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FULL PAPER

Early improvement of glycaemic control after virus clearance in patients with chronic hepatitis C and severe liver fibrosis: a cohort study

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SUMMARY

HCV has been recognized as the cause of chronic hepatitis C (CHC) since 1990. CHC is associated with progressive liver damage and extrahepatic conditions. Direct antiviral agents (DAAs), approved in 2014, have shown effectiveness in eradicating HCV in most patients. However, little is known about the effect of viral eradication on hepatic and extra-hepatic damage.

We performed a historical cohort study of patients with HCV-related liver diseases who achieved SVR from March 2015 to October 2016 at INMI Lazzaro Spallanzani liver Unit in Rome (Italy). Repeated measures of glycaemia were analysed through a multilevel analysis framework to assess short time kinetics of blood glucose level at different times after therapy and for different levels of HCV viremia.

The analysis included 205 patients. A model assessing temporal kinetics and variation of glycaemia according to HCV viremia provided evidence that blood glucose levels significantly dropped in patients with diabetes achieving SVR. Most of the variations occurred at 3-5 weeks of therapy (-17.96 mg/dL; $p<0.001$) and in coincidence with HCV clearance (-13.92 mg/dL; $p<0.001$). A weak, non-statistically significant reduction was observed in normoglycemic patients.

Our study provides evidence that DAAs therapy may significantly improve glycaemic control in patients with CHC achieving SVR even when liver diseases are already established.

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INTRODUCTION

Hepatitis C virus (HCV) is a small, enveloped, positive-sense single-stranded RNA virus associated with chronic infection in humans (chronic hepatitis C; CHC). It is transmitted through contaminated needles (Eckhatdt *et al.,* 2017); medical equipment (Lanini *et al.,* 2010); blood and blood products; from sexual intercourse (Kouyos *et al.,* 2014); from an infected mother to her baby (Canadian Pediatric Society 2008). The World Health Organization (WHO) estimates that 71 million people globally have chronic hepatitis C infection (CHC). An estimated 400,000 people die of hepatitis C each year, mostly from cirrhosis and hepatocellular carcinoma. (Lanini *et al.,* 2016)

Epidemiological studies suggest that prevalence of type 2 diabetes among patients with chronic hepatitis C (CHC) may be much higher than in the general population (White *et al.,* 2008) ranging from 13% to 67% according to liver fibrosis stage and time of infection (Serfaty *et al.,* 2017; Hammaerstad *et al.,* 2015). In particular, molecular investigation suggests that HCV can directly interfere with glucose uptake and gluconeogenesis at cellular level; thus, the clearance of HCV infection could immediately improve glucose metabolism (Shoji *et al.,* 2012).

Since 2014, direct antiviral agents (DAAs) have been approved for treatment of HCV infection. DAAs have shown excellent safety profile and extraordinary efficacy with proportions of Sustained Virologic Response (SVR) defined as undetectable HCV RNA at 12 weeks after the end of therapy - ranging from 80 to 95%. However, it is yet to be demonstrated whether SVR coincides with the eradication of infection, the improvement of liver damage and the normalization of other abnormalities seen in patients with CHC (Jacobsen *et al.,* 2017; Zanotto *et al.,* 2017). In particular, little is known about the immediate impact of SVR on glucose metabolism.

The aim of this study was to describe the blood glycaemic profile in patients with CHC and established liver damage who achieved SVR:

a) at different time points after initiation of therapy;
b) for different patterns of exposure to HCV (i.e., in patients with either detectable or undetectable HCV level in blood).

Key words:
Glycaemia, insulin resistance, metabolism, liver fibrosis, HCV, DAA.

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MATERIALS AND METHODS

Study design and setting

We performed a historical cohort study of patients with CHC and established liver disease who achieved SVR at INMI Lazzaro Spallanzani liver Unit in Rome (Italy) from March 2015 to October 2016. The paper is reported according to the STROBE statement (Vandenbroucke *et al.,* 2007).

Participants

Patients were eligible for the study if they received DAA treatment, achieved SVR (i.e., undetectable HCV RNA at 12 weeks after the end of therapy), have liver fibrosis ≥F3 and have glycaemia determination before therapy. Patients were excluded if they received interferon, received organ/cell transplant, are listed for liver transplant or have type 1 diabetes. Glycaemia values were collected before therapy, at 3-4 weeks, 2-4 months, 5-7 months and 12-24 months after DAAs. Blood HCV RNA levels (viremia) were recorded before therapy, at 4/12/24 weeks during therapy and at 12 weeks after the end of therapy.

Variables, data source and measurement

Repeated measures of glycaemia were used as the only outcome variable. Information about the patient's clinical and epidemiological features before therapy (confounders) were collected from clinical records. Glycaemia values (in mg/dL) were collected from the laboratory database. Time after DAAs was modelled as a 5-level categorical variable; i.e.: before therapy, at 3-4 weeks, 2-4 months, 5-7 months and 12-24 months after DAAs. Pattern of exposure to DAAs and HCV was modelled as a 4-level categorical variable; i.e., without DAAs and HCV detectable (before therapy); with DAAs and HCV detectable (early on therapy); with DAAs and HCV undetectable (late on therapy) and without DAAs and HCV (after the end of therapy). HCV genotype was determined using the RealTime HCV Genotype II assay (Abbott Molecular, Abbott Park, IL, USA). Plasma HCV RNA was assessed using the Abbott real time HCV RNA assay (Abbott Molecular) with a lower limit of detection of 12 IU/mL. A patient was defined as diabetic if he/she had clinical records indicating twice glucose ≥126 mg/dL or once ≥200 mg/dL (American Diabetes association, 2015).

Study size

The study was designed to have no fewer than 130 measures of glycaemia per time-point of analysis in order to guarantee the detection of variation of mean glucose levels from 110 to 100 mg/dL assuming beta 0.90, alpha 0.05, correlation 0.60 and total variance 1650.

Statistical methods

Statistical correlation due to repeated measures of dependent variables over time from the same patients were handled according to a multilevel analysis framework, already used and validated on other complex clinical datasets (Lanini *et al.,* 2017; Lanini *et al.,* 2015). Bivariable analysis was carried out by the mixed effect linear regression model (MELRM) with random intercept at patient level to select significant confounders (P<0.050). Multivariable analyses for modelling glycaemia trends were carried out by two separate MELRMs with random intercept at patient level and random slope either at time after DAAs level (the model to assess the temporal kinetics of glycaemia) or at the level of exposure to DAAs and HCV (the model to assess variation of glycaemia according to DAAs and HCV exposure). All analyses were adjusted for significant confounders, if present. Diabetes status was considered as an *a priori* potential effect modifier. Other effect modifiers were included if significant interaction was present (P<0.050). Statistical significance for trends (P-joint) and for punctual comparison (P) were calculated with the ANOVA-style joint test.

RESULTS

Participants

From March 2015 to October 2016, 326 consecutive patients with CHC and liver fibrosis ≥3 achieved SVR after therapy with DAAs. Of them 205, for a total of 817 gly-

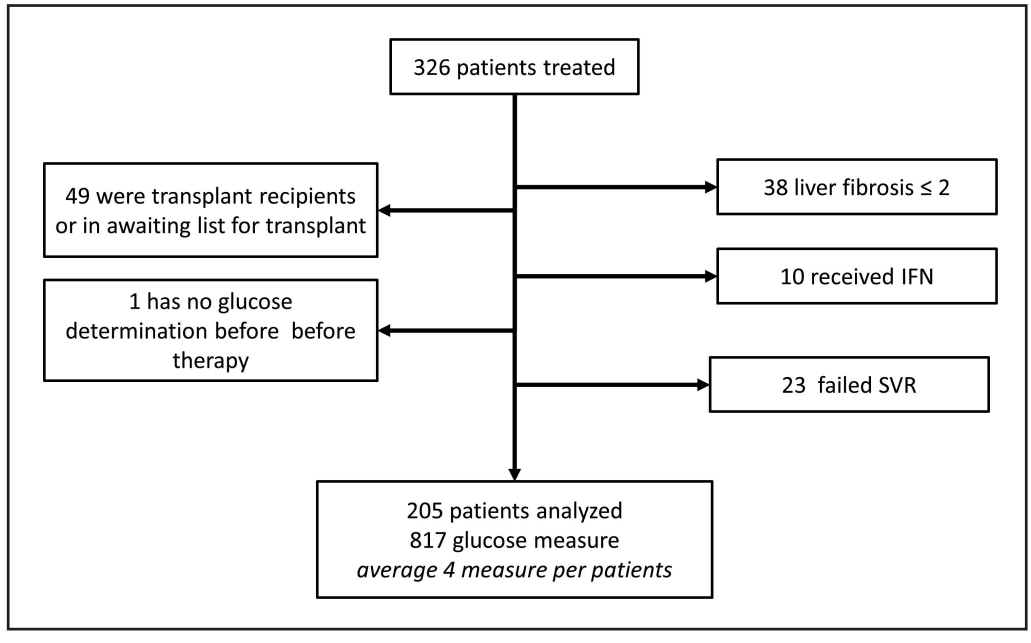

Figure 1 - *Population sample selection flow chart.*

caemia determinations (average 4 determinations per patient), were eligible for the analysis. *Figure 1* shows the patient selection flowchart. The rate of HCV clearance in the 205 patients included in the analysis was 20.98% at week 4, 78.54% at week 12 and 100% at week 24 after the start of treatment.

The patients' clinical features are reported in *Table 1*. Overall, most of the patients were aged 50-64 years (56.6%) with male predominance (63.9%); 26.3% of participants had diabetes, 42.4% were overweight (BMI≥25 kg/m^2), 15.6% were HIV Ab positive and 78.5% had liver cirrhosis. Virological testing showed that 48.8% of patients were highly viraemic (HCV RNA ≥600.000 IU/mL) before therapy; genotype 1b was the most frequent (37.6%) followed

Table 1 - *Sample description and bivariable analysis.*

Characteristics		*Sample description*			*Blood glucose (mg/dL)*			*P-value*
		Pat.	*%*	*Obs.*	*Mean*	*95% CI*		
Age	*≤49*	23	11.22%	88	96.51	84.98	108.04	0.278
	50-64	116	56.59%	463	103.22	98.14	108.30	
	≥65	66	32.20%	266	107.17	100.47	113.86	
Gender	*Male*	131	63.90%	519	106.69	101.93	111.44	0.046
	Female	74	36.10%	298	98.60	92.26	104.94	
Diabetes	*No*	151	73.66%	579	92.12	88.77	95.47	<0.001
	Yes	54	26.34%	238	134.51	129.12	139.91	
BMI	*≤24.99*	118	57.56%	478	100.59	95.58	105.59	0.054
	≥25.00	87	42.44%	349	108.16	102.29	114.04	
HIV	*Negative*	173	84.39%	674	104.49	100.29	108.68	0.404
	Positive	32	15.61%	143	100.05	90.50	109.59	
Liver disease	*Severe fibrosis*[†]	44	21.46%	137	97.86	89.29	106.43	0.131
	Cirrhosis	161	78.54%	680	105.24	100.97	109.52	
HCVRNA	*≤599,999*	105	51.22%	427	102.85	97.51	108.20	0.631
	≥600,000	100	48.78%	390	104.74	99.22	110.26	
Genotype	*1a*	56	27.32%	223	105.94	98.65	113.23	0.315
	1b	77	37.56%	299	107.24	101.03	113.46	
	2	17	8.29%	66	95.45	82.14	108.75	
	3	30	14.63%	131	97.62	87.86	107.38	
	4	25	12.20%	98	101.47	90.54	112.40	
Therapy duration	*12*	101	49.27%	371	103.22	97.68	108.76	0.787
	24[‡]	104	50.73%	446	104.28	98.94	109.62	
Ribavirin	*No*	78	38.05%	319	104.91	98.71	111.11	0.646
	Yes	127	61.95%	498	103.06	98.16	107.95	
DAAs	*SOF+RIBA*	26	12.68%	108	97.85	87.48	108.23	0.129
	SOF+SIM±RIBA	59	28.78%	213	107.63	100.51	114.76	
	SOF+DAC±RIBA	40	19.51%	169	101.64	93.22	110.06	
	2D/3D±RIBA	7	3.41%	30	85.25	66.47	104.03	
	SOF+LED±RIBA	73	35.61%	297	106.59	100.37	112.81	
Time after therapy	*Before*	205	100.00%	205	109.78	105.24	114.32	<0.001
	3-4 weeks	157	76.59%	157	105.58	100.67	110.49	
	2-4 months	153	74.63%	153	99.74	94.79	104.68	
	5-7 month	157	76.59%	157	99.09	94.18	104.00	
	12-24 months	145	70.73%	145	100.27	95.25	105.29	
Pattern of exposure to DAAs and HCV	*DAA - / HCV +*	205	100.00%	205	109.78	105.23	114.33	<0.001
	DAA + / HCV +	126	61.46%	152	103.73	98.67	108.79	
	DAA + / HCV -	153	74.63%	248	100.56	96.01	105.12	
	DAA - / HCV -	162	79.02%	212	100.13	95.48	104.78	

[†]Severe fibrosis is according to F3 Metavir score [‡]2 patients received 16 weeks therapy and 2 patients received 20 weeks therapy.

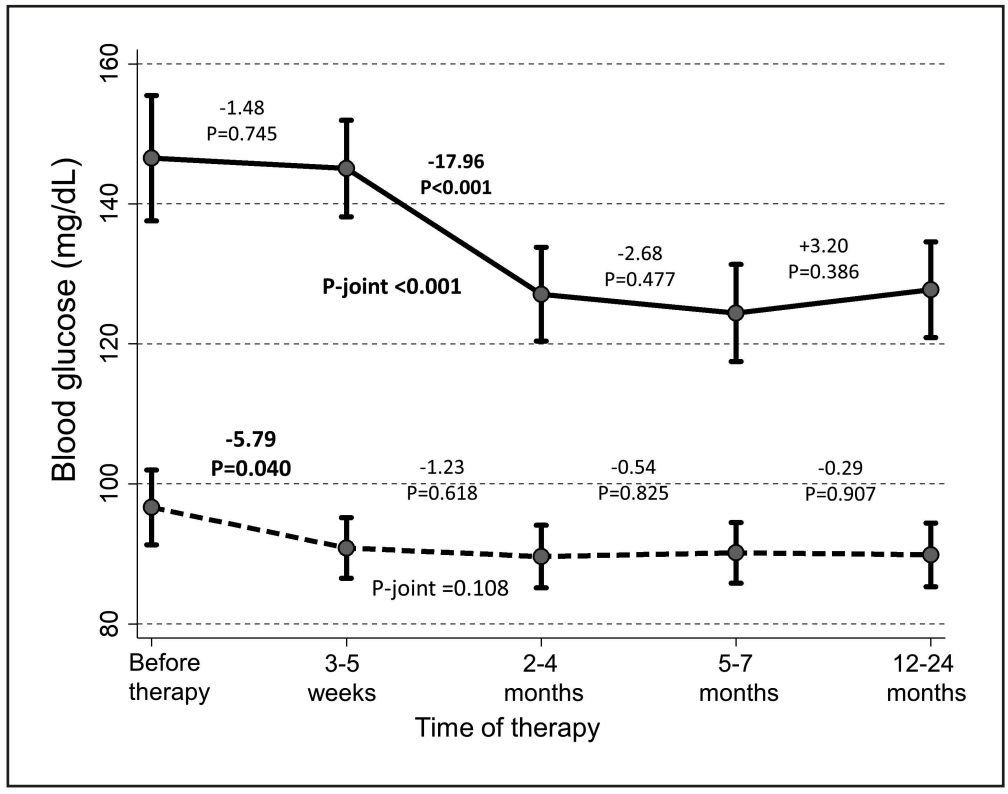

Figure 2 - *Temporal kinetics of glycaemia in diabetic (solid line) and normoglycemic patients (dashed line). Figures over the connection lines indicated the punctual estimates of glucose variations (in mg/dL) between two adjacent time points after therapy. P represents the statistic test to assess significance of variation between two adjacent time points after therapy. P-joint represents the statistic test to assess the overall temporal trend either in diabetic or in normoglycemic patients. When P-joint is above 0.050, there is no evidence for a significant variation of glycaemia over time.*

by genotype 1a (27.3%), genotype 3 (14.6%) genotype 4 (12.2%) and genotype 2 (8.3%).

Descriptive data analysis

One-hundred and two patients (49.76%) underwent 24-week treatment, 101 (49.27%) underwent 12-week treatment, 2 received 16-week therapy. Most of the patients (86.32%) received an anti-HCV therapy including at least two second-generation DAAs (i.e., sofosbuvir, daclatasvir, ledipasvir, simeprevir, ombitasvir, paritaprevir, dasabuvir). Sofosbuvir was the most used DAAs (96.2%) followed by ledipasvir (35.61%); simeprevir (28.78%); daclatavir (19.51%) and the combination ombitasvir/paritaprevir ± dasabuvir (3.41%). 62.0% of the patients received ribavirin in addition to DAAs.

Outcome data analysis (glycaemia predictors)

Bivariable analysis (*Table 1*) provided evidence that diabetes (p<0.001), time after DAAs (p<0.001), pattern of exposure to DAAs/HCV (p<0.001) and gender (p=0.046) were all significant predictor of blood glucose level. Multivariable analysis consisted of two separate models. The first model included the time after DAAs and diabetes to assess glycaemia temporal kinetics. The second model included patterns of exposure to DAAs/HCV and diabetes to assess variation of glycaemia according patients' HCV RNA level in the blood and exposure to DAAs. Both models also included an interaction term between diabetes and all other covariates. Gender was removed from final models as it was not significant after adjusting for other covariates in both models (p=0.192 and p=0.168, respectively).

Main results (glycaemia kinetics modelling)

The model to assess temporal kinetics of glycaemia provided strong evidence (p-joint <0.001) that after DAAs blood glucose levels dropped significantly in patients with diabetes. Most of the observed variation occurred at 3-5 weeks of therapy (variation -17.96 mg/dL 95% CI -25.26 to -10.41; p<0.001). In contrast, in patients without diabetes the reduction of blood glucose level was weak and not statistically significant overall (p-joint =0.108). However, the punctual variation between glycaemia at baseline and at 3-5 weeks after therapy was marginally significant (variation -5.79 mg/dL 95% CI -11.30 to -0.279 p=0.040). *Figure 2* shows the complete results of the analysis.

The model to assess variation of glycaemia according patients' virologic status and exposure to DAAs provided strong evidence (p-joint <0.001) that in patients with diabetes blood glucose level dropped significantly after HCV viremia became undetectable (variation -13.92 mg/dL 95% CI -21.30 to -6.53; p<0.001). In contrast, in patients without diabetes the reduction of blood glucose levels was weak and not statistically significant overall (p-joint =0.062). Similar to the previous model, the punctual variation between blood glucose levels before therapy and those in the earliest phase of DAAs administration was marginally significant (variation -6.52 mg/dL 95% CI -12.17 to -0.877; p=0.024). *Figure 3* shows the complete results of the analysis.

DISCUSSION

Our study indicates that, in patients with CHC, the clearance of HCV after DAAs therapy may significantly improve glycaemic control even when liver damage is already established. Our models suggest that blood glucose levels dropped significantly in coincidence with HCV RNA clearance during DAAs administration and did not climb back after DAAs withdrawal at the end of therapy. Moreover, the variations of blood glucose level during and after therapy

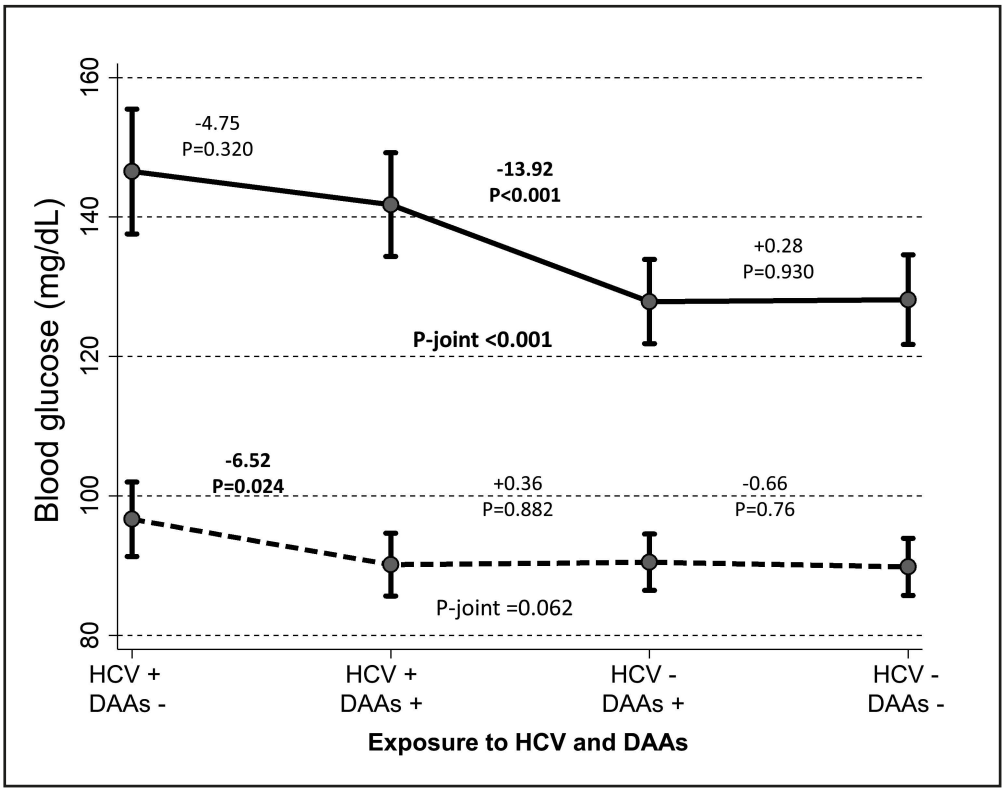

Figure 3 - *Variation of glycaemia according to patients' virologic status and exposure to DAA in diabetic (solid line) and normoglycemic patients (dashed line). Figures over the connection lines indicated the punctual estimates of glucose variations (in mg/dL) between two adjacent levels of exposure to HCV and DAA. P represents the statistic test to assess significance of variation between two adjacent levels of exposure. P-joint represents the statistic test to assess the overall significance of variation across the different levels of exposure to HCV and DAA either in diabetic or in normoglycemic patients. When P-joint is above 0.050 there is no evidence for a significant variation of glycaemia according to virologic status and exposure to DAA.*

were much more evident in diabetic than in normoglycemic patients. Since this metabolic improvement persisted after the withdrawal of DAAs at the end of therapy, it most likely rules out the current hypothesis that DAAs can, per se, affect blood glucose levels (Premji *et al.,* 2015). However, the mean levels of blood glucose remained significantly higher in diabetic than in normoglycemic patients. Our results are consistent with recent observations from molecular and clinical investigations.

Previous studies speculated that HCV could worsen glucose control by impairing human pancreatic beta-cell function (Narita *et al.,* 2004; Masini *et al.,* 2005). However, recent evidence suggests that HCV can directly affect glucose homeostasis at the level of liver cells. *In vitro* studies carried out on hepatocytes have shown that HCV transcription can directly suppress glucose cellular uptake by down-regulating GLUT2 expression, the main insulin independent glucose transmembrane carrier into the liver (Kasai *et al.,* 2009). The ability of HCV to interfere with glucose metabolism at the cellular level is supported by another study, showing that the expression of HCV core protein can unbalance HOTAIR-Sirt1 signalling, a metabolic pathway involved in glucose oxidation (Li *et al.,* 2016). Moreover, animal model studies have demonstrated that transgenic mice expressing HCV NS5A protein have an upregulation of Akt-/JNK-PEPCK signalling pathways, resulting in a significant increase of gluconeogenesis (Kuo *et al.,* 2014), Finally, molecular analyses carried out on CHC patients have suggested that HCV can directly affect the metabolic regulation of glucose homeostasis by impairing the function of histone deacetylases 9, an enzyme that regulates hepatic gluconeogenesis via deacetylation of FoxO1 (Chen *et al.,* 2015). This evidence suggests that the coincidence between reduction of blood glucose level and suppression of HCV replication, as observed in our study, may have a consistent pathophysiological basis.

The results of recent observational studies carried out on CHC patients seems to confirm the improvement of glucose metabolism in patients who clear HCV infection. A cohort study from the Veteran Affairs Health Care System showed that after SVR, diabetic patients improved glycaemic control and that the degree of improvement is proportional to the severity of metabolic impairment before anti-HCV therapy. The metabolic improvement was significant, but did not completely eliminate the need for oral antidiabetic medications (Hum *et al.,* 2017), Comparable results were also reported in a small series of cases from Egypt, where 65 patients with diabetes had 11.51 mg/dL mean reduction of blood glucose levels between baseline and 24 weeks after the end of anti-HCV therapy (Abdel Alem *et al.,* 2017). Our study confirms these observations and adds to current knowledge by providing a quantitative measure of the time and extent of such metabolic improvement. In addition, we found that suppression of HCV viral replication is a strong predictor of blood glucose level, an observation that provides additional new evidence to support the hypothesis that HCV replication (Shoji *et al.,* 2012) has, *per se*, a direct impact on glucose homeostasis.

Our study underlines that despite a significant improvement, glycaemia remained significantly higher in diabetic than in normoglycemic patients, suggesting that HCV is not the sole factor involved in the pathogenesis of diabetes in patients with CHC. Other factors may directly affect glycaemic control (Shoji *et al.,* 2012; Serfaty *et al.,* 2017): specific genetic profiles of patients, impaired liver function (Orsi *et al.,* 2017) and enhanced inflammatory response (Lee *et al.,* 2014), which persisted after HCV clearance in several patients with established liver damage (Welsch *et al.* 2017).

The prevalence of diabetes in our sample was 26.5%, much higher than expected for the Italian population in general (about 5.3%) (Istat 2017) although consistent with the expectation for patients with CHC and established liver damage. (Serfaty *et al.,* 2017). Furthermore, the relatively slow kinetics of HCV clearance (approximately 20% of patients cleared HCV RNA after 12 weeks of therapy) may be consistent with the high proportion of patients with established cirrhosis, who were prioritized for HCV treatment according to the Italian National Guidance, and the use of sofosbuvir as the only DAAs in combination with ribavirin (which were widely used in the early implementation of DAAs but are currently considered sub-optimal) (European Association for the study of the liver 2016; Gentile *et al.,* 2019).

In conclusion, DAA therapy may significantly improve glycaemic control in patients with CHC even when liver cirrhosis is already established. The metabolic benefit achieved after HCV clearance persists after the end of therapy, suggesting that all patients with CHC and diabetes should receive therapy with DAAs regardless of the stage of liver disease. Whilst HCV clearance seems to improve, but not completely resolve, impaired glucose metabolism, the actual impact of HCV clearance on patients' quality of life and longevity needs to be determined. Prospective cohort studies with large numbers of patients are required to assess whether the observed metabolic improvement can actually result in a reduction of the most relevant diabetes complications, such as microangiopathy and macrovascular diseases. Since our study sample included only Caucasian subjects, non-Caucasian populations should be included in these studies for results to be generalizable.

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Transparency declarations

All the authors declare no conflict of interest.

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