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Revisiting predictors of virologic response to PEGIFN + RBV therapy in HIV-/HCV-coinfected patients: the role of metabolic factors and elevated GGT levels

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SUMMARY. Evaluation of metabolic factors and elevated γ-glutamyltransferase (GGT) levels as independent predictors of treatment failure in a thoroughly documented cohort of HIV-/HCV-coinfected patients (HIV/HCV). Sixty-four HIV/HCV patients treated with pegylated interferon-α-2a plus ribavirin (PEGIFN + RBV) at the Medical University of Vienna within a prospective trial were included in this study. In addition, 124 patients with HIV/HCV from the AIFA-HIV and AHIVCOS cohorts were included as a validation cohort. Advanced liver fibrosis, GGT elevation, insulin resistance (IR) and low CD4+ nadir were defined as METAVIR F3/F4, GGT levels >1.5× sex-specific upper limit of normal, homoeostasis model assessment of insulin resistance >2 and CD4+ nadir <350 cells/μL, respectively. HCV-genotype 1/4 (OR26.3; $P = 0.006$), advanced liver fibrosis (OR20.2; $P = 0.009$), interleukin 28B *rs12979860* non-C/C SNP (OR8.27; $P = 0.02$) and GGT elevation (OR7.97; $P = 0.012$) were independent predictors of treatment failure, while both IR (OR3.51; $P = 0.106$) and low CD4 + nadir (OR2.64; $P = 0.263$) were not independently associated with treatment failure. A statistically significant correlation between GGT elevation and prior alcohol abuse ($r = 0.259$; $P = 0.039$), liver steatosis ($r = 0.301$; $P = 0.034$) and low-density lipoprotein-cholesterol ($r = -0.256$; $P = 0.041$) was observed. The importance of GGT elevation as an independent predictor of treatment failure was confirmed in a validation cohort (OR2.76; $P = 0.026$). While GGT elevation emerged as an independent predictor of treatment failure in both the derivation and the validation cohort, no independent associations between metabolic factors and treatment failure were observed. Thus, our findings suggest that GGT elevation is an independent predictor of treatment failure in HIV/HCV that can easily be incorporated into predictive algorithms.

Keywords: hepatitis C virus, human immunodeficiency virus, pegylated interferon, ribavirin, γ-glutamyltransferase.

INTRODUCTION

Liver disease is the second leading cause of death in patients infected with human immunodeficiency virus (HIV) [1]. When compared to hepatitis C virus (HCV) monoinfection, HIV/HCV coinfection, observed in 25–30% per cent of European and US-American HIV-positive patients [2], was found to be associated with higher rates of liver fibrosis progression [3] and markedly higher risks of cirrhosis and end-stage liver disease [4]. In addition, an

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; cART, combined antiretroviral therapy; GGT, γ-glutamyltransferase; HCV, hepatitis C virus; HCV-GT, hepatitis C virus genotype; HOMA-IR, homoeostasis model assessment of insulin resistance; IL28B, interleukin 28B *rs12979860* SNP; IR, insulin resistance; LDL, low-density lipoprotein; N(t)RTI, nucleos(t)ide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RVR, rapid virologic response; SVR, sustained virologic response.

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epidemic of acute hepatitis C, with a 14-fold increase in incidence rate in the last 13 years, has been reported among HIV-positive men who have sex with men [5,6]. This may lead to an additional increase in the prevalence of HIV/HCV coinfection among HIV-positive patients in the near future. As a result, treatment of chronic hepatitis C (CHC) in HIV-/HCV-coinfected patients (HIV/HCV) is of high priority. However, in these patients, therapy-restricting contraindications, considerably lower rates of treatment initiation and lower rates of virologic response to pegylated interferon-α plus ribavirin (PEGIFN + RBV) complicate CHC therapy in comparison with HCV monoinfection [7,8].

Thus, baseline predictors of sustained virologic response (SVR) have increasingly become of scientific interest. Besides well-established predictors of SVR in HIV/HCV, such as HCV-genotype (HCV-GT), HCV-RNA load, liver fibrosis and interleukin 28B *rs12979860* SNP (IL28B) [9,10], metabolic factors such as insulin resistance (IR) [11–13] and low-density lipoprotein (LDL)-cholesterol levels [14–16] have been proposed as predictors of SVR in HIV/HCV. The role of IR is particularly controversial, as other studies have reported negative results [15,16]. One meta-analysis [17] not including the most recent negative results reported by Butt and co-workers [15] confirmed the association between IR and virologic response in HIV/HCV [17].

In addition, low CD4+ T-lymphocyte count [18,19] and nadir [12] have been found to be associated with treatment failure in HIV/HCV.

One major limitation of previous studies investigating the role of metabolic factors and CD4+ T-lymphocyte count and nadir in the prediction of SVR in HIV/HCV has to be considered: no study included all well-established predictors of SVR. Thus, they do not allow us to draw conclusions on whether metabolic factors are independent predictors of SVR in these patients when all well-established predictors of SVR are considered.

In HCV-monoinfected patients, the association between elevated γ-glutamyltransferase (GGT) levels and virologic response has been intensively studied [20–25]. In contrast, in HIV/HCV, no association between GGT levels and virologic response has yet been reported. The aim of this study was to evaluate metabolic factors and elevated GGT levels as independent predictors of treatment failure in a thoroughly documented cohort of HIV/HCV.

PATIENTS AND METHODS

Study population

A total of 64 HIV/HCV patients treated with pegylated interferon-α-2a plus ribavirin (PEGIFN + RBV) at the Medical University of Vienna within a prospective trial were included in this study. Inclusion requirements consisted of the availability of GGT serum levels, homoeostasis model assessment of IR (HOMA-IR), HCV-GT, HCV-RNA levels, IL28B, liver biopsy, CD4+ T-lymphocyte count and nadir as well as information on virologic response. All patients had compensated liver disease, were naïve to PEGIFN-based CHC therapy and were free of malignancies as well as significant cardiac, pulmonary or renal disease.

In addition, 124 patients with HIV/HCV from the AIFA-HIV and AHIVCOS cohorts were included as a validation cohort. Requirements for the inclusion in the validation cohort consisted of the availability of GGT serum levels, HCV-GT, IL28B and liver biopsy as well as information on virologic response.

Definitions, blood sampling and IL28B testing

Prior alcohol abuse was defined as an average daily alcohol consumption >50 g for more than two consecutive years. Alcohol consumption was assessed by repeated clinical interviews and measurement of blood alcohol levels. Insulin serum levels were retrospectively assessed using stored screening serum samples obtained within 35 days prior to CHC treatment by chemiluminescence immunoassay (Siemens, Erlangen, Germany). Homoeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting glucose serum levels (mg/dL) × fasting insulin serum levels (μU/dL) × 405^{-1}. In accordance with previous studies [11] in HIV-/HCV-coinfected and HCV-monoinfected patients, IR was defined as HOMA-IR >2. Liver enzyme (ALT, AST, and GGT) elevations >1.5× sex-specific upper limit of normal (ULN) were referred to as elevated liver enzymes. HCV-GT and serum HCV-RNA levels were determined using commercially available assays [VERSANT HCV-Genotype 2.0 Assay (LiPA) (Siemens, Vienna, Austria) and COBAS TaqMan HCV Test (Roche, Vienna, Austria)]. High HCV-RNA load was defined as HCV-RNA levels $>6 \times 10^5$ IU/mL. Serum CD4+ T-lymphocyte counts were determined using standard flow fluorocytometry. A CD4+ T-lymphocyte nadir <350 cells/μL was referred to as a low CD4+ T-lymphocyte nadir, as current European AIDS Clinical Society guidelines recommend initiation of combined antiretroviral therapy (cART) in HIV/HCV with a CD4+ T-lymphocyte count <350 cells/μL. IL28B was analysed as described previously using the StepOnePlus Real Time PCR System (Applied Biosystems, Carlsbad, CA, USA) and a Custom TaqMan SNP Genotyping Assay [26].

Liver biopsy

Liver specimens were obtained percutaneously or via the transjugular route during hepatic venous pressure gradient measurements [27]. Pathologists blinded to patients' clinical data assessed liver fibrosis according to the METAVIR

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classification [28]. METAVIR F3/F4 was denoted as advanced liver fibrosis.

CHC treatment

All patients were treated with pegylated interferon-α-2a (180 μg) once a week. HCV-GT 1/4 patients received 1000–1200 mg ribavirin, while HCV-GT 2/3 patients received 800 mg ribavirin daily. Treatment duration was 48 weeks, except for HCV-GT 1/4 patients without rapid virologic response (RVR), who were treated for 72 weeks. RVR and SVR were defined as undetectable HCV-RNA at week 4 on treatment and 24 weeks after the end of PEG-IFN + RBV treatment, respectively.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 20 (SPSS Inc., Armok, NY, USA). Continuous variables were reported as mean ± standard deviation or median (interquartile range), while categorical variables were reported as number of patients with/without (proportion of patients with) a certain characteristic. Group comparisons were exclusively performed for parameters that have previously been described in the literature as predictors of SVR in either HCV-monoinfected or HIV-/HCV-coinfected patients: HCV-GT [9,10,29], HCV-RNA load [9,10], liver fibrosis [9,29], IL28B [9,10,29], IR [11–13], LDL-cholesterol levels [14–16], CD4+ T-lymphocyte count [18,19] and nadir [12], as well as GGT levels [20–25]. Student's *t*-test was used for group comparisons of continuous variables when applicable. Otherwise, the Mann–Whitney *U*-test was applied. Group comparisons of categorical variables were performed using either Pearson's chi-squared or Fisher's exact test.

A total of three binary logistic regression models were calculated. While Model 1 and Model 2 are based on the derivation cohort, Model 3 is based on the validation cohort. All baseline characteristics statistically significantly associated with treatment failure in the derivation cohort and a well-established predictor of SVR, HCV-GT, were included in Model 1. The same variables were included in Model 2, evaluating independent predictors of SVR using backward stepwise binary logistic regression analysis. All independent predictors of SVR in the derivation cohort were included in Model 3. A P value ≤0.05 was considered as statistically significant.

Ethics

This study was conducted with the understanding and the consent of each participant and in accordance with the declaration of Helsinki as approved by the local ethics committee of the Medical University of Vienna (EKN 1369/2012).

RESULTS

Patient characteristics of the derivation cohort

The majority of patients were men (70%) with a mean age of 38 ± 15.2 years. Twenty-eight per cent of patients reported prior alcohol abuse. Lipodystrophy was observed in 17% of patients with a mean BMI of 22.8 ± 3.5 kg/m^2. Elevated ALT, AST and GGT serum levels were observed in 70%, 37% and 52% of patients, respectively. The prevalence of IR was 50%, with a mean HOMA-IR of 2.67 ± 2 and mean LDL-cholesterol levels of 99.6 ± 44.4 mg/dL. HCV-GT 1/4 was observed in 66% and 63% of patients displayed high HCV-RNA load. Thirty-seven and 64% of patients had advanced liver fibrosis and IL28B non-C/C, respectively. Seventy-seven per cent of patients were on cART. The mean CD4+ T-lymphocyte count and CD4+ T-lymphocyte nadir were 529 ± 239 cells/μL and 314 ± 200 cells/μL, respectively. The prevalence of low CD4+ T-lymphocyte nadir was 64%. Sixty-three per cent of patients displayed a SVR in per-protocol analysis. SVR rates of 54% and 77% were observed in patients with HCV-GT 1/4 and 2/3, respectively. (Table 1).

Patient characteristics of the validation cohort

The prevalence rates of HCV-GT 1/4, advanced liver fibrosis, IL28B non-C/C and GGT elevation were 71%, 38%, 75% and 58%, respectively. In the validation cohort, 65% of patients achieved SVR. Patients with HCV-GT 1/4 and 2/3 displayed SVR rates of 27% and 56%, respectively.

Baseline characteristics associated with treatment failure in the derivation cohort

A higher prevalence of GGT elevation was observed in patients without SVR (71%), when compared to patients with SVR (40%, $P = 0.017$). While patients without GGT elevation displayed SVR rates of 77%, SVR rates of 55% were observed in patients with GGT elevation ($P = 0.017$). (Table 1, Figs 1 and 2).

Low-density lipoprotein-cholesterol levels (100.6 ± 46.1 *vs* 99 ± 43.9 mg/dL, $P = 0.891$) were comparable between patients with and without SVR.

While 67% of patients without SVR had IR, IR was observed in 40% of patients with SVR ($P = 0.039$). In addition, patients without IR achieved higher rates of SVR (75% *vs* 50%, $P = 0.039$).

The association of HCV-GT 1/4 with treatment failure was not statistically significant. However, a trend towards higher rates of SVR in patients without HCV-GT 1/4 was observed (77% *vs* 55%; $P = 0.077$). The prevalence of HCV-GT 1/4 infection in patients with and without SVR was 58% and 79%, respectively ($P = 0.077$).

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Table 1 Baseline characteristics of the derivation cohort and comparison of baseline characteristics of patients with and without SVR

Patient characteristics	All patients $n = 64$ (100%)	No SVR $n = 24$ (38%)	SVR $n = 40$ (63%)	*P* value
Epidemiological characteristics				
Sex, male	45/19 (70%)	18/6 (75%)	27/13 (68%)	
Age (years)	38 ± 15.2	40.7 ± 8.9	36.4 ± 8.8	
Prior alcohol abuse	18/46 (28%)	9/15 (38%)	9/31 (23%)	
BMI (kg/m^2)	22.8 ± 3.5	22.9 ± 3.7	22.7 ± 3.4	
Blood sample				
ALT (IU/mL)	70.5 (62)	72 (40)	70.5 (85)	
ALT elevation, >1.5 × UNL	45/19 (70%)	17/7 (71%)	28/12 (70%)	
AST (IU/mL)	65.4 ± 38.5	58.5 ± 25.7	69.7 ± 44.6	
AST elevation, >1.5 × UNL	19/33 (37%)	6/14 (30%)	13/19 (41%)	
GGT (IU/mL)	76.5 (105)	128.5 (149)	67.5 (57)	
GGT elevation, >1.5 × UNL	33/31 (52%)	17/7 (71%)	16/24 (40%)	**0.017**
HOMA-IR	2.67 ± 2	2.98 ± 2.04	2.5 ± 1.99	
Insulin resistance, HOMA-IR >2	32/32 (50%)	16/8 (67%)	16/24 (40%)	**0.039**
LDL-cholesterol (mg/dL)	99.6 ± 44.4	100.6 ± 46.1	99 ± 43.9	0.891
HCV infection parameters				
HCV-GT 1/4	42/22 (66%)	19/5 (79%)	23/17 (58%)	0.077
HCV-RNA (log IU/mL)	6.05 ± 0.9	6.21 ± 0.78	5.95 ± 0.96	
High HCV-RNA load, >6 × 10^5 IU/mL	40/24 (63%)	16/8 (67%)	24/16 (60%)	0.594
IL28B non-C/C	41/23 (64%)	20/4 (83%)	21/19 (53%)	**0.013**
Advanced liver fibrosis, METAVIR F3/F4	23/41 (36%)	14/10 (58%)	9/31 (23%)	**0.004**
HIV infection parameters				
Lipodystrophy	11/53 (17%)	7/17 (29%)	4/36 (10%)	
cART	49/15 (77%)	21/3 (88%)	28/12 (70%)	
PI	26/23 (53%)*	13/8 (62%)*	13/15 (46%)*	
N(t)RTI	45/4 (92%)*	20/1 (95%)*	25/3 (89%)*	
NNRTI	23/26 (47%)*	10/11 (48%)*	13/15 (46%)*	
CD4+ T-lymphocyte count (cells/μL)	529 ± 239	481 ± 163	558 ± 272	0.215
CD4+ T-lymphocyte nadir (cells/μL)	314 ± 200	248 ± 148	353 ± 218	
Low CD4+ T-lymphocyte nadir, <350 cells/μL	41/23 (64%)	20/4 (83%)	21/19 (53%)	**0.013**

*% Of patients on cART.

Statistics: Student's *t*-test was used for group comparisons of continuous variables when applicable. Otherwise, Mann–Whitney *U*-test was applied. Group comparisons of categorical variables were performed using either Pearson's chi-squared or Fisher's exact test.

Definitions: Prior alcohol abuse: average daily alcohol consumption >50 g for more than two consecutive years.

ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; cART, combined antiretroviral therapy; GGT, γ-glutamyltransferase; HCV, hepatitis C virus; HCV-GT, hepatitis C virus genotype; HOMA-IR, homoeostasis model assessment of insulin resistance; IL28B, interleukin 28B *rs12979860* SNP; IR, insulin resistance; LDL, low-density lipoprotein; N(t)RTI, nucleos(t)ide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SVR, sustained virologic response.

Bold values denote statistically signifcant *P* values.

The prevalence of high HCV-RNA (67% *vs* 60%; $P = 0.594$) did not vary significantly between virologic response groups.

Patients without SVR displayed a higher prevalence of IL28B non-C/C (83% *vs* 53%; $P = 0.013$) and advanced liver fibrosis (58% *vs* 23%; $P = 0.004$) when compared to patients with SVR. Accordingly, higher rates of SVR were observed among patients without IL28B non-CC (83% *vs* 51%, $P = 0.013$) and without advanced liver fibrosis (76% *vs* 39%, $P = 0.004$).

While the difference in CD4+ T-lymphocyte count between patients with and without SVR did not attain statistical significance (558 ± 272 *vs* 481 ± 163 cells/μL; $P = 0.215$), patients with treatment failure displayed a higher prevalence of low CD4+ T-lymphocyte nadir (83% *vs* 53%; $P = 0.026$). Accordingly, lower rates of SVR were

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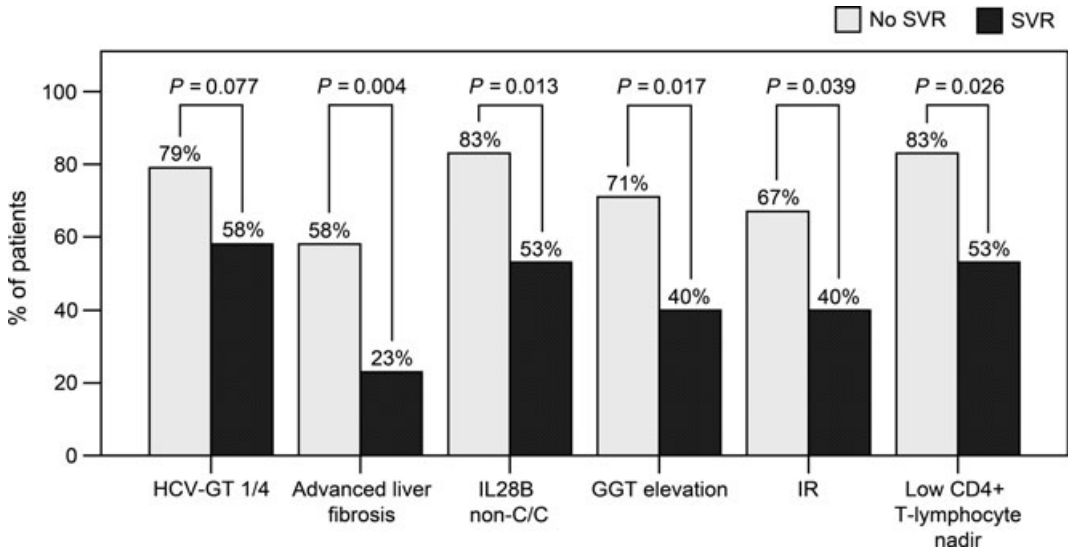

Fig. 1 The prevalence rates of certain baseline characteristics in patients with and without sustained virologic response (SVR). Statistics: Group comparisons were performed using Pearson's chi-squared test. Definitions: Advanced liver fibrosis: METAVIR F3/4; GGT elevation: >1.5 × UNL; IR: HOMA-IR >2; Low CD4+ T-lymphocyte nadir: <350 cells/μL.

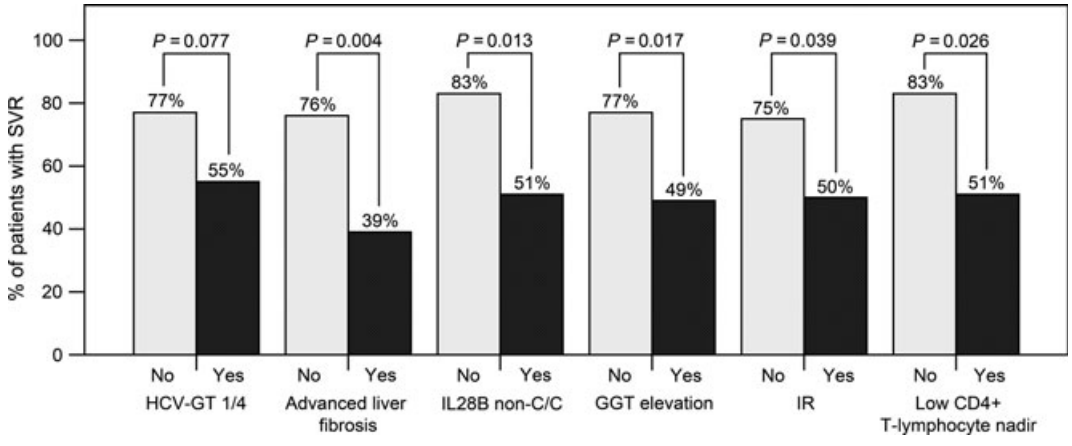

Fig. 2 Sustained virologic response (SVR) rates in patients with and without certain baseline characteristics. Statistics: Group comparisons were performed using Pearson's chi-squared test. See Fig. 1 for statistics and definitions.

observed in patients with low CD4+ T-lymphocyte nadir (51% *vs* 83%; $P = 0.026$).

Analysis of correlates of GGT elevation

A statistically significant correlation between GGT elevation and prior alcohol abuse ($r = 0.259$, $P = 0.039$), liver steatosis ($r = 0.301$, $P = 0.034$) and LDL-cholesterol ($r = -0.256$, $P = 0.041$) was observed. The correlation between GGT elevation, serum glucose ($r = 0.23$, $P = 0.067$), triglycerides ($r = 0.051$, $P = 0.069$) and HCV-GT 1/4 ($r = -0.241$, $P = 0.055$) showed only a trend towards statistical significance. (Table 2).

Multivariate analysis of baseline characteristics associated with treatment failure in the derivation cohort

All baseline characteristics statistically significantly associated with treatment failure and a well-established predictor of SVR, HCV-GT were included in a binary logistic regression model. HCV-GT 1/4 (odds ratio (OR) 26.3; 95% confidence interval (CI) 2.6–189.9; $P = 0.006$), advanced liver fibrosis (OR 20.2; 95%CI 2.1–189.9; $P = 0.009$), IL28B non-C/C (OR 8.27; 95%CI 1.4–48.81; $P = 0.02$) and GGT elevation (OR 7.97; 95%CI 1.57–40.48; $P = 0.012$) were independent predictors of SVR in the derivation cohort. In contrast, both IR (OR 3.51; 95%CI 0.77–16.09;

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Table 2 Analysis of correlates of GGT elevation in the derivation cohort

	GGT elevation, >1.5 × UNL	
Parameter	Correlation coefficient	*P* value
Epidemiological characteristics		
Age (years)	0.107	0.401
BMI (kg/m^2)	0.094	0.461
Prior alcohol abuse	0.259	0.039
Liver enzymes		
ALT elevation	0.191	0.13
AST elevation	0.142	0.316
Metabolic parameters		
Glucose (mg/dL)	0.23	0.067
Insulin (μU/mL)	−0.085	0.502
HOMA-IR	−0.051	0.69
Insulin resistance	0.094	0.461
Triglycerides (mg/dL)	0.051	0.069
Total cholesterol (mg/dL)	−0.129	0.311
LDL-cholesterol (mg/dL)	−0.256	**0.041**
HDL-cholesterol (mg/dL)	0.02	0.873
Liver biopsy		
Liver steatosis (% hepatocytes)	0.301	**0.034**
Advanced liver fibrosis	0.139	0.272
HCV infection parameters		
High HCV-RNA load	−0.04	0.751
HCV-Genotype 3	0.189	0.135
HCV-Genotype 1/4	−0.241	0.055
IL28B non-C/C	0.056	0.66
HIV infection parameters		
Lipodystrophy	0.110	0.387
Low CD4+ T-lymphocyte nadir	0.121	0.34
PI	0.195	0.195
N(t)RTI	−0.045	0.766
NNRTI	−0.126	0.403

Statistics: Spearman's rank correlation coefficient was used for correlation analysis.
Definitions: GGT elevation: >1.5 × UNL; Prior alcohol abuse: average daily alcohol consumption >50 g for more than two consecutive years; ALT elevation: >1.5 × UNL; AST elevation: >1.5 × UNL; IR: HOMA-IR >2; Advanced liver fibrosis: METAVIRF3/4; High HCV-RNA: >6 × 10^5 IU/mL; Low CD4+ T-lymphocyte nadir: <350 cells/μL.
Bold values denote statistically signifcant *P* values.

P = 0.106) and low CD4+ T-lymphocyte nadir (OR 2.64; 95%CI 0.48–14.52; *P* = 0.263) were not independent predictors of treatment failure (Model 1). (Table 3).

All independent predictors of treatment failure in Model 1 were included in another binary logistic regression model. HCV-GT 1/4 (OR 22.3; 95%CI 2.5–198; *P* = 0.005), advanced liver fibrosis (OR 27.4; 95%CI 3.4–218.7; *P* = 0.002), IL28B non-C/C (OR 7.02; 95%CI 1.38–35.71; *P* = 0.019) and GGT elevation (OR 7.13; 95%CI 1.6–31.9; *P* = 0.01) remained as independent predictors of SVR in this final model (Model 2).

Multivariate analysis of baseline characteristics associated with treatment failure in the validation cohort

HCV-genotype 1/4 OR 2.62; 95% CI 1.06–6.48; *P* = 0.037), IL28B non-C/C (OR 3.4; 95%CI 1.33–6.69; *P* = 0.011) and GGT elevation (OR 2.76; 95%CI 1.13–6.73; *P* = 0.026) were independent predictors of treatment failure in the validation cohort. In contrast, advanced liver fibrosis (OR 1.64; 95%CI 0.7–3.84; *P* = 0.259) was not an independent predictors of treatment failure (Model 3). (Table 3).

DISCUSSION

This study based on a thoroughly documented cohort of HIV/HCV provides evidence that GGT elevation is an important independent baseline predictor of treatment failure in HIV/HCV when all well-established risk factors are considered. HCV-GT, HCV-RNA load, liver fibrosis and IL28B are well-established predictors of SVR in HIV/HCV [9,10]. Medrano and co-workers [9] have proposed a prediction model comprising these four parameters with high predictive accuracy with an area under the curve of 0.85 in the validation cohort. In line with previous studies, HCV-GT 1/4, advanced liver fibrosis and IL28B non-C/C were independent predictors of treatment failure in our study. Interestingly, the prevalence of high HCV-RNA load did not vary throughout the virologic response groups. As a result, high HCV-RNA was not included in multivariate analysis. As the definition of high HCV-RNA load is empiric [10], it should be re-evaluated in a larger cohort of HIV/HCV in analogy to a study by Zeuzem and co-workers [30].

Low GGT levels were found to be an independent predictor of both RVR [20] and SVR [21–24] in various cohorts of HCV-monoinfected patients. In addition, it has been shown that GGT elevation is the strongest predictor of virologic nonresponse in HCV-GT 1 patients [25]. Recently, Everhart and co-workers [31] evaluated high serum GGT levels as a predictor of virologic response and clinical end points in 1319 patients with significant liver fibrosis included in the Hepatitis C Anti-viral Treatment Against Cirrhosis (HALT-C) trial. Interestingly, a statistically significant association between GGT elevation and virologic response, as well as any clinical end point, death or transplantation, two-point increase in Ishak fibrosis score, and death alone was observed. In the only study to date reporting data on serum GGT levels in HIV/HCV by Cacoub and co-workers [13], a trend towards higher GGT levels in HIV/HCV without SVR was observed. In our study, using a lower cut-off value for the definition of GGT elevation, GGT

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Table 3 Multivariate analysis of baseline characteristics associated with treatment failure in the derivation (Model-1 and Model-2) and validation cohort (Model-3)

	Model-1, $n = 64$		Model-2, $n = 64$		Model-3, $n = 124$	
Parameter	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
HCV-GT 1/4	26.3 (2.6–189.9)	**0.006**	22.3 (2.5–198)	**0.005**	2.62 (1.06–6.48)	**0.037**
Advanced liver fibrosis	20.2 (2.1–189.9)	**0.009**	27.4 (3.4–218.7)	**0.002**	1.64 (0.7–3.84)	0.259
IL28B non-C/C	8.27 (1.4–48.81)	**0.02**	7.02 (1.38–35.71)	**0.019**	3.4 (1.33–8.69)	**0.011**
GGT elevation	7.97 (1.57–40.48)	**0.012**	7.13 (1.6–31.9)	**0.01**	2.76 (1.13–6.73)	**0.026**
IR	3.51 (0.77–16.09)	0.106				
Low CD4 + T-lymphocyte nadir	2.64 (0.48–14.52)	0.263				

Statistics: Binary logistic regression was used for multivariate analysis. See Fig. 1 for definitions.
Bold values denote statistically signifcant *P* values.

elevation emerged as an independent predictor of treatment failure from multivariate analysis. Agundez and co-workers [32] have demonstrated that HCV-monoinfected patients with IL28B non-C/C displayed higher GGT levels. However, Everhart and co-workers [31] observed a statistically significant association between high serum GGT levels and virologic response in HCV-monoinfected patients, regardless of IL28B. The significance of IL28B as a confounding factor is limited in our study, as no correlation between IL28B non-C/C and GGT elevation was observed and GGT elevation was an independent predictor in multivariate analysis.

γ-Glutamyltransferase (GGT) elevation is commonly referred to as a biochemical marker of alcohol abuse [33], and a correlation between prior alcohol abuse and GGT elevation was observed in our study population. GGT elevation was correlated with liver steatosis and inversely correlated with LDL-cholesterol. In addition, a positive trend between GGT elevation and both serum glucose and triglyceride levels was observed. Similar metabolic impairments have previously been described in HCV-monoinfected patients with high serum GGT levels [31]. These metabolic impairments are in line with previous findings in patients with alcohol abuse [34], although in our study, all patients were abstinent for a minimum of 6 months before the initiation and during CHC therapy. Other causes of GGT elevation in HIV/HCV that might be of relevance are nonalcoholic fatty liver disease [35] and cART-induced hepatotoxicity [36]. GGT elevation, however, was not associated with BMI, IR, lipodystrophy and cART drug classes in our study population. Thus, the underlying pathophysiology of GGT elevation and a potential causal association between GGT elevation and SVR remain unclear.

Patients without SVR displayed a higher prevalence of IR than patients with SVR. In contrast, IR was not an independent predictor of treatment failure in multivariate analysis. Interestingly, a recent study by Staettermayer and co-workers [37] showed an association between IL28B non-C/C and IR in HCV-monoinfected patients. Moreover, while no statistically significant correlation between GGT elevation and neither HOMA-IR, IR nor IL28B non-CC was observed in our study population, an association between high GGT levels and HOMA-IR, as well as the prevalence of diabetes has previously been shown in HCV-monoinfected patients [31]. All studies reporting positive results on the association between IR and SVR in HIV/HCV [11–13] were lacking data on IL28B and serum GGT levels. Thus, it is conceivable that IL28B and serum GGT levels are the underlying confounding factors of the association between IR and SVR in HIV/HCV.

CD4+ T-lymphocyte nadir [12] has been found to be a predictor of SVR in HIV/HCV. While patients without SVR displayed a higher prevalence of low CD4+ T-lymphocyte nadir in our study, a low CD4+ T-lymphocyte nadir was not associated with treatment failure in multivariate analysis. The difference in the prevalence of low CD4+ T-lymphocyte nadir in patients with and without SVR might be attributed to the association between absolute CD4+ T-lymphocyte count and liver fibrosis [38,39].

To confirm the importance of GGT levels as an independent predictor of treatment failure, a large, independent validation cohort comprising 124 HIV/HCV was included in this study. The significant difference in SVR rates between the derivation and the validation cohort may be attributed to differences in statistical analysis. While per-protocol analysis was performed in the derivation cohort, intention-to-treat analysis was used in the validation cohort. Intention-to-treat analysis may provide a better estimation of the clinical value of GGT elevation as an independent predictor of treatment failure.

HCV-GT 1/4, IL28B non-C/C and GGT elevation were independently associated with treatment failure in the validation cohort. The association between advanced liver fibrosis and treatment failure was not statistically significant. Nevertheless, considering the profound evidence derived from previous studies [9,10], we do challenge the

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role of advanced liver fibrosis as an independent predictor of treatment failure.

In conclusion, our study confirmed HCV-GT 1/4, advanced liver fibrosis and IL28B non-C/C as independent predictors of treatment failure. While GGT elevation emerged as an independent predictor of treatment failure in both the derivation and the validation cohort, no independent associations between metabolic factors and treatment failure were observed. Thus, our findings suggest that GGT elevation is an independent predictor of treatment failure in HIV/HCV that can easily be incorporated into predictive algorithms.

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AUTHORS CONTRIBUTION

Authors have participated in the study concept and design (MM, TR and MPR); acquisition of data (TR, BAP, FB, MCA, and AR); measurement of insulin levels (BOP); validation cohort (RZ and MP); analysis and interpretation of data (MM, TR, MT and MPR); drafting of the manuscript (MM and TR); critical revision of the manuscript for important intellectual content (MM, TR, BAP, FB, MCA, BOP, AR, RZ, MP, MT and MPR).

AUTHORS DECLARATION OF PERSONAL INTERESTS

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