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Ribavirin for Hepatitis E Virus Infection After Organ Transplantation: A Large European Retrospective Multicenter Study

Nassim Kamar,¹ Florence Abravanel,² Patrick Behrendt,³ Jörg Hofmann,⁴ Georges Philippe Pageaux,⁵ Christelle Barbet,⁶ Valérie Moal,⁷ Lionel Couzi,⁸ Thomas Horvath,⁹ Robert A. De Man,¹⁰ Elisabeth Cassuto,¹¹ Ahmed M. Elsharkawy,¹² Annelies Riezebos-Brilman,^{13,a} Anne Scemla,¹⁴ Sophie Hillaire,¹⁵ Mhairi C. Donnelly,¹⁶ Sylvie Radenne,¹⁷ Johnny Sayegh,¹⁸ Cyril Garrouste,¹⁹ Jérôme Dumortier,²⁰ François Glowaki,²¹ Marie Matignon,²² Audrey Coilly,²³ Lucile Figueres,²⁴ Christiane Mousson,²⁵ Anne Minello,²⁶ Sébastien Dharancy,²⁷ Jean Philippe Rerolle,²⁸ Pascal Lebray,²⁹ Isabelle Etienne,³⁰ Peggy Perrin,³¹ Mira Choi,⁴ Olivier Marion,² and Jacques Izopet²; for the Hepatitis E Virus Ribavirin Study Group

¹Department of Nephrology, Dialysis and Organ Transplantation, Centre Hospitalier Universitaire (CHU) Rangueil, Institut National de la Santé et de la Recherche Médicale (INSERM) U1043, Institut Fédératif de Recherche Bio-médicale de Toulouse (IFR-BMT), University Paul Sabatier, Toulouse, France; ²Department of Virology, INSERM U1043, IFR-BMT, University Paul Sabatier, Toulouse, France; ³Department of Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, and Institute of Experimental Virology, TWINCORE, Centre for Experimental and Clinical Infection Research, a joint venture between the Medical School Hannover and the Helmholtz Centre for Infection Research, German Centre for Infection Research, Hannover, Germany; ⁴Charité Universitätsmedizin Berlin, Department of Nephrology and Intensive Care and Institute of Virology, Labor Berlin Charité-Vivantes-GmbH, Berlin, Germany; ⁵Department of Hepatology, Saint Eloi Hospital, Montpellier, France; ⁶Department of Nephrology and Clinical Immunology, Bretonneau Hospital, University Hospital, Tours, France; ⁷Aix Marseille Université, Assistance Publique Hôpitaux de Marseille, Institut Pour la Recherche Pour le Développement, Microbes, Evolution, Phylogénie et Infection, Institut Hospitalo-Universitaire-Méditerranée Infection, Hôpital Conception, Centre de Néphrologie et Transplantation Rénale, Marseille, France; ⁸Department of Nephrology and Transplantation, CHU Bordeaux, Bordeaux, France; ⁹Department of Medicine I, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ¹⁰Departments of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam, The Netherlands; ¹¹Department of Nephrology and Transplantation, CHU Nice, France; ¹²Liver Unit, University Hospitals Birmingham, Birmingham, United Kingdom; ¹³Department of Medical Microbiology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands; ¹⁴Service de néphrologie-transplantation, Hôpital Necker, Assistance publique-Hôpitaux de Paris (AP-HP), Paris et Université Paris Descartes, Paris, France; ¹⁵Department of Hepatology, Hôpital Foch, Suresnes, France; ¹⁶Department of Hepatology and Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom; ¹⁷Department of Hepatology and Liver Transplantation, CHU de la Croix Rousse, Lyon, France; ¹⁸Department of Nephrology and Transplantation, CHU Angers, Angers, France; ¹⁹Department of Nephrology, CHU Clermont-Ferrand, Clermont-Ferrand, France; ²⁰Department of Hepatology, Edouard Herriot Hospital, CHU Lyon, Lyon, France; ²¹Department of Nephrology, CHU Lille, Lille, France; ²²Assistance Publique-Hôpitaux de Paris, Nephrology and Renal Transplantation Department, Groupe Hospitalier Henri-Mondor/Albert-Chenevier, Université Paris-Est-Créteil, Département Hospitalo-Universitaire Virus-Immunité-Cancer, Institut Mondor de Recherche Biomédicale, Equipe 21, INSERM U 955, Créteil, France; ²³Centre Hépatobiliaire, Hôpital Paul Brousse, AP-HP, INSERM U1193, Université Paris-Sud Paris-Saclay, Villejuif, France; ²⁴Department of Nephrology and Clinical Immunology, CHU Nantes, Nantes, France; ²⁵Department of Nephrology, CHU François Mitterrand, Dijon, France; ²⁶Department of Hepatogastroenterology and Digestive Oncology, CHU François Mitterrand, Dijon, France; ²⁷Hôpital Claude Huriez, Services Maladies de l'Appareil Digestif, INSERM Unité 995, Lille, France; ²⁸Department of Nephrology, CHU Limoges, Limoges, France; ²⁹Department of Hepatology, Pitié Salpêtrière Hospital, Paris, France; ³⁰Department of Nephrology, CHU Rouen, Rouen, France; and ³¹Department of Nephrology, CHU Strasbourg, Strasbourg, France

(See the Editorial Commentary by Crum-Cianflone on pages 1212–4.)

Background. Ribavirin is currently recommended for treating chronic hepatitis E virus (HEV) infection. This retrospective European multicenter study aimed to assess the sustained virological response (SVR) in a large cohort of solid organ transplant (SOT) recipients with chronic HEV infection treated with ribavirin monotherapy (N = 255), to identify the predictive factors for SVR, and to evaluate the impact of HEV RNA mutations on virological response.

Methods. Data from 255 SOT recipients with chronic HEV infection from 30 European centers were analyzed. Ribavirin was given at the median dose of 600 (range, 29–1200) mg/day (mean, 8.6 ± 3.6 mg/kg/day) for a median duration of 3 (range, 0.25–18) months.

Results. After a first course of ribavirin, the SVR rate was 81.2%. It increased to 89.8% when some patients were offered a second course of ribavirin. An increased lymphocyte count at the initiation of therapy was a predictive factor for SVR, while poor hematological tolerance of ribavirin requiring its dose reduction (28%) and blood transfusion (15.7%) were associated with more relapse after ribavirin cessation. Pretreatment HEV polymerase mutations and de novo mutations under ribavirin did not have a negative impact on HEV clearance. Anemia was the main adverse event.

Conclusions. This large-scale retrospective study confirms that ribavirin is highly efficient for treating chronic HEV infection in SOT recipients and shows that the predominant HEV RNA polymerase mutations found in this study do not affect the rate of HEV clearance.

Keywords. organ transplantation; hepatitis E virus; ribavirin; sustained virological response; anemia.

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^aPresent affiliation: Department of Medical Microbiology, University Medical Centre Utrecht, The Netherlands

Correspondence: N. Kamar, Department of Nephrology and Organ Transplantation, CHU Toulouse Rangueil, TSA 50032, 31059 Toulouse Cedex 9, France (kamar.n@chu-toulouse.fr).

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In immunosuppressed patients, mainly in solid organ transplant (SOT) recipients, genotype 3 hepatitis E virus (HEV) can be responsible for chronic hepatitis that can lead to rapid liver fibrosis progression and cirrhosis [1–3]. The reduction of immunosuppressants targeting the T-cell response is considered as a therapeutic option [4, 5]. It leads to HEV clearance in one-third of patients with chronic HEV infection [5]; in the remaining patients, ribavirin monotherapy is recommended

[6]. In vitro, ribavirin inhibits HEV replication [7, 8]. In vivo, case reports and small series showed that ribavirin was efficient for treating chronic HEV infection [9–11]. A retrospective multicenter French study, which included 59 SOT recipients, confirmed this finding [12]. After a first course of ribavirin monotherapy, a sustained virological response (SVR) was observed in 78% of patients [12]. Re-treating relapses for a longer period guaranteed SVR in additional patients, thus leading to an SVR of 85% [12]. In some patients who failed to clear HEV, HEV RNA polymerase mutations were detected [13]. However, the impact of these mutations on antiviral response to ribavirin is still unknown [14, 15]. This retrospective multicenter study aims to assess the SVR in a large cohort of SOT recipients with chronic HEV infection treated with ribavirin monotherapy, to identify the predictive factors for SVR, and to evaluate the impact of HEV RNA mutations on virological response.

PATIENTS AND METHODS

Dutch, English, French, German, and Scottish transplant centers were asked to participate in this retrospective study; 30 of them agreed. We collected data from 280 SOT recipients with chronic autochthonous HEV infection who were treated with ribavirin monotherapy between September 2009 and March 2018. Chronic hepatitis was defined as a persistent replication of HEV for at least 3 months. Patients who had persistent increase in liver enzyme levels lasting for >3 months before first screening for HEV and diagnosis and who were treated before the third month after diagnosis were considered to have chronic hepatitis. Patients who were treated during the acute HEV phase and those without a complete follow-up (ie, <6 months' follow-up after ribavirin cessation) were excluded (n = 25). Hence, data from 255 patients were analyzed. Of note, data from 57 patients were already published [12]. However, in the previous publication, HEV RNA polymerase mutations were not analyzed. For this reason, data from these patients were included for the present study. Details regarding participating centers and the number of included patients per center are presented in the Supplementary Materials. The patients' characteristics are presented in Table 1. Data were recorded anonymously. According to French and German laws, anonymous retrospective studies do not require institutional review board (IRB) approval. In other countries, the study was approved by the IRB of each hospital.

Virological Parameters

As previously described [16], HEV RNA in blood was detected and quantified by real-time polymerase chain reaction (PCR) that targeted the *ORF3* gene (or *ORF2* gene in 1 center). Most samples were centralized and analyzed in the French reference center for HEV in Toulouse. The limit of detection for HEV RNA was 100 copies/mL. SVR was defined as undetectable HEV RNA in the serum at least 6 months after ribavirin therapy

Table 1. Patient Characteristics

Variable	N = 255
Age, y	51 ± 14
Sex, male/female, no.	189/66
Type of organ transplantation, no.	
Kidney	153
Liver	56
Heart	14
Kidney-pancreas	10
Lung	9
Kidney-liver	6
Heart-kidney	3
Lung-kidney	2
Heart-liver	1
Pancreas	1
History of acute rejection, %	26.3
Immunosuppression at initiation of RBV, %	
Calcineurin inhibitor	87.7
Cyclosporine A	13.2
Tacrolimus	86.8
mTOR inhibitor	24.8
Antimetabolite	64.6
Steroids	74.1
Serum creatinine level at initiation of RBV, μmol/L	134 ± 60
eGFR (MDRD) at initiation of RBV, mL/min/1.73 m ²	55 ± 23
Lymphocyte count at initiation of RBV, cells/μL	1456 ± 887
Hemoglobin level at initiation of RBV, g/dL	2.8 ± 2
rEPO use at initiation of RBV, %	1.6
Platelet count at initiation of RBV, cells/μL	186 372 ± 22 428
Anti-HEV IgG at initiation of RBV, positive/negative, no. ^a	160/42
Anti-HEV IgM at initiation of RBV, positive/negative, no. ^a	190/17
Serum HEV RNA at initiation of RBV, positive/negative, no.	255/0
HEV genotype 3, no. ^b	238
3	52
3chi	90
3efg	96
Pretreatment HEV RNA polymerase mutation, no. (%) ^c	76/112 (68)
Time between transplantation and RBV therapy, mo	74 ± 64
Time between diagnosis and RBV therapy, mo, median (range)	4 (0.5–82)

Data are presented as mean ± standard deviation unless otherwise indicated.

Abbreviations: eGFR, estimated glomerular filtration rate; HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; MDRD, modification in diet for renal disease; mTOR, mammalian target of rapamycin; RBV, ribavirin; rEPO, recombinant erythropoietin.

^aHEV serology was not done in all patients.

^bTwo hundred thirty-eight of the 255 patients were infected by HEV genotype 3. HEV genotyping was not done in the remaining 17 patients.

^cBaseline HEV RNA polymerase mutations were assessed in only 112 patients.

was completed [17]. The detection of variants in the HEV polymerase was performed by the Sanger method by sequencing a 1100-nucleotide fragment covering the entire HEV polymerase gene. A first real-time PCR assay was performed using primers designed according to the HEV subtype (Supplementary File 2). Then, 3 overlapping nested PCR assays were performed to amplify the whole gene. Nested PCR products were sequenced on both strands by the dideoxy chain termination method (Prism Ready Reaction AmpliTaq Fs and BigDye terminator, Applied

Biosystems, Paris, France) on an ABI 3130XL analyzer (Applied Biosystems, Foster City, California) using the same sense and antisense primers as used for amplification. Electrophoregraphy data were analyzed using Sequencher software version 4.8 (Gene Codes Corporation, Ann Arbor, Michigan).

The HEV polymerase sequences were determined in 112 patients before ribavirin treatment initiation and in 26 patients who remained viremic after 3 months of therapy or at relapse after ribavirin cessation. We failed to sequence HEV polymerase at baseline for 4 patients, as well as 1 patient in relapse. Regarding all remaining patients, no samples were available to sequence HEV polymerase.

Statistical Analyses

Data are presented either as mean \pm standard deviation or median (range). Proportions were compared by the χ^2 test or Fisher exact test. Quantitative variables were compared by the nonparametric Friedman test for serial measurements and either Student *t* test or the Wilcoxon test. Independent factors associated with a sustained virological response were studied using a stepwise multivariate logistic regression model that used initial inclusion criteria that had a significance of $P < .05$, using Statview software (SAS Institute, Cary, North Carolina). For this purpose, patients with an SVR were compared to those without an SVR (as defined above). A *P* value of $<.05$ was considered to be statistically significant.

RESULTS

Ribavirin Therapy

The median time between HEV diagnosis and ribavirin therapy was 3 (range, 0–82) months. The duration of ribavirin therapy varied considerably, from 0.25 to 18 (median, 3) months. Patients were given 1 month of therapy or less ($n = 12$), between 1 and 2 months ($n = 13$), 3 months ($n = 128$), 4 months ($n = 26$), 5 months ($n = 21$), 6 months ($n = 37$), 7 months ($n = 4$), 9 months ($n = 1$), 10 months ($n = 5$), 11 months ($n = 1$), 12 months ($n = 2$), 15 months ($n = 2$), 17 months ($n = 1$), or 18 months ($n = 2$). Ribavirin doses were given at the discretion of physicians and according to kidney function and ribavirin tolerance. The median dose of ribavirin was 600 (range, 29–1200) mg/day (mean, 8.6 ± 3.6 mg/kg/day). Most patients received 400 mg/day ($n = 47$), 600 mg/day ($n = 72$), or 800 mg/day ($n = 81$). Thirty-one patients were given <400 mg/day, and 32 patients received >800 mg/day. Twenty-eight percent of patients required ribavirin dose reduction due to poor hematological tolerance. In this subgroup of patients, the mean ribavirin dose decreased from 706 ± 221 mg/day to 399 ± 194 mg/day ($P < .0001$). In the whole population, mean ribavirin dose decreased slightly from 614 ± 241 mg/day to 572 ± 428 mg/day ($P = .13$). Conversely, it was increased in 10% of patients. Furthermore, no significant change in estimated glomerular

filtration rate was observed—that is, 55 ± 29 mL/minute/1.73 m^2 at the initiation of ribavirin, 55 ± 29 mL/minute/1.73 m^2 at the end of therapy, and 53 ± 27 mL/minute/1.73 m^2 at 6 months after ribavirin cessation ($P = .33$).

Virological Response

All patients were viremic at the initiation of ribavirin. One month later, HEV RNA was assessed in 221 of 255 patients and was found to still be positive in 105 patients (47.5%). At the end of ribavirin therapy, HEV RNA was still positive in the blood in only 10 patients (3.9%). Their characteristics are presented in [Supplementary Table 1](#). After ribavirin cessation, HEV relapsed in 38 patients. Hence, SVR was achieved after a first course of ribavirin in 207 of 255 patients (81.2%; [Figure 1](#)). The SVR rate according to the duration of ribavirin therapy is presented in [Figure 2](#).

Thirty-six of 48 patients who did not achieve SVR were offered a longer course of ribavirin ([Figure 1](#)). Of these, 18 achieved SVR after having been treated for 3 months ($n = 1$), 4 months ($n = 1$), 5 months ($n = 2$), 6 months ($n = 10$), 12 months ($n = 2$), and 15 months ($n = 2$). Seven other patients are still currently under therapy. The 11 remaining patients did not achieve SVR: 1 of them was unaccounted for during the follow-up 1 month after reinitiating ribavirin, another patient was considered a nonresponder and stopped ribavirin, 4 patients were given long-term ribavirin therapy because of persisting replication (1 of them, a heart transplant recipient, died from decompensated cirrhosis related to HEV), and 5 patients relapsed after having been re-treated for 5 months ($n = 1$), 6 months ($n = 1$), 10 months ($n = 2$), and 12 months ($n = 1$) and stopped ribavirin. Interestingly, 2 of the relapsed patients undergoing the second course of ribavirin cleared the virus spontaneously a few weeks after ribavirin cessation, therefore achieving SVR.

With respect to the 12 remaining patients who relapsed after the first course of ribavirin and who have not been re-treated, immunosuppression was stopped and dialysis initiated in 1 patient, which allowed him to achieve SVR. Another patient cleared the virus spontaneously 2 months after HEV relapse and before being re-treated. He achieved SVR. Eight patients were still viremic and were not offered additional therapy. Finally, 2 patients died from cardiovascular disease and lung cancer while viremic.

Hence, SVR was achieved in 22 patients who relapsed after the first course of ribavirin. Consequently, overall, SVR was observed in 229 of the 255 patients (89.8%).

Predictive Factors for SVR

Results of univariate analysis are presented in [Table 2](#). Interestingly, the detection of HEV RNA in the blood at week 4 after the initiation of therapy was not associated with more frequent relapses after ribavirin cessation. The following variables were included in a multivariate analysis model: recipients'

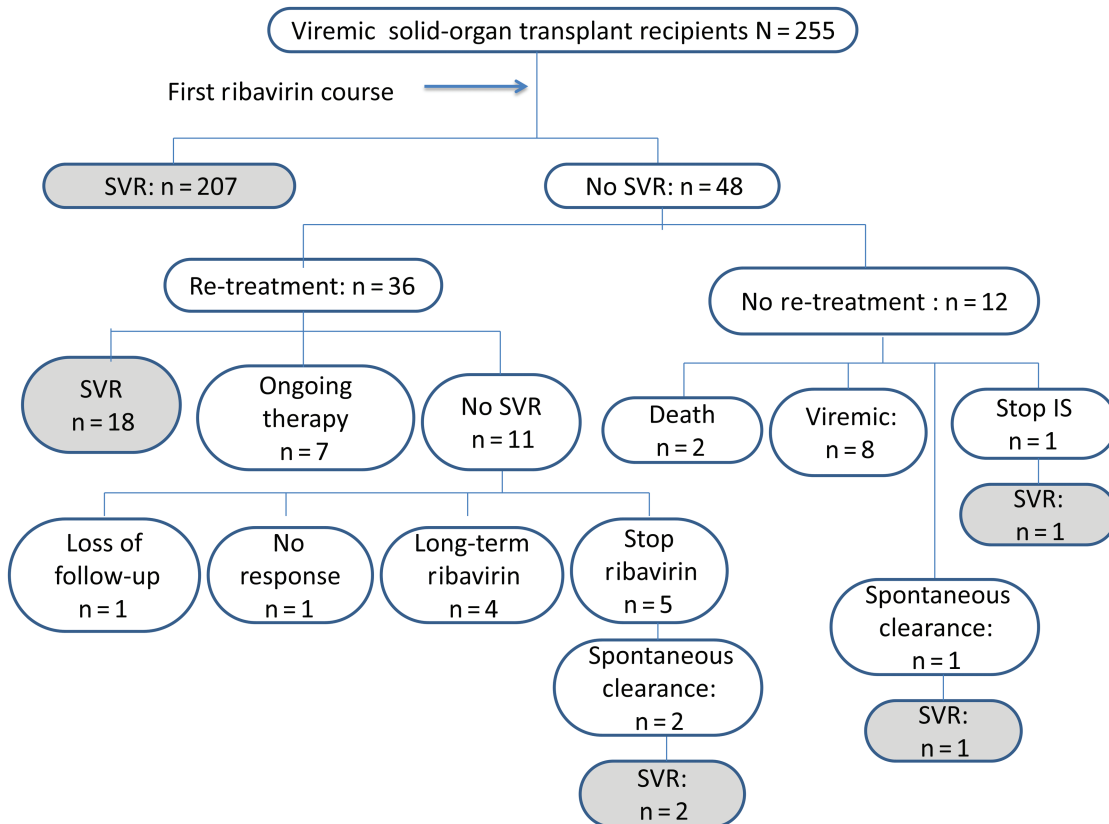


Figure 1. Outcomes of solid organ transplant recipients treated with ribavirin. Abbreviations: IS, immunosuppressants; SVR, sustained virological response.

sex, alanine aminotransferase level, total lymphocyte count, anti-HEV immunoglobulin G, and the use of tacrolimus (vs cyclosporine A) at the initiation of ribavirin, as well as the use of

recombinant erythropoietin (rEPO) during therapy, ribavirin dose reduction, and the need of blood transfusion under therapy. High lymphocyte count at the initiation of therapy was identified

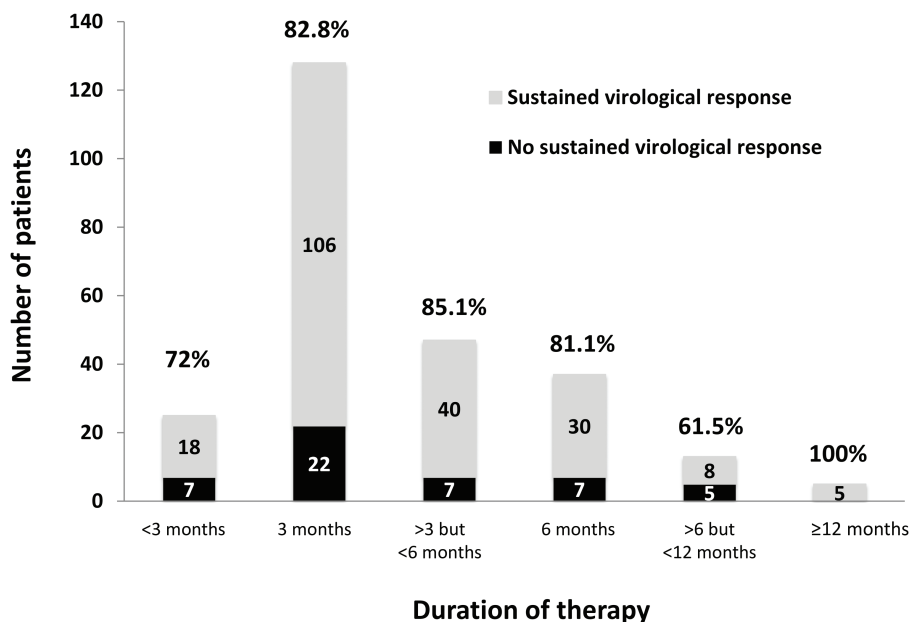


Figure 2. Sustained virological response rate according to the duration of ribavirin therapy.

Table 2. Predictive Factors for Sustained Virological Response (N = 255)

Variable	Patients With SVR (n = 207)	Patients Without SVR (n = 48)	PValue
Univariate analysis			
Age, y	52 ± 14	49 ± 13	.15
Sex, male/female, n	159/48	30/18	.05
Liver transplant recipient, yes/no, n	51/156	12/36	>.99
Rejection history, %	25.1	31.2	.46
CNIs at RBV initiation, %	87.3	89.4	.81
Tacrolimus at RBV initiation, %	84	97	.02
mTOR inhibitor at RBV initiation, %	21.2	18.8	.84
Antimetabolite at RBV initiation, %	70.2	68.1	.86
Steroids at RBV initiation, %	73.4	78.7	.58
AST level at RBV initiation, IU/L ^a	68 (19–1448)	79 (23–480)	.41
ALT level at RBV initiation, IU/L ^a	120 (10–1733)	100 (32–373)	.03
γGT level at RBV initiation, IU/L ^a	122 (19–914)	129 (22–1015)	.04
Bilirubin level at RBV initiation, μmol/L ^a	11 (2–186)	13 (4–102)	.16
Creatinine level at RBV initiation, μmol/L	136 ± 62	129.5 ± 49	.52
eGFR (MDRD) at RBV initiation, mL/min/1.73 m ²	54 ± 22	57 ± 26	.45
Hemoglobin level at RBV initiation, g/dL	12.9 ± 1.9	12.4 ± 2	.08
Lymphocyte count at RBV initiation, cells/μL	1529 ± 895	1095 ± 758	.02
Tacrolimus trough level at RBV initiation, ng/mL	6.3 ± 2.8	7.2 ± 2.9	.07
Platelet count at RBV initiation, cells/μL	185 160 ± 104 539	191 550 ± 149 320	.7
Time between transplantation and RBV, mo	76 ± 66	62 ± 50	.18
Time between diagnosis and RBV, mo ^a	4 (0.5–82)	6 (0.5–50)	.49
Duration of RBV therapy, mo ^a	3 (0.25–18)	3 (0.75–12)	.92
Duration of RBV therapy <3 mo, yes/no, n	18/189	7/41	.27
Duration of RBV therapy ≤3 mo, yes/no, n	124/83	29/19	.99
Duration of RBV therapy 3 mo, yes/no, n	106/101	22/26	.52
Duration of RBV therapy >3 and <6 mo, yes/no, n	40/167	7/41	.54
Duration of RBV therapy ≤6 mo, yes/no, n	194/13	43/5	.35
Duration of RBV therapy of 6 mo, yes/no, n	30/177	7/41	.99
Duration of RBV therapy ≥6 mo, yes/no, n	43/164	12/36	.56
Initial RBV dose, mg/d ^a	600 (29–1200)	600 (200–1000)	.47
Initial RBV dose, mg/kg/d	8.6 ± 3.7	8.6 ± 3.5	.97
Cumulative RBV dose, mg	71 280 ± 53 828	67 626 ± 44 743	.67
RBV trough level at month 1	2.14 ± 1.38 (n = 54)	2.25 ± 1.26 (n = 19)	.75
RBV dose <400 mg/d at EOT, %	20.9	22.5	.83
RBV dose reduction, %	24.3	46.8	.004
Use of rEPO during RBV therapy, %	41.9	61.7	.02
Blood transfusion, %	10.9	36.2	<.0001
Hemoglobin level at EOT, g/dL	11.7 ± 2.3	9.8 ± 2.3	.001
Positive anti-HEV IgG at RBV initiation, yes/no, n ^b	135/26	25/16	.002
Positive anti-HEV IgM at RBV initiation, yes/no, n ^b	156/12	34/5	.32
HEV RNA concentration at RBV initiation, IU/mL ^a	599 500 (200–166 000 000)	1 444 000 (200–37 000 000)	.96
HEV genotype 3chi/3efg, n	68/81	22/15	.14
Positive HEV RNA at month 1, yes/no, n ^b	82/95	23/21	.5
Pretreatment HEV polymerase mutations, yes/no, n ^b	57/25	19/11	.65
Multivariate analysis			
	OR	(95% CI)	PValue
Sex, male	1008	(.35–2.9)	.99
ALT at baseline	2.22	(.65–7.58)	.19
Cyclosporine A (vs tacrolimus)99
Anti-HEV IgG at baseline (positive)	1.7	(.6–4.8)	.31
rEPO during therapy (Y)	1.41	(.49–4.03)	.52
Higher lymphocyte count at baseline	1.001	(1–1.02)	.04
RBV dose reduction (Y)	0.34	(.14–.84)	.02
Transfusion during therapy (Y)	0.3	(.1–.84)	.02

Data are presented as mean ± standard deviation unless otherwise indicated. Numbers are bold correspond to statistically significant differences.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; EOT, end of therapy; γGT, gamma-glutamyl transpeptidase; HEV, hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M; MDRD, modification in diet for renal disease; mTOR, mammalian target of rapamycin; N, no; OR, odds ratio; RBV, ribavirin; rEPO, recombinant erythropoietin; SVR, sustained virological response; Y, yes.

^aMedian (range).

^bHEV serology at baseline, quantitative HEV RNA concentration at baseline, pretreatment HEV polymerase mutations, and HEV RNA at month 1 were not obtained for all patients.

as an independent predictive factor that was associated with SVR. Conversely, ribavirin dose reduction and blood transfusions were independent predictive factors associated with no achievement of SVR (Table 2).

Effect of HEV RNA Mutations on Virological Response

HEV RNA mutations were assessed in 112 patients before the initiation of ribavirin. HEV polymerase mutations were detected in 76 patients (67.9%). V1479I was the most common mutation detected ($n = 75$), followed by G1634R ($n = 10$). The following mutations and combinations were observed: V1479I alone ($n = 64$), V1479I and G134R ($n = 9$), V1479I and Y1320H ($n = 1$), V1479I and D138G/N ($n = 1$), and G1634R alone ($n = 1$). The presence of pretreatment mutation did not impact SVR. Pretreatment mutations were observed in 57 of 82 patients (69.5%) who achieved SVR and 19 of 30 patients (63.3%) who did not ($P = .65$). The type of mutation did not have any impact on SVR (Table 3). Among 19 patients who had pretreatment

mutations and who did not achieve SVR, 17 patients were re-treated with ribavirin: 13 achieved SVR and 4 are still under therapy. The 2 remaining patients were not re-treated: 1 of them spontaneously cleared the virus before being re-treated.

HEV polymerase mutations were assessed in 26 patients who did not achieve SVR after the first course of ribavirin. Twenty patients had de novo mutations: 5 did not have any mutation before therapy whereas all 15 remaining patients already had other mutations before therapy. Among these 20 patients who developed de novo mutations under therapy, 16 patients were re-treated with ribavirin: 12 achieved SVR and 4 are still under therapy. The remaining 4 patients were not re-treated.

Ribavirin Tolerance

Anemia was the main side effect observed under ribavirin therapy. Under ribavirin therapy, rEPO was required in 45.6% of patients. At initiation of therapy, 21.6% were already receiving rEPO at the median dose of 12 000 (range, 1000–120 000) IU/week. At the end of therapy, 38% were given rEPO ($P < .0001$, compared to baseline) at the median dose of 20 000 (range, 1000–320 000) IU/week ($P = .0003$, compared to baseline). A few patients required only transient use of rEPO. Blood transfusions were required for 15.7% of patients. In patients who required increased doses of rEPO, the introduction of rEPO, or blood transfusion, ribavirin was initiated at a slightly lower dose (581 ± 243 mg/day) compared to those who did not require any intervention (636 ± 238 mg/day) ($P = .07$). However, at baseline, they had a significantly lower eGFR rate (47.5 ± 19.3 vs 60.6 ± 22 mL/minute/1.73 m², $P < .0001$), had a significantly lower hemoglobin level (12.5 ± 1.6 g/dL vs 13.1 ± 2.25 g/dL, $P = .009$), and more frequently received often mycophenolic acid (79.3% vs 57.7%, $P = .0005$).

DISCUSSION

Ribavirin monotherapy was found to be efficient for treating chronic genotype 3 and 4 HEV infection [12]. Most results were obtained in SOT recipients, mainly from a retrospective multicenter French study that included 59 patients [12]. In the present study, we aimed to assess the efficiency and safety of ribavirin in a larger cohort of SOT recipients and to assess the impact of HEV polymerase mutations on virological response. Our findings were 4-fold: (1) After a first course of ribavirin, the SVR rate was 81.2%; this rate increased to 89.8% when some patients were offered a second course of ribavirin. (2) Increased lymphocyte count at initiation of therapy was a predictive factor for SVR, while poor hematological tolerance of ribavirin requiring its dose reduction and blood transfusion were associated with more frequent relapses after ribavirin cessation. (3) Pretreatment HEV polymerase mutations and de novo mutations under ribavirin did not have a negative impact on HEV clearance. (4) Anemia was the main adverse event.

Table 3. Hepatitis E Virus Polymerase Mutations

Mutation	Patients Who Achieved SVR After First RBV Course (n = 57)	Patients Who Did Not Achieve SVR After First RBV Course (n = 19)	P Value
Pretreatment combinations			
V1479I	49	15	.39
V1479I + G1634R	6	3	
V1479I + Y1320 H	1	0	
V1479I + D1384G/N	0	1	
G1634R	1	0	
Pretreatment mutations			
V1479I	56	19	.99
G1634R	7	3	.7
Y1320 H	1	0	>.99
D138G/N	0	1	.25
De novo mutations after therapy (n = 20)			
K1383N	1		
G1634R	3		
D1384N or G	2		
K1383N + D1384G	2		
K1383N + K1398R	1		
K1383N + G1634R	1		
K1383N + Y1587Y	1		
D1384G + K1398R	1		
D1384G + G1634R	2		
K1383N + D1384G + G1634R	2		
Y1320H + D1384G + G1634R	1		
K1383N + Y1587Y/F + G1634G/R	1		
K1383N + D1384G + Y1587F + G1634R	1		
K1383N + K1398R + Y1587F + G1634R	1		

Abbreviations: RBV, ribavirin; SVR, sustained virological response.

In the present study, in a large cohort, the SVR rate was 81.2%, and was quite similar to the one reported previously (78%) [12]. In addition, similar to our previous study, treating relapsed patients for a longer period resulted in the achievement of SVR in at least 50% of reported cases. Unfortunately, treatment failure under ribavirin can be observed. Debing et al have previously detected G1634R mutations in the HEV RNA polymerase of 2 patients who failed to clear HEV with ribavirin therapy [13]. Later on, in case reports and small case series, other mutations such as K1383N, D1384G, V1479I, and Y1587F were identified [15, 18, 19]. They were detected in some patients before ribavirin therapy or appeared under ribavirin in relapsed patients [14, 15, 18]. In the present study, HEV polymerase mutations were assessed—not in all, but in a large number of patients. As previously reported by Lhomme et al in a series that included 63 patients, pretreatment G1634R mutation did not impact SVR [14]. However, other mutations in the polymerase could play a role [20]. In the present study, these mutations, analyzed alone or in combination, did not have any impact on virological response. Furthermore, the majority of relapsed patients and partial responders to ribavirin (77%) developed de novo mutations after a first course of ribavirin therapy. Most of them achieved SVR after re-treatment. Hence, HEV RNA polymerase mutations do not seem to have an impact on HEV clearance. This is in line with in vitro data according to which mutations may improve ribavirin antiviral activity, but they may also increase HEV replication [15].

In nonresponders or subjects who relapsed after re-treatment, no existing therapy seems relevant [21], except pegylated interferon, which can only be used in liver transplantation [22] because it increases the risk of acute rejection [23]. Sofosbuvir has shown a synergistic effect with ribavirin in vitro [24]. However, this was not confirmed in vivo [25]. Similar to what has been done in some patients in the current study, it has been suggested that maintaining ribavirin long-term may have a beneficial effect on preventing the progression on liver fibrosis [26]. The main issue is the hematological tolerance of ribavirin. The reduction of ribavirin doses and blood transfusion were independent predictive factors of non-SVR. In a previous study, we did not observe a relationship between ribavirin levels and SVR [27]. In the present study, ribavirin levels were not assessed in most patients. Therefore, we were not able to assess their impact on SVR. However, as previously reported, we found that a high lymphocyte count at baseline was a predictive factor for SVR [12]. This finding is in line with previous reports suggesting that HEV persists in immunosuppressed patients [28]. Abravanel et al had previously observed that patients with persistent HEV RNA shedding in the stools despite undetectable HEV RNA in the blood at the end of scheduled duration of ribavirin therapy were at increased risk of relapsing after ribavirin cessation [29]. Very recently, Marion et al found that in patients who tested positive for HEV in the stools at the end of therapy, prolonging

the duration of ribavirin therapy significantly improved the SVR rate [30]. In the present study, HEV RNA was not systematically assessed in the stools at the end of therapy.

In our previous multicenter study, in univariate analysis, we found that positive HEV RNA in the blood after 1 month of therapy was associated with SVR [12]. This was not confirmed in the present study. Finally, in a small case series that included 35 patients, we observed that a decrease of HEV RNA concentration ≥ 0.5 log at day 7 after starting ribavirin was an independent predictive factor for SVR [27]. In the present study, data regarding HEV RNA at day 7 were not collected.

Due to its retrospective nature, our study has several limitations. The duration of ribavirin varied considerably across different centers. We cannot exclude that in some patients the duration of ribavirin was determined according to their immunological status or to the evolution of viral load under ribavirin, especially those who were given >6 months of therapy. However, only 5% of patients received ribavirin for >6 months. In addition, the SVR rate did not differ between patients who were initially given ≤ 3 months or >3 months. Because HEV RNA was not collected at day 7, and since it was not assessed in the stools at the end of scheduled therapy, we cannot recommend an optimal duration of therapy. However, based on recent results by Marion et al [30], we suggest a 3-month course of therapy after which, in case of persistent HEV RNA in the stools, we suggest prolonging therapy for 3 more months. Another limitation of this study is the lack of assessment of HEV RNA polymerase mutations in all patients. However, it has been done in 44% of them, and no impact of these mutations on virological response was observed. Finally, ribavirin trough levels were not systematically assessed in all patients. Further studies are required to better determine the optimal dose of ribavirin.

In summary, this large-scale retrospective European study confirms that ribavirin is highly efficient for treating chronic HEV infection in transplant recipients. Re-treating relapsed patients for a longer period allows clearing the virus. The predominant HEV RNA polymerase mutations found in this study do not affect the rate of HEV clearance. Finally, there is still an unmet need for those patients who fail to clear the virus with ribavirin.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Hepatitis E Virus Ribavirin Study Group. J. Bellière, O. Cointault, A. Del Bello, L. Espostio, A. L. Hebral, L. Lavyssière, S. Lhomme, J. M. Mansuy (Toulouse); H. Wedemeyer (Hannover); P. Nickel (Berlin); M. Bismuth (Montpellier); K. Stefc, M. Büchler, L. D'Alteroche (Tours); P. Colson (Marseille); S. Bufton (Birmingham); C. Ramière (Lyon); P. Trimoulet (Bordeaux); S. Pischke (Hamburg); E. Todesco; R. Sberro Soussan; C. Legendre, V. Mallet (Paris); I. Johannessen, K. Simpson (Edinburgh).

Author contributions. N. K. designed the study, collected the data, did the statistical analyses, and wrote the manuscript. O. M. collected the data. F. A. and J. I. did the virological workup and reviewed the manuscript. All other authors provided patients from different centers and reviewed the manuscript.

Potential conflicts of interest. N. K. reports personal fees from AbbVie, Amgen, Astellas, Chiesi, Fresenius, Gilead, Medical Care, Merck Sharp & Dohme, Neovii, Novartis, Roche, Sanofi, and Shire. C. L. reports lecture fees from Hansa Medical and travel support from CSL Behring. G. P. P. reports grants from Novartis and personal fees from Intercept, AbbVie, and Gilead. V. M. reports grants, personal fees, and nonfinancial support from AbbVie, as well as personal fees and nonfinancial support from Gilead. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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