Clinical Practice Guidelines

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EASL Clinical Practice Guidelines on hepatitis E virus infection☆

European Association for the Study of the Liver*

Summary: Infection with hepatitis E virus (HEV) is a significant cause of morbidity and mortality, representing an important global health problem. Our understanding of HEV has changed completely over the past decade. Previously, HEV was thought to be limited to certain developing countries. We now know that HEV is endemic in most high-income countries and is largely a zoonotic infection. Given the paradigm shift in our understanding of zoonotic HEV and that locally acquired HEV is now the commonest cause of acute viral hepatitis in many European countries, the focus of these Clinical Practice Guidelines will be on HEV genotype 3 (and 4).

Introduction

As a cause of significant morbidity and mortality, infection with hepatitis E virus (HEV) represents an important global public health problem. The European Association for the Study of Liver (EASL) invited a panel of experts in the field to develop Clinical Practice Guidelines (CPGs) with a particular focus on HEV genotype (gt) 3. The objective of these CPGs was not to draft a review article on hepatitis E but rather to define specific suggestions for the management of distinct features of HEV infection, even though the supporting evidence may be weak in many cases. In order to keep the manuscript and the reference list to a reasonable length, these CPGs frequently refer to previous review articles which summarise the evidence on distinct topics in more detail. In addition, despite the increasing knowledge, areas of uncertainty exist and unanswered questions should be defined. Therefore, clinicians, patients and public health authorities must continue to make choices on the basis of the evolving evidence.

Methodology

These EASL CPGs have been prepared by a panel of experts invited by the EASL Governing Board. The recommendations were approved by the EASL Governing Board. They are based as far as possible on evidence from existing publications and presentations at international meetings as well as, if evidence was unavailable, the experts' personal experiences and opinions. Wherever possible, the level of evidence and recommendation are cited. The evidence and recommendations have been graded according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system.[1] Thus, the strength of recommendations reflects the quality of underlying evidence. The quality of the evidence in the recommendations has been classified into one of three levels: high (A), moderate (B) or low (C). The GRADE system offers two grades of recommendation: strong (1) or weak (2). Thus, the recommendations consider the quality of evidence: the higher the quality of evidence, the more likely a strong recommendation is warranted; the greater the variability in values and preferences, or the greater the uncertainty, the more likely a weaker recommendation is warranted. It must be noted that only the review of literature was used to inform recommendations. Other criteria or support of recommendations such as cost, feasibility, acceptability, or cost-effectiveness were not considered (Table 1).

Background

HEV was discovered in the early 1980s. At that time, Soviet troops in Afghanistan were affected by large outbreaks of unexplained hepatitis (testing negative for hepatitis A virus [HAV] and hepatitis B virus [HBV]). A pooled sample of affected soldiers' stool was ingested by a Russian scientist. He developed a brisk hepatitis, and a new virus was found in his stool by electron microscopy.[2] Subsequently the viral genome was cloned and named HEV.[3]

Term	Definition
HEV	Hepatitis E virus
HEV infection	Infection caused by HEV which is either symptomatic or asymptomatic, including extrahepatic manifestations (*e.g.* neurological)
Hepatitis E	Clinical or biochemical evidence of hepatitis caused by HEV
Extrahepatic	Damage to tissues/organs outside the liver associated with/caused by HEV (see Table 2)
SVR	Sustained virologic response
R_0	**The basic reproductive rate** This term equates to the number of individuals infected by an index case with an infectious disease. If R_0 is >1 then the infection will spread through a naïve population. The R_0 of HEV in the pig population is up to 8. This means that HEV is highly infectious in the pig population, to a similar extent to measles in a measles naïve human population

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Table 1. Level of Evidence and Grade of Recommendations used in these CPGs.

Level of evidence*		Confidence in the evidence
Level A	Data derived from meta-analyses or systematic reviews or from (multiple) randomised controlled trials (RCTs) with high quality.	*Further research is unlikely to change our confidence in the estimate of benefit and risk.*
Level B	Data derived from a single RCT or multiple non-randomised studies.	*Further research (if performed) is likely to have an impact on our confidence in the estimate of benefit and risk and may change the estimate.*
Level C	Small studies, retrospective observational studies, registries.	*Any estimate of effect is uncertain.*
Recommendations		
Grade	**Wording associated with the grade of recommendation**	
1 (strong)	*"must", "should", or "EASL recommends"*	
2 (weak)	*"can", "may", or "EASL suggests"*	

* Level of evidence was graded down if there is a poor quality, strong bias or inconsistency between studies. Level was graded up if there is a large effect size.

Our understanding of HEV has changed completely over the past decade. Previously, HEV was thought to be limited to certain developing countries and was only ever seen in high-income countries in travellers returning from hyperendemic areas in Asia or Africa. We now know that HEV is endemic in most high-income countries and is largely a zoonotic infection, with pigs as the primary host.[4–6] Given the paradigm shift in our understanding of zoonotic HEV and that locally acquired HEV is now the commonest cause of acute viral hepatitis in many European countries,[7] the focus of these CPGs will be on HEV gt 3 (and 4).

Virology

HEV belongs to the *Hepeviridae*, a diverse family of viruses infecting mammals, birds and fish. Strains of HEV infecting humans belong to the *Orthohepevirus* genus which is divided into four species (A–D).[8] Human cases of hepatitis E are caused by strains within species A, which comprises eight genotypes.[9] Two of these (gt 1 and 2) only infect humans. Gt 3 and 4 are endemic in animal species such as pigs and wild boar; these strains cause zoonotic infections in humans, via consumption of contaminated meat or direct contact and other probable routes. At the molecular level, gt 3 is highly diverse and includes related viruses found in rabbits, with evidence of occasional infection with similar viruses in humans.[10] Thus far, gt 5 and 6 have only been reported in wild boar. Recently, HEV gt 7 was identified in a patient who regularly consumed camel meat and milk,[11] and, although no further human cases have been reported yet, many strains have since been identified in camels (gt 7 and 8). While HEV is primarily a hepatotropic virus, infection of other tissues, including neuronal, kidney and placental tissue, has been reported, possibly explaining some of the extrahepatic manifestations (reviewed in[12,13]).

HEV has a 7.2-kb positive-strand RNA genome which encodes three open reading frames (ORFs).[12] ORF1 encodes the functional domains involved in replication of the viral genome (the so called "replicase"), including a methyl transferase, a putative protease, an RNA helicase and an RNA-dependent RNA polymerase (RdRp). ORF2 encodes the capsid and ORF3 encodes a protein involved in release of viral particles from infected cells.

Analysis of stool samples from HEV-infected individuals demonstrated that viral particles are approximately 27–30 nm in diameter.[2] Virus excreted in bile and stool is non-enveloped; however, quasi-enveloped forms of HEV exist in the blood, with virions wrapped in membranes derived from infected cells.[14,15]

Unanswered questions and perspectives

- Are other animal homologues of HEV capable of infecting humans?
- Our understanding of the molecular virology and pathogenesis of hepatitis E is incomplete.

HEV genotypes 1 and 2

The key clinical observations from developing countries were made in the 1950s and 1970s in India. In the mid 1950s there was a major outbreak of unexplained hepatitis in Delhi,[16] and in 1978–9 in Kashmir, with high mortality observed in pregnant women.[17] These outbreaks were retrospectively confirmed as being caused by HEV, and were the first well-documented observations of excess maternal mortality associated with HEV.

HEV gt 1 and 2 are obligate human pathogens spread by the faecal-oral route via contaminated water. They cause human disease in areas with fragile sanitary infrastructure in Asia (gt 1), Africa (gt 1 and 2) and Mexico (gt 2). Sporadic cases are common, but are sometimes interspersed by large outbreaks involving thousands or tens of thousands of cases.[5] More recently, there have been ongoing, stuttering outbreaks in African refugee camps, including recent and ongoing outbreaks in South Sudan, Niger, Nigeria and Namibia.

HEV gt 1 and 2 usually cause a brief, self-limiting hepatitis in young adults that is clinically indistinguishable from other causes of acute viral hepatitis.[5] The clinical attack rate on exposure is approximately one in five.[18] Chronic infection with HEV gt 1 and 2 has not been reported so far. The mortality rate in pregnant women is approximately 25%. Deaths are caused by fulminant hepatic failure and obstetric complications such as eclampsia and haemorrhage,[5] which are associated with a high perinatal infant mortality. The cause of the excess maternal mortality is not known. Such patients need to be cared for in a high-dependency setting. Despite its possible teratogenicity there has been interest in the use of ribavirin in pregnant women with HEV infection. However, there are currently no data to support the use of ribavirin in such patients.

Some studies also show a high mortality in patients with underlying chronic liver disease who develop HEV infection. This includes a study from India which shows that the 12-month mortality rate in such patients approaches 70%.[19] However, the mortality of HEV-related acute-on-chronic liver failure

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varies widely in different studies. Studies from Asia, which mainly involved HEV gt 1 infections, reported mortality rates of between 0–67% in patients with chronic liver disease when experiencing an HEV super-infection.[20] In a cohort in India, HEV most commonly complicated patients with Wilson's disease.[21] In a recent analysis of 368 patients with acute-on-chronic liver failure, HEV-associated cases were described as having a more benign course than alcohol-associated cases.[22]

In 2005, the global burden of disease of HEV was estimated to be 20 million infections, with three million symptomatic cases and 70,000 deaths per year.[18] This estimate is problematic for two reasons: firstly, it is not a complete estimate of the worldwide burden of HEV, as it only considered infections in a limited number of developing countries where HEV gt 1 and 2 predominate. It took no account of zoonotic HEV, which is endemic in high-income countries. Secondly, the global burden estimate was based, at least in part, on seroprevalence data. These studies used 1st and 2nd generation serological assays with very poor sensitivity. For instance, a study in a rural Bangladeshi population was found to have underestimated the true seroprevalence by 100%, when a validated highly sensitive assay was used.[23] Thus, our current estimate of global burden of HEV is of limited value, and requires updating urgently.

In some countries, the epidemiology of HEV is changing, as zoonotic HEV infection has emerged. The best example of this is China where previously HEV gt 1 was the dominant circulating genotype.[24] In recent years, particularly in Eastern China, gt 1 has become much less common and gt 4 is now the most common genotype found in human cases.[5] In addition, the demographic has changed to that seen in high-income countries with zoonotic HEV gt 3 and 4, as hepatitis E is now most commonly observed in middle-aged Chinese men. The reasons for this shift from gt 1 to gt 4 are uncertain. It could reflect improvements in sanitary infrastructure, which have asserted a negative ecological pressure on HEV gt 1. An alternative possibility is that the R_0 of HEV gt 4 may be much higher than previously thought. Very recent data show that the consumption of pork is associated with HEV IgG seropositivity in areas previously considered endemic for gt 1, including Nepal[25] and South Africa.[26] The issue of co-circulating zoonotic and non-zoonotic strains in such geographical settings merits further study. In other low-income settings, zoonotic HEV seems to be the dominant genotype. A good example of this is in South America, where HEV infection is almost universally caused by HEV gt 3.[27] The epidemiology of HEV in South America is thus very similar to high-income countries with zoonotic HEV, including Europe.

Recommendations

- Travellers with hepatitis returning from areas endemic for HEV gt 1 or 2 should be tested for HEV. **(A1)**
- Pregnant women with HEV gt 1 or 2 should be cared for in a high-dependency setting, and transferred to a liver transplant unit if liver failure occurs. **(A1)**

Unanswered questions and perspectives

- There are insufficient data to support the use of ribavirin in pregnant women with HEV infection.

The rest of these guidelines is restricted to HEV gt 3 and 4 in developed countries. For more detailed guidance regarding the clinical management of outbreaks of acute HEV in resource-limited settings, please see the World Health Organization (WHO) Guidelines.[28]

Epidemiology

Based on recent seroprevalence and very recent blood donor data it is likely that there are at least two million locally acquired HEV infections in Europe every year.[7,29,30] In high-income countries, including Europe, hepatitis E is mostly a locally acquired zoonotic infection. In France there were approximately 2,000 laboratory-confirmed infections with HEV in 2014, and 99% were in non-travellers caused almost universally by HEV gt 3, with occasional cases caused by HEV gt 4. An increasing number of animals have been found to carry HEV, most of which have little relevance to human infection. Animals carrying HEV that have implications for human health are more limited and include pigs, wild boar and deer (all gt 3, or 4).[6] In addition, there are more limited data suggesting that other animals may have a role, including rabbits, camels (gt 7) and shellfish. However, the true primary host for HEV is the pig. HEV is found in pigs worldwide, but causes no symptoms. HEV is highly infectious to pigs ($R_0 = 8.8$), and once one animal in a pig herd becomes infected it is almost certain that all the animals in that herd will become infected as well.[31] The evidence suggesting a primarily porcine origin of zoonotic HEV comes from molecular epidemiological studies. These show that HEV recovered from humans has close sequence homology to HEV found in local pig and wild boar populations. In addition, seroprevalence studies have shown high HEV exposure rates in veterinarians caring for pigs and other individuals with close contact with these animals.[4–6]

Infectious HEV has been found in every step of the food chain (from slaughter house to grocery shelves) in a number of different countries including in Europe,[32] Japan and the USA. An important route of infection is by consumption of infected pig meat products which have been undercooked or consumed without cooking, *e.g.* air-dried sausage such as figatellu, which is a culinary delicacy in southern France. Small outbreaks of hepatitis E have been directly linked to consumption of products such as figatellu by analysis of HEV sequences in the patients, as well as the sausages.[33] However, there are other possible routes of human infection. When infected, pigs excrete a huge amount of HEV in the stool. This has led to environmental contamination including slurry lagoons, streams and rivers. HEV has consequently been found in shellfish and in soft fruits and salads irrigated with infected water.[5,6,34,35] Recent data shows that HEV gt 3 has found its way to the top of the aquatic food chain, as it has been found in dolphins in Cuba.[36] A study from France showed that drinking bottled water was protective against exposure to HEV, but whether domestic water supplies are a significant source of human infection remains an open question.[37] HEV gt 4 has been found in cattle in China; it was also documented in their milk and was able to survive pasteurisation.[38] This has not yet been confirmed in other regions such as Europe.[39] Finally, although HEV gt 3 is the dominant circulating genotype in Europe, gt 4 has been found in a small number of European pigs, and there are occasional human cases/clusters with gt 4 which have been noted in a number of countries,

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including Italy and France.[40] How HEV gt 4 has found its way into Europe is unknown.

The incidence of HEV infection varies between and within countries and over time (see Table S1), for reasons that are unknown. For example, the incidence of HEV infection is particularly high in France, compared to many other countries. However, a recent study has shown that human infection with HEV is not uniformly distributed in France (incidence assessed by anti-HEV IgM ranging from 0.4% to 4.6%), and is highest in the southwest and southeast of the country. These areas have such a high incidence of HEV infection that they can be considered hyperendemic.[37] The reasons for these observations are uncertain.

Studies have shown that, at least in England, Germany and Denmark, the seroprevalence of HEV declined in the last few decades of the 20th Century. These data suggest that there was a 'cohort effect', with many individuals being infected during the period following the 2nd World War.[41] More recently, in a European Centre for Disease Control (ECDC) sponsored study, countries in Europe have universally seen very significant increases in laboratory-confirmed cases of HEV infection.[7] This is partly explained by improved case ascertainment, because clinicians have become aware of the importance of locally acquired infection. In addition, there has been a significant increase in incidence in some countries, including the Netherlands, France, England, and Scotland. For example, in Scotland the number of viraemic blood donors has recently increased from 1:14,500 to 1:2,481, which has been accompanied by a 100% increase in seroprevalence in Edinburgh, mainly amongst individuals <35 years of age.[42] Temporally associated with this increase in incidence, the origin of Scottish human HEV infection appears to have changed. Previously, HEV documented in humans had close sequence homology to HEV found in Scottish pigs, but it now bears very close sequence homology to HEV found in pigs from Continental Europe. This implies there has been a recent significant change in the amount of HEV contamination of the human food chain originating from Continental Europe.[43]

In recent years, it has become apparent that there are 'hot-spots' of HEV infection in Europe. This includes southwest France (incidence 3–4%);[37] the Netherlands (1:600 blood donors viraemic, 2014);[44] Scotland (1:2,481 donors viraemic, 2016[42]); western Germany (1:616 blood donors viraemic, 2015;[45] Czech Republic (400 laboratory-confirmed cases 2015);[7] Abruzzo, central Italy (seroprevalence 49%)[46] and western/central Poland (seroprevalence 50%).[30] There may well be other areas, as yet unidentified, with high levels of circulating virus. Recently, the ECDC has taken an active role in addressing the threat of zoonotic HEV to the human population in Europe, using a 'One Health' approach. This has culminated in the establishment of 'HEVnet', which is based at The Dutch National Institute for Public Health and the Environment (RIVM) in the Netherlands.[47] The objective of this exercise is to develop a central repository for human and animal HEV sequences, together with key anonymised clinical data from human cases. 'HEVnet' is, therefore, likely to be a very important tool for improving the future understanding of HEV epidemiology.

Unanswered questions and perspectives

- How do routes of HEV infections vary by geographical location?
- Why are there 'hot-spots' of HEV infection in certain locations?
- Why has HEV infection increased in some countries in recent years?
- HEV dose-dependence of clinical and immunological presentation, as well as variable duration of serological markers of the infection, need to be carefully studied to develop and match serological assays to certain epidemiological settings.
- Does person to person spread occur with HEV gt 3 and 4?

Clinical aspects: acute infection

Acute HEV gt 3 infection is clinically silent in the vast majority of patients. Only a minority (probably less than 5%) develop symptoms of acute hepatitis with elevated liver enzymes, jaundice and non-specific symptoms such as fatigue, itching and nausea. However, HEV infection is the major cause of acute viral hepatitis in many European countries and in Germany, the UK and France there have been more reported cases of acute hepatitis E than of HAV or acute HBV infections in 2015–16.[7]

Immunocompetent patients with acute hepatitis E can clear the infection spontaneously, but there have been a few reports of cases with more prolonged viraemia. Monitoring of liver enzymes and liver function parameters is sufficient during acute hepatitis E infection, in patients who do not suffer from other chronic diseases. Progression to acute liver failure (ALF) is rare in patients with HEV gt 3 infection. However, single cases of ALF due to HEV infection have been reported in several European countries. In a German single centre study of 80 patients with ALF, HEV RNA was found in 10% of patients and HEV considered as the probable cause.[48] Patients with confirmed acute hepatitis E should be monitored for aminotransferases (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), bilirubin and INR. Once HEV infection is cleared, patients develop immunity against HEV which is not sterilising. Thus, re-infection with HEV is possible even though the likelihood of developing symptomatic hepatitis is reduced compared to non-immune individuals.

In contrast to HEV in developing countries, HEV gt 3 and 4 tend to affect older males. In one study from England the M:F ratio was 3:1, and the median age 63 years.[49] The finding that older males are most likely to develop clinically apparent acute hepatitis on exposure to HEV gt 3 and 4 is a consistent observation, but unexplained. It seems likely that this relates to host factors, rather than differential exposure, as individuals of all ages appear to be exposed to HEV. One possible explanation is that clinically apparent hepatitis is more likely to be evident in patients with subclinical hepatic steatosis/fibrosis. In a study from England, some patients with hepatitis E were heavy alcohol consumers and an excess number were diabetic, both of which are risk factors for hepatic steatosis and fibrosis.[50]

Acute hepatitis E is a concern in patients with underlying chronic liver disease. Some cases of acute-on-chronic liver failure are caused by HEV infection. This is a particular problem in elderly patients where acute hepatitis may take a more severe course. HEV infection may be less relevant in European patients with decompensated cirrhosis. Only 11/343 patients with decompensated chronic liver disease followed in France or the UK had acute hepatitis E and three of those died.[51] Of note, HEV did not alter the mortality in this study, compared to other causes of hepatic decompensation. These findings are in line with data from France showing a low prevalence of HEV infection in patients with severe acute alcoholic hepatitis.[52]

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There are only a few case reports of HEV gt 3 and 4 in pregnancy. Excess maternal mortality has not been observed.

Recommendations

- All patients with symptoms consistent with acute hepatitis should be tested for hepatitis E. **(A1)**
- EASL suggests testing for hepatitis E in patients with unexplained flares of chronic liver disease. **(C2)**

Unanswered questions and perspectives

- Why are most cases of symptomatic acute hepatitis E seen in older men?
- What is the duration of viraemia in asymptomatic HEV infections?
- What is the role of HEV in decompensated chronic liver disease?
- What is the clinical relevance of re-infection with HEV, and how commonly does this occur?

Clinical aspects: chronic infection

Immunosuppressed patients can fail to clear HEV infection.[53–55] Such patients develop chronic hepatitis, but this has only been seen in patients infected with HEV gt 3 or 4 to date.[53–55] Chronic HEV infection has been defined as a persistence of HEV replication for six months.[53] However, in an observational study performed in solid organ transplant recipients, it was observed that no spontaneous HEV clearance occurred between three and six months after infection, and spontaneous HEV clearance occurred only within the first three months after infection.[56] These data suggest that in solid organ transplant recipients, patients who are viraemic for more than three months after HEV infection can be regarded as chronically infected and considered for treatment.[56] However, in a small number of cases, spontaneous clearance has been observed between three and six months.[57]

The clinical presentation of chronic HEV infection has mainly been described in the setting of organ transplantation, but is similar in other immunosuppressed groups including patients with haematological disorders, individuals living with HIV, and patients with rheumatic disorders receiving heavy immunosuppression. In a series of 85 solid organ transplant recipients, only one-third of patients were symptomatic, with fatigue as the main symptom.[58] The majority of patients are asymptomatic and present with mild and persistent liver function test (LFT) abnormalities: in one study of chronically infected transplant recipients, the median ALT, AST and gamma-glutamyl transferase levels at diagnosis were 260 ± 38 IU/L, 155 ± 25 IU/L, and 308 ± 56 IU/L, respectively.[58] It is important to note that some patients had normal or only very slightly increased liver enzyme levels. In addition, in some patients with persistent HEV replication both anti-HEV IgG and IgM remain negative.[58] It is therefore mandatory that such patients are assessed with nucleic acid amplification techniques (NATs) using serum or plasma and, if possible, stool samples.

One-third of solid organ transplant recipients infected by HEV have resolving hepatitis and the remaining patients develop chronic hepatitis.[58,59] Other smaller studies show that progression to chronic infection occurs in less than 50% of patients.[60] In solid organ transplant recipients infected with HEV gt 3 rapid progression of liver fibrosis has been observed, leading to cirrhosis and, in some cases, decompensation and death.[53–55,59] There appears to be no difference in HEV RNA concentration between patients with or without progressive liver fibrosis.[61] Interestingly, liver fibrosis can regress after HEV clearance.[59] Extrahepatic HEV-associated manifestations, *i.e.* neurological and renal injury, have been observed both during acute and chronic HEV infection (see below).[62–64] In solid organ transplant recipients, a low lymphocyte count at diagnosis and the use of tacrolimus (rather than cyclosporine A) are associated with the development of chronic infection after exposure to HEV.[58] Among patients with HIV infection, chronic HEV infection has mostly been described in those with a $CD4^+$ T-cell count $<200/mm^3$.[65] No predictive factor(s) for the development of chronic HEV infection have been identified in other immunosuppressed groups.

Recommendations

- EASL recommends HEV testing in all immunosuppressed patients with unexplained abnormal LFTs. **(A1)**

Unanswered questions and perspectives

- What is the definition of chronic HEV infection?
- When should therapy be initiated? How long should we wait?

Extrahepatic manifestations

Extrahepatic manifestations of HEV infection are increasingly recognised (Table 2), the most important being neurological.

Neurological injury

HEV infection has been described in association with a range of neurological injuries. To date, approximately 150 cases of neurological injury in the context of HEV gt 3 infection have been described, mainly from Europe.[63] HEV-associated neurological injury has also been described in Asia in the context of HEV gt 1 infection. Most (>90%) cases have been documented in the immunocompetent, but neurological injury also occurs in the context of chronic infection with HEV gt 3. Neurological pathology that has been described in association with HEV infection includes neuralgic amyotrophy (NA), Guillain-Barré syndrome (GBS), encephalitis/myelitis, mononeuritis multiplex, Bell's palsy, vestibular neuritis, myositis and peripheral neuropathy. The best documented are NA, GBS, and encephalitis/myelitis.[63,66]

There have been several cohort and case studies of HEV infection in patients with NA. These are almost universally from Europe in the context of HEV gt 3 infection. In an Anglo/Dutch cohort study, 5/47 (10.6%) of patients with NA had evidence of HEV infection at the start of their illness.[67] Very recent data, from a multicentre study of 118 patients with NA in Europe, shows that patients with HEV-associated disease have a distinct clinical phenotype, compared to patients with NA, without evidence of HEV infection. Patients with HEV-associated NA were significantly more likely to have bilateral involvement of, and more extensive damage to, the brachial plexus. They were also more likely to have neurological damage outside the brachial plexus, particularly phrenic nerve involvement.[68] Another recent European multicentre study systematically tested over

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Table 2. Extrahepatic manifestations of acute and chronic hepatitis E.

Organ system	Clinical syndrome	Notes
Neurological	• *Neuralgic amyotrophy • *Guillain-Barré syndrome • *Meningoencephalitis • Mononeuritis multiplex • Myositis • Bell's palsy, vestibular neuritis and peripheral neuropathy	See main text
Renal*	• Membranoproliferative and membranous glomerulonephritis • IgA nephropathy	See main text
Haematological	• Thrombocytopenia • Monoclonal immunoglobulin • Cryoglobulinemia • Aplastic anaemia • Haemolytic anaemia	• Mild thrombocytopenia is common. Occasionally severe • Reported in 25% of cases of acute HEV in UK study. Significance uncertain • Occurs mainly in association with renal disease • case reports only • case reports only
Other	• Acute pancreatitis • Arthritis • Myocarditis • Autoimmune thyroiditis	• 55 cases worldwide. HEV gt 1 only. The pancreatitis is usually mild • Case reports only • Case reports only • Case reports only

* There is good evidence to support a causal role for HEV and these associated conditions. For the other extrahepatic manifestations, causality remains to be established.
HEV, hepatitis E virus.

450 consecutive patients with acute onset of non-traumatic neurological injury prospectively. Evidence of HEV infection was found in 2.4% of patients, three of whom had NA with bilateral involvement, which we now know is the clinical phenotype associated with HEV infection.[69] Finally, it is worth noting that in one centre's experience (Dalton *et al.*) the triad of bilateral shoulder pain in a middle-aged male with abnormal LFTs is highly predictive of HEV infection.[70]

There have been three case-control studies on HEV infection and GBS from the Netherlands, Bangladesh and Japan.[71–73] Collectively these studies confirm the relationship between HEV infection and GBS, as evidence of HEV infection at the start of the neurological illness was found in 5–11% of patients, significantly higher than in controls. In addition, in a very recent cohort study from Belgium, 6/73 (8%) of patients with GBS had evidence of HEV infection.[74]

There have been 12 case reports/small case series on HEV infection and encephalitis/myelitis, from Europe, Asia and the USA. Some cases had features of additional involvement of the peripheral nervous system. Five of the cases were in immunocompromised transplant recipients in the context of chronic HEV gt 3 infection. Several of these patients had a prominent ataxic component to their neurological symptomatology. These patients had poor outcomes, with long-term neurological sequelae and two deaths.[63] In one of these patients, 'quasispecies compartmentalisation' was noted, *i.e.* there was a significant difference in sequence homology in HEV RNA from the serum and cerebrospinal fluid.[75] This raises the question of whether certain strains of HEV might be neurotropic.

In all of the aforementioned studies, the patients with HEV-associated neurological injury generally had only modest abnormalities of liver function, and were mostly anicteric. Some patients had normal LFTs. Thus, the neurological symptoms and signs dominated the clinical picture. Pathogenic mechanisms are uncertain, but could be due to molecular mimicry, which would be congruent with current notions in NA and GBS, or due to direct neurotropism. It seems likely that, at least in the case of NA, GBS and encephalitis/myelitis, the relationship between HEV infection and neurological damage is causal.[63] The evidence to support causality includes the number and homogeneity of cases over time and geographical location; case-control data in GBS; documentation of HEV RNA in the serum and cerebrospinal fluid, with quasispecies compartmentalisation in some cases; intrathecal anti-HEV IgM synthesis; resolution of neurological symptoms with viral clearance;[76] *in vitro* data that show HEV can grow on a range of neurological cell lines; and *in vivo* animal studies that show HEV can cross the blood-brain barrier.[77]

Renal injury

HEV can cause glomerulonephritis in both immunocompetent and immunosuppressed patients.[64,78–81] Renal impairment has been documented in solid organ transplant recipients during acute HEV infection.[64] Cases of membranoproliferative glomerulonephritis with and without cryoglobulinaemia, as well as cases of membranous glomerulonephritis have been reported, mainly in immunosuppressed patients infected by HEV gt 3.[78–81] Cases of membranoproliferative and membranous glomerulonephritis have been documented with HEV gt 1 and 3 in the immunocompetent.[78,81] Renal function improves and proteinuria levels decrease following HEV clearance, either spontaneously or following therapy.[79,80] These data suggest the relationship between HEV infection and the associated renal injury is likely to be causal. Of note, in one case, HEV RNA was isolated from the cryoprecipitate obtained from a patient who developed HEV-associated cryoglobulinaemic glomerulonephritis.[81]

Cryoglobulinaemia

Cryoglobulinaemia has been observed in patients chronically infected by HEV: it disappears following antiviral therapy.[64,81] Anti-HEV IgG seroprevalence seems to be higher in patients with essential cryoglobulinaemia.[82,83] Finally, HEV-associated cryoglobulinaemia with arthralgia, myalgia and rash has been also reported in a liver transplant recipient.[84]

Pancreatitis

Acute pancreatitis episodes have been reported in patients infected with HEV gt 1 from Southeast Asia.[85–87] However, no cases of acute pancreatitis have been documented in patients with HEV gt 3 or 4 infections.

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Haematological disorders

Severe thrombocytopenia has been described in patients with acute HEV gt 1 and 3 infections.[66,88–91] HEV infection has been associated with a few other haematological disorders, mostly described as single case reports. These include autoimmune haemolytic anaemia, aplastic anaemia, and acute liver failure associated with pure red-cell aplasia.[92–94] Asymptomatic monoclonal paraprotein has been documented in up to 25% patients infected with HEV gt 3.[66] The clinical significance of this observation is uncertain.

Other manifestations

Several other extrahepatic disorders have been described with HEV infection. These include myocarditis,[95] thyroiditis,[96] Henoch-Schönlein purpura,[97] and myasthenia gravis.[98] A causal relationship between these associations has not been established.

Recommendations

- EASL recommends HEV testing, irrespective of LFT results, in patients presenting with NA **(B1)** and GBS **(B1)** and suggests HEV testing for patients with encephalitis/myelitis. **(C2)**
- EASL suggests testing patients with HEV infection for proteinuria. **(C2)**
- Patients with acute or chronic HEV infection who develop new onset proteinuria may be considered for a renal biopsy. **(C2)**
- EASL suggests antiviral treatment for patients with chronic HEV infection and associated glomerular disease. **(C2)**

Unanswered questions and perspectives

- What are the neurological conditions which are causally related to HEV infection?
- What are the pathogenic mechanisms of HEV-associated extrahepatic injury?
- What is the incidence of HEV-associated glomerulonephritis?
- Are there any other HEV-associated extrahepatic manifestations that remain to be discovered?
- The treatment of most extrahepatic manifestations of HEV infection remains to be determined.

Diagnosis

Laboratory diagnosis

The incubation period for hepatitis E is approximately 15 to 60 days. Around three weeks post-infection, HEV RNA is detected in blood and stool, with viraemia lasting approximately three to six weeks, and shedding of virus in stool for approximately four to six weeks. The first appearance of HEV RNA occurs shortly before the onset of symptoms. Around the time of clinical onset, biochemical markers become elevated and antibodies start to appear, with IgM antibodies appearing first, followed soon after by IgG antibodies. The IgM antibodies are relatively short-lived (usually no longer than three to four months, but may persist for up to a year); however, the IgG response is long lasting with increasing antibody avidity over time.

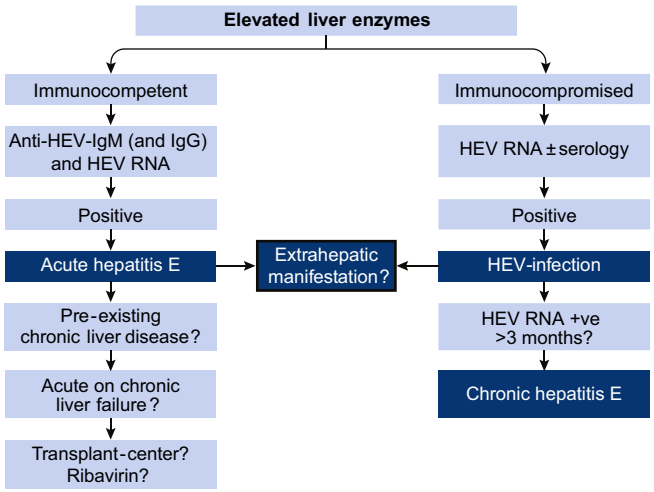

Fig. 1. Diagnostic algorithm for HEV infection. Serology and NAT testing are best used in combination, as a negative PCR does not exclude acute infection; serology is sometimes negative in the immunosuppressed patients with chronic infection. HEV, hepatitis E virus; NAT, nucleic acid amplification techniques.

Molecular analysis

Detection of HEV RNA in blood or stool is indicative of HEV infection. In immunosuppressed patients with chronic hepatitis E, anti-HEV antibodies are often undetectable, and in such cases NATs are the only reliable means of diagnosis (Fig. 1). Chronic cases of hepatitis E are defined as HEV RNA being detectable for at least three months. In such chronic cases, viral load testing is used to evaluate the response of patients to modification of immunosuppressive drug treatment or antiviral therapy, as well as to identify relapsing infections.

NATs are used for the detection of HEV RNA. An evaluation of laboratory testing for HEV RNA by NATs revealed wide variations in the performance of different assays[99] and led to the development of the 1st WHO International Standard (IS) for HEV RNA for NAT-based assays[100] and the 1st WHO international Reference Panel (IRP) for HEV gt 1–4. The availability of the WHO IS and IRP has facilitated the comparison of the results of diagnostic tests performed by different laboratories, helping to harmonise testing. The WHO IS is an important tool for defining the analytical sensitivity of assays and enables reporting using a common unit, *i.e.* International Unit (IU); this provides a system of traceability. Analytical sensitivity of NAT-based assays can be lower than 10 IU/ml.

Many different NAT-based assays have been reported for the detection of HEV RNA in serum and plasma or stool samples: these include conventional reverse transcription PCR (RT-PCR) and nested protocols, real-time RT-PCR, transcription-mediated amplification methods including, for example, reverse transcription loop-mediated isothermal amplification.[99–102] The most frequently used assays for the detection of HEV RNA target highly conserved regions of the genome, in particular the region of ORF2 that overlaps ORF3, and are able to detect all four major genotypes of HEV that infect humans.[103] The sensitivity and specificity of assays depend upon well-designed primer and probe sequences. Occasionally, however, polymorphisms have resulted in false negative results in patients with HEV infection, so improvements have been made to the robustness of existing assays.[104] Sequence analysis is used to determine HEV genotype.

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Antibody assays

Acute HEV infection can also be diagnosed by the detection of anti-HEV antibodies (IgM, IgG or both) by enzyme immunoassays in combination with HEV NAT. Serological testing alone, relies upon detection of anti-IgM and (rising) IgG titres (Table 3), since the specificity of certain assays is not optimal and anti-HEV IgM on its own is not a sufficiently robust marker for diagnosis.[105] Immunoblots are available for confirmatory testing, although they suffer from the same limitations and so have proved ineffective. Occasionally, anti-HEV IgA antibodies are used for diagnosis of acute hepatitis E; however, such assays are not widely available. Past infection is determined by the presence of anti-HEV IgG.[106] In studies investigating seroprevalence, sub-optimal performance of certain assays which lack sensitivity has previously resulted in very significant underestimates of populations' exposure to HEV.[106]

Antigen assays

Detection of HEV antigen by enzyme immunoassays may also be used to diagnose both acute and chronic infections. Older versions of the antigen assays were not as sensitive as NATs,[107] however, newer assays offer improved sensitivity.[108,109] HEV antigen levels may be lower in patients with acute hepatitis E than in patients with chronic hepatitis E, with an OD450/630 of >15 suggested to discriminate between acutely and chronically infected individuals in one study.[110] Importantly, HEV antigen may persist for several months after ribavirin-induced HEV RNA clearance of chronic hepatitis E. This observation, and experimental data, suggest that the presence of HEV antigen does not necessarily correlate with infectious virions.[110] In one recent study, it was suggested that glycosylated forms of ORF2 are excreted in the sera of infected patients at high levels; however, infectious virions are associated with the much less abundant non-glycosylated form of ORF2.[111]

Immunohistochemistry

Immunohistochemistry for HEV ORF2 protein can be used to establish a histopathologic diagnosis of hepatitis E.[112]

Table 3. Laboratory diagnosis of HEV infection.

Infection status	Positive markers
Current infection - acute	• HEV RNA • HEV RNA + anti-HEV IgM • HEV RNA + anti-HEV IgG* • HEV RNA + anti-HEV IgM + anti-HEV IgG • Anti-HEV IgM + anti-HEV IgG (rising) • HEV antigen
Current infection - chronic	• HEV RNA (± anti-HEV) ≥3 months • HEV antigen
Past infection	• Anti-HEV IgG

*Patients with re-infection are typically anti-HEV IgM negative, but IgG and PCR positive. HEV, hepatitis E virus.

Recommendations

- EASL recommends using a combination of serology and NAT testing to diagnose HEV infection. **(A1)**
- EASL recommends NAT testing to diagnose chronic HEV infection. **(A1)**

Unanswered questions and perspectives

- The role of HEV antigen in diagnosis remains to be determined.

Differential diagnosis

The differential diagnosis of HEV infection is shown (Table 4). An important differential diagnosis of acute hepatitis E is drug-induced liver injury (DILI).[113] In a cohort study of UK patients with 'criterion-referenced' DILI, it was found that in 13% the diagnosis of DILI was erroneous, as the patients had acute hepatitis E, caused by gt 3.[114] This is an easy mistake to make as polypharmacy and DILI are both most common in the elderly, as is acute hepatitis E. Therefore, it is important to note that when making a diagnosis of DILI, particularly in a patient with a predominant aminotransferase elevation, it is key to first exclude HEV infection. Another common diagnostic difficulty is distinguishing between autoimmune hepatitis and acute hepatitis E. It is not uncommon for autoimmune hepatitis to present for the first time in older patients, and be associated with non-specific 'sticky' cross-reactive antibodies, which can produce false positive HEV serology results. False positive HEV serology can occur in the context of Epstein-Barr virus infection, also due to cross-reactive antibodies.[105]

Previously, only patients who had travelled to areas in Asia and Africa that are hyperendemic for HEV gt 1 and 2 were considered for HEV testing. We now know that the vast majority of patients with hepatitis E in developed countries have locally acquired infection, so in most countries national diagnostic testing algorithms have been changed. Any patient presenting with biochemical evidence of hepatitis should be considered for HEV testing, irrespective of travel history. In some countries, patients presenting with hepatitis are only tested for HEV if the 'first-line' virological testing (for HAV, HBV and hepatitis C virus [HCV]) is negative. This is no longer appropriate, as we know that acute hepatitis E is the commonest cause of acute viral hepatitis in many countries. Therefore, all patients presenting with hepatitis should be tested for HEV at presentation (Table 5).

Recommendations

- All patients with hepatitis should be tested for HEV, as part of the first-line virological investigation, irrespective of travel history. **(A1)**
- Patients presenting with suspected DILI should be tested for HEV. **(A1)**

HEV and the blood supply

In addition to zoonotic transmission, HEV can be transmitted iatrogenically between humans through infected blood and blood products. Transfusion-transmitted HEV infection has been documented in many countries in Europe (HEV gt 3) and Japan (HEV gt 3 and gt 4). Most cases of transfusion-transmitted HEV infection are asymptomatic, and only a small minority of recipients of infected blood or blood products develop symptomatic hepatitis. When infected blood or components are given to the immunosuppressed, there is a significant risk that the recipient will develop chronic HEV infection. This is quite easily overlooked, as infected recipients have no symptoms and develop only minor persistent abnormalities in liver function, which may be delayed for months after infection. Transmission of HEV by solvent/detergent-treated plasma has been reported,

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Table 4. Differential diagnosis of hepatitis E.

Infection status	Differential diagnosis
Acute infection*	• Drug-induced liver injury • Autoimmune hepatitis • Acute hepatitis E • Sero-negative hepatitis • EBV hepatitis • Acute hepatitis B • Acute hepatitis A • Acute hepatitis C • CMV hepatitis
Chronic infection in the immunosuppressed	• Graft rejection • Drug-induced liver injury • Recurrence of primary liver pathology in liver transplant recipients • Graft *vs.* host disease • Intercurrent infections, *e.g.* sepsis • Chronic hepatitis E • EBV and CMV reactivation

*The differential diagnosis is in order of frequency of each condition seen at a rapid-access jaundice clinic in Southwest England. CMV, cytomegalovirus; EBV, Epstein-Barr virus.

and since 2015 in Europe solvent/detergent-treated plasma is tested for HEV by NAT. There are currently no reports of HEV transmission by virally-inactivated fractionated blood products (purified plasma proteins).

Transfusion-transmitted HEV infection has been most well-documented in England and Japan.[29] In a study from 2012–13, in southeast England, 225,000 blood donors were screened for HEV by PCR. Seventy-nine donors were viraemic (gt 3) and 62 infected blood components were used prior to identification of donor viraemia.[29] Follow-up of 43 recipients showed 18 (42%) had evidence of infection, which was more likely with high donor viral loads and low levels of donor IgG antibody. Three patients required intervention with reduced immunosuppression (n = 1) or ribavirin therapy (n = 2) to successfully achieve viral clearance. In this study the minimum infective dose contained a viral load of 2×10^4 IU HEV RNA, and 55% of blood components with at least this dose transmitted infection.[29] In Japan, 20 cases of transfusion-transmitted infection have been documented in recent years. In an analysis of 19 of these cases, caused mostly by HEV gt 3 and two by gt 4, the minimum infective dose was 3.6×10^4 IU HEV RNA and the rate of infection was 50%.[115] The presence of anti-HEV IgG in recipients does not necessarily protect the recipient from transfusion-transmitted infection, as low levels of antibody appear not to prevent re-infection.

As zoonotic HEV infection is very common in many developed countries and mostly asymptomatic, it is no surprise that HEV has found its way into the human blood supply. However, what has come as a surprise to many professionals involved in transfusion medicine is the very high frequency of viraemic donors in many countries (Table S1), ranging from 1:600 in the Netherlands to between 1:14,799 and 1:74,131 in Australia. These findings, together with the known adverse outcome of transfusing infected blood and components outlined above, have made HEV a 'hot-topic' in the blood transfusion community. Several countries have introduced universal, targeted or partial screening for HEV in donors, including Ireland, the UK, France, the Netherlands and Japan. In Germany, some blood transfusion companies have introduced voluntary HEV screening. In many other countries donor screening is being considered (Table S1). The screening methodology of choice is NAT, as infected donors usually have normal LFTs and are often anti-HEV IgM and IgG negative. Screening donors for HEV with NATs carries considerable cost, but a recent cost-effectiveness analysis from the Netherlands suggests that screening for HEV compares favourably in this regard to existing donor screening for HBV, HCV and HIV. In England, it has been estimated that transfusion-transmitted HEV infection comprises <1% of all human infections with HEV (the remainder being due to zoonotic transmission), and that transfusion of 13 components from different donors equates to the annual dietary risk of HEV exposure in the general population. Thus, although donor screening will be very effective at minimising iatrogenic HEV infection, it will have a relatively minor impact on the numbers of HEV infections in the population as a whole.

Although extremely rare, HEV has been transmitted by liver and kidney grafts from infected donors; there are no current recommendations for organ donor screening.

Recommendations

- Patients with abnormal LFTs after receiving blood products should be tested for HEV. **(A1)**
- EASL recommends that blood donor services screen blood donors for HEV by NAT, informed by local risk-assessment and cost-effectiveness studies, both of which may vary considerably by geographical location. **(A1)**

Unanswered questions and perspectives

- Does the infective dose of transfusion-transmitted HEV differ by immunological status of the recipient?
- Should organ donors be screened for HEV?
- Does the clinical phenotype of transfusion-transmitted HEV infection differ from zoonotic infection?
- Can HEV be effectively removed from blood products?

Treatment of acute hepatitis E

Acute HEV infection does not usually require antiviral therapy. In almost all cases HEV infection is spontaneously cleared. However, some patients may progress to liver failure, so the question of whether hepatic decompensation can be avoided by antiviral treatment arises. Moreover, early therapy of acute hepatitis E may shorten the course of disease and reduce overall morbidity, as has been shown in the treatment of acute hepatitis caused by HBV and HCV.

Very few case reports are available on ribavirin treatment for severe acute HEV infection.[116] Ribavirin therapy was associated with very rapid normalisation of liver enzymes and HEV RNA became undetectable within a few days. Cases of ribavirin treatment of both HEV gt 3 and HEV gt 1 infection have been published. Liver synthetic function rapidly improved in one case.

Corticosteroids have been used in individual cases of ALF, which were retrospectively identified as being been caused by HEV infection. Steroid therapy was associated with improved liver function parameters in these cases.[48] However, there is currently insufficient evidence to support corticosteroid treatment in patients with ALF due to HEV infection.

Statement

Acute HEV infection does not usually require antiviral therapy. **(A)**

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Table 5. Suggested testing for HEV.

Immunological status	Patients who should be tested for HEV
Immunocompetent	• Any patient with biochemical evidence of hepatitis • Suspected drug-induced liver injury • Decompensated chronic liver disease* • Neuralgic amyotrophy* • Guillain-Barré syndrome* • Encephalitis* • Patients with unexplained acute neurology and a raised ALT**
Immunocompromised (developed countries)	• As above • Persistently abnormal ALT***

* Testing should be done at disease onset, irrespective of ALT results.
** Testing should be done at disease onset, if ALT is abnormal.
*** If the ALT is above the limit of normal on more than one occasion. ALT, alanine aminotransferase; HEV, hepatitis E virus.

Recommendations

- Ribavirin treatment may be considered in cases of severe acute hepatitis E or acute-on-chronic liver failure. **(C2)**

Unanswered questions and perspectives

- Does ribavirin therapy reduce morbidity in acute hepatitis E?
- The benefit of ribavirin in patients with severe acute hepatitis E and those with HEV-associated liver failure is uncertain
- The dose and duration of ribavirin therapy in ALF are not defined
- Should corticosteroids be used in patients with HEV-associated ALF?
- Corticosteroid therapy is probably safe in the context of HEV infection, but further data are needed.

Treatment of chronic HEV infection in solid organ transplant recipients

Solid organ transplant recipients chronically infected with HEV who spontaneously achieve viral clearance have a lower tacrolimus trough level and a lower daily steroid dose compared to those who remain viraemic.[59] This has led to the notion that reducing immunosuppressive therapy, especially drugs targeting T-cells, could be a useful initial therapeutic option.[59] Adopting this approach achieves sustained viral clearance in nearly one-third of chronically infected solid organ transplant recipients (Fig. 2).[58,59]

In vitro studies show that mTOR inhibitors upregulate HEV replication,[117] whilst mycophenolate has suppressive effects on HEV.[118] To what extent these *in vitro* findings are clinically relevant remains to be determined. Although in one study of heart transplant recipients the use of mycophenolate was associated with a lower likelihood of developing chronic hepatitis E,[60] mycophenolate-treated patients can still develop chronic hepatitis E.

PEGylated-interferon-α has been successfully used to treat a small number of liver transplant recipients and a haemodialysis patient who cleared HEV after a three-month course of therapy.[119–121] However, interferon is generally contraindicated in kidney, pancreas, heart, and lung-transplant recipients because it stimulates the immune system and increases the risk of acute rejection.[122]

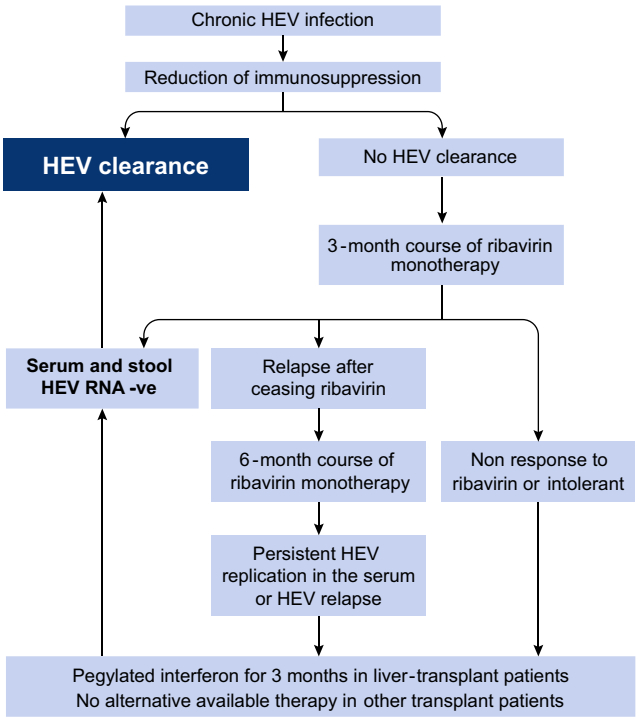

Fig. 2. Treatment algorithm for chronic HEV infection. The first therapeutic manoeuvre in transplant recipients is to reduce the dose of immunosuppression if possible. This will allow HEV to be cleared in about 30% of patients. If this is not possible, or unsuccessful, clinicians should follow the illustrated treatment algorithm. The role of other therapies such as sofosbuvir and immunoglobulins remains to be determined. HEV, hepatitis E virus.

Ribavirin monotherapy has been more extensively studied in the treatment of chronic HEV infection in solid-organ transplant recipients, with a number of case reports and case series.[123–126] Although ribavirin is the treatment of choice, its use is not backed-up by any placebo-controlled trials. Several small series have reported high sustained virological response (SVR) rates after a three-month course of ribavirin monotherapy.[126] In a multicentre retrospective study that included 59 solid-organ transplant recipients treated with ribavirin at a median dose of 600 (range, 29–1,200) mg/day for three (range, 1–18) months, the SVR was 78%. Relapsers who were retreated with ribavirin for a longer period (six months) cleared the virus and achieved SVR.[126] No difference in SVR was observed between patients who received ribavirin for three months or less and those who were given therapy for more than three months. However, the optimal duration of ribavirin therapy is still unknown.

Ribavirin treatment can be associated with side effects including dose-dependent anaemia, dry cough and skin reactions. As patients with chronic hepatitis E frequently suffer from co-morbidities associated with impaired renal function or anaemia, ribavirin should be dosed with caution. Dose adaptations that consider haemoglobin and eGFR levels are strongly recommended.[127]

The mechanism of action of ribavirin against HEV is not fully understood. It has been suggested that ribavirin inhibits HEV replication by depleting guanosine triphosphate (GTP) pools.[128] Heart transplant patients who were treated with mycophenolic acid, an inosine monophosphate dehydrogenase inhibitor that decreases GTP production, had a lower risk of developing chronic hepatitis in one study.[129] *In vitro*, mycophenolic acid and ribavirin have a synergistic anti-HEV effect.[118] Conversely, *in vivo*, in solid organ transplant recipients, the change in HEV RNA con-

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centration over time did not differ between patients given ribavirin with or without mycophenolic acid.[130] The use of mycophenolic acid as an immunosuppressant does not protect an individual transplant recipient from developing HEV infection.

Deep sequencing has identified several HEV RNA mutations. A recent study showed that ribavirin increases HEV heterogeneity, an effect that seems to be reversible. A G1634R mutation in the HEV polymerase was first described in two cases of ribavirin treatment failure.[131] However, pre-treatment G1634R mutations did not impact on SVR in a series of solid organ transplant recipients given ribavirin.[132] In another study, the G1634R mutation appeared during therapy in patients who relapsed.[133] Several other variants in the polymerase regions have been described.[133] Some of these increase ribavirin sensitivity; others increase HEV replication, while others decrease HEV replication.[133,134] Hence, the role of HEV RNA variants and their impact on HEV treatment outcome are uncertain.

A high lymphocyte count has been found to be an independent predictor of SVR in solid organ transplant recipients treated with ribavirin.[135] Persistence of HEV RNA in the stools at the end of ribavirin therapy in patients with undetectable HEV RNA in the serum is associated with increased risk of HEV viraemia after ribavirin cessation.[136] A decrease in HEV RNA concentration ≥0.5 $\log_{10}$ IU/ml at day seven has been shown to predict SVR.[130] Interestingly, in this study no association was observed between SVR and ribavirin trough level, seven days or two months after starting therapy.[130]

At present, no other antiviral therapies are known to be effective in the treatment of patients with chronic HEV infection, other than those outlined.[137] It has recently been reported that sofosbuvir, a specific and potent inhibitor of the hepatitis C virus NS5B RdRp, also has some activity against HEV RNA replication *in vitro* and that the antiviral effect is additive with ribavirin.[138] It is presently unknown if these observations made *in vitro* will translate to clinical efficacy *in vivo*.[137,139]

Treatment of chronic HEV infection in other immunosuppressed patients

The treatment of chronic HEV infection in non-transplant immunosuppressed patients, *i.e.* patients with haematological disorders or HIV, has been documented in a few case reports and small series. PEGylated-interferon-α, ribavirin or the combination of both was effective for treating HEV infection in patients with haematological disorders[140–142] and those with HIV.[76,143,144] Nine out of twelve stem-cell-transplant recipients treated with ribavirin achieved SVR.[142]

Recommendations

- EASL recommends decreasing immunosuppression at diagnosis of chronic HEV infection in solid organ transplant recipients, if possible. **(B1)**
- In patients with persisting HEV replication three months after detection of HEV RNA, EASL recommends ribavirin monotherapy for a duration of 12 weeks. **(B1)**
- At the end of the scheduled period of therapy, HEV RNA should be assessed in the serum and in the stool **(B1)**. If HEV RNA is undetectable in both, EASL suggests stopping ribavirin. **(C2)**

Statement

- The optimal treatment duration in patients who test HEV RNA positive after four or eight weeks of therapy and who are HEV RNA negative after 12 weeks of therapy is unknown. **(C)**

Recommendations

- In patients in whom HEV RNA is still detectable in the serum and/or in the stool after 12 weeks, ribavirin monotherapy may be continued for an additional three months (six months therapy overall). **(C2)**

Statement

- The optimal therapeutic approach is unknown in patients who show no response to ribavirin and/or who relapse after retreatment. **(C)**

Recommendations

- Liver transplant recipients who show no response to ribavirin can be considered for treatment with PEGylated-interferon-α. **(C2)**

Unanswered questions and perspectives

- What is the optimal ribavirin dose and duration of therapy?
- What is the best treatment in patients who show no response to ribavirin and/or who relapse after retreatment?
- What is the mechanism of action of ribavirin?
- Alternative therapies need to be developed for patients who do not achieve viral clearance with (or cannot tolerate) ribavirin or PEGylated-interferon-α.

Prevention of HEV infection

Recent evidence from a cell culture model suggests that heating virus stocks for more than two minutes at 70 °C eliminates HEV infectivity, while infective HEV could be recovered by storage at room temperature even after 28 days. A temperature of 80 °C was required to prevent HEV infection when heating for one minute.[145] However, it is unclear to what extent these *in vitro* data can be translated into food preparation practices.

Several case-control studies clearly defined consumption of undercooked meat from pigs, wild boar and deer as risk factors for HEV infection in Europe.[61] Thus, it is strongly recommended that individuals at risk of severe acute or chronic HEV infection avoid consumption of food products which may contain infectious HEV. However, recommending that the general population avoid undercooked pork is not currently justified. Immunocompetent individuals are likely to be able to tolerate exposure to HEV without any significant health threat. However, there is some evidence that consumption of pork is associated with increased mortality.[146] To what extent this increased risk can be attributed to HEV infection remains to be determined. The safety of other food products including strawberries, spinach, shellfish and camel milk requires further investigation.

The risk of patient-to-patient transmission of HEV is poorly defined. Sexual transmission of HEV has been described in

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men having sex with men,[147,148] but in another study of a cohort of patients with HIV, no evidence of sexual transmission was found.[149] As stool contains high amounts of infectious HEV particles and as stool-derived HEV has been shown to be more infectious than plasma-derived HEV[150,151] strict hygienic recommendations should be considered to prevent the spread of HEV by contaminated stool, *e.g.* in hospitals or nursing homes. HEV RNA can also be detected in urine.[152] It is unclear if HEV can be transmitted by saliva, sweat, semen or breast-milk.

A vaccine against HEV was licensed in China in 2011. This vaccine showed an efficacy of 97% for preventing episodes of symptomatic acute hepatitis,[153] with its long-term efficacy proved during further follow-up.[154] The vaccine is based on a protein containing 239 amino acids of HEV ORF2 protein (aa 368–606), derived from HEV gt 1. Surface protrusions, formed by dimerisation of HEV 239, correspond to a protruding domain of the virus capsid responsible for eliciting neutralising antibodies. Cellular immune responses are also involved in the control of HEV infection and these include both natural killer cells as well as HEV-specific T-cells.[155] The vaccine prevented symptomatic HEV gt 4 infections, suggesting cross genotype efficacy, but the vaccine does not provide sterilising immunity and, subclinical infections can still occur. While the vaccine seems to be safe in pregnant women,[156] the long-term efficacy and safety in patients with chronic liver disease and the immunosuppressed remain to be determined. A major role of the vaccine could be to prevent HEV outbreaks, *e.g.* in African refugee camps or other emergency settings. However, the vaccine is currently not licensed for this purpose in countries other than China, but efforts are currently underway to obtain WHO 'prequalification' for use in emergency settings.

Recommendations

- Immunocompromised individuals and those with chronic liver diseases should avoid consumption of undercooked meat (pork, wild boar and venison) and shellfish. **(B1)**
- EASL suggests that immunocompromised patients consume meat only if it has been thoroughly cooked to temperatures of at least 70 °C. **(B2)**

Unanswered questions and perspectives

- The risk of HEV transmission to animals by potentially contaminated animal feeds is unknown.
- The HEV infection dynamics in farm-reared animals need to be better defined.
- What is the risk of patient-to-patient transmission by exposure to contaminated body fluids/close personal contact?
- The efficacy and safety of an HEV vaccine needs to be defined in immunocompromised patients, patients with end-stage organ disease awaiting transplantation, and patients with chronic liver disease.
- The efficacy of an HEV vaccine against HEV gt 3 remains to be determined.
- The efficacy of an HEV vaccine in farm-reared animals is unknown.
- How long does immunity (both natural immunity and immunity after vaccination) against HEV last?

Conclusions

Our understanding of HEV infection has completely changed in the last decade. There are still many knowledge gaps, and it is likely that as answers to these questions become available, these CPGs will require amendment in a few years' time.[42,157–159]

Conflict of interest

H.R. Dalton reports grant support from the BMA, consultant/advisory roles with Roche, Gilead, Wantai, Merck and delivery of sponsored lectures for the Gates Foundation. Co-holder of patent Kernow CIPG with NIH and others. H. Wedemeyer reports grant support from Abbott, Roche diagnostics, AbbVie and Gilead, consultant/advisory roles for Abbott, Roche diagnostics, AbbVie and Gilead, and delivery of lectures sponsored by Abbott, AbbVie and Gilead. All other panel members report no conflicts of interest.

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

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Supplementary data

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References

Author names in bold designate shared co-first authorship

[1] Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008;336:924–926.
[2] Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, et al. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology 1983;20:23–31.
[3] Reyes GR, Purdy MA, Kim JP, Luk KC, Young LM, Fry KE, et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. Science 1990;247:1335–1339.
[4] Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis 2008;8:698–709.
[5] Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, et al. Hepatitis E. Lancet 2012;379:2477–2488.
[6] Kamar N, Dalton HR, Abravanel F, Izopet J. Hepatitis E virus infection. Clin Microbiol Rev 2014;27:116–138.
[7] Adlhoch C, Avellon A, Baylis SA, Ciccaglione AR, Couturier E, de Sousa R, et al. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. J Clin Virol 2016;82:9–16.
[8] Smith DB, Simmonds PInternational Committee on Taxonomy of Viruses Hepeviridae Study Group, Jameel S, Emerson SU, Harrison TJ, et al. Consensus proposals for classification of the family Hepeviridae. J Gen Virol 2014;95:2223–2232.
[9] Purdy MA, Harrison TJ, Jameel S, Meng XJ, Okamoto H, Van der Poel WHM, et al. ICTV virus taxonomy profile: hepeviridae. J Gen Virol 2017;98:2645–2646.
[10] Abravanel F, Lhomme S, El Costa H, Schvartz B, Peron JM, Kamar N, et al. Rabbit hepatitis E virus infections in humans, France. Emerg Infect Dis 2017;23:1191–1193.
[11] Lee GH, Tan BH, Teo EC, Lim SG, Dan YY, Wee A, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. Gastroenterology 2016;150:355–357, e353.

Please cite this article in press as: Negro F, Wedemeyer H. EASL Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol (2018), https://doi.org/10.1016/j.jhep.2018.03.005

[12] Debing Y, Moradpour D, Neyts J, Gouttenoire J. Update on hepatitis E virology: Implications for clinical practice. J Hepatol 2016;65:200–212.
[13] Pischke S, Hartl J, Pas SD, Lohse AW, Jacobs BC, Van der Eijk AA. Hepatitis E virus: Infection beyond the liver? J Hepatol 2017;66:1082–1095.
[14] Takahashi M, Tanaka T, Takahashi H, Hoshino Y, Nagashima S, Jirintai, et al. Hepatitis E Virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the coexistence of HEV antibodies: characterization of HEV virions in blood circulation. J Clin Microbiol 2010;48:1112–1125.
[15] Feng Z, Lemon SM. Peek-a-boo: membrane hijacking and the pathogenesis of viral hepatitis. Trends Microbiol 2014;22:59–64.
[16] Naidu SS, Viswanathan R. Infectious hepatitis in pregnancy during Delhi epidemic. Indian J Med Res 1957;45:71–76.
[17] Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Am J Med 1980;68:818–824.
[18] Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology 2012;55:988–997.
[19] Kumar Acharya S, Kumar Sharma P, Singh R, Kumar Mohanty S, Madan K, Kumar Jha J, et al. Hepatitis E virus (HEV) infection in patients with cirrhosis is associated with rapid decompensation and death. J Hepatol 2007;46:387–394.
[20] Kumar A, Saraswat VA. Hepatitis E and acute-on-chronic liver failure. J Clin Exp Hepatol 2013;3:225–230.
[21] Ramachandran J, Eapen CE, Kang G, Abraham P, Hubert DD, Kurian G, et al. Hepatitis E superinfection produces severe decompensation in patients with chronic liver disease. J Gastroenterol Hepatol 2004;19:134–138.
[22] Shalimar, Kedia S, Gunjan D, Sonika U, Mahapatra SJ, Nayak B, et al. Acute liver failure due to hepatitis E virus infection is associated with better survival than other etiologies in Indian patients. Dig Dis Sci 2017;62:1058–1066.
[23] Kmush BL, Labrique AB, Dalton HR, Ahmed ZB, Ticehurst JR, Heaney CD, et al. Two generations of "Gold Standards": the impact of a decade in hepatitis E virus testing innovation on population seroprevalence. Am J Trop Med Hyg 2015;93:714–717.
[24] Ren X, Wu P, Wang L, Geng M, Zeng L, Zhang J, et al. Changing epidemiology of hepatitis A and hepatitis E viruses in China, 1990–2014. Emerg Infect Dis 2017;23:276–279.
[25] Shrestha AC, Flower RL, Seed CR, Rajkarnikar M, Shrestha SK, Thapa U, et al. Hepatitis E virus seroepidemiology: a post-earthquake study among blood donors in Nepal. BMC Infect Dis 2016;16:707.
[26] Madden RG, Wallace S, Sonderup M, Korsman S, Chivese T, Gavine B, et al. Hepatitis E virus: Western Cape, South Africa. World J Gastroenterol 2016;22:9853–9859.
[27] Rendon J, Hoyos MC, di Filippo D, Cortes-Mancera F, Mantilla C, Velasquez MM, et al. Hepatitis E virus genotype 3 in Colombia: survey in patients with clinical diagnosis of viral hepatitis. PLoS One 2016;11:e0148417.
[28] WHO HEV Guidelines, 2018. in press.
[29] Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet 2014;384:1766–1773.
[30] Grabarczyk P, Sulkowska E, Gdowska J, Kopacz A, Liszewski G, Kubicka-Russel D, et al. Molecular and serological infection marker screening in blood donors indicates high endemicity of hepatitis E virus in Poland. Transfusion 2018. https://doi.org/10.1111/trf.14531, [Epub ahead of print].
[31] Bouwknegt M, Frankena K, Rutjes SA, Wellenberg GJ, de Roda Husman AM, van der Poel WH, et al. Estimation of hepatitis E virus transmission among pigs due to contact-exposure. Vet Res 2008;39:40.
[32] Berto A, Martelli F, Grierson S, Banks M. Hepatitis E virus in pork food chain, United Kingdom, 2009–2010. Emerg Infect Dis 2012;18:1358–1360.
[33] Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis 2010;202:825–834.
[34] Maunula L, Kaupke A, Vasickova P, Soderberg K, Kozyra I, Lazic S, et al. Tracing enteric viruses in the European berry fruit supply chain. Int J Food Microbiol 2013;167:177–185.
[35] Terio V, Bottaro M, Pavoni E, Losio MN, Serraino A, Giacometti F, et al. Occurrence of hepatitis A and E and norovirus GI and GII in ready-to-eat vegetables in Italy. Int J Food Microbiol 2017;249:61–65.
[36] Montalvo Villalba MC, Cruz Martinez D, Ahmad I, Rodriguez Lay LA, Bello Corredor M, Guevara March C, et al. Hepatitis E virus in bottlenose dolphins Tursiops truncatus. Dis Aquat Organ 2017;123:13–18.
[37] Mansuy JM, Gallian P, Dimeglio C, Saune K, Arnaud C, Pelletier B, et al. A nationwide survey of hepatitis E viral infection in French blood donors. Hepatology 2016;63:1145–1154.
[38] Huang F, Li Y, Yu W, Jing S, Wang J, Long F, et al. Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. Hepatology 2016;64:350–359.
[39] Baechlein C, Becher P. No evidence for zoonotic hepatitis E virus infection through dairy milk in Germany. Hepatology 2017;65:394–395.
[40] Bouamra Y, Gerolami R, Arzouni JP, Grimaud JC, Lafforgue P, Nelli M, et al. Emergence of autochthonous infections with hepatitis E virus of genotype 4 in Europe. Intervirology 2014;57:43–48.
[41] Holm DK, Moessner BK, Engle RE, Zaaijer HL, Georgsen J, Purcell RH, et al. Declining prevalence of hepatitis E antibodies among Danish blood donors. Transfusion 2015;55:1662–1667.
[42] Thom K, Gilhooly P, McGowan K, Malloy K, Jarvis L, Crossan C, et al. HEV in Scotland: evidence of recent increase in viral circulation in humans. *Euro Surveill.* in press.
[43] Ijaz S, Said B, Boxall E, Smit E, Morgan D, Tedder RS. Indigenous hepatitis E in England and wales from 2003 to 2012: evidence of an emerging novel phylotype of viruses. J Infect Dis 2014;209:1212–1218.
[44] Zaaijer HL. No artifact, hepatitis E is emerging. Hepatology 2015;62:654.
[45] Müller B, Koch H, Pichl L. PCR-Screening of blood donations for hepatitis E with the cobas HEV test performed on the new Roche cobas 8800 platform in minipools of 6. Transfus Med Hemother 2015;42:1–66.
[47] Lucarelli C, Spada E, Taliani G, Chionne P, Madonna E, Marcantonio C, et al. High prevalence of anti-hepatitis E virus antibodies among blood donors in central Italy, February to March 2014. Euro Surveill 2016;21.
[47] https://ecdc.europa.eu/en/news-events/1st-hepatitis-e-virus-network-hevnet-meeting, accessed March 2018.
[48] Manka P, Bechmann LP, Coombes JD, Thodou V, Schlattjan M, Kahraman A, et al. Hepatitis E virus infection as a possible cause of acute liver failure in Europe. Clin Gastroenterol Hepatol 2015;13:1836–1842, e1832; quiz e1157–e1838.
[49] Dalton HR, Stableforth W, Thurairajah P, Hazeldine S, Remnarace R, Usama W, et al. Autochthonous hepatitis E in Southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. Eur J Gastroenterol Hepatol 2008;20:784–790.
[50] Dalton HR, Bendall RP, Rashid M, Ellis V, Ali R, Ramnarace R, et al. Host risk factors and autochthonous hepatitis E infection. Eur J Gastroenterol Hepatol 2011;23:1200–1205.
[51] Blasco-Perrin H, Madden RG, Stanley A, Crossan C, Hunter JG, Vine L, et al. Hepatitis E virus in patients with decompensated chronic liver disease: a prospective UK/French study. Aliment Pharmacol Ther 2015;42:574–581.
[52] Haim-Boukobza S, Coilly A, Sebagh M, Bouamoud M, Antonini T, Roche B, et al. Hepatitis E infection in patients with severe acute alcoholic hepatitis. Liver Int 2015;35:870–875.
[53] Kamar N, Selves J, Mansuy JM, Ouezzani L, Peron JM, Guitard J, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med 2008;358:811–817.
[54] Kamar N, Mansuy JM, Cointault O, Selves J, Abravanel F, Danjoux M, et al. Hepatitis E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. Am J Transplant 2008;8:1744–1748.
[55] Gerolami R, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. N Engl J Med 2008;358:859–860.
[56] Kamar N, Rostaing L, Legrand-Abravanel F, Izopet J. How should hepatitis E virus infection be defined in organ-transplant recipients? Am J Transplant 2013;13:1935–1936.
[57] Meisner S, Polywka S, Memmler M, Nashan B, Lohse AW, Sterneck M, et al. Definition of chronic hepatitis E after liver transplant conforms to convention. Am J Transplant 2015;15:3011–3012.
[58] Kamar N, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C, 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.
[59] Kamar N, Abravanel F, Selves J, Garrouste C, Esposito L, Lavayssiere L, et al. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. Transplantation 2010;89:353–360.
[60] Pischke S, Stiefel P, Franz B, Bremer B, Suneetha PV, Heim A, et al. Chronic hepatitis e in heart transplant recipients. Am J Transplant 2012;12:3128–3133.

Please cite this article in press as: Negro F, Wedemeyer H. EASL Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol (2018), https://doi.org/10.1016/j.jhep.2018.03.005

[61] Legrand-Abravanel F, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. J Infect Dis 2010;202:835–844.
[62] Kamar N, Bendall RP, Peron JM, Cintas P, Prudhomme L, Mansuy JM, et al. Hepatitis E virus and neurologic disorders. Emerg Infect Dis 2011;17:173–179.
[63] Dalton HR, Kamar N, van Eijk JJ, McLean BN, Cintas P, Bendall RP, et al. Hepatitis E virus and neurological injury. Nat Rev Neurol 2016;12:77–85.
[64] Kamar N, Weclawiack H, Guilbeaud-Frugier C, Legrand-Abravanel F, Cointault O, Ribes D, et al. Hepatitis E virus and the kidney in solid-organ-transplant patients. Transplantation 2012;93:617–623.
[65] Dalton HR, Bendall R, Keane F, Tedder R, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. N Engl J Med 2009;361:1025–1027.
[66] Woolson KL, Forbes A, Vine L, Beynon L, McElhinney L, Panayi V, et al. Extra-hepatic manifestations of autochthonous hepatitis E infection. Aliment Pharmacol Ther 2014;40:1282–1291.
[67] van Eijk JJ, Madden RG, van der Eijk AA, Hunter JG, Reimerink JH, Bendall RP, et al. Neuralgic amyotrophy and hepatitis E virus infection. Neurology 2014;82:498–503.
[68] **van Eijk JJJ**, **Dalton HR**, **Ripellino P**, Madden RG, Jones C, Fritz M, et al. Clinical phenotype and outcome of hepatitis E virus-associated neuralgic amyotrophy. Neurology 2017;89(9):909–991.
[69] **Dalton HR**, **van Eijk JJ**, **Cintas P**, Madden R, Jones C, Webb G, et al. Hepatitis E infection and acute non-traumatic neurological injury: A prospective pilot multicenter study. J Hepatol 2017;67:925–932.
[70] Dalton HR, Seghatchian J. Hepatitis E virus: Emerging from the shadows in developed countries. Transfus Apher Sci 2016;55:271–274.
[71] van den Berg B, van der Eijk AA, Pas SD, Hunter JG, Madden RG, Tio-Gillen AP, et al. Guillain-Barre syndrome associated with preceding hepatitis E virus infection. Neurology 2014;82:491–497.
[72] Geurtsvankessel CH, Islam Z, Mohammad QD, Jacobs BC, Endtz HP, Osterhaus AD. Hepatitis E and Guillain-Barre syndrome. Clin Infect Dis 2013;57:1369–1370.
[73] Fukae J, Tsugawa J, Ouma S, Umezu T, Kusunoki S, Tsuboi Y. Guillain-Barre and Miller Fisher syndromes in patients with anti-hepatitis E virus antibody: a hospital-based survey in Japan. Neurol Sci 2016;37:1849–1851.
[74] Stevens O, Claeys KG, Poesen K, Saegeman V, Van Damme P. Diagnostic challenges and clinical characteristics of hepatitis E virus-associated Guillain-Barre syndrome. JAMA Neurol 2017;74:26–33.
[75] Kamar N, Izopet J, Cintas P, Garrouste C, Uro-Coste E, Cointault O, et al. Hepatitis E virus-induced neurological symptoms in a kidney-transplant patient with chronic hepatitis. Am J Transplant 2010;10:1321–1324.
[76] Dalton HR, Keane F, Bendall R, Mathew J, Ijaz S. Treatment of chronic hepatitis E in a HIV positive patient. Ann Intern Med 2011;155:479–480.
[77] Zhou X, Huang F, Xu L, Lin Z, de Vrij FMS, Ayo-Martin AC, et al. Hepatitis E virus infects neurons and brains. J Infect Dis 2017;214:361–368.
[78] Ali G, Kumar M, Bali S, Wadhwa W. Hepatitis E associated immune thrombocytopenia and membranous glomerulonephritis. Indian J Nephrol 2001;11:70–72.
[79] Taton B, Moreau K, Lepreux S, Bachelet T, Trimoulet P, De Ledinghen V, et al. Hepatitis E virus infection as a new probable cause of de novo membranous nephropathy after kidney transplantation. Transpl Infect Dis 2013;15:E211–E215.
[80] Del Bello A, Guilbeau-Frugier C, Josse AG, Rostaing L, Izopet J, Kamar N. Successful treatment of hepatitis E virus-associated cryoglobulinemic membranoproliferative glomerulonephritis with ribavirin. Transpl Infect Dis 2015;17:279–283.
[81] Guinault D, Ribes D, Delas A, Milongo D, Abravanel F, Puissant Lubrano B, et al. Hepatitis E virus-induced cryglobulinemic glomerulonephritis in a non-immunocompromised person. Am J Kidney Dis 2016;67:660–663.
[82] Pischke S, Polywka S, Haag F, Iking-Konert C, Sterneck M, Lutgehetmann M, et al. Association of hepatitis E virus and essential cryoglobulinemia? J Clin Virol 2015;67:23–24.
[83] Bazerbachi F, Leise MD, Watt KD, Murad MH, Prokop LJ, Haffar S. Systematic review of mixed cryoglobulinemia associated with hepatitis E virus infection: association or causation? Gastroenterol Rep 2017;5:178–184.
[84] Pischke S, Behrendt P, Manns MP, Wedemeyer H. HEV-associated cryoglobulinaemia and extrahepatic manifestations of hepatitis E. Lancet Infect Dis 2014;14:678–679.
[85] Sinha S, Jha R, Lakhtakia S, Narayan G. Acute pancreatitis following kidney transplantation - role of viral infections. Clin Transplant 2003;17:32–36.
[86] Bhagat S, Wadhawan M, Sud R, Arora A. Hepatitis viruses causing pancreatitis and hepatitis: a case series and review of literature. Pancreas 2008;36:424–427.
[87] Deniel C, Coton T, Brardjanian S, Guisset M, Nicand E, Simon F. Acute pancreatitis: a rare complication of acute hepatitis E. J Clin Virol 2011;51:202–204.
[88] Singh NK, Gangappa M. Acute immune thrombocytopenia associated with hepatitis E in an adult. Am J Hematol 2007;82:942–943.
[89] Colson P, Payraudeau E, Leonnet C, De Montigny S, Villeneuve L, Motte A, et al. Severe thrombocytopenia associated with acute hepatitis E virus infection. J Clin Microbiol 2008;46:2450–2452.
[90] Thapa R, Mallick D, Ghosh A. Childhood hepatitis E infection complicated by acute immune thrombocytopenia. J Pediatr Hematol Oncol 2009;31:151.
[91] Fourquet E, Mansuy JM, Bureau C, Recher C, Vinel JP, Izopet J, et al. Severe thrombocytopenia associated with acute autochthonous hepatitis E. J Clin Virol 2010;48:73–74.
[92] Abid S, Khan AH. Severe hemolysis and renal failure in glucose-6-phosphate dehydrogenase deficient patients with hepatitis E. Am J Gastroenterol 2002;97:1544–1547.
[93] Mishra P, Mahapatra M, Kumar R, Pati HP. Autoimmune hemolytic anemia and erythroid hypoplasia associated with hepatitis E. Indian J Gastroenterol 2007;26:195–196.
[94] Shah SA, Lal A, Idrees M, Hussain A, Jeet C, Malik FA, et al. Hepatitis E virus-associated aplastic anaemia: the first case of its kind. J Clin Virol 2012;54:96–97.
[95] Goyal A, Mishra DK, Kawar R, Kalmath BC, Sharma A, Gautam S. Hepatitis E associated myocarditis: an unusual entity. Bombay Hosp J 2009;51:361–362.
[96] Dumoulin FL, Liese H. Acute hepatitis E virus infection and autoimmune thyroiditis: yet another trigger? BMJ Case Rep 2012;2012.
[97] Thapa R, Biswas B, Mallick D. Henoch-Schonlein purpura triggered by acute hepatitis E virus infection. J Emerg Med 2010;39:218–219.
[98] Belbezier A, Deroux A, Sarrot-Reynauld F, Larrat S, Bouillet L. Myasthenia gravis associated with acute hepatitis E infection in immunocompetent woman. Emerg Infect Dis 2014;20:908–910.
[99] Baylis SA, Hanschmann KM, Blumel J, Nubling CMHEV Collaborative Study Group. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. J Clin Microbiol 2011;49:1234–1239.
[100] Baylis SA, Blumel J, Mizusawa S, Matsubayashi K, Sakata H, Okada Y, et al. World Health Organization International Standard to harmonize assays for detection of hepatitis E virus RNA. Emerg Infect Dis 2013;19:729–735.
[101] Baylis SA, Crossan C, Corman VM, Blumel J, Scobie L, Dalton HR. Unusual serological response to hepatitis E virus in plasma donors consistent with re-infection. Vox Sang 2015;109:406–409.
[102] Lan X, Yang B, Li BY, Yin XP, Li XR, Liu JX. Reverse transcription-loop-mediated isothermal amplification assay for rapid detection of hepatitis E virus. J Clin Microbiol 2009;47:2304–2306.
[103] Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods 2006;131:65–71.
[104] Garson JA, Ferns RB, Grant PR, Ijaz S, Nastouli E, Szypulska R, et al. Minor groove binder modification of widely used TaqMan probe for hepatitis E virus reduces risk of false negative real-time PCR results. J Virol Methods 2012;186:157–160.
[105] Hyams C, Mabayoje DA, Copping R, Maranao D, Patel M, Labbett W, et al. Serological cross reactivity to CMV and EBV causes problems in the diagnosis of acute hepatitis E virus infection. J Med Virol 2014;86:478–483.
[106] Bendall R, Ellis V, Ijaz S, Ali R, Dalton HR. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. J Med Virol 2010;82:799–805.
[107] Zhang F, Li X, Li Z, Harrison TJ, Chong H, Qiao S, et al. Detection of HEV antigen as a novel marker for the diagnosis of hepatitis E. J Med Virol 2006;78:1441–1448.
[108] Wen GP, Tang ZM, Yang F, Zhang K, Ji WF, Cai W, et al. A valuable antigen detection method for diagnosis of acute hepatitis E. J Clin Microbiol 2015;53:782–788.

Please cite this article in press as: Negro F, Wedemeyer H. EASL Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol (2018), https://doi.org/10.1016/j.jhep.2018.03.005

[109] Zhao C, Geng Y, Harrison TJ, Huang W, Song A, Wang Y. Evaluation of an antigen-capture EIA for the diagnosis of hepatitis E virus infection. J Viral Hepat 2015;22:957–963.
[110] Behrendt P, Bremer B, Todt D, Brown RJ, Heim A, Manns MP, et al. Hepatitis E virus (HEV) ORF2 antigen levels differentiate between acute and chronic HEV infection. J Infect Dis 2016;214:361–368.
[111] Montpellier C, Wychowski C, Sayed IM, Meunier JC, Saliou JM, Ankavay M, et al. Hepatitis E virus lifecycle and identification of 3 forms of the ORF2 capsid protein. Gastroenterology 2018;154:211–223, e218.
[112] Lenggenhager D, Gouttenoire J, Malehmir M, Bawohl M, Honcharova-Biletska H, Kreutzer S, et al. Visualization of hepatitis E virus RNA and proteins in the human liver. J Hepatol 2017;215:1197–1206.
[113] Davern TJ, Chalasani N, Fontana RJ, Hayashi PH, Protiva P, Kleiner DE, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. Gastroenterology 2011;141:1665–1672, e1661–e1669.
[114] Dalton HR, Fellows HJ, Stableforth W, Joseph M, Thurairajah PH, Warshow U, et al. The role of hepatitis E virus testing in drug-induced liver injury. Aliment Pharmacol Ther 2007;26:1429–1435.
[115] Satake M, Matsubayashi K, Hoshi Y, Taira R, Furui Y, Kokudo N, et al. Unique clinical courses of transfusion-transmitted hepatitis E in patients with immunosuppression. Transfusion 2017;57:280–288.
[116] Peron JM, Dalton HR, Izopet J, Kamar N. Acute autochthonous hepatitis E in western patients with underlying chronic liver disease: a role for ribavirin? J Hepatol 2011;54:1323–1324, [Author reply 1324–1325].
[117] Zhou X, Wang Y, Metselaar HJ, Janssen HL, Peppelenbosch MP, Pan Q. Rapamycin and everolimus facilitate hepatitis E virus replication: revealing a basal defense mechanism of PI3K-PKB-mTOR pathway. J Hepatol 2014;61:746–754.
[118] Wang Y, Zhou X, Debing Y, Chen K, Van Der Laan LJ, Neyts J, et al. Calcineurin inhibitors stimulate and mycophenolic acid inhibits replication of hepatitis E virus. Gastroenterology 2014;146:1775–1783.
[119] Kamar N, Rostaing L, Abravanel F, Garrouste C, Esposito L, Cardeau-Desangles I, et al. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. Clin Infect Dis 2010;50:e30–e33.
[120] Haagsma EB, Riezebos-Brilman A, van den Berg AP, Porte RJ, Niesters HG. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. Liver Transpl 2010;16:474–477.
[121] Kamar N, Abravanel F, Garrouste C, Cardeau-Desangles I, Mansuy JM, Weclawiak H, et al. Three-month pegylated interferon-alpha-2a therapy for chronic hepatitis E virus infection in a haemodialysis patient. Nephrol Dial Transplant 2010;25:2792–2795.
[122] Rostaing L, Izopet J, Baron E, Duffaut M, Puel J, Durand D. Treatment of chronic hepatitis C with recombinant interferon alpha in kidney transplant recipients. Transplantation 1995;59:1426–1431.
[123] Mallet V, Nicand E, Sultanik P, Chakvetadze C, Tesse S, Thervet E, et al. Brief communication: case reports of ribavirin treatment for chronic hepatitis E. Ann Intern Med 2010;153:85–89.
[124] Kamar N, Rostaing L, Abravanel F, Garrouste C, Lhomme S, Esposito L, et al. Ribavirin therapy inhibits viral replication in patients with chronic hepatitis E virus infection. Gastroenterology 2010;139:1612–1618.
[125] Pischke S, Hardkte S, Bode U, Birkner S, Chatzikyrkou C, Kauffmann W, et al. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. Liver Int 2013;33:722–726.
[126] Kamar N, Mallet V, Izopet J. Ribavirin for chronic hepatitis E virus infection. N Engl J Med 2014;370:2447–2448.
[127] Kamar N, Chatelut E, Manolis E, Lafont T, Izopet J, Rostaing L. Ribavirin pharmacokinetics in renal and liver transplant patients: evidence that it depends on renal function. Am J Kidney Dis 2004;43:140–146.
[128] Debing Y, Emerson SU, Wang Y, Pan Q, Balzarini J, Dallmeier K, et al. Ribavirin inhibits in vitro hepatitis E virus replication through depletion of cellular GTP pools and is moderately synergistic with alpha interferon. Antimicrob Agents Chemother 2014;58:267–273.
[129] Pischke S, Wedemeyer H. Hepatitis E virus infection: multiple faces of an underestimated problem. J Hepatol 2013;58:1045–1046.
[130] Kamar N, Lhomme S, Abravanel F, Cointault O, Esposito L, Cardeau-Desangles I, et al. An early viral response predicts the virological response to ribavirin in hepatitis E virus organ transplant patients. Transplantation 2015;99:2124–2131.
[131] Debing Y, Gisa A, Dallmeier K, Pischke S, Bremer B, Manns M, et al. A mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. Gastroenterology 2014;147:1008–1011.
[132] Lhomme S, Kamar N, Nicot F, Ducos J, Bismuth M, Garrigue V, et al. Mutation in the hepatitis E virus polymerase and outcome of ribavirin therapy. Antimicrob Agents Chemother 2015;60:1608–1614.
[133] Todt D, Gisa A, Radonic A, Nitsche A, Behrendt P, Suneetha PV, et al. In vivo evidence for ribavirin-induced mutagenesis of the hepatitis E virus genome. Gut 2016;65:1733–1743.
[134] Debing Y, Ramiere C, Dallmeier K, Piorkowski G, Trabaud MA, Lebosse F, et al. Hepatitis E virus mutations associated with ribavirin treatment failure result in altered viral fitness and ribavirin sensitivity. J Hepatol 2016;147:1008–1011.
[135] Kamar N, Izopet J, Tripon S, Bismuth M, Hillaire S, Dumortier J, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. N Engl J Med 2014;370:1111–1120.
[136] Abravanel F, Lhomme S, Rostaing L, Kamar N, Izopet J. Protracted fecal shedding of HEV during ribavirin therapy predicts treatment relapse. Clin Infect Dis 2015;60:96–99.
[137] Kamar N, Wang W, Dalton HR, Pan Q. Direct-acting antiviral therapy for hepatitis E virus? Lancet Gastroenterol Hepatol 2017;2:154–155.
[138] van der Valk M, Zaaijer HL, Kater AP, Schinkel J. Sofosbuvir shows antiviral activity in a patient with chronic hepatitis E virus infection. J Hepatol 2017;66:242–243.
[139] Donnelly MC, Imlach SN, Abravanel F, Ramalingam S, Johannessen I, Petrik J, et al. Sofosbuvir and daclatasvir anti-viral therapy fails to clear HEV viremia and restore reactive T cells in a HEV/HCV co-infected liver transplant recipient. Gastroenterology 2017;152:300–301.
[140] Alric L, Bonnet D, Laurent G, Kamar N, Izopet J. Chronic hepatitis E virus infection: successful virologic response to pegylated interferon-{alpha} therapy. Ann Intern Med 2010;153:135–136.
[141] Alric L, Bonnet D, Beynes-Rauzy O, Izopet J, Kamar N. Definitive clearance of a chronic hepatitis E virus infection with ribavirin treatment. Am J Gastroenterol 2011;106:1562–1563.
[142] Tavitian S, Peron JM, Huguet F, Kamar N, Abravanel F, Beyne-Rauzy O, et al. Ribavirin for chronic hepatitis prevention among patients with hematologic malignancies. Emerg Infect Dis 2015;21:1466–1469.
[143] Neukam K, Barreiro P, Macias J, Avellon A, Cifuentes C, Martin-Carbonero L, et al. Chronic hepatitis E in HIV patients: rapid progression to cirrhosis and response to oral ribavirin. Clin Infect Dis 2013;57:465–468.
[144] Hajji H, Gerolami R, Solas C, Moreau J, Colson P. Chronic hepatitis E resolution in a human immunodeficiency virus (HIV)-infected patient treated with ribavirin. Int J Antimicrob Agents 2013;46:595–597.
[145] Johne R, Trojnar E, Filter M, Hofmann J. Thermal stability of hepatitis E virus as estimated by a cell culture method. Appl Environ Microbiol 2016;82:4225–4231.
[146] Dalton HR, Bendall RP, Pritchard C, Henley W, Melzer D. National mortality rates from chronic liver disease and consumption of alcohol and pig meat. Epidemiol Infect 2010;138:174–182.
[147] Montella F, Rezza G, Di Sora F, Pezzotti P, Recchia O. Association between hepatitis E virus and HIV infection in homosexual men. Lancet 1994;344:1433.
[148] Payne BA, Medhi M, Ijaz S, Valappil M, Savage EJ, Gill ON, et al. Hepatitis E virus seroprevalence among men who have sex with men, United Kingdom. Emerg Infect Dis 2013;19:333–335.
[149] Keane F, Gompels M, Bendall R, Drayton R, Jennings L, Black J, et al. Hepatitis E virus coinfection in patients with HIV infection. HIV Med 2012;13:83–88.
[150] Sayed IM, Verhoye L, Cocquerel L, Abravanel F, Foquet L, Montpellier C, et al. Study of hepatitis E virus infection of genotype 1 and 3 in mice with humanised liver. Gut 2017;66:920–929.
[151] Allweiss L, Gass S, Giersch K, Groth A, Kah J, Volz T, et al. Human liver chimeric mice as a new model of chronic hepatitis E virus infection and preclinical drug evaluation. J Hepatol 2016;64:1033–1040.
[152] Geng Y, Zhao C, Huang W, Harrison TJ, Zhang H, Geng K, et al. Detection and assessment of infectivity of hepatitis E virus in urine. J Hepatol 2016;64:37–43.
[153] Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010;376:895–902.
[154] Zhang J, Zhang XF, Huang SJ, Wu T, Hu YM, Wang ZZ, et al. Long-term efficacy of a hepatitis E vaccine. N Engl J Med 2015;372:914–922.
[155] Brown A, Halliday JS, Swadling L, Madden RG, Bendall R, Hunter JG, et al. Characterization of the specificity, functionality, and durability of host T-cell responses against the full-length hepatitis E virus. Hepatology 2016;64:1934–1950.

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[156] Wu T, Zhu FC, Huang SJ, Zhang XF, Wang ZZ, Zhang J, et al. Safety of the hepatitis E vaccine for pregnant women: a preliminary analysis. Hepatology 2012;55:2038.
[157] Baylis SA, Koc O, Nick S, Blumel J. Widespread distribution of hepatitis E virus in plasma fractionation pools. Vox Sang 2012;102:182–183.
[158] Cleland A, Smith L, Crossan C, Blatchford O, Dalton HR, Scobie L, et al. Hepatitis E virus in Scottish blood donors. Vox Sang 2013;105:283–289.
[159] Stramer SL, Moritz ED, Foster GA, Ong E, Linnen JM, Hogema BM, et al. Hepatitis E virus: seroprevalence and frequency of viral RNA detection among US blood donors. Transfusion 2016;56:481–488.

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