAFLD) and has a prevalence of almost 55% in HCV infected patients, but the prevalence is significantly increased up to 85% in patients with HCV and HIV coinfection (13,14). In our retrospective study carried out on 105 HCV infected patients, liver steatosis was absent in 31.42% cases and was mild in 16.19% of them, as estimated by Fibromax Test. Hepatic steatosis accelerates hepatic fibrosis progression in HCV-infected patients to hepatic cirrhosis and may have an important contribution for development of hepatocellular carcinoma in these patients (15). Moreover, both NAFLD and VAFLD are associated with a significant risk of cardiovascular diseases and diabetes mellitus (16,17). In addition, a strong correlation has been found between the severity of hepatic steatosis and PrOD-induced therapeutic response in HCV infected patients, as regard levels of hepatic cytolysis enzymes and GGT (17).

On the other side, metabolic disturbances (such as hypolipidemia or increase of insulin resistance) are specific to HCV infection and successful viral eradication is associated with a reversal of these conditions (18). In line with previous observations, our study confirms the significant associations between interferon-free treatment in HCV infection and metabolic abnormalities. Most of the DAAs agents used for HCV infection treatment increased total cholesterol and LDL-cholesterol levels in clinical trials, irrespective of the agents used. However, different results were obtained for HDL-cholesterol levels (e.g. significantly increased during and after treatment, not affected or decreased).

Cancellation of the suppressive effect of chronic HCV infection on lipid metabolism was associated with an increase of total cholesterol up to 20 mg/dl (19). This increase was similar in patients who achieved SVR after interferon-based therapy and also after different interferon-free regimens (2,19-25). Other authors noted that the elevation of cholesterol levels was dependent of the DAAs treatment, suggesting a direct pharmacological action of the antiviral therapy on lipid metabolism (19). In our study, all elevated serum cholesterol levels were obtained after all three interferon-free therapeutic regimens: 34.97% (PrOD), 32.52% (ELB/GRA) and 25.75% (SOF/LED). Triglyceride levels

are decreased in patients who achieved SVR after interferon-free treatment (2, 20). Our results are somehow different from previous results. We have found a non-significant increase in triglycerides levels in all three regimens, with an average of 23.83% (CV = 181%) in PrOD-treated group, 26.36% (CV = 171.3%) in SOF/LED-treated group and 12.83% (CV = 42%) in ELB/GRA-treated group. However, we consider this result a bias due to the small sample size and the high inter-individual variability. In our study, we could not find a significant difference between baseline and post-treatment fasting blood glucose, also most probably due to the small sample size of each treatment group. Assessing the correlation between post-treatment metabolic parameters (cholesterol, triglycerides and blood glucose levels) and baseline host factors (liver injury), we have found a treatment-dependent relationship. Severity of liver injury, as revealed by the results of Steato Test and NASH Test, were significantly correlated with lipids alterations and the strongest correlation was obtained for SOF/LED (Pearson correlation test = 0.96 for cholesterol, 0.976 for triglycerides and 0.955 for glucose).

So far, most of the trials performed with DAAs evaluated and demonstrated therapeutic success achieved by interferon-free regimens on viral eradication and reduction of fibrosis severity. Increasingly more recent studies are focused on the metabolic effects of interferon-free therapies, but data about their impact on steatosis and steatohepatitis are still limited. Some authors reported a decreased of severity of steatosis after DAAs treatment, but the effect was dependent of different host factors, such as obesity or low HDL-cholesterol (26).

On other side, lipids alterations (total cholesterol, LDL-/HDL-cholesterol and triglycerides) are predictive factors for NAFLD and hepatic steatosis with important cardiovascular risk and atherogenesis, but also for possible evolution to HCC (27). For now, liver steatosis is a suspected adverse drug reaction noted in all international safety databases for all three interferon-free regimens assessed also in our study, but a clear causality relationship is not available. Thus, it cannot be estimated if these reports are cases of drug-induced steatosis/steatohepatitis (DIS/DISH) or otherwise host-related (28).

The major limitation of our study was the small sized single-center analysis. Further extended stud-

ies will be performed to find a possible correlation between DAAs and liver steatosis.

CONCLUSION

HCV eradication by different DAAs regimens was associated with a significant increase of total

serum cholesterol in a manner dependent of treatment and liver impairment factors (steatosis/steatohepatitis).

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