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SHORT COMMUNICATION

Determining the therapeutic range for ribavirin in transplant recipients with chronic hepatitis E virus infection

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Abstract

The aim of this study was to define the therapeutic range for ribavirin (RBV) in transplant recipients with chronic hepatitis E virus (HEV) infection. In this retrospective multicentre cohort study, data of adult transplant recipients with chronic HEV infection, who had been treated with RBV monotherapy between 01-3-2008 and 01-08-2018, were included. ROC curve analyses were performed, and the half-maximal effective RBV concentration was calculated to determine a representative therapeutic range. In 96 patients, RBV monotherapy for a median of three months resulted in a sustained virologic response in 63.5% of the patients, while 88.5% of the patients developed anaemia. RBV plasma concentrations at steady state were significantly higher in clinical responders compared with clinical non-responders: median 1.96 (IQR 1.81-2.70) versus 0.49 (IQR 0.45-0.73) mg/L, P = .0004. RBV caused a dose-dependent haemoglobin reduction with higher RBV plasma concentrations resulting in more haemoglobin reduction. The therapeutic range for RBV for chronic HEV infection in transplant recipients ranges between 1.8 and 2.3 mg/L.

KEYWORDS
hepatitis E virus, kidney, ribavirin, therapeutic drug monitoring, transplant recipients

Abbreviations: EASL, European Association for the Study of the Liver; HCV, hepatitis C virus; HEV, hepatitis E virus; HSCT, hematopoietic stem cell transplant; MPA, mycophenolic acid; RBV, ribavirin; SOT, solid organ transplant; SVR, sustained virologic response; TDM, therapeutic drug monitoring.

1 | INTRODUCTION

Ribavirin (RBV) for chronic hepatitis E virus (HEV) infection in immunocompromised patients is associated with a sustained virologic response (SVR) of around 80%.[1] The use of RBV is, however, limited by its side effects, which include haemolytic anaemia and a decrease in glomerular filtration rate (GFR).[2]

Ribavirin is mainly excreted by the kidneys and has a long half-life (approximately 300 hours). Therefore, RBV steady-state plasma concentrations are not reached until week 8. In patients infected with HCV, a relationship has been described between RBV plasma concentrations, SVR and anaemia.[3] The aim of this study was to investigate the association between RBV plasma concentrations and virologic response and anaemia in transplant recipients with a chronic HEV infection.

2 | MATERIALS AND METHODS

2.1 | Study design and setting

This was a retrospective multicentre study in which four hospitals participated. Data of adult solid organ transplant (SOT) and haematopoietic stem cell transplant (HSCT) patients diagnosed with HEV infection, who had been treated with RBV monotherapy between 01-03-2008 and 01-08-2018, were collected. For all patients, socio-demographic, clinical parameters and laboratory results were collected.

The decision to treat HEV with RBV, the starting and maintenance dose of RBV and the timing of RBV plasma concentrations measurements were determined by the treating physician. No formal therapeutic drug monitoring (TDM) protocol for RBV was implemented in any of the participating hospitals.

A waiver was given for this retrospective study by the Medical Ethics Committee of the Erasmus MC (MEC-2018-1326).

2.2 | Response assessment

A SVR was defined as an undetectable level of HEV RNA in serum at least 6 months after completion of RBV therapy.[4] Since 6 months after the completion of therapy, RBV is washed out, and we examined the relationship between viral kinetics during RBV treatment and RBV exposure to assess clinical response. Clinical response was defined as a decrease of the HEV RNA load between two measurements with at least a factor 2. Further referred to as "clinical response" in this manuscript. A rise in the HEV RNA load was defined as "clinical non-response". Declines in HEV RNA load without at least a factor 2 (n = 5) were not included. Only the first RBV plasma concentration at steady state was included in the analysis. For the determination of the lower limit of the therapeutic range, plasma concentrations of patients with at least 90 days between the diagnosis of HEV infection and initiation of RBV therapy were included.

2.3 | Toxicity assessment

Toxicity of RBV was determined based on the percentage reduction of the haemoglobin (Hb) concentration during a RBV plasma concentration measurement compared to the Hb concentrations at the initiation of RBV therapy (baseline) for each patient. Anaemia was defined as a haemoglobin concentration <8.5 mmol/L (men) and <7.5 mmol/L (women). For the toxicity analysis and determination of the upper limit of the therapeutic range, every plasma concentration was included.

2.4 | Statistical analysis

Variables are described with descriptive statistics, and differences in characteristics are described with the Mann–Whitney *U* test for quantitative data. Receiver operating characteristic (ROC) curve analyses were performed to determine a representative cut-off value for RBV pre-dose concentrations between responders and non-responders. In the analysis of a ROC curve, an area under the concentration *versus* time curve (AUC) of >0.7 is considered to be acceptable to determine a representative cut-off value. The half-maximal effective concentration (EC_{50}) was calculated with nonlinear regression of log concentration *versus* Hb reduction to determine the maximum cut-off value for the therapeutic range. The EC_{50} refers to the concentration of RBV which induces a response halfway between the baseline and maximum Hb reduction in per cent. All statistical analyses were performed using SPSS for Windows, version 24 and GraphPad Prism version 7.02.

3 | RESULTS

3.1 | Patients and ribavirin therapy

A total of 92 HEV-infected SOT and 4 HSCT recipients were included. The characteristics of the 96 patients are depicted in Table 1. RBV monotherapy for a median of 3 (range 1-44) months resulted in a SVR in 63.5% of the patients. In total, 324 RBV plasma samples were included, of which 68 samples of 40 patients were RBV steady-state plasma concentrations.

3.2 | Therapeutic effect of ribavirin

The RBV plasma concentrations at steady state were not different between patients with or without SVR (Figure S1). Whereas RBV plasma concentrations at steady state were significantly higher in the clinical response group compared with the clinical non-response group: median 1.96 (IQR 1.81-2.70) *versus* 0.49 (IQR 0.45-0.73) mg/L, *P* = .0004. The RBV dose at steady state was not significantly higher in the clinical response group compared with the clinical non-response group: median 8.44 (IQR 4.92-13.03) *versus* 8.16 (IQR

TABLE 1 Characteristics of patients with HEV infection

	Overall (n = 96)
Age, years	56 (22-84)
Gender	
Male	63 (65.6%)
Female	33 (34.4%)
Ethnicity	
Caucasian	91 (94.8%)
African	5 (5.2%)
Body weight, kilograms	74 (43.5-140)
Serum creatinine during RBV therapy, µmol/L	124 (100-165)
Kidney function during RBV therapy, ml/min/1.73 m^2	50 (37-68)
Type of organ transplant	
Kidney	42 (43.8%)
Liver	19 (19.8%)
Heart	14 (14.6%)
Lung	10 (10.4%)
Pancreas	1 (1.0%)
Kidney and pancreas	3 (3.1%)
Kidney and heart	3 (3.1%)
Stem cell	4 (4.2%)
Immunosuppressive therapy at the start of RBV	
MPA	51 (53.1%)
Glucocorticoid	61 (63.5%)
Calcineurin inhibitors	
Tacrolimus	76 (79.2%)
Cyclosporine A	3 (3.1%)
mTOR inhibitor	
Everolimus	14 (14.6%)
Sirolimus	6 (6.3%)
Tacrolimus pre-dose concentration at initiation of RBV therapy, mcg/L	5.7 (4.5-7.7)
Haemoglobin concentration at treatment initiation, mmol/L	8.1 (5.3-10.8)
Positive anti-HEV IgG at the start of RBV	70 (72.9%)
Positive anti-HEV IgM at the start of RBV	74 (77.1%)
Positive serum HEV RNA at the start of RBV	96 (100%)
Interval between diagnosis of HEV infection and start of RBV, days	120 (2-1380)
Duration RBV therapy, days	90 (26-1333)
Sustained Virologic Response	
Yes	61 (63.5%)
No	29 (30.2%)
Unknown	6 (6.3%)

Note: Continuous variables are displayed as medians and ranges. Categorical variables as counts and percentages.

Abbreviations: HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; RBV, ribavirin; RNA, ribonucleic acid.

4.88-10.51) mg/kg/day, P = .61, and total daily dose, median 600 (IQR 400-800) *versus* 600 (IQR 350-800) mg/day, P = .88 (Figure S2). No correlation was found between the RBV dose and RBV concentrations (r^2 = .040) at steady state. A worse renal function was not associated with treatment failure (defined as no decline in HEV RNA load).

The ROC curve established a cut-off point of 1.80 mg/L to achieve a clinical response (sensitivity 66%, specificity 68%,

AUC = 0.75 (95% CI 0.628 to 0.871, $P < .0001$), Figure 1A). This decreased to 1.10 mg/L with a sensitivity of 89% and specificity 65%. The ROC curve analysis revealed no differences when HSCT recipients were excluded.

3.3 | Ribavirin and toxicity

Eighty-five (88.5%) patients developed anaemia during RBV therapy. Twelve (12.5%) patients needed a blood transfusion because Hb concentrations dropped below 5.0 mmol/L. During RBV treatment, 24 (25%) patients had an increasing Hb concentration due to the use of erythropoiesis-stimulating agents or a blood transfusion. RBV caused Hb reduction regardless of the dose. Figure 1B demonstrates a stronger Hb reduction with increasing RBV plasma concentrations. Based on the EC_{50} cruve, an upper limit of the therapeutic range of 2.3 mg/L was established. A common side effect of MPA is anaemia. When assessing the upper limit of the therapeutic range according to the concomitant use of MPA, the upper limit decreased to 1.5 mg/L in patients using MPA and increased to 9.8 mg/L in patients not using MPA. Furthermore, the upper limit of the therapeutic range increased to 3.3 mg/L when this limit was assessed after excluding the HSCT recipients.

4 | DISCUSSION

Here, we show that a steady-state RBV therapeutic range of 1.8-2.3 mg/L is the optimal range for treating a chronic HEV infection in transplant recipients. Our findings are in line with those of Kamar et al who observed no association between RBV plasma levels and SVR.[5] In our study, a SVR (around 60%) was observed which was lower compared to other studies.[1,6,7] An explanation might be that at initiation of RBV therapy, the immunosuppressive therapy was not reduced sufficiently with 44.8% of the patients on triple immunosuppressive therapy. Furthermore, in our cohort MPA was used as immunosuppressive agent in almost 75% of the kidney transplant recipients, whereas MPA was used in 33% of the other transplant recipients. Debing et al showed that MPA has strong antiviral activity in vitro.[8] Differences in the use of immunosuppressive agents may have contributed to the lower SVR in our cohort.

As many centres in the world are not able to measure ribavirin concentrations, TDM is not common practice, the more so because RBV exposure appears not to be associated with SVR. However, because we observed no correlation between the RBV dose and RBV plasma concentrations at steady state, TDM could provide important information on RBV under- or overexposure. A more practical way of dosing ribavirin is to start with 10 mg/kg. Next, we recommend to measure HEV RNA quantitatively in order to identify patients with an insufficient viral response in an early phase after initiation of RBV therapy. Depending on the renal function of a patient, we propose to reduce the dose to 75% (eGFR between 30 and 50 mL/min per 1.73m^2) or 50% (eGFR between 10 and 30 mL/min per 1.73 m^2). Based on our toxicity analysis, regular monitoring of Hb, and adjusting the dose accordingly, is sufficient to prevent RBV-related toxicity. A reduction of the dose is desirable in case the Hb concentration drops > 15%. RBV should than be stopped for 2 weeks and restarted at half the initial dose.

In SOT recipients or when MPA is not used as an immunosuppressive agent, one might aim for higher RBV concentrations. Furthermore, in case of severe toxicity of RBV, a lower limit of the therapeutic range of 1.1 mg/L might be targeted. We recommend to aim for the lower limit of 1.80 mg/L when treatment-naive patients start treatment with RBV for chronic HEV.

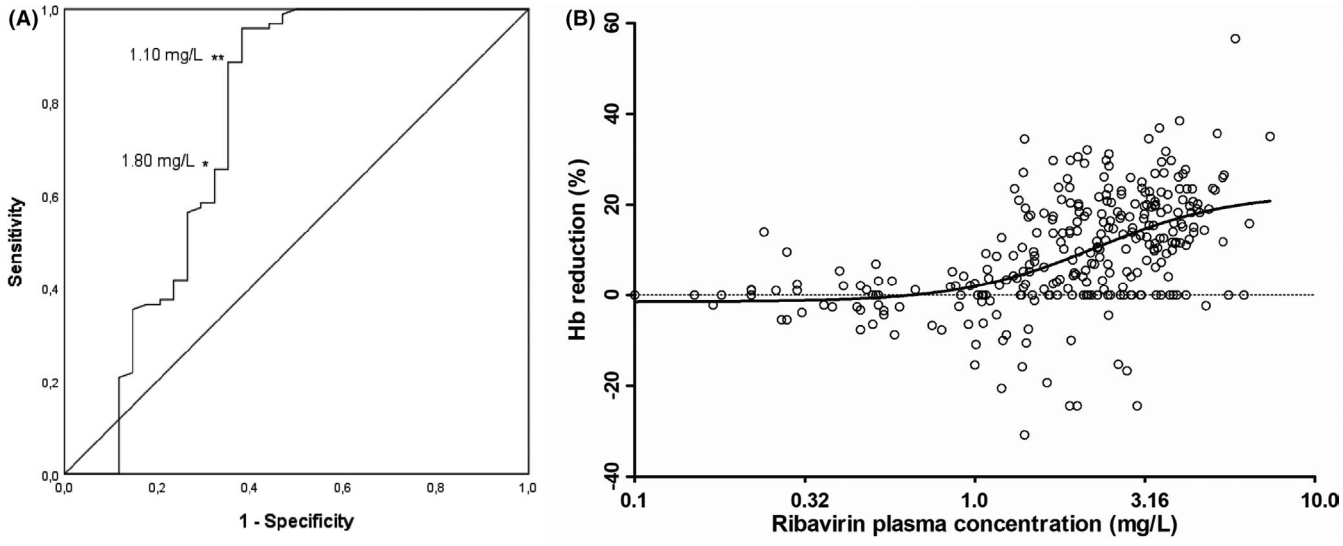

FIGURE 1 Determination of the therapeutic range for ribavirin in transplant recipients with chronic HEV infection. A, ROC curve for RBV plasma concentration as predictor of effect in chronic HEV patients treated with monotherapy ribavirin. Cut-off point * = 1.8 mg/L; cut-off point ** = 1.1 mg/L. B, Toxicity and RBV plasma concentration. EC_{50}curve: Haemoglobin reduction (%) vs log ribavirin plasma concentration (mg/L). EC_{50}, half-maximal effective concentration; calculated E_{max}value of 22.5% Hb reduction; Hb, haemoglobin

A limitation of the present study is its retrospective design. RBV dosing was clinically driven and not every plasma concentration was measured during steady state.

In conclusion, RBV monotherapy for a median of 3 months resulted in a SVR in 63.5% of the patients, with 88.5% developing anaemia. RBV plasma concentrations at steady state were significantly higher in clinical responders compared with clinical non-responders, defined as a ≥2-fold decrease in HEV RNA load. The therapeutic range for RBV for treating a chronic HEV infection in transplant recipients ranges between 1.8 and 2.3 mg/L.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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