Identification of naïve HVC-4 patients who may be treated with pegylated-interferon and ribavirin according to IL28B polymorphisms.

Identification of naïve HVC-4 patients who may be treated with pegylated-interferon and ribavirin according to IL28B polymorphisms

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Abstract

Background

The current treatment of HCV-4 patients is dual therapy with PEG-IFN and ribavirin; however, new drugs against this genotype will be available within few months. Despite the evidenced good virological response in IFN-free regimens, the high cost of these new therapies will require patient selection. In our paper we propose the use of both rs8099917 and rs12979860 IL28-B polymorphisms, in order to identify potentially categories of SVR, null-responder and relapse and consequently to choose the dual therapy or novel approach.

Methods

One hundred and sixty-nine patients with chronic hepatitis C and genotype 4 treated with pegylated interferon and ribavirin for 48 weeks were retrospectively studied. All patients were genotyped for rs8099917 and rs12979860 interleukin-28B polymorphisms.

Results

80 patients with SVR (88.8%) had the TT/CC or TT/TC (rs8099917/rs12979860) ($p < 0.001$) genotypes; the null-responders (n = 13), 9 (69.2%) showed the GG/TT allelic distribution ($p < 0.001$); relapsers showed a prevalent distribution of the TG/TC genotype (83.3%) ($p < 0.001$). The 6 (100%) breakthrough patients showed TT/TC genotype, while the partial responders patients did not show any particular IL-28B genetic profile. Genetic profiles different from TT/CC showed 94.9% negative predictive value for SVR, with 92.6% of sensitivity and 65.2% of specificity. Insulin-resistance, diabetes and liver fibrosis were not relevant in our multivariate analysis.

Conclusions

The combination of both rs8099917/rs12979860 polymorphisms is useful for early identification of SVR, null-responders and relapsers. This could be used to chose between standard dual therapy or novel approach with IFN-free regimens.

Abbreviations

HCV, hepatitis C virus;

HCV-4, hepatitis C virus genotype 4;

PEG-IFN, pegylated interferon alfa;

SVR, sustained virological response;

RVR, rapid virological response;

EVR, early virological response;

SNP, single nucleotide polymorphism;

IL, interleukin;

NR, null responder;

IR, insulin resistance;

PR, partial responder;

REL, relapser;

BT, breakthrough;

IQR, inter-quartile range;

SD, standard deviation;

PPV, positive predictive value;

NPV, negative predictive value;

DAAs, directly acting antivirals

1. Background

Hepatitis C virus (HCV) is a major cause of chronic hepatitis worldwide and the leading cause of liver transplantation in developed countries, with a global prevalence of 2%, representing 123 million infected people (Shepard et al., 2005). Hepatitis C virus genotype 4 (HCV-4) is prevailing in North and sub-Saharan Africa: Egypt has the highest HCV-4 incidence and prevalence (>13%) and HCV-4 represents 90% of all genotypes (Abdel-Aziz et al., 2000 and Nguyen and Keeffe, 2005). HCV-4 has recently spread in Europe, particularly in Italy, Greece, Spain, Netherlands and Germany, through immigration from Egypt and North Africa (Kamal, 2011) and among intravenous drug users. The treatment of HCV-4 infection has evolved in the last years: with the combined administration of pegylated interferon alfa (PEG-IFN) and ribavirin for 48 weeks, the rate of sustained virological response (SVR) ranges between 50 and 79%, being better than genotype 1 (Kamal et al., 2005). Main perspective is treatment optimization in the light of a better knowledge of host and viral factor that influence therapy responsiveness (Asselah et al., 2010). Rapid virological response (RVR) and early virological response (EVR) have been defined as the best predictive factors for SVR (Martinot-Peignoux et al., 2009). Patients with high plasma concentrations of ribavirin have higher chance for a better response to therapy (Aguilar Marucco et al., 2008, D'Avolio et al., 2011 and D'Avolio et al., 2012b), although with a greater rate of anemia (Brochot et al., 2010, D'Avolio et al., 2012a and van Vlerken et al., 2011). The influence of single nucleotide polymorphisms (SNP) rs8099917 and rs12979860 in the interleukin (IL)-28B region of chromosome 19 has recently been shown to be associated with response to treatment with PEG-IFN and ribavirin (D'Avolio et al., 2011 and Ge et al., 2009). Carriers of rs8099917 T/T and rs12979860 C/C polymorphisms showed higher rates of RVR, EVR and SVR (D'Avolio et al., 2011, D'Avolio et al., 2012b and Suppiah et al., 2009), and IL-28B genotyping could now play a decisive role in clinical practice of HCV treatment. The role of IL-28B in the treatment of HCV-4 hepatitis has recently been deepened by Asselah et al. (2012), and a strict relationship between rs12979860 polymorphism and the likelihood to reach SVR has been demonstrated.

Until now, the treatment of HCV-4 patients was performed only with PEG-IFN and ribavirin, but new drugs will be soon available against this genotype; as example, the association of sofosbuvir and ribavirin without PEG-IFN for 12 or 24 weeks, both in naïve or experienced patients has recently evidenced good virological response without significant adverse events (Ruane et al., 2013). These very promising results, however, have to be confirmed in a long-term follow up, which is currently unavailable. The main problem of this novel interferon-free treatment might possibly be a higher relapse rate after the end of therapy or drug resistance development, as observed in some regimens (Schinazi et al., 2014).

The use of this therapy is also associated with increased costs and, consequently, there will be the need of a careful discrimination of the patients who really need this new treatment from those who might benefit from standard regimen with PEG-IFN and ribavirin only.

The aim of our paper is to estimate a proportion of HCV-4 patients which could be effectively treated with standard therapy, and on the other side, to provide an early identification of patients with low probability of response, such as null-responders (NR), using a genetic combination of both rs8099917 and rs12979860 IL28B polymorphisms.

2. Methods

One hundred and sixty-nine patients with HCV-4 chronic hepatitis were retrospectively studied. Inclusion criteria were as follows: treatment-naïve patients with diagnosis of HCV-4 chronic hepatitis, without other viral co-infections (hepatitis B or HIV), and without major contraindications to the standard of care with PEG-IFN and ribavirin. Treatment with PEG-IFNα-2a at the dosage of 180 μg/week or PEG-IFNα-2b 1.5 μg/kg/week and ribavirin at 15 mg/kg/day were administered with a previewed duration of 48 weeks. Patients were genotyped for rs12979860 and rs8099917 polymorphisms. HCV-RNA was detected monthly during treatment and every 3 months during treatment follow-up. Insulin resistance (IR) was defined by a HOMA index >2. Cryoglobulins were measured as cryocrit (%) before treatment start. Liver fibrosis was measured with transient elastography (Fibroscan®) and expressed as METAVIR score. Patient follow-up was performed for 2 years after treatment completion with HCV RNA performed every 3 months.

RVR was defined as HCV-RNA undetectable after 4 weeks of therapy; EVR as HCV-RNA negative after 12 weeks; SVR as HCV-RNA undetectable 24 weeks after treatment completion. Treatment failure was defined as the lack of SVR. Four different categories of treatment failure were recognized: null-responders, when the decrease of HCV-RNA after 12 weeks of therapy was less than 2 log; partial responders (PR), if HCV-RNA decrease was more than 2 log after 12 weeks, but still detectable at week 24; relapsers (REL), when HCV-RNA undetectable at treatment completion, but positive in the follow-up without re-infection; breakthrough (BT), with HCV-RNA positive after an initial response (RVR or EVR) during the treatment. Treatment was discontinued in NRs at week 12 while in PRs at week 24. The study was conducted in compliance with the Declaration of Helsinki and with the local Review Board regulations; all patients gave written informed consent according to the local ethic committee standards.

2.1. IL-28B genotyping

Genomic DNA was isolated from blood samples. Patients who agreed to undergo genetic analyses were genotyped for rs8099917 and rs12979860 IL-28B polymorphisms with Taq Man Drug Metabolism Genotyping Assays (TaqMan MGM probes, FAM and VIC dye-labeled, Applied Biosystems by Life Technologies, Carlsbad, California, US), using a real-time polymerase chain reaction allelic discrimination

system (Bio-Rad Real-time thermal cycler CFX96) using a standard procedure (primers, probes, and PCR conditions available on request).

2.2. HCV RNA testing

HCV RNA was quantified in plasma samples with a commercially available real-time PCR system; the Cobas Ampliprep/Cobas TaqMan (HCV RNA vs. 2.0 Roche Molecular System, Branchburg, NJ, US) with a detection limit of 12 IU/mL. HCV genotping was performed with a Line Probe Assay (INNO-LiPA HCV™ II, Siemens HEALTHCARE Diagnostics).

2.3. Statistical analysis

For descriptive statistics, continuous variables were summarized as median (Inter-quartile range (IQR): 25th to 75th percentiles). Categorical variables were described as frequency and percentage. All data were assessed for normality using a Shapiro–Wilk test and categorical data were compared using a Mann Whitney or Kruskal–Wallis statistical test. To investigate continuous data, a Spearman Rank correlation was utilized. The association was calculated using the $\chi 2$-test.

Univariate analysis was performed to identify the candidate factors to be included in multivariate analysis. Multivariate linear regression analysis with stepwise forward selection was performed with p-values of less than 0.05 as the criteria for model inclusion.

Statistical analyses were conducted by using SPSS software package ver. 20.0 (Chicago, IL, USA).

2.4. Study end-points

Main goal of the study was to find out if in HCV-4 infected patients there was an association between IL-28 rs8099917 and rs12979860 polymorphisms genotypes, combined into haplotypes, and different treatment outcome, such as SVR, null responder, partial responder, relapser and breakthrough.

3. Results

3.1. Baseline characteristics of patients

Baseline characteristics of study population are reported in Table 1. We studied 169 naïve patients with HCV-4 chronic hepatitis treated with PEG-IFNα 2a/2b and ribavirin. Two principal ethnic groups were represented: Egyptians, 104 (61.5%) and Italians 57 (33.7%), 7 patients were from Morocco (4.1%) and 1 from Algeria (0.6%). Median age for all patients was 36 years (IQR, 31.5–42.5), in Egyptians was 34 (31.0–41.0), and in Italians 39 (34.5–44.0), without significant difference ($p = 0.084$). Forty-five patients had cryoglobulinemia before treatment (26.6%) without any symptoms in the majority of them (68.8%); insulin resistance was detected in 30.1% and diabetes in 14.2%. The majority of patients (57.4%) showed a mild liver fibrosis (F0–F1), whereas in 35.4% and 7.1% of them the fibrosis was moderate (F2–F3) and severe (F4), respectively.

Table 1.

Baseline characteristics and treatment outcome of study population; results are showed as median (Inter-quartile range).

N	169
Gender: male, n (%)	128 (75.7)
Age (yr)	36 (31.5–42.5)
Ethnic group, n (%)	
Egyptian	104 (61.5)
Italian	57 (33.7)
Morocco	7 (4.1)
Algeria	1 (0.6)
BMI (kg/m2)	25.4 (23.6–32.4)
BMI > 30, n (%)	14 (8.3)
Fibrosis stage [Metavir score], n (%)	
0	12 (7.1)
1	85 (50.3)
2	33 (19.5)
3	27 (15.9)
4	12 (7.1)
Stiffness [kPa]	8.9 (4–25)
Alanine aminotransferase (ALT) [IU/ml]	91.2 (19–266)
HCV genotype 4 subtypes, n (%)	
Indeterminate	61 (36.1)
a	10 (5.9)

acd	68 (40.2)
d	18 (10.6)
e	1 (0.6)
f	4 (2.4)
g	1 (0.6)
h	6 (3.6)
HCV-RNA [LogIU/ml]	5.2 (3.0–7.9)
PEG-IFN type, n (%)	
2a	109 (64.5)
2b	60 (35.5)
Treatment outcomes, n (%)	
RVR	61 (36.1)
EVR	35 (20.7)
Null-responders	13 (7.7)
Partial-responders	36 (21.3)
Relapsers	24 (14.3)
Breakthrough	6 (3.6)
SVR	90 (53.3)

3.2. IL-28B genetic profile and virological response

Fig. 1 reports IL-28 rs12979860 and rs8099917 polymorphisms distributions between Egyptians and Italians patients; this difference resulted not statistically significant ($p = 0.082$). Treatment outcomes were summarized in Table 1. The overall rate of SVR was 53.3% (50.9% in Italians, 53.8% in Egyptians, without significant difference ($p = 0.426$) between the two ethnic groups), RVR 36.1%, EVR 20.7%; NR 7.7%, PR 21.3%, relapser 14.3% and BT 3.6%.

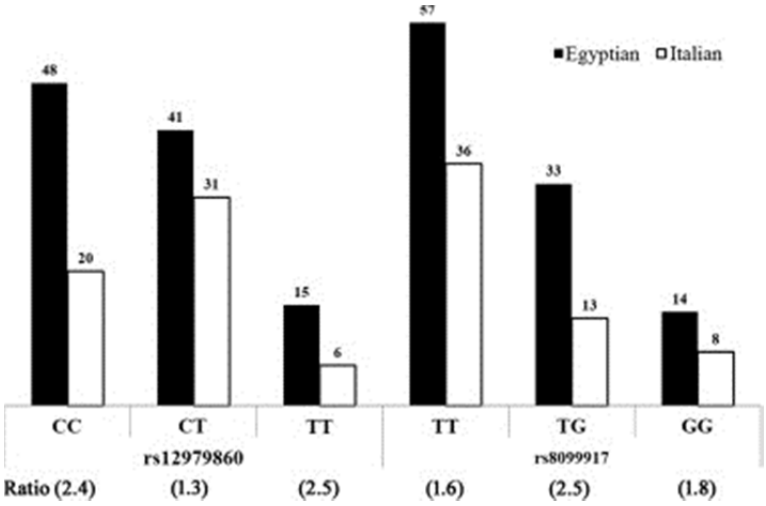

Fig. 1.

Genotype distributions of rs12979860 and rs8099917 IL28 polymorphisms in Egyptians vs. Italians patients.

We observed that RVR (p = 2.45 × 10−12) and EVR (p = 5.51 × 10−4) had the strongest significant correlation with IL-28 rs12979860 CC genotype. Conversely, SVR (p = 1.94 × 10−25) was strongly associated with rs8099917 TT genotype.

In Fig. 2 we reported rs8099917 and rs12979860 genotype distribution according to treatment outcome: we observed that among the 90 patients with SVR, 80 (88.8%) showed the TT/CC or TT/TC genotype; 31 (34.4%) the TT/TC one and 8 (8.8%) the TG/CC; only one (1.1%) presented the GG/TT genotype. Differences in SVR rate according to IL-28 haplotypes were statistically significant: TT/CC vs TT/TC (p = 0.004), TT/CC vs TG/CC (p = 0.001), TT/CC vs TG/CC (p < 0.001). Among the NR patients (n = 13), 9 (69.2%) showed the GG/TT genetic profile, 3 patients (23%) the TT/CC one and 1 patient (7.7%) the GG/TC one. All differences were statistically significant (p < 0.001). Relapsers showed a prevalent distribution of the TG/TC haplotype (83.3%), and only few patients had GG/TC or TT/TC profiles (p < 0.001). The 6 BT patients showed only TT/TC genotype, while PR patients did not show any particular IL-28B profile.

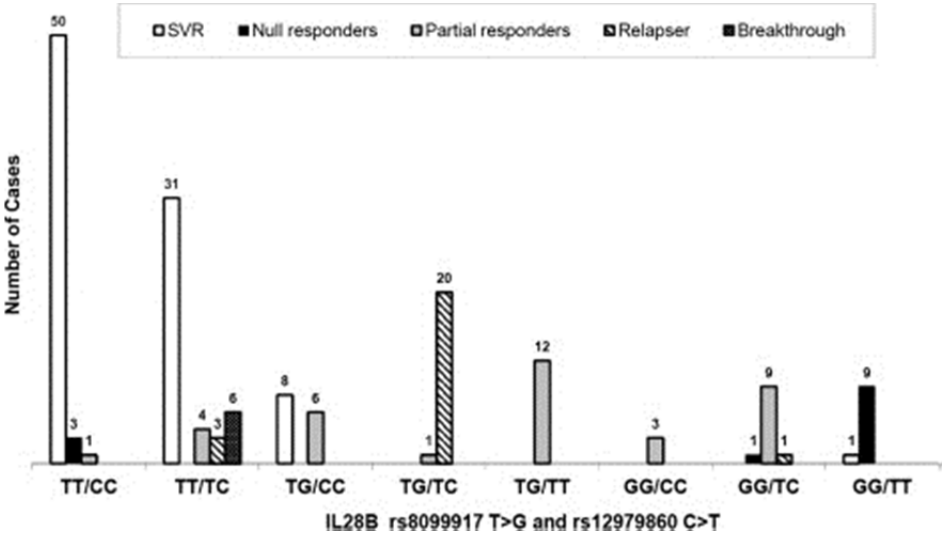

Fig. 2.

Genotype distributions of rs12979860 and rs8099917 IL28 polymorphisms according to the outcomes of the treatment.

In Table 2 results of univariate and multivariate linear regression analysis were reported. Univariate analysis for SVR identified ($p < 0.200$) the following factors: gender, viral load, ALT baseline, IL-28B TT/CC profile, PEG-IFN type and RVR; for NR only IL-28B GG/TT genotype; for relapsers, gender and IL-28B TG/TC genotype. In multivariate analysis only IL-28B profiles were statistically significant for all treatment outcomes ($p < 0.001$); RVR was significant for SVR ($p = 0.003$) and NR ($p = 0.001$).

Table 2.

Univariate and multivariate analysis between biological variables and clinical outcomes.

	SVR		NR		REL	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
	p value	p value	p value	p value	p value	p value
Age	0.399		0.757		0.701	
Gender	0.136	0.370	0.918		0.104	0.643
Ethnicity	0.441		0.105		0.505	
HCV-RNA	0.008	0.088	0.411		0.319	
ALT	0.016	0.744	0.847		0.600	
Metavir	0.238		0.503		0.323	
IL28B (917/860) TT/CC (SVR) GG/TT (NR) TG/TC (REL) TT/TC (BT)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interferon type	0.155	0.855	0.379		0.776	
RVR	<0.001	0.003	0.001	0.081	0.268	

Bold values are statistically significant by uni/multivariate analysis.

In Table 3 values of sensitivity, specificity, positive and negative predictive value (PPV, NPV) for IL-28B profiles according to respective outcomes were reported. We observed that the TT/CC profile showed a 92.6% sensitivity and 65.2% specificity for SVR, with PPV = 0.556 and NPV = 0.949; for the prediction of NR, GG/TT profile showed sensitivity and specificity of 90% and 97.5%, respectively, with PPV = 0.692 and NPV = 0.994; finally, for the prediction of relapse, the TG/TC profile showed sensitivity and specificity of 95.2% and 97.3%, respectively, with PPV = 0.833 and NPV = 0.993.

Table 3.

Predictive values of IL-28B genotypes and haplotypes (paired combination of two genotypes) for SVR, NR and REL.

	SVR			NR			Relapsers		
	917TT	860CC	917TT/860CC	917GG	860TT	917GG/860TT	917TG	860TC	917TG/860TC
PPV	0.900	0.644	0.556	0.769	0.692	0.692	0.833	1.000	0.833
NPV	0.785	0.835	0.949	0.910	0.917	0.994	0.814	0.641	0.993
Sensitivity	0.827	0.817	0.926	0.417	0.409	0.900	0.426	0.316	0.952
Specificity	0.873	0.673	0.652	0.979	0.973	0.975	0.967	1.000	0.973

PPV, positive predictive value; NPV, negative predictive value.

In Fig. 3 we described with a dichotomous tree an hypothetical decision process based on presence/absence of Il28B favorable genotype and presence/absence of RVR; we observed that patients with TT/CC genotype and RVR achieved the 100% of SVR, while patients without TT/CC genotype and without RVR obtained a very low rate of virological response (27.1%).

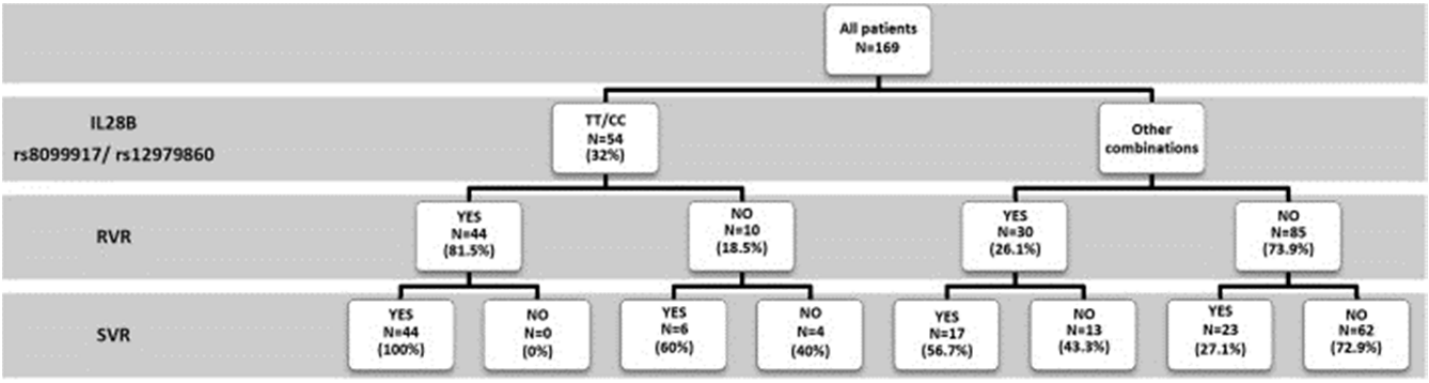

Fig. 3.

Flowchart showing the different rates of SVR according to IL28B genotype and RVR.

4. Discussion

The role of IL-28B polymorphisms on the outcome of anti-HCV treatment has been demonstrated in recent studies in which the correlation between rs12979860 and/or rs8099917 polymorphisms and SVR, RVR or EVR have been underlined (Asselah, 2010, Asselah et al., 2012, D'Avolio et al., 2011, Lange and Zeuzem, 2011, Rau et al., 2012, Tanaka et al., 2009 and Thompson et al., 2010). This correlation was highly significant for genotypes 1 and 4 and weaker in HCV genotypes 2 and 3 (Asselah et al., 2012, D'Avolio et al., 2011, D'Avolio et al., 2010, D'Avolio et al., 2012b, Stattermayer et al., 2011 and Tanaka et al., 2009).

In our study population of 169 HCV-4 infected patients, similarly to Asselah et al. (2012), we have noticed that the global SVR was 53.3%, also if the mean age in our patients was lower (36 vs 44.3 years). This finding was strictly related to the difference of Egyptian patients included in these studies. In fact, we have a great prevalence of immigrants from Egypt with predominance of younger males, but this ethnic difference did not affect the genotypes frequency of IL-28B polymorphisms.

Our study confirms previously reported data (D'Avolio et al., 2011 and D'Avolio et al., 2012b) indicating IL-28B polymorphisms as good negative predictive factors for SVR (Table 3). Moreover, rs12979860 CC patients were strongly related to RVR and EVR, confirming the rs12979860 polymorphism association with early viral clearance as for HCV-1 (Ge et al., 2009 and Prokunina-Olsson et al., 2013), while SVR was more strongly associated to rs8099917 TT, confirming the rs8099917 polymorphism association with sustained response, as for HCV-1 (Rau et al., 2012, Suppiah et al., 2009 and Tanaka et al., 2009).

This evidence was also recently confirmed in a systematic meta-analysis (Luo et al., 2012) where rs8099917 TT genotype resulted more correlated to SVR than rs12979860 CC (OR = 5.171 vs OR = 4.473 respectively).

Treatment failure, defined by the lack of SVR, is a heterogeneous classification that requires further detailed examinations: NR and PR have never been distinguished according to IL-28B polymorphisms. It is now confirmed that rs12979860 TT and rs8099917 GG genotype are related to non-response without any further discrimination according to the type of failure (Riva et al., 2012). The lack of response to HCV therapy (particularly for HCV-1 and 4) depends on treatment, ribavirin exposure, viral and host factors (Aguilar Marucco et al., 2008, Chevaliez and Asselah, 2011, D'Avolio et al., 2011, Jin et al., 2012, Loustaud-Ratti et al., 2008 and Pedersen et al., 2011), with a predominant role for genetic host factors such as IL-28B polymorphisms (Asselah et al., 2010, D'Avolio et al., 2011 and D'Avolio et al., 2012b). In our paper we focused our attention on the distinction between NR and PR patients. This is, in our opinion, a very important point for the different role of these outcomes and for the different clinical approach to be adopted in the future for HCV treatment. NR show the worse outcome and probably require novel therapies, maybe without interferon-based treatment (Di Bisceglie et al., 2006) or higher ribavirin dosage or exposure (Aguilar Marucco et al., 2008, D'Avolio et al., 2011, D'Avolio et al., 2012b, Jin et al., 2012, Morello et al., 2008 and Pedersen et al., 2011). For the first time we have observed that the NR and PR have two different IL28B genotype distributions. GG/TT genotype was significantly associated with NR, which may be considered as the more unfavourable IL-28B genetic profile. Our data showed high sensitivity and specificity for this IL-28B profile according to NR (90.0% and 97.5%, respectively; PPV and NPV: 69.2% and 99.4%, respectively). These data confirm the usefulness of the GG/TT profile to distinguish NR before treatment. Otherwise, the PR do not seem to have any significant distribution according to IL-28B profiles.

This finding could have some important clinical applications in identifying patients showing a IL-28B genetic profile prone to NR before treatment with PEG-IFN and ribavirin and then choose different therapy option based on directly acting antivirals (DAAs).

For the first time, in this study we also identified a IL-28B profile (TG/TC) related to HCV relapser after treatment completion.

Reasons of HCV relapse after therapy with PEG-IFN and ribavirin are not completely understood yet. Excluding low therapy adherence, some factors have been correlated with HCV relapse in previous studies: shortening duration of treatment was associated with reappearance of HCV-RNA, especially with 12–16 weeks of antiviral therapy in HCV genotypes 2 and 3 (Mangia et al., 2005 and Shiffman et al., 2007), while in HCV genotype 1, a high baseline viral load and overweight were related to HCV rebound (Fried et

al., 2008); the type of PEG-IFN was recently related to virological relapse, with a relapse rate in PEG-IFN 2a higher than 2b (29.3 vs 21.1, p = 0.0024) (Brochot et al., 2013). In HCV-4 possible causes of relapse have not been inquired. Interestingly, Asselah et al. (2012) evidenced a positive role of the C allele in the rs12979860 polymorphism to prevent relapse and NR. In our population, a single IL-28B combination TC/TG included 83% of relapsers and showed a good predictive value also in the multivariate analysis (Table 2); this genetic profile showed high sensitivity (95.2%), specificity (97.3%), PPV (83.3%) and NPV (99.3%), in absence of others causes of relapse that were excluded, such as poor adherence or lower doses of ribavirin/PEG-IFN. Interestingly, this TG/TC haplotype resulted to be the main factor related to relapse in our population. This information could be used to improve anti-HCV therapeutic strategy: extending the duration of therapy at 72 weeks, increasing the dosage of ribavirin and performing therapeutic drug monitoring (Brochot et al., 2010, D'Avolio et al., 2011, D'Avolio et al., 2012a, D'Avolio et al., 2012b, D'Avolio et al., 2006 and Slavenburg et al., 2011) or including DAAs.

Moreover, we have also considered in our univariate and multivariate analysis other factor previously associated with PEG-IFN non-response in genotype 4, such as insulin-resistance, diabetes and liver fibrosis (Moucari et al., 2009). But in our analysis, however, these factors became non-significant as compared with IL-28B polymorphisms and therefore they probably are unnecessary in order to the patient selection.

Combining the IL28B genotype and the RVR (Fig. 3) the clinician can direct the best therapeutic choice for patients with genotype 4; the dual therapy remains the optimal choice in patients with TT/CC genotype and RVR, in the other cases it would be appropriate to wait new DAAs, such as sofosbuvir which showed good rates of SVR in HCV-4 (Lawitz et al., 2013).

In conclusion, our study confirms that IL-28B polymorphisms have to be considered as a negative predictive factors for SVR and that using both IL-28B rs8099917/rs12979860 polymorphisms we can obtain a proper patient stratification according to the probability of response or non-response. As a consequence, the clinicians could use a novel genetic tool to redefine the initial choice of therapy with high probability of knowledge of outcome: in HCV-4 naïve patients with TT/CC genotype the main treatment choice should be the standard therapy with PEG-IFN and ribavirin for 48 weeks; the patients with GG/TT or TG/TC genotypic profiles could be treated with new drugs in IFN-free regimens.

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