Hepatitis E in Transplantation

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Abstract Hepatitis E virus (HEV) has a worldwide distribution and is known to cause acute and fulminant hepatitis. However, over the last few years, it has been shown to also cause chronic hepatitis and cirrhosis in immunosuppressed patients, especially solid-organ-transplant patients. In immunocompetent and immunosuppressed patients, HEV is also associated with extra-hepatic manifestations, such as neurological symptoms and kidney injury. Unfortunately, a diagnostic assay for HEV infection is still not available in all countries. Reduction of immunosuppression is the first-line therapeutic option for organ-transplant patients with chronic hepatitis. In addition, ribavirin is highly efficient at treating chronic HEV infection. In this comprehensive review, we summarize the current knowledge regarding HEV diagnosis, its natural history, clinical manifestations, and treatments in patients with a solid-organ transplant.

Keywords Hepatitis E virus · Transplantation · Chronic hepatitis · Extra-hepatic manifestations · Ribavirin

Introduction

Hepatitis E virus (HEV) is the most prevalent form of hepatitis virus worldwide [1••]. In developing countries, HEV was and is still is responsible for epidemics [1••]. In developed countries, until the last decade, the spread of HEV was considered to be travel-related [1••]. However, over the last few years, increased numbers of HEV cases diagnosed in developed countries, improvements in HEV diagnostic assays, and the discovery of HEV-induced chronic hepatitis and extra-hepatic manifestations have increased interest in HEV infection. Epidemiological studies have shown that HEV is prevalent in developed countries and that, in most cases, it is locally acquired [1••]. Large-animal reservoirs of HEV have also been identified [2••].

One of the most important findings over the last few years is the risk of evolution of HEV infection to chronic hepatitis in immunosuppressed patients, i.e., in patients with a solid-organ transplant (SOT), HIV-infected patients, hematological patients receiving chemotherapy, patients with a stem-cell transplant, and patients given immunotherapy [1••].

The aim of this comprehensive review is to describe the incidence, clinical manifestations, and treatment of HEV infection among transplant patients.

Hepatitis E Virus

HEV was first identified in an electron-microscopy study of stool samples from an infected volunteer in 1983. HEV is a small (27–34 nm) non-enveloped virus belonging to the Hepeviridae family. Studies have shown that the density of virions in serum and cell-culture supernatants is lower than that of virions in feces, but increases after de-lipidation [3, 4]. This suggests that virions in the circulation of infected individuals and those produced in cell culture are enveloped.
HEV strains present a high diversity and those infecting humans are classified into four major genotypes: i.e., HEV1–HEV4: these are within the Orthohepevirus genus [5]. Genotypes 1 (HEV1) and 2 (HEV2) are restricted to humans. HEV1 is present mainly in Asia and Africa, and HEV2 in Africa and Mexico. HEV1 and HEV2 are waterborne viruses in developing countries and are spread through inadequate sanitary conditions. Genotypes 3 (HEV3) and 4 (HEV4) infect humans, but also pigs, wild boar, deer, and other mammals. HEV3 is widespread whereas HEV4 is mainly present in Asia, although it was recently introduced into Europe [6]. HEV3 and HEV4 are spread by zoonosis; contamination is linked to direct contact with the animal reservoir, consumption of pig or game meats, or contamination of the environment via the animal reservoir [2••], [7]. It is not known how long HEV can survive in an infectious state in the environment or in food products. A few studies indicate that HEV could remain infectious at temperatures used in some cooking regimes (e.g., 71 °C for 5 min) [8].

**Diagnosis**

Cases of acute hepatitis E are primarily diagnosed by detecting anti-HEV antibodies, mainly anti-HEV IgM, or by detecting viral RNA in the serum and/or feces. Sensitivity of the anti-HEV IgM assays are >98 %, thus they can be used as first-line tools for the routine diagnosis of acute hepatitis E [9]. However, in immunocompromised patients, who may not mount a good antibody response to HEV, sensitivity is around 85 % for the microplate assay and 75 % for a rapid test [10]. Detection of anti-HEV IgG indicates a past infection. The seroprevalence of anti-HEV IgG among the general population in high-income countries varies widely, partly due to variations in the sensitivity of detection assays [11, 12].

Although molecular detection of HEV RNA is essential to diagnose acute hepatitis E in anti-HEV IgM-negative patients, only validated HEV RNA assays should be used. Studies have demonstrated that the performance of RT-PCR tests varies widely [13, 14]. Of note, the Food and Drug Administration has not approved any commercial serology or PCR test. Yet, HEV RNA testing should be done 3 months after diagnosis to assess for HEV clearance [15]. A quantitative HEV RNA assay of the plasma or stools is also necessary to assess the response to anti-viral therapy [16, 17].

**HEV Transmission in Transplant Patients**

Similar to the general population, the fecal-oral route is the main route of HEV transmission in transplant patients [1••]. HEV 1 and 2 are transmitted via contaminated water. However, HEV3 and 4 are transmitted by consumption of pig or game meat, or the consumption of other products present in the environment of animal sewage [1••]. More recently, the risk of transmission by blood products has been highlighted because of the high incidence of HEV RNA among blood donors [18••, 19]. Hence, for transplant patients, in addition to the classical paths of contamination, two other modes of transmission need to be considered: (i) transmission by administration of blood products and (ii) transmission via the transplanted organ.

Transplant patients often receive red blood-cell transfusions and can receive fresh plasma, especially during the perioperative period. In addition, fresh plasma can be given in desensitization protocols or to treat antibody-mediated rejection. Although there is a theoretical high risk of HEV transmission via blood products, only one case of HEV transmission via a blood transfusion to a liver-transplant patient has been reported [20]. Similarly, only one case of transmission via a liver allograft has been reported [21], and in which all of the biological markers (serology and PCR) for HEV were negative in both the donor and recipient at transplantation. However, HEV RNA genotype-3 was detected in liver tissue obtained before transplantation [21]. Although it is not recommended to screen all blood or organ donors for HEV, when there are increased liver-enzyme levels early after transplantation or after a blood transfusion, patients should be screened for HEV. In cases where HEV is diagnosed, the transplant physician can then ask to retrospectively test the organ and/or blood donors.

**Prevalence and Incidence of HEV Among Organ-Transplant Patients**

Similar to the general population, HEV seroprevalence among patients with a solid-organ transplant cannot be easily determined from the published literature because of the large variability within the studied populations and the different serological assays used in these studies. Indeed, as mentioned above, a large difference is observed in seroprevalence data according to the assay used.

The Wantai test is considered to be the most sensitive assay [11]. Studies that have used this assay report HEV seroprevalences ranging from 8.3 to 43 % among patients with a solid-organ transplant (Table 1). Among all comers, the prevalence and incidence of replicating HEV, confirmed by detecting HEV RNA in the serum, ranges from 0 to 3.2 and 1 to 3.2 %, respectively. HEV reinfection has been observed in 3 out of 91 HEV seropositive organ-transplant patients (3.3 %) [22]: one of these three patients developed chronic hepatitis. Interestingly, in these three patients, anti-HEV IgG concentration was <7 WHO IU/mL. These patients had probably a partial protection since in rhesus macaques, partial protection was observed when anti-HEV IgG was <7.1 WHO IU/mL [23].
Clinical Presentation of HEV Infection in Organ-Transplant Patients

In the general population, most patients are asymptomatic during an acute phase [24]. The main symptoms observed during this phase are jaundice, weakness, and weight loss [24]. Liver-enzyme levels are generally very high, i.e., alanine aminotransferase levels are ~3000–5000 IU/L [24]. Most transplant patients are also asymptomatic during an acute phase [25]. Fatigue is then the main symptom whereas jaundice is rarely observed. In the transplant population, the increase in liver-enzyme levels is much lower than those observed in immunocompetent patients. Mean alanine aminotransferase levels are between ~200 and 300 IU/L [25], and in some cases, liver-enzyme levels are below 2 folds the upper limit of the normal subnormal. For this reason, HEV diagnosis can be delayed in some patients. In addition, HEV may be mistaken for drug-induced liver injury [26, 27]. HEV infection can also cause extra-hepatic manifestations, which can be the main clinical presentation, i.e., neurological symptoms [28].

Natural History of HEV Infection After Organ Transplantation

In the general population, HEV often causes self-limiting infection [24]. However, HEV can also cause fulminant hepatitis in pregnant women and in patients with underlying chronic liver disease, so-called acute on chronic hepatitis [24]. In pregnant women infected by HEV genotype 1, the risk of death can be up to 30 % [24]. However, no case of fulminant hepatitis has been reported in pregnant women infected by HEV genotype 3 or 4. The risk of death after HEV infection in patients with chronic liver disease is ~70 %, whatever the genotype [29] [30]. Over the last few years, it has been shown that HEV infection can cause chronic hepatitis and cirrhosis in immunosuppressed patients, i.e., solid-organ transplant recipients [31], HIV patients [32], or hematological patients receiving chemotherapy [33]. Nearly all cases of chronic hepatitis have been described in patients infected by genotype 3 [25]: very few cases of HEV-genotype 4 have been reported, and no cases of HEV-genotype 1 or 2 [1].

The definition of HEV infection is not well established. Persistent replication of HEV in the serum and/or stools that lasts for at least 6 months has been used to define chronic hepatitis. However, an observational study performed on SOT patients showed that, without any modification to immunosuppressive therapy, no cases of spontaneous HEV clearance occur beyond 3 months after infection [15]. All patients with resolving hepatitis cleared viremia within the first 3 months after infection, typically within the first month. Based on this data, patients with ongoing replication beyond 3 months are considered to be chronically infected [15]. Recently, a single case of a liver-transplant patient who experienced spontaneous HEV clearance at 4 months after...
infection has been reported [34]; however, no data regarding the management of immunosuppressive therapy during the period of infection were provided.

In a retrospective multicenter European study that included 85 SOT patients, 29 patients (34.1 %) had spontaneous clearance of HEV, whereas the other 56 patients (65.9 %) evolved to chronic hepatitis [25]. Nine of the 85 patients (9.4 %) developed cirrhosis [25]. One of the most striking findings was the rapid progression of liver fibrosis after infection [35]. Indeed, in some cases, cirrhosis occurred within 2 to 3 years after HEV infection, suggesting that HEV is much more aggressive than other hepatotropic viruses [35]. The risk factor for the evolution to chronic hepatitis is a decreased immune response. In the first report on chronic HEV infection, it was observed that patients who evolved to chronic hepatitis had significantly lower CD3 and CD4 cell counts compared to those who did not [31]. In this multicenter retrospective study, it was shown that patients who developed chronic hepatitis: (i) had lower liver enzymes levels during infection, suggesting a lower immune response; (ii) had a shorter delay after transplantation and since the last treated acute-rejection episode (if any), and had a lower platelet count during HEV infection; and (iv) were more often treated by tacrolimus than by cyclosporine A [25]. Multivariate analysis showed that a low platelet count at infection and the use of tacrolimus were independent predictive factors for chronic HEV infection [25]. In vivo, meta-analyses have shown that the risk of acute rejection is lower in patients treated by tacrolimus compared to those given cyclosporine A, suggesting that tacrolimus is a more potent immunosuppressive drug [36]. In vitro, it has been shown that cyclosporine A, tacrolimus, and mammalian target of rapamycin inhibitors increase HEV replication [37] [38] whereas, conversely, mycophenolic acid inhibits HEV replication [37]. A lower specific anti-HEV T cell response and a lower inflammatory response were observed in patients who developed chronic HEV infection [39, 40]. Finally, greater quasispecies heterogeneity was observed in patients with persistent HEV, suggesting the appearance of persistent variants [40, 41].

**HEV Infection After Stem-Cell Transplantation**

In stem-cell-transplant patients, the incidence of positive HEV RNA in the serum ranges between 0 and 2.4 % [42-44]. The mode of transmission has not been fully studied. Recently, HEV RNA has been detected in a hematopoietic stem-cell donor [45]. Chronic HEV infections, leading in some patients to cirrhosis, have been reported in both adults and pediatric patients with a bone-marrow transplant [44] [46, 47]. Finally, two cases of HEV reactivation have been reported [33, 44].

**Extra-Hepatic Manifestations**

Besides the classical hepatic manifestations, HEV can cause extra-hepatic manifestations. Neurological manifestations are the most commonly reported extra-hepatic manifestation associated with HEV. A retrospective Anglo-French study reported an incidence of 5.5 % of neurological manifestations among immunocompetent and immunocompromised patients infected by HEV [48]. A large number of case reports, small series, and retrospective studies have reported on neurological manifestations that occurred either in immunocompetent and immunocompromised patients during an acute phase of HEV, or in immunocompetent patients with chronic hepatitis. The main neurological manifestations reported in the literature are Guillain–Barré syndrome [49•], neuralgic amyotrophy [50•], and encephalitis [51]. In some of these patients, HEV RNA was detected in the cerebrospinal fluid and these viral variants were quite different from those found in the serum at the same time, suggesting the presence of neurotropic variants [48], [51]. This observation is emphasized by the fact that immunocompetent patients with Guillain–Barré syndrome or neuralgic amyotrophy had a very mild increase in liver enzymes levels [49•, 50•].

Acute HEV infection has been associated with a decrease in kidney function in kidney- and liver-transplant patients [52, 53]. Interestingly, similar to what is known for patients infected with hepatitis C virus, HEV has been found to cause glomerular injuries and cases of HEV-induced membranoproliferative and membranous glomerulonephritis have been reported [53-55]. In some cases, cryoglobulinemia was concomitantly detected. An early case report documented HEV-associated cryoglobulinemia with arthralgia, myalgia, and a rash in a liver-transplant patient [56]. In addition, among 68 patients with essential cryoglobulinemia, 46 % (15/33) tested positive for anti-HEV IgG compared to 23 % (8/35) of patients with cryoglobulinemia with a defined cause (p = 0.04) [57], suggesting a possible relationship between HEV and the presence of cryoglobulinemia.

Several other extra-hepatic manifestations have been associated with HEV, including severe thrombocytopenia and aplastic anemia, pancreatitis (only with genotype 1), myositis, and thyroiditis [1•].

**Management of HEV Infection in Organ Transplant Patients**

No information regarding the optimal management of HEV infection during an acute phase has been reported. In patients who develop chronic HEV infection, it has been shown that decreased immunosuppression, especially drugs that target T cells, can be considered as a first therapeutic option [35] [25]. Indeed, tacrolimus levels and steroid doses were lower in transplant patients with...
chronic infection when the virus was eliminated compared to those who remained viremic [35]. Reduction of immunosuppression allowed HEV clearance in one third of cases. In the remaining patients, an initial short 3-month course of pegylated interferon was successfully used in liver-transplant patients and in a patient receiving hemodialysis after a failed transplant [58–60]. However, interferon is contraindicated in other organ-transplant patients because of the increased risk of acute rejection [61].

Several case reports and small series have reported on the beneficial effects of oral ribavirin alone to treat chronic HEV infection [62–64]. A multicenter French retrospective study reported on 59 patients with a solid-organ transplant who had been treated with ribavirin as monotherapy for HEV infection [65••]. The initial median ribavirin dose was 600 (29–1200) mg/day and was adjusted for kidney function. Seventeen patients were given 600 mg/day and 17 other patients received 800 mg/day. The median duration of ribavirin treatment was 3 (range 1–18) months. The majority of patients were treated for 3 (n = 36) or 6 (n = 12) months. A sustained virological response (SVR) was observed in 78 % of patients [65••]. Six patients had relapse of infection and subsequently received extended therapy, of which four then achieved a SVR whereas the other two cleared viremia by the end of therapy but did not have a sufficient follow-up to determine if they achieved SVR or not; anemia was the main side effect.

Two cases of ribavirin failure have been recently documented [66]. Both patients had a G1634R mutation involving viral polymerase. This mutation did not cause ribavirin resistance in vitro but did increase the replicative capacity of the virus [66]. Adjustment to dose and duration of ribavirin therapy may be necessary in patients that have this mutation.

The mechanism of action of ribavirin against HEV is not fully known. In vitro data suggest that ribavirin inhibits HEV replication through depletion of guanosine triphosphate (GTP) pools [67]. In addition, in vitro studies have shown that mycophenolic acid and ribavirin have synergistic effects against HEV [37]. This finding has not been confirmed in organ-transplant patients [16], where the decrease in viral load was similar in patients receiving ribavirin with or without mycophenolic acid [16]. In a multicenter retrospective study of ribavirin therapy, a higher lymphocyte count was identified as an independent predictive factor for a SVR [68]. In a sub-study performed in our center, we found that patients with undetectable HEV RNA in the serum but who had persisting viral shedding after cessation of ribavirin were at higher risk for a relapse after therapy [17]. A decrease in viral load, particularly a decrease of ≥0.5 log IU/mL by day 7, is an independent predictive factor for a SVR [16].

How to Manage Organ-Transplant Patients Infected by HEV?

When possible, immunosuppression should be decreased, especially calcineurin and mTOR inhibitors. HEV RNA concentration must be monitored monthly for at least 3 months. In the absence of HEV clearance after this period of reduced immunosuppression, ribavirin should be started as a monotherapy. Ribavirin doses should be adapted to kidney function to reduce adverse effects. However, ribavirin trough levels measured at day 7 and month 2 after the initiation of therapy do not predict a SVR [16]. Three months of therapy is recommended: after this time, HEV RNA should be assessed in both the serum and stool. If HEV RNA is still detectable in the stool, but no longer detected in the serum, ribavirin therapy can be extended for an additional 3 months. If HEV RNA is undetectable in both serum and stool, then ribavirin can be stopped. If a relapse occurs after ribavirin cessation, treatment can then be repeated for a longer period, i.e., 6 months. Interestingly, in patients previously successfully treated for HEV and in those who have spontaneous clearance of HEV, no reactivation occurred after kidney re-transplantation [69].

Prevention

Prevention of HEV infection mainly relies primarily on educating patients on safe dietary habits, such as avoiding eating undercooked game meat, pork, or pork sausages and to avoid drinking non-potable water. One efficient and safe vaccine is available [70] and is only licensed in China. Recent long-term data have confirmed its protective effects [71••]. Screening blood products for HEV in endemic area can be proposed. Indeed, in France, systematic screening of plasma for HEV has been established. However, it is still not the case for other blood products and in other endemic countries.

Conclusion

The interest in HEV in the transplant population has emerged within the last few years with the discovery that the virus can induce chronic hepatitis, cirrhosis, neurological manifestations, and kidney injury in this setting. However, HEV has not been systematically assessed in transplant patients with abnormal liver tests. Serological and molecular tests still need to be approved in several countries, including the USA. Although ribavirin is efficient at treating HEV infection, further studies are required to determine the optimal duration of treatment. In addition, novel therapies are required for patients where ribavirin has failed.
Compliance with Ethical Standards

Conflict of Interest  Drs Marion, Abravanel, Lhomme, Izopet, and Kamar declare no conflicts of interests.

Human and Animal Rights and Informed Consent  This article does not contain any studies with human or animal subjects performed by the author.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

2. •• Pavia N, Meng XJ, Doceul V. Zoonotic origin of hepatitis E. Curr Opin Virol. 2015;10:34–41. This is a comprehensive review summarizing current knowledge about hepatitis E virus.


