

# World Journal of *Hepatology*

*World J Hepatol* 2017 December 8; 9(34): 1261-1277



**ORIGINAL ARTICLE****Basic Study**

- 1261** Morphological alterations and redox changes associated with hepatic warm ischemia-reperfusion injury  
*Jawad R, D'souza M, Selenius LA, Lundgren MW, Danielsson O, Nowak G, Björnstedt M, Isaksson B*

**Observational Study**

- 1270** Liver decompensation predicts ribavirin overexposure in hepatitis C virus patients treated with direct-acting antivirals  
*Guardigni V, Badia L, Conti M, Rinaldi M, Mancini R, Viale P, Verucchi G*

**ABOUT COVER**

Editorial Board Member of *World Journal of Hepatology*, Balasubramaniyan Vairappan, Assistant Professor, Liver Diseases Research Lab, Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Dhanvantri Nagar, Puducherry 605006, India

**AIM AND SCOPE**

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

**INDEXING/ABSTRACTING**

*World Journal of Hepatology* is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, and Scopus.

**FLYLEAF**

I-IV Editorial Board

**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Ya-Jing Lu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xiu-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Hepatology*

**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

**EDITORS-IN-CHIEF**  
**Clara Balsano, PhD, Professor**, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor**, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

**EDITORIAL BOARD MEMBERS**  
All editorial board members resources online at <http://www.wjgnet.com>

[www.wjgnet.com/1948-5182/editorialboard.htm](http://www.wjgnet.com/1948-5182/editorialboard.htm)

**EDITORIAL OFFICE**  
Xiu-Xia Song, Director  
*World Journal of Hepatology*  
Baishideng Publishing Group Inc  
7901 Stoneridge Drive, Suite 501,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
7901 Stoneridge Drive, Suite 501,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

**PUBLICATION DATE**  
December 8, 2017

**COPYRIGHT**  
© 2017 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
<http://www.wjgnet.com/bpg/gerinfo/204>

**ONLINE SUBMISSION**  
<http://www.f6publishing.com>

Observational Study

# Liver decompensation predicts ribavirin overexposure in hepatitis C virus patients treated with direct-acting antivirals

Viola Guardigni, Lorenzo Badia, Matteo Conti, Matteo Rinaldi, Rita Mancini, Pierluigi Viale, Gabriella Verucchi

Viola Guardigni, Lorenzo Badia, Matteo Rinaldi, Pierluigi Viale, Gabriella Verucchi, Department of Medical and Surgical Sciences, University of Bologna, S. Orsola-Malpighi Hospital, Bologna 40138, Italy

Viola Guardigni, Lorenzo Badia, Gabriella Verucchi, Research Centre for the Study of Hepatitis, University of Bologna, Bologna 40138, Italy

Matteo Conti, Rita Mancini, Metropolitan Laboratory, Maggiore Hospital, Bologna 40133, Italy

ORCID number: Viola Guardigni (0000-0002-2769-7325); Lorenzo Badia (0000-0001-5694-1158); Matteo Conti (0000-0002-1579-9674); Matteo Rinaldi (0000-0002-3568-5973); Rita Mancini (0000-0002-5491-2449); Pierluigi Viale (0000-0003-1264-0008); Gabriella Verucchi (0000-0001-6546-0495).

**Author contributions:** Guardigni V carried out the statistical analysis and drafting the manuscript; Guardigni V and Badia L developed concept and design of the study; all authors were involved in data collection, interpretation of data and editing of the manuscript.

**Institutional review board statement:** Formal institutional approval was not deemed necessary since anonymised data routinely collected in our centre for clinical practice purposes were used.

**Informed consent statement:** All study participants provided informed consent prior to study inclusion.

**Conflict-of-interest statement:** The authors declare no conflict of interests.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on

different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Viola Guardigni, MD, Department of Medical and Surgical Sciences, University of Bologna, S. Orsola-Malpighi Hospital, via G. Massarenti 9, Bologna 40138, Italy. [viola.guardigni2@unibo.it](mailto:viola.guardigni2@unibo.it)  
Telephone: +39-0512-144301  
Fax: +39-0512-144301

Received: May 29, 2017

Peer-review started: June 1, 2017

First decision: July 4, 2017

Revised: August 2, 2017

Accepted: October 15, 2017

Article in press: October 16, 2017

Published online: December 8, 2017

## Abstract

### AIM

To determine whether ribavirin (RBV) concentrations differ according to cirrhosis stage among cirrhotic patients treated with interferon-free regimens.

### METHODS

We included patients with hepatitis C virus and cirrhosis [Child-Pugh (CP) A or B], Glomerular Filtration Rate  $\geq$  60 mL/min, who started therapy with DAAs and weight-based RBV between October 2014 and February 2016. RBV plasma levels were assessed during the treatment. We focused our analysis on the first 8 wk of therapy.

### RESULTS

We studied 68 patients: 54 with compensated (CP-B) and 14 with decompensated (CP-A) cirrhosis. Patients with



decompensated cirrhosis displayed significantly higher RBV concentrations than those with compensated cirrhosis at week 1, 2, 4 and 8 ( $P < 0.035$ ). RBV levels were positively correlated with Hb loss over the treatment ( $P < 0.04$ ). Majority (71%) of CP-B patients required a RBV dosage reduction during the treatment. After adjustment for confounders, Child-Pugh class remained significantly associated (95%CI: 35, 348,  $P = 0.017$ ) to RBV levels, independently from baseline per-Kg RBV dosage.

### CONCLUSION

Liver decompensation might affect RBV clearance leading to an overexposure and increased related toxicities in decompensated cirrhosis. Our findings underscore the importance of an early ribavirin therapeutic drug monitoring and suggest that an initial lower RBV dose, rather than weight-based, might be considered in those with advanced liver disease (CP-B) treated with direct-acting antivirals.

**Key words:** Hepatitis C; Direct-acting antivirals; Ribavirin; Therapeutic drug monitoring; Decompensated cirrhosis

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this study, patients with decompensated cirrhosis displayed higher plasma ribavirin concentrations in comparison to compensated patients, when treated with Interferon-free regimens for hepatitis C. Higher ribavirin levels were found to lead to greater rates of related toxicities and Child-Pugh class resulted independently associated with ribavirin plasma levels, in our population. Our findings suggest that ribavirin concentrations should be strictly monitored in subjects with advanced liver disease, during direct-acting antivirals-treatment. An early dosage adjustment of ribavirin should be performed when high levels of this antiviral are detected in patients' plasma, in order to avoid toxicities among these frail individuals.

Guardigni V, Badia L, Conti M, Rinaldi M, Mancini R, Viale P, Verucchi G. Liver decompensation predicts ribavirin overexposure in hepatitis C virus patients treated with direct-acting antivirals. *World J Hepatol* 2017; 9(34): 1270-1277 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i34/1270.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i34.1270>

## INTRODUCTION

Before the advent of direct-acting antivirals (DAAs), the association of Peg-interferon (Peg-IFN) and ribavirin (RBV) represented the standard of care (SOC) for the treatment of chronic C hepatitis, regardless of hepatitis C virus (HCV) genotype and stage of liver disease<sup>[1]</sup>. Although HCV-induced cirrhosis represents a major cause of liver-related morbidity and mortality and successful therapy of individuals with advanced fibrosis and liver cirrhosis is associated with better

outcomes [e.g., decreased incidence of hepatocellular carcinoma (HCC), decompensation]<sup>[2]</sup>, patients with cirrhosis were rarely treated because of the high risk of decompensation due to Peg-IFN administration.

New DAAs-regimens have dramatically changed this scenario, leading to the achievement of high rate of HCV eradication, even in individuals with compensated and decompensated cirrhosis. Although the role of RBV in the era of DAAs will probably decrease, it is currently still recommended for difficult-to-treat patients (i.e., experienced and cirrhotic), to optimize sustained virological response (SVR) in many treatment regimens<sup>[3]</sup>.

Monitoring of ribavirin plasma concentration, given its interindividual variability, was used during combination therapy with Peg-IFN, since RBV exposure had been shown to be associated with treatment efficacy (SVR) and side effects (i.e., anaemia)<sup>[4,5]</sup>. In particular, trough RBV concentration ( $C_{trough}$ ) at week 4 and 8 of treatment was commonly used<sup>[5-7]</sup>. Indeed, steady-state concentrations are reached after the first 4 wk of therapy according to scientific literature<sup>[8]</sup>.

Although higher RBV concentration levels have been associated to higher rates of SVR and therapeutic ranges at week 8 have been established also for first-generation DAAs-based HCV therapies (with telaprevir or boceprevir)<sup>[9]</sup>, role of RBV therapeutic drug monitoring (TDM) in the era of second-generation DAAs regimens has not been investigated and elucidated yet.

Thus far, data on ribavirin TDM among cirrhotic patients with advanced liver disease, treated by the combination of RBV and last generation DAAs are lacking.

Multiple factors are involved in RBV pharmacokinetics consequently affecting RBV plasma concentrations, such as creatinine clearance, gender, age, diet, HIV infection, weight<sup>[3,10]</sup>. Child-Pugh score, a marker of cirrhosis severity, used to estimate the prognosis in these patients, has never been investigated as a determinant of RBV plasma concentrations during HCV treatment.

The aim of our retrospective study was to determine whether ribavirin plasma levels differ according to cirrhosis stage (defined by Child-Pugh class) in a cohort of subjects with liver cirrhosis, treated for HCV with IFN-free DAA-regimens containing RBV and to define effects of plasma RBV levels.

## MATERIALS AND METHODS

We retrospectively included in this study all the patients with chronic C hepatitis and cirrhosis (Child-Pugh A or B), without significant renal impairment [Estimated Glomerular Filtration Rate (eGFR)  $\geq 60$  mL/min], who started HCV treatment with DAAs in association with ribavirin (weight-based dose  $\geq 11$  mg/kg per die) between October 2014 and February 2016 at Infectious Diseases Department of University Hospital of Bologna (Italy). We excluded from the analysis two subjects

who had less than two available plasma ribavirin level measurements throughout the antiviral therapy. Patients who received Peg-IFN in combination with DAAs and RBV were excluded from the study. All the patients were treated for 12 or 24 wk according to the national and international guidelines and were afterwards monitored for at least 12 wk after completing the therapy to assess SVR (SVR12). Patients were categorized into two groups (compensated or decompensated cirrhosis) according to their Child-Pugh score ( $< 7$  or  $\geq 7$ , respectively) to perform the analysis of interest.

For each subject, we collected the following data at baseline (corresponding with day of treatment start): Demographics (sex, age, race), medical history on previous HCV treatment (naïve or experienced), on chronic C hepatitis (HCV genotype, HCV RNA quantification) and on liver diseases (fibrosis stage, history of previous HCC, Child-Pugh class), HIV and HBV coinfections, creatinine level, eGFR, haemoglobin (Hb), bilirubin, current anti-HCV regimen (administered DAAs and Ribavirin initial dosage).

Data on RBV dose reduction over the course of treatment, therapy duration and response to the HCV treatment (as SVR12) were also recorded. Liver fibrosis was assessed according to the Metavir score either from elastographic measurements or from liver biopsy prior to HCV treatment. HCV RNA levels were detected by using Roche COBAS AmpliPrep/COBAS TaqMan HCV Test v 2.0 (Roche Molecular Systems, Inc., Pleasanton, CA). eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.

Reasons for RBV dosage adjustment were the following: Occurrence of anaemia (Hb decline of more than 20 g/L or Hb less than 100 g/L), high RBV trough concentration ( $> 2500$  ng/mL), that was considered at high risk for significant Hb decline<sup>[11,12]</sup>, and other adverse effects (e.g., cutaneous rash, itch).

All the subjects' visits and blood samples were standardized according to treatment schedule and performed at baseline and at week 1, 2, 4, 8, 12, 16, 20, 24 over treatment period. For each patient RBV concentrations were quantified using plasma samples that were collected multiple times during the treatment (average of 6.5 samples per subject), although we mainly focused our analysis on the first 8 wk of treatment because steady concentrations are usually reached between week 4 and 8<sup>[8]</sup> and because changes in RBV concentrations after week 8 (due to dosage adjustment) were expected to occur in our population.

RBV concentrations were measured in plasma samples, collected before taking the next dose of the drug (*C<sub>trough</sub>*), using a novel analytical method, validated according to the ISO 15089 predicaments. In brief, Ribavirin is extracted from plasma by means of a methanol-water (20%-80% mL/L) solution containing ZnSO<sub>4</sub> (0.1 mol/L in water). The drug is then resolved chromatographically from endogenous and exogenous isobaric interferences by means of a binary

chromatographic gradient. Specific signals for the drug are obtained in multiple reaction monitoring mode, recording the  $245.1 > 113.2$  and  $245.1 > 96.2$  m/z mass transitions. Ion extraction chromatograms enable drug determination with great specificity and sensitivity (Limit of Quantification = 1 µg/L).

### Statistical analysis

Variables were expressed as mean  $\pm$  SD or median and range interquartile. Continuous variables were compared by *t*-test or by Mann-Whitney-*U* test, when distribution was normal or not normal, respectively. Categorical variables were compared by chi-square test or Fisher's exact as appropriate. Bivariate association between RBV plasma levels and continuous variables of interest were tested by Spearman rank correlations. Linear regression was first performed to evaluate factors associated with RBV plasma levels in the univariate analysis and afterward to determine independent predictors of RBV plasma levels among variables with significant result at the univariate. All statistical analysis were performed using SPSS software version 21. Tests were considered significant for a *P* value  $< 0.05$ .

## RESULTS

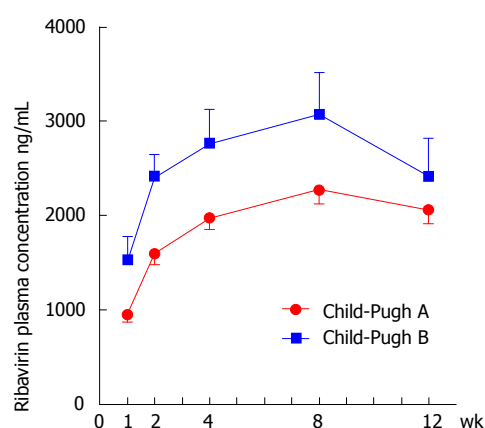
### Characteristics of population

We totally evaluated 68 cirrhotic patients who underwent anti-HCV treatment with DAAs in association with RBV in the study period: 54 had a compensated liver cirrhosis and 14 a decompensated cirrhosis (Child-Pugh A, scored  $< 7$  and B, scored  $\geq 7$ , respectively). Baseline characteristics of our population are shown in Table 1. No differences in demographics were found between the two groups. Fifty-seven point four percent of the population was composed by HIV co-infected patients who showed a greater, but not significantly, prevalence in decompensated subjects (53.7% vs 71.4%). Most of the subjects (63.2%) had been previously treated with Peg-IFN-based regimen, unsuccessfully. Genotype 1a was the most common HCV genotype (33.8%), as well as the association of Sofosbuvir (SOF, 400 mg/die) and Daclatasvir (DCV, 60 mg/die, or 30 mg/die in 13 HIV patients taking atazanavir/rtv-containing antiretroviral regimens) was the most frequently used (42.6%) in the overall population, without any difference according to Child-Pugh class. As expected, significant differences in haemoglobin and total bilirubin levels were detected, before starting antiviral therapy between the two groups: Indeed compensated cirrhotic patients showed higher mean Hb ( $146 \pm 16$  g/L vs  $129 \pm 17$  g/L,  $P = 0.002$ ) and lower median bilirubin levels [0.47, interquartile range (IQR) 2.8-8.2 mg/L vs 7.7 mg/L, IQR 5.3-16,  $P < 0.001$ ] compared to decompensated patients. Creatinine values resulted higher in compensated patients ( $P = 0.011$ ), although eGFR was similar in the two groups. 86.8% of subjects was treated for 24 wk, 13.2% for 12 wk.

Table 1 Subjects' baseline characteristics

Characteristics	Overall (n = 68)	Child pugh A (n = 54)	Child pugh B (n = 14)	P value
Age (yr), mean $\pm$ SD	55 $\pm$ 8.6	55.2 $\pm$ 9.5	54.6 $\pm$ 5	0.8
Male, n (%)	52 (76.5)	43 (79.6)	9 (64.3)	0.2
White race, n (%)	66 (97.1)	53 (98.1)	13 (92.9)	0.3
HIV infection, n (%)	39 (57.4)	29 (53.7)	10 (71.4)	0.2
HBsAg positive, n (%)	1 (1.5)	1 (1.9)	0	-
History of HCC, n (%)	6 (8.8)	4 (7.4)	2 (14.3)	0.6
HCV treatment experienced, n (%)	43 (63.2)	33 (61.1)	10 (71.4)	0.5
Liver stiffness (kPa), median (IQR)	21.4 (15.9-34.4)	21.2 (15.6-33.1)	31.1 (17.8-54.3)	0.1
HCV genotype, n (%)				0.7
1a	23 (33.8)	18 (33.3)	5 (35.7)	
1b	11 (16.2)	9 (16.7)	2 (14.3)	
2	6 (8.8)	6 (11.1)	-	
3	22 (32.4)	17 (31.5)	5 (35.7)	
4	6 (8.8)	4 (7.4)	2 (14.3)	
Weight (kg), mean $\pm$ SD	73 $\pm$ 14.1	73.2 $\pm$ 15.3	72.1 $\pm$ 8.5	0.7
Hb baseline (g/L), mean $\pm$ SD	143 $\pm$ 18	146 $\pm$ 16	129 $\pm$ 17	0.002
Bilirubin (mg/L), median (IQR)	9.5 (5.6-19)	4.7 (2.8-8.2)	7.7 (5.3-16)	< 0.001
Creatinine (mg/L), mean $\pm$ SD	8 $\pm$ 1.3	8.2 $\pm$ 1.5	7.5 $\pm$ 0.8	0.011
eGFR (mL/min per 1.73 m <sup>2</sup> ), median (IQR)	101 (90-109)	99 (88-105)	102 (98-107)	0.3
HCV-RNA baseline (IU/mL)	933559 (25667-2128936)	1228876 (410822-2940050)	246455 (126308-712307)	0.007
<sup>1</sup> DAA's regimen, n (%)				0.2
SOF, n (%)	6 (8.8)	6 (11.1)	-	
SOF + DCV, n (%)	29 (42.6)	21 (38.9)	8 (57.1)	
SOF + SMP, n (%)	1 (1.5)	-	1 (7.1)	
DCV + SMP, n (%)	4 (5.9)	3 (5.6)	1 (7.1)	
SOF + LDV, n (%)	12 (17.6)	11 (20.4)	1 (7.1)	
OBV/PTV/r + DSV, n (%)	12 (17.6)	9 (16.7)	3 (21.4)	
OBV/PTV/r, n (%)	4 (5.9)	4 (7.4)	-	

<sup>1</sup>All regimens are intended in association with ribavirin. HCC: Hepatocellular carcinoma; eGFR: Estimated glomerular filtration rate; DAAs: Direct-acting antivirals; SOF: Sofosbuvir; DCV: Daclatasvir; SMP: Simeprevir; LDV: Ledipasvir; OBV: Ombitasvir; PTV: Paritaprevir; R: Ritonavir; DSV: Dasabuvir.



**Figure 1 Ribavirin plasma concentrations over the treatment.** Error bars represent standard errors. Ribavirin plasma concentrations statistically differ between Child-Pugh A and B patients at week 1, 2, 4 and 8 (all *P* value < 0.025). Legend: Child-Pugh B patients display significantly higher ribavirin concentrations (expressed as C trough) compared to Child-Pugh A patients over the first 8 wk of treatment with direct acting antivirals.

### Ribavirin concentrations and dosage

There was no difference in initial mean dose of RBV (mg/kg) between the two groups (*P* = 0.3). Significant differences in RBV plasma concentrations between the two groups were found at each considered time-point (week 1, 2, 4 and 8) with much higher *C<sub>trough</sub>* levels among decompensated (Child-Pugh B) than among

compensated (Child-Pugh A) patients. Otherwise, no remarkable difference in overall RBV mean values (measured during all the course of treatment) was revealed (Table 2).

Figure 1 shows the significant different trends of RBV plasma concentration from baseline until week 12 in the two cirrhotic groups.

In almost three-quarters (71.4%) of Child-Pugh B patients a reduction of RBV dosage was required during the treatment period, instead only 50% of Child-Pugh A subjects experienced a dosage adjustment (*P* = 0.15), after an average of 6.8 and 7.1 wk from treatment start among Child-Pugh A and B, respectively. Most common reasons for RBV dosage reduction were onset of anaemia (36.1%) and high RBV concentration revealed by TDM (36.1%), data not shown. Although decompensated patients experienced greater Hb loss (at both week 4 and week 8) than those with compensated cirrhosis, the differences were not significant (Table 2).

Multiple factors potentially associated with RBV concentration were investigated at univariate analysis. The significant predictors of RBV concentrations (as a treatment average between week 1 and 8) were baseline RBV dose per kilogram ( $\beta$  value 205, 95%CI: 77; 332, *P* = 0.002), male gender ( $\beta$  value -539, 95%CI: -975; -103, *P* = 0.016) and Child-Pugh class ( $\beta$  value 232, 95%CI: 66; 399, *P* value= 0.007). After adjusting for all these significant variables, only baseline

**Table 2** Ribavirin dosages and plasma levels (C trough) and haemoglobin loss over the course of treatment

Parameters	Overall ( <i>n</i> = 68)	Child pugh A ( <i>n</i> = 54)	Child pugh B ( <i>n</i> = 14)	<i>P</i> value
RBV (mg/kg pre die), mean ± SD	14.4 ± 1.7	14.4 ± 1.36	14.9 ± 1.6	0.3
<sup>1</sup> RBV week 1 (ng/L) median (IQR)	9105 (6130-1310)	8750 (5700-11680)	14050 (9010-22280)	0.007
<sup>1</sup> RBV week 2 (ng/L) median (IQR)	15000 (11600-27680)	14150 (11150-20000)	23550 (17200-28930)	0.001
<sup>2</sup> RBV week 4 (ng/L) median (IQR)	18300 (12900-27680)	17850 (12200-26500)	26250 (17700-35130)	0.024
<sup>3</sup> RBV week 8 (ng/L) median (IQR)	23100 (17050-31000)	21500 (16100-27950)	33000 (19800-41380)	0.0034
Mean RBV 1, 2, 4 wk, (ng/L) median (IQR)	14240 (10770-19450)	13280 (10480-18200)	19600 (14670-26870)	0.005
Mean RBV 1, 2, 4, 8 wk, (ng/L) median (IQR)	16490 (12220-21750)	15135 (11850-19830)	21700 (15090-29280)	0.006
Mean RBV, full treatment duration (ng/L), mean ± SD	18079 ± 6609	17528 ± 6020	20327 ± 7700	0.2
RBV dosage reduction, <i>n</i> (%)	37 (54.4)	27 (50)	10 (71.4)	0.2
Weeks before reduction, median (IQR)	6.1 (4-9.8)	5 (4-9.7)	7 (4-10)	0.6
Hb loss week 4 (g/L), mean ± SD	19.6 ± 1.8	19 ± 13	21 ± 32	0.5 <sup>4</sup>
Hb loss week 8 (g/L), mean ± SD	21 ± 19	20 ± 13	25 ± 35	0.3 <sup>4</sup>

<sup>1</sup>Data for 68 subjects; <sup>2</sup>Data for 62 subjects; <sup>3</sup>Data for 95 subjects; <sup>4</sup>Analysis of covariance of Hb at week 4 and haemoglobin (Hb) at week 8, adjusted for baseline Hb levels. RBV: Ribavirin; Hb: Haemoglobin; IQR: Interquartile range.

**Table 3** Baseline predictors of average plasmatic Ribavirin levels in the first 8 wk of therapy

	Univariate analysis			Multivariate analysis		
	$\beta$ value	95%CI	<i>P</i> value	$\beta$ value	95%CI	<i>P</i> value
Baseline ribavirin dosage (mg/kg)	205	77; 332	0.002	161	36; 286	0.013
IFN-experienced	97	-303; 497	0.6			
Male gender	-539	-975; -103	0.016	-349	-765; 68	0.099
Age (yr)	13	-9; 34	0.3			
White race	424	-714; 1563	0.5			
Bilirubin (mg/L)	870	-920; 2670	3			
Creatinine (mg/L)	-2890	-16830; 11040	7			
Child-Pugh class	232	66; 399	0.007	192	35; 348	0.017
Liver stiffness (kPa)	-1.6	-14; 11	0.8			
HIV infection	76	-314; 466	0.7			

IFN: Interferon; HIV: Human immunodeficiency virus.

RBV dosage ( $\beta$  value 161.1, 95%CI: 35.6; 286,  $P$  = 0.013) and Child-Pugh class ( $\beta$  value 191.7, 95%CI: 35; 348,  $P$  = 0.017) remained significantly associated to RBV levels (Table 3). To investigate whether the association with Child-Pugh class was driven by single items of this composite index, we analysed each of them (*i.e.*, Encephalopathy, Ascites, Bilirubin, INR, Albumin at baseline) in relationship to RBV concentrations, but no significant differences in RBV overexposure were observed. Moreover, clearance of RBV (indirectly assessed by its plasma concentrations) did not seem to be related to portal hypertension signs (*e.g.*, platelet count, esophageal varices), data not shown.

Remarkably, there were no significant differences in RBV concentration (as an average value obtained from the first 8 wk measurements) between subjects who achieved a rapid virological response (RVR, namely HCV undetectability within week 4 of treatment) and those who did not (Figure 2). Furthermore, no correlation between RBV concentrations (at any time-point) and time to reach HCV undetectability was observed in our population (data not shown). The majority of patients included in this study (92.6%) achieved SVR12, without any difference in SVR rate based on Child-Pugh class

and RBV levels at week 4 and 8 (data not shown).

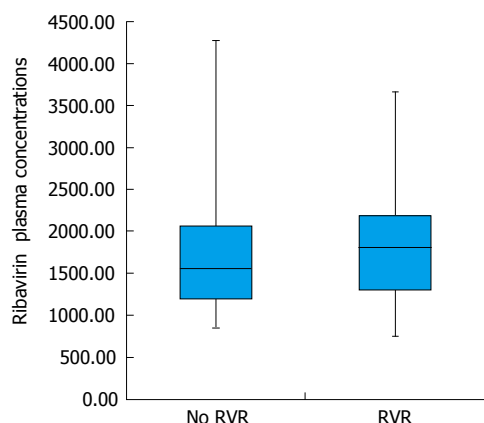
### Haemoglobin loss

As depicted in Figure 3, Hb loss (g/L) in the first two months of treatment (at the two therapy time-points: Week 4 and 8) was correlated to RBV concentrations measured at the corresponding week ( $R$  = + 0.272,  $P$  value= 0.033 at week 4,  $R$  = + 0.279,  $P$  = 0.025 at week 8), with higher RBV levels associated with greater Hb decline. The relationship between Hb loss at week 8 and RBV levels at that specific week was independent from Child-Pugh class (in a multivariate linear regression,  $P$  = 0.002).

## DISCUSSION

The advent of new IFN-free DAAs regimens has allowed to achieve very high rates of HCV eradication, even in patients with advanced liver disease. Although the role of RBV is now discussed and its relevance will probably decrease over time, this antiviral drug is still recommended in combination with DAAs in several challenging situations: For treatment-experienced but DAAs-naïve patients without cirrhosis or with compensated cirrhosis (Child-Pugh A) to reduce weeks of therapy, for subjects who failed to





**Figure 2** Average of ribavirin plasma concentrations between week 1 and 8 of treatment in association with rapid virological response. Legend: Achievement of Rapid Virological Response (*i.e.*, hepatitis C virus RNA undetectability within week 4 of treatment) was not associated with ribavirin plasma concentrations measured over the first 8 wk of treatment. RVR: Rapid virological response.

achieve SVR on prior antiviral therapy containing DAAs and for those with decompensated cirrhosis (Child-Pugh B or C) to increase SVR rates<sup>[13]</sup>.

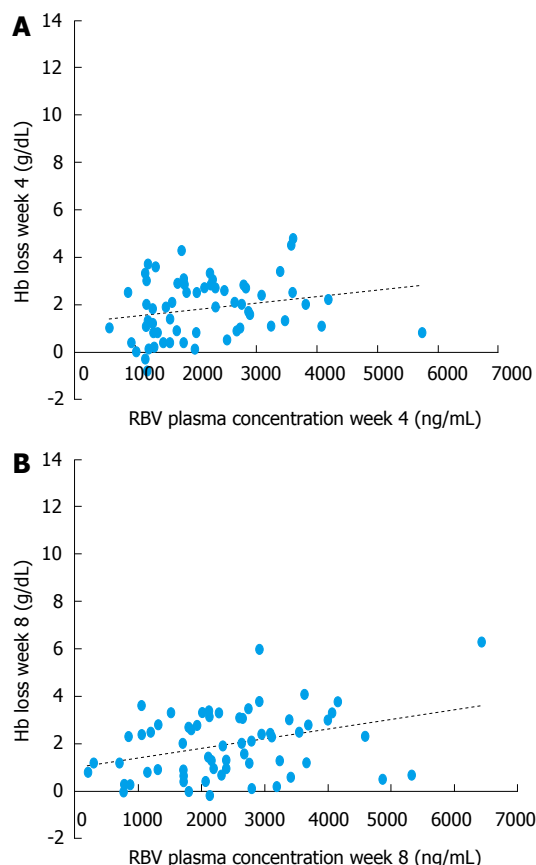
To date, specific data regarding ribavirin TDM in cirrhotic patients treated with IFN-free regimens are lacking and daily weight-based ribavirin remains the SOC. In particular, a daily dose of ribavirin higher than 10 mg/kg of body weight had been previously associated to higher rate of SVR among subjects treated with Peg-IFN and RBV<sup>[14]</sup> and it is still used with DAAs, although the use of RBV according body weight has been occasionally disapproved due to the variability in this antiviral elimination phase<sup>[4]</sup>.

In our study, patients with decompensated cirrhosis (Child-Pugh B) remarkably displayed higher plasma levels of RBV during the first 8 wk of therapy with DAAs compared to those with compensated cirrhosis (Child-Pugh A), although initial weight-dosages were similar. This raises the hypothesis that liver failure might play a meaningful role in RBV clearance, not observed before.

The fact that overall RBV mean plasma concentrations did not differ based on Child-Pugh class in our cohort might be explained by the greater rate of RBV dose reduction (but no discontinuation) and the following RBV exposure decline in those with decompensated cirrhosis, over the course of treatment.

Reduced dosages of ribavirin are currently recommended in patients with moderate or severe renal impairment (Creatinine Clearance  $\leq 60$  mL/min), since RBV exposure is predicted to increase up to 17% in this subsets of patients, due to its renal excretion<sup>[15]</sup>. Also weight, gender, age, fat meals have been shown to clearly affect RBV metabolism<sup>[3]</sup>. Gastrointestinal tract is another potential site of RBV clearance<sup>[16]</sup>.

To date, no adjustment in ribavirin dosage is required in liver dysfunction, which instead affects metabolism of many drugs<sup>[17]</sup>, since so far liver failure did not seem to impact on ribavirin clearance.



**Figure 3** Correlation between ribavirin plasma concentrations and haemoglobin loss at week 4 and 8. Legend: Higher ribavirin plasma concentrations resulted significantly correlated with greater haemoglobin loss at both week 4 (A) and week 8 (B) of therapy in the overall population. RBV: Ribavirin.

Only Glue<sup>[18]</sup> assessed the single dose pharmacokinetics of this antiviral in subjects with various degrees of chronic liver disease: In agreement to our results, they observed that  $C_{max}$  significantly increased with the severity of liver dysfunction, although there was a remarkable overlap in individual  $C_{max}$  values among the groups and they eventually did not propose any dosage reduction in these patients.

Contrarily, we first evaluated RBV pharmacokinetics in patients with liver disease over the course of the treatment in a real-world setting, obtaining then different findings. One could argue that a slower clearance of the drug in our population was due to a cirrhosis-related renal dysfunction (*i.e.*, hepatorenal syndrome), but we included only subjects with eGFR  $\geq 60$  mL/min and unexpectedly creatinine was not found to be associated to RBV concentrations over the first 8 wk at univariate analysis in our population.

Conversely, a higher Child-Pugh score determined a RBV overexposure, leading to high rate of dose adjustment in those with Child-Pugh  $\geq 7$ . To our knowledge, this is the first study exploring ribavirin TDM in decompensated cirrhotic individuals treated with new DAAs regimens and showing an overexposure in this group of patients. Indeed, while few data report RBV plasma levels in therapeutic range in patients with

Child-Pugh A treated with a fixed dose (800 mg daily) of RBV associated with SOF and DCV<sup>[19]</sup>, no information about Child-Pugh B or C patients are currently available. These findings might be useful to clinicians, since an overexposure can be modulated by lowering the initial RBV dosage from the weight-based dosage in certain patients with advanced liver disease, avoiding secondary and detrimental effects.

Furthermore, our data show a positive correlation between RBV concentrations and Hb loss, as already widely reported in literature, even in the context of IFN-free regimens<sup>[20]</sup>. This finding strengthens the importance of considering a lower RBV dosage in subjects with decompensated cirrhosis, also considering their higher rate of baseline anaemia characterizing this group of patients (confirmed also in our population). Indeed, anaemia occur in about 75% of patients with chronic liver disease and can be linked to different aetiologies (e.g., haemorrhage, splenomegaly)<sup>[21]</sup>, regardless of the use of anti-HCV drugs, such as ribavirin, and represents a condition to be taken into account in the management of cirrhotic patients undergoing anti-HCV treatment.

According to our results, higher RBV concentrations in the first weeks of therapy did not seem to enhance RVR rate in cirrhotic patients treated with IFN-free regimens and no relationship between RBV plasma concentration at week 4 or 8 and rate of SVR was found. This might appear in contrast with prior data regarding Peg-IFN/RBV<sup>[22]</sup> or, more recently, SOF/RBV treatment (e.g., FISSION, NEUTRINO and FUSION trials)<sup>[23,24]</sup>, in which RBV exposure was one of the factor associated with SVR, with suggested RBV plasma concentration threshold between 2000 and 3000 ng/mL<sup>[25,26]</sup>. In our cohort, the low rate of patients failing to achieve SVR (7.4%) could explain the lack of relationship between RBV levels and SVR rate: It is in general difficult to identify statistically significant predictive markers with such a high response rate.

This study has some limitation to be considered. The main limitation is the absence of  $C_{max}$  and AUC data, due to the observational nature of the study; however  $C_{trough}$  can be considered in clinical practice as a surrogate of plasma exposure and its values correlate with drug efficacy and toxicity in other studies<sup>[27]</sup>. Moreover, this is a retrospective study with a limited population size and this suggests caution prior to extend our results to the all the population with liver cirrhosis treated with RBV and DAAs. The inclusion of patients with mild renal impairment (eGFR 60-89 mL/min) could represent a confounding factor, although baseline creatinine was not found to affect RBV concentrations in our population.

In conclusion, liver failure might affect RBV clearance leading to an overexposure and increased related toxicities (e.g., anaemia) in subjects with decompensated cirrhosis who are now being treated with last-generation DAAs. Our findings underscore the importance of RBV plasma monitoring and early dose adjustments in these patients and suggest that an initial lower dose, rather than weight-based, of RBV might be considered

in individuals with advanced liver disease (CP B class) treated with new DAAs in order to reduce toxicities without increasing virological failure rates.

This might improve the knowledge on the tailored use of ribavirin within IFN-free regimens for HCV treatment, in subjects with advanced liver disease. Further studies will be needed to confirm our results in order to determine the optimal dosage of ribavirin in patients with advanced cirrhosis in the era of DAAs-based regimens, since difficult-to-treat patients remain a challenge in HCV treatment and maintaining the benefit of this effective antiviral is still a relevant issue.

## COMMENTS

### Background

In the era of new direct-acting antivirals (DAAs), ribavirin (RBV) is still recommended for difficult-to-treat hepatitis C virus (HCV) positive patients, but data on RBV therapeutic drug monitoring (TDM) among patients with advanced liver disease treated with RBV and DAAs are lacking.

### Research frontiers

TDM represent an innovative technique and is currently widely used approach to personalize and ameliorate anti-infective treatments.

### Innovations and breakthroughs

This is the first study showing that patients with decompensated cirrhosis displayed significantly higher RBV concentrations than those with compensated cirrhosis, when treated with new Interferon-free regimens.

### Applications

These findings might improve the knowledge on the tailored use of ribavirin within IFN-free regimens for HCV treatment in subjects with advanced liver disease, for whom a lower initial dosage of RBV might be considered, in order to reduce risks related to RBV overexposure.

### Terminology

DAAs: Direct-acting antivirals represent the current standard of care for Chronic C Hepatitis; RBV: Ribavirin is an old-fashioned antiviral used in combination with last generation DAAs for difficult-to-treat HCV patients; TDM: Therapeutic drug monitoring by means of blood measurement of a drug concentration; *C trough*: Plasma concentration of a drug measured before taking the next dose of the drug.

### Peer-review

This paper offers important approach to the topic and treatment algorithm.

## ACKNOWLEDGMENTS

The authors thank Dr. Mario Luca Morieri for his relevant contribution to this manuscript.

## REFERENCES

- 1 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]
- 2 **Van der Meer AJ**, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE, Janssen HL. Association between sustained virological response and all-cause

- mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584-2593 [PMID: 23268517 DOI: 10.1001/jama.2012.144878]
- 3 **Pradat P**, Virlogeux V, Gagnieu M-C, Zoulim F, Bailly F. Ribavirin at the Era of Novel Direct Antiviral Agents for the Treatment of Hepatitis C Virus Infection: Relevance of Pharmacological Monitoring. *Adv Hepatol* 2014 [DOI: 10.1155/2014/493087]
  - 4 **Brochot E**, Castelain S, Duverlie G, Capron D, Nguyen-Khac E, François C. Ribavirin monitoring in chronic hepatitis C therapy: Anaemia versus efficacy. *Antivir Ther* 2010; **15**: 687-695 [PMID: 20710050 DOI: 10.3851/IMP1609]
  - 5 **Kuntzen T**, Kuhn S, Kuntzen D, Seifert B, Mullhaupt B, Geier A. Influence of Ribavirin Serum Levels on Outcome of Antiviral Treatment and Anemia in Hepatitis C Virus Infection. *PLoS One* 2016; **11**: e0158512 [PMID: 27388623 DOI: 10.1371/journal.pone.0158512]
  - 6 **Loustaud-Ratti V**, Debette-Gratien M, Jacques J, Alain S, Marquet P, Sautereau D, Rousseau A, Carrier P. Ribavirin: Past, present and future. *World J Hepatol* 2016; **8**: 123-130 [PMID: 26807208 DOI: 10.4254/wjh.v8.i2.123]
  - 7 **Marucco DA**, de Requena DG, Bonora S, Tettoni C, Bonasso M, De Blasi T, D'Avolio A, Sciandra M, Siccardi M, Baietto L, Trentini L, Sinicco A, Cariti G, Di Perri G. The use of trough ribavirin concentration to predict sustained virological response and haematological toxicity in HIV/HCV-co-infected patients treated with ribavirin and pegylated interferon. *J Antimicrob Chemother* 2008; **61**: 919-924 [PMID: 18238889 DOI: 10.1093/jac/dkn013]
  - 8 **Glue P**. The clinical pharmacology of ribavirin. *Semin Liver Dis* 1999; **1**: 17-24 [PMID: 10349689]
  - 9 **De Kanter CTMM**, Buti M, DeMasi R, Ouwerkerk-Mahadevan S, Doffershoff ASM, Witek J, Drenth JPH, Zeuzem S, Burger DM. Ribavirin concentration determines treatment success of first-generation DAA-based chronic HCV therapy. *Antivir Ther* 2016; **21**: 153-159 [PMID: 26378941 DOI: 10.3851/IMP2994]
  - 10 **Hatu G**, Bailly F, Pourcelot E, Pradat P, Mialhes P, Maynard M, Parant F, Chiarello P, Livrozet JM, Zoulim F, Gagnieu MC. Lower ribavirin bioavailability in patients with HIV-HCV coinfection in comparison with HCV mono-infected patients. *BMC Infect Dis* 2014; **14**: 150 [PMID: 24650094 DOI: 10.1186/1471-2334-14-150]
  - 11 **Bonora S**, Calcagno A, Cometto C, Fontana S, Aguilar D, D'Avolio A, Gonzalez de Requena D, Maiello A, Dal Conte I, Lucchini A, Di Perri G. Short-term additional enfuvirtide therapy is associated with a greater immunological recovery in HIV very late presenters: A controlled pilot study. *Infection* 2012; **40**: 69-75 [PMID: 22135137 DOI: 10.1007/s15010-011-0223-4]
  - 12 **Rendon AL**, Nunez M, Romero M, Barreiro P, Martin-Carbonero L, Garcia-Samaniego J, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Early monitoring of ribavirin plasma concentrations may predict anemia and early virologic response in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2005; **39**: 401-405 [PMID: 16010160 DOI: 10.1097/01.qai.0000170034.90438.68]
  - 13 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C. *J Hepatol* 2017; **66**: 153-194 [PMID: 27667367 DOI: 10.1016/j.jhep.2016.09.001]
  - 14 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749 DOI: 10.1016/S0140-6736(01)06102-5]
  - 15 **Polepally AR**, Badri PS, Eckert D, Mensing S, Menon RM. Effects of Mild and Moderate Renal Impairment on Ombitasvir, Paritaprevir, Ritonavir, Dasabuvir, and Ribavirin Pharmacokinetics in Patients with Chronic HCV Infection. *Eur J Drug Metab Pharmacokinetics* 2017; **42**: 1-7 [PMID: 27165046 DOI: 10.1007/s13318-016-0341-6]
  - 16 **Dixit NM**, Perelson AS. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. *Cell Mol Life Sci* 2006; **63**: 832-842 [PMID: 16501888 DOI: 10.1007/s00018-005-5455-y]
  - 17 **Tolman KG**. Drugs and the liver. *Med J Aust* 1977; **2**: 655-656 [PMID: 607107]
  - 18 **Glue P**, Schenker S, Gupta S, Clement RP, Zambas D, Salfi M. The single dose pharmacokinetics of ribavirin in subjects with chronic liver disease. *Br J Clin Pharmacol* 2000; **49**: 417-421 [PMID: 10792198 DOI: 10.1046/j.1365-2125.2000.00186.x]
  - 19 **Parisi SG**, Loregian A, Andreis S, Nannetti G, Cavinato S, Basso M, Scaggiante R, Dal Bello F, Messa L, Cattelan AM, Palù G. Daclatasvir plasma level and resistance selection in HIV patients with hepatitis C virus cirrhosis treated with daclatasvir, sofosbuvir, and ribavirin. *Int J Infect Dis* 2016; **49**: 151-153 [PMID: 27378577 DOI: 10.1016/j.ijid.2016.06.020]
  - 20 **Rower JE**, Meissner EG, Jimmerson LC, Osinusi A, Sims Z, Petersen T, Bushman LR, Wolfe P, McHutchison JG, Kottlil S, Kiser JJ. Serum and cellular ribavirin pharmacokinetic and concentration-effect analysis in HCV patients receiving sofosbuvir plus ribavirin. *J Antimicrob Chemother* 2015; **70**: 2322-2329 [PMID: 25971261 DOI: 10.1093/jac/dkv122]
  - 21 **Gonzalez-Casas R**, Jones EA, Moreno-Otero R. Spectrum of anemia associated with chronic liver disease. *World J Gastroenterol* 2009; **15**: 4653-4658 [PMID: 19787828 DOI: 10.3748/wjg.15.4653]
  - 22 **Larrat S**, Stanke-Labesque F, Plages A, Zarski JP, Bessard G, Souvignet C. Ribavirin quantification in combination treatment of chronic hepatitis C. *Antimicrob Agents Chemother* 2003; **47**: 124-129 [PMID: 12499179 DOI: 10.1128/AAC.47.1.124-129.2003]
  - 23 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
  - 24 **Jacobson IM**, Gordon SC, Kowdley K V, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR; POSITRON Study; FUSION Study. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]
  - 25 **Maynard M**, Pradat P, Gagnieu MC, Souvignet C, Trepo C. Prediction of sustained virological response by ribavirin plasma concentration at week 4 of therapy in hepatitis C virus genotype 1 patients. *Antivir Ther* 2008; **13**: 607-611 [PMID: 18672540]
  - 26 **Maeda Y**, Kiribayashi Y, Moriya T, Maruhashi A, Omoda K, Funakoshi S, Murakami T, Takano M. Dosage adjustment of ribavirin based on renal function in Japanese patients with chronic hepatitis C. *Ther Drug Monit* 2004; **26**: 9-15 [PMID: 14749543 DOI: 10.1097/0007691-200402000-00004]
  - 27 **Arase Y**, Ikeda K, Tsubota A, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, Akuta N, Someya T, Hosaka T, Sezaki H, Kobayashi M, Kumada H. Significance of serum ribavirin concentration in combination therapy of interferon and ribavirin for chronic hepatitis C. *Intervirology* 2005; **48**: 138-144 [PMID: 15812187 DOI: 10.1159/000081741]

P- Reviewer: Ozenirler S, Solinas A S- Editor: Cui LJ L- Editor: A  
E- Editor: Lu YJ





Published by **Baishideng Publishing Group Inc**  
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

