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Short communication

Hepatitis E infection in stem cell and solid organ transplantpatients: A cross-sectional study
The importance of HEV RNA screening in peri-transplant period

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ABSTRACT

Background: 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.
Objectives: 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 (2013–2015).
Study design: 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.
Results: 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.
Conclusions: 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 immunosuppression. Prospective U.K. studies are necessary to inform screening policies in this population.

1. 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.

2. Objectives

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.

3. Study design

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

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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) according to manufacturer's instructions. Primers used were forward 5′GGT GGT TTC TGG GGT GAC3′ and reverse 5′AGG GGT TGG TTG GAT GAA3′ with probes HEV Probe 1 (FAM-TGATTCTCAGCCCTTCGC-BHQ1) and HEV Probe 2 (FAM-TGATTCTCAGCCCTTTGC-BHQ1). Conditions for amplification were 50 °C for 15 min, 95 °C for 2 min then 45 cycles of 95 °C for 15 s and 60 °C for 45 s. 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. All HEV RNA positive samples were sequenced across a 1.3 kb region of the HEV capsid protein (ORF2). cDNA was generated using the SuperScript II Reverse Transcriptase kit (Invitrogen) in accordance with the manufacture's protocol using 10μM of primer R3O (5′-AGACTCCCGGGTTTTACCTACCTTCATTTT-3′). A semi-nested PCR was carried out with 5 μl of cDNA using MyFi Mix (Bioline) reagents. Amplification was undertaken using primers R3O and ORF2-FWD1 (5′-TTGGCGTGACCAGKCCCAGCGCC-3′) in the first round of PCR and R3O and ORF2-G3 (5′-TCYAAYTAYGCYCAGTAYC-GGGT-3′) in the second round. Conditions for amplification were the same for both rounds: 92 °C for 2 min, 35 cycles at 95 °C for 15 s, 52 °C for 30 s and 72 °C for 2 min and final elongation step at 72 °C for 10 min. The PCR products were run on 1.5% agarose gel and purified using Illustra GFX PCR DNA and GEL BAND Purification kit (GE Healthcare). The purified PCR products were quantified and then sequenced on the ABI 3730 automated capillary sequencer (Apply Biosystems) using the primers R3O, ORF2-FWD1, ORF2-G3, MENG-F1 (5′-GTYATGYTY-TGCATACATGGCT-3′), MENG-R1-FWD (5′-GACAGAATTGATTTCGTC GGC-3′), MENG-R1 (5′-AGCCGACGAAATYAATTGTGTC-3′) and MENG-R0 (5′−CCCTTATCCTGCTGAGCATTCTC-3′).

Generated sequences were analysed using DNAstar (Lasergene). Alignments were undertaken to determine phylogenetic relationships between the sequences from this investigation and the designated subgenotype reference sequences [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.

4. Results

870 patients (259 HSCT, 262 L T 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. In total 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.

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 Fig. 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. Two 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 Graft vs. Host disease 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.

5. 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/

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	