Factors Associated With Chronic Hepatitis in Patients With Hepatitis E Virus Infection Who Have Received Solid Organ Transplants

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BACKGROUND & AIMS: Hepatitis E virus (HEV) infection can cause chronic hepatitis in recipients of solid organ transplants. However, the factors that contribute to chronic infection and the outcomes of these patients are incompletely understood. We performed a retrospective analysis of data from 17 centers from Europe and the United States that described the progression, outcomes, and factors associated with development of chronic HEV infection in recipients of transplanted solid organs. **METHODS:** We studied data from 85 recipients of solid organ transplants who were infected with HEV. Chronic HEV infection was defined by the persistent increases in levels of liver enzymes and polymerase chain reaction evidence of HEV in the serum and/or stool for at least 6 months. **RESULTS:** Fifty-six patients (65.9%) developed chronic hepatitis. Univariate analysis associated liver transplant, shorter times since transplant, lower levels of liver enzymes and serum creatinine, lower platelet counts, and tacrolimus-based immunosuppressive therapy (rather than cyclosporin A) with chronic hepatitis. On multivariate analysis, the independent predictive factors associated with chronic HEV infection were the use of tacrolimus rather than cyclosporin A (odds ratio [OR], 1.87; 95% confidence interval [CI], 1.49–1.97; $P = .004$) and a low platelet count at the time of diagnosis with HEV infection (OR, 1.02; 95% CI, 1.001–1.1; $P = .04$). Of patients with chronic hepatitis, 18 (32.1%) achieved viral clearance after the dose of immunosuppressive therapy was reduced. No HEV reactivation was observed after HEV clearance. **CONCLUSIONS: HEV infection causes chronic hepatitis in more than 60% of recipients of solid organ transplants. Tacrolimus therapy is the main predictive factor for chronic hepatitis. Dose reductions of immunosuppressive therapy resulted in viral clearance in more than 30% of patients.**

Keywords: Liver Disease; Virus; Transplantation; Fibrosis.

Hepatitis E virus (HEV) is a well-known cause of acute hepatitis that can be fulminant in patients with chronic liver disease and pregnant women.[1] Locally acquired HEV genotype 3 infection is increasingly recognized as a public health issue in developed countries and is believed to be a porcine zoonosis.[1] Over the past 2 years, a number of cases of chronic hepatitis caused by HEV genotype 3 have been reported in the immunosuppressed, that is, solid organ transplant recipients,[2] patients with hematologic disorders,[3,4] and patients with human immunodeficiency virus (HIV).[5] Chronic HEV infection can cause rapidly progressive cirrhosis, necessitating a liver transplant.[6–8] However, factors predicting the development of chronic infection in immunosuppressed individuals exposed to HEV are incompletely understood.

In the first report of chronic HEV infection in 14 solid organ transplant recipients, it was found that the length of time since transplant and leukocyte, total lymphocyte, and CD2-positive, CD3-positive, and CD4-positive lymphocyte count, as well as platelet count and serum creatinine level, were significantly lower in patients who developed chronic hepatitis compared with those with

Abbreviations used in this paper: HEV, hepatitis E virus; HIV, human immunodeficiency virus; PCR, polymerase chain reaction.

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resolving hepatitis.[2] In a later study of 27 solid organ transplant recipients, it was observed that serum creatinine level and platelet count were significantly lower in patients who developed chronic hepatitis.[9] In addition, the number of patients receiving tacrolimus rather than cyclosporin A at diagnosis of HEV infection was significantly higher in patients with chronic hepatitis compared with those with resolving hepatitis.[9] Very recently, in a study of 38 solid organ transplant recipients, lower alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at diagnosis of HEV infection were observed in patients developing chronic hepatitis compared with those with resolving hepatitis.[10]

However, to date, no independent factors have been identified that predict the development of chronic hepatitis in immunosuppressed patients exposed to HEV. The main limiting factor in the previous reports was the small number of patients available to study. This prompted us to collect, from numerous centers in Europe and one from the United States, all cases of HEV infection that have recently occurred in solid organ transplant patients with the aims of determining factors that predict the development of chronic hepatitis as well as the natural history and outcome of HEV infection in this setting.

Patients and Methods

To maximize the numbers of patients included in the study, all kidney and liver transplant centers in France were approached and invited to collaborate. In addition, institutions in other European countries and the United States that had previously documented cases of HEV infection in solid organ transplant recipients were also invited to participate. In total, 85 cases of autochthonous HEV infection in solid organ transplant recipients were included from the following centers: Toulouse University Hospital (France, n = 52); University Medical Center Groningen (The Netherlands, n = 5); Montpellier University Hospital (France, n = 4); Hannover Medical School (Germany, n = 3); Edouard Herriot University Hospital (Lyon, France, n = 3); Lille University Hospital (France, n = 3); Nice University Hospital (France, n = 2); Necker Hospital (Paris, France, n = 2); Saint Antoine Hospital (Paris, France, n = 2); Pitié Salpétrière Hospital (Paris, France, n = 2); Royal Cornwall Hospital (Truro, Cornwall, England, n = 1); North Central Kidney Institute, Avera Mckennan Hospital (Sioux Falls, SD, n = 1); Saint Luc Hospital (Brussels, Belgium, n = 1); Limoges University Hospital (France, n = 1); Dijon University Hospital (France, n = 1); Lyon Nord Croix Rousse Hospital (France, n = 1); and Paul Brousse Hospital (Villejuif, France, n = 1).

At Toulouse University Hospital, starting in January 2004, all solid organ transplant patients were systematically tested for HEV (immunoglobulin [Ig] M, IgG, and polymerase chain reaction [PCR]) at transplant, every year after transplant, and each time they developed abnormal liver enzyme levels. At the 16 remaining centers, starting in 2008, patients with unexplained elevated liver enzyme levels were tested for HEV serology and/or serum HEV RNA on an ad hoc basis.

Sixty-eight men and 17 women were included in this retrospective study, with an age range of 23 to 77 years (median, 48 years). The type of organ transplant and indications for transplant surgery are shown in Table 1. Initial HEV infection occurred 48 (range, 1–180) months after transplant. Data recorded included clinical and laboratory parameters at transplant and at HEV infection, any contact with animals before infection, immunosuppressive regimen at transplant (induction therapy and maintenance immunosuppressant regimen), and any episode of acute rejection before infection (number, type, treatment, and time between the last acute rejection episode and HEV infection). A number of laboratory findings were systematically recorded before HEV exposure, at initial diagnosis of HEV infection, as well as at 1, 3, and 6 months later, that is, liver enzyme levels (AST, ALT, γ-glutamyl transpeptidase, alkaline phosphate, and total bilirubin), serum creatinine level, hematologic parameters (hemoglobin level and white blood cell, lymphocyte, and platelet counts), and immunosuppressive regimen (type, dose, level). Viral serologies and/or viremia for HIV, hepatitis C virus, hepatitis B virus, HEV, and hepatitis A virus were assessed at diagnosis of HEV infection. In patients who developed chronic HEV infection, the pre-

Table 1. Type of Organ and Reason for Transplant in 85 Solid Organ Transplant Recipients With Autochthonous HEV Infection

Type of organ transplant	n	Reason for transplant	n
Kidney	47	Glomerular disease	21
		Genetic disease	11
		Uropathy and interstitial nephropathy	11
		Vascular disease and diabetes	3
		Unknown	1
Liver	26	Chronic viral hepatitis	8
		• Hepatitis B virus (n = 4)	
		• Hepatitis C virus (n = 3)	
		• Hepatitis C virus/HIV (n = 1)	
		Alcohol-related liver disease	6
		Genetic disease	4
		Autoimmune hepatitis	2
		Other causes	6
Liver-kidney	2	Alcoholic liver disease/chronic renal disease	1
		Idiopathic portal hypertension/ chronic renal disease	1
Kidney-pancreas	6[a]	Type 1 diabetes mellitus	6
Islet cell	1	Type 1 diabetes mellitus	1
Heart	2	Ischemic heart disease	2
Lung	1	Mucoviscidosis	1

NOTE. Eighty of 85 patients received 1 or 2 cadaveric donor organs. Eighty of 85 were recipients of a first transplant.
[a]These organs were transplanted simultaneously, except in 1 patient who received the pancreas allograft after the kidney allograft.

viously described data were collected until sustained viral clearance or death. Finally, we assessed clinical and biological parameters and outcome at last follow-up.

Definition of Resolving HEV Infection (Resolving Group)

Resolving HEV infection was defined as elevated liver enzyme levels plus serologic evidence of acute HEV infection (positive anti-HEV IgM and/or rising IgG antibodies) and/or PCR evidence of HEV in the serum and/or stool of less than 6 months' duration. Viral clearance was achieved spontaneously in this group and specifically did not require either anti-HEV antiviral therapy or a reduction in immunosuppressive therapy.

Definition of Chronic HEV Infection (Chronic Group)

Chronic HEV infection was defined by the presence of persistently elevated liver enzyme levels and PCR evidence of HEV in the serum and/or stool for at least 6 months. In the absence of any established definition for chronic HEV infection, we used the previously described definition that appears in prior publications.[2,5,9]

Statistical Analyses

Reported values represent either means (±SD) or medians (ranges). Proportions were compared by the χ^2 test or Fisher exact test. In each group, quantitative variables were compared by the nonparametric Friedman test for serial measurements and the Wilcoxon test. Independent factors associated with chronic HEV infection were studied using a stepwise multivariate logistic regression model that used initial inclusion criteria with a significance of $P < .05$, using StatView Software (SAS Institute Inc, Cary, NC). For this purpose, patients with resolving hepatitis (resolving group) were compared with those who developed chronic hepatitis (chronic group), as defined previously. $P < .05$ was considered statistically significant.

Results

Clinical and Laboratory Findings at Initial HEV Infection

The results of the HEV serology and PCR testing at diagnosis are shown in Table 2. When tested (n = 82), all patients were HEV PCR positive and were HEV genotype 3 (Table 2). The diagnosis of HEV infection in these cases was based on an increased IgM and IgG level. At diagnosis of HEV infection, HIV serology was positive in only 1 patient, HBV DNA was positive in 2 patients, and HCV RNA was positive in 2 patients; all of these coinfections were long-standing and previously documented (Table 1). Anti–hepatitis A virus IgG was found to be positive in 20 of 63 patients, and anti–hepatitis A virus IgM was negative in all patients.

Twenty-seven patients (32%) were symptomatic at diagnosis of HEV infection, that is, at the time of initial HEV viremia as detected by PCR. The symptoms were mostly nonspecific and included fatigue (n = 20), diarrhea (n = 5), arthralgia (n = 4), weight loss (n = 3), abdominal pain (n = 2), jaundice (n = 1), itch (n = 1), fever (n = 1), and nausea (n = 1). These symptoms were largely self-limiting and resolved within a few days in nearly all patients, including those who developed chronic hepatitis. Thirty-five percent of patients reported having been in contact with animals, mainly cats and dogs, but only 6 patients had been in contact with pigs. Compared with preinfection (4 [0.5–90] months before HEV infection) liver enzyme levels, there was a significant increase in ALT (from 42 ± 8 to 260 ± 38 IU/L, $P < .0001$), AST (29 ± 3 to 155 ± 25 IU/L, $P < .0001$), γ-glutamyl transpeptidase (90 ± 20 to 308 ± 56 IU/L, $P < .0001$), alkaline phosphate (179 ± 23 to 408 ± 96 IU/L, $P = .008$), and total bilirubin levels (11.2 ± 0.8 to 22.5 ± 3.8 μmol/L, $P = .005$). At diagnosis of HEV infection, none of the patients had documented cirrhosis.

Table 2. HEV Serologic and PCR Results at Diagnosis in 85 Solid Organ Transplant Patients With HEV Infection

HEV diagnostic test	No. tested	No. positive	Percent (%)
Anti-HEV IgM[a]	78	32	41.0
Anti-HEV IgG[a]	78	63	80.8
Serum HEV PCR	82[b]	82	100
HEV genotyping	64	59[c]	[c]

[a]A wide range of differing commercial and in-house assays were used, and these differed from center to center.
[b]Three patients were not tested for HEV RNA by PCR. The diagnosis of HEV infection in these cases was based on an increased IgM and IgG level.
[c]All 59 patients in whom HEV was genotyped were infected with HEV genotype 3. In 50 of these cases, HEV subtyping was performed and showed infection with the following subtypes: 3f, n = 37; 3c, n = 12; 3e, n = 1. In 5 patients, genotyping was not possible due to failure to amplify HEV.

Outcome of HEV Infection

Fifty-six of the 85 patients (65.9%) developed chronic hepatitis. Because the number of patients in the study from Toulouse University Hospital was much higher than from other centers, the proportion of chronic HEV infections from Toulouse was compared with those from outside Toulouse. Chronic infection occurred in 57.8% of the Toulouse patients and 78.8% of patients from the other pooled centers ($P = .07$).

Comparison of Resolving and Chronic HEV Groups

The clinical and laboratory findings of the patients from both groups at transplant and at HEV infection are presented in Tables 3 and 4. The proportion of liver transplant patients was significantly higher in the chronic group. The number of patients who had experienced an acute rejection before the acute HEV episode

Table 3. Patient Characteristics at Transplant

Variables	Resolving group (n = 29)	Chronic group (n = 56)	*P* value
Age (*y*)	49.5 ± 13.7	45.3 ± 12	NS
Male/female	24/5	44/12	NS
Transplanted organ			.04
Kidney	23	24	
Liver	4	22	
Combined liver-kidney	1	1	
Kidney-pancreas	0	6	
Islet cell	0	1	
Heart	1	1	
Lung	0	1	
Liver/nonliver transplant	5/24	23/33	.05
Induction therapy (%)	72.4	68	NS
RATG/anti-IL2R blockers	13/8[a]	28/10	NS
Calcineurin inhibitors (%)	96.5	98.2	NS
Cyclosporin A/tacrolimus	17/11	15/40	.004
Belatacept (%)	3.4	0	NS
mTOR inhibitors (%)	0	10.7	NS
Mycophenolic acid (%)	55.2	55.3	NS
Azathioprine (%)	27.6	12.5	NS
Corticosteroids (%)	89.6	87.5	NS
Acute rejection before HEV (%)	13.8	19.6	NS
Corticosteroid pulses for acute rejection (%)	13.8	19.6	NS
T cell–depleting agent for AR (%)	0	3.5	NS
Rituximab before HEV (%)	3.4	0	NS
No. of acute rejection episodes	1	1.2 ± 0.6	NS
Time between last AR and HEV (*days*)	102 ± 93	29.5 ± 31	.03

RATG, rabbit antithymocyte globulins; anti-IL2R, anti–interleukin-2 receptor blockers; mTOR, mammalian target of rapamycin; AR, acute rejection.
[a]One patient received anti-CD52 induction therapy and is included in the RATG group.

was similar in both groups. However, the time between the last acute rejection episode and HEV infection was significantly shorter in the chronic group. The time between transplant and HEV infection was also significantly shorter in the chronic group. The proportion of patients receiving tacrolimus rather than cyclosporin A was significantly higher in the chronic group at transplant and at HEV infection. At HEV infection, AST level, ALT level, the peak of AST level, the peak of ALT level, serum creatinine level, and platelet count were significantly lower in the chronic group.

Predictive Factors for Chronic HEV Infection

The following variables were included in a multivariate analysis model: the time between transplant and HEV infection; the peak AST level; the peak ALT, AST, ALT, and serum creatinine levels; platelet count; the use of tacrolimus versus cyclosporin A; and having received (or not) a liver transplant. The independent predictive factors associated with chronic HEV infection were the use of tacrolimus rather than cyclosporin A (odds ratio, 1.87; 95% confidence interval, 1.49–1.97; $P = .004$) and a low platelet count at diagnosis of HEV infection (odds ratio, 1.02; 95% confidence interval, 1.001–1.1; $P = .04$).

At diagnosis of HEV infection, the platelet count was lower in liver transplant patients (179,846 ± 79,489/mm^3) compared with non–liver transplant patients (209,600 ± 68,521/mm^3), but the difference was not statistically significant ($P = .08$). However, in both groups, the platelet count was lower in patients who developed chronic hepatitis compared with those with resolving hepatitis: 171,666 ± 81,252/mm^3 versus 218,200 ± 68,225/mm^3 in liver transplant patients ($P = .2$) and 199,161 ± 68,563/mm^3 versus 225,083 ± 67,492/mm^3 in non–liver transplant patients ($P = .2$).

Outcome of the Resolving Group

During the 6 months after diagnosis of HEV infection, there was no significant change in the type, dose, or trough levels of immunosuppressive therapy (data not shown). In the resolving group, liver enzyme levels decreased significantly within 6 months of diagnosis (Figure 1). Serum HEV RNA was still positive in 6 patients at month 1, in 1 patient at month 3, and in no patient at month 6. At last follow-up (ie, at 15 [1.5–68] months after diagnosis of HEV infection), anti-HEV IgG was positive in 12 of 25 patients and anti-HEV IgM in 12 of 24 patients. No reactivation of HEV was observed after HEV clearance. One liver transplant patient died at 32 months after HEV clearance from an unrelated cause (septic shock).

Outcome of the Chronic Group

During the first 6-month period after diagnosis of HEV infection, the type of immunosuppressive therapy remained unchanged. However, the daily dose and trough levels of tacrolimus were significantly reduced.

Table 4. Patient Characteristics at Diagnosis of HEV Infection

Variables	Resolving group (n = 29)	Chronic group (n = 56)	*P* value
Time since transplant (*mo*)	70.3 ± 52.8	41.4 ± 38	.005
Symptoms at presentation (%)	31	32	NS
AST level (*IU/L*)	107 (16–1571)	94 (21–436)	.02
ALT level (*IU/L*)	263 (24–2675)	135 (28–874)	.001
γ-glutamyl transpeptidase level (*IU/L*)	244 (28–2337)	173 (25–3482)	NS
Alanine phosphatase level (*IU/L*)	251 (66–1924)	172 (46–7775)	NS
Bilirubin level (*μmol/L*)	16 (6–75)	7 (5–277)	NS
Peak AST level (*IU/L*)	223 (31–1571)	147 (39–874)	.001
Peak ALT level (*IU/L*)	272 (29–2675)	167 (32–522)	.005
Serum creatinine level (*μmol/L*)	168 ± 69	130 ± 51	.005
Hemoglobin level (*g/dL*)	13.1 ± 1.85	12.9 ± 1.56	NS
White blood cell count (*/mm^3*)	7253 ± 2834	6122 ± 2370	NS
Lymphocyte count (*/mm^3*)	1414 ± 684	1399 ± 702	NS
Platelet count (*/mm^3*)	225,655 ± 62,521	190,384 ± 79,903	.04
Calcineurin inhibitors (%)	75.9	83.9	NS
Cyclosporin A/tacrolimus	9/13	4/43	.003
Cyclosporin A ($mg \cdot kg^{-1} \cdot day^{-1}$)	1.9 ± 0.5	2.24 ± 1.2	NS
Cyclosporin A trough level (*ng/mL*)	88 ± 82	183 ± 106	NS
Cyclosporin A C2 level (*ng/mL*)	543 ± 155	352 ± 248	NS
Tacrolimus ($mg \cdot kg^{-1} \cdot day^{-1}$)	0.06 ± 0.03	0.09 ± 0.09	NS
Tacrolimus trough level (*ng/mL*)	8.7 ± 3.2	10.1 ± 4.3	NS
Belatacept (%)	3.4	0	NS
mTOR inhibitors (%)	24	19.6	NS
Sirolimus trough level (*ng/mL*)	7.8 ± 4.3	8.7 ± 3.6	NS
Everolimus trough level (*ng/mL*)	10.5 ± 7.07	12.1 ± 6.3	NS
Mycophenolic acid (%)	79.3	69.6	NS
Mycophenolic dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	19.2 ± 7.6	20.1 ± 8.07	NS
Azathioprine (%)	0	7.1	NS
Corticosteroids (%)	72.4	69.6	NS
Corticosteroid dose ($mg \cdot kg^{-1} \cdot day^{-1}$)	0.08 ± 0.04	0.1 ± 0.09	NS
Anti-HEV IgG P/N[a] (%)	32.1	46	NS
Anti-HEV IgM P/N[a] (%)	84.6	78.8	NS
HEV RNA concentration (*copies/mL*)[b]	1,927,500 (500–71,440,000)	1,248,500 (35,600–71,000,000)	NS

C2, concentration 2 hours after intake; mTOR, mammalian target of rapamycin; N, negative; P, positive.
[a]Anti-HEV IgG and IgM were studied in, respectively, 28 and 26 patients from the resolving group and 50 and 52 patients from the chronic group.
[b]Data were available for 18 patients from the resolving group and 26 patients from the chronic group.

The daily dose of tacrolimus was reduced from 0.09 ± 0.09 $mg \cdot kg^{-1} \cdot day^{-1}$ at HEV infection to 0.06 ± 0.05 $mg \cdot kg^{-1} \cdot day^{-1}$ at month 6 ($P = .02$), and the trough levels were reduced from 10.1 ± 4.3 ng/mL at HEV infection to 7.9 ± 3.3 ng/mL at month 6 ($P = .002$). However, even though ALT levels decreased slightly within the 6 months after diagnosis of HEV infection, liver enzyme levels remained significantly higher than at baseline, that is, before the acute HEV episode (Figure 1).

Among the 56 patients from the chronic group, 18 (32.1%) achieved sustained HEV clearance following a reduction in the dose of immunosuppression; this occurred at 19.5 (10–106) months after diagnosis of HEV infection. HEV clearance occurred following reduction of tacrolimus trough level (n = 9), reduction of tacrolimus trough level and dose of mycophenolic acid (n = 2), reduction of tacrolimus trough level and withdrawal of mycophenolic acid (n = 2), reduction of cyclosporine trough level (n = 2), reduction of cyclosporine trough level and withdrawal of mycophenolic acid (n = 1), and conversion from calcineurin to mammalian target of rapamycin (mTOR) inhibitors (n = 2). No acute rejection occurred after dose reduction of immunosuppressive therapy.

Among the 38 remaining patients, because of a significant or rapid progression in liver fibrosis and/or because of the presence of HEV-related cirrhosis requiring consideration of a second liver transplant, 20 patients were given antiviral therapy to clear the virus: pegylated interferon (n = 5), ribavirin (n = 14), and combined pegylated interferon and ribavirin (n = 1). At last follow-up, 14 had achieved sustained viral clearance and 6 were still viremic and still receiving therapy.

Of the 18 remaining patients who did not receive antiviral therapy, 5 patients died while their serum HEV RNA was still positive. The causes of death were decompensated cirrhosis (n = 2; the deaths occurred at 22 and 38 months after diagnosis of HEV infection), septic shock caused by a hepatic abscess (n = 1, death occurred at 6 months after diagnosis of HEV infection), acute respiratory distress in a liver transplant patient with chronic respiratory disease (n = 1, death occurred at 21

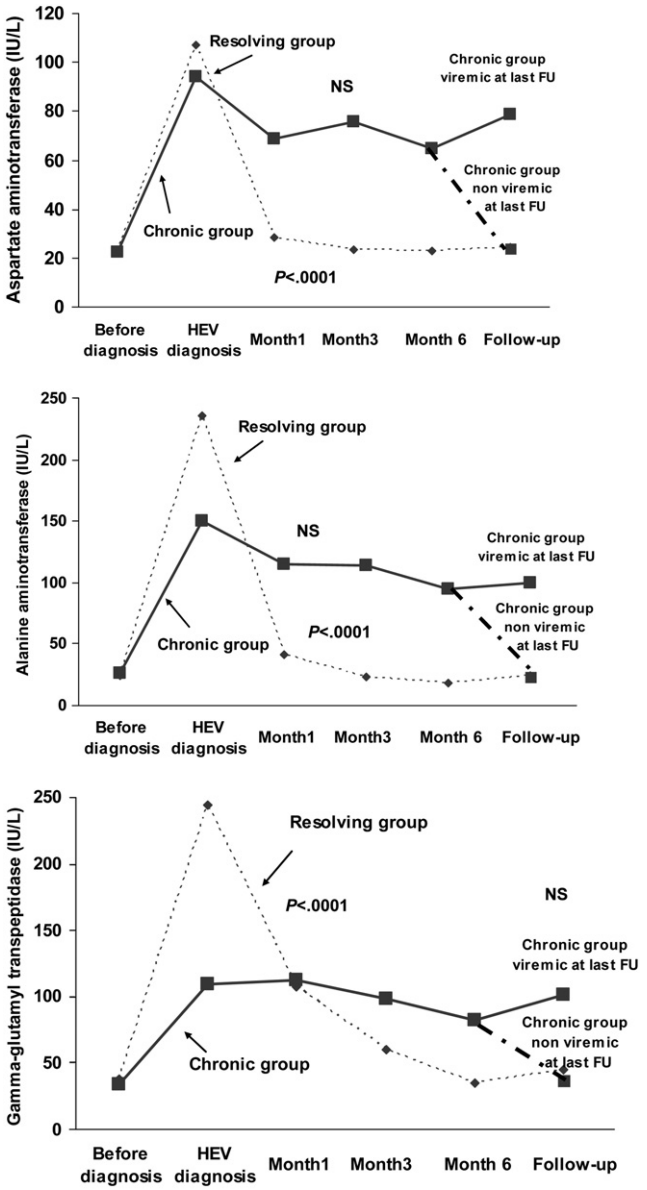

Figure 1. Outcome of liver enzyme levels in the patients with resolving hepatitis and chronic hepatitis. NS, not significant; FU, follow-up.

months after diagnosis of HEV infection), and a relapse of hepatocellular carcinoma in a liver transplant patient who was coinfected with HBV (death at 19 months after HEV infection). The 13 other patients were still viremic at last follow-up.

Eight of the 56 patients developed cirrhosis, and 2 liver transplant patients required a second liver transplant.

At last follow-up (29.5 [9–117] months after diagnosis) of the 32 initially chronic patients who achieved viral clearance either after immunosuppressant dose reduction (n = 18) or after antiviral therapy (n = 14), AST and ALT levels were, respectively, 25 (12–106) IU/L and 23.5 (8–99) IU/L. Anti-HEV IgG was positive in 20 of 30 patients, and anti-HEV IgM was positive in 27 of 30 patients.

At last follow-up (18 [6–92] months after diagnosis) for the 24 remaining chronic patients, who have either died (n = 5) or who were still viremic with (n = 6) or without (n = 13) antiviral therapy, the AST and ALT levels were, respectively, 78.5 (21–812) IU/L and 100 (17–737) IU/L. Anti-HEV IgG was positive in 12 of 18 patients, and anti-HEV IgM was positive in 17 of 19 patients.

Discussion

This retrospective multicenter study is the largest series to assess the natural history and outcome of solid organ transplant recipients infected with HEV and to determine the predictive factors for chronic hepatitis in this setting. The diagnosis of HEV infection in immunosuppressed individuals is not straightforward. Most patients have no symptoms, and clinically evident jaundice is rare. However, in contrast to HEV infection in the immunocompetent patient, in who ALT levels at presentation are often >1000 IU/L,[1] in immunosuppressed solid organ transplant recipients the degree of transaminitis is modest and often in the range of 100 to 300 IU/L. The diagnosis of HEV infection is confirmed by serology and molecular techniques. However, as our data show, serologic testing for anti-HEV antibodies has a significant false-negative rate in immunosuppressed patients, so negative results should be treated with caution.

The data presented in the current report confirm and extend previous observations in a much smaller number of solid organ transplant recipients and are 4-fold: (1) approximately 60% of solid organ transplant patients infected with HEV develop chronic hepatitis; (2) the use of tacrolimus rather than cyclosporin A as a main immunosuppressant and a low platelet count at diagnosis of HEV infection are 2 independent predictive factors for the development of chronic HEV infection; (3) nearly one-third of patients who are chronically infected with HEV achieved sustained viral response after dose reduction of immunosuppressive therapy, and this was mainly due to the reduction of therapy-targeting T cells; and (4) no reactivation was observed after HEV clearance.

In 2008, it was shown that HEV infection can cause chronic hepatitis and cirrhosis in solid organ transplant recipients.[2,6–8,11] In previous reports on small numbers of patients, it was shown that nearly 60% of solid organ transplant recipients infected with HEV developed chronic infection.[9,10] The present study, which included 85 solid organ transplant recipients from 17 different centers, confirms these previous reports and found that 65.9% of HEV infections in solid organ transplant recipients became chronic. At Toulouse University Hospital, solid organ transplant patients were systematically tested for anti-HEV IgG and IgM as well as for serum HEV RNA every year and each time a patient developed abnormal liver enzyme levels. At the other centers, testing for HEV infection was started a couple of years ago and only

patients with unexplained elevated liver enzyme levels were tested for anti-HEV IgG and IgM and/or serum HEV RNA. The difference in the screening protocol between Toulouse and the other centers may have led to an overestimation of the rate of chronic HEV infection in the patients included from these latter institutions. Patients from Toulouse comprise 61% of the patients in the current study, and the proportion of chronic HEV infections in the Toulouse cohort was 57.8%, suggesting that approximately 60% of transplant recipients who are infected with HEV evolve to having chronic hepatitis.

In the present study, 8 (14.3%) solid organ transplant recipients with chronic HEV developed cirrhosis. Two liver transplant patients required a second liver transplant. As previously reported, there was a relapse of chronic HEV infection in the second transplant in one of these 2 patients.[6] During follow-up, 6 patients died, 2 of who died from HEV-related decompensated cirrhosis.[8]

Even though all centers participating in the current study do not use tacrolimus and cyclosporin A with the same frequency, the most important independent factor predicting the development of chronic hepatitis appears to be the use of tacrolimus rather than cyclosporin A. This may be related to a greater immunosuppressive effect of tacrolimus compared with cyclosporin A. Following kidney, liver, or heart transplant, acute rejection has been found to be significantly less common in patients treated with tacrolimus compared with cyclosporin A.[12–14] Several factors identified as predictive of chronic infection in univariate analysis in the current and earlier studies[2,9,15] seem to relate to the degree of immunosuppression, that is, time between the last episode of acute rejection and HEV, time since transplant, and a low leukocyte count, a low total lymphocyte count, and low CD2-positive, CD3-positive, and CD4-positive cell counts.

Better renal function was observed in patients who developed chronic hepatitis. The reason for this observation is uncertain. It might be related to the shorter time since transplant at HEV infection in such patients, with a resulting overall lower exposure to nephrotoxic calcineurin inhibitors.[16] A higher rate of chronic hepatitis was observed among liver transplant patients. We do not have an explanation for this finding. We can speculate that the local inflammation observed in the liver allografts may be responsible for persisting HEV replication. It has been recently shown that liver transplant patients are also at higher risk for HEV infection compared with kidney transplant patients.[17]

In the present study, univariate analysis also showed that ALT and AST levels were lower at diagnosis of HEV infection in patients who developed chronic hepatitis. Similar observations have been made regarding the natural history of chronic hepatitis C infection.[18] The reason for this observation is not certain but might be explained by the greater immunosuppressive effect of tacrolimus, which may down-regulate T-cell responses against the virus. In vitro, cyclosporin A but not tacrolimus specifically inhibits the replication of HCV in cultured hepatocytes at clinically relevant concentrations.[19,20] The effect of cyclosporin A against HCV seems to be specific and independent of its immunosuppressive function.[19,20] Hence, we might speculate whether cyclosporin A can inhibit HEV replication. If so, this may be another explanation for our finding that patients treated with tacrolimus more often develop chronic hepatitis E infection.

The second independent predictive factor of chronic hepatitis is a low platelet count at diagnosis of HEV infection. A number of cases of severe thrombocytopenia associated with acute hepatitis E have been reported in nontransplant patients.[21–23] However, the relationship between HEV infection and platelet count is still unknown. We cannot exclude the possibility that low platelet count is related to advanced fibrosis and/or to persisting hypersplenism after transplant in liver transplant patients. However, at diagnosis of HEV infection, no patient was known to have cirrhosis. Furthermore, the platelet count did not differ significantly between liver and nonliver transplant patients. Finally, the platelet count was lower in patients who developed chronic hepatitis compared with those with resolving hepatitis both in liver and nonliver transplant patients. We speculate that the low platelet count may be immune mediated, as it is believed to be in other viral infections.[24] However, further studies are required to assess the effect of HEV infection on platelet count.

A decrease in dose and trough levels of immunosuppressive therapy targeting T cells (ie, cyclosporin A and tacrolimus) led to HEV clearance in one-third of chronically infected solid organ transplant patients. In the remaining chronic patients, it has been recently shown that pegylated interferon alfa[4,25–27] or ribavirin alone[28,29] can successfully treat HEV infection, producing a sustained virologic response. Several patients in the present study are still receiving, or have only just completed, antiviral therapy. A longer follow-up is required to evaluate sustained virologic response. Finally, in contrast to the findings of Le Coutre et al, who reported a case of HEV reactivation in a patient with acute lymphoblastic leukemia after allogeneic stem cell transplant,[30] we found no reactivation in the present study among patients who achieved viral clearance after immunosuppressant dose reduction or who had a sustained virologic response after antiviral therapy.

The geographical distribution of cases of HEV infection in solid organ transplant recipients included in the current study deserves some comment. The very large number of cases (52/85) from Toulouse, compared with the other centers, is a reflection of the high prevalence of HEV in this area[31] and the group's interest in HEV, including a rigorous and systematic HEV screening program of all solid organ transplant recipients using mo-

lecular techniques. Indeed, HEV seroprevalence in blood donors in the Midi-Pyrénées area (Toulouse area) is 16.6%,[31] compared with 3.2% in northern France.[32] At Toulouse University Hospital, the incidence of HEV infection after transplant is 3.2/100 person-years.[17] The prevalence of chronic HEV infection in solid organ transplant patients in Toulouse is much higher than in The Netherlands,[33] Germany,[11] or Spain.[34] We do not know why there is such a high incidence of HEV infection in the Toulouse area, but available evidence suggests that regional culinary culture could be a factor in viral exposure. A recent case-control study has shown that the consumption of game meat was the only predictive factor associated with HEV infection after solid organ transplant.[10] In southeast France, HEV infection had been reported through consumption of raw pig liver sausage.[35] In most patients the route of infection is uncertain, and further research is required. Until more data are available, we would recommend that transplant patients avoid consuming raw or undercooked pork and game.

In keeping with all retrospective studies, the present study has some limitations. These limitations mainly relate to incomplete/inconsistent laboratory monitoring. Some variables were not studied in all patients; for example, systematic follow-up for HEV serology was not performed in all patients. HEV concentrations and HEV genotypes were also not determined in all patients.

In conclusion, this is the largest series to assess the occurrence of chronic HEV infection after solid organ transplant. Approximately 60% of solid organ transplant patients infected with HEV developed chronic hepatitis. The use of tacrolimus rather than cyclosporin A and low platelet count were the main independent factors associated with chronic HEV infection.

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Conflicts of interest

The authors disclose no conflicts.