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Low IL10 serum levels as key factor for predicting the sustained virological response to IFNα/ribavirin in Brazilian patients with HCV carrying *IL28B* CT/TT genotype

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ABSTRACT

Propose: *IL28B* polymorphisms rs12979860 CC genotype was associated to protection of HCV infection and sustained virological response (SVR) in HCV infected patients treated with pegIFNα/ribavirin (IFNα/RIB), however, this polymorphism frequency varies depending on genetic components. Studies with larger number of Brazilian individuals, determining *IL28B* polymorphisms is lacking. Regarding to treatment response, the levels of IL10 seem to influence response to IFNα/RIB therapy. Thus, the *IL28B* polymorphism frequency was investigated in health controls and infected HCV patients, as well as, in patients who reach SVR *vs* Non-SVR. Also, to gain insight into the interplay between *IL28B* genotypes, IL10 levels and therapy response, a subgroup of genotyped HCV patients SVR and Non-SVR were analyzed regarding the IL10 production.

Methods: It was enrolled 487 HCV infected patients and 234 healthy individuals. Patients with response to IFNα/RIB were classified as SVR ($n = 81$) and Non-SVR ($n = 123$). TAQMAN probes were used for genotyping the SNP rs12979860, resulting in CC, CT or TT genotypes. In one hundred one patients, the levels IL10 were measured at week 4 of IFNα/RIB.

Results: CC genotype was associated to SVR ($p = 0.029$) and its frequency was higher in healthy individuals *vs* patients ($p = 0.02$). Patients carrying CT/TT with IL10 < 10 pg/mL, had a chance of 2.72 to achieve SVR in multivariate model ($p = 0.043$).

Conclusion: CC genotype was associated to SVR and protection to HCV infection. Moreover, *IL28B* genotyping and IL10 serum levels could be further explored as a useful algorithm for identify the CT/TT SVR patients.

1. Introduction

It is estimated in the that hepatitis C virus (HCV) infection incidence is increasing, currently HCV affects more than 200 million people worldwide causing a chronic liver disease, that can progress to cirrhosis and hepatocellular carcinoma demanding a high rate of liver transplantation [1]. Discovery that SNPs at Interleukin 28b gene (*IL28B*) are strong predictors markers for spontaneously clearance and successful therapy of HCV, has raised questions about the mechanisms underlying this association [2,3].

IL28B encodes IL28b also known as Interferon λ3 (IFN λ3), categorized as type III IFNs including IL28a (IFN λ2) and IL29 (IFN λ1), in humans, these genes cluster on the long arm of chromosome 19 [4]. The lambda IFNs are functionally related to type I IFNα and IFNβ, inducing cellular signaling via Jak/signal transducers and activator of transcription (STAT), up-regulating IFN-stimulated genes (ISGs), which activates several mechanisms of antiviral response [5,6]. Nonetheless, differential expression of their subunits receptors and antiviral potency denotes that IFNs type I and III do not simple overlap their actions [7].

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Nowadays the conventional therapy for hepatitis C uses ribavirin (RIB), an antiviral drug combined with pegylated IFNα (IFNα), which was used as mono-therapy scheme before RIB association [8]. The continuous virus clearance up to 6 months after treatment represents a sustained virological response and patients carrying rs12979860 CC genotype at *IL28B* have higher chance to achieve this status when compared to the CT or TT genotypes carriers [2,9,10]. In addition, depending upon virological and other host factors the treatment failure can range from 20% to 50% [10].

Either the virus persistence in chronic infection, as well as, the high failure of treatment seems to be related to the ability of HCV to scape by modulation of immune response, since in early acute phase of HCV infection the IFNs types I and III control viral replication [11]. Consequently, HCV seem to create an unique strategy to evade immune response, which should allow its co-existence, for years in the liver environment, within a permanent IFN activated system [12].

The alteration on the balance of cytokines involved in the inflammatory modulation, specifically the up regulation of Interleukin 10 (IL10) seem to be the main key for controlling the host immunity against HCV. IL10 promotes MHC II downregulation, impairing important cellular response to the virus [13,14]. Besides, the $CD8^+$ T and $CD4^+$ Th1 lymphocytes dysfunction in HCV infected patients can occur by direct cellular negative regulation [15].

In this sense, improvement of the response of the combined IFNα/RIB may be related to the RIB induction of IL10 decrease [16] and the IFNα innate immunity stimulation, favoring Th1 response with a rescue of the efficient antiviral cellular immunity. Previously studies had shown that low levels of serum IL10 before or during HCV therapy were associated to final successful virological response (SVR) [17–20]. The decrease of IL10 in sustained responders patients occurs as early as 3 days after IFNα/RIB therapy [21].

The production of IL10 in response to the IFNα/RIB and the *IL28B* genotype could be synergic immunological factors in achieving sustained virus clearance. In fact Umemura et al. [22], observed that Japanese patients carrying TT compared to TG/GG genotypes at the rs8099917 of the *IL28B* had lower levels of IL10 and showed sustained virological response [22].

In a previously study with Brazilian patients the frequency of the minor allele G from *IL28B* at the rs8099917 was lower (0.20) than the minor allele T at rs12979860 (0.55) [23], suggesting that the former is more suitable for performing studies on the therapy response. Therefore, our aim was determined the frequency of *IL28B* polymorphism in heath and HCV infected patients and also associate the levels of IL10 with the rs12979860 C/T at the *IL28B* and response to the IFNα/RIB therapy.

2. Materials and methods

2.1. Patients and treatment

The present study included patients chronically infected with HCV ($n = 487$) attended at the Gastrohepatology Service of the Oswaldo Cruz University Hospital of the University of Pernambuco and Liver Institute of Pernambuco, IFP-PE (Recife, northeastern Brazil) and blood donors ($n = 234$) matched by geographical region which were genotyped for *IL28B* at rs12979860. The patients included in this study had anti-HCV positive serology (Roche Elecsys, Rotkreuz, Switzerland) and were positive for HCV-RNA (Roche COBAS Amplicor System, Rotkreuz, Switzerland). The HCV genotyping was performed by VERSANT *HCV Genotype* 2.0 Assay (Roche Diagnosis, Rotkreuz, Switzerland). Co-infections with hepatitis A, hepatitis B viruses, and immunodeficiency virus were considered exclusion criteria. For blood donors group the inclusion criteria were to have negative serology for HCV, HBV, HIV and HTLV.

Two hundred four patients were treated with peginteferon-α associated with ribavirin (IFN-α/RIB) either 24 or 48 weeks according to HCV non-1 and 1 genotypes respectively. The therapy consisted of the administration of peg-IFN α-2a, 180 μg or alfa-2b, 1.5 μg/kg weekly, both associated with ribavirin 15 μg/kg/day. Standard definitions of treatment used were according to international practicing guidelines [24]. Patients who remained with undetectable HCV RNA 6 months after the end of therapy were consider to achieve sustained virological response (SVR), those who had undetectable HCV RNA during the treatment but had a detectable HCV RNA within 6 months of concluding the treatment were considered relapses (RL) and those who did not achieved a negative HCV RNA during the therapy were non-responders (NR). For analysis the patients were grouped as SVR and Non-SVR (NR/RL).

2.2. Sample collection, DNA extraction and genotyping of IL28B (rs12979860)

The patients attended at a regular basis in the ambulatory of IFP clinics were informed about the study and at this time samples of whole blood were draw in tubes containing EDTA solution and kept at −20 °C for 24 h until DNA extraction. The DNA was extracted from whole blood samples using the Wizard Genomic Purification Kit (Promega, Madison, WI) following the manufacturer's instructions. The SNP for *IL28B* (rs12979860) was genotyped by real-time PCR, using specific probes, performed by the TaqMan system (Applied Biosystems, Foster City, CA). The probes, primes and validated protocols for the SNPs are available at http://snp500cancer.nci.nih.gov.

2.3. Serum levels quantification of IL10

Serum IL10 was measured in 121 out of 204 patients, which had been collected the serum in the end of week 4 of treatment and had the complete analysis for treatment response. The samples were collected in tubes without additive and centrifuged at 1500*g* for 10 min and stored at −80 °C until analysis. IL10 level was measured in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, US). All samples were diluted 1:20, with the calibrator diluent, according to the manufacturer's instructions. Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (BioRad, CA, USA). The cutoff was set based on the IL10 median levels of CT/TT patients who achieved the SVR and the results of receiver operating characteristic (ROC) curve for serum levels using moderate criteria.

2.4. Statistical analysis

Statistical analysis was performed using SPSS statistical software package version 17.0 (SPSS, Inc., Chicago, IL). Categorical variables were compared using the χ^2 test or Fisher's exact test when appropriate. Two-group comparisons were performed using the Student's t test or Mann–Whitney U-test for parametrically or non-parametrically distributed data. For generate a cutoff for IL10 levels in CT/TT patients regarding therapy response a combine analysis of IL10 median values in CT/TT carrying patients and a receiving operating characteristic (ROC) curve, in these patients, using moderate criteria was calculated. Binary logistic regression was performed to identify predictors of SVR. The results are presented using odds ratio (OR) with 95% confidence interval (CI). The differences were considered statistically significant when the p-value was <0.05.