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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.

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; 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

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.

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higher chance for a better response to therapy (Aguilar Marucco et al., 2008; D'Avolio et al., 2011, 2012b), although with a greater rate of anemia (Brochot et al., 2010; D'Avolio et al., 2012a; 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; 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, 2012b; 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 χ^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