Genetic polymorphisms of IL28b gene as predictors of response to dual therapy in genotypes 1 and 4-HCV and HIV/HCV-infected patients

Laura Sticchi[1,2], Antonio Di Biagio[3], Marina Sartini[1], Emanuela Rappazzo[1], Laura A. Nicolini[3], Giovanni Cenderello[4], Caterina Valle[5], Emilio Azzola[6], Alessandro Grasso[7], Pasqualina De Leo[8], Alessandra Boldrini[9], Maurizio Setti[10], Roberta Prinapori[3], Carolina Lorusso[11], Bianca Bruzzone[2], Giancarlo Icardi[1,2]; Ligurian HIV Network (Anna I. Alessandrini, Infectious Diseases Unit, IRCCS San Martino-IST, Genoa, Italy; Valentina Bartolacci, Infectious Diseases Unit, Santa Maria di Misericordia Hospital, Albenga, Italy; Silvia Boni, Infectious Diseases Unit, Sant'Andrea Hospital, La Spezia; Martina Coppelli, Department of Health Sciences, University of Genoa, Genoa, Italy; Chiara Dentone, Infectious Disease Unit, Sanremo Hospital, Imperia, Italy; Giovanni Mazzarello, Infectious Diseases Unit, IRCCS San Martino-IST, Genoa, Italy; Claudio Viscoli, Infectious Diseases Unit, IRCCS San Martino-IST, University of Genoa, Genoa, Italy

[1]Department of Health Sciences, University of Genoa, Genoa, Italy; [2]Hygiene Unit, IRCCS AOU San Martino-IST, Genoa, Italy; [3]Infectious Diseases Unit, IRCCS AOU San Martino-IST, University of Genoa, Genoa, Italy; [4]Infectious Diseases Unit, Galliera Hospital, Genoa, Italy; [5]Molecular Biology Unit, St. Corona Hospital, Pietra Ligure, Savona, Italy; [6]Gastroenterology and Digestive Endoscopy Unit, St. Corona Hospital, Pietra Ligure, Savona, Italy; [7]Gastroenterology Unit, San Paolo Hospital , Savona, Italy; [8]Infectious Disease Unit, San Paolo Hospital , Savona, Italy; [9]Infectious Disease Unit, Sanremo Hospital, Imperia, Italy; [10]Internal Medicine and Clinical Immunology Unit, IRCCS AOU San Martino-IST, Genoa, Italy; [11]Department of Mental Health and Addiction -Local Health Unit 4, Chiavari, Genoa, Italy

SUMMARY

We describe the genotypes and allele distribution of interleukin 28B (IL28B) rs12979860 and rs8099917 single nucleotide polymorphisms (SNPs) in hepatitis C virus (HCV) G1-4 infected patients, to assess predictive ability and to determine whether the combined determination of two IL28B SNPs might improve sustained virologic response (SVR) prediction of both in HCV mono- and HIV/HCV co-infected patients. IL28B SNPs were genotyped in 269 patients, 181 mono- and 88 co-infected, treated with pegylated interferon and ribavirin. Data stratified by HCV mono- and HCV/HIV co-infected patients showed that 58% and 31% of the rs12979860CC carriers and 49% and 21% of the rs8099917TT carriers had SVR. IL28B SNPs, HCV mono-infection and HCV RNA load were associated with SVR as independent predictors in the two study groups as a whole. ROC curve analyses in the two populations separately, based on gender, age, baseline HCV RNA load and rs12979860/rs8099917 revealed similar receiver operating characteristics (ROC) areas under the curve values. Combining the determination of IL28B SNPs, rs8099917 genotyping improved the response prediction in rs12979860CT carriers only in mono-infected patients. In the era of direct-acting antiviral agents, adopting SVR baseline predictors to orientate naïve-patient management represents an important issue. A model involving IL28B SNPs appears able to predict SVR in both populations.

KEY WORDS: Hepatitis C virus-G1, Hepatitis C virus-G4, Interleukin-28B rs12979860, Interleukin-28B rs8099917, Human immunodeficiency virus.

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Corresponding author
Laura Sticchi
Hygiene Unit
IRCCS AOU San Martino Hospital-IST,
University of Genoa,
L.go R. Benzi, 10 - 16132 Genoa, Italy
E-mail: sticchi@unige.it

INTRODUCTION

Hepatitis C virus (HCV) represents a significant public health challenge with an estimated global burden of 170 million carriers worldwide, who may partly progress to cirrhosis and he-

patocellular carcinoma (Shepard *et al*., 2005). A number of new direct-acting antiviral agents (DAA) have recently been developed to improve the treatment response, in combination with pegylated interferon (Peg-IFN) and ribavirin (RBV) as triple therapy or an IFN-free regimen. However, DAAs are expensive, genotype-specific and induce the more rapid occurrence of resistant mutants (Kamal, 2014).

Therefore, the novel antiviral therapies in clinical practice need optimization, especially in discriminate patients who can still benefit from Peg-IFN/RBV therapy or those who need triple therapy or should wait for new more potent drugs (Rosso *et al*., 2014). In addition, it is noteworthy that conventional dual therapy may achieve successful treatment response in 40-50% of naïve patients with HCV1-infection with minor costs and drug-drug interactions (Simin *et al*., 2007).

For this reason, prediction of individual response before the start of therapy would be useful (Ahlenstiel *et al*., 2010). Several viral factors (such as genotype-HCV, *quasispecies* diversity, baseline viremia) and host factors (i.e. age, gender, ethnicity, liver fibrosis, body mass index, co-morbidities) have been studied as predictors of the natural course of hepatitis C and drug response.

A number of genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) near the interleukin 28B (IL28B) gene on chromosome 19 coding for IFNL-3 (rs12979860, rs8099917, rs12980275, and rs8103142) (Thomas *et al*., 2009; Ge *et al*., 2009; Rauch *et al*., 2010; Suppiah *et al*., 2009; Tanaka *et al*., 2009; Honda *et al*., 2010) strongly associated with treatment outcome and with spontaneous viral clearance in HCV-infected individuals (Balagopal *et al*., 2010; Zhang *et al*., 2011).

Other SNPs of IL28B (rs8105790, rs11881222, rs28416813, rs4803219 and rs7248668) have been studied in genotype 1-HCV-infected patients (Tanaka *et al*., 2009), although SNPs rs12979860 and rs8099917 have been found to represent the most important in different populations and the strongest predictors of sustained virological response (PVR) with the favorable variants, homozygosis for the SNPs rs12979860 (CC) and rs8099917 (TT), especially in HCV 1-infected patients treated with Peg-IFN/RBV (Suppiah *et al*., 2009; Tanaka *et al*., 2009) as well as in individuals co-infected with HIV (Rallón 2010). Recently, a new dinucleotide variant rs368234815 located near IL28B, in high linkage disequilibrium with rs12979860 creating the interferon-lambda protein (IFNL4), has been associated with HCV clearance (Prokunina-Olsson *et al*., 2013) and seemsto be better predictor than rs12979860 of treatment failure in co-infected patients (Franco *et al*., 2014).

The different distribution of IL28B SNPs in worldwide populations might explain the heterogeneous treatment response rate, extensively described in the literature (Ge *et al*., 2009; Thomas *et al*., 2009). The fact that the patient's genetic background is correlated to SVR encourages the perspective of personalized treatment decision-making and IL28B genotyping has increasingly become a significant diagnostic test for the management of HCV-infected patients, also useful in the new era of very potent DAA (Matsuura *et al*., 2014).

However, the question remains whether a cost-effective response-guided analysis should include one or more of these SNPs determinations, which IL28B variant should be chosen for diagnosis and how current and future genotyping of other polymorphisms may help evaluate the risk/benefit antiviral treatment profile in clinical practice.

This study describes the genotypes and allele distribution of IL28B rs12979860 and rs8099917 SNPs in a large cohort of HCV G1-4 infected individuals, with the aim to assess the predictive ability of these variations in patients who had received antiviral treatment, and to determine whether the combined determination of two IL28B SNPs might have improve the prediction of SVR both in HCV-mono-infected and in HIV/HCV-co-infected patients.

MATERIALS AND METHODS

Study cohort

This retrospective cohort was composed of 269 consecutive Caucasian patients with HCV G1-4 infection who were followed up at the main referral outpatient clinics of Infectious Diseases

and Gastroenterology in the Liguria Region, Northern Italy.
All patients had received a full course of combined therapy (Peg-IFN/RBV) and were tested for the IL28B SNP rs12979860 and rs8099917 genotypes, previously reported to be associated with treatment outcome (Ge *et al.*, 2009; Suppiah *et al.*, 2009; Tanaka *et al.*, 2009; Boglione *et al.*, 2014).
SVR was defined as HCV RNA undetectable by a sensitive assay at least 24 weeks after stopping therapy.
For this analysis fibrosis was classified as mild or severe by definition of stage of fibrosis in patients with a liver biopsy (F1-F2 or F3-F4), in accordance with Scheuer's scoring system, or by liver stiffness ≥8.7 kPa in those who had undergone transient elastography by FibroScan (Ziol *et al.*, 2005; Italian Association for the Study of the Liver (AISF) 2014).
All the activities were conducted in compliance with current healthcare standards and with the Declaration of Helsinki. All patients provided written informed consent according to local procedures of each clinical center. Ethical approval for conducting this study was unnecessary according to Italian legislation regarding the guidelines on observational studies, and on this basis, formal approval by local Institutional Review Boards is not required for retrospective cohort studies (Italian Medicines Agency 2008). Nevertheless, the study was notified to the Ethics Committee of the IRCCS AOU San Martino-IST Teaching Hospital of Genoa (Italy). According to Italian law, personal information concerning the subjects involved in the study was protected (Italian Law Decree n.196 2003).

HCV load and genotyping
Serum HCV-RNA load was tested using Cobas AmpliPrep/Cobas TaqMan HCV test (Roche Molecular Systems, USA) in accordance with the manufacturer's instructions, with a detection limit of 15 IU/ml; viral load was classified as low (<400000 IU/mL) or high (>400000 IU/mL) (Ladero *et al.*, 2012).
HCV genotyping was performed using a commercial real-time polymerase chain reaction (RT-PCR) hybridization assay (Versant HCV Genotype v2.0 LiPA; Siemens).

Genotyping for rs12979860 and rs8099917 SNPs
The DNA samples were genotyped for two sets of IL28B SNPs, rs12979860 and rs8099917, specifying by C or T and T or G allele, using a RT-PCR in a Rotor-Gene Thermocycler by fluorescent probes (Fast Set IL 28B, Arrow Diagnostics and Interleuchina 28B-realtime, Nuclear Laser Medicine), according to the manufacturer's instructions.

Statistical analysis
The baseline characteristics of the study cohort, genotype distributions and allele frequencies between the study groups were compared using χ^2 and Fisher exact test and the differences in numeric variables were evaluated with ANOVA test for normalized distributed variables or Wilcoxon signed-rank test for non-parametric statistical analysis as appropriate.
The odds ratio (ORs), 95% confidence intervals (CIs) and corresponding p-values were studied using logistic regression analysis to determine the predictors of treatment success. The ORs were calculated using the major homozygous allele as the reference group unless otherwise specified. The multivariate analysis was calculated using variables with p-value of <0.05 on the univariate analysis, except for age and gender variables as main characteristics for description of the study cohort.
All statistical analyses were performed using the STATA SE 9.2 program for windows (Statacorp, Texas, USA), a p-value of less than 0.05 was considered to be significant.

RESULTS

Features of the study population
Baseline characteristics of the study cohort are shown in Table 1. A total of 269 chronically HCV G1-4 infected patients were included in the study. One hundred and eighty-one and 88 individuals were HCV mono- and HCV/HIV co-infected, respectively.
The mean age was 49.7 years (standard deviation: +/-11.5) in mono-infected and 47 years (standard deviation: +/-9.9) in co-infected patients.
Among HIV/HCV co-infected subjects the median CD4+ T-lymphocyte count was 542 cells/

mm^3 (range: 52-1,719). Sixty-six (75%) patients were found with HIV RNA load <50 copies/mL. Twenty-two subjects had detectable viral load with a median of 3,300 copies/mL (range: 175-470,000). The 85% of the co-infected group was treated with combination antiretroviral therapy. Genotype 1-HCV infection was the most frequent in both groups (166/181, 91.7% HCV mono-in-

TABLE 1 - *Baseline characteristics of all HCV-monoinfected and HCV/HIV-co-infected patients according to their response to antiviral therapy.*

Variables	*HCV-monoinfected pts*			*HCV/HIV co-infected pts*		
N	*181*			*88*		
	Responders	*Non-responders*	*P value*	*Responders*	*Non-responders*	*P value*
Age (*years*) (n=181)			(n=88)			
≤50	27 (27.2)	72 (72.7)	0.06	3 (18.7)	13 (81.2)	0.84
>50	33 (40.2)	49 (59.8)		12 (16.7)	60 (83.3)	
Gender (n=181)			(n=88)			
males	24 (40.0)	52 (42.9)	0.71	4 (26.7)	16 (21.9)	0.69
females	36 (60.0)	69 (57.1)		11 (73.3)	57 (78.1)	
Plasma HCV RNA load (*IU/mL*) (n=161)	**2.2x106± 3.9x10⁶**	**4.0x10⁶± 7.8x10⁶**	**0.00**	(n=83) 1.7x10⁶± 4.1x10⁶	2.2x10⁶± 3.1x10⁶	0.14
Body mass index (*kg/m²*) (n=123)			(n=55)			0.57
<25	27 (27.2)	72 (72.7)	0.06	2 (13.3)	13 (86.7)	
>25	33 (40.2)	49 (59.7)		8 (20.0)	32 (80.0)	
Aspartate aminotransferase (*IU/L*) (n=147)	56.3±52.5	71.9±52.5	0.99	(n=74) **40.2±9.5**	**65.4±41.2**	**0.00**
Alanine aminotransferase (*IU/L*) (n=147)	**84.6±96.1**	**109.9±198.9**	**0.00**	(n=74) **55.2±21.6**	**79.9±55.1**	**0.00**
Platelet count (*x 10⁴/mm³*) (n=150)	20.6±7.6	17.9±9.3	0.12	(n=75) 22.5±44.3	16.44±76.3	0.08
White blood cell counts (*/mmc*) (n=143)	**5959.8± 1922.6**	**6231.6±5654.1**	**0.00**	(n=72) 5838.8± 1373.8	6309.8±2331.1	0.08
Degree of fibrosis (n=107)			(n=16)			
Mild	15 (53.6)	42 (53.2)	0.97	2 (66.7)	4 (30.7)	0.25
Severe	13 (46.4)	37 (46.8)		1 (33.3)	9 (69.2)	
HCV genotype (n=181)			(n=88)			
1	55 (91.7)	111 (91.7)	0.98	11 (73.3)	55 (75.3)	0.87
4	5 (8.3)	10 (8.3)		4 (26.7)	18 (24.6)	
Il-28b genotype frequency (n=181)			(n=88)			
Rs12979860						
CC	**28 (46.7)**	**20 (16.5)**	**0.00**	**9 (60.0)**	**20 (27.4)**	**0.047**
CT	**29 (48.3)**	**82 (67.7)**		**4 (26.7)**	**40 (54.8)**	
TT	**3 (5)**	**19 (15.7)**		**2 (13.3)**	**13 (17.8)**	
Rs8099917						
TT	**37 (61.6)**	**38 (31.4)**	**0.00**	11 (73.3)	42 (57.5)	0.11
TG	**20 (33.3)**	**67 (55.4)**		4 (26.6)	29 (39.7)	
GG	**3 (5)**	**16 (13.2)**		0 (0)	2 (2.7)	

NOTE. Data are no. (%) of patients or mean ± standard deviation. Statistically significant differences are shown in bold type. Fibrosis was classified as mild or severe by stage in patients with a liver biopsy (F1-F2 or F3-F4) or by liver stiffness >8.7 kPa.

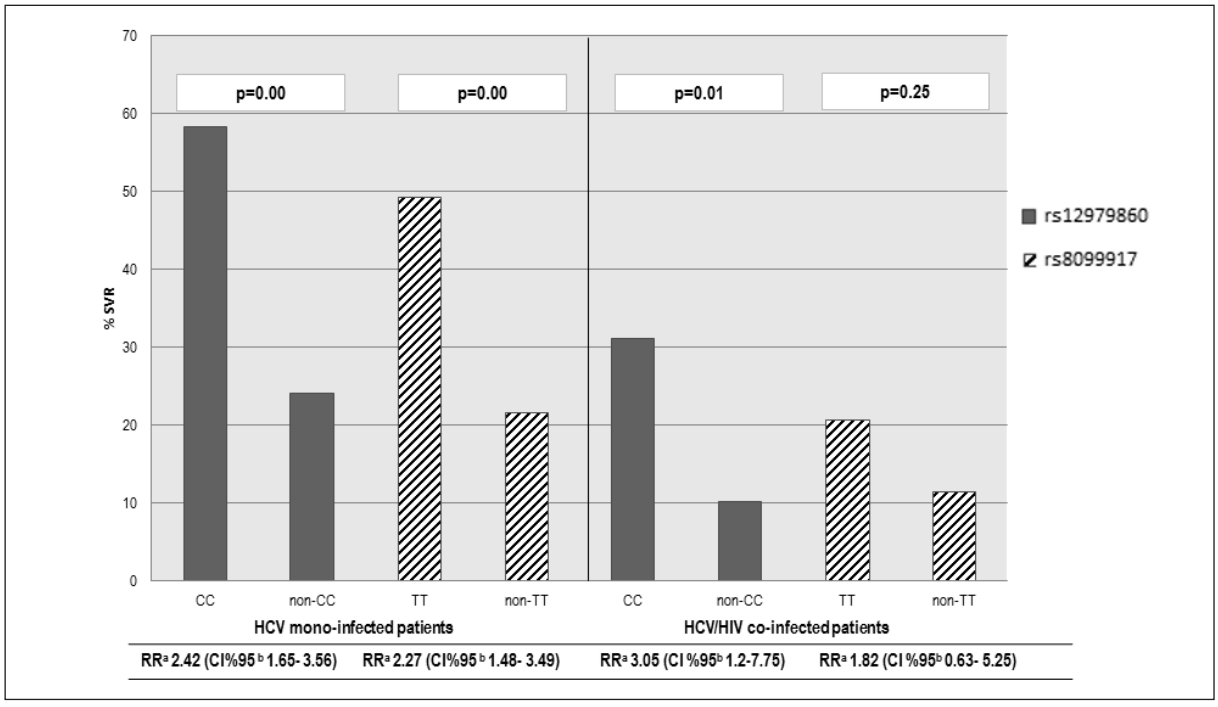

FIGURE 1 - *Sustained virologic response (SVR) rate according to rs12979860 and rs8099917 in HCV and HCV/HIV-infected.*

TABLE 2 - *Factors associated with SVR among HCV 1- and 4-infected patients.*

Variable	*N (%) of subjects with SVR*	*Univariate p*	*OR (CI 95%)*	*Multivariate p*
Age				
≤50	45 (29.22)	0.57	1.65 (0.83, 3.31)	0.15
>50	30 (26.08)			
Gender				
female	28 (29.16)	0.73	1.05 (0.54, 2.03)	0.88
male	47 (27.16)			
HCV mono-infection (*vs* HCV/HIV co-infection)				
Mono-infection	60 (33.14)	**0.01**	4.93 (2.20, 11.01)	**0.00**
Co-infection	15 (17.04)			
Body Mass Index (kg/m^2)				
≤25	26 (21.66)	0.06		
>25	20 (34.48)			
Liver fibrosis				
mild	14 (23.33)	0.64		
severe	17 (26.98)			
Baseline HCV RNA load				
<400000 IU/mL	29 (45.3)	**0.00**	3.88 (1.92, 7.80)	**0.00**
≥400000 IU/mL	43 (23.8)			
rs12979860 genotype				
CC	37 (48.05)	**0.00**	3.35 (1.56, 7.20)	**0.00**
TC/TT	38 (19.79)			
rs8099917 genotype				
TT	48 (37.50)	**0.00**	2.39 (1.10, 5.21)	**0.03**
TG/GG	27 (19.14)			

fected patients; 66/88, 75% HCV/HIV co-infected patients). Antiviral therapy with known virological outcome was performed in all patients. The overall SVR rate was 28% (33% and 17% in mono and co-infected individuals, respectively).
Mono-infected individuals, carriers of rs12979860CT genotype, were 61.3% (111/181), homozygotes for allele C were 48/181 (26.5%) and the remainder were homozygotes for allele T (12.5%). As for the rs8099917 SNP, genotypes were thus distributed: 75/181 (41.4%) carried the rs8099917TT genotype, whereas 87/181 (48.0%) and 19/181 (10.5%) were heterozygotes and homozygotes for the G allele.
In co-infected patients the IL28B genotypes were distributed as follows: 44/88 (50%) CT, 29/88 (32.9%) CC and 15/88 (17.0%) TT for the rs12979860, 53/88 (60.2%) TT, 31/88 (35.2%) TG, 2/88 (2.6%) GG for the rs8099917.

Rs12979860/rs8099917 SNPs and treatment response
Overall, SVR rates were higher in patients with the rs12979860CC and rs8099917TT genotypes compared to allele T and G carriers. Data stratified by HCV mono- and HCV/HIV co-infected patients showed that 58% and 31% of the rs12979860CC carriers and 49% and 21% of the rs8099917TT carriers had SVR, significantly more common in these patients, except to rs8099917TT in co-infected patients, probably due to the small sample size (Figure 1).
The SVR rates in favorable IL28B allele carriers were higher in HCV mono-infected than in HCV/HIV co-infected group (χ^2=5.4, p<0.05 for the rs12979860 and χ^2=10.8, p<0.001 for the rs8099917 SNPs).
Furthermore, factors associated with SVR like age, gender, HCV mono-infection (*vs* HCV/HIV co-infection), body mass index (BMI), fibrosis, higher baseline HCV RNA load and rs12979860/rs8099917 SNPs were subjected to univariate and multivariate logistic regression analysis (OR; 95% CI; P value) in HCV mono- and HCV/HIV co-infected patients as a whole.
Both in the univariate and in the multivariate analyses, rs12979860CC and rs8099917TT genotypes, HCV mono-infection and baseline HCV RNA load less than 400,000 IU/mL were significantly associated with SVR as independently predictors (p<0.05) (Table 2).

Receiver operating characteristic (ROC) curve analysis was performed separately in the two groups to compare the predictive power of a model that exclude the significant variable HCV/HIV co-infection identified in the multivariate analysis.
ROC curve analyses, which allow a comparison of predictive model based on gender, age, baseline HCV RNA load and rs12979860/rs8099917 SNPs in the two groups, revealed similar ROC areas under the curve (AUC) values (0.77 vs 0.74) (Figures 2a/2b).

Combination of rs12979860 and rs8099917
In unfavorable genotypes 1 and 4-HCV, the combined assessment of examined SNPs was evaluated, resulting in the three most prevalent genotypes (rs12979860CC/ rs8099917TT, rs12979860CT/

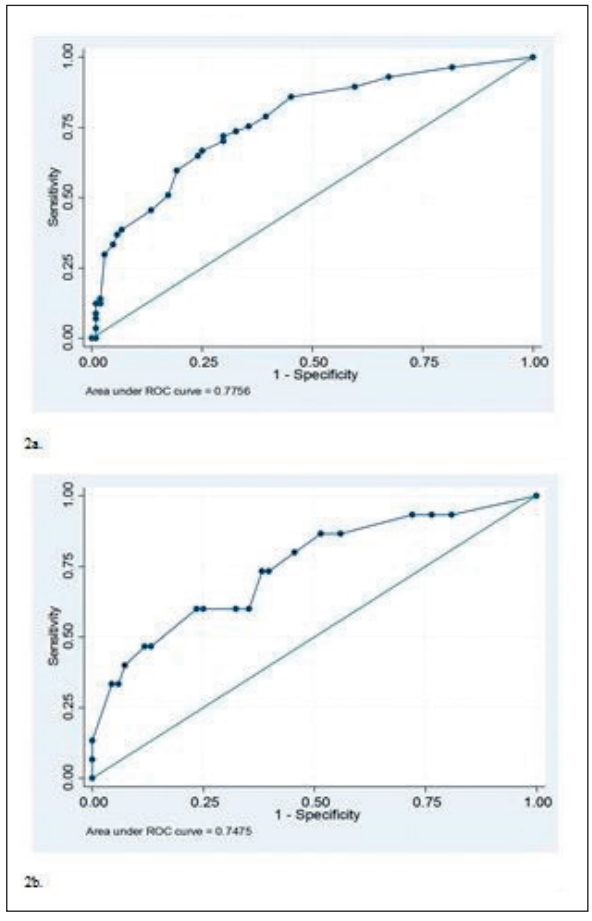

FIGURE 2 - *Association of SNPs genotyping with sustained virologic response (SVR) in 1-4 HCV- (A) and HCV/HIV-infected patients (B).*

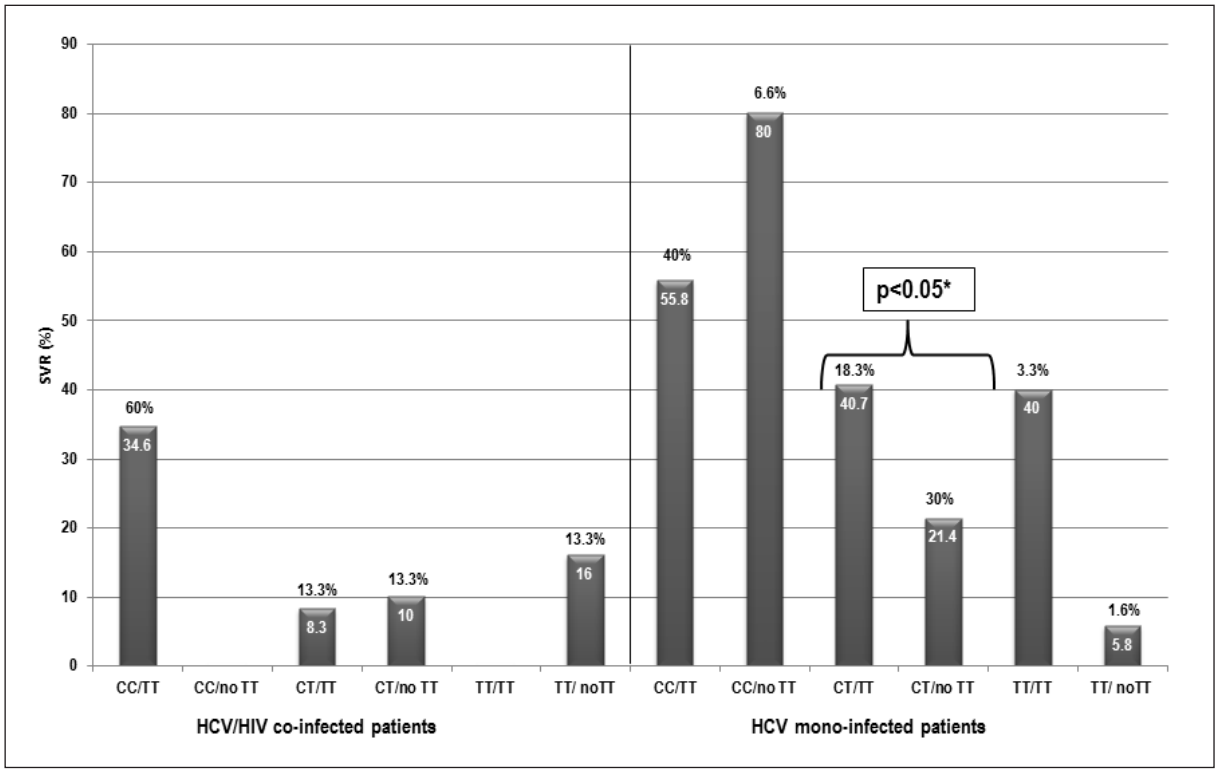

FIGURE 3 - *Association of rs1297860 and rs8099917 genotyping with SVR in HCV- and HCV/HIV-infected patients. *RR=1.95 (CI95% 1.03, 3.51)*

rs8099917noTT and rs12979860CT/rs8099917TT) with a frequency of 60%, 13% and 13% in HCV/HIV co-infected patients and 40%, 30% and 18% in HCV mono-infected patients, respectively (Figure 3).

Analyzing HCV mono-infected and HCV/HIV co-infected patients separately, the rs8099917 genotyping improved the response prediction in rs12979860CT carriers only in mono-infected patients (x_2=3.95; p=0.001), with a risk ratio of 1.95 (CI 95% 1.03, 3.51). In contrast, this positive effect was not appreciated in HCV/HIV co-infected patients (Figure 3).

DISCUSSION

In the era of new therapies recently implemented in clinical practice it should be not overlooked that a considerable number of HCV-1 infected patients are able to eliminate the virus with conventional dual therapy (Rosso *et al*., 2014). The ability to identify patients likely to achieve SVR with Peg-IFN/RBV appears clinically useful and appropriate for a cost-effective approach to identify eligible patients for triple therapy or to decide that the standard of care would be sufficient (Kamal 2014, Andriulli *et al*., 2014). Several virus and host factors (i.e. plasma HCV RNA load, fibrosis degree) significantly influence the likelihood of achieve SVR, whereas other features have less impact, such as age, gender, race and BMI (Andriulli *et al*., 2014, Fried 2002, Manns *et al*., 2001). In particular, IL28B genotyping is crucial when considering triple therapy; Ahlenstiel *et al*. suggest that triple therapy would be more valuable for carriers of the unfavorable IL28B allele, even if the role of IL28B SNPs may be weakened by the forthcoming more effective DAAs (Kamal 2014, Ahlenstiel *et al*., 2012). Genotyping HCV patients for IL28B polymorphisms, with other predictors of SVR, may be an important cost-effective screening method to optimize the risk/benefit ratio of combination therapies.

The present study describes the prevalence,

allele distribution and combined genotyping of rs1297860 and rs8099917 SNPs in different populations of HCV mono- and HIV/HCV co-infected individuals, by the simultaneous detection of the two IL28B loci.

Our results highlight the clinical utility of the IL28B gene variations in genotype 1 and 4-HCV-infected patients: the association between rs1297860CC and rs8099917TT and treatment outcome is very strong and SVR could be predicted in 58% and 49% in mono-infected, 31% and 21% in co-infected patients. These results are in line with other studies that have strongly demonstrated the effect of IL28B polymorphisms on SVR in HCV mono-infected (Lange and Zeuzem, 2011, Thompson *et al.*, 2010, Stättermayer *et al.*, 2011) and HCV/HIV co-infected patients with genotype 1-HCV (Rallon *et al.*, 2010, Pineda *et al.*, 2010, Neukam *et al.*, 2012).

Several studies have demonstrated that in addition to IL28B genotyping other baseline factors (such as HCV RNA load, age, gender, BMI, fibrosis stage, aspartate aminotransferase, alanine aminotransferase) play an important role in the selection of candidates for antiviral therapy (Wohnsland *et al.*, 2007, Kau *et al.*, 2008). Our multivariate analysis, conducted by taking into account the two study cohorts as a whole, has shown that the strongest predictor is HCV mono-infection (*vs* HCV/HIV co-infection) resulting highly significantly associated with SVR ($p<0.001$). Moreover rs1297860 and rs8099917 SNPs independently predict SVR with ORs 3.35 (CI 95% 1.56,7.20) and 2.39 (CI 95% 1.10, 5.21-22.44) respectively ($p<0.001$), compared to other parameters. Even though this study is limited by the fact that some baseline variables (pre-therapy liver biopsy or transient elastography, BMI or other biochemical data) were not available in all patients, our results are in line with other studies presenting similar data (Thomas *et al.*, 2009, Suppiah *et al.*, 2009, Tanaka *et al.*, 2009, Thompson *et al.*, 2010, Stättermayer *et al.*, 2011).

In order to clarify if it could be necessary to distinguish the HCV mono-infected and HCV/HIV co-infected population in a prediction model of response to antiviral therapy, we conducted the ROC curve analysis in the two study groups separately, showing that the mono- and co-infected populations presented similar predictive profiles. Several findings proven the role of IL28B variations on SVR in HIV/HCV co-infected patients (Rallon *et al.*, 2010, Pineda *et al.*, 2010), but further studies are required to evaluate the associations of the IL28B genotype with other factors. Indeed, the predictors of SVR seem to have singularities with respect to the mono-infected population because the status of HIV infection strongly impacts the success of HCV therapy (Pineda *et al.*, 2010, Soriano *et al.*, 2006). Moreover, some variables that may negatively impact on SVR are more common or exclusive in the setting of HIV infection, such as antiretroviral drugs interfering with hepatitis C therapy, CD4+ cell depletion, insulin resistance, liver steatosis, or more advanced fibrosis stages (Pineda *et al.*, 2010, Soriano *et al.*, 2006).

In the era of the more potent DAA, SVR rates appear to be similar in the HIV co-infected compared with HCV mono-infected patients treated with the same regimen (Sulkowski *et al.*, 2015, Osinusi *et al.*, 2015). The guidelines for the use of antiviral agents in HIV1 infected adults and adolescents maintain that "HIV/HCV co-infected persons should be treated and retreated the same as persons without HIV infection" (Dept of Health and Human Services 2015).

To define a predictive model for decision-making in treatment management, another significant issue is which IL28B variant should be chosen for diagnosis and if the determination of a single SNP can be sufficient to predict treatment outcome. In this study we analyzed the pattern of combined SNPs in HCV mono-infected and in HCV/HIV co-infected patients separately, to determine if the detection of IL28 rs8099917 SNP might improve the predictive value of rs12979860 on treatment outcome. The combined assessment of examined SNPs resulted in three most prevalent genotypes (rs12979860CC/rs8099917TT, rs12979860CT/rs8099917noTT and rs12979860CT/rs8099917TT), in agreement with the reported distribution in Caucasian (Fischer *et al.*, 2012, Galmozzi *et al.*, 2013). In HCV mono-infected patients carriers of rs12979860CT, the additional genotyping of rs8099917 SNP significantly improved SVR prediction. The rs12979860CT/rs8099917TT genotype was associated with 40.7% SVR, in contrast only 21.4% of patients with the

rs12979860CT/rs8099917noTT obtained SVR ($p<0.05$). Our finding is aligned with results reported by Fischer *et al*. in two large cohorts of 942 and 377 HCV 1-naïve patients: carriers of the rs12979860CT/rs8099917TT genotype had a 55% SVR rate compared to the 40% observed in patients with the rs12979860CT/rs8099917TG genotype ($p=0.001$). For this reason, the authors claimed that the combined determination of these two SNPs might be considered a further diagnostic procedure for improving treatment decisions (Fischer *et al*., 2012). In contrast, other authors affirmed that IL28B genotyping commercial tests should include one single polymorphism to give an unambiguous message to physicians and, moreover, that rs12979860 determination should be enough to predict treatment outcome because of its highest predictive value to SVR (Halfon *et al*., 2011). In particular, Galmozzi *et al*. in a combined analysis of IL28B SNPs in 187 HCV1-infected patients from an Italian cohort, did not confirm that carriers of rs12979860CT take advantage from the additional determination of rs8099917 for SVR prediction (rs12979860CT/rs8099917TT *vs* rs12979860CT/rs8099917TG: 43% *vs* 39%). This was probably due to differences in sample size and in viral and host characteristics between the cohorts from northern Europe studied in the Fischer *et al*. and the Italian cohort (Fischer *et al*., 2012, Galmozzi *et al*., 2013).

Within this controversial context, our study cohort, being Italian and with a relatively limited sample size, presents similar features and limits. Nevertheless, we appreciated the combined effect of different IL28B gene variants on SVR in heterozygous carriers of the rs12979860 T allele as in the northern Europe study cohorts. To the best of our knowledge, our results are the first that may support this approach.

Interestingly, this positive effect was not observed in HCV/HIV co-infected patients. Probably this is related to statistical power analysis, limiting our ability to detect significant associations between different rs12979860/rs8099917 genotypes and treatment outcome. Alternatively, it could be simply related to the status of HIV infection that strongly impacts the success of HCV therapy. However, it remains unclear whether in a larger patient cohort these associations might become significant. Further studies should probably be conducted to evaluate the impact of combined determinations of IL28B SNPs in this specific group.

In conclusion, adopting baseline predictors of SVR as a criterion to orientate the treatment management of naïve-patients represents an important issue and, although HCV mono- and HIV/HCV co-infected patients respond differently to antiviral therapy, a predictive model involving IL28B SNPs appears able to predict SVR in both populations.

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