BTS
British Transplantation Society

The Voice of Transplantation in the UK

Guidelines for Hepatitis E & Solid Organ Transplantation

First Edition

Compiled by a Working Party of
The British Transplantation Society
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British Transplantation Society Guidelines

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1 INTRODUCTION

1.1 The Need for Guidelines

The incidence and prevalence of hepatitis E virus (HEV) infection has increased in many developed countries over the last decade. It has also been recognised that HEV infection can persist in immunosuppressed individuals, leading if left untreated to chronic hepatitis and significant liver fibrosis. Transplant recipients are therefore at risk of developing persistent HEV infection. There are currently no international guidelines on the management of hepatitis E in transplant recipients. These guidelines have therefore been developed to inform clinical teams and patients about hepatitis E, to help increase the recognition of persistent hepatitis E infection, and to provide clear guidance on its management.

1.2 Process of Writing and Methodology

The British Transplantation Society formed a guideline development group in May 2016, which was chaired by Dr Stuart McPherson. The guideline was produced in line with BTS Clinical Practice Guideline development policy and the recommendations of NHS Evidence (1). A literature search was conducted by the writing team using PubMed® to identify the relevant evidence. Search terms included combinations of hepatitis E, HEV, transplant, transplantation, immunosuppression, treatment, ribavirin, antiviral, and blood transfusion.

The first draft of the guideline was written between May and December 2016 by a team that included Dr Stuart McPherson, Mr James Powell, Prof Richard Tedder, Dr Samreen Ijaz, Dr Ian Rowe and Dr Michael Ankcorn. Contributions were also received from Dr Ken Simpson and Dr Ines Ushiro-Lumb. A consensus meeting of the guideline development group was held in January 2017 to agree the recommendations and strength of grading. The preliminary draft guideline was reviewed by members of the guideline development group and revised by Dr Stuart McPherson and Dr Ahmed Elsharkawy.

The draft guidelines were edited by Dr Peter Andrews, Chair of the BTS Standards Committee, and opened for public consultation through the website of the British Transplantation Society in April 2017. Comments from organisations and individuals representing relevant patient groups were specifically encouraged. Following revision, the final guidelines were published in June 2017.

These guidelines will next be revised in 2021.

1.3 Guideline Development Group

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1.4 Declarations of Interest

Dr Stuart McPherson – speaker, consultancy or travel support from AbbVie, BMS, Gilead, MSD, Novartis and Roche

Dr Ahmed Elsharkawy - speaker, consultancy, research grant or travel support from Abbvie, Astellas, BMS, Chiesi, Gilead and MSD

Dr Ian Rowe – speaker or travel support from AbbVie, Bayer and Norgine

Mr James Powell – none

Dr Michael Ankcorn – none

Dr Samreen Ijaz – none

Prof Richard Tedder – none

1.5 Grading of Recommendations

These guidelines represent consensus opinion from experts in the field of transplantation in the United Kingdom. They represent a snapshot of the evidence available at the time of writing. It is recognised that recommendations are made even when the evidence is weak. It is felt that this is helpful to clinicians in daily practice. In these guidelines the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system has been used to rate the strength of evidence and the strength of recommendations (2). The approach used in producing the present guidelines is consistent with that adopted by Kidney Disease Improving Global Outcomes (KDIGO) (3,4). Explicit recommendations are made on the basis of the trade-offs between the benefits on the one hand, and the risks, burden, and costs on the other.

For each recommendation the quality of evidence has been graded as:

A (high)

B (moderate)

C (low)

D (very low)

Grade A evidence means high quality evidence that comes from consistent results from well performed randomised controlled trials, or overwhelming evidence of another sort (such as well-executed observational studies with very strong effects).

Grade B evidence means moderate quality evidence from randomised trials that suffer from serious flaws in conduct, inconsistency, indirectness, imprecise estimates, reporting bias, or some combination of these limitations, or from other study designs with special strength.

Grade C evidence means low quality evidence from observational evidence, or from controlled trials with several very serious limitations.

Grade D evidence is based only on case studies or expert opinion.

For each recommendation, the strength of recommendation has been indicated as one of:

Level 1 (we recommend)

Level 2 (we suggest)

Not graded (where there is not enough evidence to allow formal grading)

A **Level 1** recommendation is a strong recommendation to do (or not do) something where the benefits clearly outweigh the risks (or vice versa) for most, if not all patients.

A **Level 2** recommendation is a weaker recommendation, where the risks and benefits are more closely balanced or are more uncertain.

1.6 Abbreviations

ACLF	acute on chronic liver failure
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
G	genotype
GGT	gamma glutamyl transferase
HEV	hepatitis E virus
HIV	human immunodeficiency virus
Ig	immunoglobulin
NAAT	nucleic acid amplification test
NHSBT	National Health Service Blood and Transplant
ORF	open reading frames
PCP	papain-like cysteine protease
PEG	polyethylene glycol
RdRp	RNA dependent RNA polymerase
RNA	ribonucleic acid
SaBTO	UK Advisory Committee for the Safety of Blood, Tissues and Organs
SOHO	substances of human origin
UTR	untranslated regions

1.7 Definitions of Hepatitis E used in this Guideline

- **Hepatitis E** Clinical hepatitis caused by acute HEV infection
- **Acute HEV** Acute infection with HEV that may or may not be symptomatic
- **Persistent HEV** HEV RNA detectable for three months or more

1.8 Disclaimer

This document provides a guide to best practice, which inevitably evolves over time. All clinicians involved in this aspect of transplantation need to undertake clinical care on an individualised basis and keep up to date with changes in the practice of clinical medicine. These guidelines represent the collective opinions of a number of experts in the field and do not have the force of law. They contain information/guidance for use by practitioners as a best practice tool. It follows that the guidelines should be interpreted in the spirit rather than to the letter of their contents. The opinions presented are subject to change and should not be used in isolation to define the management for any individual patient. The guidelines are not designed to be prescriptive, nor to define a standard of care. The British Transplantation Society cannot attest to the accuracy, completeness or currency of the opinions contained herein and do not accept any responsibility or liability for any loss or damage caused to any practitioner or any third party as a result of any reliance being placed on the guidelines or as a result of any inaccurate or misleading opinion contained in the guidelines.

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2 EXECUTIVE SUMMARY OF RECOMMENDATIONS

Hepatitis E Biology and Disease

We recommend that:

- Virus specific tests, including HEV RNA and/or antigen detection, must be used to diagnose HEV infection in transplant recipients as antibody detection is unreliable in immunosuppressed individuals. (1B)

We suggest that:

- All clinicians managing transplant recipients should receive specific training about HEV (acute and persistent) as its prevalence is increasing and the clinical consequences of infection can be significant. (Not graded)

Testing of Solid Organ Donors for Hepatitis E

We recommend that:

- All solid organ donors are screened for HEV in line with the UK Advisory Committee for the Safety of Blood, Tissues and Organs (SaBTO) recommendations. (1C)

We suggest that:

- The detection of HEV viraemia in a donor is not an absolute contra-indication to use of an organ from that donor, but will inform clinical management decisions post-transplant. (2C)
- Individuals who become infected with HEV through transplantation are managed according to recommendations pertaining to other persistently infected individuals. (2C)

Prevention of Hepatitis E in Solid Organ Transplant Recipients

We recommend that:

- Individuals must receive written advice regarding the risk of HEV from undercooked meat (particularly processed pork) before and after transplantation. (1D)

Surveillance and Screening for HEV in Solid Organ Transplant Recipients

We recommend that:

- Potential recipients of solid organ transplants do not need routine screening for HEV infection. There may be specific instances where testing for HEV is indicated pre-transplantation, such as in an immunosuppressed individual with raised liver enzymes. (D1)
- Solid organ transplant recipients with liver transaminases above the upper limit of normal or symptoms suggestive of HEV infection are tested for HEV using an HEV RNA or an antigen assay. (1C)

We suggest that:

- Transplant recipients have a plasma sample taken at the time of transplantation and stored for a minimum of one year that could be tested retrospectively for HEV or other infections. (2D)

Treatment of Acute Hepatitis E in a Patient on the Transplant List

We suggest that:

- Individuals with unexplained acute on chronic or acute liver failure should be tested for HEV. (2C)
- Treatment with ribavirin is considered for patients with cirrhosis who develop hepatitis E when on the liver transplant waiting list. (2D)

Management of HEV Infection in Solid Organ Transplant recipients

Newly diagnosed or acute HEV infection

We suggest that:

- The initial management of newly diagnosed or acute HEV infection in solid organ transplant recipients includes observation and monitoring of HEV RNA levels and liver enzymes as more than 30% will spontaneously clear the infection within three months. Dynamic viral monitoring and antibody profiling may help clinical decision-making. (2C)

- A strategic reduction in immunosuppression is considered in patients with acute or persistent HEV as this may facilitate viral clearance, but the risk of rejection should be carefully assessed. (2C)
- Early treatment with ribavirin may be considered in specific cases of acute hepatitis E, such as patients who develop severe liver dysfunction (jaundice and coagulopathy) or extra-hepatic manifestations, although evidence for this recommendation is currently limited. (2D)

Persistent HEV infection

We recommend that:

- Persistent HEV infection is diagnosed when HEV RNA is detectable in blood or stool for more than three months after the onset of relevant symptoms, raised liver enzymes, or from the first positive HEV RNA test. (1C)
- Individuals with persistent HEV infection (documented or estimated duration of infection of more than three months) receive treatment with ribavirin with the aim of achieving sustained virological response (HEV RNA not detected in plasma and stool six months after completion of treatment). (1C)
- A baseline quantitative HEV RNA assessment is undertaken on both plasma and stool at the start of treatment. (1C)
- Treatment with ribavirin should continue for at least three months for solid organ transplant recipients with persistent HEV infection. For most individuals 3-6 months of ribavirin treatment will suffice. (1C)
- Monthly HEV RNA testing in plasma and stool is undertaken until a decision is made to stop treatment. (1C)
- Ribavirin is continued until stool tests are negative for HEV RNA on two occasions one month apart, as continued shedding of HEV in stool is an important factor predicting relapse after ribavirin treatment. (1C)
- A test of sustained virological response is conducted by testing plasma and stool samples for HEV RNA at three and six months after stopping antiviral therapy. (1C)
- Regular haemoglobin monitoring is conducted during ribavirin therapy as haemolytic anaemia is a common treatment-related side effect. Ribavirin dose reduction may be required during treatment to maintain an adequate haemoglobin concentration. Epoetin therapy and/or blood transfusion may be indicated to allow continued antiviral therapy without avoidable drug reduction. (1A)

- PEG-interferon should not be used as first line for the treatment of persistent HEV in transplant recipients as there is a moderate risk of precipitating organ rejection. (1D)

We suggest that:

- Assessment of the change in plasma HEV RNA after seven days of ribavirin treatment may help predict the chance of achieving sustained virological response after three months of ribavirin treatment. We therefore suggest quantitative testing of a plasma sample taken at day seven of ribavirin treatment to help determine the likely length of treatment required. (2C)
- To minimise treatment-related side-effects, the dosage of ribavirin is adapted according to creatinine clearance, estimated using the Cockcroft-Gault equation. (2C)
- Patients with persistent HEV who relapse after a first course of ribavirin are re-treated for at least six months with ribavirin at dosages toward the higher dose range, where tolerated. (2D)
- Routine baseline sequencing of HEV for mutations is not indicated prior to antiviral treatment as the significance of mutations has not been determined. (2D)
- PEG-interferon treatment may be considered in cases of ribavirin-refractory persistent HEV infection. However, patients will require very close monitoring for rejection. (2D)

3 HEPATITIS E VIRUS BIOLOGY AND DISEASE

Statements of Recommendation

We recommend that:

- Virus specific tests, including HEV RNA and/or antigen detection, must be used to diagnose HEV infection in transplant recipients as antibody detection is unreliable in immunosuppressed individuals. (1B)

We suggest that:

- All clinicians managing transplant recipients should receive specific training about HEV (acute and persistent) as its prevalence is increasing and the clinical consequences of infection can be significant. (Not graded)

3.1 Introduction

Hepatitis E virus (HEV) belongs to the genus *Hepevirus* in the *Hepeviridae* family and infects humans and a range of animal hosts (1). Studies of evolutionary history indicate that HEV has evolved through a series of events in which ancestral HEV may have adapted to a succession of animal hosts leading to human beings (2). Four major HEV genotypes infect humans (G1 to G4) and are remarkable in their associated divergences, leading HEV to be aptly described as having 'two faces' (3). The epidemiological picture, transmission routes and reservoirs, as well as clinical features and outcome differ significantly depending on the region of the world and accordingly, the HEV genotype. G1 and G2 are restricted to the human host. G1 occurs in Asia and Africa, with G2 reported from Mexico and also Africa. G3 has a worldwide distribution and is associated with infection in humans, pigs and other mammalian species; in contrast, G4 only infects humans and pigs, principally in South East Asia. G4 also occurs in pigs in India, and occasionally in Europe.

3.2 Epidemiology

Developing World

HEV G1 and G2 viruses remain major public health concerns in resource poor settings, where HEV is thought to be responsible for >50% of cases of viral hepatitis. The virus is transmitted via the faecal-oral route through the consumption of contaminated food and water. Person-to-person spread is

uncommon. There is a striking age-related clinical picture with disease mainly reported from young adults between the age of 15 and 39 years with a slight male preponderance (4). Recent studies in populations from Nepal and Bangladesh indicate antibody prevalence rates of 47% and 50% respectively with no differences noted by gender (5). Seroprevalence increased with age and demonstrated low prevalence in children <10 years. As well as sporadic infection, the virus is linked to large waterborne outbreaks that can affect many thousands of individuals (6-8). More recent outbreaks have been reported from camps for displaced persons and refugees in Africa, where significant mortality is observed in pregnant women and in children under the age of two years (9).

Developed World

In the developed world, HEV G3 and G4 are zoonotic infections, being transmitted to humans from an animal reservoir. Case control studies have indicated that the consumption of pork products (particularly processed) and game meat are associated with HEV infection (10-11). The pig remains the best studied vector and the concept of a zoonosis is supported by the close sequence homology shared between human and swine HEV sequences. Human infections are essentially 'dead end' infections with person-to-person transmission being uncommon, although a few sporadic clusters have been observed related to the consumption of undercooked meat (12-14).

Surveillance of hepatitis E shows a remarkably consistent demographic picture with the majority of clinical cases reported in males over the age of 50 years (15-17). Seroprevalence rates vary widely from 1% to 50% (3,18-19), variation traditionally attributed to the performance of the different assays used. However, it is now accepted that significant variances in seroprevalence exist between and within countries, indicating local differences in risk and exposure (18,20). Data from England collected over ten years indicate that the infection is dynamic in the population, suggesting fluctuations in risk over time (15). Estimates of the burden of infection in the general population of England suggest as many as 200,000 infections occur annually and account for around 600-800 cases of hepatitis E. What influences the fluctuations in the prevalence of viraemia and disease is unclear. There is an observed cohort effect with an increase in seroprevalence with age and low rates in children (21).

Hepatitis E Virus in England

Enhanced surveillance data from England collected over ten years indicate that the infection is dynamic in the population, suggesting fluctuations in risk over time (15). Parallel molecular characterisation indicates genotype 3 virus to be linked to indigenous infection in England with analysis indicating two phylogenetically distinct clades, group 1 and group 2. A breakdown of the

proportion of circulating virus demonstrated group 2 viruses emerged in 2008, and since 2011 these have been the dominant virus linked to indigenous infection in England (15). It is unclear what factors influence the fluctuation in the prevalence of viraemia and disease, but the year on year increase in case numbers noted since 2010 seems to be linked to the emergence of group 2 viruses.

Seroprevalence rates in the general population is high at ~13% (21) with data from modelling work and from extrapolation of HEV-infected donors indicating that up to 200 000 HEV infections occur per year and that these account for around 600-800 cases of hepatitis in England. Data from the selective screening programme implemented by NHS Blood and Transplant in March 2016 indicate that 1 in 2500 donations are HEV RNA positive (data correct in February 2017).

Case control studies using food based questionnaires show an association between the consumption of pork products and HEV infection in England (10). National surveys have shown that 93% of UK pigs are HEV antibody positive, with 20% having detectable HEV RNA in either plasma or caecal samples at time of slaughter. Sequence analysis indicate that all but one of the pigs harbour genotype 3, group 1 viruses raising the question as to the main source of HEV infection in England.

Persistent HEV infections are increasingly recognised, reflecting increased awareness and testing. PHE surveillance shows these infections occur across a broad range of immunosuppressed patient groups (solid organ transplantation, haematopoietic stem cell transplantation, haemato-oncology and HIV-infected) but also in atypical immunosuppressed patients such as those with rheumatoid arthritis or inflammatory bowel disease. As expected, the management of these patients is varied and where patients are being treated, Ribavirin is the drug of choice. Whilst clearance has been noted in the majority of treated patients, viral relapse is increasingly recognised.

3.3 Transfusion Transmitted HEV

The prevalence of asymptomatic infection in blood donors has raised the concern of infection via blood components. Studies in blood donors report a wide range of seroprevalence rates from 6% to 46% (18). With the exception of Scotland where 1 in 14520 blood donors tested were viraemic, data from Europe demonstrate a high HEV RNA prevalence rate in blood donations ranging from 1 in 762 in the Netherlands (2013/2014) to 1 in 1240-4525 in Germany (2011) and 1 in 2848 in England (2012/2013)(18). In contrast, no HEV RNA positive donations were reported in Canadian and Australian studies, with lower RNA prevalence rates seen in Japan and USA (18,22-24).

An investigation undertaken in England in recipients of HEV-containing blood components showed that 18 (42%) of 43 went on to develop HEV infection. Follow up of the HEV-infected recipients

indicated that the outcome of infection was complex, but that patients treated with medium or high doses of immunosuppressive drugs developed prolonged or persistent HEV with a delayed or absent immune response (25).

Mitigation of risk of HEV transmission through pathogen inactivation/reduction has limited efficacy. Being non-enveloped, HEV is not sensitive to solvent/detergent treatment, and pathogen reduction protocols which denature DNA and RNA have also been shown to be ineffective (26-27). Some countries have implemented universal or selective screening protocols for the provision of HEV RNA negative blood components. In the UK, universal screening of all blood components for HEV is now recommended by the UK Advisory Committee for the Safety of Blood, Tissues and Organs (SaBTO) on the basis of superior cost effectiveness of universal over selective testing for HEV.

3.4 HEV Replication

The hepatitis E virion is a 27-32nm spherical particle that is icosahedral in symmetry and has spikes on the capsid surface (28). The genome is a single-stranded, 7.2Kb RNA of positive sense with a 7-methylguanylate (7mG) cap at its 5' end and a poly-A tail at its 3' end (29). The genome has short 5' and 3' untranslated regions (UTRs) and three open reading frames (ORF). ORF1 encodes for a polyprotein with several putative functional motifs and domains including methyltransferase, papain-like cysteine protease (PCP), RNA helicase and RNA dependent RNA polymerase (RdRp), which are involved in the replication and processing of viral proteins (30). ORF2 encodes the major viral capsid protein which encapsidates the viral RNA genome and has three defined domains: the shell, middle and protruding domains (31). A number of studies investigating neutralising epitopes have mapped these to be in the protruding domain of the ORF2 protein with residues 452-617 identified to be important (32). The viral capsid is also thought to be involved with cellular proteins for the purpose of cell entry, capsid assembly and virus egress. The ORF3 encodes a small phosphoprotein which associates with the cytoskeleton and more specifically with microtubules and is thought to be essential for the release of virus from infected cells (28).

The life cycle of HEV remains poorly understood, mainly because of the lack of efficient *in vitro* culture methods. The viral particles concentrate on the surface of hepatocytes, bind to an undefined receptor and are internalised. Following uncoating, the genomic RNA is released and translated in the cytoplasm into the non-structural proteins (33). The viral polymerase RdRP then replicates the positive-sense genomic RNA into negative strand transcripts. These serve as templates for the synthesis of a 2.2kb subgenomic RNA as well as full-length positive sense transcripts. The positive sense subgenomic RNA is translated into ORF2 and ORF3 proteins (34-35). The capsid proteins

package the viral genome to assemble progeny virions. Viral egress is thought to require the cellular secretory machinery together with the ORF3 protein. More recent data have shown that virus secreted into the bloodstream is associated with the ORF3 protein and wrapped by a lipid cellular membrane, whilst virus secreted into the bile and thus in the stool is non-enveloped (36).

3.5 Clinical Features

Acute Hepatitis E

The clinical features of HEV infection range from asymptomatic infection to mild hepatitis to fulminant liver failure and are influenced greatly by genotype and by the age and gender of the patient. Symptoms, if they occur, include general malaise, abdominal pain, anorexia, nausea and fever and are followed by the onset of jaundice accompanied by dark urine, pale stools and pruritis. Most infections are self-limiting. Data reported mainly from the G1 virus in the developing world suggest a mortality rate of between 0.5 – 4% (3). This increases markedly to approximately 25% among pregnant women, particularly in the third trimester (37). Spontaneous abortion, stillbirth and neonatal death are also increased. This poor outcome of infection during pregnancy appears only to be associated with G1 infection and is seen not with G3 infection. Less than 1% of G3 infections cause clinical hepatitis and acute liver failure is very rare. Acute hepatitis E in patients with underlying liver disease may lead to decompensation and a poor outcome (38-40).

Persistent HEV Infection

Persistent infection leading to chronic hepatitis has been reported in immunosuppressed populations including solid organ transplant recipients, patients with haematological disorders receiving chemotherapy, and HIV-infected individuals (40-46). With the exception of one G4 (47) and one G7 (camelid) (48) virus, all chronic HEV infections have been G3. Persistent infections with G1 and G2 viruses have not been reported. The clinical features of persistent HEV infection are often unremarkable. Liver transaminases are usually only very modestly raised and few patients present with any symptoms (42). Once infected, 60% of solid organ transplant recipients fail to clear the virus and are at risk of developing chronic hepatitis (41). Liver biopsy shows rapid progression of liver fibrosis with 10% of patients progressing to cirrhosis over a few years (42,48). Factors such as low leucocyte, total lymphocyte and T-cell counts are associated with failure to clear HEV (42). In the HIV setting, patients who develop chronic infection have low CD4 counts (46,50-51). Viral clearance following treatment in HIV-infected individuals is associated with the recovery of CD4 levels (46,51) and can present as an immune reconstitution hepatitis (52).

Extrahepatic Manifestations

A number of extrahepatic manifestations linked both to acute and to persistent hepatitis E infection have been reported. These include thrombocytopenia, glomerulonephritis, acute pancreatitis and acute thyroiditis (53-55). A range of neuropathologies have also been described including brachial neuritis, Guillain-Barré syndrome, peripheral neuropathy, neuromyopathy, and vestibular neuritis (56-60).

3.6 Diagnosis of Hepatitis E

Acute hepatitis E cannot be clinically distinguished from other causes of acute hepatitis. Diagnosis of HEV infection can be undertaken using methods for detecting antibody, antigen and RNA.

After an incubation period of 2-6 weeks, the immune response to HEV follows a typical pattern: an initial short-lived IgM response followed by more durable IgG antibodies (61). Although there are four human HEV genotypes, they elicit very similar antibody responses and appear to represent a single serotype (62-63). Enzyme immunoassays or rapid immunochromatographic kits use a range of recombinant viral antigens for the detection of specific IgM antibodies. The anti-HEV IgM titres increase rapidly and then wane over the weeks following infection. Anti-HEV IgG antibodies are detected shortly after the IgM and continue to rise into the convalescence period, remaining detectable for months to years.

Antigen detection has been used recently for the diagnosis of HEV infection. Antigen ELISAs are less sensitive than molecular methods; however, they provide a more rapid and accessible method for identifying current HEV infection (64-67).

Detection of HEV RNA is important in the diagnosis, confirmation and monitoring of HEV infection. In patients with an acute HEV infection, peak viraemia occurs during the incubation and early phase of the disease. Viral RNA can be detected a few weeks before the onset of clinical symptoms in both blood and stool samples. Plasma HEV RNA does not persist in the immunocompetent host. The viraemia lasts on average for eight weeks, becoming undetectable in blood approximately three to four weeks after the onset of symptoms. Viral shedding in stool continues beyond plasma viral clearance in both acute and treated persistent infection (68).

The clinical diagnosis of persistent hepatitis E infection is challenging, as these infections are largely asymptomatic. Testing strategies for identifying individuals with persistent infection are not clear and mean that infection can remain undiagnosed for years. As persistent infection often occurs in

immunosuppressed individuals, the majority of whom will have complex underlying conditions and management strategies, these infections can also be misdiagnosed as drug-induced liver injury or graft versus host disease (69-70), sometimes with catastrophic consequences. Laboratory diagnosis of persistent HEV must be through detection of the virus itself, either through HEV RNA testing or HEV antigen testing, as antibody detection in the immunosuppressed population is not a reliable marker of infection.

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4 TESTING OF SOLID ORGAN DONORS FOR HEPATITIS E

Statements of Recommendation

We recommend that:

- All solid organ donors are screened for HEV in line with the UK Advisory Committee for the Safety of Blood, Tissues and Organs (SaBTO) recommendations. (1C)

We suggest that:

- The detection of HEV viraemia in a donor is not an absolute contra-indication to use of an organ from that donor, but will inform clinical management decisions post-transplant. (2C)
- Individuals who become infected with HEV through transplantation are managed according to recommendations pertaining to other persistently infected individuals. (2C)

4.1 Introduction

Although HEV is normally acquired through the oral (enteral) route, it can be transmitted at the time of solid organ transplantation, either with the transplanted organ or through blood components from an HEV-infected donor. Pre-transplant immunosuppression may allow potential recipients to become persistently infected before transplantation, and acute infection may also occur in the potential transplant recipient. Peri-transplant haematological support may also confer additional HEV risk. Case reports confirm transmission of HEV following liver and renal transplantation (1,2). The actual risk of transplant-transmitted HEV infection is not known, but data presented at the UK Advisory Committee for the Safety of Blood, Tissues and Organs (SaBTO) in November 2016 suggest that, based on a prevalence study of an English blood donor population, around two organs (deceased or living) donors per year in the UK would have HEV viraemia at the time of donation (3).

4.2 Solid Organ Donors

Given the potential for donor-transmitted HEV infection, SaBTO has considered the issue and made recommendations covering blood components, organs, cells and tissues extending the scope of HEV screening from that provided for in earlier recommendations (3,4). With regards to solid organ donation, SaBTO has recommended that 'Although the risk of transmission via donated organs … is very low, the Committee recommends that all organ donors be individually screened for hepatitis E

viraemia. The detection of viraemia is unlikely to be an absolute contra-indication to use of an organ from a donor, but will inform clinical management decisions post-transplant. The Committee recognises that there are operational challenges in implementing such testing, particularly of deceased donors, and that these will need to be addressed.' Plans are underway with NHSBT to organise testing arrangements but no date for formal implementation has been set.

4.3 Living Solid Organ Donors

In the setting of living donation, it is recommended that potential donors be provided with dietary advice regarding avoidance of HEV infection and that screening with HEV-NAAT be undertaken within four weeks of organ donation. The responsibility for undertaking HEV-NAAT lies with the centre assessing the potential living donor. Samples should be tested as a single sample NAAT and achieve the same sensitivity delivered by NHSBT. If HEV viraemia is detected in the potential donor, then living organ donation should be deferred until such time that laboratory testing confirms spontaneous resolution of HEV infection (plasma and stool HEV RNA not detected) in the otherwise healthy potential donor. It is, however, recognised that in situations of great urgency, such as paediatric living liver donation, that life saving donation may still be considered from individuals known to be viraemic, although the risk to the donor in this situation is not known. The effect of active HEV infection on liver regeneration after living donation is not known.

4.4 HEV Donor Testing

In the setting of deceased organ donation, the responsibility for undertaking donor HEV testing and reporting results to transplant centres lies with NHS Blood and Transplant. However, it is recognised that time constraints arising from the organ donation process mean that HEV-NAAT results will not, under normal circumstances, be available prior to organ retrieval and transplantation ,but should be available between 24 and 28 hours after transplantation to inform ongoing clinical management of the recipient. Although the potential for donor-transmitted HEV infection will exist, the risk is considered small both in terms of the likely incidence of such an event and the consequences of transmitted infection in the post-transplant setting when this is detected early and managed appropriately.

The risks of HEV transmission must be compared to the risk of remaining on the waiting list. For most if not all recipients, the absence of HEV-NAAT test results will not, and should not, influence decisions regarding the use of organs. The absence of HEV results prior to transplant is analogous to

current post-hoc testing for *Trypanosoma cruzi* (Chagas disease) and malaria infection in those donors with identifiable risks. However, the identification of deceased donors with HEV viraemia is important, even after transplantation has occurred, because it allows for appropriate post-transplant recipient monitoring, the possible modification of immunosuppressive therapy, or the use of antiviral therapy for those recipients that do not spontaneously clear the virus. This situation where a HEV viraemic donor provides an organ for a non-viraemic recipient is analogous to that of the solid organ transplant recipient who is a cytomegalovirus (CMV) donor positive/recipient negative mismatch.

4.5 Management of Transplant Recipients who Receive an Organ from an HEV Viraemic Donor

The most appropriate management of the transplant recipient who receives an organ from a donor that is later identified to have HEV viraemia, and is therefore at risk of becoming persistently infected with HEV, is not known. In the absence of evidence to the contrary, it is therefore recommended that individuals who become persistently infected with HEV through transplantation be managed according to recommendations pertaining to other persistently infected individuals, summarised in chapter 7 of this document.

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5 PREVENTION OF HEPATITIS E INFECTION IN SOLID ORGAN TRANSPLANT RECIPIENTS

Statements of Recommendation

We recommend that:

- Individuals must receive written advice regarding the risk of HEV from undercooked meat (particularly processed pork) before and after transplantation. (1D)

5.1 Dietary Advice

It has been established that HEV G3, the prevalent genotype in developed countries, is a dietary-acquired zoonosis with a number of animal species being the reservoir including pigs, wild boar, deer and rabbits. In Europe a large proportion of asymptomatic pigs are infected with HEV G3 (up to 90% of UK pigs at slaughter have serological evidence of past HEV infection (1-7)), and consumption of raw or inadequately cooked pork or game meat from an animal viraemic at the time of slaughter is believed to be the major source of infection. Clear evidence for pork as a source of HEV infection comes from a case-controlled study which showed that HEV infection occurred in 54% of individuals who consumed Figatellu, a traditional French pig liver sausage that is known to harbour HEV and is consumed raw (8). A recent study from the UK found that 6 of 63 pork sausages had detectable HEV RNA (9), again suggesting porcine meat products as a potential source of infection. Moreover, a case-controlled study from the UK found that consumption of processed pork products was associated with an increased risk of acquiring HEV (10).

HEV G3 has been found in other species including deer, wild boar and rabbits, and consumption of infected meat from these animals could also serve as a source of infection (11,12). Other food products including shellfish may be contaminated by pig effluent and irrigation water and lead to HEV infection; however, transmission from these sources remains less clearly defined (13). One model showed that the yearly risk of acquiring HEV from a dietary source was one in 500 to 1000 (14), suggesting that transplant recipients have a significant risk of acquiring HEV from diet. This study also demonstrated that less than a third of the infections harboured a virus that could have come from a UK farmed pig.

Studies have demonstrated that infectious HEV can survive for long periods (more than one month) in food products, particularly when stored at 4°C (15). HEV is also not easily inactivated by cooking and can remain infectious when cooked at temperatures of less than 80° C for less than two minutes

(15). HEV is also not inactivated when heated to 56° C or 60 °C even for long periods (1 hour), which is the approximate temperature of the central portion of meat when cooked rare (15-17). Therefore, considerable evidence implicates the consumption of undercooked meat (predominantly porcine) products in the recent rise in the cases of HEV in Europe. Ensuring pork, and other meat that may be from HEV-infected animals, is adequately cooked is one method that could reduce the risk of HEV infection in transplant recipients and the general population.

In contrast to the above, the major route of transmission of HEV G1 and G2 is the faecal-oral route. In some developing countries, particularly Asia and the Far East, HEV G1 and G2 are endemic. HEV is a major cause of acute viral hepatitis in these regions, and large waterborne outbreaks due to HEV have been described (18). Although HEV G1 and G2 is not associated with chronic infection, acute HEV has a significant mortality, ranging from 0.5-4% (19). Transplant recipients travelling to countries where HEV is endemic should be advised to maintain good hand hygiene, drink boiled or bottled water only, and avoid raw or undercooked meat.

5.2 Transfusion of Blood Products

The first known transmission of HEV from a blood component in the UK was reported in 2006 (20). HEV RNA was subsequently detected in pools of blood from English, Welsh and Scottish donors and there have been a small number of documented cases of HEV infection specifically linked to transfusion of blood components from an infected donor (14,21,22). It is now known that HEV G3 can become a persistent infection in immunosuppressed individuals with the potential for associated morbidity (23). Therefore, there is potential for iatrogenic transmission of HEV to transplant recipients who frequently require transfusion of blood products peri-transplantation.

In order to try and quantify the overall risk of transmission of HEV from transfusion of blood products, a large well-conducted study retrospectively screened 225,000 blood donations from the South East of England for HEV RNA (14). 79 donors were viraemic with HEV G3, giving an overall prevalence of 1 in 2848. Of those blood products that were viraemic with HEV the transmission rate was 42%, which equates to an approximate rate of transmission of 1 in 5000 transfusions.

Similar prevalences of HEV viraemia among donor blood products have been reported from other European countries (24-27). As transplant recipients frequently receive multiple transfusions peri-operatively, the risk of acquiring HEV is significant. One model, using the likelihood of infection of 1 in 5000, suggested that there would be a one in 150 risk of transfusion-associated HEV if an individual were exposed to components from 20 donors over one year (14). The current risk may be

higher still; screening data of blood donors in England in 2016 demonstrated a HEV RNA prevalence of 1 in 1875 donors tested (data from Feb-Sep 2016, NHSBT, personal communication).

From April 2017, donated blood in the UK has been universally screened for HEV using HEV-NAAT testing, as a cost-effectiveness analysis demonstrated that universal testing was more cost effective that selective testing. This is likely to significantly reduce the risk of transfusion acquired HEV.

5.3 Immunisation against HEV

Prevention of HEV infection is potentially possible through immunisation. Recombinant vaccines developed from genotype 1 HEV have shown efficacy in trials in China (28,29). One of these (Hecolin®, Xiamen Innovax Biotech) is licensed for use in China, but not elsewhere in the world. Its efficacy for the prevention of other HEV genotypes has not been established. Further studies in developed countries, including the UK, would be required to test the efficacy of this vaccine, its durability of immune response, and ultimately its cost-effectiveness before further use could be recommended.

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6 SURVEILLANCE AND SCREENING FOR HEV IN SOLID ORGAN TRANSPLANT RECIPIENTS

Statements of Recommendation

We recommend that:

- Potential recipients of solid organ transplants do not need routine screening for HEV infection. There may be specific instances where testing for HEV is indicated pre-transplantation, such as in an immunosuppressed individual with raised liver enzymes. (D1)
- Solid organ transplant recipients with liver transaminases above the upper limit of normal or symptoms suggestive of HEV infection are tested for HEV using an HEV RNA or an antigen assay. (C1)

We suggest that:

- Transplant recipients have a plasma sample taken at the time of transplantation and stored for a minimum of one year that could be tested retrospectively for HEV or other infection. (2D)

6.1 Screening for HEV in Patients awaiting Transplantation

Screening for HEV in patients awaiting transplantation would involve testing for HEV in all potential transplant recipients. This might be considered worthwhile if there were documented cases of HEV reactivation after transplantation when patients were treated with immunosuppressive drugs that could be prevented, such as is seen with hepatitis B; or if there were health consequences from asymptomatic HEV infection whilst waiting for transplantation.

Observational studies to determine the risk and consequences of infection after transplantation, including the theoretical possibility of reactivation, have been performed. The largest of these included 700 patients from Toulouse, where HEV is hyperendemic. Of these, 14.1% (99 individuals) were seropositive pre-transplantation. Reactivation was not observed in this cohort, although there was an appreciable incidence of de novo infection (1).

There is a single case report of apparent reactivation of HEV in a patient with acute lymphoblastic leukaemia following stem cell transplantation (2). Reactivation is not considered to be likely as there is no evidence of long-term sanctuary sites. However, viral infection can persist for some time after clearance of plasma viraemia in an immunosuppressed host, as evidenced by continued faecal

shedding. Enhanced immunosuppressive treatment, for example in controlling rejection episodes, may bring about the return of plasma viraemia. A seropositive individual who has recovered from infection in the past is not at risk of reactivation, although reinfection in the face of immunosuppression has been documented.

The majority of HEV infections in immunocompetent individuals are asymptomatic and achieve prompt seroconversion (3). There are no apparent health consequences of this asymptomatic infection and this does not lend support to screening all individuals for HEV pre-transplantation. However, there may be specific instances where testing potential transplant recipients for HEV before transplantation may be appropriate, such as in individuals who are immunosuppressed pre-transplant but have raised liver enzymes, or individuals with clinical features to suggest current or recent HEV infection, as this may alter their management.

6.2 Post-transplant Screening or Surveillance for HEV in Solid Organ Transplant Recipients

A number of studies have tried to estimate the prevalence of HEV in transplant recipients. Studies using sensitive assays (Wantai HEV test) have reported seroprevalence rates between 8.3% and 43% for anti-HEV antibodies in transplant recipients, the spread probably reflecting true geographic differences in prevalence (4). The prevalence of detectable HEV RNA among transplant recipients in these studies, indicating current viraemia, ranged from 0-3.2% (4). However, studies reported to date have been conducted in geographically distinct populations using different methodologies and may not be generalisable to all transplant populations. It is also likely that the incidence of infection and prevalence rate of viraemia have changed over the last decade, with a demonstrable recent increase in incidence (5). A recent study at a single centre in the UK found the point prevalence of HEV viraemia in transplant recipients was 16/2418 (0.7%) (manuscript submitted).

It has been previously estimated that the annual risk of acquiring HEV in the UK is approximately 1 in 500 to 1000 (6). The transplant patient may acquire HEV in two ways: through diet, which is a continuing cumulative risk measured by the attack rate in the population; and through the receipt of substances of human origin (SOHO) including organs and blood components, which is a temporally constrained risk, but also defined by the attack rate in the population. For solid organ recipients where the SOHO risk is usually small, survival for a year after transplantation will accrue a dominant dietary risk. Exposure to SOHO from 13 donors will carry the same risk as one year of dietary exposure (7).

Given that HEV can become chronic in transplant recipients and can cause associated morbidity, should screening or surveillance be offered to transplant recipients? Two simple options for screening/surveillance could potentially be offered:

1. Test all transplant recipients annually using HEV RNA or HEV Antigen testing
2. Test transplant recipients for HEV RNA who have raised liver enzymes or symptoms suggestive of HEV (e.g. neurological symptoms)

There are no studies assessing the efficacy of these potential screening/surveillance options. The potential advantage of testing all transplant recipients for HEV is that this method should identify all patients who develop persistent HEV. However, this would be costly. The potential option of testing patients with unexplained raised liver enzymes or symptoms suggestive of HEV should diagnose the majority of patients with chronic HEV, as most patients will have raised liver enzymes at some point during the infection. However, some patients with chronic HEV have been reported with normal or minimally raised liver enzymes and these could potentially be missed using this approach. The natural history of patients with persistent HEV who maintain normal liver enzymes is not known, and in particular their risk of developing clinically significant liver fibrosis has not been determined. Overall, there is insufficient evidence to support annual testing for HEV in transplant recipients and this would be a costly intervention. However, we recommend that transplant recipients with raised liver enzymes or symptoms suggestive of HEV are tested for HEV RNA.

We suggest that transplant recipients have a plasma sample taken at the time of transplantation and stored for a minimum of one year that could be tested retrospectively for HEV or other infections. A plasma sample is the preferred analyte for NAAT testing because it stabilises viral nucleic acid. However, if a serum sample is already being routinely archived in transplant recipients that will be adequate for HCV testing.

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7 TREATMENT OF ACUTE HEPATITIS E IN A PATIENT ON THE TRANSPLANT LIST

Statements of Recommendation

We suggest that:

- Individuals with unexplained acute on chronic or acute liver failure should be tested for HEV. (2C)
- Treatment with ribavirin is considered for patients with cirrhosis who develop hepatitis E when on the liver transplant waiting list. (2D)

7.1 Acute HEV in Cirrhotic Patients on the Transplant List

Acute HEV infection in a patient on a transplant waiting list might be identified through abnormalities in 'routine' liver enzymes or due to a symptomatic presentation with liver dysfunction. Symptomatic presentation is recognised to be more frequent in older patients, often with underlying chronic liver disease or alcohol excess (1). In the presence of pre-existing cirrhosis and liver dysfunction acute symptomatic HEV infection can precipitate acute on chronic liver failure (ACLF), which is associated with a mortality of approximately 50% at three months after hospital admission (2). In this scenario there is a rationale to treating HEV and there are small case series supporting this approach. These series describe patients with cirrhosis and liver dysfunction that worsened with HEV infection and were subsequently treated with ribavirin. The largest series to date included six such patients (3). Two patients had pre-existing liver failure (manifest by ascites and hepatic encephalopathy) and these patients died despite treatment. The other four patients survived and cleared HEV infection. Treatment with ribavirin appeared safe, although dose reduction in ribavirin was required for anaemia in some patients, with treatment duration ranging up to one month (3).

The natural history of HEV infection in immunocompetent individuals is for spontaneous seroconversion and it is unclear whether ribavirin treatment speeds this process. These uncontrolled retrospective analyses cannot define whether virological or clinical outcomes were improved by treatment. As such, there is insufficient evidence to recommend treatment with ribavirin in all patients on the transplant list. However, treatment may be considered for patients awaiting liver transplantation, where the associated mortality risk is appreciable.

HEV infection occurring in a patient on the liver transplant waiting list is not an absolute contraindication to transplantation.

7.2 Treatment of Patients with Acute Liver Failure due to Acute Hepatitis E

Acute liver failure is a syndrome characterised by severe liver dysfunction (jaundice, coagulopathy and hepatic encephalopathy) on a background of a previously 'normal' liver. HEV is a common cause of acute hepatitis, but rarely causes fulminant liver failure. In a study of 681 patients with acute liver failure from the USA, only 0.4% of patients were HEV IgM positive suggesting acute HEV as the cause of their liver failure (4). Another study from the UK retrospectively tested 80 patients with acute liver failure and found four cases (5%) of HEV, of which two had been labelled as drug-induced liver injury (5). As there is significant geographical variation in the incidence of HEV infection, it is likely that this variability will also be seen in the incidence of HEV-associated acute liver failure.

There are reports of successful transplantation for HEV-associated acute liver failure (6,7). The role of ribavirin in the setting of patients with HEV-associated acute liver failure is not known. Treatment with ribavirin could be considered in patients with HEV viremia and liver failure, but as acute kidney injury frequently accompanies liver failure, careful monitoring and use of ribavirin at reduced dosages (discussed below) would be required. However, it is not known if this affects virological or clinical outcomes.

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8 MANAGEMENT OF HEV INFECTION IN SOLID ORGAN TRANSPLANT RECIPIENTS

Statements of Recommendation

Newly Diagnosed or Acute HEV Infection

We suggest that:

- The initial management of newly diagnosed or acute HEV infection in solid organ transplant recipients includes observation and monitoring of HEV RNA levels and liver enzymes as more than 30% will spontaneously clear the infection within three months. Dynamic viral monitoring and antibody profiling may help clinical decision-making. (C2)
- A strategic reduction in immunosuppression is considered in patients with acute or persistent HEV as this may facilitate viral clearance, but the risk of rejection should be carefully assessed. (2C)
- Early treatment with ribavirin may be considered in specific cases of acute hepatitis E, such as patients who develop severe liver dysfunction (jaundice and coagulopathy) or extra-hepatic manifestations, although evidence for this recommendation is currently limited. (2D)

Persistent HEV Infection

We recommend that:

- Persistent HEV infection is diagnosed when HEV RNA is detectable in blood or stool for more than three months after the onset of relevant symptoms, raised liver enzymes, or from the first positive HEV RNA test. (1C)
- Individuals with treatment for persistent HEV infection (documented or estimated duration of infection of more than three months) receive treatment with ribavirin with the aim of achieving sustained virological response (HEV RNA not detected in plasma and stool six months after completion of treatment). (1C)
- A baseline quantitative HEV RNA assessment is undertaken on both plasma and stool at the start of treatment. (1C)
- Treatment with ribavirin should continue for at least three months for solid organ transplant recipients with persistent HEV infection. For most individuals 3-6 months of ribavirin treatment will suffice. (1C)

- Monthly HEV RNA testing in plasma and stool is undertaken until a decision is made to stop treatment. (1C)
- Ribavirin is continued until stool tests are negative for HEV RNA on two occasions one month apart, as continued shedding of HEV in stool is an important factor predicting relapse after ribavirin treatment. (1C)
- A test of sustained virological response is conducted by testing plasma and stool samples for HEV RNA at three and six months after stopping antiviral therapy. (1C)
- Regular haemoglobin monitoring is conducted during ribavirin therapy as haemolytic anaemia is a common treatment-related side effect. Ribavirin dose reduction may be required during treatment to maintain an adequate haemoglobin concentration. Epoetin therapy and/or blood transfusion may be indicated to allow continued antiviral therapy without avoidable drug reduction. (1A)
- PEG-interferon should not be used as first line for the treatment of persistent HEV in transplant recipients as there is a moderate risk of precipitating organ rejection. (1D)

We suggest that:

- Assessment of the change in plasma HEV RNA after seven days of ribavirin treatment may help predict the chance of achieving sustained virological response after three months of ribavirin treatment. We therefore suggest quantitative testing of a plasma sample taken at day seven of ribavirin treatment to help determine the likely length of ribavirin treatment. (2C)
- To minimise treatment-related side-effects, the dosage of ribavirin is adapted according to creatinine clearance, estimated using the Cockcroft-Gault equation. (2C)
- Patients with persistent HEV who relapse after a first course of ribavirin are re-treated for at least six months with ribavirin at dosages toward the higher dose range, where tolerated. (2D)
- Routine baseline sequencing of HEV for mutations is not indicated before antiviral treatment as the significance of such mutations has not been determined. (2D)
- PEG-interferon treatment may be considered in cases of ribavirin-refractory persistent HEV infection. However, patients will require very close monitoring for rejection. (2D)

8.1 Management of Acute HEV Infection Post-transplantation

There are no published randomised controlled studies assessing the optimal management of acute HEV infection in the transplant setting. All data on the management of acute infection post-transplantation come from case series and observational studies. One of the largest and most informative studies was published in 2011. This assessed the outcomes of presumed acute HEV infection in 85 solid organ recipients presenting to 17 centres in Europe and the United States (1). This study found that the majority of patients were asymptomatic (32%) or had self-limiting symptoms (most commonly fatigue, diarrhoea or arthralgia). Only one patient developed jaundice and none developed liver failure. There was a significant rise in liver enzymes (ALT, AST, GGT, ALP and bilirubin) in the patients compared with pre-HEV infection liver enzyme levels. Overall, 34% of patients spontaneously cleared the infection in the first six months after infection and liver enzymes returned to pre-infection levels. The majority of those with spontaneous clearance were non-liver transplant recipients. None of the patients in this study received antiviral therapy, but tacrolimus levels were slightly lower at month six compared with time of HEV infection (7.9 vs 10.1, p=0.002) during the six months of follow up, suggesting clinicians had reduced the patients' immunosuppression. A further 21% of patients achieved clearance of HEV with specific reduction in immunosuppression after six months. Therefore, the current natural history data suggest that acute HEV infection in solid organ transplant recipients does not require specific antiviral treatment at the outset in the majority of cases, as a significant proportion of patients will spontaneously clear the infection.

Thus the initial management of acute infection in solid organ transplant recipients should include careful observation and monitoring of HEV RNA levels, serology, and liver enzymes. Where possible, a reduction in immunosuppression should be considered (discussed below). If HEV RNA clearance from the blood and stool has not been achieved by three months then persistent infection is likely to occur and the patient should be managed as having persistent HEV infection. There may be specific cases where early antiviral therapy with ribavirin is indicated, such as patients who develop severe liver dysfunction (jaundice and coagulopathy), although evidence for this is currently limited.

It has recently been recognised that HEV infection (acute and persistent) has been associated with extrahepatic syndromes, particularly neurological manifestations, such as Guillain-Barré syndrome, neuralgic amyotrophy and encephalitis (2). Renal (glomerulonephritis with and without cryoglobulinaemia), cardiac (myocarditis), and autoimmune extrahepatic manifestations (e.g. thyroiditis and thrombocytopenia) have also been described (2). Although it has not been proven, a few case reports have suggested that ribavirin treatment may improve the natural history

of these extrahepatic manifestations (3-5). Therefore, early ribavirin treatment could be considered for patients with suspected extrahepatic manifestations due to HEV, although convincing evidence is currently lacking.

8.2 Treatment of Persistent Hepatitis E Post-transplantation

Following acute infection with HEV G3, the infection persists in approximately 60% of solid organ transplant recipients leading to persistent HEV infection (6). This can cause a chronic hepatitis that can progress rapidly (3-5 years) to cirrhosis in approximately 15% of infected solid organ transplant recipients (6). Therefore, persistent HEV infection should be actively treated with the aim of clearing HEV from the blood and stool.

When should treatment be started?

Chronic HEV infection is conservatively defined as the finding of detectable HEV RNA in the blood and/ or stool for greater than six months. However, it has now been demonstrated that spontaneous clearance of HEV rarely occurs between three and six months of infection (6). Therefore, efforts to treat HEV should begin after three months of infection. Practically, it can be difficult to determine exactly when HEV is acquired, as it is asymptomatic in the majority of infected individuals and the stage at which the initial detection of HEV RNA occurs is variable. Fatigue is the most common symptom and jaundice is rare (6). Neurological symptoms can also occur (2). As HEV infection is frequently asymptomatic in transplant recipients, clinicians should have a high index of suspicion for the infection and should investigate raised liver enzymes of any degree with reflex HEV testing. Typically ALT levels are between 200-300 U/L in transplant recipients with HEV infection, but patients with may also present with minimally raised liver enzymes or enzymes within the upper normal range (6). Persistent HEV infection can be misdiagnosed as drug-induced liver injury (7), rejection (in liver transplants) (8) or graft versus host disease, so careful assessment is needed of all liver enzyme abnormalities in transplant recipients.

Individuals with persistent HEV infection (documented or estimated duration of infection of greater that three months) should be treated with the aim of achieving a sustained virological response (HEV RNA non detected in plasma and stool six months after completing treatment). In order to help define the length of infection a review of the patient history, anti-HEV status and previous liver enzymes should be undertaken. If available, analysis of stored specimens from before the first finding of HEV RNA in the blood may also be helpful.

Modification of immunosuppression

Persistent HEV infection occurs mainly in heavily immunosuppressed individuals, particularly those on T cell suppressing drugs. Reduction of immunosuppression can lead to clearance of HEV infection in approximately 30% of individuals with persistent HEV (1). Different classes of immunosuppressant drugs have different effects on HEV replication (Table 1) so a strategic modification of immunosuppression may facilitate clearance of HEV.

Table 1. Effect of immunosuppression on HEV replication *in vitro* and *in vivo*

Drug	*In vitro* effect	*In vivo* effect	References
Mycophenolate	Inhibition of HEV replication	Unclear. No effect seen in small studies	(9) (10) (11)
mTOR inhibitors	Potentiation of HEV replication	Higher HEV RNA levels in patients with persistent HEV on mTOR inhibitors	(10) (12) (13)
Calcineurin inhibitors	Potentiation of HEV replication	Unclear. Tacrolimus may be associated with persistence of HEV	(1) (11)
Corticosteroids	No effect	Unknown	(11)

All data assessing the impact of modification of immunosuppression in transplant recipients come from uncontrolled studies and case series. In the absence of large well designed studies assessing the effect of immunosuppression on HEV infection some *in vitro* studies have assessed the effect of immunosuppressants on HEV replication in cell culture models. Overall, these have shown that ciclosporin increased HEV viral replication in a dose-dependent manner and may therefore potentiate infection with HEV (11). Tacrolimus also increased HEV replication in a cell culture model; however, this effect was only seen at high dosages. mTOR inhibitors also appeared to potentiate HEV replication (13). Corticosteroids had no effect on HEV replication in these models (11). Interestingly, mycophenolate was shown to inhibit HEV replication (11). This effect was potentiated with the addition of ribavirin. However, the underlying mechanism of the inhibitory effect of

mycophenolate on HEV replication was shown to be a reduction in hepatocyte levels of GTP, which might not occur physiologically *in vivo* (12).

The largest study assessing transplant recipients with acute HEV to date found that patients treated with ciclosporin were more likely to have spontaneous clearance than those treated with tacrolimus. (1). For patients with established persistent HEV, one small study (28 patients on mycophenolate and 7 not on mycophenolate) reported that there was no difference in the rate of HEV clearance in transplant recipients treated with ribavirin whether or not they were on immunosuppression containing mycophenolate (10), suggesting that the observed *in vitro* inhibitory effect of mycophenolate of HEV may not be significant in patients with HEV. However, this was an uncontrolled study so it remains unclear whether mycophenolate is beneficial in patients with persistent HEV infection. The same study also demonstrated that patients treated with mTOR inhibitors had higher HEV RNA at baseline and throughout ribavirin treatment than those treated without mTOR inhibitors, suggesting that mTOR inhibitors may be associated with persistence of HEV (10).

Overall, current evidence suggests that calcineurin and mTOR inhibitors may contribute to persistence of HEV replication in hepatocytes and the development of persistent HEV, whereas corticosteroids appear to have no effect on viral replication, and mycophenolate may have an inhibitory HEV replication *in vitro*. Therefore, strategic modification of immunosuppression might help with viral clearance. Further studies are awaited to help define the role of modification of immunosuppression in persistent HEV. It is important to recognise that changes in immunosuppression can precipitate rejection in more immunogenic individuals so the risk of rejection versus the potential benefits of modification of immunosuppression must be carefully balanced.

Antiviral therapy

Ribavirin

Ribavirin is an antiviral medication that has been used for many years in combination with pegylated interferon for the treatment of hepatitis C. More recently, ribavirin has been shown to have antiviral activity against HEV in *in vitro* replicon models (14,15). The exact mechanism of antiviral activity of ribavirin is unknown. Ribavirin has been shown to reduce HEV replication *in vitro* by reducing intracellular pools of GTP, but it is not known whether this occurs *in vivo* in HEV-infected

hepatocytes. (15). Ribavirin is a guanosine analogue and may also act as a nucleoside inhibitor, inhibiting replicating HEV RNA (14).

The first report of the use of ribavirin monotherapy for the treatment of persistent HEV was in 2010 (16). In that report, two patients with persistent HEV were treated with ribavirin 12 mg/kg for 12 weeks, and both cleared HEV RNA from blood and stool by four weeks and remained HEV RNA negative during follow up. The largest single study of ribavirin in persistent HEV was reported by Kamar *et al* in 2014 (17). This was a retrospective multicentre case series of 59 transplant (37 kidney, 10 liver, 5 heart, 2 lung and 5 kidney/pancreas) recipients with persistent HEV (median duration of infection nine months; although five patients had documented viraemia of less than three months) treated with ribavirin (median dose 600 mg) for a median length of treatment of three months (range 1-18 months). A sustained virological response (serum HEV RNA negative six months after treatment) was achieved in 78% of cases. Of the 10 patients who relapsed, six were retreated and five of these achieved a sustained virological response. Immunosuppressive regimens were not altered during their ribavirin treatment. Anaemia, a well-recognised side effect of ribavirin, was the most frequent adverse effect reported in this study.

Since the study by Kamar *et al,* other uncontrolled studies have confirmed their findings (Table 2). A systematic review conducted in 2015 reported the outcomes of 105 patients (including the 59 patients treated in the Kamar *et al* study) treated with ribavirin for persistent HEV from 19 case series/reports (91% post-transplant) and found an overall sustained virological response rate of 74% with ribavirin treatment (18). There was a wide variation in the dosage of ribavirin (200-1200 mg), length of treatment (median three month range 1-18) and duration of infection (median 16 month range 1-84) in these studies. A sustained virological response rate of 63% was seen in two recent studies after three months of ribavirin treatment where a more standardised protocol was used (9,10). Table 2 shows a summary of all the studies reported to date where more than 10 patients have been included.

It remains unknown what the optimum treatment duration and dose of ribavirin should be. More recent studies have aimed to more clearly define treatment duration and have looked for factors that may predict outcome from treatment. In hepatitis C it is well known that the viral kinetics on treatment with PEG-interferon and ribavirin predict the overall outcome from treatment. Patients treated with PEG-interferon and ribavirin for HCV who achieve a 'rapid virological response' (HCV RNA not detected after 4 weeks) were significantly more likely to achieve a sustained virological response than patients who had a slower viral response (19). Given these findings, Kamar *et al* assessed the impact of early virological response on outcome in 35 solid organ transplant recipients

with persistent HEV who were treated with ribavirin for three months. In that study, the overall SVR rate was 63% (10). Importantly, they found that a decrease in HEV RNA after seven days was an independent predictor of sustained virological response to ribavirin treatment. A fall in HEV RNA of 0.5 log copies/mL at day 7 of ribavirin treatment had a positive predictive value of 88% for SVR and a fall of 1 log copies/mL had a 100% positive predictive value for SVR. Therefore, this study suggested that virological response at day seven could be incorporated into treatment algorithms to help determine treatment course length (i.e. those with a favourable response at day seven could be treated for three months with ribavirin and those with a slower virological response probably require a longer course, such as six months). However, this was a small study and these findings require validation.

Table 2. Overview of the studies of ribavirin for the treatment of chronic HEV where more than 10 participants were included

n	Study type	Dose (mg) Median (range)	Duration (months)	SVR	Predictor of response	Ref
59	Multicentre case series	600 (29-1200)	Median 3 (1-18)	78%	Higher lymphocyte count at start of ribavirin predicted SVR	(17)
35	Retrospective observational	600 (200-1000)	3	63%	Decrease in HEV RNA by 0.5 log copies/mL at day 7 predicted SVR	(10)
24	Retrospective observational	600 (200-800)	3	63%	Presence of HEV in stool at 3 months predicted relapse	(9)
105	Systematic review	200-1200	Median 3 (1-18)	74%		(18)
63 (40 included in (17))	Retrospective observational	200-1000	Median 3 (3-18)	67%	Trend towards reduced SVR in patients with HEV G1634R variant	(21)
11	Case series	600-1000	5 (n=10) 1.5 (n=1)	82% (100% in those with complete follow up)		(22)

SVR = sustained virological response (HEV RNA negative six months post-treatment)

In that study they also assessed the impact of ribavirin dosage on SVR. Patients were treated on a ribavirin dosage that was adapted according to the estimated glomerular filtration rate and ranged from 200-1000 mg/day (median 600 mg). Interestingly ribavirin levels were assessed at day seven and there was no association between ribavirin levels and sustained virological response rates.

One of the major sources of HEV viral replication is the gastrointestinal tract (20). Another recent study assessed the kinetics of HEV shedding in stool in 24 transplant recipients treated with ribavirin for three months to determine if this had an impact on treatment response (9). The overall sustained virological response rate was 63%. Interestingly, all patients were plasma HEV RNA negative after three months of treatment with ribavirin, but five patients were still excreting HEV RNA in the stool at the end of treatment and these all relapsed. Therefore, persisting HEV in the stool, even after clearance from the blood suggests ongoing HEV infection. This study suggests that measurement of stool HEV RNA, in addition to the assessment of HEV RNA in the blood during treatment can help determine the appropriate length of treatment.

Although the finding of negative HEV in blood and stool at the end of treatment is strongly predictive of achieving a sustained virological response with ribavirin treatment, a small proportion (3/24 [12.5%]) of patients relapsed after three months of ribavirin treatment even with negative HEV RNA in blood and stool at the end of treatment (9). It is possible that current assays for the detection of HEV have insufficient sensitivity and that these patients had persisting low level viraemia or had intermittent or low level shedding of virions in the stool that was not detected by the HEV RNA test used. Moreover, patients with HEV treated with ribavirin can develop mutations conferring resistance to ribavirin, which may contribute to treatment failure (21).

Detection thresholds for definition of HEV clearance are currently unknown. In the absence of robust data in this area it is currently felt that plasma clearance should be defined by testing using a validated assay with a Poisson sensitivity of at least 100 IU/ml or better. Stool clearance should be defined by testing a 10% extract of faecal material using a validated assay with a Poisson sensitivity of 100 IU/ml or better. There should be an aspiration to achieve better sensitivity and these suggestions are liable to change as more is learnt in this area

A suggested algorithm for the treatment of HEV is shown in Figure 1.

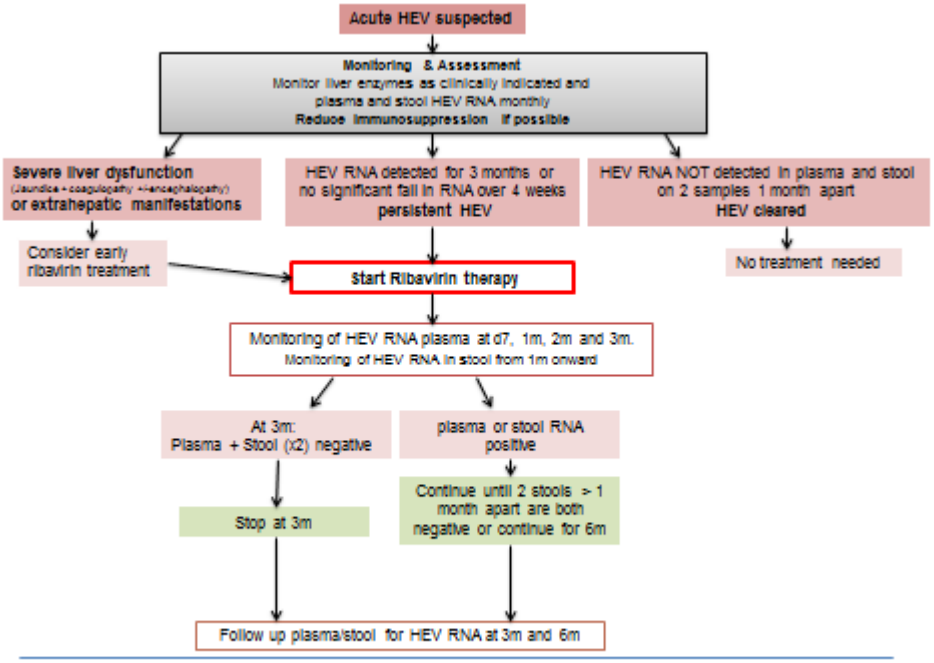

Figure 1. A suggested algorithm for the treatment of HEV in solid organ transplant recipients

Dosage of Ribavirin

It remains unknown what the optimum dosage of ribavirin is for the treatment of persistent HEV. Interestingly, one small study found no relationship between ribavirin levels at day seven and outcomes from treatment in patients with persistent HEV (10). To date, all the reported studies have used variable dosing ranging from 200 mg to 1200 mg per day with a median dosage of 600 mg/day. The pharmacokinetics of ribavirin are variable in transplant recipients and clearance depends particularly on renal function (23). In order to reduce the side effects of ribavirin and achieve a steady state of ribavirin in the therapeutic range, the starting dose can be adapted based on the creatinine clearance (the eGFR can be unreliable in transplant recipients so use creatinine clearance is preferred). Table 3 shows a suggested dosing regimen for ribavirin according to the creatinine clearance calculated by the Cockcroft-Gault equation, which can be used in transplant recipients treated with ribavirin (23). Two of the reported studies of ribavirin in persistent HEV have used this dosing regimen, and this regimen goes some way to standardizing the dosing of ribavirin until further studies clarify the optimum dosing (9, 10).

Table 3. Suggested starting dosage of ribavirin in transplant recipients based on the Creatinine Clearance measured by the Cockcroft-Gault equation (23)

Dosage to achieve steady state levels of ribavirin of	**Creatinine Clearance by Cockcroft-Gault equation (mL/min)**				
	100	**80**	**60**	**40**	**20**
10 µmol/L	810	690	570	450	330
14 µmol/L	1140	970	800	630	460
Practical starting total daily ribavirin dosage (mg)	**1000**	**800**	**600-800**	**600**	**400**

Note: ribavirin is usually prescribed using twice daily dosing

The most frequent side effect of ribavirin is a haemolytic anaemia, which required intervention in approximately 40% (dose reduction, epoetin or blood transfusion) of those subjects included in the previous systematic review (18). Therefore, patients require regular monitoring of haemoglobin on treatment, initially every two weeks until the haemoglobin has reached nadir then monthly thereafter. Dose reduction of ribavirin is recommended when the haemoglobin falls below 100 g/L. Although epoetin has been used in some previous studies, there is no evidence to date that its use has an impact on sustained virological response. For patients who develop very severe anaemia use of epoetin or even blood transfusion may be indicated.

Treatment failure with ribavirin treatment

Approximately 40% of transplant recipients with persistent HEV relapse after three months of treatment with ribavirin (9,10). There are a number of potential reasons for treatment failure. The need for dose reduction as a result of side effects is a likely explanation in many cases, as ribavirin needs to reach sufficient levels to have antiviral activity in all sites where the virus replicates in order to clear the infection. As discussed above, another probable reason for failure is that some patients receive an insufficient duration of treatment to ensure HEV is cleared from both blood and stool. This could potentially be minimised by ensuring that patients have necessary assessments of plasma and stool HEV RNA to ensure negativity before completing treatment with ribavirin. It has also

recently been shown that a G1634R mutation in the RdRp domain of the ORF1 protein is associated with ribavirin treatment failure (24,25). Interestingly this mutation appears to increase the fitness of HEV *in vitro* compared to the wild-type virus and may therefore contribute to the relative 'ribavirin resistance' in some patients with persistent HEV (25,26). When studied in a cohort of 63 patients treated with ribavirin for persistent HEV the G1634R mutation was present at baseline in 36.5% of patients. This mutation was found in 31% of those who had sustained virological response with ribavirin and 47.6% of those who failed treatment (p=0.2) suggesting it may have a minor effect on treatment response, but other factors are also likely to be important (21). The potential use of this and other mutations to help tailor treatment in patients with persistent HEV requires further study.

The majority of patients who relapse will respond to a longer course of treatment with ribavirin, even those harboring the G1634R mutation (21). Therefore, retreatment with a longer course of ribavirin should be considered for patients who relapse, and treatment continued until the HEV RNA is negative in blood and stool on two tests at least one month apart. Re-treatment for six months with ribavirin will be sufficient for many patients to achieve a sustained virological response, but some patients will require longer treatment (27). There are occasional reports of very resistant cases of HEV with multiple mutations that confer resistance to ribavirin who have persisting HEV viraemia despite continued treatment (26). This emphasises the importance of ensuring the patient receives effective treatment on their first course of treatment where possible.

PEG-interferon

There are a few reports of successful treatment of persistent HEV with PEG-interferon, summarised in a recent systematic review (18). Overall, this review highlighted eight patients (six who had undergone solid organ transplantation) treated with PEG-interferon for a median of three months (range 3-12), of which 75% had a sustained virological response. Worryingly, two patients (25%) experienced acute rejection during treatment. PEG-interferon is well known to increase the risk of rejection in transplant recipients. In a randomised controlled trial of PEG-interferon +/- ribavirin for hepatitis C in liver transplant recipients, 10% of patients had acute rejection necessitating treatment withdrawal and augmentation of immunosuppression (28). This may not be a direct effect of the PEG-interferon, but could be due to a reduction in calcineurin inhibitor levels associated with clearance of the virus so careful monitoring of immunosuppressant levels is needed. Acute rejection can have significant implications for solid organ recipients, particularly non-liver transplant patients, and as such the use of PEG-interferon is not recommended as a first line treatment for HEV. PEG-interferon treatment could be considered in cases of ribavirin refractory HEV, particularly if

associated with ribavirin resistance mutations. However, patients will require very close monitoring for rejection.

Sofosbuvir

Sofosbuvir is a pangenotypic nucleotide analog licensed for the treatment of HCV. One recent study suggested the sofosbuvir inhibited HEV RNA replication in an experimental model of HEV (29). The inhibitory effect on HEV replication was additive when combined with ribavirin. However, the anti-HEV activity was markedly lower than its anti-HCV effect suggesting that standard dosing of sofosbuvir in patients with HEV may be insufficient to have an antiviral effect. One case report using sofosbuvir at a dose of 400 mg per day in combination with ribavirin in an allogeneic stem cell transplant recipient demonstrated antiviral activity against HEV *in vivo*; however, treatment was insufficient to clear the virus (30). In contrast, another report found no change in HEV RNA levels in an immunosuppressed post-liver transplant patient with HCVG3/HEVG3 co-infection who was treated with sofosbuvir and daclatasvir (without ribavirin) (31). Further clinical studies are required.

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