Clinics and Research in Hepatology and Gastroenterology (2019) **43**, 108—111

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

RESEARCH LETTER

Hepatitis E virus infection in heart transplant recipients, Southeastern France

KEYWORDS
Hepatitis E;
Heart transplantation;
Ribavirine;
Autochthonous;
Zoonosis

Hepatitis E virus (HEV) is an emerging concern in France where infections are most often related to consumption of pig liver sausages [1]. This virus was recently identified as the first cause of acute infectious hepatitis, with an estimated incidence of 68,007 cases per year (5^{th}—95^{th} percentiles of the output distribution, 46,032—101,279), and it is responsible for 546 hospitalizations (540—553) and 20 deaths (18—22) per year [2]. Although classically asymptomatic or spontaneously resolving in immunocompetent people, HEV genotype 3 infection can become chronic and cause cirrhosis among severely immunocompromized patients, most often solid organ-transplant recipients (SOTR) [3]. This has been mostly documented in kidney-transplant recipients (KTR) and liver-transplant recipients (LTR), but far more rarely post-transplantation of other organs, including heart [3,4]. We report here four HEV infections and HEV seroprevalence in heart-transplant recipients (HTR).

This retrospective study was conducted on blood samples collected from 159 HTR between June 2013 and April 2018 (58 months) and tested in public hospitals of Marseille, south eastern France. This cohort consisted in patients followed-up for a heart transplantation for whom a least one HEV serology, including both IgG and IgM determinations, was performed in our institution. Liver biochemical markers were determined at each medical consultation, each month the first year post-transplantation, then every two months and every three months the second and third year post-transplantation, respectively. HEV serologies were carried out using Wantai assays (Beijing, China) until January 2017, then Euroimmun assays (Lübeck, Germany). Serum HEV RNA was detected using in-house real-time and conventional reverse transcription-polymerase chain reaction (PCR) assays targeting open reading frame 2 (ORF2) of the HEV genome that encodes the major capsid protein, as described previously [5]. HEV sequence obtained corresponded to nucleotides 6055—6231 of the reference HEV genome GenBank accession no.AB369687.1. The HEV genotype was determined by phylogenetic analysis with the MEGA6 software (http://www.megasoftware.net/) using the Neighbor-Joining method and the Kimura 2-parameter method (see supplementary Figure S1).

Case-patient No. 1, a 64-year-old man, was followed-up in November 2013 for heart transplantation performed three years earlier for ischemic cardiopathy. Only overweight and psoriasis were noted during clinical assessment. His immunosuppressant therapy contained tacrolimus (3.5 mg/L), mycophenolate mofetil (2 g/d) and prednisone (10 mg/d). Systematic testing revealed liver enzymes disturbance (ALT, 149 IU/L, Fig. 1b), which allowed diagnosing HEV infection. HEV RNA was still detected in serum in September 2014, indicating chronic hepatitis E. Ribavirin was introduced (800 mg/d) for 12 weeks. No results for HEV RNA testing was available until September 2017. At this time, HEV RNA was not detected in serum and liver parameters were within the normal range. Case-patient no.2, a 52-year-old man, was admitted in July 2014 for follow-up of a corticosteroid-induced diabetes due to heart transplantation performed 3 years earlier for familial dilated cardiomyopathy. He was immunosuppressed by everolimus (3 mg/d), tacrolimus (2 mg/d) and prednisolone (5 mg/d). Hepatic parameters were slightly disturbed (ALT, 139 IU/L, Fig. 1b), which led to search for and detect IgM anti HEV and HEV RNA in the patient's serum. Three months after HEV diagnosis, HEV RNA was strongly positive in the patient's serum, suggesting chronic HEV infection. Ribavirin (800 mg/d) was administered for 12 weeks. On Febuary 2015, HEV RNA was negative and liver parameters did normalize. Case-patient No. 3, a 53-year-old man, was monitored in February 2017 for heart transplantation performed 13 years earlier for dilated cardiomyopathy. He was clinically asymptomatic and was receiving mycophenolate mofetil (155 mg/d) and ciclosporin (2000 mg/d). Systematic testing revealed a dis-

https://doi.org/10.1016/j.clinre.2018.09.010

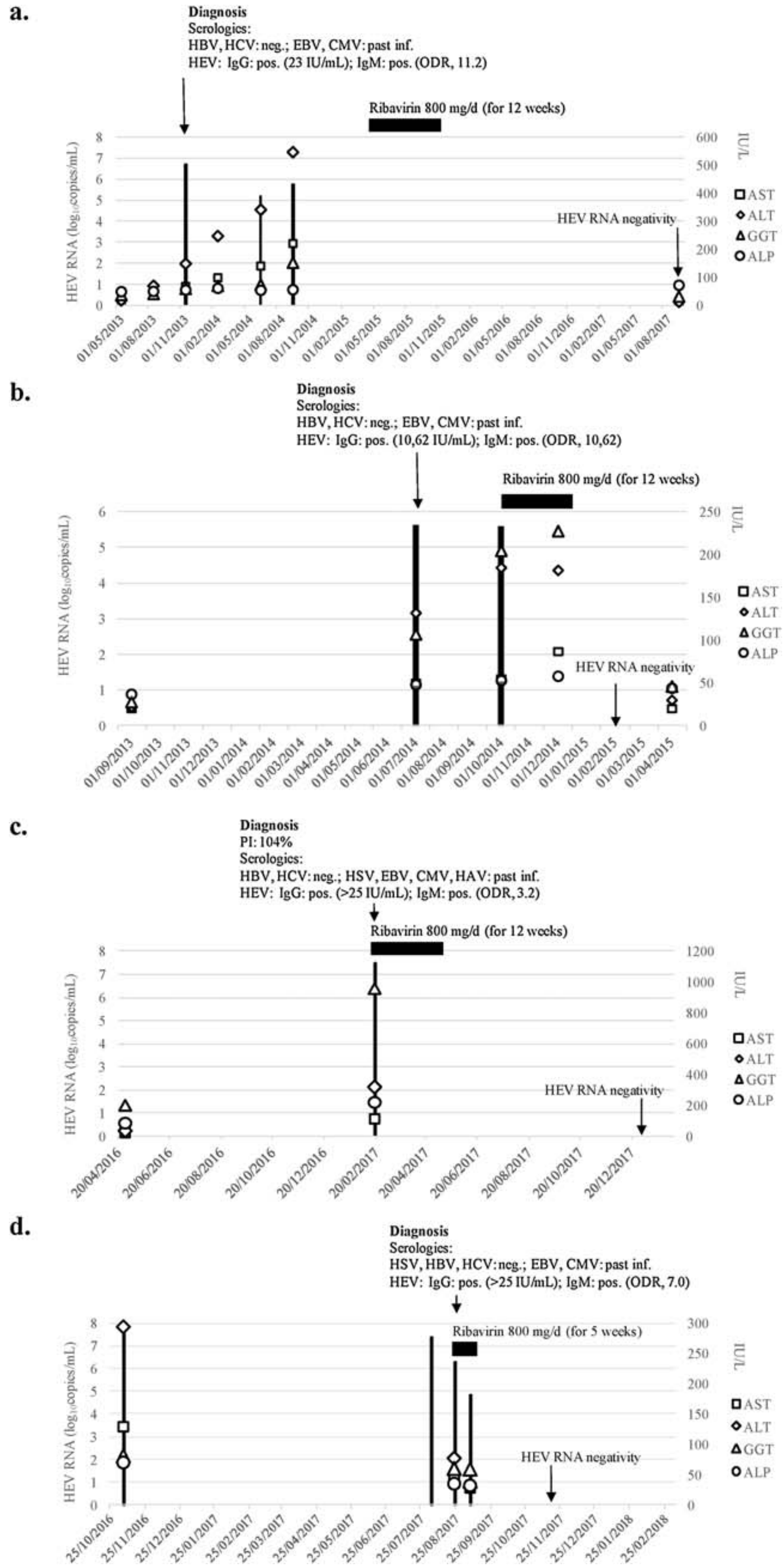

Figure 1 Course of virological and biochemical parameters and ribavirin therapy in case-patients No. 1 (a), 2 (b), 3 (c), and 4 (d). ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; CMV: cytomegalovirus; EBV: Epstein-Barr virus; GGT: gammaglutamyltransferase; HAV: hepatitis A virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HEV: hepatitis E virus; HSV: herpes simplex virus; inf., infection; neg., negative; ODR, serum-to-threshold optical density ratio; PI: prothrombin index; pos.: positive.

turbance of liver enzymes (ALT, 318 IU/L) (Fig. 1c), which led to diagnose HEV infection. No previous serum was available to determine if this infection was chronic, but ALT were within normal values 10 months earlier. Ribavirin was introduced (800 mg/d, 3 months) and HEV RNA was negative seven months post-end of treatment. Case-patient No. 4, a 22-year-old man, was admitted in August 2017 for routine follow-up of heart transplantation performed 2 years earlier for dilated cardiomyopathy. He had no clinical symptoms. His immunosuppressant treatment contained everolimus (150 mg/d), mycophenolate mofetil (315 mg/d), and tacrolimus (8 mg/d). Sytematic testing revealed a discrete liver enzymes elevation (alanine aminotransferases (ALT), 76 IU/L; Fig. 1d), which led to test for and diagnose HEV infection based on anti-HEV IgM and HEV RNA-positivity in serum. Retrospective testing showed HEV RNA-positivity and liver cytolysis 9 months earlier, indicating chronic hepatitis E. Ribavirin was instaured (400 mgx2/d, 5 weeks), which was associated with viral clearance as assessed 6 weeks post-treatment. These four patients did not recently receive blood products nor did travel abroad. Three ate figatellu, a pig liver sausage, which is a major source of autochthonous HEV infections in France [6,7]. Consistently, phylogeny identified HEV genotype 3f and 3i, which are the most frequent ones in French autochthonous cases, in three and one patient, respectively (GenBank-no.MH165304-MH165305; MH557093-MH557094) (Supplementary Figure S1) [5,8]. Mean ($\pm$standard deviation) pairwise nucleotide identity between the four sequences was $85 \pm 5\%$ (range, 78—92%), indicating that HEV strains were different and reflecting genetic diversity of HEV circulating in southeastern France [5].

The four cases were among the 159 heart-transplant recipients for whom 344 HEV serologies were performed between June 2013 and April 2018 (58 months) in public hospital of Marseille, southeastern France. Mean age of these patients was 49 ± 14 years (range, 19—85 years), and 78% of them were men. HEV serology was performed a mean number of 2.1 ± 1.2 times (1.0—7.0) per patient during the study period; 58 patients (36%) were tested only once, 49 twice (31%), 33 thrice (21%), and 19 (12%) more than thrice. Case-patients no.2 and no.3 had been tested HEV-negative once and case-patient no.4 had been tested HEV-negative at three occasions before being diagnosed as HEV-infected; case-patient no.1 had not been tested prior diagnosis. IgG-positive/IgM-negative patterns were found with the Wantai assay for 45.3% of the patients [57 ± 12 years (24-85)]. This is higher than anti-HEV IgG prevalence previously assessed among KTR and blood donors in our geographical area with the same assay (35% ($P = 0.09$; Chi-square test) [9] and 30—40% [10], respectively).

The five-year incidence of HEV infection among HTR in our center was 2.5%. This is in the same range than the HEV diagnosis incidence we previously described among 578 KTR, which was 1.0% over a 27 month-period. In both cohorts, liver enzyme testing was systematic [5,11]. These data point out that HEV infection is common among SOTR in south eastern France, consistently with what was observed among blood donors sampled in 2011-2013, of whom 3.2% had IgM to HEV and 0.9‰ were HEV RNA-positive [10,12]. A greater HEV incidence was described among HTR than KTR and/or LTR in the Netherlands during 2000-2011 (1.9% in 259 HTR vs. 1.0% in 300 LTR and 0.2% in 574 KTR) whereas in Germany HEV incidence was similar in 274 HTR (1.5%) and 226 LTR (0.9%) [4,13,14].

Chronic HEV (defined by the persistence of a viremia for more than 6 months) infection was demonstrated here in two cases, strongly suspected in one case, and not assessable in the fourth case. This highlights that HEV infection often becomes chronic in HTR, as previously observed in other HTR cohorts and other SOTR [3—5,13]. Ribavirin was initiated in the four present cases and associated with HEV infection resolution in absence of major side effect, as observed in a majority of patients in previous studies [3] including among HTR [4,13]. Nonetheless, severe anemia as major side effect indicating ribavirin dose reduction, erythropoietin use, or blood transfusion were previously described to be required in 12—54% of SOTR [3]. Moreover, failure of ribavirin to clear HEV was described in 25% of chronically-infected HTR in previous studies [4,13], and even in one patient in association with sofosbuvir that was described to inhibit HEV replication in vitro [15]. Overall, previous data strongly warrant testing for HEV infection in HTR with liver cytolysis. Moreover, education regarding the risk of acquiring HEV through pig liver sausage consumption should be systematic for those patients.

Funding

This work was supported by the French Government under the ''*Investissements d'avenir*'' (Investments for the Future) program managed by the *Agence Nationale de la Recherche* (ANR, Fr: National Agency for Research) (reference: Méditerranée Infection 10-IAHU-03).

Ethics

All data have been generated as part of the routine work at *Assistance Publique-Hôpitaux de Marseille* (Marseille university hospitals), and this study results from routine standard clinical management.

Disclosure of interest

The authors declare that they have no competing interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.clinre.2018.09.010.

References

[1] Colson P, Raoult D. Autochthonous hepatitis E: a common and fatal but neglected emerging disease in France. Clin Microbiol Infect 2017;23:898—9.

[2] Van Cauteren D, Le Strat Y, Sommen C, Bruyand M, Tourdjman M, Da Silva NJ, et al. Estimated annual numbers of foodborne pathogen-associated illnesses, hospitalizations, and deaths, France, 2008—2013. Emerg Infect Dis 2017;23:1486—92.

[3] Kamar N, Dalton HR, Abravanel F, Izopet J. Hepatitis E virus infection. Clin Microbiol Rev 2014;27:116—38.
[4] Koning L, Pas SD, de Man RA, Balk AH, de Knegt RJ, ten Kate FJ, et al. Clinical implications of chronic hepatitis E virus infection in heart transplant recipients. J Heart Lung Transplant 2013;32:78—85.
[5] Moal V, Legris T, Burtey S, Morange S, Purgus R, Dussol B, et al. Infection with hepatitis E virus in kidney transplant recipients in southeastern France. J Med Virol 2013;85:462—71.
[6] Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis 2010;202:825—34.
[7] Kupferschmidt K. Europe's new hepatitis problem. Science 2016;353:862—3.
[8] Lhomme S, Abravanel F, Dubois M, Chapuy-Regaud S, Sandres-Saune K, Mansuy JM, et al. Temporal evolution of the distribution of hepatitis E virus genotypes in Southwestern France. Infect Genet Evol 2015;35:50—5.
[9] Rossi-Tamisier M, Moal V, Gerolami R, Colson P. Discrepancy between anti-hepatitis E virus immunoglobulin G prevalence assessed by two assays in kidney and liver transplant recipients. J Clin Virol 2013;56:62—4.
[10] Mansuy JM, Gallian P, Dimeglio C, Saune K, Arnaud C, Pelletier B, et al. A nationwide survey of hepatitis E viral infection in French blood donors. Hepatology 2016;63:1145—54.
[11] Moal V, Motte A, Kaba M, Gerolami R, Berland Y, Colson P. Hepatitis E virus serological testing in kidney transplant recipients with elevated liver enzymes in 2007—2011 in southeastern France. Diagn Microbiol Infect Dis 2013;76:116—8.
[12] Gallian P, Lhomme S, Piquet Y, Saune K, Abravanel F, Assal A, et al. Hepatitis E virus infections in blood donors, France. Emerg Infect Dis 2014;20:1914—7.
[13] Pischke S, Stiefel P, Franz B, Bremer B, Suneetha PV, Heim A, et al. Chronic Hepatitis E in Heart Transplant Recipients. Am J Transplant 2012;10:3128—33.
[14] Pas SD, de Man RA, Mulders C, Balk AH, van Hal PT, Weimar W, et al. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. Emerg Infect Dis 2012;18:869—72.
[15] Todesco E, Mazzola A, Akhavan S, Abravanel F, Poynard T, Roque-Afonso AM, et al. Chronic hepatitis E in a heart transplant patient: sofosbuvir and ribavirin regimen not fully effective. Antivir Ther 2018 [Epub ahead of print].

Léa Luciani[a]
Pierre Deharo[b]
Sarah Aherfi[a]
Virginie Chalvignac[c]
Patrick Borentain[d]
Philippe Colson[a,*]

[a] *Aix-Marseille université, institut de recherche pour le développement (IRD), Assistance publique — hôpitaux de Marseille (AP—HM), microbes, evolution, phylogeny and infection (MEΦI), institut hospitalo-universitaire (IHU) — Méditerranée Infection, 19—21, boulevard Jean-Moulin, 13005 Marseille, France*
[b] *Assistance publique — hôpitaux de Marseille (AP—HM), centre hospitalo-universitaire Timone, service de cardiologie, 264, rue Saint-Pierre, 13385 Marseille cedex 05, France*
[c] *Assistance publique — hôpitaux de Marseille (AP—HM), centre hospitalo-universitaire Timone, service de chirurgie cardio-thoracique, 264, rue Saint-Pierre, 13385 Marseille cedex 05, France*
[d] *Assistance publique — hôpitaux de Marseille (AP—HM), centre hospitalo-universitaire Timone, service d'hépatologie-gastrologie-entérologie, 264, rue Saint-Pierre, 13385 Marseille cedex 05, France*

* Corresponding author at: IHU — méditerranée infection, AP—HM, 19—21, boulevard Jean-Moulin, 13005 Marseille, France.
E-mail address: philippe.colson@ap-hm.fr (P. Colson)

Available online 27 November 2018