Association of ITPA gene polymorphisms and the risk of ribavirin-induced anemia in HIV/HCV co-infected patients on HCV combination therapy

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Running headline: ITPA and RBV-induced anemia in HIV/HCV co-infection

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Abstract

Polymorphisms of the ITPA gene have been associated with anemia during combination therapy in HCV mono-infected patients. Our aim was to confirm this association in HIV/HCV co-infected patients.

Methods

In this prospective, observational study, 73 HIV/HCV co-infected patients treated with Peg-IFN plus RBV were enrolled. Two SNPs within or adjacent to ITPA gene (rs1127354 and rs7270101) were genotyped. The associations between the ITPA genotype and anemia or treatment outcome were examined.

Results

Fifty-nine patients (80.8%) had CC at rs 1127354 whereas 14 (19.2%) had a CA/AA ITPA genotype. Percent decreases from baseline hemoglobin level were significantly greater in patients with CC than in those with CA/AA genotype at week 4 (P = 0.0003), week 12 (P < 0.0001), week 36 (P = 0.0102), but not at the end of treatment. RBV dose reduction was more often needed in patients with CC than in those with CA/AA genotype (OR = 11.81; 95%CI: 1.45-256.17, P = 0.0039) as was erythropoietin therapy (OR = 8.28; 95%CI: 1.04-371.12, P = 0.0057). Risk factors independently associated with percent hemoglobin nadir decrease were: RBV dose reduction (OR = 11.72; 95CI: 6.82-16.63, P < 0.001), baseline hemoglobin (OR = 1.69; 95CI: 0.23-3.15, P = 0.024), and BMI (OR = -0.7, 95CI: -1.43-0.03, P = 0.061). ITPA polymorphism was not an independent predictor of sustained virological response.

Conclusions

Polymorphisms at rs 1127354 in the ITPA gene influence hemoglobin levels during combination HCV therapy and the need for RBV dose reduction and erythropoietin use in HIV/HCV co-infected patients.

Word count: 250
Key words: Inosine triphosphatase, Inosine triphosphate, pegylated interferon, ribavirin, anemia, hemolysis, erythropoietin, polymorphism, HIV/HCV co-infection, sustained virological response.
Introduction

Hepatitis C virus (HCV) infection is one of the most common co-morbid conditions in patients with human immunodeficiency virus (HIV) infection [25]. Its prevalence is especially high in Southern European countries, and its importance is highlighted by the fact that end-stage liver disease is an important cause of death in HCV/HIV co-infected patients [2, 25]. The standard of care in HCV infection in HIV/HCV co-infected patients is combined therapy with pegylated interferon (PEG-IFN) plus ribavirin (RBV), although the success rate of such combination antiviral therapy is around 30% in patients with genotype 1 HCV infection [5, 33]. Furthermore, HCV therapy is difficult to tolerate with significant associated morbidity. Among the important adverse effects that may compromise the effectiveness of HCV therapy, hematologic toxicity is common and may lead to RBV dose reductions that may affect treatment efficacy [5, 33]. Among HCV therapy-associated hematologic adverse effects, anemia is particularly important because of its implications for treatment outcomes and its frequency.

Anemia in the setting of HCV therapy may be caused by both components of treatment: a bone marrow blockade by PEG-IFN and hemolytic anemia induced by RBV [3, 8]. Recently, two functional variants in the inosine triphosphatase (ITPA) gene, which encodes for ITPA on chromosome 20, have been associated with RBV-induced anemia in HCV mono-infected patients treated with PEG-IFN plus RBV [9]. We sought to confirm such an association in a cohort of HIV/HCV co-infected patients treated with PEG-IFN plus RBV, trying to replicate the association between the functional ITPA variants and hemoglobin decrease over the course of HCV therapy. Our working hypothesis was that ITPA gene polymorphisms are associated with RBV-induced anemia in HCV/HIV co-infected patients treated with PEGIFN + RBV.

Patients and Methods

Study Population. The cohort of patients in the present study is derived from a well-characterized cohort of 389 HIV/HCV co-infected patients on active follow-up at the
Hospital de la Santa Creu i Sant Pau in Barcelona. All the patients consented to the provision of genetic material as part of their co-infections assessment. To be included in the study all the patients had to be stable, either treated or untreated, with respect to HIV infection. Non-responders to previous IFN-based therapies were not included in the study. Patients with chronic renal disease or creatinine clearance ≤50 mL/min, hemoglobin ≤11.5 g/dL, neutrophil ≤1,500/mm³ or platelet ≤70,000/mm³ at baseline were also excluded. Subjects who were hospitalized or had a frank cognitive impairment such as delirium or dementia on enrolment were not eligible. Patients with opportunistic infections, neoplasms or fever of undetermined origin were not considered for HCV therapy. The diagnosis of AIDS was based on the 1993 revised case definition of the Centers for Disease Control and Prevention [6]. All the patients were negative for hepatitis B surface antigen, did not have evidence of other liver diseases, and had not received other therapies, except for combined antiretroviral therapy. All patients had had abnormal levels of serum alanine amino transferase (ALT) for more than 6 months and were positive for anti-HCV antibody and serum HCV-RNA. The study was approved by the Ethics Committee of the Hospital de la Santa Creu i Sant Pau.

HCV RNA levels

Plasma HCV-RNA was measured using a real-time PCR assay (COBAS TaqMan, Roche, Barcelona, Spain), which has a detection limit below 15 IU/ml. HCV genotyping was performed using a commercial real-time PCR hybridization assay (Versant HCV Genotype v2.0 LIPA; Siemens, Barcelona, Spain).

Liver fibrosis staging

The extent of liver fibrosis was measured using transient elastography by FibroScan (Echosens, Paris, France). The median value of all tests per patient is expressed in kiloPascal (kPa) units. Cirrhosis, corresponding to METAVIR score F4, was defined for liver stiffness values of 14 kPa or higher.

HCV combination therapy

Treatment regimens included PEG-IFN alpha 2α at standard doses (180 µg per week) plus weight-adjusted RBV (1000 mg/day for patients weighting <75 kg and 1200...
mg/day for patients weighting > 75 kg). Patients with HCV genotypes 1 or 4 received either 48 or 72 weeks of treatment; patients with HCV genotype 3 received 24 or 48 weeks of treatment, according to virological response at week 4 (patients with positive HCV viral load at week 4 had six months more of treatment). Therapy was stopped in patients with a suboptimal virological response at weeks 12 (HCV viral load decrease under two logs with respect to baseline values) and 24 (positive HCV viral load). Sustained virological response (SVR) was defined as undetectable HCV RNA in serum at the end of follow-up (24 weeks after cessation of treatment). Patients in whom qualitative serum HCV RNA test result was positive at 24 weeks were considered non responders, and therapy was stopped.

PEG-IFN and RBV dose modification followed the standard criteria and procedures [16]. Specifically, RBV dose was cut by 200 mg in patients receiving 1000 mg or 1200 mg, when hemoglobin decreased < 12 g/dL, and by another 200 mg when it was below < 10 g/dL. RBV treatment was stopped when hemoglobin decreased to <8.5 g/dL. Patients with baseline hemoglobin (Hb) concentrations of < 13 g/dl were given reduced doses of RBV (200 mg lower than the standard dose determined by body weight) to prevent early discontinuation of therapy.

Recombinant human erythropoietin (r-huEPO) was administered in the event of: 1) A decline in Hb from baseline of more than 4 g/dL, or 2) Hb concentration of ≤ 10 g/dL, or 3) Symptomatic anemia which may occur at any Hb concentration following a rapid fall in Hb concentration. Treatment with r-HuEPO was continued until either of the following: resolution of anemia-related symptoms or recovery of Hb concentrations to the lower of 12 g/dL or 1.0 g/dl below pre-treatment values.

**IL28B and ITPA genotyping.** Genomic DNA was automatically extracted from blood samples using the QIAsymphony SP equipment (Quiagen, Hilden, Germany). We analyzed two functional polymorphisms (rs1127354 and rs 7270101) in the ITPA gene as well as polymorphism rs12979860 in the IL28 gene by real-time PCR on an ABI PRISM 7000 Sequence Detection System using the TaqMan SNP genotyping Assays (Applied Biosystems, Foster City, CA, USA), according to manufacturer’s instructions.
Homozygous and heterozygous sequenced samples of each SNP were included as internal controls. Definition of clinical endpoints. The primary genetic association analyses considered association of polymorphisms in the ITPA gene with the following: 1) The percent reduction in Hb level from baseline to week 4 (continuous variable); 2) The absolute Hb reduction from baseline to week 4 (continuous variable); 3) Hb reduction > 3 g/dl; 4) Hb reductions over the course of therapy, both defined quantitatively (absolute and percent decreases) and qualitatively (> 3 g/dl reduction); 5) The need for RBV dose reduction, 6) The need for r-huEPO therapy, and 7) Rate of SVR. Statistical analyses. Data are expressed as mean and standard deviation (SD) or as otherwise specified. Continuous variables were assessed with the Mann–Whitney test for two groups or a nonparametric analysis of variance by applying a rank transformation on the dependent variable (Rank-ANOVA) for more than two groups with Bonferroni alpha-adjustment for post-hoc comparisons. Categorical data such as genotype and allele frequencies were compared by use of the Fisher’s exact test. The level of significance was established at 0.05 and all reported P values are two-sided. All analyses were performed with the SAS version 9.1.3 software (SAS Institute Inc., Cary, NC). Stepwise logistic regression analysis was used to examine the association of SVR, anemia, and other parameters with polymorphisms in the ITPA gene. The variables selected to enter into stepwise regression were those that correlated significantly with ITPA gene polymorphisms (after Bonferroni correction for multiple testing).

Results

Study population. Among 389 patients with HIV/HCV co-infection, 73 received therapy with PEG-IFN) plus RBV for a median time of 9.6 ± 3.7 months (range: 3-18 months). Fifty-six patients (76%) had a complete HCV treatment course. The mean duration of treatment in the 17 patients (24%) who did not complete treatment was 5.8 ± 1.1 months (range: 3-8 months). Baseline characteristics of the 73 patients and
their genotypes at rs 1127354 in the ITPA gene are shown in Table 1. There were 22 cirrhotic patients without differences between groups (OR = 1.10 [95%CI: 0.27-5.43], P = 0.8566).

There were no statistically significant differences between treated and not treated for HCV co-infected patients in terms of genotypes at rs 1127354 in the ITPA gene (OR = 1.55; 95%IC: 0.80-3.17, P = 0.2240). There were no differences either between both groups in terms of genotypes at rs 7270101 in the ITPA gene (OR = 1.25; 95%IC: 0.28-7.88, P = 0.9736).

Duration of HCV infection and HCV genotypes are shown in Table 1. There were no significant differences between treated patients according to genotype at rs 1127354 in the ITPA gene and HCV genotype (Table 1). There were no differences either between groups according to genotype at rs 1127354 in the ITPA gene and genotype of the IL28 gene (44.1% vs. 28.6% for CC genotype, OR = 0.51; 95%CI: 0.11-2.04, P = 0.4488), and genotype at rs 7270101 in the ITPA gene (27.2% vs. 14.3% for AC/CC genotype, OR = 0.45; 95%CI: 0.04-2.39, P = 0.4938) (Table 1).

Most of the patients were virologically well-controlled in terms of HIV infection (79.4% undetectable HIV-1 RNA). There were no differences between both groups of patients in terms of antiretroviral drug exposure (Table 2). There were not differences between HCV-treated patients according to genotype at rs 1127354 in the ITPA gene and ABC-based cART (38.9% vs. 37.7%, P = 0.8205). Twelve patients had low hemoglobin levels at baseline and were given a RBV dose decreased by 200 mg. There were 8 patients with CC and 4 with CA + AA genotypes of the ITPA gene (P = 0.2271) (Table 1).

**Sustained virological response and IL28B and ITPA polymorphism.** Thirty-nine patients (53.4%) achieved SVR. SVR was associated with HCV genotype (88.9% for genotype 3 and 41.8% for genotypes 1 & 4; OR = 11.13; 95%CI: 2.20-105.90 , P = 0.0013), IL28B genotype at rs 12979860 (70.0% for CC and 41.9% for CT/TT genotypes, respectively; OR = 3.24; 95%CI: 1.09-9.92, P = 0.0311), and with genotype at rs 1127354 in the ITPA gene (78.6% for CA+AA and 47.4% for CC genotypes, respectively; OR = 4.06; 95%CI: 0.92-24.52, P = 0.0371). SVR was not associated with genotypes at rs 7270101 in the ITPA gene (OR = 1.52, 95%CI: 0.45-5.32, P = 0.6305). A multivariable
analysis was performed taking SVR as the dependent variable and age, sex, BMI, baseline HCV RNA, HCV genotype, RBV dose reduction, platelet count, baseline fibrosis, IL28B genotype, and ITPA genotype as independent variables. Independent predictors of SVR were: HCV genotype, age, baseline HCV RNA, and RBV dose reduction (Table 3).

**Decrease in hemoglobin levels during PEG-IFN + RBV therapy.** Figure 1 shows the percent decreases in Hb levels between 59 patients with CC and 14 with CA/AA genotypes of the ITPA gene. Hb decreased more in patients with CC than CA/AA genotypes at week 4 (-2.6 ± 1.3 vs. -0.90 ± 0.9, P = 0.0002) and week 12 (-3.9 ± 1.8 vs. -1.7 ± 0.7, P = 0.0003). The Hb nadir was reached earlier in patients with CC genotype (13.7 ± 9.9 weeks) than in patients with CA/AA genotype (25.0 ± 10.5 weeks) (P = 0.0004). The percent decrease in Hb level at week 4 was -17.4 ± 10.3% for patients with CC genotype whereas it was -6.1 ± 6.9% for those with CA/AA genotype (P = 0.0003). At week 12, the percent decrease in Hb was -23.4 ± 10.9% for patients with CC genotype whereas it was -11.9 ± 5.2% for those with CA/AA genotype (P = 0.0003). At week 36, the percent decrease in Hb levels was -18.6 ± 15.3% for patients with CC genotype whereas it was -7.0 ± 11.7% for those with CA/AA genotype (P = 0.0192), whereas at week 48 the respective decreases were -15.4 ± 17.5% and -12.7 ± 10.9% (P = 0.6605). The percentage of patients who presented a decrease of Hb ≥ 3 g/dl from baseline at each time point is shown in Figure 2. Genotypes at rs 7270101 in the ITPA gene were not associated with Hb decrease measured in any form. Genotypes at rs 1127354 in the ITPA gene were not associated with maximal white blood cell count decrease (-3.48 ± 1.69 vs. -2.86 ± 1.19 x 10^3/mm^3, respectively for CC and CA/AA genotypes, P = 0.2910).

**Modification of RBV during PEG-IFN + RBV therapy.** RBV dose was reduced ≥ 200 mg in 15 patients (34.2%), because of Hb decrease. During the first 12 weeks of therapy, the proportion of patients receiving the full dose of RBV was higher for patients with the CA/AA than for those with the CC genotype (100% vs. 54.2%, OR = 11.81; 95%CI: 1.45-256.17, P = 0.0039). Therefore, none of CA/AA carriers needed to reduce RBV dosage. Patients who needed RBV dosage modification showed a significant decrease
in percentages of SVR (32.0% vs. 64.6%, OR: 3.88; IC95% 1.25-12.50; P = 0.0163).

Among the 48 patients who did not require a reduction of RBV dosage, 64% (31/48) had SVR. Although no significant differences were observed between the CA/AA and CC genotypes (78.6% vs. 58.8%, OR 2.57; IC95%: 0.57-16.64, P = 0.3201), a higher percentage of SVR was observed in patients with CA/AA genotype. Genotypes at rs 7270101 in the ITPA gene were not associated with modification of RBV dose.

**Administration of erythropoietin (r-huEPO) during PEG-IFN + RBV therapy.** Twenty patients (27.4%) needed administration of r-huEPO because of anemia. The Hb level at baseline (15.4 ± 1.5 vs. 14.4 ± 1.5 g/dl, P = 0.0189), and the percent decrease at week 4 (-20.3 ± 11.3% vs. -12.6 ± 9.3%, P = 0.0064) and at week 12 (-32.1 ± 10.7% vs. -16.1 ± 7.4%, P < 0.0001) of patients who did or did not receive r-huEPO was statistically different. Forty-one percent of patients with CC genotype needed r-huEPO, whereas none of the patients with CA/AA genotype needed r-huEPO (OR = 8.28; 95%CI: 1.04-371.12, P = 0.0057).

**Factors influencing decrease in hemoglobin levels during PEG-IFN + RBV therapy.**

To determine the factors associated with Hb decrease during PEG-IFN plus RBV therapy in HIV/HCV co-infected patients, a logistic regression analysis was performed taking maximum percent Hb decrease as the dependent variable and age, sex, BMI, baseline Hb level, baseline platelet count, RBV dose (reduced vs. not reduced), and genotypes at rs 1127354 in the ITPA gene as independent variables. Independent predictors of Hb decrease were: RBV dose reduction, baseline hemoglobin, and BMI (Table 4).

**Discussion**

Our study shows a strong association of the development of RBV-induced anemia, measured in any form, with polymorphisms in the rs 1127354 of the ITPA gene in HIV/HCV co-infected patients treated with PEG-IFN plus RBV. This finding is similar to the associations found in clinical trials of treated HCV mono-infected patients [20, 31, 32]. This finding suggests that, whatever the operating protective mechanism is, the
toxic effects of RBV triphosphate on the red blood cells may be modulated by functional polymorphisms in the ITPA gene. Moreover, this is in agreement with the dose-dependent mechanism through which RBV causes its toxic effects on red blood cell membrane [24]. Although the effect was most evident early in treatment, it persisted throughout. The protective effect of polymorphisms in ITPA resulted in no need for RBV dose reductions and thus a greater cumulative RBV exposure. Additionally, patients with the protective genotype had no need for r-huEPO. Adverse effects of combination antiviral therapy for HCV infection are the most common cause of treatment discontinuation and can jeopardize treatment adherence, thus compromising the effectiveness of treatment. The rates of treatment discontinuation in mono-infected patients range from 24.5% to 27%, and discontinuation usually occurs within the first 6 months of treatment, anemia being the cause of discontinuation in one third of these patients [11]. Therefore, anemia is not only highly incidental, but is also of significant magnitude, and in two studies of combination HCV antiviral therapy, Hb decreased by at least 3 g/dl in 54% of the patient population and by more than 25% from baseline in approximately 28% of patients [29]. Furthermore, in HIV/HCV co-infected patients, combination therapy for HCV infection is associated with more profound anemia than seen in mono-infected patients [29]. In fact, the Hb decreased by at least 3 g/dl in 50.6% of patients in the present study, and a decrease of ≥ 25% of baseline Hb level was observed in 67.1%, figures similar to those reported by others [12]. This is most likely due to a higher prevalence of pre-treatment anemia in co-infected patients as well as to the potential need for treatment with antiretrovirals or other medications that may cause anemia. Apart from its effects on therapy discontinuation and adherence to HCV combination therapy, anemia is the main cause of RBV dose reduction. RBV-induced anemia requires RBV dose modification in 9% to 22% of HCV mono-infected patients [10, 17]. Usually, dose reduction is needed early in treatment (during the first 12 weeks) and this reduction in RBV dose appears to be critical to achieve SVR [10, 28]. It is recommended to avoid dose reduction in patients in whom the response rate is lower and in whom a maximal effort is required to increase the individual’s chance of
response. This group includes, among others, patients with HIV/HCV co-infection [10, 33]. Among co-infected patients, RBV dose reduction because of anemia is needed in around a third of patients [4, 12], figures similar to the 34.2% found in our study. The association of RBV dose reduction and anemia is so strong that it prevented ITPA being and independent predictor of anemia in our multivariate model, because of co-linearity between both variables.

To avoid both discontinuation and RBV dose reduction, r-huEPO has been successfully used both in mono-infected and co-infected patients to stimulate erythropoiesis, otherwise compromised in HCV-infected patients [1, 21]. As r-huEPO therapy, which is needed in about a third of treated co-infected patients (27% among our patients) [21], is a surrogate marker of RBV-induced anemia in treated HIV/HCV co-infected patients, the difference in its use with respect to polymorphism at rs 1127354 in the ITPA gene found in our work was expectable. However, even with these correcting measures, the Hb level between CC and CA/AA patients was significantly different until week 48.

Although anemia complicating combination HCV therapy may be caused by PEG-IFN or RBV, the driving force is hemolytic anemia caused by RBV triphosphate accumulation in erythrocytes, which in turn induces oxidative damage to membranes, eventually leading to extravascular hemolysis [8, 24]. The mechanism of protection from hemolysis by increased ITP intra-erythrocytic levels is poorly understood, but it has been suggested that increase in intracellular ITP may in turn cause a decrease in intracellular phosphate concentration, which may prevent the conversion of RBV into RBV triphosphate or, alternatively, that ITP complexes with RBV triphosphate thus conferring protection against hemolysis [10, 31]. Recent evidence indicates that ITP protects against RBV-induced anemia by substituting for GTP (depleted by RBV) in the biosynthesis of ATP [13]. Since ITP intracellular levels are dependent on ITPA activity, which in turn is modulated by functional polymorphisms in the ITPA gene [26], these polymorphisms may in the end determine the degree of protection against RBV-induced hemolytic anemia in HIV/HCV co-infected patients, as has been recently reported [19, 23], and our work suggests.
Data regarding the association of ITPA polymorphism and combination HCV treatment outcome are quite controversial, some studies showing such an association [14], whereas others [7, 19, 23, 31, 32] do not see any association between ITPA gene, anemia and SVR. This association may only be the reflection of decreased treatment efficacy due to dose reduction of RBV in patients with severe anemia, because the potential of RBV dose reduction to limit treatment efficacy is well known [28]. This is probably the case in our study since ITPA polymorphism was not an independent predictor of SVR.

Treatment with inhibitors of the HCV serine protease together with peg-interferon plus ribavirin is associated with anemia beyond that seen with peg-interferon plus ribavirin therapy [18, 22]. In this setting, two Japanese studies have shown that ITPA polymorphisms are still able to influence hemoglobin levels during triple HCV therapy [7, 30].

However, the present results have inherent limitations. Firstly, this is a cross-sectional study and, therefore, no causal relationships can or should be drawn. Second, we have not measured ITPA expression, nor have we looked at ITP intracellular levels in erythrocytes. Notwithstanding that, several mutations leading to ITPA deficiency, which is a benign cell enzymopathy characterized by accumulation of ITP in erythrocytes, and increased toxicity of purine analogue drugs have been well characterized [27]. Among them, mutation at rs 1127354 of the ITPA gene causes a substantial reduction in ITPA activity, and homozygosity for P32T mutation causes non-detectable ITPA activity [9]. Altogether these data indicate that there is a good correlation between ITPA gene polymorphisms, ITPA functional activity and, therefore, ITP accumulation in red blood cells [15, 26]. Third, the number of patients included in the present study is relatively small.

In summary, polymorphism at rs 1127354 in the ITPA gene is strongly associated with RBV-induced anemia in HIV/HCV-co-infected patients treated with PEG-IFN plus RBV. This finding has the potential to inform clinical decision-making, especially in patients who need aggressive dose escalation strategies with RBV or those who are at high risk...
of anemia or related morbidity, such as older patients, patients with chronic renal
dysfunction or hemoglobinopathies.
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Conflict of interest
No author declared any conflict of interest.

Contributorship statement
PD, JMG, and MB conceived the research, designed the database and wrote the article.
JS performed the genetics for the study. AF and JM retrieved and processed the blood samples. GM, MMG, CP, JM and KL enrolled the patients and monitored them throughout HCV therapy.
Figure 1. Percent decrease in hemoglobin levels according to ITPA genotype over the course of HCV combination therapy

CCr = Patients with CC genotype who had RBV dose reduced and/or r-huEPO administered

CCf = Patients with CC genotype who did not have RBV dose reduced and/or r-huEPO administered

Error bars express standard error of the mean. Hb level at each time point was measure twice with a 24 hr. interval.

* P < 0.05 between CA/AA and CC genotypes

Figure 2. Percentage of patients with a decrease of ≥ 3 g/dl of hemoglobin according to genotype at rs 1127354 in the ITPA gene per week of treatment. Patients in the CC group include all patients irrespective of RBV dose reduction or r-huEPO administration.
References


Table 1. Baseline characteristics of HIV-HCV co-infected patients treated with peg-interferon and ribavirin

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<td>6.2 ± 0.7</td>
<td>6.2 ± 0.7</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&amp;4, (%)</td>
<td>55 (75.3)</td>
<td>46 (77.9)</td>
<td>9 (64.3)</td>
</tr>
<tr>
<td>3, (%)</td>
<td>18 (24.7)</td>
<td>13 (22.1)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>Fibrosis, kPa</td>
<td>10.7 ± 11.8</td>
<td>10.5 ± 11.5</td>
<td>11.8 ± 13.5</td>
</tr>
</tbody>
</table>

All parameters in mean ± standard deviation unless otherwise specified. Kg = kilogram, m² = squared meters, g = grams, dl = deciliter, l = liter, IU = international units, kPa = kilopascals, ITPA = inosine triphosphatase, BMI = body mass index, MsM = Men who have sex with men, HTSX = heterosexuals, IDU = Intravenous drug users, WBC = white blood cell, g = grams, l = liter, dl = deciliter, AST = aspartate aminotransferase, ALT = alanine aminotransferase, HCV = hepatitis C virus.
Table 2. HIV infection parameters and antiretroviral drug exposure in HIV-HCV co-infected patients treated with peg-interferon and ribavirin

<table>
<thead>
<tr>
<th>Number</th>
<th>Total</th>
<th>ITPA genotypes at rs 1127354</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>CA + AA</td>
</tr>
<tr>
<td>ART concomitant to HCV therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI-based, %</td>
<td>73</td>
<td>29 (39.7)</td>
<td>24 (40.7)</td>
</tr>
<tr>
<td>NNRTI-based, %</td>
<td>59</td>
<td>34 (46.6)</td>
<td>26 (44.1)</td>
</tr>
<tr>
<td>None, %</td>
<td>14</td>
<td>6 (8.2)</td>
<td>6 (10.2)</td>
</tr>
<tr>
<td>3 NRTIs, %</td>
<td>14</td>
<td>3 (4.1)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>INsTI-based, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>NRTI backbone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF + TDF, %</td>
<td>73</td>
<td>34 (46.6)</td>
<td>23 (38.9)</td>
</tr>
<tr>
<td>ABC + 3TC, %</td>
<td>59</td>
<td>18 (24.6)</td>
<td>18 (30.5)</td>
</tr>
<tr>
<td>None, %</td>
<td>14</td>
<td>14 (19.2)</td>
<td>12 (20.3)</td>
</tr>
<tr>
<td>ABC + TDF, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>2 (3.4)</td>
</tr>
<tr>
<td>AZT + 3TC, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>ddi + 3TC, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ddi + ABC, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>3TC alone, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>ABC alone, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>AZT-based, %</td>
<td>14</td>
<td>1 (1.4)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>ABC-based, %</td>
<td>14</td>
<td>29 (39.7)</td>
<td>23 (38.9)</td>
</tr>
<tr>
<td>Individual antiretroviral exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT, m</td>
<td>73</td>
<td>30.1 ± 43.9</td>
<td>27.4 ± 36.9</td>
</tr>
<tr>
<td>3TC/FTC, m</td>
<td>59</td>
<td>72.3 ± 52.9</td>
<td>69.4 ± 54.9</td>
</tr>
<tr>
<td>d4T, m</td>
<td>14</td>
<td>36.9 ± 40.5</td>
<td>38.2 ± 41.1</td>
</tr>
<tr>
<td>ddi, m</td>
<td>14</td>
<td>27.3 ± 38.7</td>
<td>26.0 ± 39.0</td>
</tr>
<tr>
<td>ddC, m</td>
<td>14</td>
<td>3.8 ± 10.8</td>
<td>4.6 ± 11.9</td>
</tr>
<tr>
<td>ABC, m</td>
<td>14</td>
<td>26.9 ± 42.7</td>
<td>29.7 ± 43.4</td>
</tr>
<tr>
<td>TDF, m</td>
<td>14</td>
<td>32.1 ± 35.7</td>
<td>27.2 ± 34.5</td>
</tr>
<tr>
<td>EFV, m</td>
<td>14</td>
<td>34.6 ± 48.8</td>
<td>31.8 ± 48.9</td>
</tr>
<tr>
<td>NVP, m</td>
<td>14</td>
<td>8.9 ± 22.7</td>
<td>9.6 ± 24.9</td>
</tr>
<tr>
<td>NRTIs, m</td>
<td>14</td>
<td>229.6 ± 135.8</td>
<td>222.7 ± 141.7</td>
</tr>
<tr>
<td>PIs, m</td>
<td>14</td>
<td>46.9 ± 51.1</td>
<td>44.3 ± 51.9</td>
</tr>
<tr>
<td>RAL, m</td>
<td>14</td>
<td>2.5 ± 9.2</td>
<td>2.9 ± 10.2</td>
</tr>
<tr>
<td>HIV RNA, log10 copies/ml</td>
<td>14</td>
<td>1.6 ± 0.7 (1.3-1.3)</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Current CD4 count, cells/mm³</td>
<td>14</td>
<td>635 ± 314</td>
<td>629 ± 340</td>
</tr>
<tr>
<td>Undetectable HIV RNA, %</td>
<td>14</td>
<td>58 (79.4)</td>
<td>46 (77.9)</td>
</tr>
<tr>
<td>CD4 nadir, mean ± SD, cells/mm³</td>
<td>14</td>
<td>229 ± 162</td>
<td>219 ± 144</td>
</tr>
<tr>
<td>CD4 nadir &lt; 100 cells/mm³, %</td>
<td>14</td>
<td>16 (27.2)</td>
<td>13 (27.7)</td>
</tr>
</tbody>
</table>

All parameters in mean ± standard deviation unless otherwise specified. HIV = human immunodeficiency virus, ART = antiretroviral therapy, PIs = protease inhibitors, NNRtIs = non-nucleoside reverse transcriptase inhibitor, NRTIs = nucleoside reverse transcriptase inhibitors, INsTI = integrase strand transfer inhibitor, AZT = zidovudine, 3TC = lamivudine, FTC = emtricitabine, d4T = stavudine, ddi = didanosine, ddC = zalcitabine, ABC = abacavir, TDF = tenofovir, EFV = efavirenz, NVP = nevirapine, RAL = raltegravir, m = months. ml = milliliters.
Table 3. Independent predictors of sustained virological response in 73 HIV/HCV co-infected patients treated with peg-interferon plus ribavirin

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% Confidence intervals</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV genotype</td>
<td>24.83</td>
<td>2.64-233-07</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>9.02</td>
<td>1.49-54.61</td>
<td>0.03</td>
</tr>
<tr>
<td>Baseline HCV RNA</td>
<td>0.35</td>
<td>0.12-0.98</td>
<td>0.05</td>
</tr>
<tr>
<td>RBV dose reduction</td>
<td>0.10</td>
<td>0.02-0.54</td>
<td>0.01</td>
</tr>
</tbody>
</table>

HIV = human immunodeficiency virus, HCV = Hepatitis C virus, RNA = ribonucleic acid, RBV = ribavirin

Table 4. Independent predictors of maximum percent decrease in Hb in 73 HIV/HCV co-infected patients treated with peg-interferon plus ribavirin

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% Confidence intervals</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBV dose reduction</td>
<td>11.72</td>
<td>6.82-16.63</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Baseline Hb level</td>
<td>1.69</td>
<td>0.23-3.15</td>
<td>0.024</td>
</tr>
<tr>
<td>BMI</td>
<td>0.7</td>
<td>-1.43-0.03</td>
<td>0.061</td>
</tr>
</tbody>
</table>

HIV = human immunodeficiency virus, HCV = Hepatitis C virus, RNA = ribonucleic acid, RBV = ribavirin, Hb = hemoglobin, BMI = body mass index