Digestive and Liver Disease 57 (2025) 378–384

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Hepatitis E virus infection in immunosuppressed patients and its clinical manifestations

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ARTICLE INFO

Article history:
Received 6 May 2024
Accepted 24 June 2024
Available online 14 July 2024

Keywords:
Hepatitis E virus
Immunosuppression
Transplantation

ABSTRACT

Background & Aims: Hepatitis E virus (HEV) is a main cause of acute hepatitis globally. However, immunosuppressed patients regularly develop chronic courses. The aim of this study was to analyse the current status of HEV diagnostics, characterize clinical manifestations and identify risk factors for complicated HEV infections.

Methods: In this retrospective study at two large hospitals, 512 patients with borderline and positive anti-HEV-IgM and 94 patients with positive HEV-PCR between January 1999 and May 2023 were included.

Results: Detection by anti-HEV-IgM-ELISA led to a positive HEV-PCR in only 17.9 %. Amongst patients with positive HEV-PCR, 61 had underlying immunosuppression and 23 were patients after solid organ transplantation (SOT). All 13 patients with chronic HEV infections were immunosuppressed. Generally, immunosuppression led to higher HEV-RNA concentrations and a higher probability of receiving immediate treatment. However, all fulminant courses with liver failure happened in patients without immunosuppression. Immunocompetent patients showed symptoms more frequently and primarily had higher bilirubin levels indicating more severe liver damage. A risk factor for delayed or failed viral clearance after SOT was the administration of mTOR inhibitors.

Conclusions: Fulminant HEV infections happen primarily in immunocompetent patients. Nevertheless, immunosuppressed patients bear the risk of undetected, prolonged HEV infections, reflected by the rare occurrence of symptoms.

1. Introduction

The hepatitis E virus (HEV) typically causes a short, self-limiting acute hepatitis. Particularly in acute infections caused by HEV genotypes 1 and 2, fulminant disease progression might occur, which can lead to acute liver failure and to a mortality rate of up to 30 % in pregnant women [1,2]. In industrialized countries, HEV genotype 3 is more prevalent and, unlike genotypes 1 and 2, is primarily not transmitted via the faecal-oral route [3]. It usually follows a zoonotic transmission mainly through consumption of undercooked meat products [4]. In patients with impaired immunocompetence, an HEV infection (particularly with genotype 3) can lead to a chronic infection due to unsuccessful viral clearance, which increases the risk to develop liver fibrosis and in consequence liver cirrhosis [4]. Therefore, it is essential to screen for HEV in immunocompromised patients presenting with elevated liver transaminases. HEV-NAT (e.g. PCR) should be performed, as detectable antibody responses might show insufficient sensitivity in immunosuppressed patients [5].

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https://doi.org/10.1016/j.dld.2024.06.020

According to the EASL guidelines [6], a conservative treatment approach is recommended in less severe cases. In patients with persistent infection or liver failure, a cautious reduction of immunosuppression and additional administration of ribavirin should be considered. Ribavirin is a drug combining direct antiviral with immunomodulatory capacities [7,8], which ultimately leads to a viral clearance in 85–90 % in those patients [6,7,9]. However, in patients after solid organ transplantation (SOT), who represent the majority of patients with persistent infection [10], treatment should be introduced with great caution because any change in immunosuppression comes with an elevated risk of acute transplant rejection. Ribavirin can also cause serious side effects like hemolytic anemia [4,7], which necessitates a prompt reduction in dosage or drug discontinuation.

In this study, a comprehensive analysis of all cases with hepatitis E virus infection over a period of almost 25 years was conducted at two supraregional hospital centers. We aimed to optimize the identification of HEV infections in immunocompromised patients and to identify characteristics for high-risk disease progression.

2. Materials and methods

2.1. Study population, design and aims

We included all patients in our retrospective study that had borderline and positive anti-HEV-IgM and/or positive HEV-PCR results between January 1999 and May 2023 at the University Hospital Regensburg (with a dedicated organ transplant program) and the Hospital Ingolstadt. Detailed analyses were then conducted on all patients who had the HEV infection confirmed by HEV-PCR.

The primary aim of the study was to optimize the identification of patients with HEV infection by analysing HEV diagnostics and clinical manifestations. The secondary aim was to identify high-risk constellations for complicated HEV infections.

All data collection was performed using the clinics' patient databases. Each patient was treated according to local standards. Initially, HEV diagnostics were analysed. Next, HEV-PCR positive patients were categorised according to their underlying diseases and possible immunosuppression. Clinical information was then collected before and during the infection course, with collection of additional follow-up patient data.

The retrospective study was authorized by the Ethics Committee of the University of Regensburg (approval number 19–1570_2–101).

2.2. HEV diagnostics and sequencing

HEV diagnostics were performed in clinical practice as determined by the treating physician at the respective study centre, usually due to disturbed liver parameters following liver-related symptoms or as part of the regular follow-up in patients with immunosuppression, always in accordance with the current guidelines.

For anti-HEV-IgM and anti-HEV-IgG, immunoblot and ELISA kits from Mikrogen (Neuried, Germany) were used at the University Hospital Regensburg and the Hospital Ingolstadt used ELISA kits from EUROIMMUN (Lübeck, Germany). HEV-PCR from serum, plasma or faeces samples and HEV sequencing were performed as recently described [11]. In 2016, the RT-qPCR protocol was adapted. Both protocols show comparable sensitivity with a 95 %-LoD of 1758 IU/ml (3000 copies/ml) [12] and 1214 IU/ml (1400 copies/ml) [13,14].

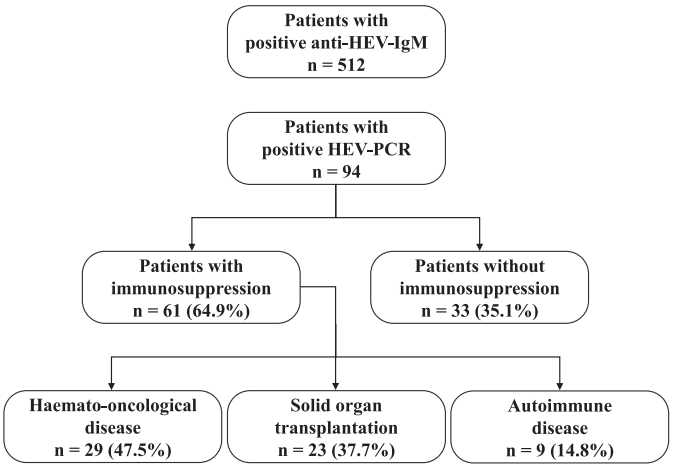

Fig. 1. Composition of the study population regarding immunosuppression.

2.3. Case definitions

The different disease courses and aspects of HEV infection were defined as the following:

- Chronic HEV infection: detectable HEV-RNA for at least 12 weeks.
- Recurrent HEV infection: detectable HEV-RNA after at least one negative HEV-PCR.
- Fulminant HEV infection: medical treatment on an intensive care unit due to liver failure with positive HEV-RNA and no other leading cause.
- HEV-related liver cirrhosis: proof of liver cirrhosis in patients with chronic HEV infection without any other liver disease present prior to diagnosis or during the course of HEV infection.
- Acute HEV infection, lost to follow-up: positive HEV-PCR for less than 12 weeks without a negative HEV-PCR.
- Chronic HEV infection, lost to follow-up: positive HEV-PCR for at least 12 weeks without a negative HEV-PCR.
- Follow-up: interval from first positive HEV-PCR to last documented visit in the clinic.

2.4. Statistical analysis

For statistical analysis, GraphPad Prism v10 (Boston, USA) and IBM SPSS v29 (Armonk, USA) were used. Statistical tests were performed as indicated in the respective figure and table legends. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Distribution of the study population

Between January 1999 and May 2023, borderline and positive anti-HEV-IgM was detected in 512 patients by ELISA or immunoblot. In a total of 94 patients, the infection was detected by HEV-PCR, who were then included in further analyses.

As shown in Fig. 1, 61 HEV-PCR positive patients (64.9 %) were on some form of immunosuppression and 33 patients (35.1 %) were immunocompetent. Patients with immunosuppression were further subdivided into 29 patients (47.5 %) with a hemato-oncological disease, 23 patients (37.7 %) after SOT and nine patients (14.8 %) with autoimmune disease.

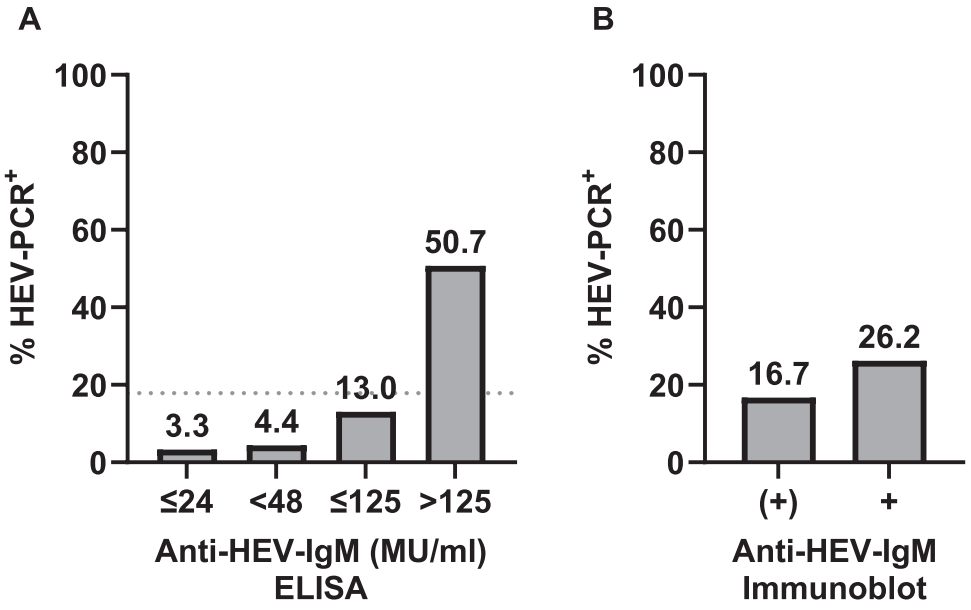

Fig. 2. Percentage of positive HEV-PCR in patients with borderline and positive anti-HEV-IgM. (A) 310 patients with borderline and positive anti-HEV-IgM-ELISA had the HEV infection tested by HEV-PCR. Breakdown in the ELISA categories: borderline (20 to 24 MU/ml), weakly positive (>24 to <48 MU/ml), positive (48 to 125 MU/ml) and positive above detection range (>125 MU/ml). The dashed line indicates the percentage of all positive results combined (17.9 %). MU = Mikrogen Units. (B) Patients with borderline and positive anti-HEV-IgM-Immunoblot. (+) = anti-HEV-IgM-borderline, + = anti-HEV-IgM-positive.

3.2. Accuracy of HEV infection diagnosis by anti-HEV-IgM-ELISA

In total (see Fig. 2A), 310 patients were tested by HEV-PCR to verify a borderline or positive anti-HEV-IgM-ELISA result. When higher anti-HEV-IgM concentrations were measured by ELISA, HEV-PCR positive results became more likely. Of the patients with borderline anti-HEV-IgM, 3.3 % had a positive HEV-PCR. In patients with weakly positive versus positive anti-HEV-IgM levels, the HEV-PCR positive percentages were 4.4 % and 13.0 %, respectively. In patients with positive anti-HEV-IgM above the detection range, 50.7 % had a positive HEV-PCR. Across all subgroups, the percentage was 16.5 %. After exclusion of borderline results, HEV-PCR was positive in 17.9 %.

Before the anti-HEV-IgM-ELISA was available, patients were tested by immunoblot. Here, borderline positive and positive results led to a detection by HEV-PCR in 16.9 % and 26.2 % of cases, respectively (see Fig. 2B).

3.3. General characteristics of patients with positive HEV-PCR

Comparing immunocompetent patients to patients with immunosuppression (see Table 1), no significant differences in sex, age, body mass index, underlying liver disease or diabetes mellitus were observed. As expected in Germany, of the 39 patients with genotyped HEV infection, 97.4 % were infected with genotype 3; only one patient showed an infection with genotype 1 after travelling from Pakistan. Remarkably, patients with immunosuppression were significantly more likely to be infected with HEV subtype 3c (63.6 % to 92.9 %, $p = 0.0423$) and revealed an HEV-RNA concentration at initial diagnosis that was nearly 50 times higher (3.0×10^4 to 1.4×10^6 copies/ml, $p < 0.0001$).

When HEV infection was diagnosed, immediate treatment was initiated in 15.2 % of immunocompetent patients, compared to 59.0 % of immunosuppressed patients ($p < 0.0001$). There was also a significant difference in the development of chronic infections which, as expected, all occurred in immunosuppressed patients. More than one in five patients could not clear the infection within 12 weeks (0.0 % to 21.3 %, $p = 0.0034$). In contrast, fulminant courses of the infection with severe hepatitis and liver failure only occurred in immunocompetent patients (9.1 % to 0.0 %, $p = 0.0407$). There was no significant difference in the development of recurrences, however, all five patients were immunosuppressed (0.0 % to 8.2 %, $p = 0.1582$). All recurrent episodes showed lower HEV-RNA concentrations and transaminases compared to the initial infection. An HEV infection-related liver cirrhosis was only evident in one immunosuppressed patient with a long-lasting chronic infection (0.0 % to 1.6 %, $p = 1.0000$). As liver cirrhosis and positive HEV-PCR were diagnosed, stored samples from the clinic's archive were retrospectively tested. Here, HEV-PCR positivity was detectable over a course of 6 years. No other reason for chronic liver damage was identified. Follow-up was shorter in immunocompetent patients compared to immunosuppressed patients (655 to 1343 days, $p = 0.0001$). Additionally, the percentage of patients lost to follow-up was higher (15.2 % to 1.6 %, $p = 0.0191$).

3.4. Clinical manifestations at detection of HEV infection

A comparison of symptoms reported by patients when diagnosed with HEV infection showed major differences between immunocompetent and immunosuppressed patients (see Table 2). In total, only 24.2 % of the immunocompetent patients described no symptoms at all compared to 73.8 % in immunosuppressed patients ($p < 0.0001$). The most common symptoms stated were fatigue (29.8 %), abdominal pain (12.8 %), darkened urine (12.8 %) and jaundice (11.7 %), with statistically significant differences between the two groups in reporting fatigue (54.5 % to 16.4 %, $p = 0.0002$), abdominal pain (24.2 % to 6.6 %, $p = 0.0221$), darkened urine (36.4 % to 0.0 %, $p < 0.0001$), jaundice (30.3 % to 1.6 %, $p < 0.0001$), pale stools (24.2 % to 0.0 %, $p = 0.0001$), pruritus (15.2 % to 1.6 %, $p = 0.0191$) and muscular pain (12.1 % to 0.0 %, $p = 0.0134$).

As shown in Table 2, immunocompetent HEV-PCR positive patients presented with anti-HEV-IgM positive results 90.0 % of the time, compared to only 75.7 % in immunosuppressed patients ($p = 0.2008$). Anti-HEV-IgG were positive in 71.6 % of all patients (76.7 % to 67.6 %, $p = 0.4317$). Immunosuppressed patients presented more frequently with a reduced leukocyte count (7.0/nl to 5.5/nl, $p = 0.0022$) and lower haemoglobin levels (13.5 g/dl to 12.4 g/dl, $p = 0.0158$), whereas immunocompetent patients

Table 1
General patient characteristics of the study population. Hepatic steatosis was graded by ultrasound. Immunocompetent and immunosuppressed patients were compared using Fisher's Exact Test or Mann-Whitney Test.

	Total (n = 94)	Immunocompetent (n = 33)	Immunosuppressed (n = 61)	p-value
Sex ♂ / ♀	67 / 27	25 / 8	42 / 19	0.6337
Age				
total, mean (95% CI)	48.0 (44.4 - 51.6)	49.0 (43.2 - 54.9)	47.5 (42.8 - 52.1)	0.9733
< 19, n (%)	8 (8.5)	2 (6.1)	6 (9.8)	0.7084
19-40, n (%)	21 (22.3)	9 (27.3)	12 (19.7)	0.4424
41-60, n (%)	41 (43.6)	13 (39.4)	28 (45.9)	0.6638
> 60, n (%)	24 (25.5)	9 (27.3)	15 (24.6)	0.8075
BMI, mean kg/m^2 (95% CI)	26.2 (24.9 - 27.5)	27.8 (25.7 - 29.9)	25.4 (23.9 - 27.0)	0.0823
Underlying liver disease, n (%)	34 (36.2)	9 (27.3)	25 (41.0)	0.2611
Hepatic steatosis				
none, n (%)	36 (47.4)	15 (51.7)	21 (44.7)	0.6387
mild, n (%)	12 (15.8)	3 (10.3)	9 (19.1)	0.3541
moderate, n (%)	17 (22.4)	5 (17.2)	12 (25.5)	0.5721
severe, n (%)	11 (14.5)	6 (20.7)	5 (10.6)	0.3159
not determined, n	18	4	14	
Diabetes mellitus, n (%)	18 (19.1)	6 (18.2)	12 (19.7)	1.0000
HEV genotype				
1a, n (%)	1 (2.6)	1 (9.1)	0 (0.0)	0.2821
3a, n (%)	2 (5.1)	1 (9.1)	1 (3.6)	0.4899
3c, n (%)	33 (84.6)	7 (63.6)	26 (92.9)	**0.0423**
3f, n (%)	1 (2.6)	1 (9.1)	0 (0.0)	0.2821
3 (other), n (%)	2 (5.1)	1 (9.1)	1 (3.6)	0.4899
not determined, n	55	22	33	
HEV titre, median copies/ml (IQR)	4.9 x 10^5 (1.6 x 10^4 - 8.7 x 10^6)	3.0 x 10^4 (3.1 x 10^3 - 3.3 x 10^5)	1.4 x 10^6 (2.8 x 10^5 - 1.7 x 10^7)	**< 0.0001**
Treatment initiated at diagnosis, n (%)	41 (43.6)	5 (15.2)	36 (59.0)	**< 0.0001**
Recurrence of HEV infection, n (%)	5 (5.3)	0 (0.0)	5 (8.2)	0.1582
HEV-related liver cirrhosis, n (%)	1 (1.1)	0 (0.0)	1 (1.6)	1.0000
Chronic HEV infection, n (%)	13 (13.8)	0 (0.0)	13 (21.3)	**0.0034**
Fulminant HEV infection, n (%)	3 (3.2)	3 (9.1)	0 (0.0)	**0.0407**
Days of follow-up, mean (95% CI)	1101 (850 - 1353)	655 (322 - 988)	1343 (1009 - 1677)	**0.0001**
Lost to follow-up, n (%)	6 (6.4)	5 (15.2)	1 (1.6)	**0.0191**

Table 2
Symptoms reported and laboratory parameters at initial diagnosis of HEV infection. Immunocompetent and immunosuppressed patients were compared using Fisher's Exact Test or Mann-Whitney Test.

	Total (n = 94)	Immunocompetent (n = 33)	Immunosuppressed (n = 61)	p-value
Symptoms reported				
Fatigue, n (%)	28 (29.8)	18 (54.5)	10 (16.4)	**0.0002**
Abdominal pain, n (%)	12 (12.8)	8 (24.2)	4 (6.6)	**0.0221**
Darkened urine, n (%)	12 (12.8)	12 (36.4)	0 (0.0)	**< 0.0001**
Jaundice, n (%)	11 (11.7)	10 (30.3)	1 (1.6)	**< 0.0001**
Loss of appetite, n (%)	9 (9.6)	6 (18.2)	3 (4.9)	0.0620
Nausea/vomiting, n (%)	8 (8.5)	4 (12.1)	4 (6.6)	0.4450
Pale stools, n (%)	8 (8.5)	8 (24.2)	0 (0.0)	**0.0001**
Pruritus, n (%)	6 (6.4)	5 (15.2)	1 (1.6)	**0.0191**
Fever, n (%)	6 (6.4)	3 (9.1)	3 (4.9)	0.6616
Muscular pain, n (%)	4 (4.3)	4 (12.1)	0 (0.0)	0.0134
Arthralgia, n (%)	2 (2.1)	2 (6.1)	0 (0.0)	0.1208
Rash, n (%)	1 (1.1)	1 (3.0)	0 (0.0)	0.3511
None, n (%)	53 (56.4)	8 (24.2)	45 (73.8)	< 0.0001
Laboratory parameters				
Anti-HEV-IgG, % positive	71.6	76.7	67.6	0.4317
Anti-HEV-IgM, % positive	82.1	90.0	75.7	0.2008
AST, median U/l (IQR)	124 (59 - 387)	182 (62 - 492)	111 (55 - 339)	0.4106
ALT, median U/l (IQR)	281 (95 - 649)	489 (100 - 961)	232 (92 - 476)	0.1149
Gamma-GT, median U/l(IQR)	195 (95 - 329)	169 (85 - 302)	222 (98 - 380)	0.6335
Alkaline phosphatase, median U/l (IQR)	147 (111 - 230)	138 (97 - 181)	168 (112 - 261)	0.3274
Cholinesterase, median U/l (IQR)	8103 (5682 - 11325)	6594 (5672 - 11022)	8774 (5598 - 11633)	0.2557
Bilirubin (total), median mg/dl (IQR)	0.8 (0.5 - 1.7)	1.4 (0.8 - 9.5)	0.6 (0.4 - 1.2)	**0.0001**
Bilirubin (direct), median mg/dl (IQR)	0.4 (0.3 - 1.6)	1.0 (0.3 - 8.8)	0.4 (0.2 - 0.6)	**0.0036**
Albumin, median g/l (IQR)	36.3 (32.1 - 41.3)	35.6 (27.5 - 40.9)	37.1 (33.6 - 41.7)	0.1948
Creatinine, median mg/dl (IQR)	0.90 (0.75 - 1.32)	0.83 (0.71 - 1.08)	0.91 (0.75 - 1.46)	0.2561
CRP, median mg/l (IQR)	4.3 (2.9 - 12.3)	5.1 (2.9 - 22.4)	3.9 (2.9 - 11.9)	0.4116
Leukocytes, median /nl (IQR)	6.1 (4.7 - 8.0)	7.0 (6.0 - 8.5)	5.5 (3.5 - 7.4)	**0.0022**
Haemoglobin, median g/dl (IQR)	12.7 (11.4 - 13.8)	13.5 (12.1 - 15.1)	12.4 (11.2 - 13.5)	**0.0158**
Thrombocytes, median /nl (IQR)	176 (119 - 247)	205 (114 - 264)	163 (122 - 231)	0.3133
INR, median (IQR)	1.01 (0.96 - 1.17)	1.03 (0.93 - 1.22)	1.01 (0.96 - 1.11)	0.7312
PTT, median s (IQR)	30.9 (28.0 - 36.8)	31.5 (28.0 - 39.8)	30.8 (28.0 - 35.3)	0.5712

Table 3
Comparison of acute and chronic HEV infected patients with immunosuppression. Statistical significance was tested by Fisher's Exact Test or Mann-Whitney Test.

	Total (n = 61)	Acute (n = 48)	Chronic (n = 13)	p-value
Origin of immunosuppression				
Solid organ transplantation, n (%)	23 (37.7)	15 (31.3)	8 (61.5)	0.0586
Haemato-oncological disease, n (%)	29 (47.5)	25 (52.1)	4 (30.8)	0.2192
Autoimmune disease, n (%)	9 (14.8)	8 (16.7)	1 (7.7)	0.6688
Median HEV, copies/ml (IQR)	1.4 x 10^6 (2.8 x 10^5 - 1.7 x 10^7)	1.1 x 10^6 (7.6 x 10^4 - 1.5 x 10^7)	7.6 x 10^6 (8.3 x 10^5 - 6.4 x 10^7)	0.0863
Treatment initiated at diagnosis				
None, n (%)	25 (41.0)	22 (45.8)	3 (23.1)	0.2060
Change of immunosuppression only, n (%)	3 (4.9)	3 (6.3)	0 (0.0)	0.5926
Ribavirin only, n (%)	18 (29.5)	14 (29.2)	4 (30.8)	1.0000
Change of immunosuppression and ribavirin, n (%)	15 (24.6)	9 (18.8)	6 (46.2)	0.0670

showed markedly higher levels of total bilirubin (1.4 mg/dl to 0.6 mg/dl, $p = 0.0001$) and direct bilirubin (1.0 mg/dl to 0.4 mg/dl, $p = 0.0036$).

3.5. Comparison of acute and chronic HEV infections in immunosuppressed patients

In total, 13 patients (21.3 % of immunosuppressed patients; 13.8 % of all patients) developed a chronic infection course (Table 3). The origin of immunosuppression in patients who developed chronic courses missed statistical significance, but there was a clear accumulation of patients after SOT (31.3 % to 61.5 %, $p = 0.0586$). Patients with chronic infections had an HEV-RNA concentration in blood that was almost seven times higher at initial diagnosis (1.1×10^6 to 7.6×10^6, $p = 0.0863$).

The therapy initiated at the time of diagnosis of HEV infection was similar in both groups. Immunosuppression was reduced in 4.9 % of the patients, whereas 29.5 % received ribavirin. 24.6 % received a combination of both treatment options and 41.0 % initially did not receive any treatment.

Further breakdown of the patients by type of SOT showed 11 patients (47.8 %) with a liver transplant, seven patients (30.4 %) with kidney transplants, two patients (8.7 %) with a history of both liver and kidney transplantation and three patients (13.0 %) with a heart transplant (see Table 4). Regarding the development of a chronic HEV infection, there were no significant differences in the type of SOT or in the days between infection and SOT. When comparing the respective immunosuppressive regimes, there were no differences concerning mycophenolate mofetil, calcineurin inhibitors and prednisolone, whereas SOT patients that developed a chronic HEV infection were more frequently on an mTOR inhibitor-based regime (0.0 % to 37.5 %, $p = 0.0316$). The initial treatment strategy with diagnosis of HEV infection or the daily dose of ribavirin did not affect the development of chronic disease courses, with three patients (13.0 %) receiving no treatment at all, 12 (52.2 %) receiving a reduction in immunosuppression and 19 (82.6 %) starting ribavirin treatment at the beginning.

4. Discussion

The current opinion is that HEV infections with genotypes 3 and 4 mainly impact patients with impaired immunocompetence who are predisposed to prolonged or complicated courses of infection [3]. The results of our bicentric study provide helpful insights into the optimization of diagnostic screening in immunocompromised patients and indicate that liver damage caused by unrestricted immunity should not be underestimated in daily clinical care.

As expected, most patients of the study cohort showed an infection with HEV genotype 3, with the exception of one patient who had previously spent extended time in Pakistan and whose infection was typed as HEV subtype 1a. In that healthy and immunocompetent patient, the infection was quickly resolved. Further analysis of genotype 3 infections revealed that in 84.6 % of cases the infection was based on subtype 3c, which was identified more frequently in immunocompromised patients. There is still disagreement about the exact correlation between the subtype of genotype 3 and clinical infection course, although some studies have shown a more severe course and higher mortality in infections caused by HEV group 1 (subtypes 3efg) compared to HEV group 2 (subtypes 3abchijklm) [11,15].

It is important to emphasize once again the significance of comprehensive diagnostics concerning the frequently underestimated HEV infection when a patient presents with elevated liver transaminases. Especially in immunosuppressed patients, virus detection by NAT should always be attempted, as anti-HEV-IgM was not positive in nearly one-fourth of the patients in our study. Furthermore, confirmation of active infection in patients with positive anti-HEV-IgM succeeded in only 17.9 %, proving both sensitivity and specificity of antibody diagnostics for the detection may be considerably limited in certain patient cohorts, as previously discussed [5].

It is crucial that a test algorithm is reliably implemented to rapidly identify patients infected with HEV. As our study shows, patients often present with unspecific symptoms such as fatigue or abdominal pain, and more than half of them describe no irregularities at all. Signs of parenchymatous damage such as jaundice and changes in urine and stool colour occur in more than 10 % of patients, although this is distributed very inhomogeneously between immunosuppressed and immunocompetent patients. Around one in three patients without impaired immunity presented with signs of intrahepatic damage, compared with only 1.6 % of immunocompromised patients, in whom around three in four did not even describe any symptoms.

Our results therefore indicate that HEV-infected immunocompetent patients suffer significant liver damage. This is also supported by the finding that immunocompetent patients show significantly higher bilirubin levels and that all registered liver failures occurred in patients with unrestricted immunocompetence. It should therefore be emphasized that acute liver damage occurs mainly in immunocompetent patients, which also supports the assumption that the HEV itself is not cytopathic and that the damage occurs mainly due to the antiviral immune response [2,16–18].

Of interest, therapy at diagnosis of HEV infection was started at a higher frequency than expected. 15.2 % of all immunocompetent patients received initial therapy with ribavirin. In immunosuppressed patients, the percentage of immediate therapy was 59.0 %. Thereof, 4.9 % underwent an adjustment of immunosuppression only, while 29.5 % received ribavirin and 24.6 % a reduction of immunosuppression in combination with ribavirin. These results underline the importance of interpreting the recommendations of the current EASL guidelines [6] in the overall context, as HEV frequently occurs in patients with severe comorbidities and therefore a wait-and-see approach is often not applicable.

Table 4
Risk factors for chronic HEV infections in patients after solid organ transplantation (SOT). Statistical significance was tested by Fisher's Exact Test or Mann-Whitney Test.

	Total (n = 23)	Acute (n = 15)	Chronic (n = 8)	p-value
Type of SOT				
Liver, n (%)	11 (47.8)	8 (53.3)	3 (37.5)	0.6668
Kidney, n (%)	7 (30.4)	4 (26.7)	3 (37.5)	0.6570
Liver + Kidney, n (%)	2 (8.7)	2 (13.3)	0 (0.0)	0.5257
Heart, n (%)	3 (13.0)	1 (6.7)	2 (25.0)	0.5257
Days since SOT, mean (95% CI)	1707 (1001 - 2412)	1745 (884 - 2606)	1634 (64 - 3204)	0.7284
Immunosuppressive agents at infection				
Mycophenolat mofetil, n (%)	19 (82.6)	14 (93.3)	5 (62.5)	0.1028
Calcineurin inhibitors, n (%)	22 (95.7)	15 (100.0)	7 (87.5)	0.3478
Tacrolimus, n (%)	19 (82.6)	12 (80.0)	7 (87.5)	1.0000
Ciclosporin A, n (%)	3 (13.0)	3 (20.0)	0 (0.0)	0.2885
mTOR inhibitors, n (%)	3 (13.0)	0 (0.0)	3 (37.5)	**0.0316**
Everolimus, n (%)	2 (8.7)	0 (0.0)	2 (25.0)	0.1107
Sirolimus, n (%)	1 (4.3)	0 (0.0)	1 (12.5)	0.3478
Prednisolone, n (%)	17 (73.9)	11 (73.3)	6 (75.0)	1.0000
Daily dose in mg				
Mycophenolat mofetil, mean (95% CI)	1498 (1050 - 1946)	1490 (882 - 2098)	1520 (865 - 2175)	0.9820
Tacrolimus, mean (95% CI)	4.71 (2.30 - 7.12)	4.77 (1.15 - 8.40)	4.58 (1.53 - 7.63)	0.5081
Prednisolone, mean (95% CI)	7.09 (4.02 - 10.16)	7.68 (3.18 - 12.18)	6.00 (1.08 - 10.92)	0.5897
Treatment initiated				
None, n (%)	3 (13.0)	2 (13.3)	1 (12.5)	1.0000
Reduction in Immunosuppresion, n (%)	12 (52.2)	7 (46.7)	5 (62.5)	0.6668
Ciclosporin A, n (%)	1 (4.3)	1 (6.7)	0 (0.0)	1.0000
Mycophenolat mofetil, n (%)	7 (30.4)	5 (33.3)	2 (25.0)	1.0000
Prednisolon, n (%)	3 (13.0)	2 (13.3)	1 (12.5)	1.0000
Tacrolimus, n (%)	3 (13.0)	1 (6.7)	2 (25.0)	0.5257
Everolimus, n (%)	2 (8.7)	0 (0.0)	2 (25.0)	0.1107
Ribavirin, n (%)	19 (82.6)	12 (80.0)	7 (87.5)	1.0000
Daily dose in mg				
Ribavirin, mean (95% CI)	552.9 (375.7 - 730.2)	591.7 (348.7 - 834.7)	460.0 (161.0 - 759.0)	0.7017

There were no specific risk factors for the development of chronic infections in our study population apart from immunosuppression and an almost seven-fold higher HEV-RNA concentration at initial diagnosis, which failed to reach statistical significance. Neither patient characteristics, the underlying immunosuppression nor the therapy initiated affected the time to clear the virus. A subgroup analysis of patients after SOT, who account for the majority of chronic HEV infections, showed that the use of mTOR inhibitors is a risk factor for a chronic infection, which is consistent with previous studies [19–21]. Of note, the time from transplantation to diagnosis was relatively high compared to existing studies. In our study, a mean of over 140 months was observed, in contrast to 74 months in the recent study by Kamar et al. [7], possibly caused by our high rate of liver transplant recipients, which usually receive a lower dosed immunosuppression compared to other SOT patients. Importantly, only five patients developed a relapse and the recurrent episode had a weaker clinical course in all patients. Notably also, all patients with recurrent infection were immunocompromised and the presence of anti-HEV-IgG did not provide immunity against infection, as recently discussed [22].

The main limitations of our study are the retrospective study design and the small number of patients who ultimately had a HEV-PCR-confirmed infection and were then included in the main analyses. As only existing clinical data was available for analysis, important aspects of the infection like the immediate phase prior to diagnosis remain unclear. No conclusions could therefore by drawn about the intervals between the initial infection, the onset of symptoms and the final diagnosis. Confirmatory studies should be performed in the future. It should also be noted that particularly in the early years of our study, diagnostics did not follow a standardised algorithm.

In summary, the diagnosis of an active HEV infection remains a relatively rare diagnosis in Germany. However, seroepidemiological studies have shown a considerably high incidence and high prevalence of anti-HEV-IgG in the German general population [23]. Therefore, it still must be assumed that the majority of infections remains undetected. In clinical practice, it is essential to distinguish between the different infection courses and the underlying conditions. In patients with immunosuppression with limited lymphocytic response, infections can lead to protracted, chronic courses, which can ultimately lead to fibrosis or even cirrhosis as a result of permanent chronic low grade inflammation. Due to the relatively disguised clinical course, particular caution is required to optimize the initiation of a therapy if viral clearance has failed. However, immunocompetent patients with clinical and laboratory signs of acute liver damage usually receive a wait-and-see therapeutic procedure and are often underestimated. Our data show that HEV genotype 3 should not be neglected as a cause of fulminant infections with liver failure.

Our study allows emphasizing the following clinical recommendations:

i) Every patient with clinical or laboratory signs of hepatitis should receive comprehensive HEV diagnostics including detection of anti-HEV-IgM and direct verification by NAT. Immunosuppressed patients should always be tested by NAT.
ii) Patients without any detectable immunosuppression or serious comorbidity do not initially require treatment, as the infection is usually cleared within a few weeks. However, they should be monitored regularly to detect fulminant infection courses as soon as possible.
iii) Patients with immunosuppression present with unspecific or largely without any clinical symptoms. Nevertheless, liver enzymes are elevated in over 90 % of cases. Particular attention is required in these patients. Frequent follow-ups are essential to start treatment in a timely manner, which should take the patient's individual medical situation into account. Depending on the primary disease, eradication of HEV without therapy can be attempted but should not exceed 12 weeks as prolonged HEV infection courses bear the risk of serious liver parenchyma injury.

Authors contributions

Study conceptualization: P.K., M.K. and J.M.W.; data collection: P.K. and M.K.; study analysis: P.K. and M.K.; study supervision: J.M.W.; resources, data interpretation and critical feedback: S.B., A.K., F.H., J.M., E.K.G., H.J.S., and J.J.W.; manuscript draft and revisions: P.K., M.K. and J.M.W.; manuscript finalisation: all authors.

Conflict of interest

The authors have no conflicts of interest to declare.

Financial support statement

J.M.W. received funding by grant We-4675/6-1 from the Deutsche Forschungsgemeinschaft (DFG) in Bonn, Germany.

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