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Monitoring hepatitis E virus fecal shedding to optimize ribavirin treatment duration in chronically infected transplant patients

To the Editor:

Hepatitis E virus genotype 3 (HEV3) and 4 (HEV4) can progress to chronic hepatitis in immunosuppressed patients.[1] Ribavirin therapy has been shown to be efficient for treating chronic HEV infection in solid-organ-transplant recipients.[2,3] Eighty percent of patients achieved a sustained virological response 24 weeks (SVR24) after ribavirin cessation.[3] However, the optimal duration of ribavirin therapy is still undetermined. A rapid decrease of HEV RNA in blood under therapy was associated with SVR24.[4] It has also been suggested that the presence of HEV polymerase mutations, such as the 1634R mutant, could influence the response to ribavirin therapy.[5,6] We previously showed that persistence of HEV RNA in the feces at the end of ribavirin therapy in patients with undetectable HEV RNA in blood was also associated with a higher risk of HEV infection relapse.[7] This prompted us to prolong the duration of ribavirin therapy in patients who had undetectable HEV RNA in the serum but persistent detectable HEV RNA in the stools at the end of the scheduled duration. Herein, we retrospectively compared the SVR24 in solid-organ-transplant recipients for whom ribavirin treatment was prolonged when HEV RNA was still detectable only in the stools, to the SVR24 in a historical group of patients in whom ribavirin was systematically stopped at the end of the scheduled duration.

Between September 2009 and September 2017, 67 solid-organ-transplant patients developed a chronic HEV infection that required ribavirin therapy in our institution. In 48 of them (72%), blood and feces samples were available at the end of scheduled ribavirin therapy. The initial scheduled duration of ribavirin therapy was 12 weeks in all patients. Until the end of 2013, ribavirin was systematically stopped at 12 weeks. As of 2014,[7] ribavirin was stopped at 12 weeks if HEV RNA was undetectable in both serum and feces. Conversely, ribavirin therapy was prolonged for 12 additional weeks or more if necessary, if HEV RNA was still detected in the stools at the end of the initial scheduled duration. According to French public health law, this retrospective non-interventional study does not require specific written informed consent (CSP Art L 1121–1.1).

Stool samples were diluted in 5 ml of EMEM, vortexed and frozen at −20 °C. Then, the samples were thawed and centrifuged at 3,560g for 10 minutes. The supernatant was finally filtered. HEV RNA in blood and stools filtrate were detected and quantified by real-time PCR that targeted the *ORF3* gene, as previously described.[8] The detection threshold was 60 IU/ml in blood and 60 IU/g in stools.

The detection of 1634R variants in the HEV polymerase was performed by the Sanger method as previously described.[5] The HEV polymerase sequences of all patients were determined before ribavirin treatment initiation and at the time of relapse. In two patients we failed to sequence HEV polymerase at baseline.

Fisher's exact test was used to compare proportions and the Mann-Whitney U test was used to compare continuous variables. A Bonferroni correction was applied for multiple comparisons. A p value <0.05 was considered statistically significant. Patient characteristics are presented (Table 1). Forty-eight patients were initially given ribavirin for 12 weeks at the median dose of 600 [600–800] mg/d (mean dosage: 9.7 ± 3.1 mg/kg). At the end of initial ribavirin therapy, all patients had undetectable HEV RNA in the serum, but 13 had still detectable HEV RNA in the stools. Ten relapses were observed within the 24 weeks following ribavirin cessation. Hence, the overall SVR24 after first-line therapy was 79.2%.

Among the 35 patients who had undetectable HEV RNA in the serum and the stools, 3 relapses were observed within 12 weeks after ribavirin cessation (Fig. 1). Hence, in this subgroup, the SVR24 was 91.4% (32 out of 35). Re-genotyping by sequencing a 345-nucleotide fragment within the *ORF2* gene and within the polymerase showed identical strains before treatment and at relapse, ruling out a re-infection. Among the 13 patients who had undetectable HEV RNA in the serum but still detectable HEV RNA in the feces, 7 patients relapsed. Thus, in this subgroup, the SVR24 was 46.1% (6 out of 13). The difference in SVR24 was statistically significant between patients who had persistent HEV RNA detection or not in the stools ($p = 0.002$).

The proportion of patients with persistent detection of HEV RNA in the stools at 12 weeks of ribavirin therapy was significantly higher in patients who relapsed (7 out of 10 patients, 70.0%) compared to those who achieved SVR24 (6 out of 38 patients, 15.6%) ($p = 0.002$). A persistent HEV shedding at 12 weeks of ribavirin predicted the relapse with a sensitivity of 70.0% (34.8–93.3) and a specificity of 84.2% (68.8–94.0). The 1634R variants were assessed in 46 out of the 48 patients at baseline. In 5 of them, 1634R variants were detected at baseline. All 5 achieved SVR24. Among the 10 patients who relapsed, 1634R variants were assessed in 9 patients at relapse and detected in 6 of them (66.7%).

Thirteen patients had persistent HEV shedding at the end of 12-weeks ribavirin therapy (Fig. 1). In six patients who were treated for HEV infection before 2014, ribavirin therapy was stopped at 12 weeks. All of them relapsed after ribavirin cessation, leading to an SVR24 of 0%. Conversely, for the 7 other patients who were treated after 2014, ribavirin therapy was prolonged for 12 additional weeks. All of them had undetectable HEV RNA in the serum and stools at the end of the extended therapy. Six achieved SVR24 and one patient relapsed. Hence, the SVR24 was 85.7% in patients who prolonged ribavirin

Keywords: Transplantation; Hepatitis E virus; Ribavirin; Duration; Stools; Sustained virological response.

Table 1. Patient characteristics.

	Total population (n = 48)	Patients with sustained virological response (n = 38)	Relapsers (n = 10)	*P* value*
Gender, male/female (ratio)	36/12 (3)	30/8 (3.75)	6/4 (1.5)	0.24
Age, years	54 [46–64]	55 [46–65]	51 [45–62]	0.60
Time from transplantation to HEV diagnosis, months	37.5 [15.0–115.5]	37.5 [14.5–123.8]	37.5 [20.3–68.8]	0.72
Time between HEV diagnosis and ribavirin treatment, months	4.0 [3.0–6.0]	4.0 [3.0–5.3]	3.8 [3.0–23.5]	0.18
Type of transplanted organ, n (%)				0.06
Kidney	29 (60)	26 (68)	3 (30)	
Liver	13 (27)	9 (24)	4 (40)	
Heart	3 (6)	2 (5)	1 (10)	
Lung	1 (2)	–	1 (10)	
Kidney-pancreas	2 (4)	1 (3)	1 (10)	
Immunosuppressive regimen				
Tacrolimus, n (%)	36 (75)	29 (76)	7 (70)	0.70
Trough level at ribavirin initiation, ng/ml	5.8 [4.8–7.5]	5.8 [4.8–7.2]	7.0 [4.4–9.1]	0.46
Trough level at the end of treatment, ng/ml	4.3 [2.7–6.0]	4.2 [2.6–5.8]	6.0 [3.2–14.2]	0.22
mTOR inhibitors, n (%)	15 (31)	12 (32)	3 (30)	1
Mycophenolic acid, n (%)	36 (75)	27 (71)	9 (90)	0.41
Steroids, n (%)	37 (77)	30 (79)	7 (70)	0.67
Induction therapy, n (%)				0.25
Polyclonal antibodies	17 (35)	11 (29)	6 (60)	
Anti–IL2 receptor blockers	20 (42)	17 (45)	3 (30)	
None	11 (23)	10 (26)	1 (10)	
Initial ribavirin dose, mg/kg	9.6 [7.8–12.2]	9.7 [7.7–12.3]	9.4 [8.3–11.4]	0.80
Reduction in ribavirin dose, n (%)	12 (25)	7 (18)	5 (50)	0.09
Ribavirin dose at the end of treatment, mg/kg	8.4 [6.2–10.6]	8.7 [6.2–12.4]	7.1 [3.2–9.3]	0.06
Ribavirin duration, months	3.0 [3.0–3.0]	3.0 [3.0–3.75]	3.0 [3.0–3.0]	0.32
Biological parameters at ribavirin initiation				
AST level, IU/L	68 [55–93]	68 [54–99]	75 [51–89]	0.95
ALT level, IU/L	100 [63–141]	118 [74–164]	60 [43–89]	**0.01**
GGT level, IU/L	140 [76–234]	136 [77–233]	200 [63–277]	0.64
AP level, IU/L	161 [99–314]	148 [96–303]	234 [164–456]	0.17
Bilirubin level, µmol/L	11.3 [8.0–17.0]	11.0 [7.8–16.3]	15.0 [9.0–29.0]	0.15
Serum creatinine level, µmol/L	128 [87–162]	124 [83–155]	133 [111–192]	0.27
eGFR, ml/min	49.5 [39.5–71.5]	49.5 [40.5–74.3]	47.5 [27.3–57.3]	0.26
Hemoglobin level, g/dl	12.7 [11.5–13.8]	13.0 [11.9–13.9]	11.1 [10.2–12.6]	**0.01**
Platelet count, G/L	172 [142–221]	168 [145–232]	172 [125–192]	0.45
Lymphocyte count, /mm^3	1,201 [870–1,690]	1,367 [987–1,752]	811 [580–1,352]	**0.03**
Anti–HEV antibodies at ribavirin initiation				
IgG, positive/negative	40/8	32/6	8/2	0.67
IgM, positive/negative	46/2	36/2	10/0	1
Baseline plasma HEV RNA concentration, $\log_{10}$ IU/ml	5.8 [5.3–6.4]	6.0 [5.2–6.4]	5.5 [5.3–5.9]	0.43
Decreased HEV RNA concentration on day 7, $\log_{10}$ IU/ml	0.72 [0.46–1.09	0.77 [0.56–1.20]	0.42 [0.37–0.48]	**0.002**
Decreased HEV RNA concentration >0.5 $\log_{10}$ IU/ml by day 7, n (%)	25/36 (69)	28/35 (80)	1/7 (15)	**0.002**
Positive plasma HEV RNA 1 month after ribavirin initiation, n (%)	31 (65)	25 (66)	6 (60)	0.73
Positive stools HEV RNA 1 months after ribavirin initiation, n (%)	36 (75)	27 (71)	9 (90)	0.41
Positive plasma HEV RNA 3 month after ribavirin initiation, n (%)	0	0	0	–
Positive stools HEV RNA 3 months after ribavirin initiation, n (%)	13 (27)	6 (16)	7 (70)	**0.002**
Detection of 1634R variant at baseline, n (%)	5/46** (11)	5/37 (14)	0/9 (0)	0.57
Detection of 1634R variant at relapse, n (%)			6/9 (67)	

ALT, alanine aminotransferase; AP, alkaline phosphate; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); GGT, gamma-glutamyltransferase; HEV, hepatitis E virus; mTOR, mammalian target of rapamycin.

*Comparison between patients with sustained virological response and relapsers. Fisher's exact test was used to compare proportions and the Mann-Whitney U test was used to compare continuous variables. *p*-values in bold are < 0.05.

**In 2 patients we failed to sequence HEV polymerase.

($p = 0.005$), compared to those who stopped ribavirin despite persistent HEV shedding.

The 3 patients who relapsed despite undetectable HEV RNA in the serum and the stools after 12 weeks of ribavirin, were re-treated for 24 weeks (Fig. 1). Two of them achieved SVR24. The third one relapsed after ribavirin cessation. Ribavirin was re-initiated. He still had detectable HEV RNA in the blood at a low concentration (~3.0 $\log_{10}$IU/ml).

Two out of the 6 patients with persistent HEV shedding who had only received 12 weeks of therapy died before re-treatment, from acute mesenteric ischemia and a metastatic esophageal carcinoma. Four patients were re-treated for 24 weeks and 3 of them achieved an SVR24. In the last patient, ribavirin had to be stopped prematurely at 8 weeks because of hematologic side effects. A treatment with interferon alpha alone was initiated.

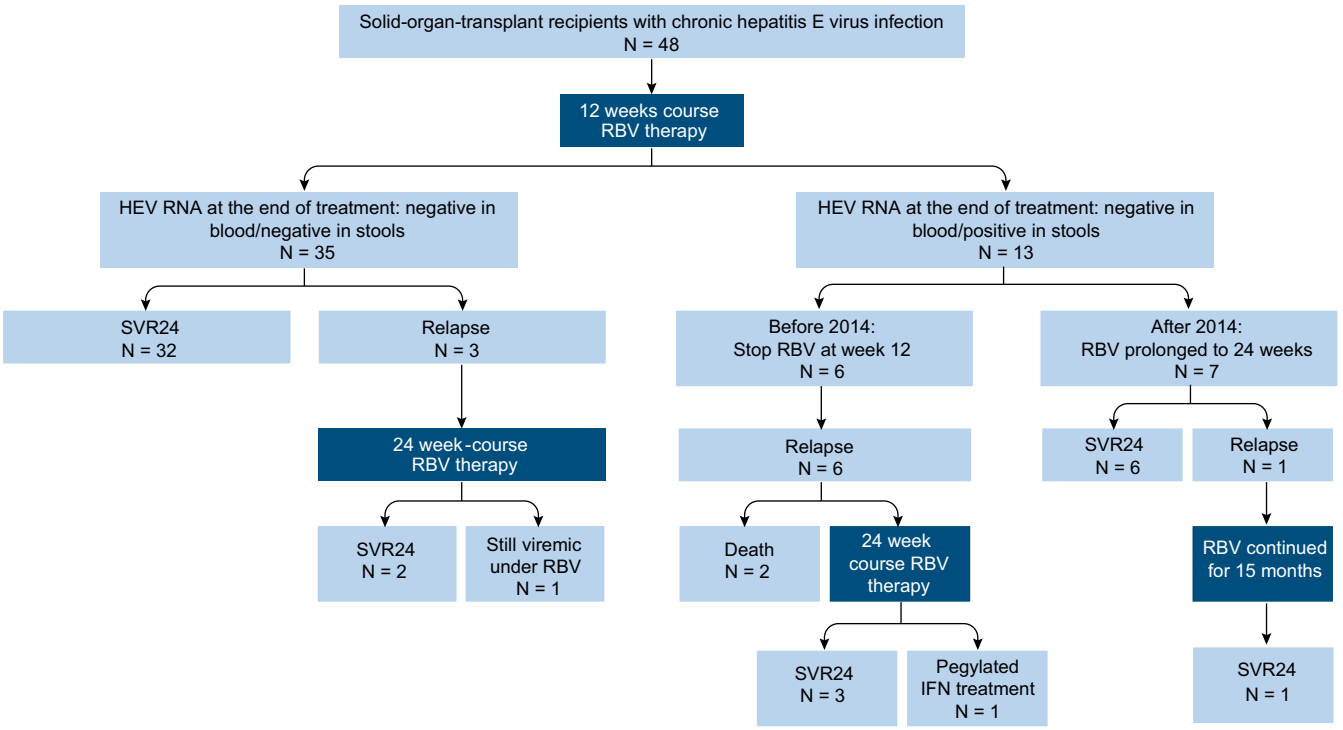

Fig. 1. Outcome of Hepatitis E virus-infected solid-organ-transplant recipients treated by ribavirin. HEV, hepatitis E virus; SVR, sustained virological response; RBV, ribavirin.

Several small series and case reports reported that ribavirin monotherapy was efficient for treating HEV infection.[3,6,9] This was confirmed in a retrospective multicenter study that included 59 solid-organ-transplant recipients.[3] The SVR24 was 78%. Ribavirin had been given at different durations, *i.e.* 3 (1–18) months. No statistically significant difference in SVR was observed between patients given ribavirin for 3 months or less and those who received it for more than 3 months. Consequently, recent European guidelines recommend the use of ribavirin monotherapy for 12 weeks as the first-line anti-HEV therapy, after reducing immunosuppression (if possible), in patients with chronic HEV infection.[10] However, the optimal duration of ribavirin in this setting is not well established. Our group had previously shown that a high lymphocyte blood count at the initiation of ribavirin was associated with SVR.[3,4] A decrease in HEV RNA concentration ≥0.5 $\log_{10}$ at day 7 of treatment has been identified as an independent predictive factor for SVR24 in solid-organ-transplant recipients.[4] Conversely, persistent viral shedding in feces at the end of therapy was associated with significantly more HEV relapse.[7] In the present study, we showed that prolonging ribavirin treatment in patients with persistent HEV RNA detection in stools prevents HEV relapse after ribavirin cessation.

In accordance with our previous reports,[3] we observed that relapsers had a significantly lower total lymphocyte count at ribavirin initiation compared to non-relapsers. Their alanine aminotransferase level was also significantly lower than in non-relapsers. This is probably related to a greater immunosuppression, which may downregulate T-cell responses against the virus. A decreased concentration of HEV RNA in the blood of >0.5 $\log_{10}$IU/ml by day 7 was significantly associated with less relapse after ribavirin cessation. Interestingly, the presence of 1634R variant of HEV polymerase before therapy did not impact the SVR since all patients with pre-treatment 1634R variants achieved SVR. However, as previously described, 1634R variants were detected in relapsers, and most of them achieved SVR after prolonged ribavirin therapy.[5] Hence, the significance of the detection of these variants is still unknown.

All patients with persistent HEV shedding in whom ribavirin was stopped at the scheduled time, *i.e.* 12 weeks, relapsed after cessation. Conversely, all patients but one (85.7%) with persistent HEV shedding at the end of the initially scheduled duration and in whom ribavirin therapy was prolonged, achieved SVR. In all patients, ribavirin was prolonged for 12 weeks, leading to total treatment duration of 24 weeks. The last patient was re-treated for a duration of 15 months and finally achieved SVR.

Finally, the optimal ribavirin dose to be used for treating HEV infection is still undetermined.[11] In the present study, there was a trend toward lower daily ribavirin dose and more dose reductions in relapsers. However, in a previous study, no correlation between ribavirin trough level and SVR was observed.[4] Further ribavirin pharmacokinetic studies are required to determine the optimal ribavirin dose and levels to treat HEV infection.

In conclusion, our study shows that monitoring HEV RNA in the stools can be a useful tool in determining the optimal regimen of ribavirin therapy.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Data collection and statistical analyses: OM; patient follow-up: OM, ADB, LE, ALH, LL, OC, DR and NK; virological work-up: SL, FA and JI; paper preparation and review: OM, SL, FA, JI and NK; study design: NK.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2018.09.011.

References

[1] Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, et al. Hepatitis E virus infection. Nat Rev Dis Primers 2017;3:17086. https://doi.org/10.1038/nrdp.2017.86.
[2] Kamar N, Marion O, Izopet J. When should ribavirin be started to treat hepatitis E virus infection in transplant patients? Am J Transplant 2016;16:727. https://doi.org/10.1111/ajt.13567.
[3] Kamar N, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. N Engl J Med 2014;370:1111–1120. https://doi.org/10.1056/NEJMoa1215246.
[4] Kamar N, Lhomme S, Abravanel F, Cointault O, Esposito L, Cardeau-Desangles I, et al. An early viral response predicts the virological response to ribavirin in hepatitis E virus organ transplant patients. Transplantation 2015;99:2124–2131. https://doi.org/10.1097/TP.0000000000000850.
[5] Lhomme S, Kamar N, Nicot F, Ducos J, Bismuth M, Garrigue V, et al. Mutation in the hepatitis E virus polymerase and outcome of ribavirin therapy. Antimicrob Agents Chemother 2015;60:1608–1614. https://doi.org/10.1128/AAC.02496-15.
[6] Debing Y, Gisa A, Dallmeier K, Pischke S, Bremer B, Manns M, et al. A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. Gastroenterology 2014;147. https://doi.org/10.1053/j.gastro.2014.08.040, 1008–1011.e7; quiz e15–16.
[7] Abravanel F, Lhomme S, Rostaing L, Kamar N, Izopet J. Protracted fecal shedding of HEV during ribavirin therapy predicts treatment relapse. Clin Infect Dis 2015;60:96–99. https://doi.org/10.1093/cid/ciu742.
[8] Abravanel F, Sandres-Saune K, Lhomme S, Dubois M, Mansuy J-M, Izopet J. Genotype 3 diversity and quantification of hepatitis E virus RNA. J Clin Microbiol 2012;50:897–902. https://doi.org/10.1128/JCM.05942-11.
[9] Pischke S, Hardtke S, Bode U, Birkner S, Chatzikyrkou C, Kauffmann W, et al. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. Liver Int 2013;33:722–726. https://doi.org/10.1111/liv.12114.
[10] European Association for the Study of the Liver. EASL Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol 2018;68:1256–1271. https://doi.org/10.1016/j.jhep.2018.03.005.
[11] De Winter BCM, Hesselink DA, Kamar N. Dosing ribavirin in hepatitis E-infected solid organ transplant recipients. Pharmacol Res 2018;130:308–315. https://doi.org/10.1016/j.phrs.2018.02.030.

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Individual surveillance using model-based hepatocellular carcinoma risk estimates in chronic hepatitis C patients after antiviral treatment

To the Editor:

We read with interest the article by Ioannou and colleagues in which they discussed using a risk-based model to estimate the risk of hepatocellular carcinoma (HCC) after antiviral treatment for hepatitis C virus (HCV) infection.[1] A shortened surveillance interval (6-month *vs.* 3-month) after sustained virologic response (SVR) has been suggested in order to diagnose HCC-related tumors at a lower stage and a smaller size, allowing for curative treatment which improves survival and reduces recurrence rates after treatment of HCC.[2]

However, the cost-effectiveness of frequent HCC surveillance for individuals after antiviral treatment remains unclear, particularly during the era of direct-acting antiviral therapy when the number of HCV-infected patients who achieve SVR has increased dramatically. Therefore, it is very important to accurately stratify the risks of HCC development in patients who received antiviral treatments using a simple and noninvasive method to guide individualized monitoring. In this article, Ioannou and colleagues have performed an excellent study using data obtained from a US cohort of the Veterans Affairs