OPEN

Hepatitis E Virus Infection, a Risk for Liver Transplant Recipients in Sweden

Miriam Frankal, MD, PhD-student,[1,2,3] Catarina Skoglund, RN, PhD-student,[4,5] Maria Castedal, MD, PhD,[4,5] Martin Lagging, MD, PhD,[1,6] and Heléne Norder, PhD[1,6]

Background. Following exposure to hepatitis E virus (HEV), liver transplant (LT) recipients have an increased risk of developing chronic infection, which may rapidly progress to severe liver damage if not treated. The prevalence of HEV infection after LT is unclear and likely varies geographically. The aim of this study was to investigate the prevalence of acute and chronic HEV infection among LT recipients in an HEV endemic region. **Methods.** During 2013 to 2015, 109 of 152 prospectively enrolled patients listed for LT received a liver graft and completed the study protocol. They were evaluated for anti-HEV IgM, HEV IgG, and HEV RNA at the time of LT assessment and 3 and 12 mo post-LT. Medical records were reviewed. **Results.** Twelve (11%) LT recipients acquired markers of HEV infection during the study period. Seven patients (6%) had detectable HEV RNA, 1 before LT and 3 at the 3-mo and another 3 at the 12-mo follow-up post-LT. All resolved their infections without treatment and had undetectable HEV RNA at the succeeding follow-up. Another 5 (5%) patients developed anti-HEV antibodies without detectable HEV RNA as an indication of HEV infection during follow-up. Signs and symptoms of HEV infection were subtle, and none were diagnosed in routine clinical care. **Conclusion.** A substantial proportion of LT recipients in Sweden are at risk of acquiring HEV infection, both before and after LT. The results highlight the frequency of silent, spontaneously resolving HEV infections and do not support universal screening of LT recipients in Sweden, despite HEV being a potentially treatable infection.

(*Transplantation Direct* 2022;8: e1409; doi: 10.1097/TXD.0000000000001409).

INTRODUCTION

Infection with hepatitis E virus (HEV) primarily results in an acute, self-limiting hepatitis in immunocompetent patients. In immunosuppressed patients, it has a propensity to evolve into a chronic infection, which, if left untreated, subsequently may lead to severe liver damage, including cirrhosis, within only a few years.[1-3] The first cases of chronic HEV infection were reported in 2008.[4] Liver transplant (LT) recipients acquiring an acute HEV infection reportedly progress to a chronic infection in up to 60% of cases because of immunosuppression.[4] Despite these dire consequences, only 30% of patients with chronic HEV infection are symptomatic, with fatigue being the most common manifestation.[4] Icterus is rare, and the majority presents with only mild elevations of liver transaminases.[5] If possible, chronic infection can be

Received 15 August 2022. Revision received 8 September 2022.

Accepted 20 September 2022.

[1] Department of Infectious Diseases/Virology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

[2] Region Västra Götaland, Södra Älvsborg Hospital, Clinic of Infectious Diseases, Borås, Sweden.

[3] Department of Research, Education and Innovation, Region Västra Götaland, Södra Älvsborg Hospital, Borås, Sweden.

[4] Region Västra Götaland, Sahlgrenska University Hospital, Transplant Institute, Gothenburg, Sweden.

[5] Department of Surgery, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

[6] Department of Clinical Microbiology, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden.

This study was in part funded by grants from the Regional Research Sources (FoU), Sweden (grant VGFOUREG-647241), The Local Research and Development council, Södra Älvsborg (grants VGFOUSA- 603751 and VGFOUSA 4180219), and ALF (Avtal om Läkarutbildning och Forskning) funds (grant ALFGBG-717711). The writing and preparation of this paper was funded in part by grants from the Gothenburg Society of Medicine (GLS 687811) and from the Research Council Södra Älvsborg, Södra Älvsborg Hospital, Borås, Sweden.

M.C. and H.N. were involved in study design, data analysis, interpretation of data, and writing of the article. M.F., C.S., and M.L. were involved in data analysis, interpretation of data, and writing of the article. All authors approved the final version of the article.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

Correspondence: Miriam Frankal, MD, PhD-student, Department of Infectious Diseases, Södra Älvsborg Hospital, Brämhultsvägen 53, 501 82 Borås, Sweden. (miriam.frankal@vgregion.se).

The authors report no conflict of interest.

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001409

treated by abating immunosuppression, after which 30% of the patients clear viremia. If this is not possible or insufficient, patients are treated with ribavirin.[6]

Five genotypes, HEV1–4 and 7, are known to infect humans. HEV1 and 2 are endemic in Asia and Africa, where they spread by fecal contaminated water in areas with poor sanitation. HEV3 and 4 are prevalent in Europe, North America, and Asia; they are mainly transmitted zoonotically via consumption of contaminated food.[7] Infection with these 2 genotypes and HEV7 may develop into chronic HEV infection, especially in immunocompromised persons.[8] HEV3, divided into 2 different major subgroups, 3I (abchij) and 3II (efg), is prevalent in Sweden, where the anti-HEV IgG seroprevalence is approximately 17% among blood donors.[9] The route of transmission is mainly fecal-oral, but blood transmission has been reported[7] also in Sweden.[10] Blood products are screened for HEV in several European countries but not in Sweden. Patients receiving an LT may require large volumes of transfused blood; hence, they have increased risk of exposure to blood products containing HEV. Additionally, screening for HEV infection is not part of the routine clinical pre- or post-LT care for Swedish LT recipients, and because of the discrete signs and symptoms, there is a risk that an HEV infection remains undiagnosed.

Globally, anti-HEV antibodies in samples from adult LT recipients assessed by different serological assays vary significantly and range from 3% to 42%,[11-20] whereas the prevalence of HEV infection with detectable HEV RNA ranges from 0.1% to 1.4%.[11,12,14,21-26] Most previous studies of HEV and LT have been retrospective and cross-sectional, and only a handful of studies have prospectively followed patients with repeated testing during the peritransplant period. Reekie et al investigated stored samples from day 0, 30, 60, and 90 post-LT and found HEV RNA prevalence of 1.15% (3/262).[24] Legrand-Abravanel et al reported annual incidence of 2.1% for de novo infections and 3.3% for reinfections studying solid organ transplant recipients,[27] as well as 4.8 cases of HEV infection/100 person-years among LT recipients.[28]

The aim of the current study was to investigate the incidence and prevalence of acute and chronic HEV infection among LT recipients in Sweden by repeated sampling during the pre- and post-LT period, with prospective analyses of HEV RNA unbiased as per protocol, regardless of alanine aminotransferase (ALT) levels or serological responses.

MATERIALS AND METHODS

Patients and Controls

Patients

Patients 18 y or older undergoing LT were prospectively asked to participate at the pre-LT evaluation at Sahlgrenska University Hospital in Gothenburg, Sweden and, if they consented, were enrolled during the study period (March 2013–May 2015). One hundred fifty-two patients were enrolled, whereof 109 were included in the analyses, having met all the inclusion criteria and completed the blood sampling in accordance with the study protocol (Figure 1). Ten patients underwent LT but were excluded from the analysis because of death (3), missing samples (6), or withdrawal of consent (1). Two had positive anti-HEV IgG before LT, none were reactive in IgM, and none had detectable HEV RNA. The deceased patients died within the follow-up and at 2, 4, and 6 mo post-LT. Of these, 1 had no available serum samples, and the remaining 2 had only pre-LT samples, whereof 1 was positive for anti-HEV IgG antibodies. Six patients were excluded because of missing samples, 3 had missing samples at the 3-mo follow-up, and another 3 patients at the 12-mo follow-up post-LT. One withdrew consent before any blood tests were drawn.

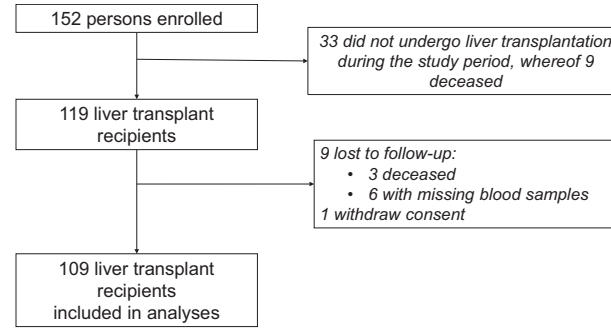

FIGURE 1. Flow chart of patient selection and loss to follow-up.

Medical records were reviewed regarding signs and symptoms of HEV infection, comorbidities and risk factors. For the included patients, the mean age at LT was 54 y (SD 11.6), and 76 (70%) were men; the most common cause for LT was hepatocellular cancer (HCC) and hepatitis C virus (HCV) infection (Table 1).

The standard immunosuppressive protocol during the study period was induction therapy with a single iv bolus dose of methylprednisolone (SoluMedrol, Pfizer) 500 to 1000 mg along with basiliximab (Simulect, Novartis) 20 mg IV before liver reperfusion and at postoperative day 4 (POD4). A few patients received instead antithymoglobulin (ATG-Fresenius, Fresenius Medical Care) IV as induction therapy for various reasons. Maintenance therapy started with delayed tacrolimus (TAC) introduction on POD3 at a low starting dose (2–3 mg BID). The target TAC trough level was 5 to 8 ng/L for the first 3 postoperative months and 3 to 5 ng/L thereafter. Mycophenolate mofetil (Cellcept, Roche) was given to all patients with a starting dose of 1 g BID. Autoimmune patients also received oral prednisolone 20 mg/d, gradually tapered to 5 mg daily after 3 mo (28).

TABLE 1.

Characteristics of 109 liver transplant recipients

Characteristics	n (%)
Male sex	76 (70)
Liver disease causing liver transplantation[a]	
Hepatocellular cancer	46 (42)
with Hepatitis C	29
with alcohol-related liver disease	11
with hepatitis B	5
with autoimmune hepatitis	2
Hepatitis C	38 (35)
Primary sclerosing cholangitis	21 (19)
Alcohol-related liver disease	19 (17)
Hepatitis B	5 (5)
Autoimmune hepatitis	4 (4)

[a]50 (46%) patients had >1 disease reported.

Controls

Five hundred Swedish blood donors were used as controls. They were previously sampled (2012) and analyzed in a study evaluating different anti-HEV serological assays.[9] In the group of blood donors, 64% were men with a mean age of 43 y (SD 13.2).[9]

Sampling

Serum samples were collected from all enrolled patients before LT (median 28 d pre-LT, interquartile range IQR: 1–72 d pre-LT) and at the 2 per-protocol samplings 3- and 12-mo post-LT. The patient enrollment was performed during the LT evaluation at the transplant center after the patient had been accepted for LT. At this time, a serum sample was drawn. For most but not all patients, the sampling was repeated at the day of surgery, but this was sometimes missed in the acute and stressful situation before the LT. Samples were stored at –20 °C until analyzed.

Detection of Anti-HEV Antibodies and HEV RNA

All serum samples were analyzed for anti-HEV IgM and IgG using the HEV IgM/HEV IgG test (DiaPro, Milan, Italy) according to the manufacturer´s instructions[9] and for HEV RNA by PCR. Samples with signal/cutoff (S/CO) ≥1.7 for anti-HEV IgG and S/CO ≥1.5 for anti-HEV IgM were considered positive.[9] All serum samples were analyzed for HEV RNA twice in duplicate by RT-qPCR and seminested PCR as previously described.[9,29] The infecting HEV strain was typed by sequencing the PCR products as previously described.[30]

Case Definition

Patients with HEV RNA were considered infected with HEV, independent of the presence of anti-HEV IgM and IgG antibodies. Patients without anti-HEV IgM and IgG at inclusion who seroconverted to anti-HEV IgM and IgG during follow-up were considered to fulfill the serological criteria of an acute HEV infection. Furthermore, patients who had anti-HEV IgG at inclusion and later showed at least a 3-fold increase in S/CO levels of anti-HEV IgG were also considered having serological signs of having acquired HEV during the study period, which had boosted the immune response. Chronic infection was defined as continuously detectable HEV RNA for ≥3 mo.[31]

Normalized ALT and Aspartate Aminotransferase

Normalized ALT was defined as the ratio between the measured ALT and the upper limit of normal (men 1.1 µkat/L and women 0.75 µkat/L in the present study). Similarly, normalized aspartate aminotransferase (AST) was defined as the ratio between the measured AST and the upper limit of normal (men 0.75 µkat/L and women 0.6 µkat/L). Thus, normalized ALT or normalized AST values above 1.0 are considered abnormal irrespective of gender or analysis method utilized.

Ethical Considerations

The study conformed to the guidelines of the 1975 Declaration of Helsinki. The ethical committee in Gothenburg, Sweden, approved the study (DNR: 534-16 and 737-12). Written informed consent was obtained from each patient when they were enrolled in the study.

Statistical Methods

Categorical variables were presented as a number and percentage. Age was reported as mean and SD. Fisher's exact test and an odds ratio (OR) with 95% confidence interval (CI) were used to analyze the prevalence of anti-HEV IgG. When testing age differences between the groups, a *t* test was performed. A subgroup analysis including the patients older than 47 y (the median age) was performed. In addition, an age adjusted analysis was made using logistic regression presenting adjusted OR (95% CI) and *P* value. Statistical analyses were performed using SPSS, version 25, and SAS, version 9.4, with a *P* value <0.05 considered as significant.

RESULTS

Prevalence of Anti-HEV IgG Antibodies Before Liver Transplantation

Anti-HEV IgG was found in baseline samples from 14 of 109 patients (13%), 10 of whom were men. This prevalence was not significantly different from that among blood donors 84 of 500 (17%). However, after adjusting for age, the analysis showed a significantly lower anti-HEV IgG prevalence among LT recipients with an adjusted OR of 0.24 (95% CI, 0.12-0.47; $P < 0.0001$) as LT recipients were significantly older than the blood donors (mean 54 versus 43 y, respectively; $P < 0.0001$). In the subgroup analysis for the patients older than 47 y (the median age), the difference in anti-HEV IgG prevalence remained significantly lower among the LT recipients (15% [13/87] versus 33% [72/221]; $P = 0.002$).

Patients With Detectable HEV RNA

Seven (6.4%) LT recipients had detectable HEV RNA during the study period (Figure 2). One unknowingly had an ongoing HEV infection before and at the time of LT. Three patients acquired HEV infections early post-LT, with detectable HEV RNA at the 3-mo follow-up sampling, and the remaining 3 cases were detected at the 12-mo post-LT study visit. All had undetectable HEV RNA at their subsequent follow-up visit. The 3 patients who had detectable HEV RNA at the 12-mo follow-up, and, hence, no further planned study visit, were sought out for resampling outside of the study as a part of routine clinical follow-up resulting from the detection of HEV RNA. The sampling was performed after 18, 21, and 23 mo, respectively, with undetectable HEV RNA. One of the 7 patients with detectable HEV RNA had preexisting anti-HEV IgG antibodies at baseline. Despite the presence of anti-HEV IgG, this patient developed detectable HEV RNA at the 3-mo follow-up. The anti-HEV IgG level remained relatively unchanged without development of detectable anti-HEV IgM antibodies throughout the course of infection. The remaining 6 patients with detectable HEV RNA did not develop an IgM and IgG response during the study period, possibly secondary to ongoing immunosuppressive therapy. The HEV strains from 5 patients could be genotyped by sequencing; all were HEV3, subtype HEV3c/i. The remaining 2 patients had HEV RNA repeatedly at low levels in the qPCR with mean Ct (cycle threshold) values of 38 and 44, respectively, which can be considered somewhat dubious and does not allow for confirmatory sequencing and genotyping.

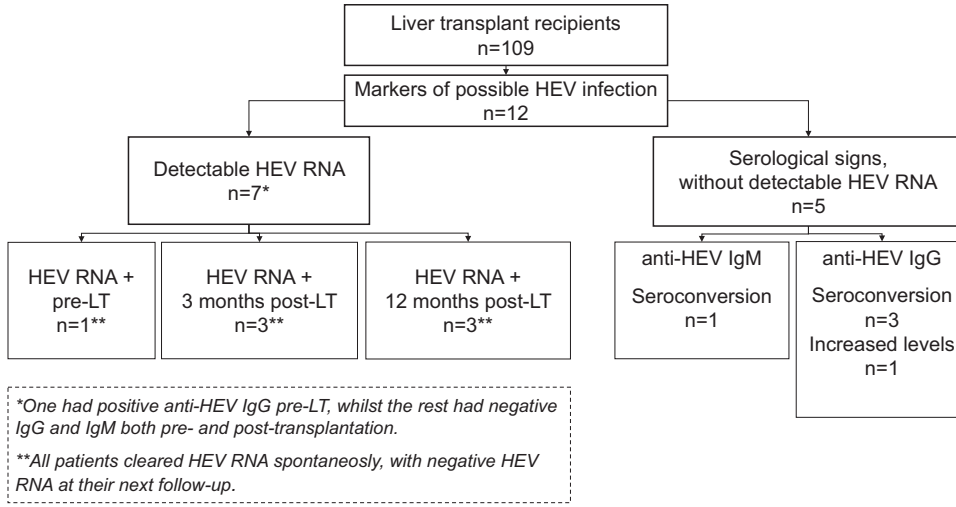

FIGURE 2. Markers of possible HEV infection in 109 LT recipients. Patients were tested for anti-HEV IgM, IgG, and HEV RNA pre-LT and at 3 and 12 mo post-LT. Seven patients had ongoing infection with detectable HEV RNA, and additionally, 5 patients showed serological signs of possible HEV infection without detectable HEV RNA. HEV, hepatitis E virus; LT, liver transplant.

TABLE 2.

Characteristics of liver transplant recipients with detectable HEV RNA

Characteristic	Pat A	Pat B	Pat C	Pat D	Pat E	Pat F	Pat G
Sex, age (y)	♀ 54	♂ 46	♂ 60	♂ 60	♀ 60	♂ 56	♀ 55
Cause of LT	CC	HCC HBV	HCV	HCC HCV ArLD	HCC HCV	HCC HCV	HCV
HEV RNA							
Pre-LT	–	–	–	–	–	–	**+**
3 mo	**+**	**+**	**+**	–	–	–	–
12 mo	–	–	–	**+**	**+**	**+**	–
Clinical follow-up				–	–	–	
(mo after LT)				(23)	(18)	(21)	
HEV genotype	3 c/i	–	3 c/i	3 c/i	3 c/i	–	3 c/i
Anti-HEV IgM/IgG							
Pre-LT	IgM– IgG–	IgM– IgG–	IgM– **IgG+**	IgM– IgG–	IgM– IgG–	IgM– IgG–	IgM– IgG–
3 mo	IgM– IgG–	IgM– IgG–	Ig M– **IgG+**	IgM– IgG–	IgM– IgG–	IgM– IgG–	IgM– IgG–
12 mo	IgM– IgG–	IgM– IgG–	Ig M– **IgG+**	IgM– IgG–	IgM– IgG–	IgM– IgG–	IgM– IgG–
nALT							
3 mo	**2.7**	**1.3**	0.6	0.3	0.6	**2.1**	0.8
12 mo	**1.2**	0.6	0.7	0.6	0.4	0.8	0.4
nAST							
3 mo	**1.5**	0.6	0.72	0.8	0.7	**2.5**	**1.6**
12 mo	**1.1**	0.5	0.9	0.8	0.6	1.0	0.8
Bilirubin							
3 mo	9.6	6.4	**33**	14	5	**111**	9
12 mo	6	9	22	12	6	**27**	10
Evolution of the infection	acute	acute	acute	acute	acute	acute	acute
Immunosuppressive therapy							
Maintenance immunosuppression	TAC+ MMF	TAC+ MMF	TAC+ MMF	TAC+ MMF	TAC+ MMF	TAC+ MMF	TAC+ MMF
Switch			EVE+ MMF (4)		EVE+ MMF (3)		
(mo after LT)							
Rejection therapy	Yes, iv steroids	No	No	Yes, iv steroids	No	Yes, iv steroids	No
(mo after LT)	(1)			(6)		(2)	
Blood transfusion	71 units (0–1)	2 units (0–1)	8 units (0–1)	0 units	4 units (0–1)	5 units (0–1)	7 units (0–1)
(mo after LT)							

Bold indicates the values that are higher than the normal limit, the pathological values, or the positive anti-HEV antibodies.
♀, female; ♂, male; ArLD, alcohol-related liver disease; CC, cryptogenic cirrhosis; EVE, everolimus; HBV, hepatitis B virus; HCC, hepatocellular cancer; HCV, hepatitis C virus; HEV, hepatitis E virus; LT, liver transplantation; MMF, mycophenolate mofetil; nALT, normalized alanine aminotransferase; nAST, normalized aspartate aminotransferase; Pat, patient; TAC, tacrolimus.

Patients Acquiring Anti-HEV Antibodies Without Detectable HEV RNA

Five (4.6%) of the LT recipients acquired anti-HEV antibodies during the study period, indicative of HEV infection, but without detectable HEV RNA (Figure 2). One seroconverted to positive anti-HEV IgM at the 3-mo follow-up, whereas anti-HEV IgG was undetectable at all time-points. Two patients seroconverted to positive anti-HEV IgG at the

3-mo follow-up and additionally 1 at the 12-mo follow-up. None of these latter patients had detectable anti-HEV IgM antibodies in any sample. Furthermore, 1 patient with pre-LT anti-HEV IgG antibodies showed markedly increased levels of anti-HEV IgG at the 3-mo follow-up.

Description of the Patients With HEV Infection and Possible Risk Factors

In total, we identified 11 patients (7 of them were women) with markers of HEV infections post-LT and 1 pre-LT (characteristics detailed in Tables 2 and 3). Three recipients had received rejection therapy before their HEV infection, with IV methylprednisolone, and increased maintenance immunosuppression. Ten of our 12 patients received blood transfusion in proximity to the LT. Most transfusions were received perioperatively; none of the patients received blood transfusion the months before LT. In total, during the study period, the median number of units per patient was 5 (0, 0, 2, 4, 5, 5, 5, 5, 7, 8, 71, 132 units, respectively, for each patient) (Tables 2 and 3). Data on risk factors such as food consumption habits, for example, consumption of pork or game meat, or traveling abroad were not given in the medical records. When reviewing the medical records in retrospect, there were no remarkable symptoms or abnormal laboratory findings that could be specifically related to acute HEV infection for any of the patients.

DISCUSSION

This study showed a high prevalence of HEV infections during the first year after LT in Sweden, compared with studies from other regions.[11,12,14,21-26] The discrepancy can partly be explained by variations in geographical distribution and various genotypes of HEV but also by methodological differences, such as different assays and study designs. Still, other similar studies reported a lower HEV RNA prevalence of 1.15%[24] and an annual incidence of HEV infection of 4.8% among LT recipients.[28] An additional explanation to our relatively high prevalence may be the unbiased screening for HEV RNA in the present study. If HEV RNA screening had been limited to patients with abnormal ALT levels, 6 of 7 patients would have remained undetected. None presented overt signs or symptoms prompting HEV testing, and none were diagnosed in routine clinical care. Hence, there is a substantial risk for doctor's delay in the absence of protocol sampling and analysis. Our results indicate that unbiased screening for HEV RNA in samples from immunosuppressed patients enables identification of HEV infections that might otherwise be overlooked.

Surprisingly, none of the enrolled patients developed chronic infection; all spontaneously cleared viremia without treatment. Several studies have reported chronic HEV infections[1,12,22,27] with rates as high as 60% in transplant

TABLE 3.

Characteristics of liver transplant recipients acquiring anti-HEV antibodies, without detectable HEV RNA

Characteristic	Pat H	Pat I	Pat J	Pat K	Pat L
Sex, age (y)	♀ 57	♂ 66	♀ 71	♀ 56	♀ 51
Cause of LT	HCV	PSC	PSC, UC	HCC, HCV	PLD, cholangitis
Anti-HEV IgM/ IgG					
Pre-LT	IgM–	IgM–	IgM–	IgM–	IgM–
	IgG–	IgG–	IgG–	IgG–	**IgG+**
3 mo	**IgM+**	IgM–	IgM–	IgM–	IgM–
	IgG–	**IgG +**	IgG–	IgG–	**IgG+++**
12 mo	**IgM+**	IgM–	Ig M–	IgM–	IgM–
	IgG–	**IgG+**	**IgG+**	**IgG+**	**IgG+++**
HEV RNA					
Pre-LT	–	–	–	–	–
3 mo	–	–	–	–	–
12 mo	–	–	–	–	–
nALT (≤1)					
3 mo	**1.1**	0.7	0.7	0.3	0.4
12 mo	0.6	0.7	0.8	0.7	0.3
nAST (≤1)					
3 mo	**1.7**	1.0	0.8	**1.4**	0.5
12 mo	**1.1**	0.7	0.9	**1.5**	0.6
Bilirubin (5–25)					
3 mo	**120**	5	7	21	8
12 mo	**75**	6	6	13	8
Immunosuppressive therapy					
Maintenance immunosuppression	TAC+ MMF	TAC+ MMF+ prednisolone	TAC+ MMF	TAC+ MMF	TAC+ MMF
Switch, (mo after LT)	Single TAC (1)		EVE+ MMF (5)		
Rejection therapy	No	No	No	No	No
Blood transfusion (mo after LT)	104 units (0–1) 28 units (1–3)	No	1 unit (0–1) 4 units (11)	5 units (0–1)	5 units, (0–1)

Bold indicates the values that are higher than the normal limit, the pathological values, or the positive anti-HEV antibodies.
♀, female; ♂, male; EVE, everolimus; HCC, hepatocellular cancer; HCV, hepatitis C virus; HEV, hepatitis E virus; LT, liver transplantation; MMF, mycophenolate mofetil; nALT, normalized alanine aminotransferase; nAST, normalized aspartate aminotransferase; Pat, patient; PLD, polycystic liver disease; PSC, Primary sclerosing cholangitis; TAC, tacrolimus; UC, ulcerative colitis.

recipients.[5] The conflicting results with our study might partly be explained by differences in methodology with regard to sampling. Because immunosuppressed patients may be asymptomatic and often lack antibody responses despite HEV infections,[5,18,22] studies testing for HEV RNA only upon detection of anti-HEV IgM or ALT flares likely underestimate the frequency of silent, self-limiting infections. The long duration of viremia during chronic infections increases the likelihood of detecting HEV RNA; acute infections can pass unobserved with spontaneous viral clearance. Another explanation can be disparities regarding immunosuppressive therapy, which affects the risk of developing chronic HEV infection.[5] Higher risk has been demonstrated when treated with TAC, which all our patients received, than the relatively less immunosuppressive drug, cyclosporine. However, all patients also received mycophenolate mofetil, which has been shown to be associated with clearance of HEV infection[32] and inhibit HEV replication in cell culture.[33] More potent maintenance immunosuppression or need for rejection therapy could also affect the risk of developing a chronic infection. During the study period, the Gothenburg transplantation center had a less aggressive treatment strategy than many other centers in Europe and United States. Between 2010 and 2015, Gothenburg had a target TAC trough level that was significantly lower than many other centers, and since 2010, steroids were no longer included in the standard protocol (unless autoimmune hepatitis or primary sclerosing cholangitis was the indication of LT). The relatively mild immunosuppressive strategy can be a contributing factor explaining why our patients spontaneously cleared their HEV infection.

The seroprevalence of 13% of anti-HEV IgG pre-LT suggests considerable HEV exposure in Sweden among LT candidates. We found a significantly lower prevalence pre-LT among LT recipients than blood donor controls, with an age adjusted OR of 0.24. Possible causes could be that patients with liver failure often lack the ability to mount detectable antibody responses[34] or could be life style factors that entail reduced exposure to HEV compared with the general population, for example, dietary advice regarding avoidance of undercooked meat. Previous studies on anti-HEV IgG seroprevalence among LT recipients in Europe, Japan, and United States showed large variability and ranged from 2.9% to 42%.[11-20] In Swedish cohorts, fairly high-prevalence rates have been reported, with 30% among patients infected with HCV,[35] and 24% in patients admitted to surgical wards.[36] It is well established that seroprevalence varies geographically, but the differences in-between studies can also be explained by different serological assays.[9] The anti-HEV prevalence is known to increase with increasing age, which makes it difficult to compare studies if not adjusting for age. Because patients with liver failure or immunosuppressed patients often lack antibody responses,[34] performing serological monitoring alone is insufficient to detect HEV infections, and molecular techniques such as qPCR should be routinely performed on samples from these patients.

Transfusion transmission of HEV is prevalent globally.[7] In our study, 10 of 12 patients with markers of HEV infection received blood transfusion in proximity to the LT. To further analyze if those patients were transfusion-transmitted, the donated blood would need to be analyzed, which was not possible in this study. Some patients who acquired antibodies may have received passive antibodies from the blood donor. If the antibodies had been passively transfused, one would expect the antibody levels to fall and not be detectable within 12 mo. The patients who acquired anti-HEV antibodies in this study had the same level of antibodies at 3 and 12 mo of follow-up, indicating that they had a prior infection rather than passively acquired antibodies. Five patients acquired anti-HEV antibodies without detectable HEV RNA. It is possible that they had a recent short viremia that passed unobserved, and at the following sampling, HEV RNA has cleared spontaneously, and the patient has developed antibodies as a sign of recent infection. It is also possible that those results are unspecific, false-positive serological reactions. The timing of sampling in this study was chosen to coincide with the patients' routine scheduled clinical follow-up at the transplantation clinic during 1 y. Testing would have been more standardized if the baseline test was performed on the day of surgery, but we believed the risk of missing to draw a sample in this acute and sometimes stressful situation would be higher than during the LT evaluation. Waiting 9 mo between visits entails a risk that infections can pass unobserved in-between the follow-ups and that this study actually underestimated the true rate of HEV infections. With a more frequent sampling and an extended follow-up period, additional infections may have been identified. In addition, retrospective review of the medical records gave limited information on symptoms and potential risk factors, for example, dietary factors, although, reviewing the records, we noted episodes of increased liver enzymes and also unspecific symptoms, which unfortunately did not result in HEV RNA evaluation. However, these are very common among LT recipients and may be multifactorial in nature; therefore, this retrospective review of the medical records made it difficult to analyze whether increased liver enzymes or other symptoms were signs of HEV infection or were caused by other factors.

In conclusion, we observed that a substantial proportion of LT recipients in Sweden is at risk of acquiring HEV infection, both before and after LT. We found 7 LT patients with detectable HEV RNA, but surprisingly, none of them developed chronic infection. The HEV infections were discrete and spontaneously resolved without clinical intervention. The results in the present study highlight the frequency of silent, spontaneously resolving HEV infections but do not support universal screening of LT recipients in a Swedish cohort.

ACKNOWLEDGMENTS

We acknowledge the assistance of the nurses who recruited patients and collected blood samples: Christina Wibeck, Ingela Broman, and Marita Rosenberg. We acknowledge Marie Karlsson, who analyzed the samples at the laboratory. We thank Mattias Molin for statistical consultation.

REFERENCES

1. Kamar N, Mansuy JM, Cointault O, et al. Hepatitis E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. *Am J Transplant.* 2008;8:1744–1748.
2. Haagsma EB, van den Berg AP, Porte RJ, et al. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl.* 2008;14:547–553.
3. Gerolami R, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med.* 2008;358:859–860.
4. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med.* 2008;358:811–817.

5. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology.* 2011;140:1481–1489.
6. Behrendt P, Steinmann E, Manns MP, et al. The impact of hepatitis E in the liver transplant setting. *J Hepatol.* 2014;61:1418–1429.
7. Kamar N, Izopet J, Pavio N, et al. Hepatitis E virus infection. *Nat Rev Dis Primers.* 2017;3:17086.
8. Ma Z, de Man RA, Kamar N, et al. Chronic hepatitis E: advancing research and patient care. *J Hepatol.* 2022;77:1109–1123.
9. Norder H, Karlsson M, Mellgren A, et al. Diagnostic performance of five assays for anti-HEV IgG and IgM in a large cohort study. *J Clin Microbiol.* 2016;54:549–555.
10. Waldenstrom J, Castedal M, Konar J, et al. Chronic hepatitis E infection with an emerging virus strain in a heart transplant recipient successfully treated with ribavirin: a case report. *J Med Case Rep.* 2015;9:180.
11. Inagaki Y, Oshiro Y, Tanaka T, et al. A nationwide survey of hepatitis E virus infection and chronic hepatitis E in liver transplant recipients in Japan. *EBioMedicine.* 2015;2:1607–1612.
12. Haagsma EB, Niesters HG, van den Berg AP, et al. Prevalence of hepatitis E virus infection in liver transplant recipients. *Liver Transpl.* 2009;15:1225–1228.
13. Buti M, Cabrera C, Jardi R, et al. Are recipients of solid organ transplantation a high-risk population for hepatitis E virus infection? *Liver Transpl.* 2010;16:106–7; author reply 108.
14. Pischke S, Suneetha PV, Baechlein C, et al. Hepatitis E virus infection as a cause of graft hepatitis in liver transplant recipients. *Liver Transpl.* 2010;16:74–82.
15. Pisano MB, Balderramo D, Wassaf MM, et al. Hepatitis E virus infection in patients on dialysis and in solid organ transplant recipients in Argentina: exploring associated risk factors. *Arch Virol.* 2017;162:787–792.
16. Riveiro-Barciela M, Buti M, Homs M, et al. Cirrhosis, liver transplantation and HIV infection are risk factors associated with hepatitis E virus infection. *PLoS One.* 2014;9:e103028.
17. Mrzljak A, Dinjar-Kujundzic P, Vilibic-Cavlek T, et al. Hepatitis E seroprevalence and associated risk factors in Croatian liver transplant recipients. *Rev Soc Bras Med Trop.* 2019;52:e20190302.
18. Buffaz C, Scholtes C, Dron AG, et al. Hepatitis E in liver transplant recipients in the Rhone-Alpes region in France. *Eur J Clin Microbiol Infect Dis.* 2014;33:1037–1043.
19. Koning L, Charlton MR, Pas SD, et al. Prevalence and clinical consequences of hepatitis E in patients who underwent liver transplantation for chronic hepatitis C in the United States. *BMC Infect Dis.* 2015;15:371.
20. Sherman KE, Terrault N, Barin B, et al.; HIV-TR Investigators. Hepatitis E infection in HIV-infected liver and kidney transplant candidates. *J Viral Hepat.* 2014;21:e74–e77.
21. Soothill G, Hessey S, Erotocritou M, et al. Diagnostic utility of hepatitis E virus antigen-specific ELISA versus PCR testing in a cohort of post liver transplant patients in a large university hospital. *J Clin Virol.* 2018;106:44–48.
22. Pas SD, de Man RA, Mulders C, et al. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. *Emerg Infect Dis.* 2012;18:869–872.
23. Ankcorn MJ, Ijaz S, Poh J, et al. Toward systematic screening for persistent hepatitis E virus infections in transplant patients. *Transplantation.* 2018;102:1139–1147.
24. Reekie I, Irish D, Ijaz S, et al. Hepatitis E infection in stem cell and solid organ transplantpatients: a cross-sectional study: the importance of HEV RNA screening in peri-transplant period. *J Clin Virol.* 2018;107:1–5.
25. Sinakos E, Gioula G, Liava C, et al. Prevalence of hepatitis E in liver transplant recipients in Greece. *Epidemiol Infect.* 2018;146:1619–1621.
26. Galante A, Pischke S, Polywka S, et al. Relevance of chronic hepatitis E in liver transplant recipients: a real-life setting. *Transpl Infect Dis.* 2015;17:617–622.
27. Abravanel F, Lhomme S, Chapuy-Regaud S, et al. Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. *J Infect Dis.* 2014;209:1900–1906.
28. Legrand-Abravanel F, Kamar N, Sandres-Saune K, et al. Hepatitis E virus infection without reactivation in solid-organ transplant recipients, France. *Emerg Infect Dis.* 2011;17:30–37.
29. Norder H, Sundqvist L, Magnusson L, et al. Endemic hepatitis E in two Nordic countries. *Euro Surveill.* 2009;14:19211.
30. Roth A, Lin J, Magnius L, et al. Markers for ongoing or previous hepatitis E virus infection are as common in wild ungulates as in humans in Sweden. *Viruses.* 2016;8:259.
31. Kamar N, Rostaing L, Legrand-Abravanel F, et al. How should hepatitis E virus infection be defined in organ-transplant recipients? *Am J Transplant.* 2013;13:1935–1936.
32. Pischke S, Stiefel P, Franz B, et al. Chronic hepatitis E in heart transplant recipients. *Am J Transplant.* 2012;12:3128–3133.
33. Wang Y, Zhou X, Debing Y, et al. Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. *Gastroenterology.* 2014;146:1775–1783.
34. Andersson D, Castedal M, Friman V. Are liver transplant recipients protected against hepatitis A and B? *Transplant Proc.* 2013;45:1193–1197.
35. Mellgren A, Karlsson M, Karlsson M, et al. High seroprevalence against hepatitis E virus in patients with chronic hepatitis C virus infection. *J Clin Virol.* 2017;88:39–45.
36. Karlsson M, Norder H, Bergstrom M, et al. Hepatitis E virus genotype 3 is associated with gallstone-related disease. *Scand J Gastroenterol.* 2019;54:1269–1273.