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Telaprevir-S isomer enhances ribavirin exposure and the ribavirin-related haemolytic anaemia in a concentration-dependent manner

Amedeo De Nicolò [a,1,*], Lucio Boglione [a,1], Alessia Ciancio [b], Jessica Cusato [a], Silvia Strona [b], Chiara Simona Cardellino [a], Adnan Mohamed Abdi [a], Giuseppe Cariti [a], Giulia Troshina [b], Gian Paolo Caviglia [b], Antonina Smedile [b], Mario Rizzetto [b], Giovanni Di Perri [a], Antonio D'Avolio [a]

[a] Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin, Italy
[b] Unit of Gastroenterology, University of Turin, Department of Medical Sciences, S. Giovanni Battista (Molinette) Hospital, Turin, Italy

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ABSTRACT

The standard-of-care for the treatment of genotype-1 chronic hepatitis C is based on the combination of direct acting antivirals, such as boceprevir and telaprevir, with ribavirin and pegylated-interferon alfa. These triple regimens give a higher response rate than dual therapy, but on the other hand show a more than 10% higher rate of anaemia. Not enough focus has been given to the interaction between telaprevir and RBV. In this work, we aimed to study and deepen this relationship by comparing ribavirin plasma and intra-erythrocytic concentrations at one month of triple and dual therapy (17 vs. 119 patients). Moreover, we determined telaprevir isomers concentrations and tested them for correlation with ribavirin concentrations and haemoglobin loss at one month of treatment. Finally, all drugs concentration data were tested for their correlation with the renal function during treatment.

The comparisons of ribavirin concentration and toxicity data were repeated on a sub-group of 9 patients who had been treated 1 year before with dual therapy and then re-treated with triple therapy.

The observed ribavirin plasma and intra-erythrocytic concentrations in triple therapy were significantly higher compared to dual therapy, both in whole group and sub-group comparison. Ribavirin concentrations were significantly correlated to the haemoglobin loss and telaprevir-S isomer concentrations ($r^2 = 0.317$ $P_{value} = 0.023$ and $r^2 = 0.388$ $P_{value} = 0.008$, respectively). Renal function had a significant decrease from the baseline value, but was not significantly correlated with drugs concentrations.

These results highlight for the first time that, in the context of triple therapy with telaprevir, ribavirin exposure is related to the telaprevir-S isomer plasma concentration.

We conclude that the addition of telaprevir to the dual therapy increases ribavirin exposure and haemoglobin loss: this effect could probably be managed through the therapeutic drug monitoring of ribavirin and telaprevir-S concentrations.

1. Background and aims

The new standard of care for the treatment of genotype 1 HCV infection consists in the association of PEG-IFNα and ribavirin (RBV) with either telaprevir (TLV) or boceprevir (BOC) (Ghany et al., 2011). Despite inducing better response rates, these new triple regimens are associated with increased side-effects and adverse events. The development of anaemia was found to be particularly frequent and severe in recipients of TLV or BOC-based regimens when compared to PEG-IFNα/RBV intakers (Jacobson et al., 2011). The mechanism of anaemia onset is not still completely understood, but is probably related to the concomitant administration of all three drugs; as a matter of a fact, both PIs and PEG-IFN may be responsible for bone marrow suppression (Ronzoni et al., 2013), while RBV leads to haemolysis (De Franceschi et al., 2000). The risk of anaemia onset is also modulated by genetic factors, mainly through the Inosine Triphosphatase (*ITPA*) (Asselah et al., 2010; D'Avolio et al., 2012; D'Avolio et al., 2013a,b,c; De Nicolò et al.,

Abbreviations: CHC, chronic hepatitis C; HCV, hepatitis C virus; PEG-IFNα, pegylated interferon alfa; DAAs, Directly Acting Antivirals; RBV, ribavirin; TLV, telaprevir; BOC, boceprevir; PIs, protease inhibitors; ITPA, inosine triphosphatase; IQR, inter quartile range; TDM, therapeutic drug monitoring; PK, pharmacokinetic; SNP, single nucleotide polymorphism; eGFR, estimated glomerular filtration rate.

* Corresponding author. Tel.: +39 011 4393979; fax: +39 011 4393882.
E-mail address: amedeo.denicolo@unito.it (A. De Nicolò).

[1] Both authors equally contributed to this work.

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2013a; Tanaka et al., 2011) gene polymorphisms. However, the weight of these factors in the new triple therapy is not still assessed.

Hammond et al. described increased plasma and intracellular RBV concentration in 5 patients receiving PEG-IFNα/RBV/TLV triple therapy (compared to PEG-IFN/RBV), suggesting that the additional rate of anaemia may be attributable to increased RBV exposure (Hammond et al., 2013). However, these data were not standardized from a genetic point of view and were observed in only 5 patients, comparing them with a different group of patients treated with dual therapy.

In an exploratory study of Toyota et al. (Toyota et al., 2013) 15 patients received the TLV monotherapy for 24 weeks: only 7 patients (46.7%) showed mild anaemia, but none moderate or severe, without any interruption of the treatment due to anaemia related-symptoms. Interestingly, in this report, the mean value of haemoglobin after 12 weeks of treatment was >12 g/dL, confirming the hypothesis that the onset of early anaemia during the triple therapy could be due to an interaction between TLV and RBV/PEG-IFN (Toyota et al., 2013).

Recently Boglione et al. (Boglione et al., 2014) showed an increased early plasma RBV concentration in 9 patients receiving PEG-IFNα/RBV/TLV triple therapy when compared to patients treated with PEG-IFN/RBV at the same timing (week 2).

Moreover, Karino et al. (Karino et al., 2013) observed a correlation between TLV weigh-based dose, the estimated glomerular filtration rate (eGFR) and RBV concentrations at 1, 2 and 4 weeks after the beginning of the treatment: they formulated the hypothesis that TLV could decrease renal function therefore increasing RBV concentrations. However, all these studies did not observe any correlation between TLV and RBV concentrations. Furthermore, the study population had some bias due to genetic differences between patients.

The aim of this paper is to verify the presence of a "boosting" effect of TLV on RBV plasma and intra-erythrocytic concentrations in a cohort of patients treated with PEG-IFN/RBV and TLV and then to investigate on the concentration-dependence of this effect.

Moreover, we deepened the comparison evaluating the RBV exposure in a subgroup of 9 patients first treated with dual therapy and then retreated with the triple one, in order to obtain genetically unbiased data.

2. Patients and methods

2.1. Patients

Seventeen genotype-1 HCV infected patients who gave informed consent as requested by ethics committee guidelines were enrolled at "Amedeo di Savoia" and "San Giovanni Battista" Hospitals in Turin, Italy, from the beginning of 2013. The inclusion criteria were: having failed a previous treatment with PEG-IFN/RBV therapy, being retreated with TLV/PEG-IFN/RBV, being mono-infected and finally not assuming any concomitant interacting drug.

From this group, 9 patients were previously treated with dual therapy in the same hospitals in the previous 3 years. For one of these patients the intra-erythrocytic RBV concentration in the previous treatment was not available. Pharmacokinetic and hematologic toxicity data (haemoglobin loss from baseline value) from the group of 17 TLV treated patients were compared with those of a larger cohort of 119 patients previously treated with only PEG-IFN and RBV.

Peg-IFNα 2a was administered once weekly at a fixed dose of 180 μg, while the 2b type was administered once weekly at the dose of 1.5 μg/kg: the same administration profile was maintained for both dual and triple therapy.

TLV was administered at a fixed dose of 750 mg q8 h (2250 mg/day) for all patients.

Anaemia was defined as haemoglobin loss of $\geqslant 3$ g/dL as compared to baseline values and/or haemoglobin level $\leqslant 10$ g/dL. WHO classification of anaemia grade was used (grade 0 $\geqslant$ 11 g/dL; 1 $\leqslant$ 11 g/dL $\geqslant$ 9.5 g/dL; 2 $\leqslant$ 9.5 g/dL $\geqslant$ 8 g/dL).

2.2. Plasma and intra-erythrocytic RBV concentrations measurement

RBV plasma concentrations have been measured at the end of dosing interval (C_{trough}) at week 4 of therapy, with a chromatographic method previously published and routinely used in our laboratory (D'Avolio et al., 2006) while another HPLC-UV method already published was used to obtain the quantification of intra-erythrocytic concentration of total RBV (D'Avolio et al., 2012). The same analytical methods have been used to obtain all RBV concentration levels in both triple and dual therapy treated patients. For TLV isomers quantification, a previously published UPLC-Tandem Mass spectrometric method was used (D'Avolio et al., 2013b).

2.3. ITPA genetic data

A venous blood sample was obtained from each patient (3 mL EDTA), together with the sampling for pharmacokinetic analyses. Whole blood was stored at −80 °C and DNA extracted using the QIamp DNA Mini Kit (Quiagen, Valencia, CA). The purified and elute DNA was directly used for the PCR real-time (BIORAD, Milano, Italia) reaction. The allelic discrimination analysis was performed using the TaqMan assays (Applied Biosystems, Foster City, CA).

The Single Nucleotide Polymorphisms (SNPs) rs1127354, rs7270101 and rs60551702 were considered in this analysis. Patients carrying at least one "protective" allele for at least one of these SNPs was considered "resistant" to anaemia, as proposed in our previous paper (D'Avolio et al., 2013c).

2.4. eGFR evaluation

The estimated glomerular filtration rate was evaluated before the beginning and after 4 weeks of treatment.

For its evaluation, the MDRD (Modification of Diet in Renal Disease) formula was considered ($GFR = 175 \times \text{Serum Creatinine}^{-1.154} * \text{age}^{-0.203} \times 1.212$ if patient is black $*$ 0.742 if female).

2.5. Statistical analysis

Statistical analysis was performed with SPSS statistical software version 20.0 (IBM).

Differences in RBV concentration at one month of the two different therapies, triple and dual, have been tested for significance using a not-parametric Wilcoxon test for paired cases, because of the small sample size and the impossibility to assess the normality.

The correlations between pharmacokinetic factors and toxicity data were investigated considering the Pearson correlation test. Differences in plasma concentrations of RBV or TLV isomers between anaemic and not-anaemic patients were evaluated through Mann–Whitney test.

Only data with a P_{value} minor than 0.05 were considered as significant.

3. Results

General population characteristics are resumed in Table 1A and B.

All patients were treated with the same RBV weight-based dose, both in new and previous dual treatment; in the subgroup, 4 out of 9 changed IFN type (2a vs 2b).

Table 1
Population baseline characteristics for each group of patients. *P* values are referred to difference between triple and double therapies.

Characteristics	Triple therapy-treated (n = 17)	Double therapy-treated (n = 119)	*P* values
A – All patients comparison			
Age (years)	52 IQR (45–56)	45 IQR (36–52)	0.857
Sex (M/F)	15/2	75/44	0.055
Metavir (F0/F1/F2/F3/F4)	0/2/0/3/12	16/53/10/19/21	–
ITPA "protective" allele carriers	29.4%	30.2%	0.994
Peg-IFN (2a/2b)	10/7	82/37	0.857
Body weight at baseline (kg)	77.0 IQR (70.50–88.00)	70.00 IQR (60.75–80.00)	0.986
Body mass index (kg/m^2)	27.58 IQR (24.39–31.05)	24.60 IQR (21.96–26.77)	0.157
Weight-based dose (mg/kg/day)	14.11 (12.98–15.19)	13.88 (12.79–15.16)	0.925
Hemoglobin at baseline (g/dl)	16.20 IQR (15.20–17.00)	14.70 IQR (13.80–15.80)	0.181
Previous outcome	Not-responders: 12 (70.6%) – Relapsers: 3 (17.6%) – Drop-out: 2 (11.8%)		
Characteristics	Triple therapy re-treatment (n = 9)	First double therapy (n = 9)	*P* values
B – Same patients comparison			
Peg-IFN (2a/2b)	4/5	8/1	0.131
Weight-based dose (mg/kg/day)	14.28 (13.16–15.81)	14.70 (13.17–16.26)	0.523
Hemoglobin at baseline (g/dl)	15.80 IQR (14.85–16.70)	15.70 IQR (14.85–16.70)	0.996
Previous outcome	Not-responders: 6 (66.7%) – Relapsers: 1 (11.1%) – Drop-out: 2 (22.2%)		

Differences in age and metavir score for the 9 patients compared in dual and triple therapy have been considered as not significant, because of the brief period of time (3 years) between first treatment and triple therapy retreatment.

No significant differences in the percentage of patients carrying *ITPA* "protective" mutations emerged in the whole group comparison (triple vs dual 29.4% vs 30.2%, respectively, P_{value} = 0.994). During the first month of therapy, both dual and triple, there were no therapy discontinuations or RBV dose reductions among patients and none of them received recombinant human erythropoietin (EPO).

3.1. RBV and TLV pharmacokinetics (all patients)

The summary of pharmacokinetic and toxicity data is reported in Table 2.

Considering the RBV administration profile between triple therapy and the dual therapy, no differences were observed.

Median RBV plasma concentration at one month of treatment in the 17 patients undergoing triple therapy with TLV was 1985 ng/mL (IQR 1497–2379), while the median concentration at the same timing in the dual therapy treated patients was 1745 ng/mL (IQR 1315–2346): however, this difference was not statistically significant.

The intra-erythrocytic concentration of RBV in triple therapy treated patients was 406227 ng/mL (IQR 276644–426973), while the corresponding concentration in dual therapy was 288107 ng/mL (IQR 212889–391141). This difference was statistically significant (P_{value} = 0.040). In the group of TLV treated patients, median TLV-S and TLV-R plasma concentrations were 1539 ng/mL (IQR 1046–2129) and 966 ng/mL (IQR 588–1261), respectively .

In the group of triple therapy treated patients, TLV-S plasma concentrations at 1 month were statistically correlated with RBV ones (P_{value} = 0.008, r^2 = 0.388, Fig. 1). TLV-R concentrations did not result significantly correlated with RBV ones (P_{value} = 0.120, r^2 = 0.163).

3.2. RBV and TLV pharmacokinetics (sub-group of patients)

The summary of pharmacokinetic and toxicity data is reported in Table 3.

Focusing on RBV plasma concentrations in the 9 patients who have comparable data for both therapies, the median data were 1985 ng/mL (IQR 1427–2513) and 1397 ng/mL (IQR 1146–1579), respectively (Fig. 2A). This difference was statistically significant (P_{value} = 0.015).

Moreover, in those 8 patients whose intra-erythrocytic RBV concentration data were available, the median data were 423897 ng/mL (IQR 370174–426973) and 305858 ng/mL (IQR 206699–334506), respectively (Fig. 2B, P_{value} = 0.012).

TLV-S and TLV-R median plasma concentrations were 1539 ng/mL (IQR 990–2339) and 904 ng/mL (415–1108), respectively.

In this group, difference between RBV plasma concentration in triple therapy and previous dual treatment were evaluated and

Table 2
Summary of the data from 17 patients treated with triple therapy (on the left) and data from 119 patients treated with double therapy, at 1 month of treatment. P values are referred to difference between triple and double therapies.

	Triple vs Double therapy (All patients)		
	Triple therapy (IQR)	Double therapy (IQR)	*P* values
Rate of anemia	88.2% (15 out of 17)	37.0% (44 out of 119)	<0.001
Plasma RBV concentration (ng/mL)	1985 (1497–2379)	1745 (1315–2346)	0.058
Intra-RBC RBV concentration (ng/mL)	406227 (276644 – 426973)	288107 (212889–391141)	0.040
Plasma TLV-S concentration (ng/mL)	1539 (1046–2129)	–	–
Plasma TLV-R concentration (ng/mL)	966 (588–1261)	–	–
Plasma TLV-R/-S concentration ratio	1.71 (1.35–1.94)	–	–
eGFR at one month of triple therapy (mL/min)	98.7 (74.9–108.6)	–	–
eGFR% decrease from the baseline	−13.2% (−19.9% – −5.6%)	–	0.002*
Hb loss (g/dL)	−3.8 g/dL (−4.6 –−3.2)	−2.2 g/dL (−3.4 – −1.1)	<0.001
Hb% loss	−23.6% (−29.2% – −19.7%)	−15.2% (−22.9% – −7.7%)	<0.001

* *P* value referred to the difference between eGFR values at one month of triple therapy and its baseline.

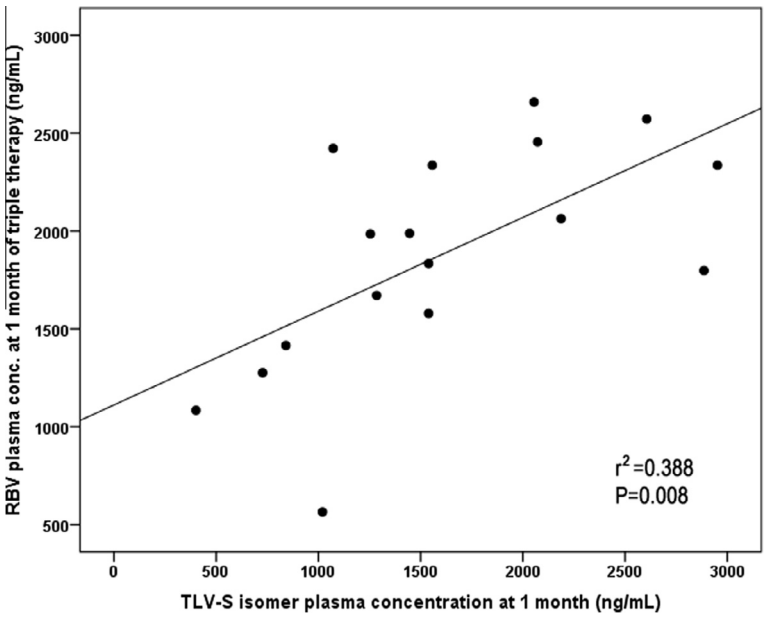

Fig. 1. Relationship between TLV-S isomer and RBV plasma concentrations: the concentrations of both compounds were determined in the same samples at 1 month of triple therapy and resulted significantly correlated ($P = P_{value}$). Data were generated using SPSS software.

Table 3
Summary of the data from 9 patients treated with triple therapy (on the left) and data from the same patients treated with double therapy, at 1 month of treatment. *P* values are referred to difference between triple and double therapies.

	Re-treatment vs Previous double therapy (same patients)		
	Triple therapy (IQR)	Double therapy (IQR)	*P* value
Rate of anemia	88.9% (8 out of 9)	33.7% (3 out of 9)	0.063
Plasma RBV concentration (ng/mL)	1985 (1427–2513)	1397 (1146 – 1579)	0.015
Intra-RBC RBV concentration (ng/mL)	423897 (370174–426973)	305858 (206699 – 334506)	0.012
Plasma TLV-S concentration (ng/mL)	1539 (990–2339)	–	–
Plasma TLV-R concentration (ng/mL)	904 (415–1108)	–	–
Plasma TLV-R/-S concentration ratio	1.87 (1.50–2.02)	–	–
eGFR at one month of triple therapy (mL/min)	94.3 (79.9–114.9)		
eGFR% decrease from the baseline	−15.7% (−19.2% – −8.2%)		0.017*
Hb loss at 1 month (g/dL)	−3.8 g/dL (−4.8 – −3.2)	−2.9 g/dL (−4.8 – −2.1)	0.091
Hb% loss at 1 month	−25.7% (−29.7% – −19.7%)	−17.8% (−29.3% – −14.4%)	0.089

* *P* value referred to the difference between eGFR values at one month of triple therapy and its baseline.

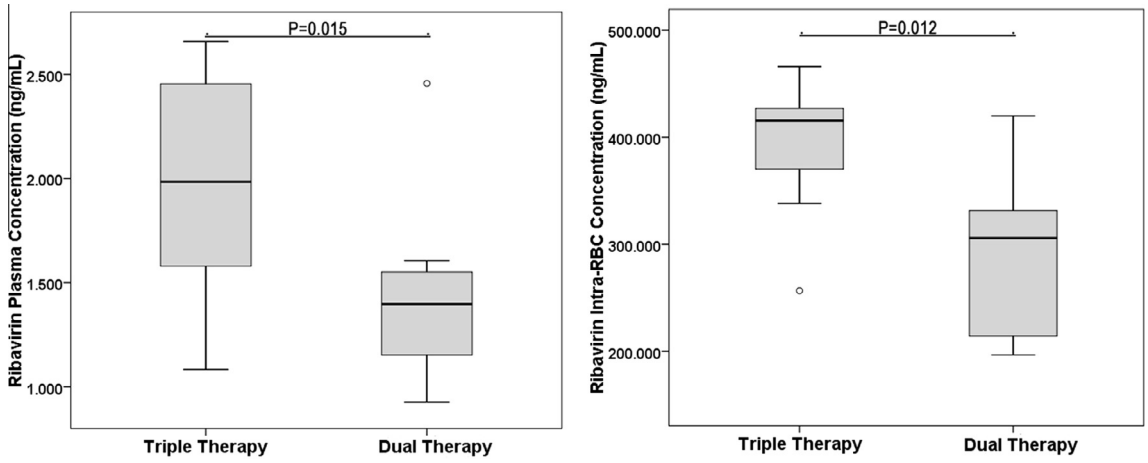

Fig. 2. Comparison of RBV pharmacokinetics in triple and dual therapy. Distribution of plasma (A, left, 9 patients) and intra-RBC (B, right, 8 patients) concentrations are reported at one month of triple therapy and dual therapy in the same subgroup of patients ($P = P_{value}$). Data were generated using SPSS software.

tested for correlation with TLV-S and -R isomers concentrations: in this case, only TLV-S reached a "borderline" correlation ($P_{value} = 0.052$), while TLV-R resulted not correlated ($P_{value} = 0.255$, $r^2 = 0.209$).

3.3. Anaemia (all patients)

Taking into account the 17 patients treated with TLV, 15 (88.2%) developed anaemia, while there were only 44 (37.0%) among the 119 patients treated with dual therapy ($P_{value} < 0.001$).

According to WHO classification, 93, 20 and 6 out of 119 patients treated with dual therapy experienced grade 0, 1 and 2 anaemia, respectively. While 13 and 6 experienced grade 0 and 1 among the 17 patients treated with triple therapy. Anaemia onset was not significantly correlated with the metavir score, either in dual or triple therapy groups.

The median haemoglobin loss in the first month of triple therapy was −3.8 g/dL IQR −4.6 – −3.2 (−23.6% IQR −29.2–19.7 from the baseline), while at the same timing of dual therapy it was −2.2 IQR −3.4 – −1.1 (−15.2% IQR −22.9 – −7.7 from the baseline). This difference was statistically significant ($P_{value} < 0.001$) (Fig. 3). This observation was also confirmed with the percent haemoglobin loss, thus removing the bias due to haemoglobin differences at the baseline between the groups ($P_{value} < 0.001$).

3.4. Anaemia (sub-group of patients)

Focusing on triple therapy re-treatment toxicity and considering the 9 patients which have comparable data with the previous dual therapy, 8 developed anaemia (88.9%), while in the previous treatment they were only 3 (33.7% $P_{value} = 0.063$). According to the WHO grading, in triple therapy 6 and 3 patients had grade 0 and 1 anaemia, respectively; in dual therapy, 7 and 2 patients had grade 0 and 1 anaemia, respectively. The median haemoglobin loss from the baseline at one month of triple therapy was −3.8 g/dL IQR −4.8 – −3.2 (−25.7% IQR −29.7% – −19.7% from the baseline), while the loss at the same timing in the previous treatment was −2.9 g/dL IQR −4.8 – 2.1 (−17.8% IQR −29.3% – −14.4% from the baseline): the difference was not statistically significant ($P_{value} = 0.091$).

3.5. PK and toxicity after one month

A significant correlation between plasma concentrations of RBV and percent haemoglobin loss was observed at one month of triple therapy ($r^2 = 0.317$, $P_{value} = 0.023$) (Fig. 4). Similarly, the same correlation was observed in the considered 119 patients treated with dual therapy ($P_{value} = 0.040$): moreover, in this group the intra-erythrocytic RBV concentration has been correlated with the haemoglobin loss ($P_{value} = 0.046$).

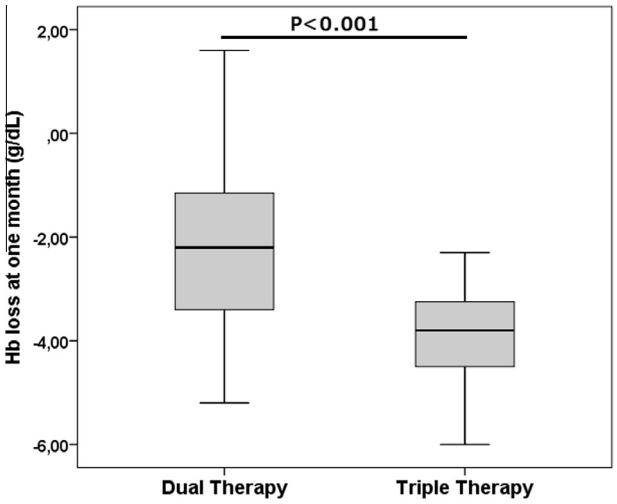

Fig. 3. Comparison of toxicity in triple and dual therapy. Distribution of haemoglobin loss at one month of dual (17 patients) and triple therapy (119 patients) in the whole group (Hb = Haemoglobin; $P = P_{value}$). Data were generated using SPSS software.

In the context of triple therapy with TLV, patients who developed anaemia had significantly higher RBV concentrations than the other patients ($P_{value} = 0.017$).

This difference was also evident in the subgroup of 9 patients in their treatment with dual therapy ($P_{value} = 0.001$, Fig. 5A). The same phenomenon was observed in triple therapy, but without a statistical significance, since only 1 patient did not experience anaemia (Fig. 5B).

In the whole group, TLV-S and TLV-R plasma concentrations were not significantly correlated with haemoglobin loss (either absolute or percent, $P_{values} > 0.2$), but pointed out a slight difference between patients who developed anaemia and those who did not (2 vs 15, $P_{value} = 0.06$).

This evidence was confirmed also in the subgroup of retreated patients.

3.6. Renal function during treatment

In the 17 patients treated with triple therapy the median eGFR at baseline was 103.1 mL/min (IQR 84.6–121.3) while after one month it was 98.7 mL/min (IQR 74.9–108.6). The median percent difference in the eGFR between baseline and one month of treatment was −12.8% (−19,3 – −6.6). This decrease in eGFR resulted statistically significant ($P_{value} = 0.002$). However no significant correlations between eGFR at baseline or at one month of therapy with concentrations of RBV or TLV have been observed.

Moreover the decrease of eGFR at one month of treatment was not significantly correlated with the weigh-normalized dose of TLV neither with its isomers concentrations ($P_{value} = 0.123$ for TLV dose; $P_{value} = 0.079$ and $P_{value} = 0.068$ for TLV-S and -R isomers, respectively). Both eGFR values at baseline and at one month of treatment were not significantly correlated ($P_{value} = 0.129$ and $P_{value} = 0.068$, respectively) with the percent haemoglobin loss; also the decrease of eGFR at one month lacks of correlation ($P_{value} = 0.483$).

4. Discussion and conclusions

As observed in the development clinical trials of TLV, TLV-treated patients had a higher rate of anaemia when compared with patients treated with the standard therapy, independently from *ITPA* genotype specific stratification.

It seems plausible that the rapid onset of anaemia in our TLV-recipients is attributable to the early higher RBV plasma exposure. In fact, the only other factor which changed in some cases (in the sub-group) between the first treatment with dual therapy and the retreatment with triple therapy was PEG-IFNα type: PEG-IFN, however, was reported to have a delayed effect (later than one month) on the development of anaemia through the inhibition of erythropoiesis, then it is very unlikely to be involved (Lin and Yin, 2009; McHutchison et al., 2006; Ronzoni et al., 2013).

Whether the increase of RBV concentrations is secondary to the intake of fat-rich food (which is recommended for TLV assumption and also known to increase RBV absorption) or, instead, it is the result of a yet undisclosed drug-drug interaction between TLV and RBV, is still to be established.

According to available data, TLV monotherapy was not associated with significant hematologic changes (Gonzalez de Requena et al., 2006; Hezode et al., 2009; Reesink et al., 2006). In a phase 1b placebo-controlled randomized study at the dose of 750 mg q8 h for 14 days, as well as in a phase 2 single-arm open-label study, in which patients received TLV monotherapy 750 mg q8 h for 24 weeks, the mean Hb value after 4 weeks of therapy was

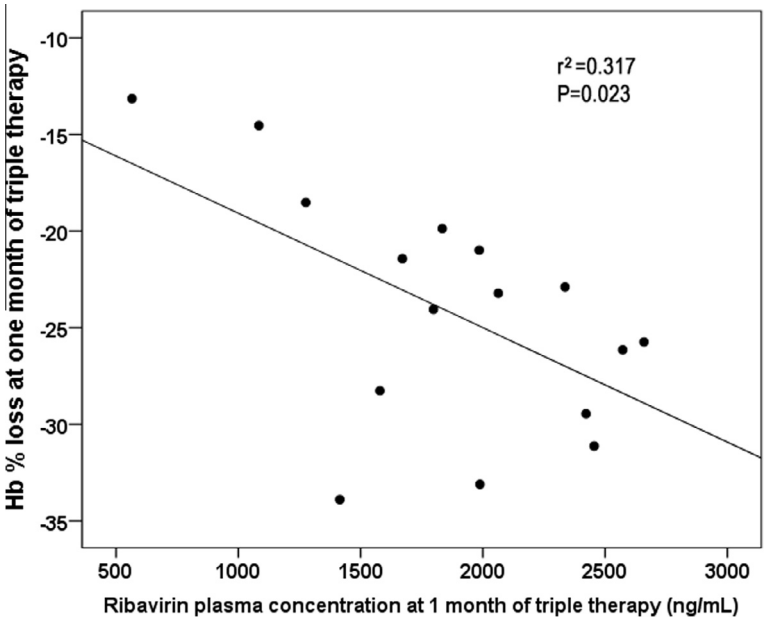

Fig. 4. Relationship between RBV pharmacokinetics and toxicity. Correlation between RBV plasma concentrations and haemoglobin % loss at one month of triple therapy ($r^2 = 0.317$, $P = P_{value}$). Data were generated using SPSS software.

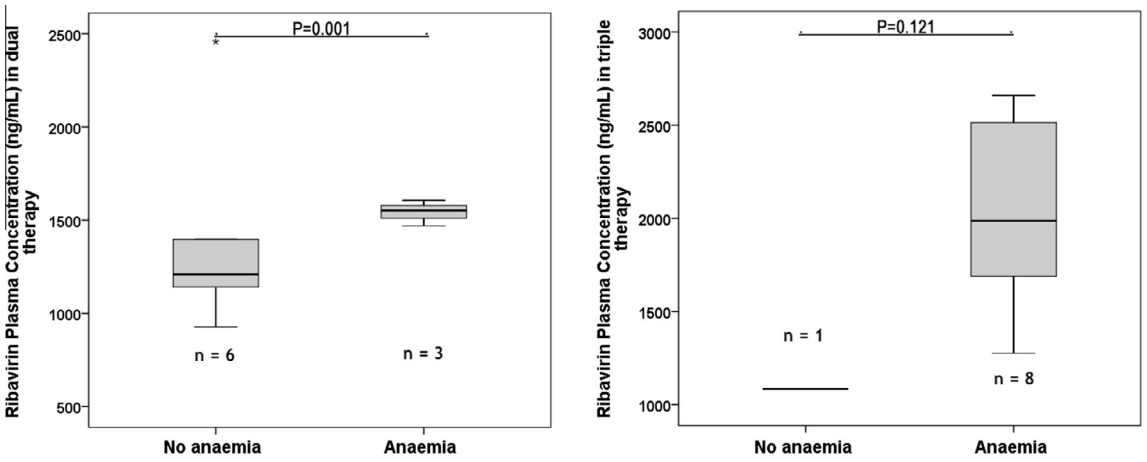

Fig. 5. RBV Pharmacokinetics and toxicity compared in both therapies. Comparison between RBV plasma concentrations in patients who did not develop anaemia and who developed it, in the sub group of 9 patients. The comparison is shown in previous dual (A, left) and triple (B, right,) therapy. Data were generated using SPSS software.

>12 g/dL and only mild anaemia was observed in 7 patients after 24 weeks. Moreover, anaemia was more frequently observed in the treatment with PEG-IFN/RBV/TLV (27%) than that with PEG-IFN/TLV (9%) (Hezode et al., 2009; Reesink et al., 2006).

The observed data from our study not only confirm reports from previous papers (Boglione et al., 2014; Hammond et al., 2013; Karino et al., 2013; Mauss et al., 2014) in terms of toxicity evaluation, but also highlight the relationship between TLV-S isomer, RBV plasma concentrations and haemoglobin loss.

From this study we observed how the TLV-S, and not TLV-R, plasma concentration is the real pharmacokinetic factor correlated with RBV concentration in triple therapy and for the first time we showed how RBV increase in triple therapy is dependent on the plasma concentration of TLV-S isomer.

This evidence is very important, as, to date, studies including the evaluation of TLV concentrations considered its total amount (-S and -R amount together) and are referred to SVR and renal impairment (Fukuda et al., 2013; Furusyo et al., 2014; Karino et al., 2013).

So, new studies (and possibly TDM, too) should be performed considering also TLV-S plasma concentrations, probably for both SVR and anaemia.

Regardless the intimate mechanism increasing RBV pharmacokinetic exposure, TLV-S isomer and RBV plasma concentrations resulted to be significantly correlated.

Probably, this phenomenon could be due to a decrease in the glomerular filtration rate, as proposed by other authors (Karino et al., 2013; Mauss et al., 2014; Tempestilli et al., 2013). In fact, as reported by Mauss et al. (Mauss et al., 2014) in a large cohort of patients with additional risks of renal impairment, the triple therapy with TLV leads to a marked reduction of eGFR within the first 12 weeks of treatment. This scenario seems to be confirmed by our data, with a median decrease of nearly 13% in eGFR at 1 month of therapy. On the other hand, unlike Karino et al. (Karino et al., 2013) we observed that the decrease of renal function was not correlated neither with TLV and/or RBV pharmacokinetics, TLV weight-adjusted dose or percent haemoglobin loss.

However, our sample is less numerous, different in ethnicity and, moreover, timings for the evaluation of eGFR and toxicity are different.

From our data, it is evident that the increased anaemia rate with TLV is the consequence of higher RBV plasma and intra-erythrocytic concentrations (Boglione et al., 2014; Karino et al., 2013).

This issue could be important in the management of the triple therapy with TLV; the tolerability improvement of this regimen is critical for treatment completion and virological response, and our data showed the possibility to reduce RBV dosage, maintaining good plasma levels. These hypothesis seems to be supported by previous clinical studies as ADVANCE, ILLUMINATE and SPRINT. These studies did not show any difference on SVR in patients with anaemia and RBV-dose reduction (Sherman et al., 2011; Zeuzem et al., 2011).

Moreover, the most common reasons for discontinuing the triple therapy are side-effects and hematologic toxicity, therefore more tolerable regimens are required for naïve patients or with non advanced fibrosis stage (Chen et al., 2013). For patients with advanced fibrosis and cirrhosis we need to consider the higher rate (51%) of serious adverse events in this population, with a discontinuation rate of nearly 12%: more specifically, the most common events seem to be the incidence of grade 2 (32%), 3 and 4 (14%) anaemia, erythropoietin administration (19%) and blood transfusion (12%) (Hezode, 2012).

Therefore we highlight that the prevention of anaemia and the individualization of therapy with TLV is pivotal in these patients, according to our experience. First of all, a stratification of risk of anaemia onset is needed before the treatment begins, performing the *ITPA* gene polymorphisms genotyping (D'Avolio et al., 2013a,b,c) and testing the patients for their baseline renal function, as it can be a predictor of anaemia (Romero-Gomez et al., 2013): in patients with a higher baseline risk of early anaemia onset, we suggest a RBV starting dose of 200 mg lower than that based on the patients weight; second, the therapeutic drug monitoring (TDM) of RBV plasma concentration (as well as the monitoring of eGFR changes) as early as 1 or 2 weeks after the start of treatment with TLV, should be used as a guide for the early dose adjustment of RBV, avoiding the onset of anaemia (D'Avolio et al., 2011; D'Avolio et al., 2012a); third, the TDM of RBV concentrations should be repeated after TLV suspension to re-adjust again the RBV dosage according to its plasma levels.

This is surely the newest and the most comprehensive approach in order to anticipate the onset of anaemia, very different from the current standard management. In fact, recently, Romero-Gómez et al. (Romero-Gomez et al., 2013), other than mentioning the involvement of renal function as a baseline factor in determining the onset of anaemia, reviewed the most effective strategies to manage it during triple therapy, but they focused on the weight-based dose of RBV and the strict monitoring of haemoglobin levels. Conversely, our hint is to modify RBV dosage according to plasma concentration measurement preventing the anaemia onset. Recently, some cut-off values of RBV concentration have been determined as predictive for the onset of early anaemia (Caviglia et al., 2012; De Nicolò et al., 2013a) and for EVR (De Nicolò et al., 2013b), in patients infected with HCV genotype 1 or 4 treated with dual therapy: from these works it was evident that, according to the patient genetics, plasma concentrations at 2 weeks minor than 1700 ng/mL are associated with minor risk of developing anaemia; moreover, concentrations of at least 1800 ng/mL after 4 weeks are associated with higher probability to reach EVR. This type of study should be repeated with triple therapy treated patients, in order to determine specific cut-off values in a genotype-stratified population.

While these cut-off values are determined for dual therapy, they could actually be a theoretical reference for the evaluation of RBV concentrations also in triple one. In fact, all the evidences seem to point out the RBV increased concentration as the cause of the higher onset of anaemia.

For all these reasons, further studies with larger cohorts of patients should be performed to deepen the role of TLV in increasing RBV concentrations (the decrease of eGFR could be only one of many different mechanisms underlying this interaction); then, the identification of new RBV plasma concentration cut-off values, to prevent toxicity and to optimize viral response, will be pivotal to confirm the usefulness of TDM-guided approach to the triple therapy with TLV.

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Transparency declarations

Authors do not declare any conflicts of interest.

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