Hepatitis E infection in stem cell and solid organ transplant patients: a cross-sectional study

The importance of HEV RNA screening in peri-transplant period

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Abstract:

Hepatitis E Virus (HEV) is a common cause of acute viral hepatitis worldwide. Typically associated with a self-limiting illness, infection may persist in immunosuppressed populations with significant morbidity and mortality. Based on clinical data published world-wide, UK blood safety guidance recommends the universal screening for HEV RNA of blood donors and donors of tissue, organs and stem cells.

This cross-sectional study aimed to determine the point prevalence of HEV viraemia and clinical course of viraemic patients in the peri-transplant period in solid organ transplant (SOT) and haematopoietic stem cell transplant (HSCT) recipients transplanted over a 3-year period (2013 to 2015). Nucleic acid extracts of whole blood from patients undergoing SOT or HSCT were tested by an in-house real-time reverse-transcriptase polymerase chain reaction assay for HEV RNA. Samples were tested at baseline (time of transplant), 30, 60 and 90 days post-transplant.

870 patients (259 HSCT, 262 liver and 349 kidney transplant) were included with 2554 samples meeting the inclusion criteria. No kidney transplant patients had HEV viraemia at time of testing. One HSCT and three liver transplant patients were found to be HEV RNA positive. Overall this represented 0.46% of the patients testing positive for HEV viraemia.

Conclusion: Prevalence of HEV viraemia in SOT and HSCT patients in U.K. although higher than in the general population is low at baseline and remains low throughout the early post-transplant phase. Clearance of viraemia can be maintained despite ongoing immunospuupression. Prospective U.K. studies are necessary to inform screening policies in this population.

Key Words:

Hepatitis E, Transplants, Blood transfusion

Background: Hepatitis E Virus (HEV) is a common cause of infectious hepatitis (1). Of the four major genotypes infecting humans, genotype 3 (G3) has been solely implicated in HEV cases in England. HEV infection can persist in immunosuppressed patients, leading to chronic hepatitis, cirrhosis (2,3) or development of other syndromes, including neurological disorders (4,5). Epidemiologic data demonstrate a recent increased incidence in the U.K. (2) and variable prevalence across Europe (6). HEV prevalence of 0.04% in blood donors from England (7) led to introduction of universal screening for HEV RNA in donors of blood, tissue, stem-cells and organs (8). However, currently, there are no data on prevalence or course of HEV infection in transplant recipients in the UK.

Aims: To determine point prevalence and clinical course of HEV viraemia in SOT and HSCT recipients to inform policy for HEV screening in the peri-transplant period.

Methods: Patients receiving HSCT, liver (LT) or kidney (RT) transplant between January 2013 - December 2015 were identified from databases at the Royal Free Hospital. Stored extracted citrated blood samples at baseline, 30, 60 and 90 days post-transplant (+/- 7 days) were identified. These time points were deemed to cover the peri-transplant period with a low likelihood of a patient becoming viraemic and clearing the infection between samples. RNA extraction was by easyMAG (BioMérieux, France). HEV reverse-transcription real-time RT-qPCR was performed on samples stored at -20°c using a Superscript III RT PCR kit (ThermoFisher Scientific, USA) on an ABI Prism 7500 thermocycler (Applied Biosystems, USA). This in-house technique is a validated modification of a previously published method (9). The assay targets a region within ORF 2/3 of the HEV genome. HEV RNA positive control material was produced from pooled serum of viraemic individuals, provided by the Public Health England Reference Laboratory (PHE).

Samples were considered positive for HEV RNA if the cycle threshold was <45 cycles with an exponential amplification curve. All positive samples were further tested for verification, viral RNA quantification and typing as previously described (10). Patients with HEV were managed according to standard clinical practice. Data on patients with viraemia are presented in accordance with the Declaration of Helsinki.

Results: 870 patients (259 HSCT, 262 LT and 349 RT) met the inclusion criteria. HSCT comprised 111 allogeneic (90/111 non-myeloablative), 145 autologous HSCT and three CD34 top-up procedures. LT comprised 259 deceased-donor, two live-donor and two domino-LT patients. RT comprised 241 deceased, 38 live-unrelated, 63 live-related donors and one with unknown donor status. There were seven simultaneous liver-kidney transplant recipients. All patients received non-HEV screened blood products. 2554 samples met the inclusion criteria, 42 of which were unavailable for testing. The distribution and point prevalence of HEV viraemia at each time point tested is shown in Table 1.

Table 1: Number of samples tested per time point and HEV viraemic prevalence.

Transplant Type		Baseline samples	30 day samples	60 day samples	90 day samples	Total
HSCT	Samples Meeting Inclusion Criteria	154	117	104	93	468
	Samples Tested	153	117	104	93	467
	Samples Positive	1	1	1	1	
	Prevalence (%)	0.65	0.85	0.96	1.08	
LT	Samples Meeting Inclusion Criteria	231	231	189	139	790
	Samples Tested	230	227	187	137	781
	Samples Positive	1	1	2	2	
	Prevalence (%)	0.43	0.44	1.07	1.46	
RT	Samples Meeting Inclusion Criteria	289	342	337	328	1296
	Samples Tested	270	337	336	320	1263
	Samples Positive	0	0	0	0	
	Prevalence (%)	0	0	0	0	

Four patients (one HSCT and three LT, Table 2) were found to have HEV viraemia. This represents 0.39% of the HSCT and 1.15% of the LT patients. Details of HEV infection are provided in Table 2 and Figure 1. No RT recipients were found to be HEV RNA positive. Sequence analysis indicated all HEV to be genotype 3 viruses.

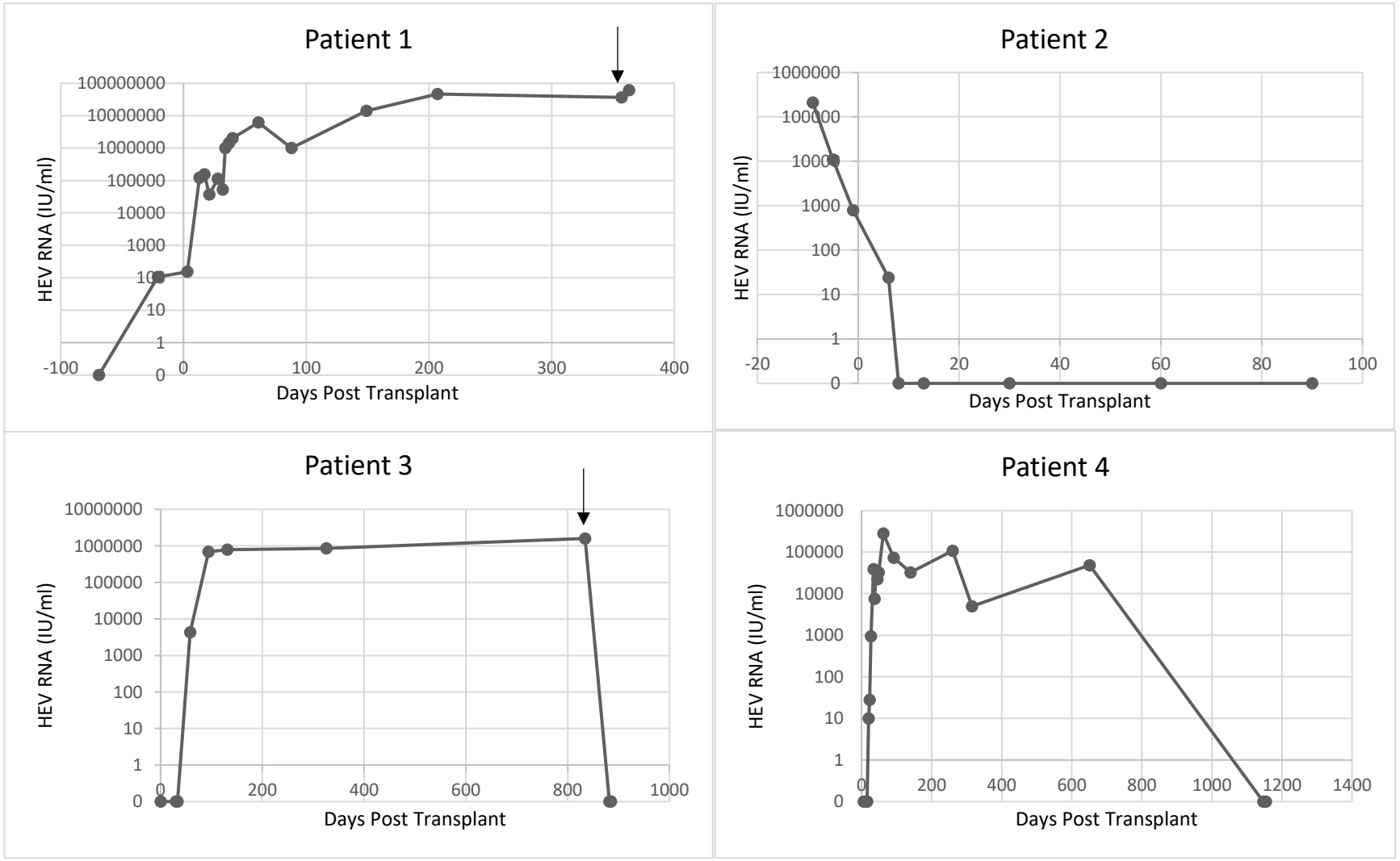
The 4 patients with HEV viraemia had variable clinical courses, described in Table 2. 2 patients were infected with HEV prior to transplant; in Patient 2 this was known and treatment was initiated prior to transplant. In Patient 1 infection was not known until 12 months post-transplant; in this time he developed neurological complications, consistent with encephalitis, suspected to be a sequel of HEV infection. He died due to sepsis and GVHD shortly after HEV infection was diagnosed. All blood products transfused to patient 1 were tested retrospectively for HEV and were negative. Of the 2 patients who became HEV viraemic post-transplant; Patient 3 was successfully treated with ribavirin, while Patient 4 spontaneously cleared the infection over 3 years post-transplant.

Legend - Table 2: Clinical data regarding HEV positive patients

Patient	Age	Sex	Transplant type	Transplant Indication	Infection Acquired	Transfusion Burden	Treatment	Outcomes	Liver Histology
1	60	M	RIC-allo	Acute Myeloid Leukaemia	Between day 69 and day 21 pre-transplant.	During AML: 14 apheresis platelets, 3 pools of platelet, 22 units red cells. Post-transplant: 3 red cell units, 4 apheresis platelets, 2 platelet pools ALL units HEV negative: tested retrospectively.	Treated with ribavirin – initiated 1-year post-op. Immunosuppression not reduced due to concomitant severe GVHD (liver, skin). Unable to clear virus.	Neurological symptoms - MRI brain showed features in keeping with a viral encephalitis. Died of complications related to HSCT- severe GVHD and recurrent sepsis.	Post HSCT: Irregular biliary epithelium with infiltration by small lymphocytes, ductopenia and cholestasis. Appearance in keeping with GVHD.
2	49	M	LT	Acute Liver Failure due to HEV on a background of moderate liver disease.	Prior to day 9 pre-transplant.	Peri- transplant: 2 platelet pools, 8 units FFP, 7 red cell units	Treated with ribavirin pre transplant to reduce viral load at transplant and for 1-month post-transplant. Immunosuppression as per normal regimen for LT.	Liver function tests normal 1-year post-transplant. Patient well at last review 18 months post-op.	Pre-transplant: severe cholestatic hepatitis/submassive necrosis in keeping with acute hepatitis E infection. Evidence of underlying NASH.

3	66	M	LT	Alcoholic Liver Disease	Between day 33 and 58 post-transplant.	Peri-transplant: 8 platelet pools, 8 units FFP, 2 units cryoprecipitate, 17 red cell units	Treated with ribavirin 10 months post-transplant. Toxicity: Anaemia requiring transfusion.	Transaminases normal post treatment HEV RNA not detected post Ribavirin treatment.	Post-transplant: Moderate chronic hepatitis with mild fibrosis. Features in keeping with chronic HEV infection.
4	33	M	LT	Acute Liver Failure due to Isoniazid and Rifampicin	Between day 16 and 20 post-transplant.	Peri-transplant: 7 platelet pools, 8 units FFP, 3 units cryoprecipitate, 19 red cell units	No treatment. At 10 months VL declined to 100000 IU/ml. At 36 months virus was cleared.	Clinically well. LFTs normal at last follow up. Not treated. Cleared.	Post-transplant: Moderate mixed inflammation of portal ducts. No viral inclusion identified. Appearance is of moderate cellular rejection.

Figure 1: HEV RNA by time post-transplant. Arrows show point at which ribavirin therapy was started for patients 1 and 3.



Discussion: In our cohort, HEV prevalence was 0.39% in HSCT, 1.15% in LT and 0% of RT patients between days 0-90 post-transplant. This is lower than 2-2.4% reported in HSCT (11) or 4.28% reported in SOT (12). This is in part related to geographic difference in viraemic prevalence and partly to methodologic differences between studies. Prevalence in blood donors varies across Europe (6); in the U.K. approximately 1/2800 donors are HEV RNA positive (7). The prevalence of 1.15% in LT patients (or 0.76% if excluding Patient 2, who received LT because of HEV related acute hepatic failure) and 0% in RT patients is lower than reported by other studies, some of which have used serology to diagnose HEV infections or screened over a longer period (12). Our study focussed on the peri-transplant period because of the implications of HEV positivity in the post-transplant recovery period. Although the results of our study are low in absolute terms, prevalence of HEV viraemia in LT, RT and HSCT patients in this study is high compared to UK blood donors (0.04%).

The source of infection on a population wide basis is usually dietary (13). In transplant recipients the risk of infection via infected blood components or transplanted organs is an important consideration. Patient 1, despite a high transfusion burden, acquired HEV infection from his food, as did Patient 2. The source of infection in patients 3 and 4 cannot be ascertained as their blood components or transplanted organs were not tested. Modelling has suggested that transfusion burden must exceed 13 units of blood components to equal the risk of dietary transmission attained in 1 year (14). HEV screening of blood donors in England (6) reduces iatrogenic transmission but does not influence dietary risk. Given this, and the fact that Patients 1 and 2 acquired their infection pre-transplant, a strategy of pre-transplant screening of recipient and donor can inform strategies for pre-emptive treatment of HEV pre-transplant and post-transplant monitoring.

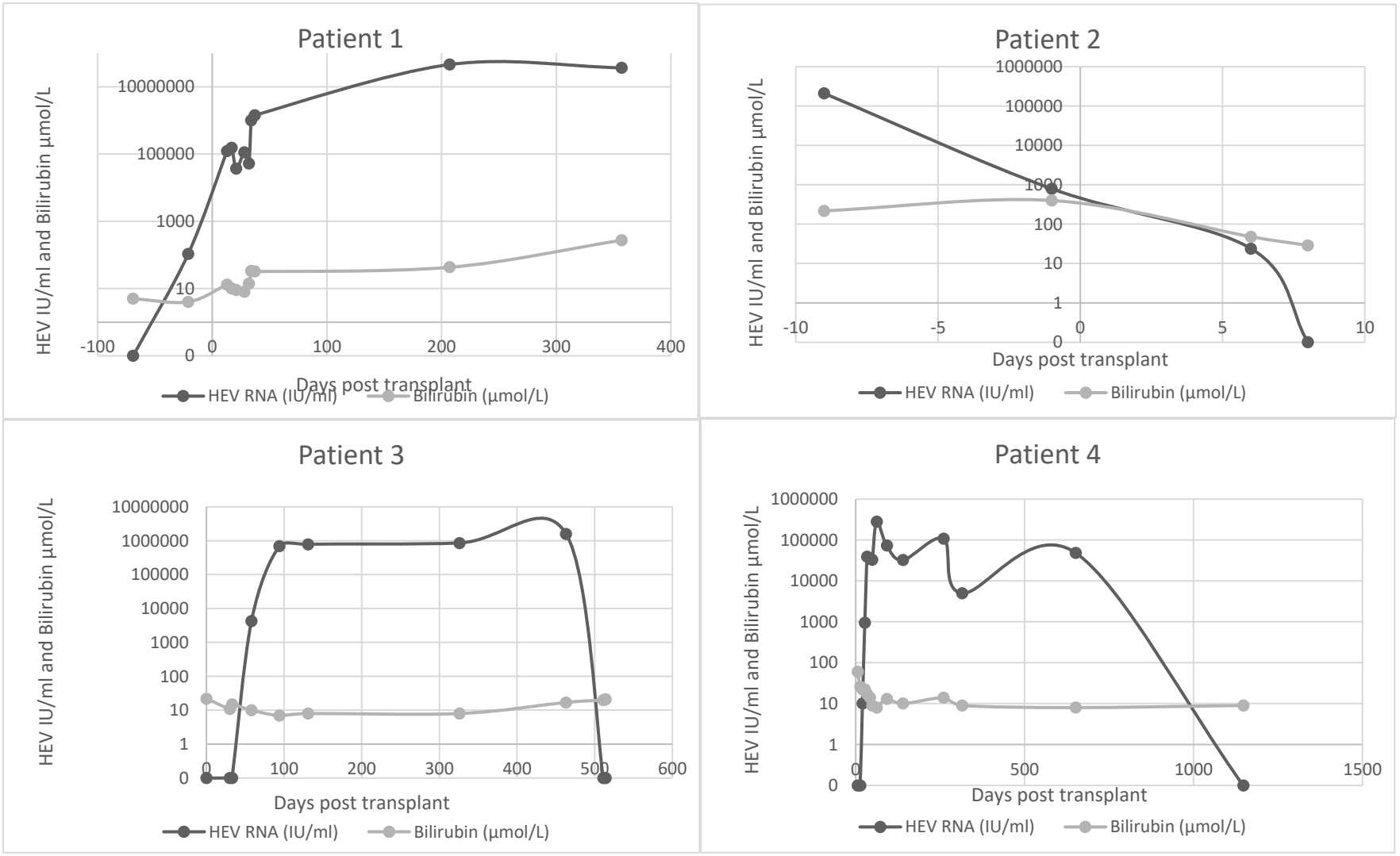
The four patients presented here demonstrate a range of clinical courses. Patient 1 developed progressive central and peripheral neurological impairment. Immunosuppressed patients presenting with neurological symptoms later diagnosed as secondary to HEV have been described (8,9). His response to ribavirin could not be documented as he died within 2 weeks of treatment. Patients 2

and 3 cleared HEV rapidly with ribavirin despite continuation of immunosuppression. Patient 4 had a fluctuating viral load and spontaneously cleared HEV from blood after three years. The factors underlying this are unclear. In the rare case where HEV viraemia is discovered pre-transplant, ribavirin pre-transplant can successfully eradicate viraemia. Serum bilirubin was not a reliable indicator of the severity of HEV viraemia (Figure 2).

This study has limitations; it was a single centre, retrospective survey, 2 of 4 patients were not tested for iatrogenic infection and the study addressed only the peri-transplant timing. However, the large cohort of unselected HSCT, LT and RT patients from a U.K. regional centre and >98% of eligible samples undergoing analysis provide a sound basis for estimating the risk and course of HEV in the first 90 days post-transplant using blood/organs not screened for HEV.

In summary, we present data from a retrospective analysis of a large cohort of 870 patients post HSCT or SOT. In this cohort prevalence of HEV is low, although 10 to 50 fold higher in HSCT and LT patients respectively that in the general population. No RT patients developed viraemia. The patient most exposed to blood products did not acquire HEV through transfusion. These data lend weight to recently published guideline recommending baseline screening of donor, but not routine baseline or ongoing screening of the recipient; and to the need for dietary advice in vulnerable populations (15).

Figure 2: HEV RNA and serum bilirubin levels



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