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Role of *ITPA* and *SLC28A2* genes in the prediction of anaemia associated with protease inhibitor plus ribavirin and peginterferon in hepatitis C treatment

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ABSTRACT

Background: Anaemia is a common side-effect of ribavirin (RBV) use that overwhelms management of hepatitis C when protease inhibitors are added.
Aim: To assess the pharmacogenomic impact of candidate genes SLC28A2, SLC28A3 and ITPA on anaemia in patients receiving triple therapy.
Methods: Patients ($n = 161$) with chronic hepatitis C genotype 1 treated with telaprevir ($n = 95$) or boceprevir ($n = 66$) were included. Using RT-PCR we genotyped ITPA (rs1127354, rs7270101) and SLC28A3 (rs56350726, rs10868138) and SLC28A2 (rs11854484). Clinically significant anaemia (CSA) was diagnosed when at least one of the following criteria was observed: (a) haemoglobin <8.5 g/dL during treatment; (b) blood transfusion required; (c) erythropoietin administered.
Results: CSA occurred in 44% (69/157) of patients and was associated with SLC28A2 rs11854484 [CC/CT genotypes: 33% (26/78) vs. TT genotype: 56% (36/64); $p = 0.006$]. Further, the needed for blood transfusion was related to genotype [CC: 0% (0/18) vs. CT: 13% (8/61) vs. TT: 27% (17/64); $p = 0.016$]. Similarly, ITPA rs1127354 genotypes [AA/AC: 19% (3/16) vs. CC: 45% (61/135; $p = 0.060$] were linked to CSA. In multivariate analysis, SLC28A2 rs11854484 TT genotype (OR:2.33;95%CI:1.10–4.95; $p = 0.027$), female sex (OR:2.54;95% CI:1.13–5.71;$p = 0.024$) and Hb drop at week 4) OR: 1.36; 95CI%: 1.11–1.67; $p = 0.003$) were independently associated with CSA. Similarly, ITPA rs1127354 genotypes [AA/AC: 16% (3/19) vs. CC: 63% (85/134); $p = 0.0001$] and ITPA rs6051702 genotypes [CC/CA: 46% (26/57) vs. CC: 65% (60/93); $p = 0.023$] were related to Hb drop of >3g/dL at week 4.
Conclusions: In patients receiving first generation protease inhibitors, genotype SLC28A2 rs11854484 predicts CSA, and helps to identify a subgroup of patients with better tolerance of triple therapy.

1. Introduction

Haemolytic anaemia is a common side-effect of ribavirin-containing antiviral treatment of hepatitis C infection [1]. Adding non-structural 3/4A protease inhibitors to peginterferon + ribavirin was shown to achieve higher rates of sustained virological response (SVR) but also increased anaemia rate and its clinical sequelae, This required more frequent modifications of ribavirin dosage, erythropoietin administration and blood transfusions [2,3]. High anaemia

Abbreviations: CNT, concentrative nucleoside transporter; GWAS, genome-wide association studies; Hb, haemoglobin; HCV, hepatitis C virus; ITPA, inosinetriphosphatase; SVR, sustained virological response.

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rates could be related not just to a boosting effect by protease inhibitors [4] but also to a direct effect of these drugs on the erythrocyte life cycle.

Genome-wide association studies (GWAS) have shown that some polymorphisms in inosinetriphosphatase pyrophosphohydrolase (ITPA) gene protected against ribavirin-induced haemolytic anaemia [5] and decreased ribavirin dose reduction rates in patients treated with peginterferon + ribavirin [6]. Further, some *ITPA* polymorphisms have been associated with anaemia in telaprevir-based triple therapy [7]. Despite on-treatment anaemia being associated with higher SVR rates during peginterferon + ribavirin therapy, ITPase deficiency protects against ribavirin-induced anaemia but is not associated with SVR [8]. Conversely, the family of concentrative nucleoside transporters (CNTs), encoded by SLC28 genes, mediates sodium-dependent uptake of nucleosides, and their analogues including ribavirin. CNT2 (encoded by SLC28A2) [9] and CNT3 (encoded by SLC28A3) [10] are the main uptake transporters of ribavirin in the small intestine. Mutant SLC28A3 haplotype has been associated with a lower incidence of severe haemolytic anaemia in patients receiving peginterferon + ribavirin [11].

In the current study, we assessed the interaction between these candidate genes (*SLC28A2* and *ITPA*), and anaemia in patients with chronic hepatitis C infection receiving protease inhibitor (PI)-based triple therapy.

2. Materials and methods

2.1. Hepatitis C treatment

In this prospective multicentre study, 161 patients with chronic hepatitis C infection receiving triple therapy were enrolled. All patients received boceprevir (800 mg t.i.d. over 44 weeks following a 4-week lead-in phase with peginterferon + ribavirin) or telaprevir (750 mg t.i.d over 12 weeks), both combined with peginterferon + ribavirin over 48 weeks [12]. As such, the patients were segregated into two groups: (a) patients receiving telaprevir from treatment commencement (95/161); (b) patients with a 4-week lead-in phase with peginterferon + ribavirin followed by the addition of boceprevir (66/161). Adverse events, mainly anaemia and rash, were managed according to standard guidelines [2,13].

2.2. Selection criteria

Inclusion criteria were: patients between 18 and 70 years of age, persistent positive antibodies to HCV and HCV-RNA positive. Exclusion criteria were: chronic hepatitis B and D infection; HIV infection; autoimmune hepatitis; Wilson's disease; primary biliary cirrhosis; clinical or biochemical evidence of hepatic decompensation (ascites, variceal bleeding, spontaneous bacterial peritonitis or encephalopathy).

All patients provided written informed consent for the collection and storage of peripheral blood mononuclear cells, as well as host DNA testing for research purposes consistent with the current study. All data were coded to maintain anonymity. The database for this analysis included clinical and demographic data extracted from the original clinical database. The study was approved by the Ethics Committees of each participating Hospital Centre. The study was conducted in accordance with the provisions of the declaration of Helsinki, and Good Clinical Practice guidelines.

2.3. Clinical endpoints

Clinically-significant anaemia was considered when at least one of the following was noted: (a) haemoglobin (Hb) <8.5 g/dL during therapy; (b) blood transfusion required; (c) erythropoietin administered. Also, anaemia was considered when Hb dropped after 4 weeks of PI exposure (week 4 for telaprevir and week 8 for boceprevir). Hb levels were routinely monitored every 4 weeks. The anaemia was managed according current recommendations [2].

2.4. DNA isolation and genotyping

Genetic polymorphisms in terms of single nucleotide polymorphisms (SNPs) of the *ITPA* gene (rs1127354 and rs7270101), *SLC28A2* (rs11854484) and *SLC28A3* (rs56350726, rs10868138) were determined using the StepOnePlus Real Time PCR System (Applied Biosystems, Foster City, USA) with a TaqMan SNP Genotyping Assay we developed for the applied biosystems using published sequences from the NCBI Entrez SNP Database (www.ncbi.nlm.nih.gov/sites/entrez). Alleles and genotype frequencies distribution of all SNPs were in accordance with Hardy–Weinberg equilibrium.

2.5. Histological features

The minimum length of the liver biopsy was 15 mm and at least 10 complete portal tracts were necessary for inclusion into the study analyses. Histological evaluation was carried out by the same pathologist at each of the Hospital Centres using Scheuer scoring: [14] F0 (none portal fibrosis), F1 (some-most portal fibrosis), F2 (few bridging fibrosis), F3 (many bridging fibrosis), F4 (cirrhosis). In patients with biochemical, endoscopic or ultrasonographic diagnosis of cirrhosis, the diagnosis was confirmed by transient elastography.

2.6. Statistical analysis

Statistical analyses and graphic presentation of the data were performed throughout with SPSS (version 19.0; SPSS Inc., Chicago, IL). All values are presented as means ± SD. Comparisons between groups were with the Mann–Whitney *U* test, the Student *t*-test or ANOVA for continuous variables, and the χ^2 or the Fisher exact probability test for categorical data. The observed distribution of homozygous and heterozygous patients was compared to the expected distribution according to the NCBI SNP database. Variables that showed a probability value of $p < 0.10$ in univariate analysis were entered into backward logistic regression analysis to identify independent factors predictive of anaemia and Hb drop. The multivariate models were constructed sequentially with variables entered individually. A significance level of 0.05 was used to remove the variables from the model, except age and sex variables which were included in all the models. Values were considered to be statistically significant when $p < 0.05$.

3. Results

3.1. Prediction of clinically significant anaemia

Clinically significant anaemia was detected in 44% (69/157) of the overall cohort, while rash was present in (35%; 55/157) and pruritus in 45.2% (71/157). Anemia was not influenced by telaprevir (47%; 49/104) or boceprevir use (38%; 20/53) ($p = 0.263$) even stratifying by SNPs, neither genotype 1a (32%) nor 1b (46%) ($p = 0.181$). More elderly patients had anaemia than those younger (58 ± 8.2 vs. 54 ± 8.3 years; $p = 0.002$), and more frequently in females than males (62% vs. 36%; $p = 0.002$). Patients developing anaemia had lower baseline Hb values compared to those who did not (14.1 ± 1.9 g/dL vs. 15.1 ± 1.6 g/dL; $p = 0.001$). In univariate analysis, *SLC28A2* rs11854484 genotypes (CC/CT 33% vs. TT 56%; $p = 0.006$) was protective against clinically-significant anaemia and *ITPA* rs1127354 genotypes (AA/AC 19% vs. CC 45%; $p = 0.060$) showed a similar trend. In multivariate analysis, *SLC28A2* rs11854484 TT