

## Hepatitis C Eradication With Direct Acting Antivirals Leads to Significant Increase in Serum Cholesterol Levels

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### ABSTRACT

**INTRODUCTION:** Patients with chronic hepatitis C have low serological levels of lipoproteins, low-density lipoprotein cholesterol and total cholesterol. However, chronic hepatitis C is known to increase cardiovascular risk. Lipid dynamics after treatment with direct acting antivirals remains unclear, but an overall decrease in cardiovascular mortality is expected after hepatitis C virus clearance.

**AIMS AND METHODS:** In this unicentric retrospective cohort study we evaluated lipid profile behaviour after virus clearance with direct acting antivirals. 100 patients were included, excluding patients with known hypercholesterolemia or previously treated with statins. Lipid profile was assessed before, at the end-of-treatment and one year after end-of-treatment.

**RESULTS:** Overall, there was a significant increase in low-density

lipoprotein cholesterol (baseline  $87.6 \pm 37.6$  mg/dL, end-of-treatment  $111.1 \pm 44.2$  mg/dL,  $p$ -value  $< 0.01$ ) and total cholesterol (baseline  $159.7 \pm 41.7$  mg/dL, end-of-treatment  $187.4 \pm 46.0$  mg/dL,  $p$ -value  $< 0.01$ ) at the end-of-treatment. Changes in low-density lipoprotein cholesterol and total cholesterol from end-of-treatment to 1 year after end-of-treatment were minimal. There were no significant changes in high-density lipoprotein cholesterol and in triglycerides. The number of patients with low-density lipoprotein cholesterol  $> 100$ mg/dL almost duplicated at the end-of-treatment (32.4% to 62.3%). Potential positive predictive factors for a significant low-density lipoprotein cholesterol increase were liver cirrhosis and low baseline low-density lipoprotein cholesterol levels.

**CONCLUSION:** This study suggests that measurement of lipid profile upon completion of treatment with direct acting antivirals is essential to prevent the underdiagnosis of dyslipidemia and avoid hindering cardiovascular risk improvement after treatment.

**Key words:** Hepatitis C; Direct acting antivirals; Cholesterol; Low-density lipoprotein; Dyslipidemia

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### INTRODUCTION

Chronic hepatitis C (CHC) has a global prevalence estimated on 1% in 2015 by the World Health Organization<sup>[1]</sup> and is a leading cause of cirrhosis, hepatocellular carcinoma (HCC), and liver transplant<sup>[2]</sup>. There is also evidence that hepatitis C virus (HCV) induces steatosis and multiple metabolic alterations, including hyperuricemia, hypozincemia, reversible hypocholesterolemia, and insulin resistance<sup>[3]</sup>. Although patients with CHC have low serological levels of lipoproteins, low-density lipoprotein cholesterol (LDL-C) and total cholesterol, an increased lipid accumulation in the liver and blood

vessels has been found<sup>[4]</sup>.

HCV influences host lipid metabolism by using host lipids in its replication cycle. At least three mechanisms influencing cholesterol and lipogenesis pathways in hepatocytes are known: (1) enhanced lipogenesis by upregulating de novo synthesis of fatty acids and cholesterol; (2) impaired lipoprotein degradation through mitochondrial lipid  $\beta$ -oxidation; and (3) impaired lipoprotein export from the liver, by reducing microsomal triglyceride transfer protein. Hepatic steatosis arises from these conditions<sup>[4,5]</sup>.

Despite the favorable lipid profile in CHC, many studies have shown that HCV infection can contribute to accelerated atherosclerosis and increase the risk of cardiovascular and cerebrovascular accidents<sup>[2,4]</sup>. Patients with CHC have higher hypertension rates, prevalence of chronic kidney disease, stroke and ischemic heart disease, increased intima-media thickness and higher rates of congestive heart failure and carotid atherosclerosis. This increased cardiovascular risk results not only from virus-induced metabolic modifications, but also by direct viral invasion of cardiovascular tissues and systemic inflammation<sup>[5,6]</sup>. It has been proved that HCV eradication significantly improves cardiovascular risk, mainly by reducing the incidence of metabolic syndrome and its complications, such as chronic renal disease and coronary heart disease<sup>[8]</sup>.

Treatment of CHC has advanced dramatically in recent years with direct acting antivirals (DAAs), resulting in shorter treatment duration, improved safety profile, higher tolerance, and higher sustained viral response (SVR) rates<sup>[2]</sup>. However, HCV clearance by both peginterferon-based therapy and DAAs increases total cholesterol and LDL-C in the blood. In some cases, with the reversal of hypolipidemia, lipid levels may increase to levels associated with increased cardiovascular risk<sup>[2,3]</sup>.

According to European guidelines, LDL-C remains the primary target of therapy in most strategies of dyslipidemia management. It can be directly determined in the blood or calculated using Friedewald's formula, which uses triglycerides (TG), total cholesterol and high-density lipoprotein cholesterol (HDL-C) values. LDL-C targets depend on patient's overall cardiovascular risk, being < 70 mg/dL, < 100 mg/dL and < 115 mg/dL, for those with very high, high and moderate cardiovascular risk, respectively<sup>[7]</sup>.

Although it has been demonstrated that a significant increase occurs in C-LDL from baseline to end of treatment (EOT) with DAAs<sup>[2,3,9,10]</sup>, studies with longer follow-ups and determining which patients have a higher risk of developing hypercholesterolemia after DAAs are lacking. Moreover, contrasting results concerning TG profile before and after HCV clearance were described<sup>[2,3]</sup>.

We hypothesize that lipid profile will significantly change after HCV clearance by DAAs, prompting the need for a closer monitoring and lipid-lowering medication in some patients, to avoid hindering cardiovascular risk improvement after HCV clearance. In this retrospective cohort study, we assessed lipid profile before, at EOT and one year after EOT with DAAs. We also evaluated some potential risk factors for dyslipidemia after HCV clearance.

## METHODS

### Patients and data collection

A total of 443 patients were treated with DAAs for chronic HCV infection between February 2015 and June 2018 in the Gastroenterology department of our hospital. Electronic records were reviewed to look for patients with a lipid panel before and after therapy. Patients administered a lipid-lowering agent during

the study period, patients who did not achieve SVR, patients with other liver diseases, and patients co-infected with hepatitis B virus or human immunodeficiency virus were excluded. From patients without exclusion criteria, 100 patients had measurements of total cholesterol levels before and at the EOT, and 49 of these patients also had total cholesterol measurements one year after EOT. Concerning C-LDL, 53 patients had measurements at baseline and at EOT, and 28 of these patients also had C-LDL measurements one year after EOT.

Demographic and clinical information was collected for all patients, including sex, age, body mass index (BMI), presence of hepatic steatosis, liver stiffness score, smoking status, arterial hypertension and diabetes diagnosis. HCV genotype, DAA regimen and SVR at week 12 were also recorded.

Arterial hypertension was defined as systolic blood pressure  $\geq$  140 mmHg, diastolic blood pressure  $\geq$  90 mmHg, or taking antihypertensive drugs. Diabetes was defined as hemoglobin A1c  $\geq$  6.5% or taking anti-diabetic medication. Overweigh was defined as BMI  $\geq$  25 kg/m<sup>2</sup>. Hepatic steatosis was assessed by either an abdominal ultrasound or a controlled attenuation parameter (CAP) compatible with liver steatosis. Liver stiffness was assessed by FibroScan® and classified from F0-F1 (< 7 Kpa, absent to mild fibrosis) to F4 (> 13 Kpa, cirrhosis). Total cholesterol, HDL-C and TG were directly measured and LDL-C was determined by Friedewald's formula. We considered a LDL-C cutoff value of 100 mg/dl to differentiate patients with increased LDL-C, based on European guidelines.

The following antiviral therapies against HCV were used, with or without concomitant ribavirin: sofosbuvir/ribavirin (SOF/RBV), sofosbuvir/ledipasvir (SOF/LED), sofosbuvir/daclatasvir (SOF/DAC), ombitasvir/ paritaprevir/ritonavir  $\pm$  dasabuvir, sofosbuvir/velpatasvir (SOF/VEL), or glecaprevir/pibrentasvir (GLEC/PIBR), depending on HCV genotype, liver fibrosis, and according to the available drugs/vigent guidelines at the time.

### Statistical analysis

Continuous data were reported as means  $\pm$  SDs. Paired t-test was used to compare means of variables measured before and after HCV treatment, including the change in total cholesterol, C-LDL, C-HDL and TG. A probability value (p-value) of less than 0.05 was considered statistically significant. All data manipulation and statistical analysis were performed using Stata™ software (Version 14; Stata Corp, College Station, TX).

## RESULTS

### Baseline characteristics

A total of 100 CHC treated patients met the inclusion criteria. The baseline characteristics of enrolled patients are shown in Table 1. Most patients were infected with HCV genotypes 1 (73%) and 3 (17%). Liver cirrhosis, assessed by FibroScan®, was present in 43% of patients. Mean LDL-C pre-treatment was 87.6 mg/dl, with only 32.4% of patients having LDL-C > 100 mg/dl before HCV eradication. The most frequent DAA regimen was SOF/LED (77%) and 31% were concomitantly treated with RBV.

At baseline, mean total cholesterol levels were significantly lower ( $p$ -value = 0.04) in patients infected with HCV genotype 3 ( $140.8 \pm 34.9$  mg/dL) than those infected with other genotypes ( $163.6 \pm 42.1$  mg/dL). Although baseline LDL-C was lower in genotype 3 ( $80.5 \pm 29.0$  mg/dL) than in other genotypes ( $89.9 \pm 38.7$  mg/dL), this difference is not statistically significant ( $p$ -value = 0.40).

**Cholesterol**

Overall, there was a significant increase in total cholesterol (baseline 159.7 ± 41.7 mg/dL, EOT 187.4 ± 46.0 mg/dL, *p*-value < 0.01) and LDL-C (baseline 87.6 ± 37.6 mg/dL, EOT 111.1 ± 44.2 mg/dL, *p*-value < 0.01) at the EOT. There were no significant changes in HDL-C (baseline 48.0 ± 18.6 mg/dL, EOT 49.7 ± 17.5 mg/dL, *p*-value = 0.43).

Increases in total cholesterol (baseline 155.2 ± 37.0 mg/dL, 1 year 186.3 ± 41.0 mg/dL, *p* < 0.01) and LDL-C (baseline 87.5 ± 34.9 mg/dL, 1 year 110.6 ± 39.2 mg/dL, *p* < 0.01) from baseline to 1 year after EOT were also significant. Changes in total cholesterol (EOT 179.8 ± 50.9 mg/dL, 1 year 186.3 ± 41.0 mg/dL, *p*-value=0.14) and LDL-C (EOT 106.1 ± 47.7 mg/dL, 1 year 115.6 ± 36.5 mg/dL, *p*-value = 0.09) from EOT to 1 year after EOT were minimal. Figure 1 evidences these effects of HCV eradication on serum lipids values. The number of patients with LDL-C > 100mg/dL has almost duplicated at the EOT (32.4% to 62.3%).

There were no significant changes in HDL-C at the EOT (baseline 48.0 ± 18.6 mg/dL, EOT 49.7 ± 17.5 mg/dL, *p*-value=0.43) and 1 year after EOT (baseline 48.0 ± 18.6 mg/dL, EOT 49.7 ± 17.5 mg/dL, *p*-value=0.43).

**Triglycerides**

There were no significant changes in TG at the EOT (baseline 115.1 ± 71.5 mg/dL, EOT 103.7 ± 53.0 mg/dL, *p*-value=0.12) and 1 year after EOT (baseline 112.6 ± 49.8 mg/dL, EOT 116.6 ± 53.8 mg/dL, *p*-value=0.68).

**Potential risk factors for significant increase in LDL-C**

Analyses of potential risk factors for a significant increase in LDL-C from baseline to EOT are shown in Table 2. Lower baseline LDL-C and presence of liver cirrhosis were considered risk factors for a significant increase in LDL-C at the EOT. HCV genotype 1, absence of diabetes and absence of liver steatosis were also associated with a significant increase in LDL-C at the EOT.

**DISCUSSION**

**Cholesterol and triglycerides**

We found a significant increase in LDL-C and total cholesterol at the EOT. Moreover, we observed that increased values in LDL-C and total cholesterol at the EOT will predict these values 1 year after treatment. Concerning TG and HDL-C, we found no significant changes with HCV treatment.

Our study is one of the first to assess lipid profile for a follow-up period longer than the EOT. Based on our results, serum cholesterol and LDL-C levels increase with HCV treatment and remain stable within the first year.

Because HCV infection increases cardiovascular risk and HCV eradication significantly reduces this increased risk<sup>[2,4,5,6,8]</sup>,

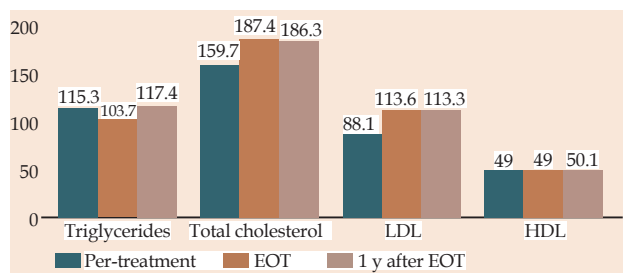


Figure 1 Effects of hepatitis C eradication on lipids.

Table 1 Baseline patient characteristics (n= 100).

Male (n=71)	71%
Mean age ± SD	51.0 ± 8.6
<b>HCV genotype</b>	
1 (n=73)	73%
2 (n=2)	2%
3 (n=17)	17%
4 (n=8)	8%
Hepatic steatosis (n=19)	27.1% †
Genotype 1 (n=15)	29.4% †
Genotype 3 (n=3)	25% †
<b>Liver stiffness</b>	
F0-F1 (n=36)	36%
F2 (n=12)	12%
F3 (n=9)	9%
F4 (n=43)	43%
Diabetes (n=7)	7%
Hypertension (n=25)	25%
Overweight (n=46)	59.7% †
Smoker (n=57)	57%
Mean LDL-C pre-treatment	87.6 ± 37.6 mg/dl
LDL-C >100mg/dl pre-treatment (n=24)	32.4% †
Mean ALT pre-treatment	98.3 ± 60.6 U/L

† Not all patients have records.

Table 2 Patients characteristics and LDL-C changes

	Baseline LDL-C (mg/dL)	EOT LDL-C (mg/dL)	p value
<b>Sex</b>			
Female (n=18)	82.3 ± 33.7	114.5 ± 46.4	0.03
Male (n=35)	90.6 ± 39.7	109.7 ± 43.6	< 0.01
<b>Hepatic steatosis†</b>			
Present (n=10)	94.2 ± 31.0	96.2 ± 55.6	0.9
Absent (n=26)	97.7 ± 40.5	114.7 ± 39.3	0.01
<b>Baseline LDL-C</b>			
> 100 mg/dl (n=16)	130.4 ± 31.4	132.6 ± 42.2	0.8
≤ 100 mg/dl (n=37)	69.1 ± 21.6	102.0 ± 42.2	< 0.01
<b>BMI†</b>			
≥ 25 kg/m <sup>2</sup> (n=26)	90.9 ± 36.7	110.6 ± 46.3	0.03
< 25 kg/m <sup>2</sup> (n=16)	91.1 ± 47.0	122.6 ± 47.5	0.02
<b>HCV genotype</b>			
1 (n=35)	87.4 ± 40.2	113.3 ± 48.5	< 0.01
2 (n=2)	101.1 ± 24.5	115.3 ± 6.1	0.62
3 (n=10)	82.1 ± 28.7	95.8 ± 30.1	0.29
4 (n=6)	93.5 ± 44.0	123.8 ± 44.4	0.31
<b>Liver stiffness</b>			
F0-F1 (n=15)	101.7 ± 40.7	117.3 ± 27.1	0.07
F2 (n=8)	99.5 ± 47.4	112.5 ± 71.7	0.49
F3 (n=7)	94.2 ± 31.7	111.4 ± 42.6	0.36
F4 (n=23)	72.3 ± 29.3	106.8 ± 44.3	< 0.01
Diabetes (n=3)	79.9 ± 36.8	117.5 ± 41.7	0.38
No diabetes (n=50)	88.1 ± 38.0	110.9 ± 44.7	< 0.01
Hypertension (n=8) †	78.8 ± 24.2	118.2 ± 18.0	0.01
No hypertension (n=22) †	90.7 ± 44.5	107.9 ± 44.5	< 0.01
Smoker (n=28)	83.3 ± 38.8	110.3 ± 44.6	< 0.01
Non-smoker (n=25)	92.5 ± 36.4	112.3 ± 44.5	0.02

† Not all patients have records

measurement of lipid profile upon completion of DAAs treatment is essential to prevent the underdiagnosis of dyslipidemia to avoid hindering cardiovascular risk improvement after HCV clearance.

#### Potential risk factors for significant increase in LDL-C

Positive predictive factors for a significant LDL-C increase with HCV treatment were liver cirrhosis (baseline  $99.3 \pm 39.4$  mg/dL, EOT  $114.7 \pm 44.7$  mg/dL,  $p$ -value = 0.04) and baseline LDL-C levels  $\leq 100$  mg/dL (baseline  $69.1 \pm 21.6$  mg/dL, EOT  $102.0 \pm 42.2$  mg/dL,  $p$ -value < 0.01). With these results we infer that the worse the liver disease, the less likely it is to balance the lipid profile after HCV eradication.

Although absence of liver steatosis was also associated with a significant increase in LDL-C, we believe our findings are probably due to missing data (30% of patients had no data concerning the steatosis status before HCV treatment). Furthermore, liver steatosis was mainly assessed by ultrasound, which is an insensible method. Although liver biopsy is the gold standard, it is an invasive procedure with risks. Therefore, in a future prospective study, liver steatosis could be assessed by CAP before and after HCV treatment, to improve our knowledge regarding steatosis and the risk of rebound hypercholesterolemia after virus eradication.

When comparing the most frequent genotypes, 1 and 3, it seems that the increase in LDL-C with HCV eradication is higher in genotype 3. This is in accordance with higher LDL-C increase in non-steatosis group, since in our cohort we had more liver steatosis in genotype 1 than genotype 3. However, it is widely known that genotype 3 is associated with a higher degree of steatosis occurrence<sup>[4,5]</sup>. The main methodology used for liver steatosis assessment was not the most suited, as abovementioned. Moreover, the pool of patients infected with genotypes 2 to 4 is insufficient to adequately assess which HCV genotypes are more related to hypercholesterolemia after HCV eradication. Therefore, we believe further studies evaluating the association between LDL-C increases after DAAs with HCV genotypes are required before taking any conclusion.

Apart from missing data concerning liver steatosis, BMI was also missing in 23 patients. This is also true for diabetes, since we only had 3 diabetic patients in our study. Consequently, no inferences should be made by regarding obesity and diabetes as predictive factors for a significant LDL-C increase with HCV treatment.

#### Other limitations

Due to the retrospective design of our study, we believe we might have a selection bias: patients with a higher cardiovascular risk might have more frequent blood lipid measurements and thus, were more prone to be included in this study. Additionally, we were only able to assess LDL-C changes between baseline and EOT in 53 patients and in 28 patients from EOT to 1 year after treatment.

#### List of abbreviations

CHC: Chronic hepatitis C; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; LDL-C: low-density lipoprotein; DAAs: Direct

acting antivirals; SVR: Sustained viral response; TG: triglycerides; HDL-C: high-density lipoprotein; EOT: End of treatment; BMI: Body mass index; CAP: Controlled attenuation parameter; SOF: Sofosbuvir; RBV: Ribavirin; LED: Ledipasvir; DAC: Daclatasvir; VEL: Velpatasvir; GLEC: Glecaprevir; PIBR: Pibrentasvir

## CONCLUSIONS

In patients with CHC without a previous diagnosis of hypercholesterolemia, total cholesterol and LDL-C levels significantly increase after HCV clearance, remaining stable within the first year. The main positive predictive factors for a significant LDL-C increase with HCV treatment were liver cirrhosis and baseline LDL-C levels  $\leq 100$  mg/dl. To prevent the underdiagnosis of dyslipidemia, measurement of lipid profile upon completion of DAAs treatment is vital to avoid hindering cardiovascular risk improvement after HCV clearance.

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